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# A SYSTEMS BIOLOGY PERSPECTIVE ON THE CONSEQUENCES OF ANEUPLOIDY IN HUMAN CELLS


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Milena Dürrbaum contributed to this work by performing the transcriptome analysis presented in figure 4E and supplementary table 1. In addition she contributed to the discussion of the manuscript.

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# 1 Abbreviations

| | |
|---|---|
| AGO | Argonaute protein |
| APC/C | Anaphase-Promoting Complex/ Cyclosome |
| ATM | Ataxia Telangiectasia Mutated |
| ATP | Adenosintriphosphat |
| ATR | Ataxia Telangiectasia and Rad3-Related Protein |
| BRAF | B-Raf Proto-Oncogene |
| BRCA2 | Breast Cancer 2 |
| BUB1 | Budding Uninhibited By Benzimidazoles 1 |
| BUB3 | Budding Uninhibited By Benzimidazoles 3 |
| BUBR1 | Budding Uninhibited by Benzimidazole-Related 1 |
| CCR4-NOT | Negative Regulator Of Transcription |
| CDC20 | Cell Division Cycle 20 |
| CDC23 | Cell Division Cycle 23 |
| CDC28 | Cell Division Cycle 28 |
| CDK4 | Cyclin-Dependent Kinase 4 |
| cDNA | complementary Deoxyribonucleic Acid |
| CHIP | Chromatin Immunoprecipitation |
| CHK1 | Checkpoint Kinase 1 |
| CHK2 | Checkpoint Kinase 2 |
| CIN | Chromosomal Instability |
| CIN70 | Chromosomal Instability transcriptional signature 70 |
| cDNA | complementary Deoxyribonucleic Acid |
| DCP2 | Decapping MRNA 2 |
| DDX6 | DEAD-Box Helicase 6 |
| DGCR8 | DiGeorge Syndrome Critical Region 8 |
| eIF4A | Eukaryotic Translation Initiation Factor 4A |
| eIF4G | Eukaryotic Translation Initiation Factor 4 Gamma |
| ERBB2 | Erb-B2 Receptor Tyrosine Kinase 2 |
| ESR | Environmental Stress Response |
| FISH | Fluorescence In Situ Hybridization |
| GAL1 | Galactokinase |
| GIN | Genomic Instability |
| HBV | Hepatitis B Virus |
| HCV | Hepatitis C Virus |
| HMGA2 | High Mobility Group AT-Hook 2 |
| HPV | Human Papillomavirus |
| HSF1 | Heat Shock Transcription Factor 1 |
| HSP70 | Heat Shock Protein 70kDa |
| HSP90 | Heat Shock Protein 90kDa |
| HSPA8 | Heat Shock Protein Family A (HSP70) Member 8 |
| iASPP | Inhibitor of Apoptosis-Stimulating Protein of p53 |
| kb | kilobase |

| | |
|---|---|
| KRAS | Kirsten Rat Sarcoma Viral Oncogene Homolog |
| LATS1 | Large Tumor Suppressor Kinase 1 |
| LATS2 | Large Tumor Suppressor Kinase 2 |
| MAD1 | Mitotic Arrest Deficient 1 |
| MAD2 | Mitotic Arrest Deficient 2 |
| p38/MAPK | p38 Mitogen-Activated Protein Kinase |
| MCM | Minichromosome Maintenance Complex |
| MDM2 | Mouse Double Minute 2 |
| MEF | Mouse Embryo Fibroblast |
| MHC | Major Histocompatibility Complex |
| miRNA | microRNA |
| miRNAome | microRNAome |
| MPS1 | Monopolar Spindle 1 |
| MS | mass spectrometry |
| MYC | V-Myc Avian Myelocytomatosis Viral Oncogene Homolog |
| NCBI | National Center for Biotechnology Information |
| NCI-60 | National Cancer Institute -60 human tumor cell lines |
| NGS | Next-Generation Sequencing |
| p53 | Tumor Protein P53 |
| p62/ SQSTM1 | 62 kDa protein/ Sequestosome 1 |
| PABP | Poly(A) Binding Protein |
| PAN2 /3 | PAB1P-dependent poly(A)-nuclease 2 /3 |
| RISC | RNA-inducing Silencing Complex |
| RNA | Ribonucleic Acid |
| RPKM | Reads Per Kilobase per Million |
| SAC | Spindle Assembly Complex |
| SILAC | Stable Isotope Labeling with Amino acids in Cell culture |
| SNP | Single Nucleotide Polymorphism |
| STAG2 | Stromal Antigen 2 |
| TCGA | The Cancer Genome Atlas |
| TFEB | Transcription Factor EB |
| TOP | Terminal Oligopyrimidine Tract |
| UBP6 | Deubiquitinating Enzyme 6 |
| UPR | Unfolded Protein Response |
| UTR | Untranslated Region |
| VHL | von Hippel-Lindau tumor suppressor |
| XRN1 | 5'-3' Exoribonuclease 1 |

## 2 Summary

Aneuploidy describes an abnormal unbalanced karyotype that has severe consequences for the cellular physiology and is associated with diseases such as cancer. In model cell lines, aneuploidy leads to impaired proliferation, disturbed proteostasis and specific transcriptome as well as proteome changes. Yet, the common molecular mechanisms underlying the response to aneuploidy are not well understood.

The work presented in this thesis was set out to study the response to aneuploidy and to elucidate the involved pathways from a systems biology perspective. We previously generated whole-chromosome aneuploid model cell lines with one or two extra chromosomes and post-tetraploid cell lines with a complex aneuploid karyotype.

Large-scale analysis of transcriptome data revealed that aneuploidy results in a conserved and distinct transcriptional response independent of the cell type, origin and type of aneuploidy. This response is characterized by the deregulation of similar pathways. In addition, we identified 23 aneuploidy markers that are similarly deregulated in a panel of aneuploid cell lines.

In the second study, we found that the transcriptional response to aneuploidy is remarkable similar to the transcriptome changes of cells with deficient heat shock transcription factor (HSF1). Indeed, we demonstrated that aneuploid cells suffer from proteotoxic stress with compromised activity of HSF1 and reduced HSP90 folding capacity. Global proteome analysis uncovered that the HSP90 client proteins are downregulated and that the overall aneuploid proteome resembles the proteome changes after HSP90 inhibition.

In the third study, we demonstrated that also the microRNAome (miRNAome) is altered in response to aneuploidy. Integrated miRNAome, transcriptome and proteome analysis indicated that the deregulated miRNAome negatively affects cellular development, growth and proliferation in the analyzed aneuploid cell lines. Besides the negative effect on the aneuploid cell physiology, the commonly upregulated microRNA (miRNA) hsa-miR-10a-5p may positively affected the survival of aneuploid cells by protecting them from stress- induced shut down of ribosomal protein translation.

In the fourth study, we followed the fate of surviving cells after whole-genome doubling, which is detrimental for the majority of the cells. The resulting complex aneuploid cells exhibit high chromosomal and genomic instability as well as a higher tolerance to mitotic errors. Transcriptome analysis revealed abnormalities in the p53 pathway suggesting an underlying mechanism to the tolerance and survival of complex aneuploid cells.

Taken together, this systems biology perspective on the response to aneuploidy revealed common affected pathways by the deregulated miRNAome, transcriptome and proteome. Moreover, our results suggest that it is the proteotoxic stress, which underlies the conserved transcriptome and proteome changes. In addition, we show that the deregulated miRNAome might contribute to the previously observed proliferation defect of aneuploid cells. Thus, the “omics” perspective on the consequences of aneuploidy as presented in this thesis benefits the aneuploidy research not only by systematic and global comparison of the aneuploid cell physiology in many different cell lines, but also by identifying potential molecular mechanisms in an unbiased manner. Besides a deeper

understanding of the aneuploid cell physiology, the results in this thesis deliver important indications for new approaches in therapeutic cancer research.

# 3 Zusammenfassung

Aneuploidie beschreibt einen veränderten, unnatürlichen Karyotyp, welcher weitreichende Auswirkungen auf die Physiologie der Zelle hat. Aneuploidie tritt häufig im Zusammenhang mit Krankheiten auf. Das bekannteste Beispiel ist Trisomie 21, auch Down-Syndrom genannt, aber auch Krebszellen weisen einen aneuploiden Karyotyp auf. In Modellsystemen führt Aneuploidie zu beeinträchtigter Zellproliferation, gestörter Proteostase und spezifischen Veränderungen des Transkriptoms und Proteoms. Die zugrundeliegenden Mechanismen für diese Auswirkungen von Aneuploidie sind jedoch nicht völlig verstanden.

Die vorliegende Arbeit hatte zum Ziel die Konsequenzen von Aneuploidie systembiologisch zu untersuchen und die involvierten Signalwege aufzuklären. Hierfür wurde in vorangegangenen Forschungsarbeiten sowohl aneuploide Zelllinien mit einem oder zwei zusätzlichen Chromosomen, sowie Zelllinien mit einem komplexen aneuploiden Karyotyp generiert.

Transkriptom Datenanalysen zeigten, dass Aneuploidie zu konservierten Veränderungen des Transkriptoms führt, unabhängig von dem Zelltyp, des Ursprungs oder der Art der Aneuploidie. Charakteristisch für diese Transkriptomveränderungen ist die Deregulation gleicher zellulärer Signalwege. Zusätzlich konnten wir 23 Aneuploidiemarker identifizieren, welche die gleiche Deregulation in einer Reihe von aneuploiden Zelllinien zeigten.

In der zweiten Studie fanden wir heraus, dass die Transkriptionsveränderungen in aneuploiden Zellen ähnlich zu den Veränderungen von Zellen mit defektem Hitzeschock Transkriptionsfaktor (HSF1) sind. Tatsächlich konnten wir zeigen, dass aneuploide Zellen unter proteotoxischem Stress stehen und beeinträchtigte HSF1 Aktivität sowie reduzierte HSP90 Proteinfaltungskapazität aufweisen. Proteomanalysen zeigten zudem, dass HSP90 Klientenproteine nach unten reguliert sind und das gesamte aneuploide Proteom dem der HSP90 Inhibition gleicht.

In der dritten Studie demonstrierten wir, dass auch das miRNAom in aneuploiden Zellen verändert ist. Analysen von miRNAom, Transkriptom und Proteom wiesen darauf hin, dass das deregulierte miRNAom die zelluläre Entwicklung, Wachstum und Proliferation negativ beeinträchtigt. Abgesehen davon, scheint die in der Mehrheit der aneuploiden Zelllinien hoch regulierte hsa-miR-10a-5p die Zellen vor Stress-induzierter Abschaltung der Translation ribosomaler Proteine zu schützen.

In der vierten Studie charakterisierten wir überlebende Zellen nach einer zelltoxischen Genomduplikation. Die resultierenden komplex-aneuploiden Zellen wiesen eine hohe chromosomale und genetische Instabilität, sowie Toleranz für mitotische Fehler auf. Transkriptomanalysen zeigten Abnormalitäten im p53 Signalweg, was auf den zugrundeliegenden Mechanismus des Überlebens dieser Zellen hinweist.

Durch unsere systembiologische Perspektive auf die Konsequenzen von Aneuploidie fanden wir heraus, dass gleiche Signalwege durch die Deregulation von miRNAom, Transkriptom und Proteom betroffen sind. Zudem legen unsere Resultate nahe, dass der proteotoxische Stress den konservierten Veränderungen von Transkriptom und Proteom zugrunde liegt. Des Weiteren zeigten wir, dass das deregulierte miRNAom möglicherweise zu dem Proliferationsdefekt aneuploider Zellen beiträgt. Die in der

vorliegenden Arbeit beschriebene „omics“ Perspektive auf die Konsequenzen von Aneuploidie trägt nicht nur durch systematische und globale Untersuchung der aneuploiden Zellphysiologie zum Verständnis von Aneuploidie bei, sondern auch durch die unvoreingenommene Identifizierung potentieller molekularer Mechanismen. Neben dem vertieften Verständnis für die aneuploide Zellphysiologie, liefert diese Forschungsarbeit neue Ansatzpunkte für die therapeutische Krebsforschung.

# 4 Aims

Aneuploidy describes an abnormal karyotype and is detrimental for cell physiology. The aneuploid karyotype is highly variable as structural and numerical aneuploidy can occur and may affect different chromosomes to a different extent. Yet, the observed phenotypes in aneuploid model cell lines appear remarkably similar. This suggests that the molecular determinants of the physiological response are common to the majority of aneuploid cells. In this thesis, I set out to identify the common determinants of the response to aneuploidy by applying in-depth analysis of transcriptome, proteome and microRNAome data to multiple different aneuploid model cell lines.

First, I asked whether there is a similarity in the transcriptional response to aneuploidy among different cell lines, different chromosomes and different origins of aneuploidy. To address this question, we acquired transcriptome data of human aneuploid model cell lines previously generated in our research group. In addition, I used publicly available transcriptome data from model aneuploid cell lines generated in other laboratories. To uncover similarities and differences in the transcriptional response to aneuploidy, I systematically analyzed and compared the aneuploid transcriptomes. To elucidate the triggers of the global gene expression changes, I compared the transcriptional response to aneuploidy to transcriptional changes in response to various cellular stresses.

Second, we addressed the hypothesis that the presence of additional chromosomes affects cellular proteostasis. To test this hypothesis, we investigated the capacity of aneuploid cells to fold proteins under normal and stress conditions. We further determined the molecular cues of the compromised proteostasis in aneuploid cells. To elucidate the effects on the proteostasis network from a systems perspective, I analyzed the large-scale transcriptomics and proteomics datasets available in our laboratory.

Third, we investigated another category of post-transcriptional regulators of the response to aneuploidy – miRNAs. We hypothesized that the observed global gene expression changes as well as the molecular and phenotypic consequences of aneuploidy might be at least partially governed by miRNA regulation. To gain a comprehensive insight into the miRNA-target landscape in aneuploid cells, we sequenced miRNAs as well as mRNAs and combined this dataset with previously derived proteome data. I performed sequencing data analysis and compared the miRNA deregulation of different aneuploid cell lines to identify similarities and differences in response to various types of aneuploidy. To unravel the contribution of miRNAs to the cellular response to aneuploidy, I performed integrated analysis of miRNA and their target mRNA and protein expression levels.

The fourth aim was to analyze the long-term consequences of tetraploidy that often results in high genomic instability and complex aneuploidy. Since high rates of genomic instability are lethal to tetraploid cells, we hypothesized that the rare surviving cells have evolved mechanisms to tolerate genomic instability. Therefore, we followed the fate of rare tetraploid surviving cells and monitored their mitotic efficiency and fidelity. Further, we analyzed the levels and proficiency of the key factors that could restrict cell cycle progression. Finally, to gain a systems perspective on compromised pathways, I performed in-depth analysis of the transcriptome of post-tetraploid cells.

# 5 Introduction

## 5.1 The leap of big data – a new perspective on aneuploidy

### 5.1.1 Technological advances enable new systematic insights into biological problems

Cells are the smallest entities of life and although diverse in function and appearance, share common properties: the information of the building blocks of a cell is encoded in the DNA as genes, the transcripts of these genes are stored in RNA molecules and the translated building blocks of a cell are composed of proteins. In a human cell, the DNA encodes for approximately 20,000 genes (Pertea and Salzberg, 2010). From these genes, up to 16,000 protein-coding RNA molecules and more than 10,000 proteins can be detected in human cells (Nagaraj et al., 2011). The entire complexity of a cell might be orders of magnitudes higher. For instance, protein modifications, diverse subcellular locations and assembly to different complexes resulting in differential functions of a protein add an additional complexity (Harper and Bennett, 2016). Moreover, the functionality of a cell is intrinsically determined by the differential gene expression and the different protein levels. Therefore, differences in the composition of all mRNAs (transcriptome) and all proteins (proteome) of a cell, but also the quantitative levels of mRNAs and proteins give important insights into the cellular state and the differences for example between tissue cells or health and disease states. Nowadays, technological advances accelerate our understanding of the cellular system in health and disease. Yet, the ever increasing amount, but also the complexity of the biological data bears a major challenge to researchers and the term “big data” has entered biological science (Check Hayden, 2015).

Big data science is characterized by the same properties as big data in other areas. That is, big data does not only refer to a growing amount of information, but also indicates a wide variety of the data types and a high velocity at which the information is gathered (Gandomi and Haider, 2015). Depending on the field the definitions slightly differ and sometimes include characteristics of big data analytics such as cross-relation of the information types in order to generate new information. For example in market research, cross-relation of data from social networks, tweets and blogs with consumer product preferences allows to assess the target group needs and to optimize business strategies. In big data science, the thousands of molecules in a biological system, such as the cell, make up the volume of information. Moreover, the state of these molecules, for example the phosphorylation state of a protein, adds an additional layer to the data complexity and makes up the variety of the data that can be gathered from a cell. Cross-relation of the information types, such as the expression of RNA and the level of the corresponding protein generates new information similar as in the market research example.

In summary, the mass of DNA (genome), RNA (transcriptome) and proteins (proteome) and also the low molecular weight components (metabolome) describe a biological cell from a holistic view- the “omics” perspective (Figure 1). To gain an “omics” perspective of a cell, simultaneous measurement of thousands of molecules is required. Nowadays, high-throughput technologies such as next-generation sequencing (NGS) and

mass spectrometry allow qualitative and quantitative analysis of the "omics" of a cell at reduced time and cost.

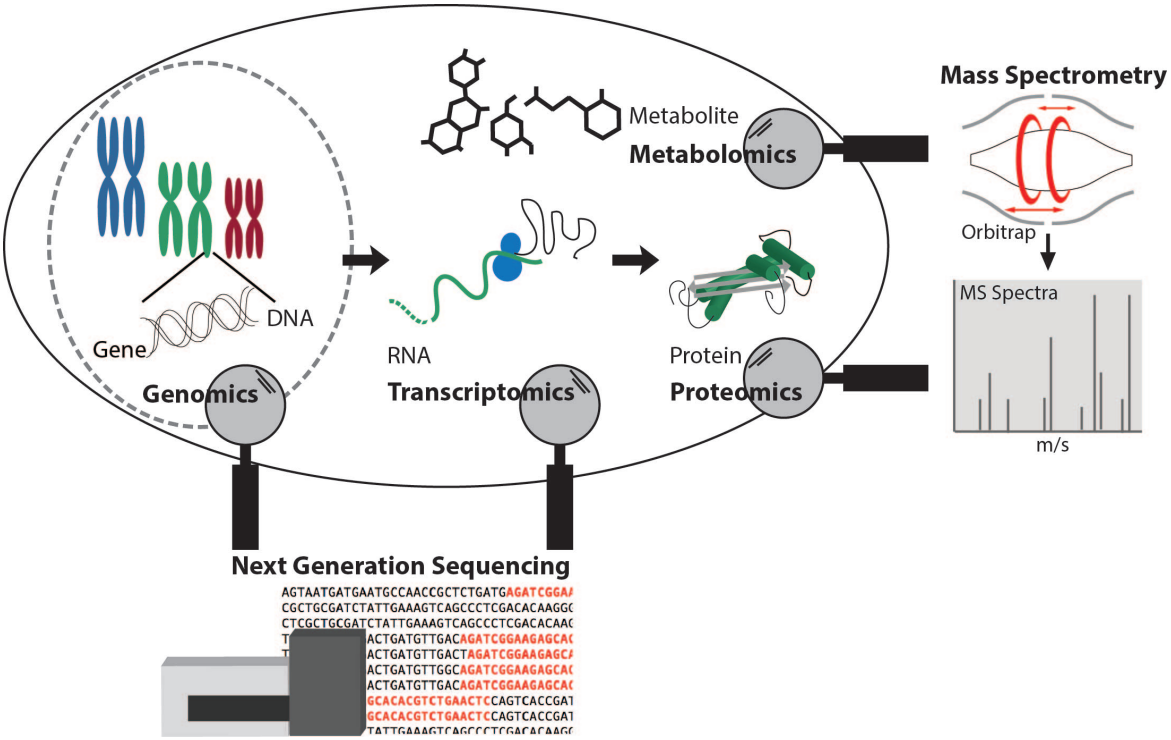


**Figure 1. The "omics" perspective of a cell.** The DNA, packed in chromosomes, encodes the majority of the genes of a cell. In genomics, the DNA is analyzed by next-generation sequencing. Genes are transcribed into RNA. In transcriptomics, the quantity and quality of RNA molecules is measured by next-generation sequencing. RNA molecules are translated into proteins. In proteomics, all proteins of a cell are measured by mass spectrometry. For instance performing mass spectrometry on an Orbitrap results in mass over charge mass spectrometry spectra. In addition, new "omics" fields emerged. For instance, the measurement of the metabolites of a cell by mass spectrometry.

*Technological advances in genomics and transcriptomics*

The essential method to assess the genome or transcriptome of a cell is DNA sequencing. The sequencing methods have been rapidly advancing in the last ten years, largely fueled by the Human Genome Project that was completed in 2003. The human genome was first fully sequenced by the Sanger chain termination sequencing method described in 1977. Even though Sanger sequencing evolved for instance by capillary electrophoresis systems, the required cloning steps and the low parallelization limited the speed and accuracy of this sequencing method (reviewed in (Mardis, 2013)). This limitation promoted the development of NGS methods. The advances of NGS over Sanger sequencing is best illustrated by the fact that whereas the Human Genome Project took 15 years for its completion with Sanger sequencing, the Illumina next-generation sequencer released in 2014 sequences 45 genomes in one day (Illumina, 2016). The introduction of NGS platforms has also revolutionized transcriptome profiling. Before NGS sequencing became broadly available, microarrays were the tool of choice for the quantification of RNA molecules. However, microarrays are limited by the extent of known RNA molecules, which are hybridized on an array, as well as by the dynamic detection range (Wang et al.,

2009b). In NGS-based transcriptome profiling, the RNA molecules are reverse transcribed to cDNAs that can be sequenced with the same methods as DNA.

A number of different NGS platforms exist today that differ in their sequencing approach and therefore also in the possible sequence length, error rate, speed and application range (reviewed in (Mardis, 2013; Goodwin et al., 2016)). One of the widely used sequencing systems is the Illumina system that belongs to the group of short read next-generation sequencers. In short read sequencing approaches the DNA or cDNA is fragmented and the fragments are ligated to universal adapters that allow amplification and later sequencing. The fragments are immobilized by adapter hybridization to linkers that are covalently attached to either beads or a solid surface. Amplification of the fragments creates clusters of each single DNA fragment amplifying the sequencing signal for the sequencing step. The two major methods for short read sequencing of genomes or transcriptomes are sequencing by ligation and sequencing by synthesis. The latter is employed on Illumina platforms and involves DNA polymerase and step-wise incorporation of nucleotides into a complementary elongating strand. The identity of the incorporated nucleotide is read out by ion concentration or fluorophore release. This method allows low and high coverage sequencing of genome and transcriptome as well as exome-sequencing, chromatin immunoprecipitation (CHIP) and DNA methylation sequencing. While the accuracy rate is above 99.5%, the major drawback is the increasing error rate with increasing read length. In third generation sequencers this major limitation of short read sequencers is overcome by new sequencing approaches such as in the nanopore sequencer (Munroe and Harris, 2010). After the sequencing step, the sequence of each fragment has to be mapped to a reference genome or assembled to the original DNA or RNA.

Sequencing of DNA or cDNA reveals the sequence identity of the genome or transcriptome of an analyzed sample. For example, mapping the reads to a reference genome can identify structural variations. Moreover, if the read depth is high enough, single nucleotide polymorphisms can be identified. It should be noted that DNA microarray technologies are still widely used for the identification of common polymorphisms or known structural variations. In addition to qualitative measurements, NGS methods allow also the quantification of the sequenced molecules, i.e. RNAs. In this case, sequenced reads are mapped to a reference genome. The number of reads that map to a transcript is linearly related to its abundance in the cell. Since the number of reads scales also with transcript length, normalization to length and total number of mapped reads resulting in reads per kilobase (kb) of transcript per million mapped reads (RPKM) is widely applied (Mortazavi et al., 2008). With the advances of sequencing technologies also the data analysis strategies such as correction for technical and biological variability and statistical testing have evolved. For instance, systematic variability between the sequencing runs and between library preparations of different samples has to be accounted for (Marioni et al., 2008; Bullard et al., 2010). While technical variability can be modeled by Poisson distribution, the variability between biological replicates would be underestimated by the Poisson model (Anders and Wolfgang, 2010). Therefore, to test whether a difference in read count is greater than expected by random variation a binominal distribution is employed to model the variance. Since the number of biological replicates is small in most of the sequencing studies, methods such as DeSeq2 (Love et al.,

2014) overcome this limitation by pooling information about the variances of different genes with the same read count. Applying these normalization and dispersion estimate methods to the raw read counts enables quantification of the RNA molecule levels in a sample. Thus, novel normalization methods have been developed in parallel to sequencing techniques allowing nowadays a robust quantification of genome and transriptome.

*Technological advances in proteomics*

Proteins execute structural, as well as enzymatic and signaling functions in the cell. They are biochemically diverse and dynamic by nature, differing not only in the amino acid sequence, but also in their secondary, tertiary and quaternary structures. Profiling of the identity and quantity of almost all proteins in a cell can be achieved by high-resolution mass spectrometry based proteomics. The most widely used approach is the "bottom-up" approach, in which proteins of a sample are digested into short peptides. The identity of each peptide is determined by mass spectrometry and used to reassemble the proteins. To deconvolute the complex peptide mixture a chromatographic separation is employed before the mass spectrometric analysis. For the peptide identification in the mass spectrometer, mass-over-charge ratios of molecules are determined. To this end, the Orbitrap mass analyzer is frequently used where ions are trapped on orbital trajectories along a spindle-form like electrode. Their oscillation frequency solely depends on the m/z (m = mass; z = charge) and is recorded as an induced current in two outer electrodes. While the frequency provides information on the identity of the ion, the amplitude of the induced current indicates the amount of that ion. In a complex protein sample, peptides with similar masses but different amino acid sequences exist. Therefore, peptides are fragmented (MS2 or tandem MS) and the exact sequence is determined based on the fragment masses. The combined information of the full ion MS1 spectra and the fragmented ion MS2 spectra facilitates the bioinformatic identification (via search engines like Mascot and Andromeda) of the peptides from which the abundance of individual proteins are calculated.

Mass spectrometry can be used to quantify the protein abundance (reviewed in (Aebersold and Mann, 2003; Cox and Mann, 2011)). One of the most frequent approaches is stable isotope labeling in cell culture (SILAC). This involves incorporation of a heavy isotope labeled arginine or lysine into one condition, which has similar biochemical properties as the light isotope labeled amino acids that are incorporated into the other condition. Subsequently, the samples are pooled and subjected to MS where they can be distinguished by the mass over charge offset in the MS1 spectrum. The relative abundances between the treated and untreated conditions are determined by peak intensities. With a state of the art mass spectrometer and bioinformatics data analysis pipeline, it is possible to measure more than hundred thousand different peptides including their posttranslational modifications. This allows the identification and quantification of over 10,000 proteins in cellular systems and has hugely contributed to our understanding of living organism since its broader application in recent years.

*Big data science complexity*

The technological advances in the fields of genome, transcriptome and proteome profiling results in vast amounts of information. The majority of the data is accessible for researchers in public databases such as the NCBI Human Genome Resources (https://www.ncbi.nlm.nih.gov/genome/), NCBI Gene Expression Omnibus (https://www.ncbi.nlm.nih.gov/geo/) or The Human Proteome Atlas (http://www.proteinatlas.org/). Previously it used to be challenging to acquire the data; today's challenge is to make sense of the data: to analyze, integrate, visualize and communicate the findings buried in numerous data sets. This is best exemplified with the international cancer genome sequencing efforts such the "International Cancer Genome Consortium" and the component of it, the "The Cancer Genome Atlas" (TCGA) Project of the U.S. National Cancer Institute (https://cancergenome.nih.gov/). One of the goals of these projects, to fully catalogue cancer genomes and common genomic alteration, has been reached. With the announced completion, nearly 10 million cancer-related mutations could be catalogued by 2015 (Ledford, 2015). However, the hope that this would provide the ultimate understanding of cancer has not been fulfilled. Instead, the sequencing projects uncovered a much higher complexity of the genomic alterations than ever anticipated. For instance, intra- and inter-tumor heterogeneity, tumor subtype specificity and driver versus passenger mutations complicate a clear picture of the cancer landscape (Vogelstein et al., 2013). Thus, the analysis of the generated data is one of the major challenges of scientists nowadays (Adams, 2015). Moreover, the effects on cellular pathways must be anticipated in order to understand the biological relevance of certain mutations. Therefore, integration with other "omics" data such as proteomics and metabolomics is necessary to interpret the impact of genomic alterations in cancer. In addition, clinical data will help the transition from knowledge to clinical application. For example, correlation of certain mutation types with a population subgroup helps to individualize the treatment. Along these lines, an additional challenge lies in the communication of the information on cancer genome alterations to the clinic. Bioinformatics tools to visualize the information, user interfaces to easily access and filter the data are therefore indispensable.

In summary, progress in high-throughput technologies to sequence genome and transcriptome as well as measure protein levels has accelerated the information gathered about complex biological systems, such as the human genome or a cancer cell. Understanding the relevance of the information and setting the information into the biological context remains a challenge to researchers.

### 5.1.2 A big data science perspective on aneuploidy in health and disease

Technological advances of high-throughput sequencing methods facilitate not only the detection of sequence aberrations such as variations smaller than 1 kb (e.g. single nucleotide polymorphisms (SNPs) or small indels), but also structural variations ranging from 1 kb to several mega base pairs (e.g. insertions, deletions, inversions, translocations as well as copy number variations (Feuk et al., 2006; MacDonald et al., 2013)). Moreover, it is nowadays possible to infer copy number variations as large as a chromosome arm or even gain or loss of a whole chromosome, since specialized algorithms retrieving this information from DNA sequencing data have been developed in the last years (for example (Bao et al., 2014; Holt et al., 2014)). Large-scale genome alterations are also referred to as aneuploidy. Whereas whole-chromosome aneuploidy refers to gain or loss of entire chromosomes, segmental aneuploidy describes alterations in chromosomal regions such as a chromosome arm and structural aneuploidy stems from copy number variations (Figure 2). The ability to detect aneuploidy in a fast, reliable and cost effective manner has become important because of their detrimental effects for human development and the association with cancer and neurodegenerative diseases.

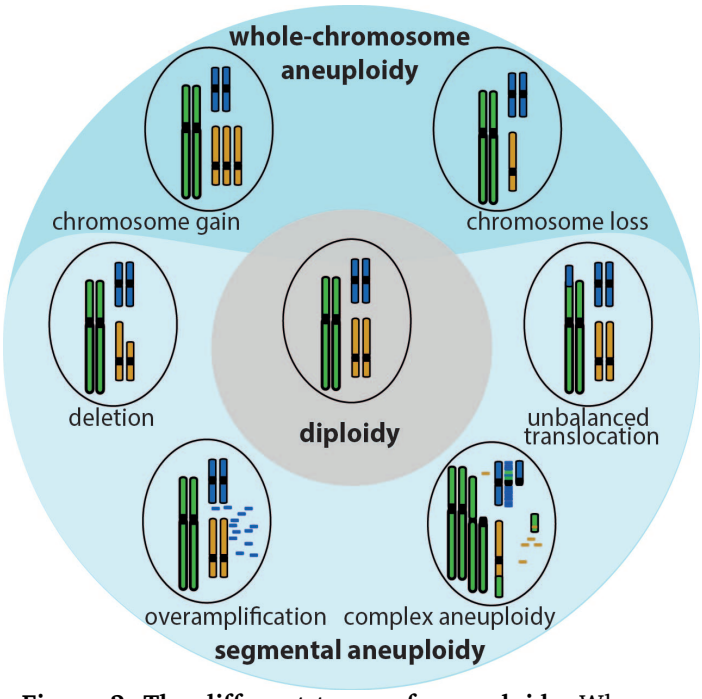


**Figure 2. The different types of aneuploidy.** Whereas the diploid karyotype is balanced, the unbalanced aneuploid karyotype is characterized by either numerical or structural chromosome changes. Whole-chromosome aneuploidy describes the gain or loss of one or more chromosomes. Segmental aneuploidy describes the deletion, amplification or unbalanced translocation of chromosome segments. Complex aneuploidy is characterized by complex segmental changes of different chromosomes, often combined with numerical chromosome changes.

*Constitutional aneuploidy in humans*

In a multicellular organism, aneuploidy can either affect all cells, so called constitutional aneuploidy, or a subset of the cells, called somatic aneuploidy. In case of constitutional aneuploidy, aneuploidization occurs during the germ development (reviewed in (MacLennan et al., 2015)). In addition, mitotic errors during early embryogenesis result in mosaic aneuploidy with intermixed diploid and trisomic cells that are for example commonly observed in trisomy 21 patients (reviewed in (Papavassiliou et al., 2014)). Estimated 10-30% of fertilized oocytes harbor an abnormal copy number and

the percentage increases with maternal age in humans (MacLennan et al., 2015). In addition, *in vitro* fertilized embryos have high rates of chromosomal aneuploidy. Aneuploidy is the leading cause of spontaneous miscarriages and only gain of a copy of chromosome 13, 18, 21, X and Y or loss of chromosome X or Y are compatible with a viable birth. Due to the severe developmental defects, only approximately 10% of children with trisomy 13 (Patau syndrome) or trisomy 18 (Edwards syndrome) survive to one year after birth (Rasmussen et al., 2011). Individuals with trisomy of chromosome 21, also known as Down syndrome, survive until adulthood, but suffer from multiple defects such as intellectual disability, increased rates of congenital heart disease, epilepsy and acute leukemia (Asim et al., 2015).

To improve the success of fertilization, prenatal or pre-implantation screening of embryos for aneuploidy is offered. Prenatal screening has greatly benefited from continued improvements of NGS technologies. Noninvasive prenatal screening methods subject cell free fetal DNA in the maternal blood to massive parallel shotgun sequencing, where the number of sequences mapping to maternal chromosomes versus cell free DNA is predictive of aneuploidy (Gregg et al., 2014; Rink and Norton, 2016). Other screening methods such as fluorescence *in situ* hybridization (FISH) and array comparative genomic hybridization are being replaced by NGS techniques (Fiorentino et al., 2014; Zheng et al., 2015). Comparative analysis of the methods proved high concordance and enhanced precision for the sequencing methods, thus paving their way for routine clinical application.

*Somatic aneuploidy in humans*

Whereas constitutional aneuploidy is detrimental for life, somatic aneuploid cells were identified in the liver and brain of healthy individuals (Duncan et al., 2012a). Indeed, the percentage of aneuploid hepatocytes ranges from one third to one half in healthy liver samples. Studies in mice and human suggest that hepatocytes become polyploid during early postnatal development and some cells subsequently become aneuploid through aberrant mitosis and chromosome missegregation (Margall-Ducos et al., 2007; Duncan et al., 2010; 2012a). In this context aneuploidy seems to be beneficial. One hypothesis poses that aneuploid cells allow phenotypic variability and increase the adaptive potential of liver tissue to chronic injury or metabolic stresses (Duncan et al., 2012a). Similarly, aneuploidy was detected in the human developing brain tissue and across adult brain tissue in non-neuronal cells and mature neurons by spectral karyotyping or FISH (Rehen, 2005; Yurov et al., 2007; Pack et al., 2014). Surprisingly, only 2.2% and 4% of aneuploid cells were detected in the human brain and in human hepatocytes, respectively, using single cell NGS, a method that determines whole chromosome aneuploidy and complex aneuploidy in somatic cells in an unbiased manner (Knouse et al., 2014). The authors attribute the deviations between the results from single cell sequencing and previous reports to the frequent artifacts in FISH assays. Nevertheless, high rates of structural aneuploidy and copy number variations can be identified in neurons (Cai et al., 2014). Thus, the higher resolution of genome-wide sequencing compared to classical approach such as FISH suggests that while somatic whole-

chromosome aneuploidy is not as widespread as previously assumed, large structural copy number variations occur frequently in neurons.

*Aneuploidy in ageing and neurodegeneration*

Besides these instances of somatic aneuploidy in healthy tissue, aneuploidy has been associated with declining cellular fitness and ageing. Evidence comes from mouse, where the ageing is associated with increased incidence of aneuploidy in the brain (Faggioli et al., 2012). Further, mouse models of the mutant spindle assembly checkpoint protein BUBR1 that leads to high chromosome missegregation rates develop aneuploidy. These mice develop premature ageing-associated phenotypes such as short lifespan, loss of subcutaneous fat, impaired wound healing and vascular ageing (Baker et al., 2004; Matsumoto et al., 2007). Concordantly, overexpression of BUBR1 preserves genomic stability and extends lifespan in mice (Baker et al., 2012). Whether aneuploidy is a passenger of the ageing process due to defects in genome maintenance or whether aneuploidy plays an active role is not completely understood. Interestingly, increased aneuploidy is often observed in Alzheimer´s disease, as FISH analysis revealed high rates of aneuploidy of chromosome 11, 17, 18, 21 and X with a prevalence for chromosome 21 and X (Iourov et al., 2009; Yurov et al., 2014). Intriguingly, the amyloid precursor protein (APP) that has a pivotal role in Alzheimer´s disease is encoded on chromosome 21. Overexpression of APP leads to increased levels of neurotoxic amyloid peptide Ab42 that is associated with plaque formation in Alzheimer's disease. Aneuploidy of chromosome 21 may therefore promotes this disease. Yet, single cell NGS did not confirm higher rates of aneuploidy in 893 neurons derived from 10 individuals with Alzheimer´s disease (van den Bos et al., 2016). Despite these recent results, the fact that the majority of individuals with Down syndrome are diagnosed with an early onset Alzheimer´s disease by the age of 40 (Gardiner et al., 2010) hints to an association of trisomy of chromosome 21 and Alzheimer´s disease or impaired neuronal function. Further NGS and functional studies on a larger cohort will be necessary to elucidate the exact association between aneuploidy and ageing as well as neurodegenerative diseases.

*Aneuploidy and cancer*

Already a century ago, Theodor Boveri postulated that unbalanced chromosome numbers might contribute to malignant tumor development (Boveri, 1914). His hypothesis has gained significant experimental support recently and aneuploidy is nowadays considered a hallmark of cancer (Sheltzer and Amon, 2011). Primary evidence stems from cytogenetic studies of patient derived tumor samples revealing that 90% of solid tumors and 70% of blood cancers show some degree of aneuploidy (Weaver and Cleveland, 2006; Mitelman et al., 2016). Although cytogenetic techniques are widely applicable in hospital settings, their low resolution limits the detection of structural aneuploidy. The complex landscape of somatic copy number variations in cancer has been revealed by high-throughput methods such as SNP-Arrays and NGS. On average as much as 25% of each

genome is affected by somatic copy number variations of a whole chromosome arm in an analysis of 3131 cancer samples by SNP arrays (Beroukhim et al., 2014).

The role of aneuploidy in tumorigenesis is not entirely clear. One central question is, whether aneuploidy is a driver of tumor development or arises as a side effect of the relaxed checkpoints and increased instability. From an "omics" perspective, this question has been tackled by identifying the relation between copy number variations and well-known tumor suppressors or oncogenes, as well as by studying the evolutionary timeline of aneuploidy in cancer. Large-scale analysis of copy number variations in cancers found 33% of amplifications associated with validated oncogenes such as MYC, Cyclin D1, CDK4 and KRAS. Additionally, 11% of deletions were found in regions with tumor suppressor genes (Beroukhim et al., 2014). This suggests that at least a subset of copy number alterations might have a causal role in tumorigenesis by affecting the expression of oncogenes and suppressors. For this analysis, only experimentally validated oncogenes and tumor suppressors have been explored. Yet, new data analysis methods can predict new drivers and suppressors by the analysis of somatic mutation patterns across large numbers of cancers (Davoli et al., 2013). Therein, the analysis of 8,200 tumor samples estimated 320 suppressors and 250 oncogenes. Intriguingly, the distribution and potency of these drivers and inhibitors of tumorigenesis is predictive for the pattern of aneuploidy observed in a range of tumor samples (Davoli et al., 2013; Zack et al., 2013). The potency of a gene to support or impair tumor development might pose a selective pressure for a gain or loss of the associated genomic region. Thus, aneuploidy might promote tumor development by affecting the gene copy numbers of drivers and suppressor.

Tumor development is driven by clonal evolution defined by acquisition of genetic variations and natural selection for beneficial traits (Stratton et al., 2009). However, the largely heterogeneous tumor cells hamper the evolutionary study of aneuploidy in cancer. Single-cell sequencing has advanced the study of tumor evolution and gives also insights into the occurrence of aneuploidy during tumor development. Copy number profiling of 100 single cells by NGS revealed defined aneuploid subpopulations within the tumor mass (Navin et al., 2011). Interestingly, the aneuploid subpopulation has separated in early tumor development from the diploid subpopulation based on chromosome breakpoint phylogeny analysis. Recent single-cell sequencing results further support the view that aneuploidy occurs early in tumor development and that mutations accumulate gradually, but account for the genetic diversity (Wang et al., 2015). While these results suggest that aneuploidy rather develops early in tumorigenesis, the complexity of aneuploidy and the rate by which aneuploidy changes, termed chromosomal instability (CIN), correlates with tumor aggressiveness (Carter et al., 2006; Birkbak et al., 2011). Indeed, elevated expression of a gene group that correlates with aneuploidy and CIN is sufficient to predict poor prognosis (Carter et al., 2006; Birkbak et al., 2011). In summary, compelling evidence indicates that aneuploidy is not a mere passenger of tumorigenesis, but causally contributes to tumor development and malignancy.

The manifold occurrences of aneuploidy in health and disease illustrate that the impact of aneuploidy on cell physiology and ultimately on cellular fitness is largely context dependent. Constitutional aneuploidy is detrimental for development, whereas somatic aneuploidy is found in both healthy cells and disease states. On one hand, aneuploidy

might serve as a source for genetic variation that allows faster adaptation to adverse condition, hence providing a selective advantage. For example, aneuploidy in liver was suggested as a mechanism to rapidly adapt to chronic live injury in mice (Duncan et al., 2012b). Similarly, aneuploid cancer cell lines are more tolerant to stress conditions (Rutledge et al., 2016). On the other hand, studies in various aneuploid model systems demonstrate that aneuploidy itself is largely detrimental for cells. To understand the context dependent effects of aneuploidy, it is essential to gain an in-depth knowledge of the molecular consequences of aneuploidy *per se*. Aneuploidy can occur in many variations affecting different chromosomes and genomic regions. Therefore, high-throughput system biology approaches allow the parallel and holistic study of diverse aneuploidy types and careful analysis of the data allows deducing the common consequences of aneuploidy on the cell physiology.

## 5.2 Routes to aneuploidy

To maintain a balanced karyotype, faithful segregation of the duplicated chromosome sets to the two daughter cells is crucial. Abnormalities in the karyotype in form of whole chromosome aneuploidy originate mainly from errors in chromosome segregation. At the beginning of mitosis accurate chromosome segregation is initiated by separation of centrosomes, the microtubule organizing centers of the cell and by nuclear envelope breakdown (Magidson et al., 2011). Each of the sisters of the chromatid pair that is held together by sister chromatid cohesion attaches via its kinetochore to microtubules nucleating from the centrosomes, thereby forming a bipolar mitotic spindle (Figure 3A). In order for each chromatid to be pulled to opposite sides of the cell, each kinetochore must attach to microtubules emanating from opposite centrosomes. Only if correct kinetochore-microtubule attachments are established, the mitotic spindle assembly checkpoint (SAC) allows separation of the chromatids to opposite spindle poles.

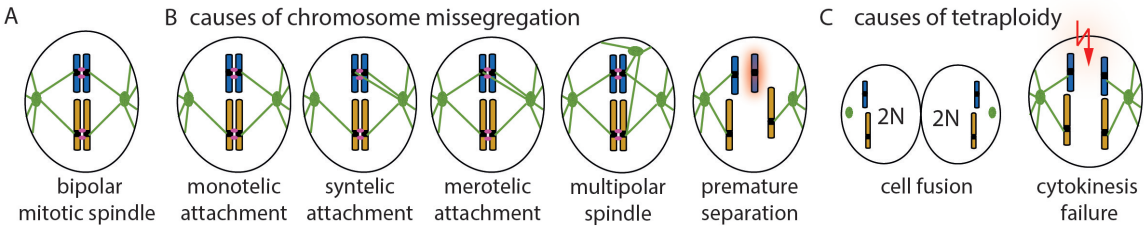


**Figure 3. Causes of aneuploidy. A** Normal, bipolar mitotic spindle. **B** Causes of chromosome missegregation include abnormal mitotic spindle formation. This can be the result of monotelic, syntelic and merotelic microtubule-kinetochore attachments. In addition, multipolar spindle geometry and premature separation of sister chromatids results in chromosome missegregation. **C** Aneuploidy may derive from a tetraploid intermediate. Causes of tetraploidy include fusion of two cells or failure of cytokinesis.

*Aneuploidy through chromosome missegregation*

Chromosome missegregation mainly stems from abnormalities in mitotic spindle formation and subsequent bypass or failure of the mitotic checkpoint. In case of a single unattached kinetochore (monotelic attachment, Figure 3B) the SAC core components of the mitotic checkpoint proteins MAD1, MAD2, BUB1, BUB3, BUBR1, Aurora B and MPS1 are recruited to the unattached sites (Heinrich et al., 2013). Two sister kinetochores attached to microtubules originating from the same centrosome (syntelic attachment, Figure 3B) are sensed by the chromosomal passenger complex that, via the activity of Aurora B kinase, destabilizes mal-attachments, thus facilitating correction and at the same time creating unattached kinetochores, which in turn trigger the recruitment of mitotic checkpoint proteins (reviewed in (Nezi and Musacchio, 2009; Wang et al., 2014)). As a result the co-activator CDC20 of the anaphase-promoting complex / cyclosome (APC/C) is inhibited and the mitotic progression is delayed. Recent data suggests that the protein abundances are critical to maintain a functional checkpoint as the reduction of MAD2 and MAD3 (BUBR1) to 60-30% of the normal abundance noticeably weakens the SAC function in fission yeast (Heinrich et al., 2013). Thus, depletion of one of the checkpoint components such as one *MAD2* allele in human colorectal cancer cell lines (Michel et al.,

2001) or *BUBR1* deletion in HeLa cells (Lampson and Kapoor, 2004) or mice (Jeganathan et al., 2007) is sufficient to compromise the mitotic checkpoint. Moreover, mutations of SAC components have been associated with increased rates of chromosome missegregation in cancer (Li and Benezra, 1996; Cahill et al., 1998; Percy et al., 2000; Wang et al., 2000; 2002; Kim et al., 2005). Increased rates of chromosome missegregation might also explain why offsprings of mice with heterozygous knockout of Mad1 or Mad2 show higher incidences of spontaneous tumors (reviewed in (Giam and Rancati, 2015)). In addition, mutations in *BUBR1* have been associated with the mosaic variegated aneuploidy syndrome that is characterized by CIN, mosaic aneuploidy and predisposition to childhood cancer. Thus, an impaired SAC should be in principle sufficient to drive aneuploidy and cancer development.

Although an impaired SAC induces aneuploidy, sequencing of cancer cell lines and tumor tissues revealed that SAC mutations are not among the core driver mutations in cancer (Sjöblom et al., 2006; Wood et al., 2007; Greenman et al., 2007; Jones et al., 2008; Parsons et al., 2008; Bleeker et al., 2009). This suggests that aneuploidy in cancer arises by other means. Merotelic attachments, where a single kinetochore attaches to two microtubules from opposite spindle poles, do not efficiently activate the mitotic checkpoint and therefore often result in lagging chromosomes and subsequent aberrant karyotypes in the daughter cells (Cimini et al., 2001; 2004). Intriguingly, lagging chromosomes are frequent in cancer cells with high rates of chromosome missegregation (Thompson and Compton, 2008), suggesting that merotelic attachments are a source of chromosome missegregation in cancer. Although merotelic attachments can be corrected in early anaphase, increased incidences or reduced correction efficiency of merotelic attachments elevate the frequency of lagging chromosomes (reviewed in (Cimini, 2008). One source for an increased frequency of merotelic attachments is a multipolar spindle formation by multiple centrosomes (Ganem et al., 2009; Silkworth et al., 2009). In fact, supernumerary centrosomes are frequent in cancer and were associated with increased incidences of chromosome missegregation (Lingle et al., 2002; Sato et al., 2001; Silkworth et al., 2009; Ganem et al., 2009). Additionally, decreased correction efficiency of merotelic attachments by hyperstabilization of incorrect microtubule-kinetochore attachments increases the rates of lagging chromosomes (Bakhoum et al., 2009; Kabeche and Compton, 2012). For instance, MAD2 overexpression stabilizes merotelic kinetochore attachments (Kabeche and Compton, 2012) and is associated with elevated rates of lagging chromosomes in cancer (Tanaka et al., 2001; Li et al., 2003; Sotillo et al., 2007; 2010; Hisaoka et al., 2008; Wang et al., 2009a; Kato et al., 2011). Thus, the major source of missegregated chromosomes is attributed to merotelic attachments.

Besides mal-attachments in early mitosis, premature separation of chromatids results in chromosome missegregation (Figure 3B). Sister chromatids are held together by sister chromatid cohesion that dissociates at chromosome arms in early mitosis, maintaining the cohesion at the centromeres. Centromeric cohesion facilitates correct bipolar kinetochore-microtubule attachments and metaphase alignment and is resolved only after the SAC is satisfied. Mutations in proteins of the cohesion complex, such as the inactivation of the cohesion subunit STAG2 in human cell lines mediates premature chromatid separation and hence chromosome missegregation (Solomon et al., 2011). Knock out of mouse Stag1 results in increased aneuploidy (Remeseiro et al., 2012).

Moreover, *STAG2* mutations or low expression have been identified in various human cancers (Solomon et al., 2011; 2013; Kim et al., 2012; Wilson et al., 2012).

In summary, many different mutations of SAC components can lead to increased chromosome missegregation and aneuploidy. Yet, these mutations are rarely found in cancer. Mutation in the cohesion subunit STAG2 presents one of the few links of mutations and aneuploidy in cancer. Merotelic attachments are most likely the major source of chromosome missegregation and aneuploidy in cancer.

*Aneuploidy through tetraploid intermediate*

Multiple observations in aneuploid cancers and cell lines revealed that aneuploidy might derive from a tetraploid intermediate state during cancer progression. First, in several aneuploid cancers, a subpopulation of tetraploid cells was reported (Wijkstrom et al., 1984; Kallioniemi et al., 1988; Levine et al., 1991; Dutrillaux et al., 1991; Michels et al., 2000; Barrett et al., 2003; Olaharski, 2006). Second, there is a clear correlation between the tetraploidy status and the progression to aneuploidy as well as p53 inactivation during neoplastic progression in Barrett's esophagus (Galipeau et al., 1996). Third, recent cancer sequencing projects provided compelling evidence that 37% of cancers underwent a whole-genome duplication at some point of tumorigenesis (Zack et al., 2013). More directly, tetraploid p53-nulls cells induced tumorigenesis when injected into nude mice, while diploids did not lead to tumor formation (Fujiwara et al., 2005). Lastly, experiments in cell lines demonstrated that induced cytokinesis failure leads to an unstable tetraploid intermediate that can evolve to aneuploid progenitor cells ((Vitale et al., 2010; Lv et al., 2014) and chapter 5.6 in this thesis).

Tetraploid cells emerge from three different mechanisms: cell fusion, cytokinesis failure or incomplete mitosis and endoreduplication (reviewed in (Davoli and de Lange, 2011)). Cell fusion can be induced by viral infection (Duelli et al., 2007) and results in a binucleated intermediate that gives rise to tetraploid daughter cells (Figure 3C). All human oncogenic viruses such as papillomavirus (HPV), hepatitis B virus (HBV), hepatitis C virus (HCV) and others induce cell fusion (reviewed in (Gao and Zheng, 2011)). Together with the notion that tetraploid cells are frequent in virus-induced tumors, virus-induced tetraploidization presents one path to near-tetraploid tumors.

Failure in cytokinesis, that is a failure to separate the two daughter cells, also results in a binucleated intermediate and mononucleated tetraploid cells in the subsequent mitosis (Figure 3C). Deregulation of multiple mitotic factors induces cytokinesis failure. For instance, impairment of the actin polymerization in mitosis by inhibition of the large tumor suppressor 1 kinase LATS1 leads to cytokinesis failure. Moreover, LATS1 is lost in a variety of cancers (reviewed in (Davoli and de Lange, 2011)).

Endoreduplication is the third mechanisms by which tetraploid cells can arise. During endoreduplication, the cells do not progress into mitosis, but skip it and enter a second S-phase in which the genome is duplicated. One trigger of endoreduplication by mitotic bypass is the prolonged telomere damage signal in the absence of a functional DNA damage checkpoint as seen in mouse embryonic fibroblasts (Davoli et al., 2010). In the absence of telomerase, telomeres shorten with each cell division. Short telomeres are

recognized by ATM/ATR kinases and lead to p53 or Rb-mediated apoptosis or senescence. Dysfunctional short telomeres are observed in early stage tumors and were proposed to be the source of DNA damage signals (De Lange, 2005). Given that p53 or Rb mutations or loss are common in cancer, prolonged ATM/ATR signaling inducing endoreduplication and mitotic failure might be a common cause of tetraploid cells in early tumor development (Davoli and de Lange, 2012).

*Sources of structural aneuploidy*

Structural aneuploidies such as deletions, insertions, translocations and duplications result mainly from improper repair of DNA damage or from unscheduled recombination events. Recurrent genomic alterations may result from misalignment and cross-over of genomic sequences with a high sequence identity (known as non-allelic homologous recombination) (reviewed in (Colnaghi et al., 2011)). Depending on the location and sequence orientation, non-allelic homologous recombination can result in deletion, duplication or inversion of DNA sequences. Genomic alterations that are not recurrent and occur at diverse breakpoints mainly arise during double strand break repair through non-allelic homologous end-joining that is inherently error prone. Joining the double strand break ends to incorrect sequences or at incorrect positions results in insertions and deletions (Hastings et al., 2009). More complex genomic alterations are characterized by multiple breakpoints flanked by unaffected stretches of genome between the rearranged regions and can be mediated by multiple mechanisms such as microhomology-mediated double strand break repair, fork stalling template switching mechanism or breakage fusion bridge cycle (Stankiewicz and Lupski, 2010). In the latter, chromosomes with damaged or shortened telomeres are fused by non-homologous end joining to other chromosomes forming an unstable dicentric chromosome (De Lange, 2005; Jafri et al., 2016). As kinetochores from opposite spindle poles attach to the centromeres during anaphase, the dicentric chromosome may break due to opposing forces. The resulting new ends may lead to another breakage fusion bridge cycle, thus promoting genomic instability (GIN). Besides telomere associated GIN, common fragile sites in the genome are prone to double strand breaks. These sites are particularly susceptible to fork stalling during replication stress and contribute to genome instability and structural aneuploidy (Bignell et al., 2010; Dereli-Öz et al., 2011; Barlow et al., 2013). Segmental rearrangements also occur due to lagging chromosomes. Chromosomes that lag behind during chromosome separations may become trapped in the cleavage furrow. This might either lead to their breakage, where the daughter cells inherit parts of the chromosome, or to the formation of a micronucleus (Janssen et al., 2011; Zhang et al., 2015). Recent studies have demonstrated that the isolated chromosomes in micronuclei may be subjected to complex rearrangements and fragmentations characteristic for a process called chromothripsis (Zhang et al., 2015). Chromothripsis in micronuclei might be due to defective and delayed DNA replication (Crasta et al., 2012). That is, chromosomes from micronuclei can be reincorporated into the main nucleus in the subsequent mitosis, thus resulting in structural aneuploidy in the daughter cells.

In summary, multifold routes can result in aneuploidy, such as mutations and deregulation of various factors or mitotic errors. This presents a challenge to study the effect of aneuploidy, as it is often impossible to separate the causes and the consequences of aneuploidy from each other. Thus, novel cellular model systems may provide an important and resourceful tool for studying the consequences of aneuploidy *per se*.

## 5.3 Model systems of aneuploidy

Since the 21st century the study of the molecular consequences of aneuploidy in cancer or trisomy syndromes has been of great interest. To directly interrogate aneuploidy in the disease context, patient-derived cell lines or aneuploid preimplantation embryos have been available for research (Biancotti et al., 2010). However, the use of patients cell lines has marked limitations, because it has not been possible to obtain the corresponding control diploid cell lines. Moreover, the *in vivo* origin may limit the dissection of the primary molecular changes driven by aneuploidy from its secondary effects that may arise through adaptation of the aneuploid cells to the specific tissue niche. Especially in cancer-derived aneuploid cell lines, the additional molecular changes driven by mutations make it difficult to address the consequences to aneuploidy *per se*. Therefore, several aneuploid yeast strains, human cell lines and mouse models were established to study the effects of immediate and long-term effects of aneuploidy.

Since chromosome missegregation is the major source of aneuploidy, induction of chromosome missegregation is often employed to study the short-term effects of aneuploidy on the cellular physiology. For instance, depletion of the SAC components MAD2 or BUB1 results in missegregation of chromosomes in the majority of retinal pigment epithelial cells (Santaguida et al., 2015). Similarly, chemical inhibition of the SAC kinase MPS1 results in CIN (Hewitt et al., 2010; Santaguida et al., 2010). Using the same principle, chromosomally unstable mouse models were generated to study the effects of enhanced chromosome missegregation *in vivo* (reviewed in (Giam and Rancati, 2015) and (Simon et al., 2015)). Instead of using SAC depletion or inhibition, chromosome missegregation can be induced by the impairment of the mitotic spindle apparatus by drug treatments such as nocodazole or monastrol (Elhajouji et al., 1997; Thompson and Compton, 2008). Common to all these methods is that the induction of chromosome missegregation generates aneuploid cells with random and undefined karyotypes. Similar random aneuploidy fission or budding yeast can be generated through meiosis of yeast strains with an odd ploidy, such as tetraploidy or pentaploidy (Niwa et al., 2006; Pavelka et al., 2010). Yet, to attribute the aneuploidy response to specific karyotype changes, defined aneuploid cells are required. For example, microcell-mediated chromosome transfer has been developed allowing the addition of a specific chromosome to a cell (Fournier, 1981). The resulting aneuploid cell lines enable the study of the consequences of a defined aneuploidy in comparison to the parental cell line that serves as a perfect matched control (Upender et al., 2004; Stingele et al., 2012). The occurrence of Robertsonian metacentric chromosomes in mice has been used to generate trisomic mouse embryonic fibroblasts (Gropp et al., 1983; Williams et al., 2008). Robertsonian fusions arise as a fusion of non-homologous chromosomes at the centromeres. Crossing of mice with different Robertsonian chromosomes results at a low frequency in progenies with trisomies due to meiotic non-disjunction. In budding yeast, defects in nuclear fusion of the karyogamy gene mutants can be used for chromosome transfer (Conde and Fink, 1976; Torres et al., 2007). During these abortive matings, chromosome transfer from one mating partner to the other occurs in rare cases. In another approach, targeted addition or removal of a chromosome can be achieved by modification of the centromere (Reid et al., 2008; Anders et al., 2009). Therein, a conditional centromere can transiently block sister

chromatid separation leading to yeast cells disomic for a specific chromosome (Anders et al., 2009). Similarly, loss of a specific chromosome can be achieved by transcription from a GAL1 inducible promoter adjacent to centromere sequences.

The above-described aneuploid model systems mostly present random or defined simple aneuploidies. However, aneuploidy in cancer can be highly complex and may arise through a tetraploid intermediate state (see chapter 5.2). To study the fate of tetraploid cells, whole-genome doubling is induced by treatment with actin inhibitors such as cytochalasin D that lead to cytokinesis failure (Fujiwara 2005). These tetraploid cells are chromosomally unstable and usually become aneuploid through multipolar mitosis (Vitale et al., 2010).

In summary, multiple different methods can be applied to generate random, defined or complex aneuploidies in different model organisms. Depending on the method applied, the generated aneuploid model cells are inheritably stable or exhibit genome instability. In this thesis, defined whole-chromsome aneuploid model cell lines, generated by microcell-mediated fusion have been used to study the consequences of aneuploidy (Donnelly et al., 2014; Dürrbaum et al., 2014). In addition, aneuploid post-tetraploid cells have been used to determine the consequences of a complex aneuploid karyotype derived from an intermediate tetraploid karyotype (Kuznetsova et al., 2015).

## 5.4 The “omics” landscape of aneuploid cells

### 5.4.1 Effects of aneuploidy on the genome

Aneuploidy in cancer cells appears in form of changes in the numbers of chromosomes but also as diverse structural changes, such as deletions, duplications, unbalanced inversions and translocations (Mitelman et al., 2016). The data obtained in large-scale cancer genome sequencing efforts present a comprehensive landscape of the genomic changes in cancer revealing the overwhelming complexity of structural changes and somatic mutations in cancer (Vogelstein et al., 2013). While the sequencing data uncovers that the number of mutations per tumor may reach up to 100,000 in melanomas and glioblastomas (Alexandrov et al., 2013), it is not entirely understood how tumors acquire these high numbers of mutations. According to the view that cancer develops in a multistage process, somatic mutations would accumulate and are selected during tumorigenesis ((Vogelstein and Kinzler, 1993) reviewed in (Jeggo et al., 2016)). The complexity of aneuploidy correlates with poor prognosis of cancer patients (Sheltzer, 2013). In addition, aneuploidy and somatic mutations coincide in tumors (Duesberg et al., 1998; Lengauer et al., 1997; 1998). These two observations suggest that aneuploidy itself might drive the accumulation of somatic mutations. In support, single cell sequencing of breast cancer cells revealed that while copy number changes and rearrangements occur early in tumor development, somatic mutations appear gradually (Wang et al., 2015). Intriguingly, at least two studies show that the mutation rates are increased near the breakpoints (De and Babu, 2010; Drier et al., 2013), suggesting that aneuploidy may promote genomic variations.

Aneuploidy has been correlated with karyotype instability in human colon cancer cell lines (Lengauer et al., 1997) and in p53 knock out colon cancer cell lines (Thompson et al., 2010). Moreover, the higher the aneuploidy the more unstable is the karyotype in transformed Chinese hamster embryo cells (Duesberg et al., 1998). These observations indicate that aneuploidy promotes karyotype instability. In support, budding yeast cells with an extra chromosome exhibit an increased chromosome missegregation frequency (Sheltzer et al., 2011). Disomic yeasts also show a higher mutation rate and defects in recombination and DNA repair, suggesting a link not only between aneuploidy and CIN, but also between aneuploidy and GIN. Human cell lines with an artificially introduced extra chromosome also display signs of CIN and/or GIN, such as chromosome aberrations in primary human fibroblasts with an extra copy of chromosome 8 (Nawata et al., 2011) and higher rates of chromosome missegregation in human colorectal cancer cells with an extra copy of chromosome 7 or 13 (Nicholson et al., 2015).

While aneuploid model cell lines have been previously linked to CIN and GIN, only advanced sequencing technologies allow a systematic study of the effects of aneuploidy on the genome. Recent results from mate-pair NGS of trisomic and tetrasomic human cell lines revealed *de novo* chromosomal rearrangements that did not occur in the parental control (Passerini et al., 2016). Further SNP analysis of clonal cell lines originating from a trisomic cell line confirmed the increased occurrence of *de novo* chromosomal rearrangements in comparison to the clones originating from the diploid parental cell line. Analysis of the protein levels of DNA replication factors revealed a down-regulation specifically for the replicative helicases MCM2-7 (Passerini et al., 2016). Depletion of these

factors in diploid cells leads to a similar GIN phenotype as observed in aneuploid cells. Moreover, overexpression of MCM subunits rescues DNA damage foci formation in the aneuploid cells. Intriguingly, downregulation of DNA replication proteins was also observed as a response to chromosome missegregation due to SAC attenuation (Ohashi et al., 2015). The damage observed in these cells seems not to stem from the chromosome missegregation itself, but from reduced replication activity in the surviving cells. Thus, these results suggest that replication stress in aneuploid cells leads to GIN. In summary, cumulative evidence indicates that the presence of extra chromosomes promotes GIN at least in aneuploid model cell lines.

### 5.4.2 The presence of extra chromosomes affects the transcriptome and proteome

The consequences of aneuploidy on the cell physiology are determined by transcriptome and proteome changes. Continued improvement of technologies facilitates the documentation of the effects of aneuploidy in cancer but also in model cell lines on mRNA and protein level and allows a new perspective on the cellular response to aneuploidy.

It is now well established that the addition of extra chromosomes results in correlative expression changes according to the gene copy number in yeast (Chikashige et al., 2007; Torres et al., 2007), mammalian cell lines (Upender et al., 2004; Williams et al., 2008; Nawata et al., 2011; Stingele et al., 2012), *A. thaliana* (Huettel et al., 2008; Sheltzer et al., 2012) and maize (Birchler, 2013). In a few cases a broad range of expression changes was reported, which, however, may be a consequence of differences in the data analysis strategies (reviewed in (Dürrbaum and Storchova, 2015a)). For instance, 25-56% of the genes encoded on trisomic chromosome 21 are expressed at diploid levels in mouse model of trisomy 21 or patient derived cells, although the averaged gene expression of chromosome 21 genes is elevated above diploid level (Aït Yahya-Graison et al., 2007; Chou et al., 2008; Biancotti et al., 2010; Vilardell et al., 2011; Wang et al., 2011). All of the above-cited studies investigated the effects of extra chromosomes on the transcriptome, whereas the consequences of monosomies have not been not broadly investigated so far. The few studies available derive from Drosophila, where monosomy of chromosome X and 4 are buffered by two evolved dosage compensation mechanisms (Larsson et al., 2001; Johansson and Stenberg, 2007; Laverty et al., 2010). In addition, segmental monosomies are also partially dosage compensated in Drosophila as suggested by the only minor reduction in expression levels (Stenberg et al., 2009). From these studies, it appears that also loss of chromosomes or chromosome parts is detrimental, at least for Drosophila. However, whether the response to monosomies in mammalian cells is similar remains to be investigated.

The analysis of the transcriptional response to aneuploidy in cancer is complicated by the intertwined appearance of somatic mutations with aneuploidy, epigenetic alterations and the high intra-tumoral heterogeneity (Vogelstein et al., 2013). Moreover, different data analysis strategies make it difficult to compare the results between the studies (reviewed in (Dürrbaum and Storchova, 2015a)). Correlations at single gene level appear to be less robust and probably reflect the complex genomic and epigenomic alterations in cancer. However, if distinct regions are summarized and correlated, a weak

but consistent correlation of gene copy number and expression can be found. Moreover, using a novel method that corrects for major non-genetic factors in meta-analysis of 77840 cancer expression profiles clearly demonstrates a correlation between gene copy number alterations and gene expression (Fehrmann et al., 2015).

Protein expression scales with the gene copy number changes in disomic yeast and tri- or tetrasomic mammalian cells (Pavelka et al., 2010; Torres et al., 2010; Stingele et al., 2013). Yet, certain proteins are dosage compensated; that is, their expression is lower than expected and more similar to wild type levels (Torres et al., 2007; Pavelka et al., 2010; Stingele et al., 2012; Dephoure et al., 2014; Donnelly et al., 2014). Specifically, the expression levels of subunits of macromolecular complexes are subjected to dosage compensation in disomic yeast as well as in human cells with extra chromosomes. In addition, abundance of many protein kinases is attenuated to normal levels as well. Especially for the formation of macromolecular complexes balanced concentration of the complex subunits is important. This was elegantly demonstrated by the “genetic tug of war method” that identified 115 dosage sensitive genes. Most of them are protein complex members (Makanae et al., 2013). In this study, target genes were cloned into a plasmid, which additionally harbors positive selection markers for growth on limited media. The limit for overexpression of a specific gene was assessed by its toxic effect preventing high plasmid copy numbers. Whereas an increase up to 100 copy numbers would not affect the cells for over 80% of genes, for 115 genes, enriched for members of protein complexes, the permissive plasmid copy number was limited and lower than expected. When the binding partners of the dosage sensitive genes were simultaneously overexpressed, the sensitivity could be rescued. This suggests that in aneuploid cells, the members of protein complexes are especially sensitive to copy number changes. However, the mechanism of dosage compensation is not understood.

In cancer, the correlation of gene copy number variations with protein abundance is rather weak, with a Spearman rank correlation of 0.1 in lung cancer (Li et al., 2014) and 0.22 and 0.28 in two breast cancer cell lines (Geiger et al., 2010). In the latter, only 4.8% and 7.8% of the protein levels could be directly correlated to the corresponding copy number changes. Yet, the few studies and the small sample size of aneuploid cancer proteomics do not allow making general conclusions on the effect of aneuploidy on the proteome. In cancer, the weak correlation in tumors is probably due to the additional aberrations of genes and pathways in cancer that makes the relation of an abnormal karyotype with the proteome much more complex.

### 5.4.3 Aneuploidy affects genome-wide expression

Aneuploidy does not only lead to changes in transcriptome and proteome of the genes encoded on the extra DNA, but also to extensive genome-wide expression changes (Upender et al., 2004; Torres et al., 2007; Sheltzer et al., 2012; Stingele et al., 2013; Gemoll et al., 2013) reviewed in (Dürrbaum and Storchova, 2015a)). Similarly, about 90-98% of deregulated genes were encoded on the disomic chromosomes in cells derived from trisomy 13 or 18 amniotic fluid samples and trisomy 21 chorionic villus samples (Hervé et al., 2016). It was first observed in aneuploid yeast strains that the

transcriptional changes are similar to a previously identified environmental stress response of yeast (Torres et al., 2007). Therein, differentially expressed genes were associated with RNA processing and ribosomes as well as nucleic acid metabolism and carbohydrate metabolism. Later studies in mammalian aneuploid model cell lines and aneuploid cells from Down syndrome patients indicated that the genome-wide transcriptome deregulation affects common gene ontology pathways (Sheltzer et al., 2012; Stingele et al., 2012; Foijer et al., 2013). A more systematic study revealed that aneuploid cell lines exhibit a similar pathway deregulation independent of the type of the aneuploid chromosome, the cell line or the laboratory in which the cell line or transcriptome data were generated (Dürrbaum et al., 2014). Similar to the response in aneuploid yeast cells, gene annotations associated with DNA and RNA pathways were consistently downregulated, while gene annotations associated with extracellular regions and lysosomes were upregulated. Of note, the expression pattern on single gene level is diverse and overlaps poorly. Thus, while there are similarities in the specific affected pathways, each cell line has different determinants of pathway deregulation.

In summary, large-scale analysis of genome, transcriptome and proteome allow to comprehensively assess the quantitative and qualitative response to aneuploidy on molecule level. Yet, the fact that primary and secondary effects are most likely reflected in the steady state “omics” data makes it difficult to dissect causes and consequences. Particular in cancer studies, multiple other molecular changes and an evolutionary adaptation process to aneuploidy probably mask the primary consequences of aneuploidy. In-depth data analysis and comparison of the aneuploidy response on transcriptome and proteome level might give further insights into the processes altered in aneuploid model cells.

## 5.5 Physiological consequences of aneuploidy

### 5.5.1 Aneuploidy affects proliferation and cell metabolism

Most aneuploidies are not compatible with life and account for one third of spontaneous miscarriages in humans (reviewed in (Hassold et al., 2007)). In surviving embryos, aneuploidy is the leading cause of mental retardation and developmental disabilities as seen in individuals with trisomy of chromosome 21. If isolated, induced pluripotent stem cell from Down syndrome fibroblasts have longer doubling times and an increased G1-cell fraction (Li et al., 2012; Jiang et al., 2013). Intriguingly, loss of the aneuploid chromosome results in proliferation improvement compared to the trisomies. This is in line with the observed proliferation defects of cells with extra chromosomes such as in aneuploid fission and budding yeast strains, mouse embryonic fibroblasts and human cell lines (Williams et al., 2008; Torres et al., 2007; Stingele et al., 2012).

What are the underlying mechanisms of the reduced proliferative capacity? As an immediate response to chromosome missegregation during mitosis, the proliferation is limited by p53 pathway activation (Li et al., 2010; Thompson and Compton, 2010; Janssen et al., 2011; Ohashi et al., 2015). Cells in which the SAC is compromised by mutation or depletion of individual SAC components show increased levels of chromosome missegregation followed by nuclear accumulation of p53 (Li et al., 2010). Along these lines, triggering erroneous microtubule-kinetochore attachments via monastrol treatment induces chromosome missegregation and p53 activation (Thompson and Compton, 2010; Janssen et al., 2011). The p53 activation mechanisms remain unclear; on one hand monastrol induced chromosome missegregation correlates with increased number of DNA damage foci and activation of the DNA damage response pathway involving ATM and p53 (Janssen et al., 2011). On the other hand, another study did not detect any signs of DNA damage, but found the p53 activation after chromosome missegregation depended on the p38 stress-response kinase (Thompson and Compton, 2010). Recent work suggests that p53 is activated by differential phosphorylation of histone 3.3 at Serine31 (Hinchcliffe et al., 2016). The authors demonstrated that histone 3.3 phosphorylation marks missegregated chromosomes. The subsequent genome-wide spreading of the phosphorylation signal induces p53. Concordantly, masking the phosphorylation diminishes p53 activation. Activation of the p53 pathway subsequently limits the propagation of cells with missegregated chromosomes. Induction of chromosome missegregation in p53 deficient cells allows accumulation of aneuploid progeny. This is true also *in vivo*, since p53 null mice with mutations in the SAC component Cdc20 have a higher survival rate than p53 positive mice and show an increased rate of aneuploid cells and thymic lymphoma development (Li et al., 2010). Thus, p53 is the key factor limiting the propagation of aneuploids.

Interestingly, the growth impairment in chronic aneuploid model cell lines seems to be p53 independent, since activation of p53 has not been observed in these cells so far (Tang et al., 2011), our unpublished data)). Characteristic for the slower growth of aneuploid model cell lines is a delay in G1 phase of the cell cycle (Torres et al., 2007; Stingele et al., 2012). More detailed analysis in aneuploid yeast cells revealed that defective G1 cyclin accumulation underlies the prolonged G1 phase, which can be rescued by overexpression of the G1 cyclin CLN2 (Thorburn et al., 2013). Similarly, the global

transcriptome analysis of different aneuploid model cell lines indicates a downregulation of cell cycle associated factors (Dürrbaum et al., 2014). However, the exact molecular mechanisms of the growth defect in aneuploid cells remain elusive.

An open question is whether the growth delay is governed by the copy number changes of specific genes encoded on the aneuploid chromosome or whether the cumulative deregulation of gene expression in aneuploid cells affects proliferation. The former might be the case for budding yeast with extra chromosome VI, which survives only at a very low frequency (Campbell et al., 1981; Dutcher, 1981). This lethality was linked to an extra copy of the β-tubulin gene, since an excess of β-tubulin over α-tubulin is not well tolerated (Katz et al., 1990). However, this seems to be rather an exception and the observation that the cell cycle delay of yeast aneuploid cells correlates with the size of the additional chromosome speaks for a cumulative gene expression effect (Thorburn et al., 2013). In support, overexpression of individual genes, previously identified to slow growth at high copy numbers, does not result in marked growth impairment similar as observed in aneuploid yeast cells (Bonney et al., 2015). Only the combined overexpression of highly dosage sensitive genes results in growth impairment. These results suggest that hindered proliferation of aneuploid cell lines is a result of the cumulative effects of aneuploidy.

The addition of extra chromosomes also affects cellular metabolism. For instance, aneuploid mouse embryonic fibroblasts display metabolic alterations such as increased glutamine consumption, ammonium and lactate production, which points to alterations in the energy metabolism (Williams et al., 2008). Aneuploid yeast strains have increased glucose uptake, but less biomass production per glucose molecule (Torres et al., 2007). Whether and how these metabolic alterations are connected to the slow proliferation remains elusive.

Interestingly, under environmental stress conditions aneuploidy confers a growth advantage in comparison to euploidy. Despite growth defects under normal conditions, aneuploid budding yeast strains show a growth advantage under various non-standard conditions such as the tumorigenic compound 4-nitroquinoline-N-oxide (Pavelka et al., 2010). Similarly, colorectal cancer cell lines with an extra chromosome are less sensitive to serum deprivation, hypoxia or cytostaticum 5-fluorouracil and grow slightly better than diploid cells under the same stress conditions (Rutledge et al., 2016). Hence, although the addition of extra chromosomes negatively affects proliferation, under stress conditions that affect the proliferation of wild type cells, aneuploid cells are less sensitive.

In summary, aneuploidy has detrimental effects on cellular growth and alters cellular metabolism. The fact that impaired growth is a shared phenotype of many different aneuploid cells suggests common molecular triggers. Yet, there are remaining open questions: What are the molecular causes of the compromised growth capacity? How is this phenotype linked to the other physiological consequences of aneuploidy in model cells? And is slower growth a mere byproduct of the negative effects of aneuploidy on the cell physiology or is it the cause for the observed phenotypes?

### 5.5.2 Aneuploidy affects the proteostasis network

Cellular functions strongly rely on a functional balance of the proteome that is described as protein homeostasis, or proteostasis. Proteostasis not only includes regulated protein synthesis and degradation, but also the maintenance of protein stoichiometry and individual protein levels. To preserve proteostasis, multifold pathways and mechanisms have evolved that build a proteostasis network. Involved are the three major mechanisms of protein synthesis, protein folding and protein degradation for clearance of misfolded or dispensable proteins (Figure 4). Disturbance of proteostasis has been linked to aging (reviewed in (Taylor and Dillin, 2011; Labbadia and Morimoto, 2015)) and neurodegenerative diseases such as Alzheimer´s disease, in which characteristic protein aggregates accumulate (reviewed in (Soto and Estrada, 2008)).

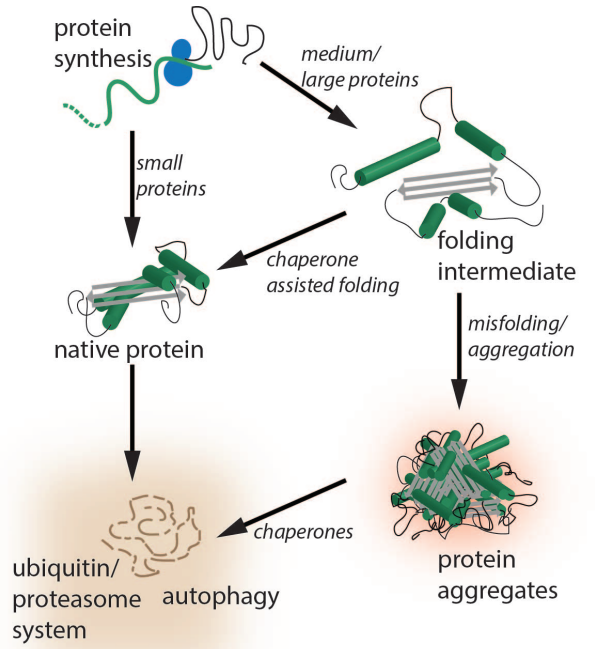


**Figure 4. The proteostasis network.** Protein homeostasis is achieved by balanced protein synthesis, efficient protein folding and degradation of protein products. Medium and large proteins undergo chaperone-assisted folding. Misfolded or insoluble proteins aggregate. Protein aggregates are cleared either by autophagy or the ubiquitin/ proteasome system.

There are several lines of evidence that aneuploidy results in proteotoxic stress, a state where the cellular proteostasis is impaired. First, aneuploid cells are sensitive to impairment of the first step of proteostasis, the protein synthesis machinery. Inhibition of protein synthesis by cycloheximide, hygromycin, radicicol or rapamycin treatment impairs proliferation in aneuploid yeast strains stronger than in euploid cells (Oromendia et al., 2012; Pavelka et al., 2010; Torres et al., 2007). Second, aneuploidy renders cells susceptible to functional impairment of the protein quality control. Protein quality control has a central function in the proteostasis network, as chaperone-assisted folding of newly synthesized proteins into their functional 3-dimensional shape, assembly of multiprotein complexes and reversion of protein misfolding or aggregation are crucial for proteostasis (reviewed in (Hartl et al., 2011; Balchin et al., 2016)). Inhibition of HSP90 by 17-AAG leads to higher cell death rate in aneuploid mouse embryonic fibroblasts than in the diploid control cells (Tang et al., 2011). Moreover, this sensitivity seems to be dependent on aneuploidy as highly aneuploid cancer cell lines exhibit a greater sensitivity to HSP90 inhibition than near-euploid cancer cell lines (Tang et al., 2011). Investigations of the HSP90 activity using a sensor based on the human tyrosine kinase Src in aneuploid yeast cells revealed reduced protein folding capacity (Oromendia et al., 2012). Similar impairment of the protein

folding capacity was observed in aneuploid human cell lines (Donnelly et al., 2014). Third, accumulation of HSP104 positive inclusions and ubiquitin-positive foci were detected in aneuploid yeasts (Oromendia et al., 2012) and in human aneuploid cell lines (Stingele et al., 2012). While these inclusions reflect compromised proteostasis in aneuploid cells, the exact source is not clear. Aneuploid yeast cells challenged with aggregation prone von-Hippel-Lindau protein (VHL) show a higher percentage of protein aggregates than the haploid control strains, indicating a defect in proteosomal clearance of protein aggregates (Oromendia et al., 2012). Conversely, increased autophagy factor levels and the co-localization of ubiquitin-positive inclusions with the cytosolic receptor for selected autophagy p62 (SQSTM1) indicate that autophagy is activated to facilitate clearance of the protein aggregates in aneuploid human cells (Stingele et al., 2013). Similarly, activation of autophagy was detected in breast epithelial cell lines with extra chromosomes (Ariyoshi et al., 2016). In addition, aneuploidy induced by impairment of SAC components leads to ER expansion and increased lysosome and aggresome formation, indicating increased autophagy (Ohashi et al., 2015).

In summary, aneuploid cells exhibit multiple features of proteotoxic stress, which go in hand with an increased sensitivity to stresses that affect protein homeostasis such as protein translation inhibition, protein quality control inhibition as well as to heat shock stress (Donnelly and Storchova, 2015). It is important to note that it is difficult to dissect the causes from consequences of proteotoxic stress. As little as 0.1% of misfolded proteins can impair the overall protein quality control and the fitness as demonstrated in yeast (Geiler-Samerotte et al., 2011). Therefore, it is likely that already initial low levels of misfolded proteins in aneuploid cells trigger multifold secondary changes in the proteostasis network. Moreover, misfolded proteins and unstable subunits of molecular complexes may titrate away components of the protein folding machinery, thus causing even more misfolded proteins in a self-promoting cycle. For instance, in *C. elegans* the toxicity of misfolded, aggregated mutant proteins was increased in a background in which destabilized polymorphic proteins compete for the protein folding machinery (Gidalevitz et al., 2009). Although it seems clear that the proteostasis network in aneuploid cells is compromised, many open questions remain to be addressed: What is the primary cause of impaired proteostasis? What is the underlying cause of the protein folding defect? And how does this affect or relate to other observed phenotypes of aneuploid cells?

## 5.6 Consequences of whole-genome doubling

Doubling of the whole-genome presents an alternative route to aneuploidy in cancer (see chapter 5.2). Proliferating tetraploid cells appear to be chromosomally unstable (Fujiwara et al., 2005; Storchova et al., 2006) and evolve to a variable aneuploidy, mostly to near-triploidy and near-tetraploidy (Davoli and de Lange, 2012; Lv et al., 2014; Kuznetsova et al., 2015). Yet, survival of tetraploid cells is largely p53 dependent and only abrogation of p53- or Rb- signaling allows further proliferation (Lanni and Jacks, 1998; Andreassen et al., 2001; Castedo et al., 2006; Ganem et al., 2009; Kuffer et al., 2013).

p53 activation in tetraploid cells has been attributed to different cues. For example, altered centrosome numbers affecting the spindle geometry and centrosome integrity was suggested to activate the p38/MAPK pathway, which activates p53 (Vitale et al., 2008). In an alternative scenario, erroneous bipolar or multipolar mitosis in tetraploid cells results in p53 activation. Indeed, cytokinesis failure induced tetraploid cells to undergo multipolar mitosis or erroneous bipolar mitosis and subsequently arrest or die upon p53 activation (Kuffer et al., 2013). Lagging chromosomes emerging from aberrant mitosis can be damaged during cytokinesis (Janssen et al., 2011) or through merotelic attachments (Guerrero et al., 2010). These might in turn activate DNA damage signaling and lead to downstream p53 activation. However, DNA damage could not be observed after multipolar or bipolar mitosis with p53 accumulation (Kuffer et al., 2013). Instead, markers for oxidative DNA damage coincided with p53 activation. Chromosome missegregation induces oxidative stress and activation of the ATM-CHK2 pathway in diploid cells (Li et al., 2010). Therefore, oxidative stress from abnormal mitosis can be one activator of p53.

Apart from p53, other regulators of the tetraploid cell cycle arrest were identified in an unbiased RNAi screen (Ganem et al., 2014). This method identified the kinase LATS2, a core component of the Hippo pathway. Further experiments revealed that LATS2 interacts with MDM2 that targets p53 for destruction, thereby stabilizing p53. This response depends on RAC1 activation and RHOA reduction, vice versa RAC1 inhibition or RHOA activation diminished Hippo pathway signaling. A possible upstream trigger might be the increased centrosome mass, since increased microtubule dynamics stimulates RAC1 activity (Waterman-Storer et al., 1999; Godinho and Pellman, 2014).

Mutations in p53 are common in cancer, which enables bypassing the proliferative limit of tetraploid cells. Yet, the exact molecular cues for post-tetraploid cell survival are not clear. What is the fate of rare survivors and what are the long-term effects of whole-genome doubling? Scant data is available, which suggests that evolved tetraploid cells show higher tolerance to CIN by providing karyotypic heterogeneity for further evolution (Dewhurst et al., 2014). In our current study we analyzed the evolutionary fate of post-tetraploid human cells and its association with p53 signaling suppression (Kuznetsova et al., 2015).

## 5.7 miRNAs – a new “omics” layer

The complex network that regulates gene expression and thereby cellular phenotypes has been recently extended by the discovery of its regulation via small RNA molecules, so called miRNAs. To date, 1881 miRNAs are identified in humans (mirbase release June 2014 http://www.mirbase.org/) and over 60% of protein coding genes are predicted to be regulated by miRNAs (Friedman et al., 2009). The first discovered miRNA negatively regulates translation of the lin-14 gene by complementary base paring in the 3’UTR, thereby controlling cell differentiation in *C. elegans* (Lee et al., 1993; Wightman et al., 1993). Since the general regulatory function of this new class of RNAs was recognized in 2001 (Lagos-Quintana et al., 2001; Lee and Ambros, 2001), the discovery of new miRNAs, their function and their association with various diseases grew exponentially according to the PubMed statistics. Whether and how miRNAs functions in the context of aneuploidy *per se* has not been studied so far.

### 5.7.1 Biogenesis and function of miRNAs

*miRNA biogenesis*

The genomic locations of miRNAs are equally distributed between intergenic or intragenic regions. That is, miRNAs are encoded between two protein-coding genes or within a protein-coding gene, respectively (Hinske et al., 2010) (Figure 5). Most intragenic miRNAs are encoded within the intron, only about 5% are exonic. While intergenic miRNAs have their independent promoter, intragenic miRNAs can either be transcribed with the host gene promoter or from an independent intergenic or from an upstream intronic promoter (Monteys et al., 2010; Ozsolak et al., 2008). Some intragenic miRNAs show correlative expression with their host genes (Rodriguez et al., 2004). Similarly, miRNAs clustering in close proximity to each other are generally co-transcribed, as for example the deeply conserved clusters mir-100, let-7 and mir-125 (Altuvia, 2005). However, this is not always the case, supposedly because of independent

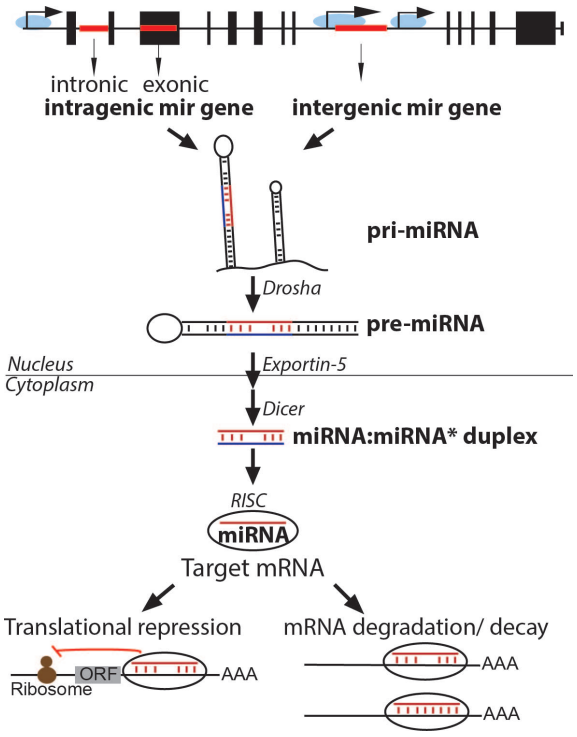


**Figure 5. The miRNA biogenesis pathway.** miRNAs are encoded within a gene (intragenic) or between two genes (intergenic). Intragenic mir genes lie either within the exon sequence (exonic) or intron sequence (intronic). Drosha cleaves the transcribed pri-miRNA into the pre-miRNA. Pre-miRNAs are exported to the cytoplasm by Exportin-5 and further processed by Dicer into a miRNA:miRNA duplexes. The guide strand is then incorporated into the RISC. Complementary binding to the target mRNA leads to translational repression and/or mRNA decay.

transcriptional regulation or alternative splicing (Monteys et al., 2010; Ramalingam et al., 2013). The majority of miRNAs are transcribed by RNA Polymerase II that generates a capped and polyadenylated primary miRNA (pri-miRNA). In addition, transcription by RNA Polymerase III was reported for a subset of miRNAs (Borchert et al., 2006; Ozsolak et al., 2008). The pri-miRNA is subsequently processed by the RNA III-type endonuclease Drosha with its essential co-factor DiGeorge Syndrome Critical Region 8, DGCR8 (reviewed in (Ha and Kim, 2014)). Intronic miRNA processing may occur after the incorporation into the spliceosome complex, but before splicing catalysis, thus not affecting the host gene transcription (Kim and Kim, 2007). Yet, alternative pathways have been suggested, such as splicing-mediated miRNA biogenesis that bypasses the Drosha processing (Berezikov et al., 2007; Westholm and Lai, 2011). Following preprocessing, precursor miRNAs (pre-miRNA) are exported to the cytoplasm by Exportin-5 and subsequently processed into their mature form. This is mediated by RNA III-type endonuclease Dicer that binds either the 5’ and/or 3’ overhang and cleaves a 22nt long RNA duplex (Park et al., 2011). The RNA duplex is then loaded onto the Argonaute protein (AGO), thus forming the pre-RNA-inducing silencing complex, which matures by unwinding of the RNA duplex and removal of the passenger strand. A member of the HSP70 family, HSPA8, together with HSP90 mediates the conformational change of AGO proteins in an ATP-dependent manner to facilitate its binding of the RNA duplex in Drosophila (Iwasaki et al., 2010). This is interesting given the reduced heat shock protein levels in human aneuploid model cell lines (see chapter 5.5.2). Within the mature RNA-inducing silencing complex (RISC), the mature single-stranded miRNA guides the complex to target mRNAs, where complementary sequence binding elicits miRNA function.

*miRNA functional mechanism*

There are two possible mechanisms how miRNAs affect gene expression (reviewed in (Iwakawa and Tomari, 2015)). First, stringent complementary binding to the target mRNA results in mRNA decay. Second, partial base pairing promotes translational repression of the target mRNA. miRNAs target mRNAs via a 5’end 7-8 nt long seed region and partial complementary positions of the 3’ end (John et al., 2004; Lewis et al., 2005; Saito and Sã Trom, 2010). Whereas it has been accepted for a long time that miRNAs recognize the 3’UTR of a target mRNA, miRNA binding sites in the coding region and 5’UTR have been identified in the past years (Schnall-Levin et al., 2011; Zhou et al., 2009; Hafner et al., 2010; Liu et al., 2013).

Target mRNA decay is mediated by recruitment of the deadenylase complexes to the RISC. Therein, the protein GW182 interacts with AGO and serves as an essential hub for the deadenylating protein complexes CCR4-NOT and PAN2-PAN3, as well as the poly(A)-binding protein PABP (Zekri et al., 2009; Braun et al., 2013; 2011; Fabian et al., 2011). Subsequent to deadenylation, the 5’cap structure of the mRNA is removed by the catalytic subunit of the decapping complex DCP2 and the decapping activators DCP1, RCK/p53/DDX6, which are recruited onto the CCR4-NOT complex (Nishimura et al., 2015). The final step in mRNA decay is the exonucleolytic mRNA degradation by the exoribonuclease 1, XRN1 (Braun et al., 2012).

Translational repression of target mRNA is less well understood. Several mechanisms have been proposed, but recent evidence indicates that miRNAs inhibit cap-dependent translation at the initiation step. Translation initiation is mediated by the interaction of the poly(A)-binding protein PABP and the 5’-cap bound eukaryotic translation-initiation factor 4G, eIF4G, which results in the formation of a circular mRNA (Derry et al., 2006). Displacement of PABP by GW182 was suggested to disrupt the mRNA circularization, thereby preventing mRNA translation (Zekri et al., 2013; Iwakawa and Tomari, 2015). Another suggested mechanism includes dissociation of the RNA helicase eIF4A subunits from the translation initiation complex ((Fukao et al., 2014), reviewed in (Fukao et al., 2015; Iwakawa and Tomari, 2015)). Yet, the detailed molecular mechanism remains to be clarified in the future.

A longstanding debate in the field concerns the relative contribution of mRNA decay and translational repression to the miRNA-dependent post-transcriptional regulation (Guo et al., 2010; Huntzinger and Izaurralde, 2011; Hu and Coller, 2012; Eichhorn et al., 2014). Quantitative approaches comparing gene and protein expression after deletion of specific miRNAs revealed that target mRNA and protein levels are changed, although for a subset of targets only proteins were affected with little or no change on mRNA level (Baek et al., 2008; Selbach et al., 2008). Another approach uses polysome profiling and ribosome profiling for determination of the mRNA translation efficiency. Comparison of the target translation efficiency with total mRNA and protein levels demonstrated that mRNA decay explains the majority of the target repression, whereas translational repression contributes only with 10-25% to the overall repression activity (Hendrickson et al., 2009; Guo et al., 2010). Kinetic studies of translational repression and mRNA decay of a reporter mRNA revealed that translational repression precedes mRNA decay (Djuranovic et al., 2012). Extended studies on the mRNA decay dynamics in various mammalian cell types showed that translational repression occurs fast, but only with a weak repression effect (Eichhorn et al., 2014). By the time translational repression shows its effect, mRNA degradation is dominating the miRNA-mediated repression effect. Thus, miRNA-target interaction results mainly in mRNA decay. Of note, the effects of miRNAs on a specific target are seldom the result of a one-to-one interaction, but rather reflect the cumulative effect of the entire miRNAome. Hence, one miRNA not only targets multiple genes, but also one gene is targeted by multiple miRNAs (Zhou et al., 2013). Synergistic miRNA repression of specific targets was shown in gastric cancer (Hashimoto et al., 2013). Moreover, competing endogenous RNAs can act as sponges and titrate miRNAs away from their targets, for instance, HMGA2 competes for the let-7 family in lung cancer (Kumar et al., 2013). Therefore, a profiling of the complete set of miRNAs in a cell, the miRNAome, is essential to fully understand the function of miRNAs in the biological context.

### 5.7.2 Association of miRNAs with aneuploidy in cancer

The miRNAome is tissue specific and its function was associated with a variety of essential cellular processes such as cell proliferation or cell death (Lagos-Quintana et al., 2002; Landgraf et al., 2007; Hwang and Mendell, 2006). Thus, it is not surprising that miRNA deregulation is associated with complex diseases such as cancer (Calin and Croce, 2006; Dalmay and Edwards, 2006). Accelerated by advanced sequencing methods, cancer

specific miRNA expression profiles and oncogenic as well as tumor suppressive miRNAs have been identified (for example (Akao et al., 2006; Dvinge et al., 2013; Schee et al., 2013; Busch et al., 2016)). Deregulation of miRNAs occurs at different steps of miRNA biogenesis and stems from multiple sources. For instance, transcriptional regulation of the factors involved in pri-miRNA processing, nuclear export or pre-miRNA processing as well as a hypoxia activated stress response may affect miRNA maturation (reviewed in (Lin and Gregory, 2015)). Further, epigenetic regulations, transcription factor activity or genetic alterations can regulate the expression of a miRNA itself.

Genomic alterations were associated with the deregulation of miRNA expression in a number of cancer studies. Frequent loss of genomic region 13q14 was associated with downregulation of mir-15a and mir-16a in chronic lymphocytic leukemia (Calin et al., 2002). Similarly, systematic analysis demonstrated an association of let-7 family downregulation with the copy number changes and focal deletions in medulloblastoma, breast and ovarian cancer (Wang et al., 2012). Not only small genomic alterations, but also the gain of an extra chromosome arm, such as 1q in cervical cancer, have been related to altered miRNA expression (Wilting et al., 2012). Genome-wide analyses of regions encoding for miRNAs show a high frequency of copy number abnormalities in ovarian cancer, breast cancer, melanoma and lung cancer (Zhang et al., 2006; Czubak et al., 2015). These results suggest that the alteration of miRNA expression originates from segmental or structural aneuploidy at least in some cancers. Mapping miRNAs to the human genome revealed that about 50% map to common fragile sites and cancer-associated genomic regions, which are regions with common breakpoints, frequent loss of heterozygosity or amplifications (Calin et al., 2004). These sites are associated with GIN in cancer, thus suggesting that miRNA deregulation due to genomic alterations might be the result of ongoing GIN in cancer. Two studies report no correlation between the copy number status and miRNA expression in haematopoetic cancer and acute myeloid leukemia (Ramsingh et al., 2013; Veigaard and Kjeldsen, 2014). This indicates that genomic copy number alterations might be just one of multiple causes of miRNA deregulation in cancer.

Another association of aneuploidy and miRNAs in cancer might lie in the influence of miRNAs on GIN and CIN. For example, overexpression of miR-28-5p leads to the reduction of MAD2 levels, thus affecting the mitotic checkpoint and ultimately promoting CIN in four different human cell lines, among them a human colorectal cancer cell line (Hell et al., 2014). Conversely, inhibition of miR-28-5p restored chromosomal stability in renal carcinoma cells and an *in vivo* mouse model. In another study, deletion of a specific miRNA subset correlates with the occurrence of chromosomal aberrations across ovarian tumor samples (Choi et al., 2014): miR-1255b, miR-148b*, and miR-193b* specifically repress homologous recombination in G1 phase, thereby maintaining the balance between homologous recombination and non-homologous end joining. Depletion of these miRNAs was suggested to allow homologous recombination in G1, leading to mitotic recombination and loss of heterozygosity.

In summary, miRNA deregulation can result from segmental and structural aneuploidy in cancer. In turn, miRNAs might promote GIN and CIN in some cases. The detrimental effects of aneuploidy on proliferation in model systems may be largely attributed to the gene dosage effects (see chapter 5.5.1). However, cancer cells are

characterized by sustained growth, hence they have seemingly overcome the adverse effects of aneuploidy (Hanahan and Weinberg, 2011). An intriguing possibility is that miRNAs might play a role in this adaptation process, for example by regulating the expression of dosage sensitive genes. A recent study suggests that miRNAs more frequently target dosage sensitive genes (Li et al., 2015). In this case, an integration of miRNA expression, target gene expression and copy number alterations revealed that miRNAs target amplified genes, thereby regulating their expression to normal levels in breast cancer, glioblastoma and ovarian cancer samples. miRNA-mediated alleviation of the consequences of copy number changes might be favored not only because of the dosage sensitivity of the repressed genes, but also to avoid negative effects on the proteostasis network. Thus, miRNA expression in cancer might contribute to the adaptation of cancer to the consequences of gene copy number alterations. However, the causal association of aneuploidy and miRNAs has not been studied in detail so far. In the cancer context, the complex molecular and genomic alterations diminish our chances to clearly map the link between aneuploidy and miRNA expression. Investigation of the miRNAome of different human aneuploid model cell lines in comparison to their diploid counterpart might help us to understand whether and how the miRNAome affects the cellular response to aneuploidy.

# 6 Results

## 6.1 Unique features of the transcriptional response to model aneuploidy in human cells

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In this study we systematically investigated the effect of aneuploidy on the transcriptome in different human cell lines. Aneuploidy affects multiple different chromosomes either in form of whole-chromosome aneuploidy or as a complex aneuploidy with additional variable genomic aberrations and may originate from different sources. Compelling evidence from budding yeast suggests that the consequences of aneuploidy are caused by the cumulative changes in gene expression levels *per se* (Bonney et al., 2015; Thorburn et al., 2013; Torres et al., 2007). However, the transcriptional landscape of aneuploid human cells and their relation to known phenotypic consequences have not been comprehensively studied. Therefore, we analyzed the transcriptome of human aneuploid model cell lines of different types and origin.

We found a common transcriptional response despite the different identity of the aneuploid chromosomes and variable cell types. The common response is characterized by the deregulation of similar pathways, in particular the up-regulation of categories associated with the endoplasmic reticulum, Golgi apparatus, lysosomes and vacuoles, membrane metabolism and MHC protein complex as well as antigen processing. Commonly downregulated annotation categories include DNA replication, DNA repair, transcription or RNA splicing. Moreover, this aneuploidy response pattern is similar in cell lines with complex aneuploidy that were derived from a tetraploid intermediate. Whereas common stress responses differ from the aneuploidy response pattern, the transcriptional changes due to impaired translation and autophagy partially resemble the aneuploid transcriptome changes. Despite similar pathway deregulation in all aneuploids, the genes that contribute to the overall deregulation differ between the aneuploid cell lines. Only 23 genes show a similar deregulation in all aneuploid cell lines; several of these genes were previously linked to cancer.

The identified common transcriptional response to aneuploidy indicates that the characteristic phenotypic consequences of aneuploidy such as impaired proliferation and activation of autophagy may originate from a similar transcriptional program. Aneuploidy in cancer is usually complex, affecting multiple different genomic regions. A common transcriptional response to aneuploidy in model cell lines might help to better understand the adaptations to the aneuploid karyotype in cancer, which most likely results in an evolved transcriptional response. Further, the 23 similarly deregulated genes might serves as markers for aneuploidy to assess tumor malignancy.

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# Unique features of the transcriptional response to model aneuploidy in human cells

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## Abstract

**Background:** Aneuploidy, a karyotype deviating from multiples of a haploid chromosome set, affects the physiology of eukaryotes. In humans, aneuploidy is linked to pathological defects such as developmental abnormalities, mental retardation or cancer, but the underlying mechanisms remain elusive. There are many different types and origins of aneuploidy, but whether there is a uniform cellular response to aneuploidy in human cells has not been addressed so far.

**Results:** Here we evaluate the transcription profiles of eleven trisomic and tetrasomic cell lines and two cell lines with complex aneuploid karyotypes. We identify a characteristic aneuploidy response pattern defined by upregulation of genes linked to endoplasmic reticulum, Golgi apparatus and lysosomes, and downregulation of DNA replication, transcription as well as ribosomes. Strikingly, complex aneuploidy elicits the same transcriptional changes as trisomy. To uncover the triggers of the response, we compared the profiles with transcription changes in human cells subjected to stress conditions. Interestingly, we found an overlap only with the response to treatment with the autophagy inhibitor bafilomycin A1. Finally, we identified 23 genes whose expression is significantly altered in all aneuploids and which may thus serve as aneuploidy markers.

**Conclusions:** Our analysis shows that despite the variability in chromosome content, aneuploidy triggers uniform transcriptional response in human cells. A common response independent of the type of aneuploidy might be exploited as a novel target for cancer therapy. Moreover, the potential aneuploidy markers identified in our analysis might represent novel biomarkers to assess the malignant potential of a tumor.

## Background

Aneuploidy, or a change in cellular chromosome numbers, has profound effects on the physiology of all eukaryotic cells analyzed to date [1]. Aneuploid yeasts are characterized by slow growth, altered sensitivity to various stresses and increased genomic instability [2-4]. At the same time, aneuploidy drives genetic variability and cellular adaptation capacity in yeast [5,6]. Plants are in general more tolerant to gene dosage changes, yet aneuploidy often impairs their vigour and alters their phenotype [7]. Aneuploid mammals are rarely viable and the sporadic survivors are affected on multiple levels. In humans, aneuploidy is responsible for a substantial proportion of spontaneous abortions and the rare survivors with trisomy of chromosome 13, 18 and 21 (Patau, Edward and Down syndrome, respectively) are severely handicapped; only trisomy 21 is compatible with survival until adulthood [8]. Aneuploidy is also linked to cancer, as nearly 90% of solid tumors and 75% of hematopoietic cancers show abnormal chromosome dosage [9]. Recently it has been shown that the occurrence of aneuploid cells increases with aging [10] and an increased incidence of aneuploidy in the brain has been linked to neurodegenerative diseases [11].

The exact mechanisms underlying the detrimental effects of aneuploidy remain unclear, but it has been convincingly shown that they are caused by the expression of the extra genes on the supernumerary chromosome [3]. In most aneuploid cells the chromosome dosage changes lead to correlating changes in mRNA (e.g. [3,12-15]) as well as on protein levels [6,15,16]. These analyses further

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revealed that besides the increased abundance of transcripts and proteins originating from the aneuploid chromosome, the expression of multiple other genes is altered as well [3,13,15,17]. This is likely a consequence of two different phenomena. First, an increase in gene copy number of a transcription factor or other regulatory factor might affect transcription levels of genes on other chromosomes [18]. Second, specific pathways might be activated in a cellular response to aneuploidy. Recent attempts to uncover the consequences of aneuploidy suggest that aneuploidy indeed instigates a specific response in eukaryotic cells [3,15,19]. Haploid yeast strains carrying an additional chromosome (hereafter referred to as disomes) exhibit a common transcriptional signature that has been previously identified in budding yeast as a so called environmental stress response that is triggered upon various exogenous stresses, such as oxidative stress, heat shock or slow growth [3,20]. A similar response was identified in a study comparing transcriptome data from disomic and complex aneuploid strains of budding yeast, partial aneuploids of fission yeast, aneuploid *Arabidopsis thaliana* plant cells, mouse cell lines with Robertsonian translocations that lead to trisomies, and human cells from patients with trisomy syndromes [19]. Additionally, comparative transcriptomics and proteomics of model human trisomic and tetrasomic cells identified a common pattern in the transcriptional response to aneuploidy [15]. These studies pointed out similarities in the response to aneuploidy in most eukaryotes. At the same time, the results showed that the response in mammalian cells diverges from the response in other model organisms, because the correlation of the transcription changes in aneuploid mammalian cells with transcriptional changes in other aneuploid species is rather modest [19]. Moreover, as there is no equivalent of the environmental stress response identified so far in mammalian cells, it remains unclear what triggers the transcriptional changes.

To obtain a comprehensive insight into the changes specific for human cells, we evaluated in detail multiple tri- and tetrasomic cell lines as well as model complex aneuploid human cell lines with hypotetraploid karyotypes more similar to cancer cells. Remarkably, we found that the cellular responses to complex multichromosomal aneuploidy and trisomy or tetrasomy of one single chromosome closely correlate. As the identified pathway pattern resembles the cellular stress response, we compared the aneuploidy response to the transcriptional changes in human cells subjected to various stress conditions. Additionally, we have also identified 18 genes that were upregulated and 5 genes downregulated in all analyzed aneuploid cell lines and might thus serve as markers of aneuploidy. This is the first study which compares a variety of different human cell lines with aneuploidy of different types and origins. By uncovering a uniform aneuploidy response pattern our results outline the cellular consequences of an abnormal karyotype in human cells.

## Results

### Common cellular response to trisomy and tetrasomy in human cell lines

Recently, we established *de novo* human aneuploid cell lines that were derived from diploid and chromosomally stable cells by a micronuclei-mediated chromosome transfer [15]. We performed transcriptional analysis of the original diploid cell lines and their trisomic and tetrasomic derivatives (HCT116: trisomy of chromosome 3 – 3/3; tetrasomy of chromosome 5 - 5/4; RPE1: trisomy of chromosome 5 and 12 - 5/3 12/3, trisomy of chromosome 21 - 21/3, see Methods for further details) and calculated the aneuploid-to-diploid fold change for all detected mRNAs. Similarly, we calculated the relative aneuploid-to-diploid ratio to determine the transcriptional changes in trisomic human cells lines generated from the diploid colorectal adenocarcinoma cell line DLD1 by introduction of an extra copy of chromosome 3, 7 or 13 [13]. To identify the similarities and differences among the transcriptional profiles of the model aneuploid cell lines in response to aneuploidy, we used an algorithm called 2-dimensional annotation analysis that quantifies the relative up- and downregulation of cellular pathways. Hereby, abundance changes of all factors assigned to each pathway are compared to the overall abundance distribution and relative values are calculated [21]. Additionally, significantly up- and downregulated pathways were validated by the annotation enrichment tool DAVID, which employs a different algorithm for the analysis (data not shown) [22]. As expected, pathways altered and enriched in the 2-dimensional annotation analysis were also identified as enriched by DAVID in all cell lines.

Our analysis identified a specific pattern of pathways that are altered in all compared model trisomic and tetrasomic cell lines (Table 1). Among the most upregulated categories we identified the endoplasmic reticulum (ER), Golgi apparatus, lysosomes and vacuoles, membrane metabolism and the MHC protein complex and antigen processing. In contrast, DNA and RNA metabolic pathways – e.g. DNA replication, repair, transcription or RNA splicing - were significantly downregulated (Figure 1A, Additional file 1: Figure S1A). Remarkably, although similar pathways are up- and downregulated in all aneuploid cell lines, we noticed that the specific factors that significantly contribute to the differentiated regulation are variable. For example, the members of the proton oligopeptide cotransporter family SCL15 (Gene Ontology Cellular Component “membrane, lysosome, lytic vacuole”) show variable expression levels in the analyzed aneuploid cell lines: SLC15A3 is upregulated in all of the HCT116 derived aneuploid cell lines, but not in the RPE1 derived

**Table 1 Pathway classes recurrently and significantly altered in aneuploid cell lines**

| | Replication | Transcription | Ribosome | Golgi | ER | Lysosomes | Membrane metabolism | Vacuole | Mitochondria | Spliceosome | MHC class proteins |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **HCT116 5/4** | - | - | - | + | + | + | + | + | - | - | + |
| **HCT116 3/3** | - | - | - | + | + | + | + | + | - | - | |
| **HCT116 5/4*** | - | - | - | + | + | + | (+) | + | - | / | + |
| **HPT1** | - | - | + | - | + | + | + | + | / | - | + |
| **HPT2** | - | - | - | + | + | + | + | + | + | - | + |
| **RPE1 12/3 5/3** | - | - | + | + | + | + | + | + | + | + | + |
| **RPE1 21/3*** | - | - | - | + | + | + | + | + | - | - | + |
| **DLD1 3/3** | - | - | - | + | / | / | + | + | - | - | / |
| **DLD1 7/3** | - | - | / | / | / | / | / | + | / | - | / |
| **DLD1 13/3** | - | - | - | + | / | / | + | + | - | - | / |
| **HE35 8/3_1** | +/− | - | - | - | - / | / | +/− | / | + | + | / |
| **HE35 8/3_2** | - | - | - | + | + | / | +/− | / | +/− | + | + |
| **HE35 8/3_3** | - | −/+ | - | + | + | + | +/− | + | (+) | + | / |

Asterisks indicate the cell lines expressing H2B-GFP.
/ = annotation not significantly altered.
+ = annotation category upregulated.
- = annotation category downregulated.

aneuploid cell lines, whereas SLC15A4 is upregulated only in HCT116 H2B-GFP 5/4 and SLC15A1 is upregulated in three out of seven aneuploid cell lines derived from HCT116 and RPE1 (Additional file 2: Table S1).

Interestingly, DLD1 with trisomy of chromosome 7 shows only partial overlap with the other aneuploid cell lines: pathways related to splicing and to DNA and RNA metabolism were similarly downregulated, and vacuole was upregulated, whereas additional changes in the transcription profile differed from the other trisomic and tetrasomic cell lines (Additional file 1: Figure S1B, Table 1). We also determined the response to aneuploidy in three clones with trisomy of chromosome 8 (here labeled as HE35 8/3_1, 2, and 3) derived from human primary embryonic fibroblasts HE35 by microcell-mediated chromosome transfer [17]. Using the 2-D annotation analysis algorithm, we observed similar changes in pathway regulations in the HE35-derived trisomies as in the other tri- and tetrasomic cell lines (Figure 1B, Additional file 1: Figure S1C, Table 1). Taken together, the results indicate that all the analyzed human cells with *de novo* created trisomies and tetrasomies elicit a nearly identical pattern of pathway changes regardless of the identity of the supernumerary chromosome and the cell line.

### Complex aneuploidy triggers the same pathway changes as low-complexity aneuploidy

Next, we asked whether similar pattern of changes in gene expression could be observed in cells with complex aneuploidy. This type of karyotypic changes might more closely resemble the situation observed in tumors that often harbor multiple changes in chromosome numbers and structures, including deletions, translocations and amplifications [23]. To this end, we used cell lines derived from HCT116 that underwent a transient tetraploidy induced by cytokinesis failure (Figure 2A). As tetraploidy leads to catastrophic mitosis and chromosomal instability, the majority of cells die soon after the tetraploidization [9,24], and the chromosome numbers of the few survivors are remarkably altered (Figure 2B). We analyzed the transcriptional changes identified in two clonal survivors, cell lines HPT1 and HPT2, by calculating the fold change of the transcripts in comparison to the original HCT116. This first global analysis of the transcriptional response in human cells with model complex aneuploidy reproduced the pattern of pathway changes observed in the tri- and tetrasomic cell lines (Figure 2C,D, Additional file 1: Figure S1D, E, Table 1, Additional file 3). Importantly, the duration of the cell cycle of the complex aneuploid cell lines is indistinguishable from diploids (Additional file 1: Figure S2). Previously, it has been proposed that the transcriptional changes identified in aneuploid cells are caused by the slow growth, which is typical for model trisomic cell lines [3,14,15]. Thus, our finding indicates that the response to aneuploidy in mammalian cells is not always associated with a slower progression through the cell cycle, and thereby the slower proliferation cannot be the only cause of the identified transcriptional changes.

Distance matrix of the Spearman rank correlation of all analyzed cell lines confirmed the high similarity among the transcriptional changes triggered by aneuploidy

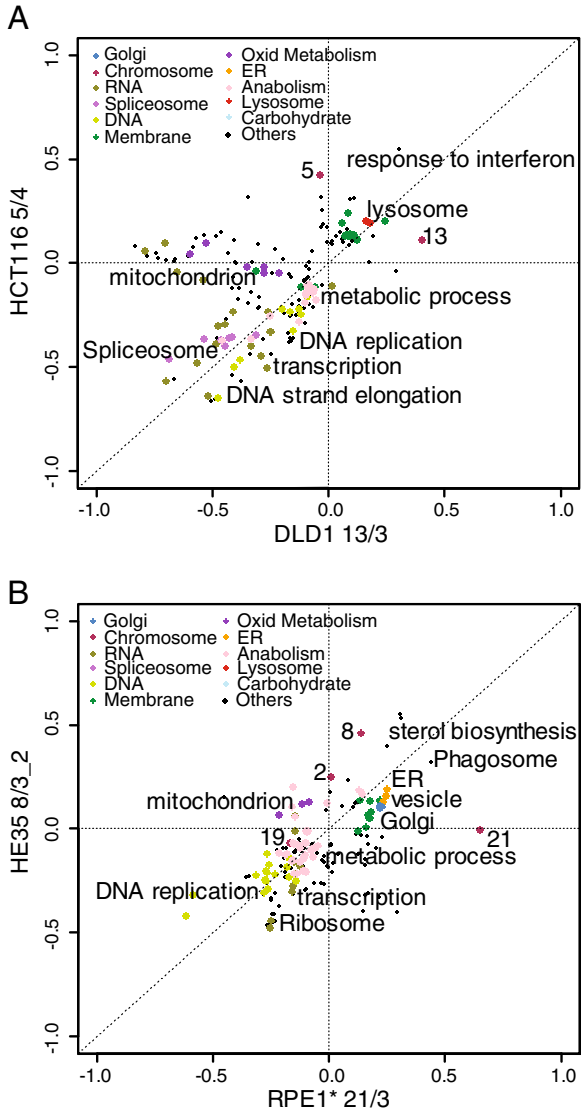


**Figure 1 Pathway alterations in tri- and tetrasomic cell lines.** **A**. 2-dimensional pathway analysis comparison of HCT116 with tetrasomy of chromosome 5 and DLD1 with trisomy of chromosome 13. Each dot represents an annotation as defined by GO and KEGG databases, color-coding marks related annotations as stated in the inset legend. The label "Others" indicates annotations that were not generalized in larger categories (see Methods). Examples of specific pathways categorized by the color- code are annotated. **B**. 2-dimensional pathway analysis comparison of HE35 (clone 2) with trisomy of chromosome 8 and RPE* with trisomy of chromosome 21. Asterisks indicate the cell lines with H2B-GFP.

(Figure 2E). We call the identified common pattern of transcriptional changes the Aneuploidy Response Pattern (ARP). Our results demonstrate that trisomy can serve as a valuable model for analysis of complex aneuploidy. Moreover, the identification of ARP suggests that chromosomal imbalance itself might be exploited as a novel target for cancer therapy regardless the type of aneuploidy and the mechanism by which it was generated.

### Comparison of the aneuploidy response pattern with transcriptional responses to stress stimuli

What triggers the specific transcriptional response to aneuploidy in human cells? Current analysis in budding yeast suggests that mutations interfering with cell proliferation result in similar gene expression changes as aneuploidy [19]. Alternatively, the transcription changes may be triggered by cellular stress caused by the presence of an extra chromosome. Thus, we asked whether there are stress stimuli that trigger reprogramming of gene expression similar to the ARP. To this end, we compared the transcription profiles of HCT116 cells exposed to sub-lethal concentrations of hydrogen peroxide, nitric oxide, hydroxyurea, actinomycin D and bafilomycin A1, or grown under hypoxic conditions as well as in medium with either low or high glucose (for further details see Methods). Strikingly, the majority of the stressors triggered pathway changes that are remarkably different from the ARP (Figure 3A, Additional file 1: Figure S3). In contrast, we observed a partial similarity between the ARP and the transcriptional changes in HCT116 treated with actinomycin D, a polypeptide antibiotic that inhibits the activity of RNA polymerases (Figure 3B,D). The 2-D annotation analysis revealed that treatment with actinomycin results in downregulation of DNA- and RNA- metabolism as well as in upregulation of ER, membrane metabolism and lysosome. This might indicate that the downregulation of DNA- and RNA metabolism in aneuploid cell lines is due to transcriptional inhibition and its consequences. However, the overlap includes only a few genes with a low Spearman correlation coefficient and thus the distance of the Spearman rank correlation is higher compared to the distances between the aneuploid cell lines (Figure 3D). Remarkably, treatment with bafilomycin A1 that inhibits vacuolar-ATPases showed transcriptional changes nearly identical to the ARP (Figure 3C,D). Bafilomycin A1 impairs vesicle fusion [25] and thus inhibits the final steps of autophagy due to the failure of autophagosome-lysosome fusion. As in aneuploids, the transcriptional changes include downregulation of DNA and RNA metabolism, whereas membrane associated annotations are upregulated. In contrast, treatment with bafilomycin does not lead to upregulation of ER, Golgi or lysosomal pathways (Figure 3C). The similarity with transcriptional effects of autophagy-inhibiting drug suggests that one of the main consequences of aneuploidy in human cells is the overload of the autophagic pathway. Taken together, our findings imply that the transcriptional changes in response to aneuploidy differ from most of the common stress responses, but show shared

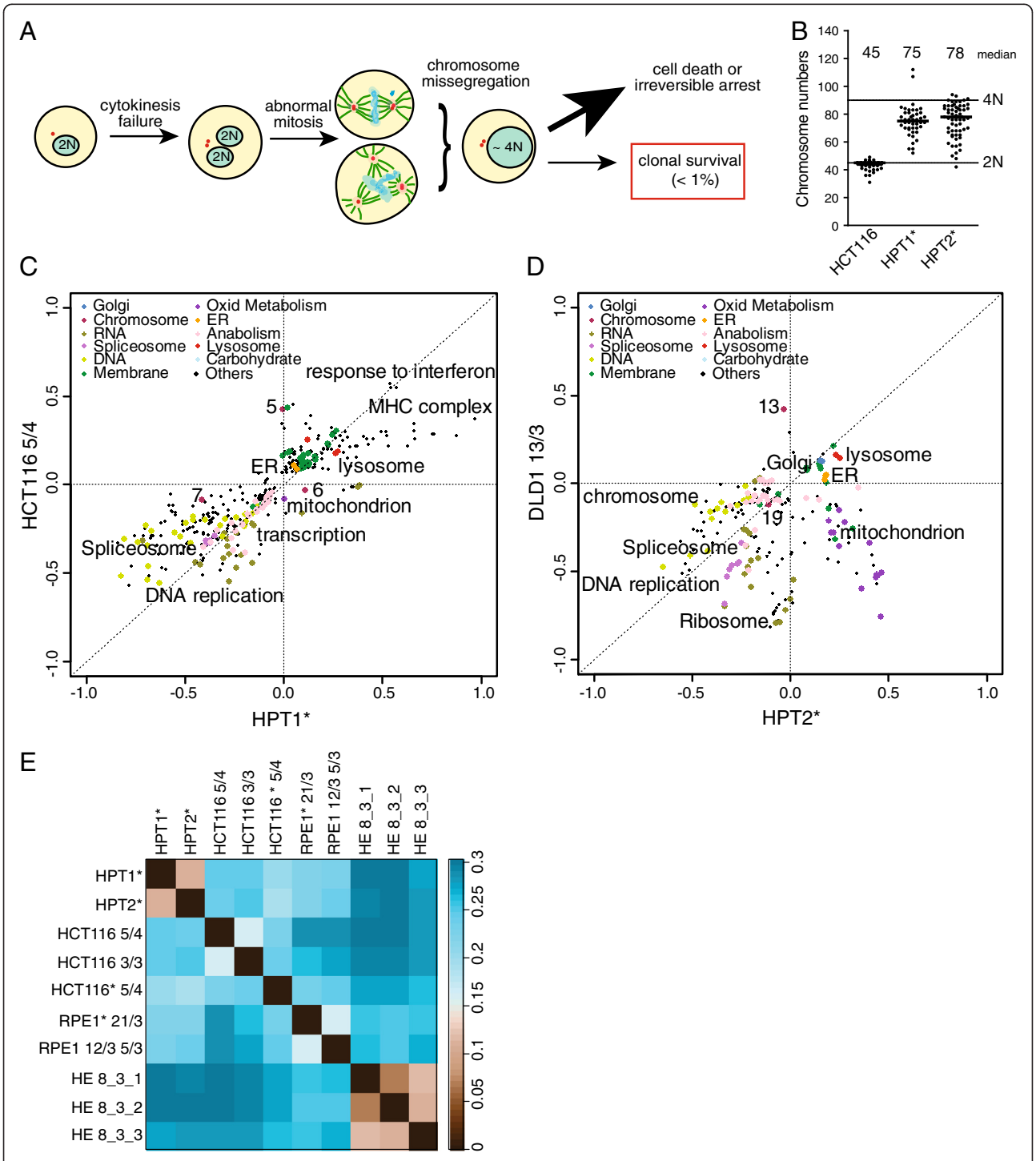


**Figure 2 Complex aneuploidy triggers similar pathway changes as trisomy or tetrasomy. A**. Schematic depiction of the construction of aneuploid cell lines with complex karyotype changes. **B**. Chromosome numbers in clonal cell lines originating from an unstable tetraploid intermediate. The numbers above the plot indicate the median chromosome number. **C**. 2-dimensional pathway analysis comparison of the complex aneuploid cell line HPT1* and HCT116 5/4. **D**. 2-dimensional comparison of complex aneuploid cell line HPT2* and DLD1 13/3. **E**. Distance matrix of the Spearman rank correlation of all analyzed aneuploid cell lines. Large distances present a low correlation and vice versa. The Spearman distance metric is defined as distanceSpearman = (1- correlationSpearman)/2 in the distanceMatrix function as part of the R Class Discovery package. The function calculates the distance of the correlation between column vectors, each of which represents different microarray experiments. Asterisks indicate the cell lines with H2B-GFP.

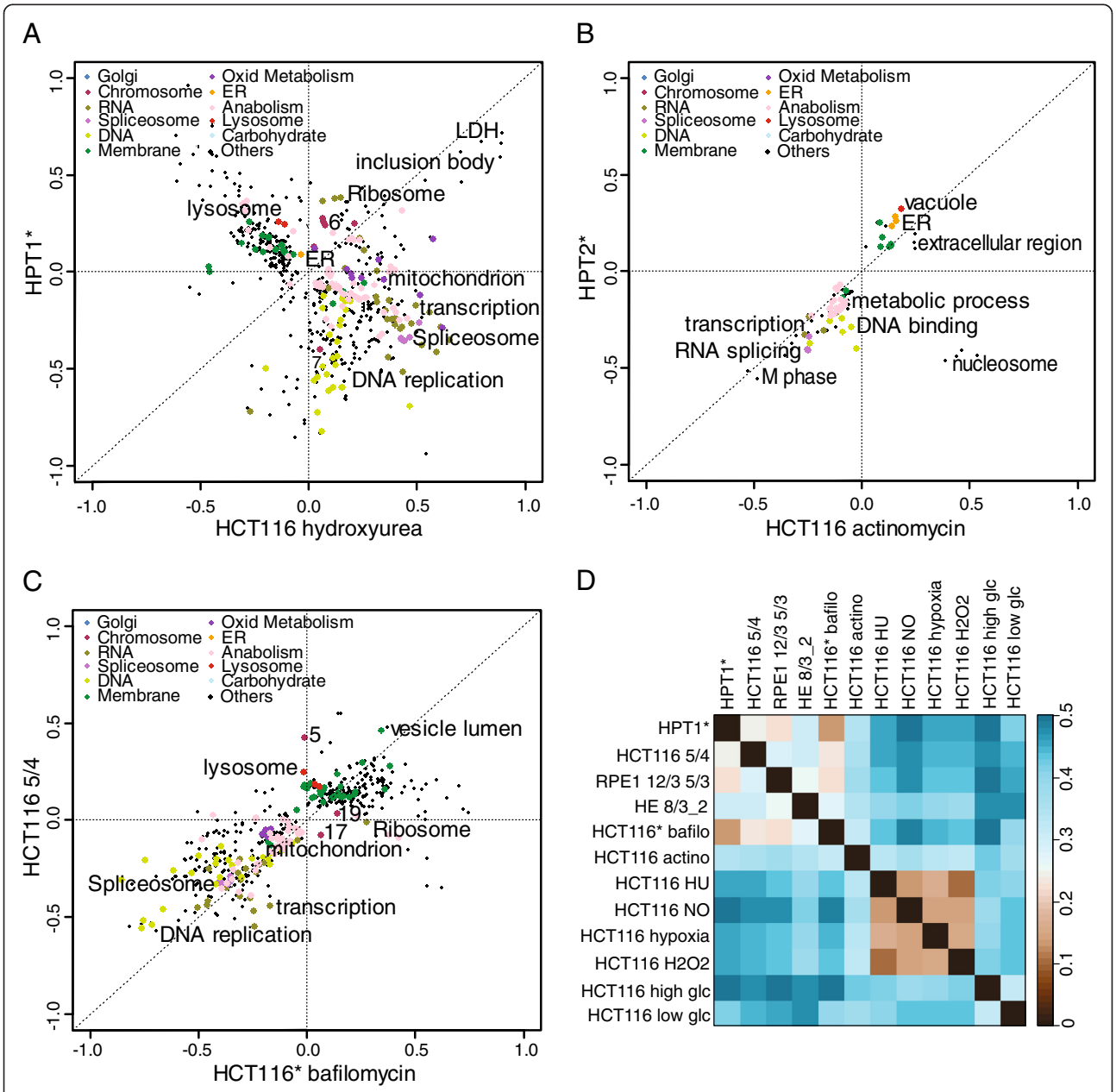


**Figure 3 Comparison of the Aneuploidy Response Pattern with transcriptional responses to stress stimuli. A**. 2-dimensional pathway analysis comparison of HCT116-derived complex aneuploid cell line HPT1* and HCT116 treated with hydroxyurea. **B**. 2- dimensional pathway analysis comparison of HCT116-derived complex aneuploid cell line HPT2* and HCT116 treated with actinomycin D. **C**. 2-dimensional pathway analysis comparison of HCT116 with tetrasomy of chromosome 5 and HCT116 grown in medium with bafilomycin A1 **D**. Distance matrix of the Spearman rank correlation of aneuploid cell lines and HCT116 grown in stress-inducing conditions. Asterisks indicate the cell lines with H2B-GFP. Abbreviations: bafilo = bafilomycin; actino = actinomycin; HU = hydroxyurea; NO = nitric oxide; H2O2 = hydrogen peroxide; glc = glucose.

features with response to conditions limiting autophagy and transcription.

### Markers of aneuploidy

Our results imply the existence of factors with recurrent expression changes triggered by aneuploidy that might serve as general markers of aneuploidy in human cells. To identify genes that are consistently up or downregulated in aneuploid cells, we merged all available datasets of aneuploid cell lines created in our laboratory. This yielded 18 genes whose expression is more than 1.4 fold increased and 5 genes whose expression is more than 1.4 fold decreased in all analyzed aneuploids (Figure 4, Table 2). The changes in mRNA levels are consistent with proteomics measurements in 7 out of 8 proteins for which the data are available [15] (Table 2). Importantly,

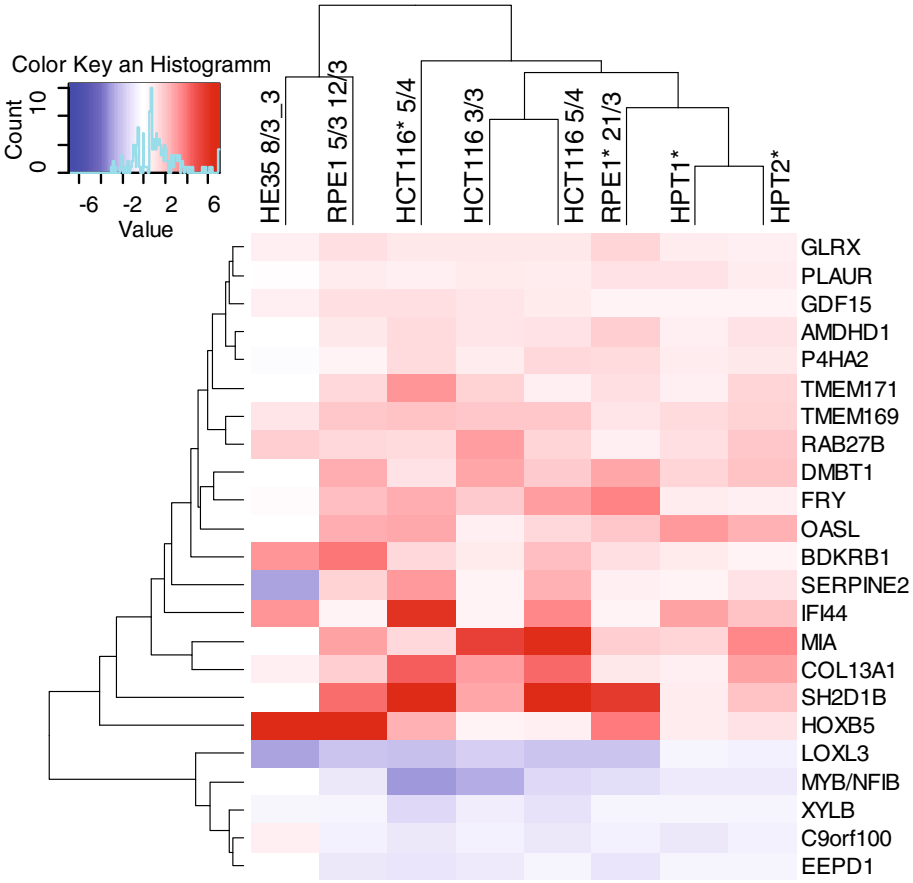


**Figure 4 Expression levels of the putative aneuploidy markers.** Heatmap of fold change ratios (aneuploid/diploid) of the 18 upregulated and 5 downregulated genes, hierarchical clustering of Euclidean distance. Asterisks indicate the cell lines with H2B-GFP.

expression of only one of these 23 genes (FRY) is similarly upregulated by the stress stimuli, suggesting that the recurrent expression changes are indeed in response to aneuploidy. Using quantitative real-time PCR we validated the expression levels of four of the identified upregulated genes in eight aneuploid cell lines and corresponding diploid controls (RAB27B, COL13A1, HOXB5, GLRX). This approach confirmed overexpression of all four genes in response to aneuploidy. Interestingly, none of the tested transcripts was confirmed in one of the complex aneuploid cell line, HPT1 (Figure 5). These candidate markers could potentially enable the discrimination of tumors with low frequency of aneuploid cells from tumors with high levels of aneuploidy and thus higher malignant potential. Further experiments will be required to determine whether the candidate markers can be used for clinical purposes for example by standard immunohistochemistry.

## Discussion

Significant recent progress in the analysis of aneuploidy in eukaryotes has revealed multiple novel features linked to aneuploidy such as growth defects, abnormal protein homeostasis and increased genomic instability. These features appear to be conserved among eukaryotes, an observation which is further supported by the finding that the transcriptional response to aneuploidy in different species remarkably correlates [19]. We analyzed in detail the transcriptional changes in aneuploid human cells by comparing the calculated aneuploid-to-diploid ratio. The comparison of eleven aneuploid model cell lines derived from four different diploid progenitor cell lines by either chromosome transfer of seven different chromosomes or by clonal propagation from unstable tetraploid progenitors revealed a striking similarity among the cell lines. The identified signature of pathways that was altered in all analyzed cell lines, which we term the aneuploidy response pattern - ARP - is characterized by upregulation of the ER and Golgi related pathways, lysosome and lytic vacuoles, MHC protein complex and antigen processing, whereas DNA and RNA metabolism and ribosome-related pathways were always downregulated (Figures 1, 2, Tables 1, 2). Our results reveal that the transcriptional changes to aneuploidy in human cells are not mediated by a canonical p53-dependent stress response, because DLD1 and its

**Table 2 List of consistently up- and downregulated transcripts (cut off 1.4 fold change)**

| | Gene | Chromosome | Function | Protein levels | Link to cancer? |
|---|---|---|---|---|---|
| 1 | PLAUR | 19 | Plasminogen activator, urokinase receptor | 4/5 | Ovarian and colorectal cancer |
| 2 | RAB27B | 18 | Secretory GTPase | 4/4 | Breast cancer |
| 3 | P4HA2 | 5 | Procollagen-proline | 4/6 | Metastasis |
| 4 | FRY | 13 | Regulator of actin cytoskeleton | 0/3 | |
| 5 | BDKRB1 | 14 | Bradykinine receptor | NA | Breast and lung cancer |
| 6 | HOXB5 | 17 | Transcription factor | NA | Leukemia, ovarian carcinoma and others |
| 7 | GDF15 | 19 | Growth differentiation factor | 1/2 | Ovarian and prostate cancer |
| 8 | OASL | 12 | 2′-5′-oligoadenylate synthetase-like | NA | |
| 9 | SERPINE2 | 2 | Serpine protease inhibitor | 4/4 | Metastasis |
| 10 | IFI44 | 1 | Interferon-induced protein 44 | NA | |
| 11 | AMDHD1 | 12 | Protein with amidohydrolase domain | NA | |
| 12 | TMEM171 | 5 | Transmembrane protein 171 | NA | |
| 13 | GLRX | 5 | Glutaredoxin | 4/4 | Pancreatic cancer |
| 14 | TMEM169 | 2 | Transmembrane protein 169 | NA | |
| 15 | DMBT1 | 10 | Membrane glycoprotein | NA | Multiple cancers |
| 16 | COL13A1 | 10 | Collagen type XIII, alpha1 | 1/1 | |
| 17 | SH2D1B | 1 | Signal transduction control | NA | |
| 18 | MIA | | Melanoma inhibitory activity | NA | Neuronal tumors |
| | | | | | |
| 1 | XYLB | 3 | Xylulokinase homologue | NA | |
| 2 | LOXL3 | 2 | Lysyl oxidase homologue | NA | |
| 3 | MYB/NFIB fusion | 6 | Myb – NFIB fusion | NA | |
| 4 | EEPD | 7 | Endonuclease/exonuclease/phosphatase domain containing | NA | |
| 5 | ARHGEF39 | 9 | Rho guanine nucleotide exchange factor 39 | NA | |

The column "Protein levels" shows how often corresponding changes of the protein levels were identified by Stingele et al. [15] (number of cell lines with altered protein expression/number of cell lines where the protein was quantified; NA – proteomics data not available). The rows 1-18 show upregulated genes; the rows 1- 5 (below) show downregulated genes.

aneuploid derivatives carry mutant p53. A remarkable feature of the identified transcriptional changes is that although similar pathways are up- and downregulated in individual aneuploids, the individual genes whose expression is altered are often variable among the different cell lines. Furthermore, several pathways were up- or downregulated in the analyzed cells that were not recurrent and thus cannot be regarded as a general consequence of aneuploidy *per se*. These changes are probably specific to the type of extra chromosomes as well as to the clonal selection. We also show for the first time that the pathway changes in human cells with complex, hypotetraploid karyotypes correlate with the changes identified in simple trisomies. Thus, the analysis of model trisomic cell lines might provide important insights into the role of the more complex aneuploidy that is frequently found in cancer.

Two of the analyzed cell lines (trisomy 7 in DLD1 and one of the three clones of trisomy 8 in HE35) showed only a partial aneuploidy response pattern (Additional file 1: Figure S1B). There are several possible scenarios to explain this finding. First, loss of some chromosome parts or a mosaic aneuploid population could lead to a weakening of the ARP. However, the transcription levels of the transcripts coded on the extra chromosomes were elevated in these cell lines as expected, which excludes this possibility. Alternatively, features specific to the supernumerary chromosome can be responsible for the difference, such as in case of chromosome 7. This has been previously observed in budding yeast, where some disomes, e.g. disome of chromosome 1, do not trigger the environmental stress response pattern (ERS) otherwise observed in other disomic strains [3]. In support of this possibility is the observation that chromosome 7

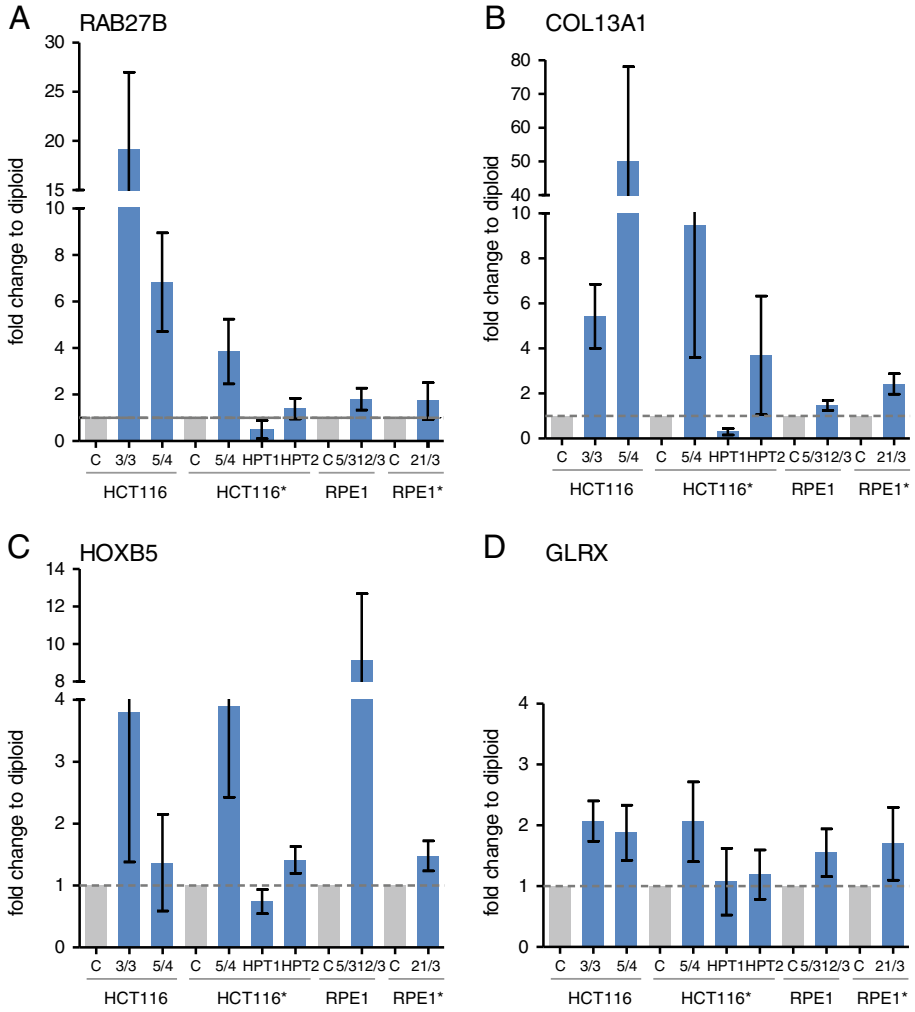


**Figure 5 Quantitative real-time PCR analysis of putative aneuploidy markers. A**. Fold change mRNA expression levels of COL13A1 quantified by real-time PCR. **B**. Fold change mRNA expression levels of RAB27B. **C**. Fold change mRNA expression levels of HOXB5. **D**. Fold change mRNA expression levels of GLRX. Asterisks indicate the cell lines expressing H2B-GFP.

is one of the few chromosomes which is more often gained than lost in chromosomally unstable cancer cells [26]. Thus, an extra copy of chromosome 7 likely represents a lesser burden for the cells than most of the other chromosomes. Finally, the deviation from the ARP might arise due to a mutation occurring during the clonal selection after the chromosome transfer. This is likely the case in HE35 8/3, where two clones correlate stronger with other aneuploids than the third trisomy 8 clone (Figure 2E). Despite these exceptions, our results show that aneuploidy triggers a uniform transcriptional response in human cells that is independent of the identity and quantity of the extra chromosomes and their combination as well as of the cell line type.

The ARP is characterized by strong downregulation of DNA and RNA metabolism, which correlates with the slow growth observed in nearly all aneuploid cells analyzed to date. Indeed, many factors required for DNA replication are less abundant, such as the heterohexameric replicative helicase MCM (Additional file 2: Table S1), and this may partially explain the previously observed slow progression through the S-phase [15]. However, the complex aneuploid cell lines progress through interphase at a rate closely matching the rate of the corresponding diploid cell lines and yet the levels of factors involved in replication are similarly decreased (Additional file 1: Figure S2). Therefore, we conclude that the proliferation impairment cannot be the only cause of ARP.

DNA repair is significantly downregulated, which might lead to increased accumulation of DNA damage. The abundance changes in the DNA replication and repair factors might explain how aneuploidy increases genome instability, as has been observed in yeast [4] as well as in human aneuploids (S.S., V.P., Z.S., unpublished results).

Further research should elucidate what are the causes of the consistent downregulation of DNA metabolism pathways and what consequences it brings upon aneuploid cells.

Among the most upregulated pathways we observed the Golgi network, ER related pathways, lysosomes and lytic vacuoles, membrane metabolism and the MHC protein complex and antigen processing. The function of MHC protein complexes is to display fragments of proteins from within the cell to immune cells [27]. Currently we do not understand the reasons for the increased levels of the MHC protein complex transcripts in aneuploid cells. We hypothesize that increased protein expression and degradation elevates the peptide presentation by MHC complex, thus elevating the immunogenicity of aneuploid cells, similarly as it has been recently observed in murine aneuploid tumors [28]. In the future it will be important to confirm this observation on the protein level and to determine whether the MHC proteins are correctly localized and functional.

Elevated expression of ER related genes might suggest an expansion of the ER that was shown in budding yeast to occur in order to alleviate ER stress [29]. This phenomenon is accompanied by elevated lipid biosynthesis, but unchanged amounts of ER chaperones [29], which closely resembles the transcriptional changes observed in aneuploid cell lines. This suggests, together with the fact that mouse trisomic MEFs are more sensitive to the Hsp90 inhibitor 17-AAG [30], that aneuploid cells suffer from protein folding defects, most likely due to the saturation of the cellular folding capacity [31]. ER stress is often observed in various cancers, where it is usually attributed to tumor microenvironments characterized by hypoxia, nutrient limitation and low pH [32]. It will be interesting to test whether chromosomal copy number changes also contribute to the ER stress in cancer cells.

Previously, it was proposed that the transcriptional response to aneuploidy is indicative of stress and slow growth [1]. Therefore, we were interested whether treatment with stress inducing agents will elicit similar response as the presence of an extra chromosome. Since chromosome missegregation was shown to trigger oxidative stress [24,33], we analysed the transcriptional response to oxidative stress inducers nitric oxide and hydrogen dioxide. Aneuploidy was also recently linked to DNA damage and replication stress [34], therefore we analyzed the response to the replication inhibitor hydroxyurea and transcription inhibition by actinomycin D. Further, aneuploidy was shown to trigger proteotoxic stress and to activate autophagy [3,15,30,31]. Therefore, we compared the response to aneuploidy to the transcriptional changes elicited by cells grown under hypoxic conditions, which is known to result in energy and ER stress with subsequent proteotoxic stress. Similarly, we tested the transcriptional changes in cells where autophagy was inhibited, a condition known to trigger proteotoxic stress. The comparison with transcription profiles of cells grown on high or low glucose was of particular interest, because aneuploid cells show changes in the metabolic pathways and higher energy demands [3,14,15]. Remarkably, we found that none of the stress conditions triggers response similar to ARP.

The only significant exception was the striking similarity between the ARP and the transcriptional changes observed in cells treated with sub-lethal concentrations of bafilomycin A1 (Figure 3). Treatment with bafilomycin A1 leads to the accumulation of vesicles and membranes and reduces cell proliferation. The similarity of cellular response to inhibition of autophagy and to aneuploidy might suggest that aneuploidy inhibits autophagy. This however is not true as we previously documented by an observation that autophagic flux (the dynamic flow through autophagy) is in aneuploids as efficient as in diploids [15]. Inhibition of basal autophagy by bafilomycin A1 treatment causes proteotoxic stress [35]. Current data suggest that aneuploidy leads to proteotoxic stress as well [31]. We propose that the similarity between the transcriptional response to treatment with bafilomycin A1 and the ARP is because both reflect transcriptional changes in cells suffering from proteotoxic stress. In general, the effect of bafilomycin A1 further emphasizes the role of autophagy in mammalian aneuploids. Aneuploidy leads to activation of autophagy in human and mouse cells [15,30] and aneuploid cells are more sensitive to the autophagy inhibiting drug chloroquine [30]. The consistent upregulation of lysosomes and lytic vacuoles appears to be specific for mammalian cells as it was not observed in aneuploid yeasts and plants [19]. In this context it is interesting that there is nearly no correlation of ARP and transcriptional changes upon low glucose condition, which is known to trigger autophagy (Additional file 1: Figure S3). We believe that this is because autophagy in aneuploids is not activated by energy or nutrition deprivation, but by different, so far unidentified triggers [36]. Partial overlap of ARP was also observed with the response to treatment with the drug actinomycin D that represses transcription by RNA polymerase and, among other effects, leads to an imbalance of ribosomal subunits [37]. Transcription-related pathways are also downregulated in aneuploids, suggesting the possibility that some of the phenotypic features of aneuploid cells might be caused by transcriptional deficiency.

Our work has also allowed the identification of several genes that were consistently up- or downregulated in aneuploid cell lines, but not in the HCT116 cells under stress stimuli. Several of the identified factors have been previously linked to cancer (Table 2). For example the plasminogen activator and urokinase receptor PLAUR/UPAR is frequently overexpressed in ovarian and

colorectal cancers, where it facilitates cell motility and metastatic potential and emerges as a marker of malignancy [38]. P4HA2 is a precursor of collagen and a metastasis marker [39] and HOXB5 is a homeobox transcription factor whose overexpression has been found in several malignant tumors [40]. Transcription of the TGFβ superfamily cytokine GDF15 (growth differentiation factor 15) is increased in multiple solid tumors, where it promotes tumor growth and confers resistance to several drugs such as bortezomib. Recent studies proposed GDF15 as a prognostic marker for ovarian and pancreatic carcinoma [41], two types of malignancies with frequent aneuploidy and chromosomal instability [9]. Glutaredoxin (GLRX) protects cells from oxidative stress and serves as a potential marker of malignancy in pancreatic carcinoma. Interestingly, some of the factors such as a xylulokinase homologue (XYLB), the 2′-5′-oligoadenylate synthetase like (OASL) and the interferon-induced protein (IFI44) have not been so far linked to cancer and may represent markers of aneuploidy that are not linked to malignancy.

Aneuploidy is associated with poor prognosis and increased drug resistance in tumors [42]. This suggests an interesting possibility that some of the genes that are expressed at higher levels in response to aneuploidy in non-transformed cells may subsequently enhance the malignant potential of these cells, whereas higher expression of others might represent a barrier for carcinogenesis. The expression of the marker genes, if confirmed in a wide range of aneuploid cell lines and in cancer cells, might provide an excellent tool for cancer biology and treatment, because it may allow distinguish tumors with high aneuploidy from predominantly diploid tumors. Moreover, pathways that are activated or inhibited by aneuploidy may serve as novel targets for cancer treatment. This has been recently demonstrated by the fact that aneuploid cells are more sensitive to drugs inhibiting HSP90, autophagy inhibitors and inhibitors of the AMPK kinase, which strongly correlates with the identified increased requirements for autophagy, protein folding and energy metabolism in aneuploid mammalian cells ([15,30] and this work). Further research should elucidate the efficacy of this approach. Our work for the first time identifies global changes in a broad spectrum of human aneuploid cell lines and may therefore help to generate new hypothesis for cancer treatments.

## Conclusions

In this study we identified a transcriptional aneuploid response pattern (ARP), a set of transcriptional changes, common in a broad range of human aneuploid model cell lines. This, for the first time, shows that complex aneuploidy, which is frequently found in cancer cells, exhibits the same transcriptional pathway changes as simple trisomy and tetrasomy. A general response to aneuploidy, as identified here in a variety of aneuploid model cell lines, might serve as a novel therapeutic target in cancer treatment. Further, we found 23 genes consistently deregulated in our model aneuploid cell lines. A confirmation of these markers in aneuploid cells might open new strategies for identifying aneuploid tumors. Since the fraction of aneuploid cells in tumor correlates with malignant potential and poor prognosis in cancer, simple and reliable biomarkers for aneuploidy may help for determining the appropriate cancer therapy.

## Methods

### Cell lines

Following model aneuploid cell lines were used for data analysis: parental cell line HCT116 (human colon carcinoma cell line): HCT116 3/3 (trisomy 3), HCT116 5/4 (tetrasomy 5) [43]; parental cell line HCT116 H2B-GFP: HCT116 5/4 (tetrasomy 5) [15], HPT1, HPT2 (hypertetraploids with complex karyotypes, A.Y.K. unpublished data); parental cell line RPE1 (human retinal pigment epithelial cell line, hTERT immortalized): RPE15/3 12/3 (trisomy 5, 12); parental cell line RPE1 H2B-GFP: RPE1 21/3 (trisomy 21) [15]; parental cell line DLD1 (human colon adenocarcinoma cell line): DLD1 3/3 (trisomy 3), DLD1 7/3 (trisomy 7), DLD1 13/3 (trisomy 13) [13], parental cell line HE35 (human embryonic cell line): HE35 8/3, clones 1–3 [17].

### Cell cultures

The HCT116- and RPE1- derived tri- and tetrasomic cell lines have been described previously [15]. The post- tetraploid cell lines HPT1 and HPT2 were generated by expansion of individual tetraploids formed by induced inhibition of cytokinesis through dihydrocytochalasin treatment. The DNA content was determined by flow cytometry, standard karyotyping, chromosome painting and array comparative genomic hybridization as in [15]. Cells were maintained in 10 cm dishes in Dulbecco's Modified Eagle Medium (DMEM) at 37°C and 5% CO2. Culture medium was supplemented with growth factors from 5% Fetal Calf Serum (FCS) and 1% Penicillin/ Streptavidin to avoid bacterial growth and contamination. Specific antibiotics were added to the medium when necessary to maintain the supernumerary chromosomes. Cell lines were cultured at 70- 90% confluence for a maximum of 10 passages.

Material for transcription analysis was obtained at the earliest possible time point, after sufficient amount of cells was achieved, approximately 25 generations after the chromosome transfer or tetraploidization, respectively. Similar approach was taken for the analysis of trisomy 8 HE35-derived clones [17]. The mRNA of

DLD1-derived aneuploids was isolated from multiple different passages [13].

### Cell growth analysis

Freshly cultured cells that carry H2B-GFP to fluorescently label the chromosomes were seeded 24 h before the experiment. Time laps movies were taken by imaging asynchronous cells in a 10 min or 4 min interval for 72 or 48 h, respectively. The time in interphase was measured as the time from nuclear envelope reformation to nuclear envelope break down.

### Quantitative real-time PCR

Total RNA was extracted with the RNeasy Mini Kit (Qiagen), treated with DNAse (recombinant DNase, Roche) and subsequently transcribed into cDNA (Transcriptor First Strand cDNA Synthesis Kit, Roche Diagnostics). Specific primers were designed using PrimerBlast; the sequences are listed below. Quantitative PCR was conducted using the Light Cycler 480 System (Roche Diagnostics) with the KAPA SYBR FAST master mix optimized for Roche Light Cycler 480 (KapaBiosystems). Absolute quantification with an external standard was performed and negative non-template controls were tested in all experiments. The specificity of the primer product amplification was confirmed in each run by melting curve analysis. mRNA expression was normalized to the control gene coding for ribosomal protein L30 (RPL30) and fold change to corresponding diploid mRNA expression was calculated. Primers: COL13A1 – forward: *GGGGGAAGCAGGACTAGATG*, reverse: *CCTGAAGCT CCGGGTAGTC*, RAB27B - forward: *TGCGGGACAAGA GCGGTTCCG*, reverse: *GCCAGTTCCCGAGCTTGCCG TT*, HOXB5 - forward: *TCCACAAATCAAGCCCTCCA*, reverse: *GTCCGGGCCATTTGGATAAC*, GLRX – forward: *AACGGTGCCTCGAGTCTTTA*, reverse: *CCTATGAGATC TGTGGTTACTGC*.

### mRNA expression analysis by microarrays

Genome-wide expression profiling of HCT116- and RPE1-derived aneuploid cells lines was conducted in three replicates by IMGM laboratories GmbH (Martinsried, Germany) as previously described [15]. cDNA was hybridized on Agilent Whole Human Genome Oligo microarrays (4x44K format) for HCT116 diploid and HCT116 5/4, or Agilent SurePrint G3 Human GE microarrays (8x60K) for the other cell lines according to a One-Color based hybridization protocol. Raw data was background normalized. The data has been deposited in the NCBI Gene Expression Omnibus (GEO, http://www.ncbi.nlm.nih.gov/geo/) accessible through GEO Series accession number GSE47830 and GSE47836.

Microarray data of trisomic and diploid colorectal cancer cell lines DLD1 [13] were kindly provided by Thomas Ried (National Institutes of Health, Bethesda, Maryland, USA). All other mRNA expression data were obtained from NCBI's Gene Expression Omnibus (http://www.ncbi.nlm.nih.gov/geo/). HCT116 cell line grown under high and low glucose conditions: accession number GSE31084; HCT116 after stress treatment: accession number GSE3176; HCT116 cell line treated with actinomycin D: with GSE12459, human embryonic diploid and trisomic cells: accession number GSE28076.

### Microarray normalization

Bioinformatics analysis of the microarray data was performed using Perseus (1.2.6.16) as part of the MaxQuant Software Package [21] and R on the free open source integrated development environment R Studio. The gBG SubSignal (green Background Subtracted Signal) from background-normalized cDNA microarray data was used for further normalization. The background-subtracted raw intensities were log2 transformed and global normalization of the log transformed raw data was performed by subtracting the median of the overall signal intensities for one experiment from each signal in this experiment. Probe sets for one gene were summarized by taking the median. The median of replicative probes signal intensities was calculated for each cell line. For comparison of each aneuploid cell line with the corresponding diploid cell line gene, expression fold change ratios were calculated.

### Data analysis

The Student's *t*-test was performed to verify the statistical significance of the fold change in mRNA expression between the signal intensities of aneuploid and diploid cell line. To correct the test statistics for multiple comparisons, false discovery rate control of the p-values was applied. Both local and frequent FDR were calculated with the "fdrtool" package [44]. For further analysis, a fold change cut off 1.4 in mRNA expression was applied. To investigate the correlation between the cell lines, a distance matrix of the Spearman rank correlation coefficient was calculated in R using the "ClassDiscovery" package in the OOMPA library and "mclust" package. Distance of the correlation was visualized in a colored matrix in the "spatstat" package. To investigate a global response to aneuploidy on gene level, data sets were filtered for those genes more than 1.4 fold up- or down-regulated in all HCT116 and RPE1 derived cell lines. The resulting gene lists were analyzed for their function and relation using the DAVID functional annotation tool [22], KEGG pathway and the NCBI Gene database.

### Pathway enrichment analysis

Pathway enrichment analysis was conducted in Perseus using the 2-dimensional annotation enrichment tool [21]. Thereby, enriched GO and KEGG annotations were

identified by testing whether genes in an annotated category have a significant preference to be altered compared to the global fold change data distribution. Significance was tested by a *t*-test followed by a false discovery rate correction in the Benjamini-Hochberg procedure (FDR cut off 0.02). Expression values for significant enriched annotations were summarized in an annotation score from −1 to 1 to represent levels of up- or downregulation. Significant and enriched annotations were summarized in larger, general categories for visualization purposes. 2-dimensional annotations enrichments of two cell lines were plotted in R. Highly up- or downregulated pathways were cross validated by submitting the fold change cut off datasets to the annotation enrichment tool DAVID [22].

### Statistical analysis

Commonly used R packages were "lattice" [45], "genefilter" [46], "fdrtool" [44] and "calibrate" [47]. In addition, Perseus (1.2.6.16) as part of the MaxQuant Software Package [21] was used for microarray analysis. All statistically evaluated experiments were performed in at least three independent biological replicates. The final plots were prepared in GraphPad Prism 5 software or R.

### Availability of supporting data

The data sets supporting the results of this article are available in the NCBI Gene Expression Omnibus repository (http://www.ncbi.nlm.nih.gov/geo/) with the accession numbers GSE47830 and GSE47836.

## Additional files

**Additional file 1: Figure S1.** Significantly altered pathways in aneuploid model cell lines. **Figure S2**. Time in interphase of the HCT116*- derived cell lines. **Figure S3**. Significantly altered pathways in response to stress stimuli compared to the ARP.

**Additional file 2: Dataset 1 Gene expression data of HCT116 and RPE1 derived aneuploid cell lines compared to the diploid cell lines.**

**Additional file 3: Dataset 2 Data derived from the 2- dimensional annotation analysis.**

#### Competing interests

The authors declare that they have no competing interests.

#### Authors' contributions

ZS initiated the study. AYK, SS and VP generated the cell lines and prepared the samples for analysis. MD performed the bioinformatics analysis and the quantitative RT PCR. MD, AYK, GS and ZS discussed and designed the data analysis strategies. ZS wrote the manuscript. All authors read and discussed the final manuscript.

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## 6.2 HSF1 deficiency and impaired HSP90-dependent protein folding are hallmarks of aneuploid human cells

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In this study we investigated the effects of aneuploidy on the proteostasis network of human cell lines. It was previously shown that aneuploid cell lines exhibit features of proteotoxic stress such as sensitivity to inhibition of protein synthesis and folding as well as accumulation of cytoplasmic protein deposits and autophagy activation (Oromendia et al., 2012; Stingele et al., 2013; Tang et al., 2011; Torres et al., 2007). However, the underlying molecular mechanisms of these phenotypes have not been clear.

To study the effect of aneuploidy on proteostasis network functions such as protein folding, we investigated the cellular sensitivity to protein folding stress as well as abundance and activity of the chaperone system. Aneuploid cell lines show impaired folding of a firefly luciferase sensor protein under normal conditions. In particular, the HSP90-dependent protein folding capacity appears to be compromised. Furthermore, viability of aneuploid cells is specifically sensitive to HSP90 inhibition, but not to other protein folding stresses. This goes in hand with reduced levels of HSP90 family expression. Moreover, global proteome analysis revealed that the abundance of HSP90 clients is reduced and that the proteome of aneuploid cells shows similar changes to the proteome after HSP90 inhibition. As an underlying cause we identified reduced expression levels and activity of HSF1. Overexpression of HSF1 mitigated the protein folding defect as well as the sensitivity to HSP90 inhibition. Moreover, aneuploid clones with an endogenous extra copy of HSF1 proliferated markedly better than other aneuploid cell lines. Finally, the overall transcriptional profile of aneuploid cells resembles the transcriptional profiles of cells with functional deficiency of HSF1.

Compromised HSF1 activity was identified as the key causative factor of impaired proteostasis, but also contributes to the phenotypic alterations of aneuploid cells. HSF1 overexpression is the first aneuploidy-tolerating mutation identified in higher eukaryotes so far. Given that *HSF*1 region is frequently amplified in cancer, this reveals a new link between aneuploidy and HSF1 in tumorigenesis.

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# HSF1 deficiency and impaired HSP90-dependent protein folding are hallmarks of aneuploid human cells

Neysan Donnelly[1], Verena Passerini[1], Milena Dürrbaum[1,2], Silvia Stingele[1] & Zuzana Storchová[1,2,*]

## Abstract

**Aneuploidy is a hallmark of cancer and is associated with malignancy and poor prognosis. Recent studies have revealed that aneuploidy inhibits proliferation, causes distinct alterations in the transcriptome and proteome and disturbs cellular proteostasis. However, the molecular mechanisms underlying the changes in gene expression and the impairment of proteostasis are not understood. Here, we report that human aneuploid cells are impaired in HSP90-mediated protein folding. We show that aneuploidy impairs induction of the heat shock response suggesting that the activity of the transcription factor heat shock factor 1 (HSF1) is compromised. Indeed, increased levels of HSF1 counteract the effects of aneuploidy on HSP90 expression and protein folding, identifying HSF1 overexpression as the first aneuploidy-tolerating mutation in human cells. Thus, impaired HSF1 activity emerges as a critical factor underlying the phenotypes linked to aneuploidy. Finally, we demonstrate that deficient protein folding capacity directly shapes gene expression in aneuploid cells. Our study provides mechanistic insight into the causes of the disturbed proteostasis in aneuploids and deepens our understanding of the role of HSF1 in cytoprotection and carcinogenesis.**

**Keywords** aneuploidy; cancer; HSF1; HSP90; protein folding
**Subject Categories** DNA Replication, Repair & Recombination; Molecular Biology of Disease; Protein Biosynthesis & Quality Control
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## Introduction

Aneuploidy is defined by karyotypes that differ from multiples of the haploid chromosome set. Aneuploidy is not well tolerated in higher eukaryotes and represents one of the leading causes of spontaneous abortions in humans, with the rare surviving newborns suffering from multiple defects (Colnaghi *et al*, 2011). Moreover, aneuploidy is prevalent in cancer, where nearly 80% of solid tumors and approximately 60% of hematopoietic cancers show karyotypes differing from normal diploidy (Stankiewicz & Lupski, 2010). At the cellular level, aneuploidy is often associated with global pathway deregulation, impaired proliferation, increased energetic and metabolic demands and altered sensitivity to cytotoxic drugs (Upender *et al*, 2004; Niwa *et al*, 2006; Torres *et al*, 2007; Williams *et al*, 2008; Pavelka *et al*, 2010; Nawata *et al*, 2011; Stingele *et al*, 2012). The cellular response to aneuploidy is highly conserved from yeast to human and remarkably, appears to be largely independent of the exact karyotypic composition (Sheltzer *et al*, 2012; Stingele *et al*, 2012; Durrbaum *et al*, 2014). Experiments in budding yeast have shown that the mere presence of a transcriptionally silent additional chromosome does not lead to any apparent phenotypes; hence, taken together, it is the gene expression of the aneuploid genome that determines the phenotypic changes observed in aneuploids (Torres *et al*, 2007). However, in spite of these important insights, the molecular mechanisms underlying these global cellular changes are not yet fully understood.

Recent progress in understanding the cellular effects of aneuploidy was facilitated by analysis of model aneuploid cells with defined karyotypic changes such as aneuploid budding and fission yeast strains, fruit flies with segmental aneuploidy, mouse embryonic fibroblasts with unbalanced Robertsonian translocations and trisomic and tetrasomic human cell lines (Upender *et al*, 2004; Niwa *et al*, 2006; Torres *et al*, 2007; Williams *et al*, 2008; Stenberg *et al*, 2009; Pavelka *et al*, 2010; Nawata *et al*, 2011; Stingele *et al*, 2012). Analysis of disomic budding yeast revealed increased sensitivity to drugs that interfere with protein synthesis and degradation (Torres *et al*, 2007). Moreover, the proliferation defect of some disomies can be ameliorated by mutation of the Ubp6 protein, a deubiquitinating enzyme that was proposed to negatively regulate protein degradation (Torres *et al*, 2007, 2010). In line with these findings in yeast, aneuploidy in murine and human cells imposes profound changes in protein homeostasis (proteostasis). Human trisomic cells show an accumulation of cytoplasmic foci positive for both ubiquitin and SQSTM1/p62, a marker of selective autophagy (Stingele *et al*, 2012). Further, primary trisomic MEFs are sensitive to chemical inhibition of the chaperone HSP90 as well as to the inhibitor of autophagy chloroquine (Tang *et al*, 2011). In agreement with this finding,

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chromosomally unstable aneuploid cancer cell lines are more sensitive to HSP90 inhibition than chromosomally stable cell lines (Tang *et al*, 2011). A recent study demonstrates that aneuploid budding yeast harbor protein aggregates and that protein folding of HSP90 clients is compromised in these cells (Oromendia *et al*, 2012). These results suggest that the protein expression from supernumerary chromosomes places a burden on cellular proteostasis and that the HSP90 machinery might be particularly affected. However, the status of HSP90 function and the protein folding capacity of aneuploid cells in higher eukaryotes were so far unknown.

Here, we demonstrate for the first time that protein folding is significantly impaired by aneuploidy in human cells. Taking advantage of trisomic and tetrasomic human cells that we constructed using micronuclei-mediated chromosome transfer into the human near-diploid colorectal cancer cell line HCT116 and human immortalized retinal pigment epithelial cell line RPE-1 (Stingele *et al*, 2012), we show that in particular HSP90-mediated protein folding is compromised. Intriguingly, we found that HSF1-dependent activation of the heat shock response (HSR) is impaired, suggesting a mechanism by which aneuploidy impairs protein folding capacity. Importantly, endogenous or exogenous overexpression of HSF1 counteracts the effects of aneuploidy on HSP90-dependent protein folding, thereby identifying enhanced expression of HSF1 as the first aneuploidy-tolerating genetic modification in human cells. Finally, we demonstrate that the functional HSP90 and HSF1 deficiency has marked consequences for protein abundance and shapes the patterns of gene expression observed in aneuploid cells.

## Results

### Trisomic and tetrasomic human cell lines show defects in protein folding

Using model trisomic and tetrasomic human cell lines, we previously found an increased amount of cytoplasmic ubiquitin-positive foci in aneuploids in comparison to cognate diploids (Stingele *et al*, 2012). The accumulation of ubiquitinated proteins might be either due to a defect in their removal or due to their increased production in aneuploid cells. However, human aneuploid cells activate the catabolic pathway of autophagy (Stingele *et al*, 2012), and proteasome activity is not impaired by aneuploidy (Supplementary Fig S1A–C). This suggests that ubiquitinated proteins accumulate at higher rates, possibly due to an overwhelmed or impaired protein folding capacity, as previously proposed (Oromendia *et al*, 2012; Donnelly & Storchova, 2014). To directly test whether trisomic or tetrasomic human cells display protein folding defects, we employed a set of three Firefly luciferase-based sensor proteins comprising wild-type Firefly luciferase (FlucWT) and single and double mutant luciferase, FlucSM and FlucDM, that are highly sensitive to changes in the protein folding environment (Gupta *et al*, 2011). First, we performed luciferase refolding assays after transiently expressing FlucWT in diploid HCT116 cells and HCT116* cells, which stably express histone H2B-GFP, and their respective aneuploid derivatives. To this end, we subjected transfected cells to heat shock at 43°C for 2 h, which is sufficient to denature > 70% of luciferase, but does not result in toxicity. We then monitored refolding at 37°C over 4 h by measuring luminescence. We observed a significant impairment of FlucWT refolding in cells with trisomy and tetrasomy of chromosome 5 compared to their respective parental cell lines (Fig 1A and B). Next, we examined the effect of aneuploidy on the more sensitive mutants FlucSM and FlucDM. The mutations disrupt the stability of the Fluc protein, but do not affect its enzymatic activity. We thus hypothesized that the effect of aneuploidy on luciferase refolding should be even more pronounced in cells transfected with the destabilized mutant proteins. Indeed, the relative ability to refold both FlucDM (Fig 1C and D) and FlucSM (Supplementary Fig S1E and F) was almost threefold reduced in aneuploid cells in comparison to the parental HCT116. Thus, aneuploidy causes protein folding defects in human cell lines.

The HSP90 chaperone is required for the refolding of heat-denatured luciferase, and the destabilizing mutations in FlucSM and FlucDM lead to an indispensable requirement for HSP90 for folding even in the absence of stress (Schneider *et al*, 1996; Gupta *et al*, 2011). Thus, whereas FlucWT activity is not impaired by HSP90 inhibition, the luminescent signal of FlucSM and FlucDM decreases upon treatment with 17-N-allylamino-17-demethoxygeldanamycin (17-AAG), a derivative of the antibiotic geldanamycin that binds the ATP pocket of the chaperone HSP90 (Supplementary Fig S1D). To specifically investigate HSP90-dependent protein folding capacity in aneuploid cells, we transfected diploid and aneuploid HCT116 cells with the Fluc sensors and measured luminescence in response to treatment with 17-AAG. Strikingly, we observed a consistent and significant decrease in FlucDM activity following treatment with 17-AAG in aneuploid cells compared to the control HCT116 cells (Fig 1E). These findings indicate that HSP90 function is indeed limiting in aneuploids. To exclude the possibility that the aneuploidy-induced protein folding defect is specific for aneuploid derivatives of HCT116 or for cells carrying extra copies of chromosome 5, we analyzed two additional aneuploid cell lines derived from RPE-1 (trisomy 21 and trisomy of chromosomes 5 and 12). Similarly as in HCT116-derived aneuploids, we observed consistently lower relative levels of FlucDM activity in aneuploid cells in contrast to diploid RPE-1 cells after treatment with 17-AAG (Fig 1F). These observations provide direct evidence that impaired protein folding and defective HSP90 function are common features of human aneuploid cells.

### Aneuploid cells are more sensitive to inhibition of HSP90 but not to other inducers of protein folding stress

To elucidate in more detail the protein folding defect in human aneuploid cells, we analyzed their sensitivity to drugs that either directly inhibit molecular chaperones or impose a severe strain on the protein folding machinery. To this end, we measured cell viability after treatment with the HSP90 inhibitor 17-AAG, the HSC70/HSP70 inhibitor VER 155008, L-azetidine-2-carboxylic acid (AZC), a toxic L-proline analog that leads to the misfolding of newly synthesized polypeptides (Qian *et al*, 2010), and heat shock (45°C, 15 h). We observed a significant sensitivity of trisomic and tetrasomic HCT116 and RPE-1 cells to 17-AAG as measured by both cell viability as well as colony-forming assays (Fig 2A and B). In contrast, the response to the other compounds was less uniform: While aneuploid RPE-1 cells were more sensitive to HSC70/HSP70 inhibition by VER 155008 than diploids, aneuploid HCT116 cells were slightly more resistant to this treatment than controls (Fig 2C). Cell

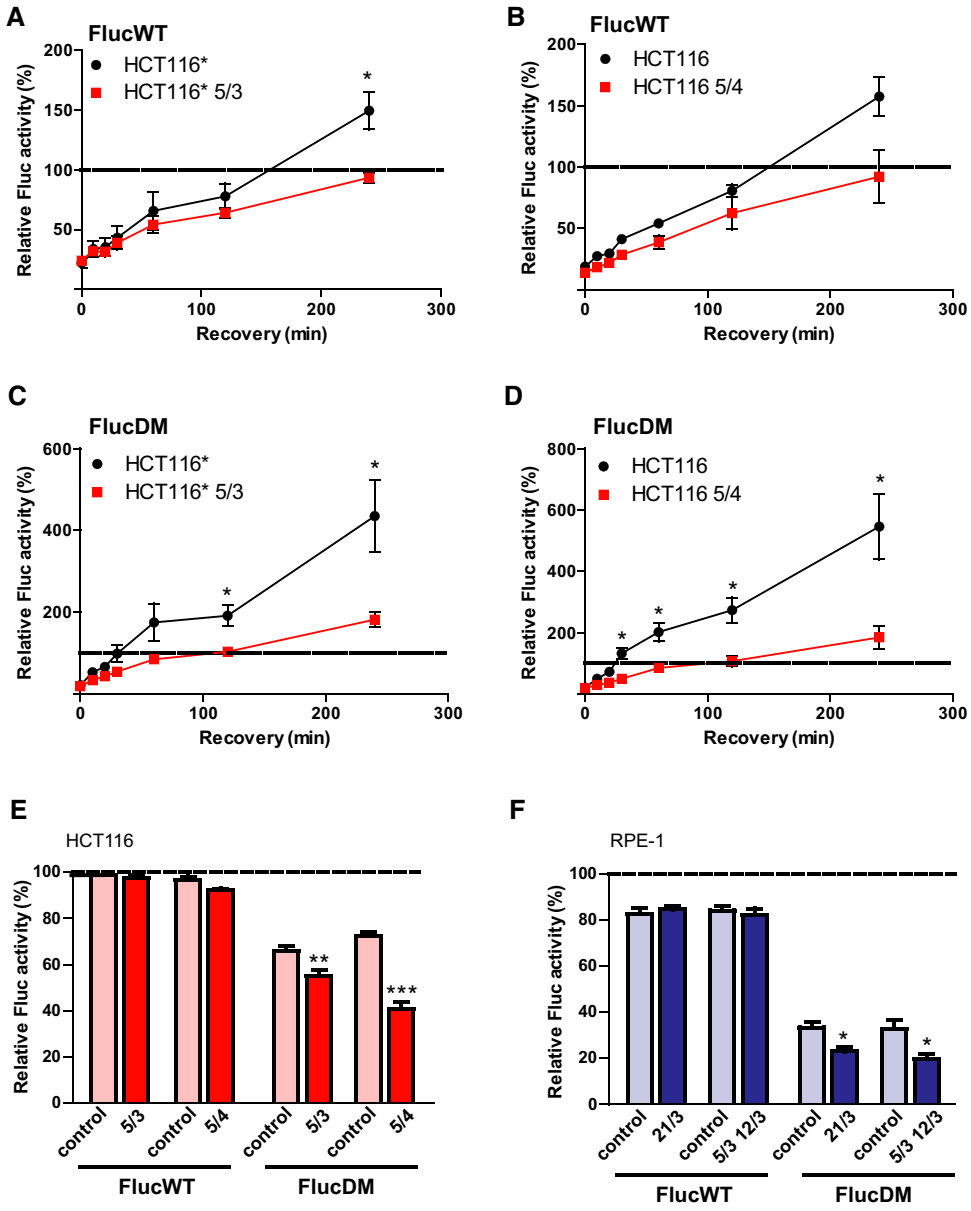



**Figure 1. Trisomic and tetrasomic human cell lines show defects in protein folding.**

A–D Refolding of the sensor proteins upon heat shock in control cells and in respective aneuploids. HCT116 and HCT116* stably expressing histone H2B-GFP and their aneuploid derivatives were transfected either with FlucWT-mCherry (A, B) or FlucDM-mCherry (C, D) and subjected to heat stress for 2 h at 43°C. Controls were maintained at 37°C. Luminescence readings were taken immediately from heat-stressed cells (0 min) and at indicated time points after recovery at 37°C. The luminescence values of control cells maintained at 37°C were set to 100% (indicated by dotted line).

E, F Refolding of the sensor proteins upon HSP90 inhibition in control cells and in respective aneuploids. FlucWT-mCherry or FlucDM-mCherry was expressed in parental and aneuploid HCT116 or HCT116* (E) and RPE-1 or RPE-1* (F) cell lines for 36 h. Cells were then incubated with either solvent control (DMSO) or 50 nM 17-AAG for 8 h followed by measurement of luminescent activity. The depicted values show the percentage of luminescence in cells treated with 17-AAG relative to DMSO-treated cells (which were set to 100%).

Data information: All plots show the means of at least three independent experiments ± SEM. *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$; non-parametric *t*-test.

line-specific responses were also observed after heat shock: Only HCT116* 5/3 cells were highly sensitive to heat shock, while the other aneuploidies either showed a similar response as controls or even a marked resistance (Fig 2D). Finally, aneuploid cells were not significantly more sensitive to treatment with AZC (Supplementary Fig S2A and B). We conclude that aneuploidy exerts profound effects on cellular proteostasis, but only the increased sensitivity to HSP90 inhibition was common to all aneuploid cells that we tested. Thus, in line with previous observations from aneuploid yeast (Oromendia *et al*, 2012) and murine cells (Tang *et al*, 2011), a specific impairment of HSP90-mediated protein folding represents a general and conserved consequence of aneuploidy.

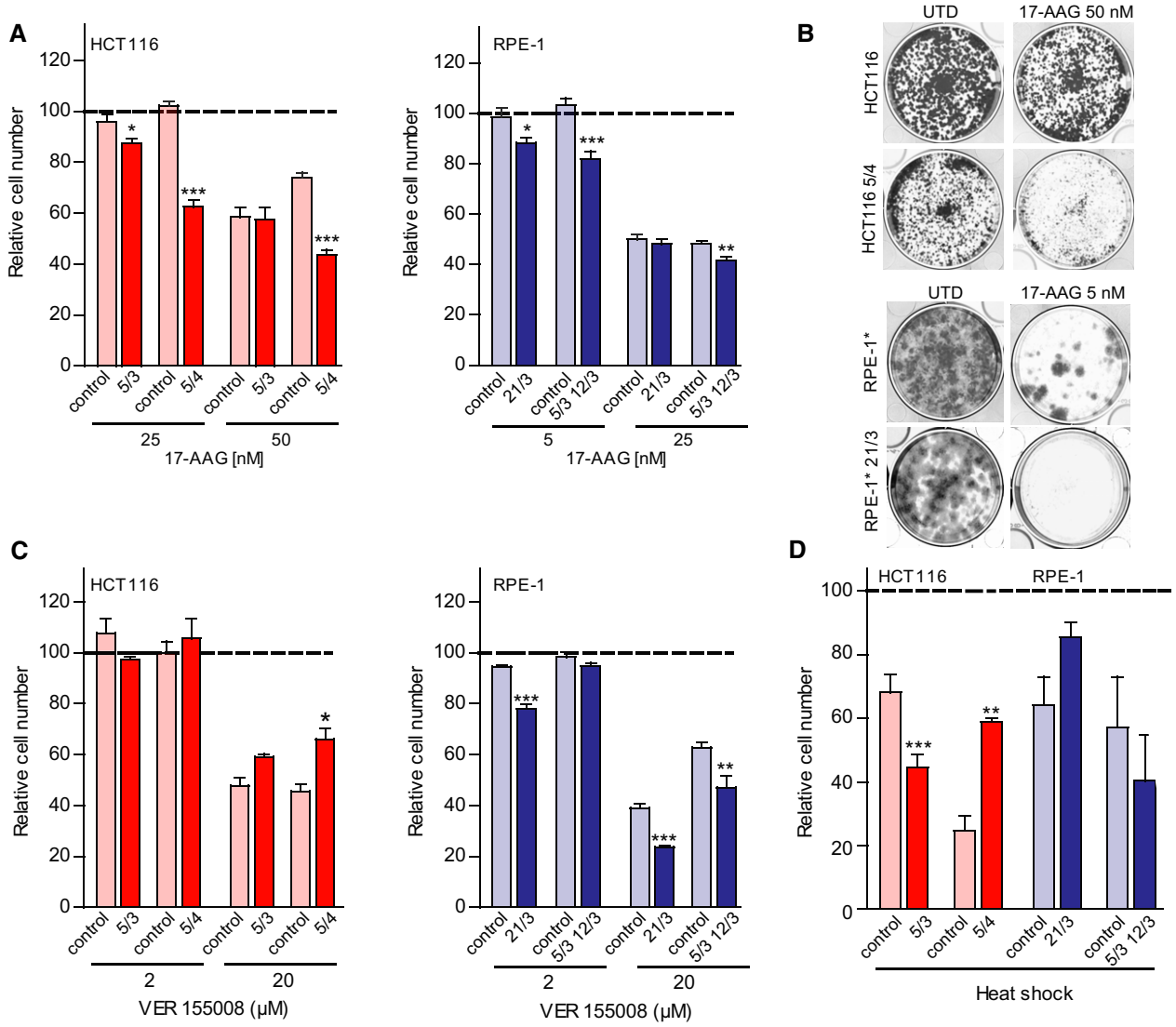



**Figure 2. Sensitivity to inhibition of HSP90 but not to other inducers of protein folding stress increases in aneuploid cells.**

A Wild-type, trisomic and tetrasomic cells were treated with the indicated concentrations of 17-AAG, and cell number was determined 72 h thereafter. Cell number is shown as the percentage of the DMSO-treated control.

B Colony formation efficiency of aneuploid and parental RPE-1 treated with either solvent control (DMSO) or 17-AAG at indicated concentrations. Cells were stained with crystal violet after 2 weeks.

C Wild-type, trisomic and tetrasomic cells were treated with the indicated concentration of VER 155008, and cell number was determined 72 h thereafter. Cell number is shown as the percentage of the DMSO-treated control.

D Wild-type, trisomic and tetrasomic cells were subjected to heat stress for 15 h at 45°C, and cell number was determined. Cell number is shown as the percentage of the untreated control (maintained at 37°C).

Data information: All plots show the means of at least three independent experiments ± SEM. *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$; non-parametric *t*-test.

### The basal and stress-induced activity of HSF1 is impaired in human aneuploid cells

Our results suggest that aneuploid cells may be compromised in their ability to mount a robust HSR when challenged with stress. Moreover, the impaired ability to refold luciferase evident already at very early time points of recovery (10, 20 min; Fig 1C and D) may point to a reduction in steady-state protein folding capacity. We hypothesized that the HSP90-specific protein folding defect might be due to changes in expression levels of factors involved in the maintenance of cellular proteostasis. Analysis of the expression changes in heat shock protein families (Kampinga *et al*, 2007) from our previously obtained global transcriptome and proteome data (Stingele *et al*, 2012) revealed a small but statistically significant reduction in both mRNA and protein abundance for the HSP90 family across a panel of six different aneuploid cell lines (Supplementary Fig S3A). Protein levels of chaperonins were also slightly decreased, although mRNA levels were not significantly changed

(Supplementary Fig S3A). Analysis by immunoblotting revealed that the levels of HSP27, HSP70, and HSP90 were modestly, but consistently and significantly reduced in the majority of aneuploid cell lines analyzed (Fig 3A and B). The protein folding capacity of molecular chaperones is regulated, in part, by binding to co-chaperones and other co-factors. Phosphorylation at the C-termini of HSP70 and HSP90 enhances binding to the co-chaperone HOP, thereby increasing productive protein folding and cellular proliferation rates (Muller *et al*, 2013). However, the ratios of phosphorylated HSP90 and HSP70 over total levels were unchanged when comparing parental cell lines and HCT116 aneuploid derivatives (Supplementary Fig S3C and D). Our finding that the chaperone expression is impaired in aneuploid cells prompted us to analyze the expression of the heat shock response transcription factor and master regulator of chaperone expression HSF1 as well. The immunoblotting revealed a consistent reduction in protein levels in all four cell lines tested (Fig 3A and B). Notably, the transcription of the *HSF1* gene is not altered in aneuploid cells, as we observed only negligible changes in HSF1 mRNA levels in qPCR experiments (Supplementary Fig S3B).

In light of our observations regarding HSF1 protein levels, we asked whether the induction of HSF1 activity, that is, the ability to mount a HSR, was also impaired in aneuploid cells. To this end, we expressed a HSP70-luc construct that contains the *HSP70* promoter fused to luciferase in diploid and aneuploid cells (Williams *et al*, 1989). We then treated the cells with 17-AAG and the proteasome inhibitor MG132, compounds that induce acute proteotoxic stress and are well-characterized activators of HSF1-dependent transcription (Mathew *et al*, 2001; Sharma *et al*, 2012). We observed that the parental cell lines induced the expression of the HSP70-luc sensor two- to threefold soon after the proteotoxic treatment and up to sevenfold in response to prolonged treatment (Fig 3C and D). In contrast, the ability of HCT116-derived and RPE-1-derived aneuploids to induce HSP70-luc was on average reduced to 50 and 60% of the control at early and later time points, respectively (Fig 3C and D). Interestingly, at the later time point, RPE-1 21/3 had recovered the ability to activate the *HSP70* promoter. This is in line with the relatively mild decrease in HSF1 and chaperone levels in this cell line and with its relatively modest sensitivity to 17-AAG (Figs 2A and 3A and B). These observations might be explained by the small size of chromosome 21; hence, RPE-1 21/3 is burdened with the least amount of extra genetic material of all the aneuploid cell lines analyzed in this study. Consistent with these findings, we also observed an impaired ability to induce HSP70 expression after acute heat shock in both HCT116- and RPE-1-derived aneuploid cells (Supplementary Fig S3E). The decrease in HSF1 expression observed in aneuploid cells is relatively small, and therefore, we asked whether it is sufficient to cause the observed impairment in maintenance of proteostasis and protein folding. To address this concern, we transfected the control cell lines with siRNA to partially deplete HSF1 to 75 and 50%, respectively (Fig 3E). Indeed, consistent with previous results (Chen *et al*, 2013), this partial and transient depletion of HSF1 rendered cells sensitive to treatment with 17-AAG, thus suggesting a striking dosage sensitivity of the cellular response to proteotoxic stress (Fig 3F). Therefore, we conclude that the protein folding defect engendered by aneuploidy may be caused by inhibitory effects on basal and induced HSF1 activity.

### Endogenous or exogenous overexpression of HSF1 ameliorates the negative effects of aneuploidy on protein folding

If the reduced protein folding capacity of aneuploid cells is due to a deficiency in HSF1 function, aneuploid cells with increased levels of HSF1 may be protected against this impairment. In fact, increased expression of HSF1 due to gene amplification is frequent in cancer, similarly as gain of chromosome 8 or its long arm where the *HSF1* gene is located on 8q24.3 (Beroukhim *et al*, 2010). Thus, we reasoned that cells that gained chromosome 8 with the resulting increased expression of HSF1 might escape the defects in HSP90 function and protein folding caused by gain of a chromosome without HSF1. To test this possibility, we generated four clonal cell lines derived from individual HCT116 cells upon micronuclei-mediated transfer of chromosome 8. Using chromosome painting, we confirmed the presence of an extra copy of chromosome 8 in all imaged cells from all four clonal cell lines (HCT116 8/3 c1-c4; Supplementary Fig S4A). Analysis of the expression of HSF1 protein revealed an increased abundance according to expected gene copy number, that is, approximately 1.5-fold higher relative to diploid HCT116, in the clonal cell lines c1, c2, and c4. Interestingly, HSF1 levels were not substantially changed in c3 (Fig 4A and B). This was likely due to a loss of the distal region of chromosome 8 where HSF1 is located (Supplementary Fig S4B, our unpublished data). Thus, we reasoned that comparison of c1, c2, and c4 with c3 would enable us to directly test whether increased levels of HSF1 protect cells from the protein folding defects caused by the introduction of the extra copy of chromosome 8. We first analyzed the expression of HSP90. Similarly to other trisomies and tetrasomies that we analyzed (Fig 3A and B, and Supplementary Fig S3A), introduction of chromosome 8 elicited a slight decrease in HSP90 levels in c3 (Fig 4A and B). Strikingly, however, in c1, c2, and c4, we observed no decrease in HSP90 levels relative to control HCT116 (Fig 4A and B). Next, we tested the sensitivity of FlucDM to 17-AAG treatment in the four clones. The luminescent output of FlucDM was significantly lower after treatment with 17-AGG in HCT116 8/3 c3 compared to control HCT116. In contrast, the relative decreases in luminescence in response to 17-AAG treatment were comparable to the control in the trisomic cell lines c1, c2, and c4 (Fig 4C). Moreover, whereas c3 exhibited a sensitivity to 17-AAG that was comparable with the other trisomies, c1, c2, and c4 were as resistant to 17-AAG as the parental HCT116 (Fig 4D). Thus, the increased levels of HSF1 counteract the negative effect of aneuploidy on HSP90 expression and on protein folding.

To directly determine a role for increased HSF1 activity in mitigating the effects of aneuploidy on HSP90 and protein folding, we performed transient transfections with a constitutively active truncated HSF1 allele (ca-HSF1) (Zuo *et al*, 1995). Immunoblotting confirmed efficient expression of ca-HSF1 upon transient transfection in HCT116 8/3 c3 aneuploids as well as in HCT116 5/4 and RPE-1 21/3 and increased the expression levels of its downstream targets (Fig 4E). Importantly, the transient ca-HSF1 expression significantly improved the survival of aneuploid cells in the presence of 17-AAG as well as protected the folding of the FlucDM sensor against its effects (Fig 4F and G). Our observations suggest that cellular sensitivity to 17-AAG is finely tuned to the levels of HSF1. To test the generality of this conclusion, we transfected the control HCT116 and

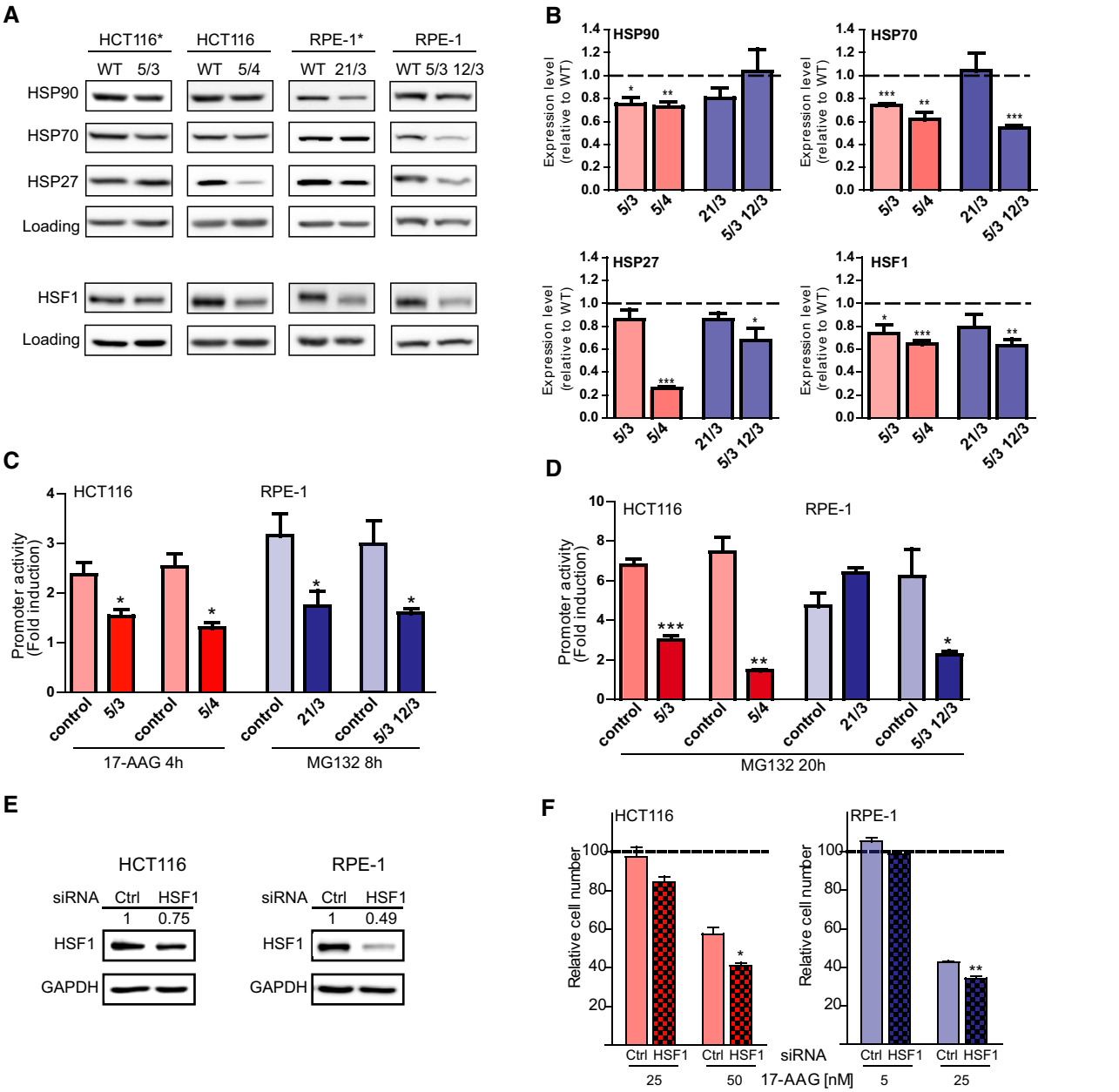



**Figure 3. The basal and stress-induced activity of HSF1 is impaired in human aneuploid cells.**

A, B Western blot analysis for HSP27, HSP70, HSP90 (the used antibody recognizes both constitutive and inducible forms of HSP90) and HSF1 in parental and aneuploid cell lines (A). Loading control: GAPDH; HSC70 (constitutively expressed chaperone) in RPE-1 5/3 12/3 and corresponding control (note that GAPDH is encoded on chromosome 12). Shown are representative images of at least 3 independent experiments. In panel B the quantification of the signal intensities from the Western blots shown in (A) are depicted, calculated relative to control cells (which were set to 1).

C, D HSP70-luc plasmid was expressed in parental and aneuploid HCT116 and RPE-1 cell lines for 36 h. Cells were then incubated with solvent control (DMSO), 2 μM 17-AAG or 5 μM MG132 for the indicated times. The depicted values show the fold induction in 17-AAG- or MG132-treated cells compared to DMSO-treated cells (which were set to 1).

E HCT116 (left panel) and RPE-1 (right panel) cells were transfected with siRNA targeting HSF1 or the GL2 subunit of luciferase as a control (ctrl). Cell extract was prepared 72 h after transfection and subjected to immunoblotting for HSF1 and GAPDH as a loading control. Quantification of the signal normalized to the loading control is shown above the images.

F HCT116 (left panel) and RPE-1 (right panel) cells transfected with siRNA targeting HSF1 or the GL2 subunit of luciferase as a control (ctrl). Forty-eight hours after transfection cells were incubated with the indicated concentrations of 17-AAG, and cell number was determined 72 h thereafter. Cell number is shown as the percentage of the DMSO-treated control.

Data information: All data are the mean of at least three independent experiments ± SEM. *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$; non-parametric *t*-test.
Source data are available online for this figure.

RPE-1 cells with ca-HSF1 (Fig 4H). In agreement with our hypothesis, the transient overexpression of ca-HSF1 also significantly protected the control cell lines against 17-AAG-associated toxicity (Fig 4I and J). Taken together, these results show that increasing the levels of the HSR master regulator HSF1 is sufficient to counteract the impaired HSP90 function of human aneuploid cells.

Since trisomy in human cells leads to proliferation defects, we asked whether the difference in HSF1 expression is reflected by changes in proliferative capacity. In line with our hypothesis that HSF1 mitigates some of the phenotypes caused by aneuploidy, we observed that HCT116 clones with high HSF1 expression proliferated markedly faster than HCT116 8/3 c3 (Supplementary Fig S4E). This also suggests that the decreased protein folding capacity contributes to the proliferation defects observed in trisomic cells.

Chromosome 8 carries approximately 4,170 open reading frames, among them the *CCNE2* gene encoding cyclin E2 that plays a critical role in the G1 and in the G1-S transition and is often overexpressed in cancers, and *MYC* encoding the c-Myc transcription factor, a critical oncogene which upregulates a large number of genes involved in cell proliferation (Dang, 1999; Hwang & Clurman, 2005). We thus asked whether increased levels of c-Myc or cyclin E2 may be involved in improved proliferation rates in c1, c2 or c4. Western blotting revealed that cyclin E2 levels were increased on average 1.23-fold, and c-Myc levels were either unchanged or slightly reduced in all four HCT116 8/3 cell lines (Supplementary Fig S4C and D), in line with our previous finding that the abundance of some proteins is lower than expected based on the corresponding copy number changes in aneuploid cells (Stingele *et al*, 2012). As expected, based on these observations, Spearman correlation analysis revealed that only HSF1 levels highly correlated with the sensitivity of trisomic cells to HSP90 inhibition, their protein folding capacity as well as with proliferation rate (Supplementary Fig S4F). Taken together, both endogenous and exogenous overexpression of HSF1 ameliorates the adverse effects of aneuploidy on HSP90-dependent protein folding and proliferation in human cells.

### Impaired HSP90 function in aneuploid cells affects the abundance of HSP90 client proteins

HSP90 plays a critical role in the folding of a wide variety of client proteins, in particular protein kinases as well as steroid hormone receptors and subunits of macromolecular complexes (Rohl *et al*, 2013). Thus, we asked whether the defect in HSP90 activity in aneuploid cells leads to a decreased abundance of client proteins that rely on HSP90. To this end, we compared recently reported data that elucidate the global HSP90 interactome and that classify interactors based on the strength of their interaction with HSP90 (Taipale *et al*, 2012) to the transcriptome and proteome changes that we observed in human aneuploid cell lines (Stingele *et al*, 2012). Our analysis revealed that the abundance of proteins that strongly interact with HSP90 was significantly lower in two out of the four aneuploid cell lines tested (HCT116* 5/4 and HCT116 5/4). In contrast, the abundance of non-interacting proteins was not affected in any of the analyzed cell lines (Fig 5A, Supplementary Datasets S1 and S2). Additionally, mRNA levels of strong interactors were unchanged, indicating that only the protein levels are affected (Fig 5A). Because this dataset of interactors may not represent a comprehensive list of all HSP90 clients, we also compared protein expression data from aneuploids with respect to another database of HSP90-interacting proteins generated by the Picard laboratory (http://www.picard.ch/Hsp90Int/index.php). Again, we observed a significant reduction in the expression of HSP90 interactors in two out of four aneuploid cells lines (HCT116 5/4 and RPE-1* 21/3; Fig 5B, Supplementary Dataset S3). Taken together, the reduced abundance of HSP90-interacting proteins in three out of four aneuploid cell lines supports the hypothesis that the HSP90 machinery is impaired in aneuploid cells and suggests that this impairment directly contributes to the altered protein composition of aneuploid cells.

### The global expression changes in aneuploids resemble the cellular responses to HSP90 and HSF1 deficiency

HSP90 represents a critical hub in cell signaling through its chaperoning of a wide array of kinases and other proteins. Indeed, pharmacological inhibition of HSP90 results in significant alterations in the activity of multiple signaling pathways (Sharma *et al*, 2012). Our previous analysis of the changes in pathway regulation in human aneuploid cells identified a specific set of pathways that are up- or downregulated in response to aneuploidy, and these pathways appear to be conserved (Sheltzer *et al*, 2012; Stingele *et al*, 2012; Durrbaum *et al*, 2014). However, it remains unclear which molecular processes are responsible for these protein expression changes observed in aneuploid cells. Thus, we first asked whether the impairment in HSP90 function contributes to the changes of protein abundance in aneuploids. We compared the quantitative proteome changes in aneuploids cells with the proteome changes occurring upon pharmacological inhibition of HSP90 for 24 h (Sharma *et al*, 2012). The analysis of proteome changes upon HSP90 inhibition was performed in HeLa cells that are extensively aneuploid. Therefore, the proteome of treated cells was normalized to the proteome of untreated HeLa cells. Using 2-dimensional annotation enrichment analysis that enables direct comparison of relative pathway enrichments (Cox & Mann, 2012), we found an overlap between the proteome changes due to aneuploidy and proteome changes due to HSP90 inhibition (Supplementary Dataset S4), in particular among the downregulated pathways, which includes pathways of DNA and RNA metabolism, such as DNA repair and replication and RNA splicing, as well as cell cycle pathways (Fig 5C, Supplementary Fig S5A and B).

HSF1 predominantly regulates the expression of genes involved in proteostasis as part of the heat shock response, but has recently been shown to control the transcription of multiple additional target genes (Mendillo *et al*, 2012). We therefore compared the transcriptional profile of aneuploid cells with the transcriptional profile of a human hepatocellular carcinoma cell line (HCC) in which HSF1 was depleted by RNAi (Chuma *et al*, 2014). Comparison of the pathway enrichment in aneuploid cells and in cells depleted of HSF1 revealed a striking similarity in both downregulated and upregulated pathways (Fig 5D, Supplementary Fig S5C, Supplementary Dataset S4). Similar comparison with cells depleted of c-Myc showed no similarities between the pathways changes. This analysis suggests that the transcriptional activity of c-Myc does not affect the pathways that are deregulated by aneuploidy and supports the notion that the observed effect is specific for HSF1 (Supplementary Fig S5D). These results suggest that functional deficiency in HSF1 is a major determinant of the previously identified transcriptional aneuploidy response

**Figure 4. Endogenous or exogenous overexpression of HSF1 ameliorates the negative effects of aneuploidy on protein folding.**

A, B Western blot analysis of HSF1 and HSP90 expression in HCT116 8/3* c1-c4 (A). Loading control: GAPDH. Shown are representative images of at least 3 independent experiments. Quantification of the signal intensities from the Western blots (B), calculated relative to control cells (which were set to 1).

C FlucDM-mCherry was expressed in parental HCT116* and HCT116* 8/3 c1-c4 for 36 h. Cells were then incubated with either solvent control (DMSO) or 50 nM 17-AAG for 8 h followed by measurement of luminescent activity. The depicted values show the percentage of luminescence in cells treated with 17-AAG relative to DMSO-treated cells (which were set to 100%).

D Parental HCT116* and HCT116* cells trisomic for chromosome 8 (HCT116 8/3* c1-c4) were treated with the indicated concentrations of 17-AAG, and cell number was determined 72 h thereafter. Cell number is shown as the percentage of the DMSO-treated control (which was set to 100%).

E Western blot analysis of HSF1 expression and its downstream targets in the indicated aneuploid cells transfected with ca-HSF1. Loading control: GAPDH. Shown are representative images of at least 3 independent experiments. Quantification of the signal intensities normalized to the loading control is shown above the images.

F RPE-1* 21/3 cells were transiently transfected with either pCDNA or ca-HSF1 by electroporation. Forty-eight hours post-transfection cells were incubated with the indicated concentrations of 17-AAG, and cell number was determined 72 h thereafter. Cell number is shown as the percentage of the DMSO-treated control.

G FlucDM-mCherry was co-expressed with either pCDNA or ca-HSF1 in the indicated cell lines for 36 h. Cells were then incubated with either solvent control (DMSO), 50 nM 17-AAG (HCT116), or 5 nM 17-AAG (RPE-1) for 8 h followed by measurement of luminescent activity. The depicted values show the percentage of luminescence in cells treated with 17-AAG relative to DMSO-treated cells (which were set to 100%).

H Western blot analysis of HSF1 expression and its downstream targets in the indicated aneuploid cells transfected with ca-HSF1 using electroporation. Loading control: GAPDH. Shown are representative images of at least 3 independent experiments. Quantification of the signal intensities normalized to the loading control is shown above the images.

I Control HCT116* cells were transiently transfected with either pCDNA or ca-HSF1 by electroporation. Fourty-eight hours post-transfection cells were incubated with the indicated concentrations of 17-AAG and cell number was determined 72 h thereafter. Cell number is shown as the percentage of the DMSO-treated control.

J Control RPE-1* cells were transiently transfected with either pCDNA or ca-HSF1 by electroporation. Fourty-eight hours post-transfection cells were incubated with the indicated concentrations of 17-AAG and cell number was determined 72 h thereafter. Cell number is shown as the percentage of the DMSO-treated control.

Data information: The data are the mean of at least three independent experiments ± SEM. *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$; non-parametric *t*-test.

pattern (Stingele *et al*, 2012; Durrbaum *et al*, 2014). Taken together, our analyses suggest that the functional HSF1 and HSP90 deficiency caused by aneuploidy determines the global transcriptome and proteome changes in these cells.

## Discussion

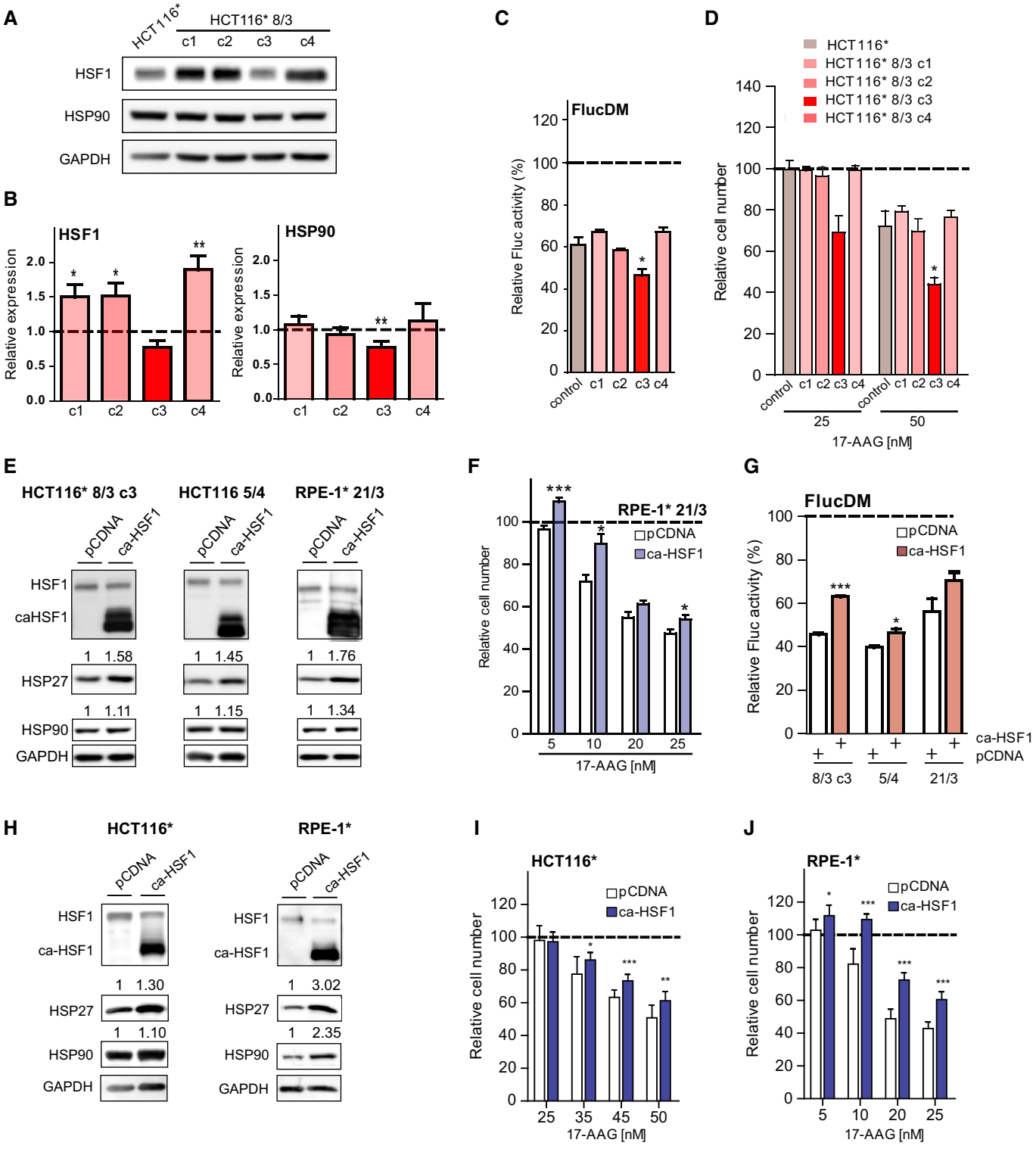
Aneuploid cells often suffer from low proliferation rates and exhibit hallmarks of cells undergoing proteotoxic stress as evidenced by their sensitivity to conditions that compromise or overburden protein folding (Torres *et al*, 2007; Tang *et al*, 2011; Oromendia *et al*, 2012; Stingele *et al*, 2012). Here, we directly demonstrate for the first time that human aneuploid cells suffer from a protein folding defect and show that in particular HSP90-dependent protein folding is affected. Additionally, we identify a pronounced impairment in the ability of aneuploids to trigger a full heat shock response, suggesting that the functionality of heat shock-associated factors, in particular, the responsible transcription factor HSF1, is compromised. Importantly, we demonstrate that increasing the gene copy number of *HSF1* counters the effects of aneuploidy on HSP90 expression and protein folding. Finally, our analysis suggests that the observed functional deficiency in HSP90 and HSF1 contributes to the transcriptome and proteome changes observed in aneuploid cells. Thus, we propose that the cellular defects associated with aneuploidy may be direct consequences of impaired protein folding capacity.

### Aneuploidy impairs protein folding

Both disomic budding yeast and tri- and tetrasomic human cells accumulate cytoplasmic protein deposits (Oromendia *et al*, 2012; Stingele *et al*, 2012). Previously, it has been proposed that the increased protein expression in aneuploid cells leads to a saturation of protein folding capacity and to low-level but chronic protein misfolding (Oromendia *et al*, 2012; Donnelly & Storchova, 2014). The misfolding, in turn, leads either to the aggregation, or destabilization and degradation of proteins with high or specific demands on the chaperone machinery. Indeed, we show that the presence of even one extra chromosome significantly impairs cellular protein folding in human aneuploid cells (Fig 1). This is mainly due to a defect in HSP90-dependent protein folding, whereas targeting the early steps in protein folding immediately after release from the ribosome, through AZC or HSP70 inhibition, does not preferentially impair the viability of human aneuploid cells (Figs 1 and 2, and Supplementary Fig S2). We hypothesize that the toxicity associated with impairment of proper protein folding at an early stage is determined by how efficiently and quickly cells can dispose of terminally misfolded proteins. Since both proteasome activity and autophagic degradation are elevated in mammalian aneuploid cells (Supplementary Fig S1B and C and Tang *et al*, 2011; Stingele *et al*, 2013), this may explain why they are not more sensitive or even slightly more resistant to such impairment. In contrast, we propose that the sensitivity to HSP90 inhibition observed in all the aneuploids analyzed in this study, regardless of the identity of the supernumerary chromosome(s) or the cell line rather reflects the loss-of-function of HSP90 clients and of HSP90-dependent processes. Therefore, our data together with previous observations in trisomic MEFs and disomic budding yeast (Tang *et al*, 2011; Oromendia *et al*, 2012) make a compelling argument that aneuploidy leads to a specific functional deficiency in HSP90-mediated protein folding.

In seeking to determine an explanation for why the HSP90 chaperone machinery is particularly affected by aneuploidy, we discovered that HSP90 family proteins were downregulated at both the mRNA and protein levels across a panel of six aneuploid cell lines. Intriguingly, this downregulation correlated with a decrease in total HSF1 levels in all four aneuploid cells lines tested and an impaired ability to induce HSF1 activity in response to acute proteotoxic stress (Fig 3). We emphasize, however, that the response to acute proteotoxic stress was not completely abolished, but rather delayed




and diminished. Notably, while a delay and decrease can be detected in aneuploid budding yeast strains, this effect appears to be more modest (Oromendia *et al*, 2012). Interestingly, elevated levels of HSP72, but not HSP90 or other heat shock factors, were identified in aneuploid murine fibroblasts compared to diploid controls (Tang *et al*, 2011). The differential regulation of HSP72 and HSP90 suggests that the activation of HSP72 in murine aneuploids is not due to elevated HSF1-dependent transcription, but rather modulated by other means that are specific to HSP72. Despite this difference, aneuploidy renders murine fibroblasts sensitive to the HSP90 inhibitor 17-AAG similarly as human aneuploids, thus further strengthening the notion that HSP90-mediated protein folding is specifically

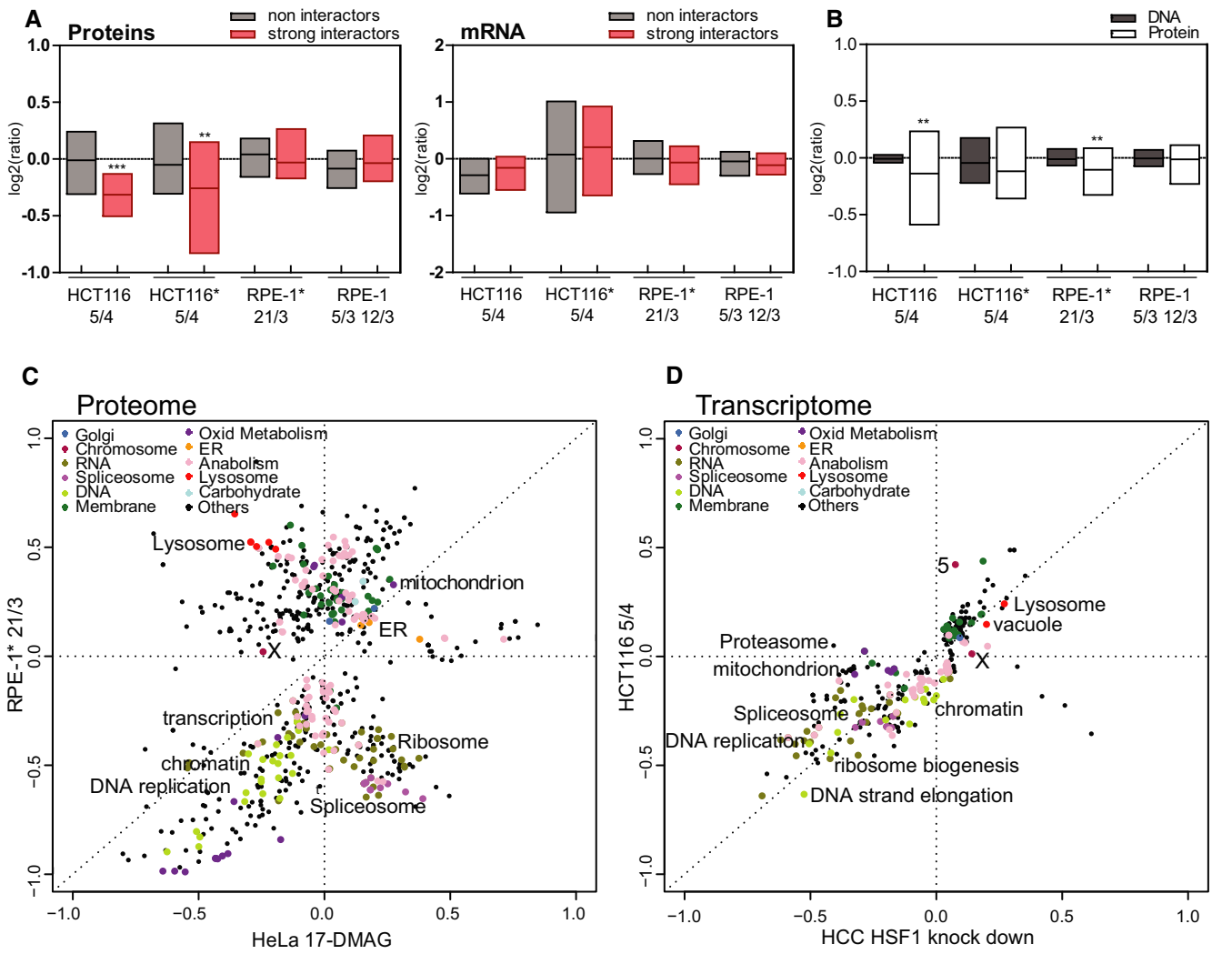



**Figure 5. The proteome and transcriptome changes in aneuploid cells resemble the cellular response to protein folding deficiency.**

A Relative abundance (calculated aneuploid/diploid ratio) of proteins that were identified as non-interactors and strong interactors of HSP90 in human cells (left panel). Relative abundance (calculated aneuploid/diploid ratio) of corresponding mRNAs (right panel). *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$; Mann–Whitney *U*-test.

B Relative abundance (calculated aneuploid/diploid ratio) of factors that were identified to interact with HSP90. *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$; Mann–Whitney *U*-test.

C Changes in pathways identified in the proteome of cells upon inhibition of HSP90 compared to the proteome changes in trisomic cells using the 2D-annotation enrichment analysis.

D Changes in pathway regulation identified in the transcriptome of HCC cells upon HSF1 knockdown compared to transcriptome changes in trisomic cells using the 2D-annotation enrichment analysis.

limiting for aneuploid cells. In future, it will be important to address by what mechanism aneuploidy impairs HSF1 function and why HSP90-dependent protein folding is particularly affected. We propose two hypothetical mechanisms for how HSF1 function might be impaired by aneuploidy. First, the metastable protein HSF1 may be incorporated into the ubiquitin-positive cytoplasmic deposits in aneuploid cells, and thereby rendered inactive. Alternatively, the HSF1 protein may be subject to post-translational inhibitory regulation that is elevated in aneuploids. Interestingly, overexpression of model β-sheet proteins in human cells also impairs the induction of cellular stress responses (Olzscha *et al*, 2011). Uncovering the similarities and differences in these two models of chronic proteotoxic stress will improve our understanding of the mechanisms involved in the maintenance of protein homeostasis.

We found that increased copy numbers of HSF1 can alleviate the protein folding defect and the impaired response to proteotoxic stress in aneuploid cells. This was confirmed in two different scenarios, by transfer of chromosome 8 and by transient overexpression of transgenic HSF1 (Fig 4). We employed overexpression of the upstream regulator of the heat shock response to ensure integrated and balanced expression of HSP90 and its co-chaperones. This is essential because the concentration of essential co-chaperones is limiting for HSP90 activity (Li *et al*, 2012), and the chaperone-dependent processes often require all components of a given chaperone

system (Rampelt *et al*, 2012). It should be noted that the transformed cell line HCT116 contains segmental aneuploidies, specifically copy gain on the long arms of chromosomes 8, 10, 16, and 17 and a loss of the Y-chromosome. These copy number changes are preserved in all created trisomic and tetrasomic cell lines (Stingele *et al*, 2012). However, our results suggest that an additional increase in copy numbers of HSF1 is necessary to rescue the defects arising in response to another whole chromosomal aneuploidy in *de novo* created trisomic cells so that the activity of HSF1 is sufficient to override the negative effects of aneuploidy on the protein folding machinery. More generally, our results suggest that in the context of the cellular response to severe proteotoxic stress (e.g. HSP90 inhibition), augmentation of HSF1 levels and/or activity fulfills a powerful cytoprotective function.

Previously, it was shown that a loss-of-function mutation in the gene encoding the deubiquitinating enzyme Ubp6 also markedly alleviated the negative effects of aneuploidy including impaired proliferation and accumulation of cytoplasmic protein deposits in budding yeast (Torres *et al*, 2010; Oromendia *et al*, 2012). Here, we have identified the first aneuploidy-tolerating genetic modification in human cells. These results support the interesting possibility that the adverse effects of aneuploidy can be suppressed either by enhancing protein degradation or by increasing cellular protein folding capacity. It is generally accepted that the sensitivity of cancer cells to HSP90 inhibitors stems from their reliance on heat shock proteins to chaperone the high number of overexpressed or mutated oncoproteins, and from the role of chaperones in protecting against general cellular stress associated with tumorigenesis (Dai *et al*, 2007). Our findings together with previous work (Tang *et al*, 2011) may provide an additional rationale for why inhibitors of protein degradation and protein folding emerge as a potentially effective cancer therapy and suggest that levels of HSF1 protein and/or activity may be important determinants of sensitivity to 17-AAG.

#### Consequences of the protein folding defects for aneuploids

Chemical or genetic impairment of HSP90 leads to the destabilization of multiple protein kinases and other proteins with critical roles in diverse cellular processes (Taipale *et al*, 2012). We found that the proteome of human aneuploid cells resembles the proteome of HeLa cells treated with the HSP90 inhibitor, 17-DMAG. In particular, pathways involved in DNA metabolic processes, chromatin modification, and transcription were downregulated in both conditions, whereas the overlap among upregulated pathways was rather modest. Even more similarities in both upregulated and downregulated pathways were revealed by comparison between transcriptional aneuploidy response patterns and the transcriptome changes in response to HSF1 depletion in the human HCC cell line (Chuma *et al*, 2014). Although HSF1, the major heat shock transcription factor, is most known for its role in regulating the expression of chaperones and proteins involved in the maintenance of proteostasis, recent discoveries have revealed its role in the regulation of a plethora of cellular processes (Mendillo *et al*, 2012). Based on our observations, we propose that the reduced HSP90 activity and the resulting decrease in stability of HSP90 client proteins partially underlies the pathway downregulation observed in aneuploid cells. Simultaneously, the impaired HSF1 activity affects, both directly, through the reduced expression of HSF1 target genes and indirectly, through the reduced protein folding capacity of aneuploid cells, the transcription of many targets. Taken together, we propose that the proteotoxic stress imposed by the presence of extra genetic material is a major determinant of the changes in gene expression in aneuploid cells.

We hypothesize that the HSP90 defect may have additional consequences for aneuploid cells. Two phenomena are worthy of particular mention. First, there is now a large body of evidence to suggest that HSP90 acts as a buffer against phenotypic variation by masking the effects of genetic polymorphisms (Jarosz *et al*, 2010). Second, HSP90 inhibition itself leads to chromosomal instability in budding yeast (Chen *et al*, 2012). Taken together with our results, we propose that aneuploidy is likely to further accelerate both the rate and manifestation of genetic change and our data suggest a general mechanism whereby changes in DNA copy number can lead to further genetic alterations.

#### Relevance for cancer and other pathologies

Aneuploidy is a hallmark of cancer, where it correlates with malignancy, drug resistance, and poor prognosis. However, trisomy and tetrasomy markedly impair cellular functions including proliferation, suggesting that aneuploidy-tolerating changes might be necessary to facilitate the growth of aneuploid cancer cells. Further, whether a similar impairment of protein folding capacity also occurs in cells of trisomy syndromes is currently not known and should be addressed in future. Intriguingly, somatic trisomy of chromosome 8 is frequently found in myeloid lineage disorders, some lymphomas and solid tumors such as breast and ovarian cancer. Interestingly, 8q24, where the *HSF1* gene is located, is one of the most commonly amplified regions in cancer cells (Beroukhim *et al*, 2010; Davoli *et al*, 2013), and chromosome 8 is the largest somatic chromosome whose trisomy is compatible, although extremely rarely, with postnatal survival (Ganmore *et al*, 2009; Beroukhim *et al*, 2010). Indeed, we show that the presence of *HSF1* on chromosome 8 protects against some of the adverse effects of aneuploidy. HSF1 is a critical facilitator of malignant proliferation, a role which it performs by supporting many important cellular processes (Dai *et al*, 2007). An additional role of HSF1 in promoting carcinogenesis may be to protect cancer cells from the proteotoxic stress induced by aneuploidy. Our results lend strong support to this notion and suggest a causal link between two recurring features of cancer cells: aneuploidy and altered HSF1 activity.

## Materials and Methods

#### Cell lines and culturing conditions

The HCT116- and RPE-1-derived tri- and tetrasomic cell lines have been constructed by micronuclei-mediated chromosome transfer as described previously (Stingele *et al*, 2012). Parental cell line HCT116 (human colon carcinoma cell line): HCT116 3/3 (trisomy 3), HCT116 5/4 (tetrasomy 5) (Haugen *et al*, 2008); parental cell line HCT116* stably expressing histone H2B-GFP: HCT116* 5/3 (trisomy 5), HCT116* 5/4 (tetrasomy 5), (Stingele *et al*, 2013), HCT116 8/3

c1-c4; parental cell line RPE-1 (human retinal pigment epithelial cell line, hTERT immortalized): RPE-1 5/3 12/3 (trisomy 5, 12); parental cell line RPE-1* stably expressing histone H2B-GFP: RPE-1* 21/3 (trisomy 21). Cells were grown in DMEM GlutaMax (Gibco) supplemented with 10% FBS and 5% Pen/Strep under standard conditions.

#### Transfections and luciferase assays

Cells were transfected with a total of 1 or 1.5 μg of the indicated plasmids in 12-well plates using X-tremeGENE HP DNA transfection reagent (Roche) according to the manufacturer's protocol. Cells were trypsinized, counted, and seeded into 96-well plates 24 h after transfection and then allowed to recover for 24 h. Then, cells were treated with either solvent control (DMSO), 5 or 50 nM 17-AAG (8 h), 2 μM 17-AAG (4 h), or 5 μM MG132 (8 or 20 h). 17-AAG and MG132 were purchased from Enzo Life Sciences and Tocris Bioscience, respectively. To measure luminescence, 30 μl of SteadyGlo reagent (Promega) was added directly to the wells of the 96-well plates and the plates were shaken for 10 s to ensure mixing and cell lysis. Luminescence was measured on a Tecan plate reader after 15-min incubation in the dark.

#### Proteasome activity assay

Cells were seeded at $2 \times 10^4$ per well in triplicates in 96-well plates. Forty minutes later, Proteasome-Glo™ Chymotrypsin-Like Cell-Based Assay (Promega) was added according to the manufacturer's protocol. Luminescence was detected using a Fluoroskan Ascent FL plate reader operated by the Ascent software. For evaluation the mean with SEM of biological triplicates was calculated.

#### Western blotting

Exponentially growing cells were harvested and lyzed in RIPA buffer supplemented with protease inhibitors (Roche). 20 μg of protein were then resolved on 10% polyacrylamide gels and transferred to nitrocellulose membranes using the semi-dry technique. After blocking in low fat 5% milk in TBS-T, membranes were incubated with the following primary antibodies: HSP90 (1:1,000; Cell Signaling #4877), HSC70 (1:1,000; Enzo Life Sciences ADI-SPA-815), HSP70 (1:1,000; Enzo Life Sciences ADI-SPA-810), HSP27 (1:1,000; Enzo Life Sciences ADI-SPA-800), HSF1 (1:1,000; Enzo Life Sciences ADI-SPA-901), cyclin E2 (1:1,000; Cell Signaling #4132), c-Myc (1:200, Santa Cruz Biotechnology sc-40), GAPDH (1:2,000; Cell Signaling #2,118). The antibodies against phospho-HSP70 and phospho-HSP90 were a kind gift from Petr Müller, Masaryk University, Brno. After incubation with HRP-conjugated secondary antibodies, HRP substrate was added and luminescent signals were quantified using a LAS 3000 instrument (FujiFilm). Protein bands were quantified using ImageJ software.

#### Colony formation assays

Cells were seeded at 1,000 per well in 6-well plates 24 h before the treatment. Subsequently, cells were continuously treated with 17-AAG (5 or 25 nM) or DMSO for 10–12 days. Colonies were fixed with methanol:glacial acetic acid (1:1) and stained with 0.02% crystal violet for 15 min before washing with tap water and air-drying.

#### Cell viability assays

Cells were seeded at 2,000 per well in 96-well plates 24 h before the treatment. Subsequently, cells were treated with the indicated drugs at the indicated concentrations and cell viability was determined after 72 h, unless otherwise stated. Viability was determined using the CellTiterGlo luminescent cell viability assay (Promega) according to the manufacturer's instructions.

#### Chromosome transfer and preparation of chromosome spreads and paints

Chromosome transfer and the preparation of spreads and paints were performed as previously (Stingele *et al*, 2012).

#### RNA isolation and qPCR

Total RNA was isolated using the RNeasy kit (Qiagen) and reverse-transcribed into cDNA using the First Strand cDNA synthesis kit (Roche). qPCR was performed with a HSF1 assay from Qiagen (Cat. No. 330001 PPH00164F) on a LightCycler 480 (Roche) instrument using the KAPA SYBR FAST master mix. Absolute quantification was performed with an external standard, and the specificity of the amplicons was confirmed by melting curve analysis. HSF1 mRNA expression was normalized to ribosomal protein L27 (RPL27) as a housekeeping gene (de Jonge *et al*, 2007).

#### siRNA transfections

Cells were transfected at 50% confluency using 800 pmol siRNA and Oligofectamine according to the manufacturer's instructions. Transfections were conducted in OptiMEM for 4 h. The siRNA sequences used were acquired from Eurofins Genomics and are as follows: HSF1 (5′ CGGAUUCAGGGAAGCAGCUGGUGCA 3′; (Jacobs & Marnett, 2009); GL2 (5′ CGUACGCGGAAUACUUCGATT 3′).

#### Electroporation

Cells were electroporated using the Amaxa Nucleofector II apparatus and following the manufacturer's instructions and protocols for HCT116 cells and RPE-1 cells, respectively. Briefly, 1 million cells were resuspended in Cell Line Nucleofector Solution V containing 2 μg of either pCDNA or ca-HSF1 plasmid and transferred to cuvettes. HCT116 cells were electroporated using the D-032 Program and for RPE-1 cells the Program was U-017.

#### Transcriptome and proteome data analysis

The analyses of quantitative transcriptome and proteome data were performed as previously described (Durrbaum *et al*, 2014). The data were retrieved from the Gene Expression Omnibus database with the following accession numbers: knockdown of HSF1 in HCC: GSE47639; knockdown of c-myc in HeLa, BT-474, MCF-7, MDA-MB-231: GSE5823; HCT116- and RPE-1-derived trisomies and tetrasomies: GSE47830 and GSE39768.

### Statistical analyses

All data related to viability, Fluc folding and protein expression were analyzed using Student's *t*-test; $*P < 0.05$; $**P < 0.01$; $***P < 0.001$. All statistically analyzed experiments were performed at least three times.

**Supplementary information** for this article is available online: http://emboj.embopress.org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## Author contributions

ND and VP performed experiments, SS performed initial experiments and analyzed the proteasome activity; MD contributed the bioinformatics analysis; ZS and ND conceived the study and wrote the manuscript, all authors analyzed the data and commented on the manuscript.

## Conflict of interest

The authors declare that they have no conflict of interest.

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## 6.3 microRNA deregulation contributes to the cellular response to aneuploidy

In this study we investigated the contribution of miRNA deregulation to the consequences of aneuploidy in human cell lines. Aneuploidy results in profound changes of the cellular physiology as well as of the transcriptome and proteome (Donnelly and Storchova, 2015; Dürrbaum et al., 2014; Oromendia et al., 2012; Passerini et al., 2016; Stingele et al., 2012; 2013; Torres et al., 2007). Given that miRNAs post-transcriptionally regulate up to 60% of the transcriptome (Friedman et al., 2009) and their deregulation affects diverse cellular processes (Hwang and Mendell, 2006; Landgraf et al., 2007), we asked whether miRNAs play a role in the response to aneuploidy.

To investigate the miRNAome in aneuploid cells we sequenced small RNA of seven different aneuploid model cell lines and their corresponding parental cell lines. We found that the miRNAome in cells with extra chromosomes is genome-wide altered. Moreover, the deregulated miRNAome is rather a response to aneuploidy *per se* than caused by the specific extra chromosomes. Strikingly, the deregulated miRNAome affects common cellular functions such as cellular development, growth and proliferation. Integrated analysis of RNA sequencing data and previous acquired proteome data revealed that the majority of miRNA targets are negatively affected by the deregulated miRNAs. We found hsa-miR-10a-5p upregulated in 5 out of 7 sequenced cell lines and validated its overexpression in 15 different aneuploid cell lines. We show that this upregulation of hsa-miR-10a-5p governs resistance to shut down of ribosomal protein translation upon starvation.

Our analysis reveals for the first time, that the addition of extra chromosomes affects genome-wide miRNA expression. The downregulation of miRNA targets associated with cellular growth and proliferation indicates a direct contribution of miRNAs to the adverse effects of aneuploidy on cell physiology. Moreover, the commonly upregulated hsa-miR-10a-5p presents an adaptation mechanism of aneuploid cells that enhances translation under stress conditions such as starvation. Future studies will reveal the broader relevance of this adaptation to aneuploidy.

# The deregulated microRNAome contributes to the cellular response to aneuploidy

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*Manuscript in preparation*

Aneuploidy, or abnormal chromosome numbers, severely alters cell physiology and is widespread in cancers and other pathologies. In model cell lines, aneuploidy impairs proliferation, leads to proteotoxic as well as replication stress and triggers conserved transcriptome and proteome changes. The underlying regulatory mechanisms of the response to aneuploidy remain elusive. In this study we show that the expression of microRNAs is greatly altered in cells with additional chromosomes. We demonstrate that the alterations occur in response to aneuploidy *per se* and independently of the identity of the extra chromosome. Further, we found that the deregulated microRNAome contributes to the cellular response to aneuploidy by negatively affecting cell development, growth and proliferation. Strikingly, we discovered hsa-miR-10a-5p upregulated in the majority of analyzed cell lines. We show that hsa-miR-10a-5p overexpression provides resistance to starvation- induced shut down of ribosomal protein translation. Thus, the changes of the microRNAome contribute on one hand to the adverse effects of aneuploidy on the cell physiology, but on the other hand also to the adaptation to aneuploidy by supporting the survival under adverse conditions such as starvation.

## Introduction

A balanced karyotype is essential for cell viability and therefore aneuploidy, characterized by unbalanced changes in chromosome numbers and sub-chromosomal structural variations, has often profound detrimental consequences for cell physiology. In humans, aneuploidy is the major cause of spontaneous abortions and the few trisomies compatible with life result in severe developmental defects (Colnaghi et al., 2011). Aneuploidy in somatic cells is frequently associated with cancer, as 70% of haematopoietic and 90% of solid cancers show an abnormal karyotype (Mitelman et al., 2016; Weaver and Cleveland, 2006). Recently developed aneuploid model systems that allow studying the consequences of aneuploidy have accelerated our understanding of the effects of aneuploidy *per se*. The physiological changes in response to an unbalanced karyotype are multifold, including impaired proliferation, replication stress and proteotoxic stress that is characterized by changes in protein stoichiometry, reduced protein folding capacity and chaperon levels as well as by activation of autophagy (Ariyoshi et al., 2016; Donnelly et al., 2014; Nawata et al., 2011; Niwa et al., 2006; Ohashi et al., 2015; Oromendia et al., 2012; Passerini et al., 2016; Pavelka et al., 2010; Stingele et al., 2013; Torres et al., 2007). The physiological response to aneuploidy goes hand in hand with a conserved transcriptional response that is manifested in a conserved pathway deregulation (Dürrbaum et al., 2014; Sheltzer et al., 2012). What triggers these changes in gene transcription and how exactly this specific response is modulated has not been clarified so far.

Given that the triggers of the transcriptional deregulations remain unclear, we asked whether microRNA (miRNA) regulation is involved in the response to aneuploidy. miRNAs are small non-coding RNA molecules that posttranscriptionally regulate gene expression of over 60% of protein coding genes (Friedman et al., 2009). The regulatory function of miRNAs is mediated by their binding to the target 3'untranslated region (UTR) via partially complementary sequences, thereby inducing translational repression and/or mRNA decay. Alternatively, some miRNAs bind to sites within the coding region or the 5'UTR (Liu et al., 2013; Schnall-Levin et al., 2011). One mRNA can be affected by multiple miRNAs that cooperatively translationally repress or degrade the target mRNA or compete for target regulation (Selbach et al., 2008). In turn, one single miRNA can repress hundreds of mRNAs and lead to large-scale transcriptome changes that for example play a key role in stem cell differentiation (Shenoy and Blelloch, 2014). Moreover, the complex miRNA-target network allows to fine-tune diverse cellular processes by modulating the amount of transcripts translated particularly upon stress conditions or changes in the environment (Hwang and Mendell, 2006; Leung and Sharp, 2010).

In the disease context such as in cancer, alterations of miRNA expression are common and specific miRNA deregulation profiles are sufficient to distinguish cancerous from non-cancerous tissues and to predict invasiveness and aggressiveness of various cancers (Kurozumi et al., 2016; Thomas et al., 2015; Yeh et al., 2016). The wide miRNA deregulation in cancers has been attributed to genomic copy number changes. For instance, the gain of arm of chromosome 1 relates to miRNA expression changes in cervical cancer (Wilting et al., 2012) and downregulation of let-7 family was associated with copy number changes in medulloblastoma, breast and ovarian cancer (Wang et al., 2012). In some cancers, deregulated miRNAs promote genomic and chromosomal instability by targeting the mitotic checkpoint or DNA damage repair components (Choi et al., 2014; Hell et al., 2014). Yet, beyond these few examples, we lack a deeper understanding of how aneuploidy in cancer and miRNAs are associated. Moreover, the relation of miRNA and aneuploidy *per se* has not been studied so far.

We have asked whether aneuploidy *per se* results in miRNA deregulation and whether this deregulation is specific to the numerical chromosome changes or general to aneuploidy. To this end, we have used a series of human trisomic and tetrasomic cell lines and their isogenic counterparts that were established previously in our laboratory (Stingele et al., 2012). Indeed, we found that chromosome copy number changes strongly deregulate the expression of more than 25% of miRNAs with only a few individual miRNAs commonly altered among different aneuploidies. Most of the identified miRNA deregulations negatively affect cell growth and proliferation, thus suggesting that miRNAs suppress proliferation of aneuploid cells. Most aneuploids strongly overexpress the miRNA hsa-miR-10a-5p that acts also via the 5'UTR of target mRNAs. We show that this provides the aneuploid cells with resistance to starvation by insuring sustained translation of ribosomal mRNAs. Our analysis of the global miRNAome changes in response to chromosome gain reveals a complex interplay of the gene expression regulatory mechanisms that shape the global transcriptome and proteome dynamics.

## Results

### *Deregulation of miRNAome in human aneuploid model cell lines*

In order to determine the effects of a chromosome gain on miRNA expression in human cells, we used a series of cells derived from HCT116 and RPE1 cell lines that contain one or more extra copies of chromosomes ((Donnelly et al., 2014; Passerini et al., 2016; Stingele et al., 2012), see Material and Methods for more details). We subjected four HCT116- derived cell lines trisomic for chromosome 3, 8 or 18 and tetrasomic for chromosome 5 as well as the parental HCT116 cell line to small RNA sequencing. In addition, we sequenced three RPE1- derived cell lines trisomic for chromosome 7, 21 and a cell line trisomic for both chromosome 5 and 12 as well as the parental RPE1 cell line. We have used the miRBase repository (http://www.mirbase.org, (Kozomara and Griffiths-Jones, 2013)), where 1881 mature human miRNAs are listed to date, as the miRNA data source for mapping. Mapping of the raw sequences to the human genome and subsequent identification of miRNAs with mirdeep2 (Friedländer et al., 2011) resulted in at least 554 and up to 719 identified mature miRNAs in the sequenced cell lines (Table 1).

**Table 1. Detected mature miRNAs**

| | HCT116 | HCT116 5/4 | HCT116 18/3 c2 | HCT116* | HCT116* 3/3 c11 | HCT116* 8/3 c7 | RPE1 | RPE1 5/3 12/3 | RPE1* | RPE1* 7/3 | RPE1* 21/3 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| mature miRNAs in miRBase | **1881** | | | | | | | | | | |
| mature miRNAs detected by mirdeep2 | 719 | 554 | 592 | 747 | 572 | 562 | 650 | 628 | 647 | 663 | 646 |
| miRNAs with detected read count in every biological replicate | 626 | 486 | 510 | 647 | 485 | 490 | 566 | 564 | 562 | 575 | 564 |

While the numbers of identified miRNAs were similar for all sequenced aneuploid cell lines, the extent of miRNA deregulation differed. We analyzed the differential miRNA expression in aneuploid cell lines in comparison to their diploid counterparts by applying DESeq2 for normalization and statistical testing (Love et al., 2014). Under the assumption of a negative binominal distribution, DESeq2 applies generalized linear models to test for differential expression. To estimate the variability, the assumption that genes with similar abundance show similar variance is made. Since miRNAs with very low expression levels are characterized by low read counts and an inherent large variability on the logarithmic scale, these miRNAs are pre-filtered (Love et al., 2014). This filtering reduced the number of miRNAs in the aneuploid cell lines with a read count greater than 10 to a range from 231 to 293 (Table 2). The RPE1- derived aneuploid cell line with three copies of chromosome 21 and carrying H2B-GFP (RPE1* 21/3) showed the lowest number of significantly deregulated miRNAs (log2 fold change +/-0.6, adjusted p-value $<0.05$) with 23 significantly deregulated miRNAs. The highest number of 74 deregulated miRNAs was

found in RPE1 5/3 12/3 and HCT116 5/4 (Table 2). The percentage of deregulated miRNAs ranged from 9.6% (in RPE1* 21/3) up to 31.2% (in RPE1 5/3 12/3).

**Table 2. Deregulated miRNAs and mRNAs in aneuploid cell lines**

| | HCT116 5/4 | HCT116 18/3 c2 | HCT116* 3/3 c11 | HCT116* 8/3 c7 | RPE1 5/3 12/3 | RPE1* 7/3 | RPE1* 21/3 |
|---|---|---|---|---|---|---|---|
| miRNAs with a mean count >=10 | 249 | 280 | 279 | 293 | 237 | 247 | 231 |
| miRNAs significantly deregulated log2 fold change >0.6 or <-0.6 (padj. <0.05) | 74 | 57 | 27 | 44 | 74 | 33 | 23 |
| percentage of significantly deregulated miRNAs | 29.7 | 20.4 | 9.7 | 15.0 | 31.2 | 13.4 | 10.0 |
| mRNAs with a mean count >=10 | 13887 | - | - | 13873 | 13278 | - | 13101 |
| mRNAs significantly deregulated log2 fold change >0.6 or <-0.6 (padj. <0.05) | 2885 | - | - | 410 | 2210 | - | 434 |
| percentage of significantly deregulated mRNAs | 20.8 | - | - | 3.0 | 16.6 | - | 3.3 |

There was only a weak correlation between the percentages of deregulated miRNAs with the amount of extra DNA (Suppl. Fig 1B). Thus, the altered miRNA expression in response to aneuploidy is largely independent of the amount of added extra DNA suggesting that the alteration in the miRNAome is a general response to aneuploidy.

### *Effects of the deregulated miRNAome on the aneuploid transcriptome*

To directly compare the changes in miRNA expression with the mRNA expression, we analyzed the transcriptome of HCT116 5/4, HCT116* 8/3 c7, RPE1 5/3 12/3 and RPE1* 21/3 by RNA sequencing. The percentage of deregulated mRNAs (log2 fold change +/- 0.6, adjusted p-value <0.05) highly differed among the cell lines, with 3.3% in RPE1* 21/3, 16.6% in RPE1 5/3 12/3, 3.0% in HCT116* 8/3 c7 and 20.8% in HCT116 5/4 (Table 2). Thus, the percentage of deregulated mRNAs in specific cell lines shows a similar trend as the percentage of deregulated miRNAs. However, when comparing these percentages with the above-described percentages of deregulated miRNAs, we found that a larger fraction of miRNAs (ranging from 9.6 to 35%) than mRNAs (ranging from 3.0 to 20.8%) was altered in all cell lines. Thus, addition of extra chromosomes affects miRNA expression to a larger extent than it affects the mRNA expression.

We asked to what extend the transcriptional response to aneuploidy can be explained by miRNA regulation. To model the miRNA-target network, we retrieved reported miRNA targets from miRTarBase (v6.1) for all deregulated miRNAs of each cell line (Vergoulis et al., 2012). We then filtered these miRNA targets for those that were altered in response to aneuploidy. To simplify the complex multiple miRNAs to one target relation, we analyzed whether these deregulated mRNA targets were inversely expressed to their related

miRNAs. Compared to the total number of deregulated mRNAs, 22% of mRNAs are potentially targeted by deregulated miRNAs, judged by their inverse expression in HCT116 5/4 (Table 3). The percentage is lower in RPE1* 21/3, RPE1 5/3 12/3 and HCT116* 8/3 c7, were 18%, 16% and 3% of deregulated mRNAs are potentially targeted by the deregulated miRNAs, respectively. Thus under these approximations, only a minor fraction of the mRNA changes in response to aneuploidy might be directly influenced by miRNA regulation.

**Table 3. Percentage of mRNA targets that are inversely expressed to the deregulated miRNAs**

| Cell line | Deregulated mRNAs | Deregulated miRNAs | Number of targets of deregulated miRNAs | Number of DE miRNA targets that are deregulated on mRNA level | Number of DE miRNA targets that are deregulated on mRNA level and show inverse expression |
|---|---|---|---|---|---|
| HCT116 5/4 | 3342 | 74 | 9111 miRNA-target interactions<br>6644 unique targets | 1280<br>38.30% | 904 miRNA-target interactions<br>729 unique targets 21.81% |
| RPE1 5/3 12/3 | 2484 | 74 | 6487 miRNA-target interactions<br>4605 unique targets | 604<br>24.30% | 406 miRNA-target interactions<br>345 unique targets 16.34% |
| RPE1* 21/3 | 788 | 23 | 6036 miRNA-target interactions<br>5078 unique targets | 214<br>27.20% | 142 miRNA-target interactions<br>127 unique targets 18.02% |
| HCT116 8/3 c7 | 250 | 44 | 965 miRNA-target interactions<br>877 unique targets | 12<br>4.80% | 7 miRNA-target interactions<br>7 unique targets 2.8% |

Finally, we asked whether the deregulated miRNAs would specifically target and regulate the expression of genes encoded on the aneuploid chromosomes to a near diploid expression (so called dosage compensation), which occurs for a small subset of genes (Stingele et al., 2012). However, our analysis of the target genes encoded on the aneuploid chromosomes did not indicate any role of miRNAs in dosage compensation (data not shown).

*Cell line specific and general miRNA deregulation in response to aneuploidy*

Comparison of the significantly altered miRNAs in aneuploid cell lines revealed a pattern of miRNA expression changes that is largely distinct for each specific cell line (Fig. 1A). Interestingly, the *Euclidean* distance clustering was not defined by the parental cell type,

but rather by distinct and cell line specific miRNA expression clusters of each aneuploid cell line. HCT116 5/4 showed the most prominent clusters of down- and upregulated miRNAs and appeared as an outlier in the *Euclidean* distance cluster. The upregulated miRNA cluster in HCT116 5/4 partially overlapped with the upregulated miRNAs in RPE1 5/3 12/3; among them most prominently the miRNA family miR-192/215 and the nearby located miR-194 and miRNAs hsa-miR-29b and hsa-miR-29c, which are encoded in a cluster on chromosome 1. Majority of the deregulated miRNA clusters overlapped only poorly with the other aneuploid cell lines. However, some similarities in the deregulated miRNAs were found. For instance, hsa-miR-21-5p and hsa-miR-24-2-5p showed similar upregulation in all HCT116-derived cell lines (Fig. 1B). Moreover, five out of seven sequenced aneuploid cell lines shared the upregulation of hsa-miR-10a-5p and hsa-miR-139-5p (Fig. 1C). Intriguingly, this upregulation did not affect the entire miRNA family and seems to be specific for the individual miRNA: while hsa-miR-10a-5p was upregulated in five out of seven of the analyzed cell lines, the family member hsa-miR-10b-5p was upregulated only in RPE1 5/3 12/3. Similarly, hsa-miR-22-5p was upregulated in five out of seven aneuploid cell lines. Moreover, hsa-miR-374b-5p was downregulated in all cell lines except RPE1 5/3 12/3 (Fig. 1D). In summary, there are vast dissimilarities in the deregulation patterns between the aneuploid cell lines and only a few miRNAs show common differential expression changes.

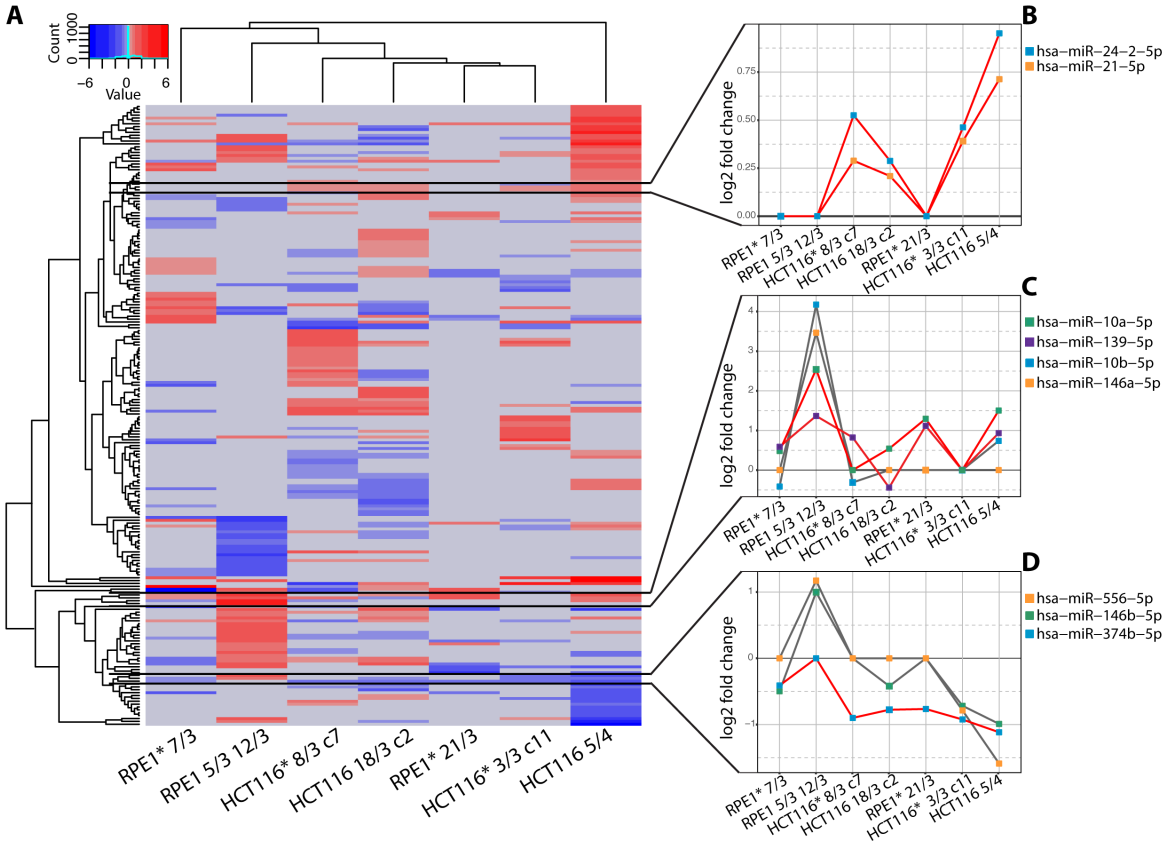


**Figure 1. miRNAome in aneuploid model cell lines. A** Heatmap of significantly altered miRNAs in aneuploid cell lines (adjusted p-value <=0.05). Blue indicates downregulation, red upregulation. Row and column dendrograms show *Euclidean* distance between miRNA expression profiles and cell lines, respectively. Commonly deregulated miRNA clusters are highlighted by black lines. **B** miRNA expression profile of miRNA cluster upregulated in all HCT116-derived aneuploid cell lines. **C** miRNA expression profile of miRNA cluster containing hsa-miR-10a-5p and hsa-miR-139-5p that are upregulated in 5 out of 7 sequenced aneuploid cell lines (red line). Grey lines indicate miRNAs in the same cluster that are not commonly deregulated. **D** miRNA expression profile of miRNA cluster containing hsa-miR-374b-5p that is down regulated in 6 out of 7 sequenced aneuploid cell lines (red line). Grey lines indicate miRNAs in the same cluster that are not commonly deregulated. Cell lines ordered according to *Euclidean* distance. Asterisks indicate cell lines with H2B-GFP.

The presence of extra chromosomes in cells generally leads to an elevated expression of the genes that are located on the supernumerary chromosomes; thus the abundance of transcripts largely scales with the gene copy numbers (Dürrbaum and Storchova, 2015; Nawata et al., 2011; Sheltzer et al., 2012; Stingele et al., 2012; Torres et al., 2007; Upender et al., 2004; Williams et al., 2008). To determine whether this holds also true for miRNA expression, we ordered the miRNAs according to their chromosome location. We then compared the distribution of the miRNA expression from the aneuploid chromosomes to miRNA expression from the diploid chromosomes and tested whether higher expression of the aneuploid distribution occurs just by chance. The aneuploid chromosome-specific miRNA expression was significantly higher than the diploid miRNA expression only for RPE1* 7/3, RPE1* 21/3 and for the miRNAs encoded on chromosome 12 in RPE1 5/3 12/3 (Fig. 2A–C). In all other aneuploid cell lines, the miRNA expression distribution did not scale with the number of chromosome copies, although the miRNAs encoded on chromosome 5 in HCT116 5/4 and RPE1 5/3 12/3 showed an elevated median expression

in comparison to the disomic chromosomes (Fig 2C–G). Due to the relatively small number of deregulated miRNAs that were encoded on the aneuploid chromosomes, we cannot rule out that the shift of the expression towards higher levels occurs just by chance (Suppl. Table 1). In addition, the median miRNA expression is often higher than expected also for some disomic chromosomes (Suppl. Fig 2A). For instance, the median expression of miRNAs encoded on chromosome 11 and 17 are significantly increased in HCT116 5/4. Similar effect was observed in other analyzed aneuploid cells lines, where the median expression of miRNAs encoded on one or more disomic chromosomes is significantly higher or lower than expected. In summary, although 10-31% of miRNAs were strongly deregulated in response to aneuploidy in each individual cell line, only a few miRNAs showed a common deregulation pattern. These miRNAs were mostly upregulated, but their upregulation could not be explained by gene copy number changes, as they were largely not encoded on any of the aneuploid chromosomes. This suggests that not the gene copy number changes are responsible for the deregulation of these specific miRNAs, but rather that the increased expression reflects the cellular response to abnormal chromosome numbers.

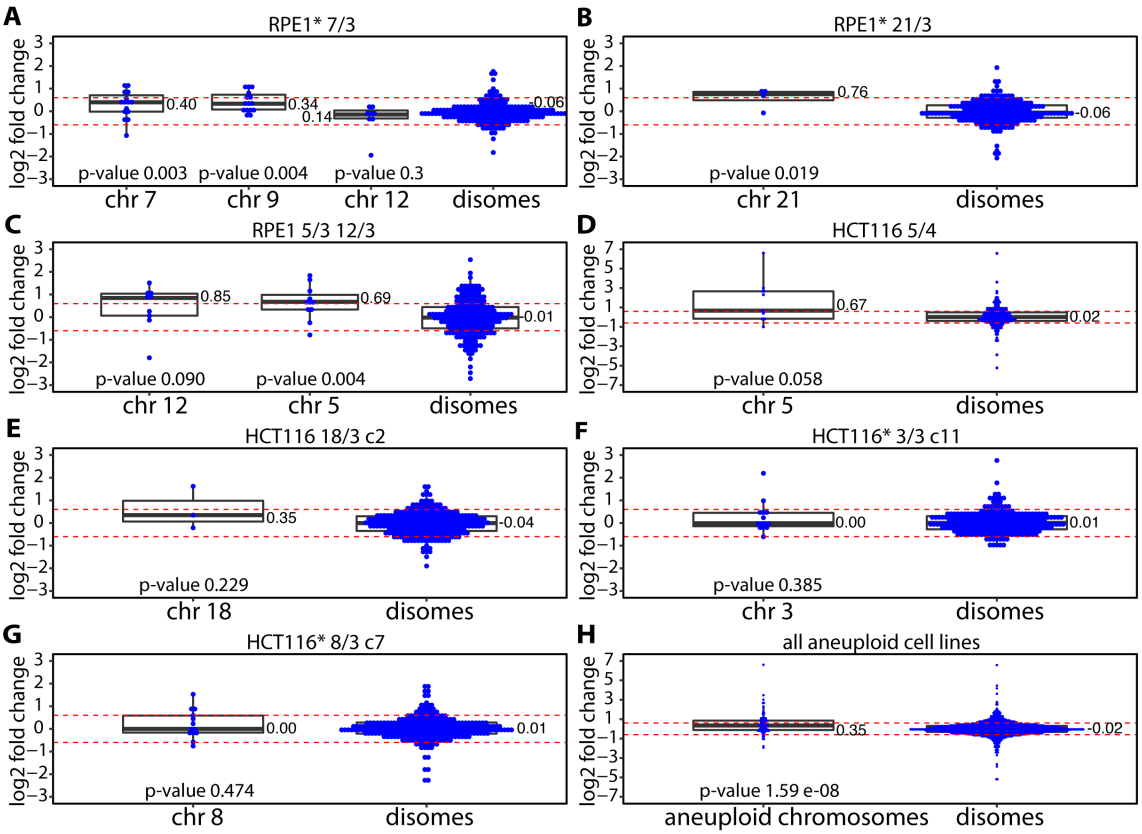


**Figure 2. Expression of miRNAs encoded on the aneuploid chromosomes versus miRNAs encoded on disomic chromosomes.** Each blue dot represent one miRNA with its log2 fold change expression normalized to the corresponding parental cell line. Boxplots present 75%, 50% and 25% quantile, with median value shown. **A** RPE1* 7/3 that has additional copy number changes of chromosome 9 and 12 **B** RPE1* 21/3 **C** RPE1 5/3 12/3 **D** HCT116 5/4 **E** HCT116 18/3 c2 **F** HCT116* 3/3 c11 **G** HCT116* 8/3 c7 and **H** miRNAs of all aneuploid chromosomes compared to all disomic miRNAs. Note that the number of miRNAs does not correlate with the median expression fold change of the aneuploid chromosomes. Significance tested with Mann-Whitney-Wilcoxon test. Asterisks indicate cell lines with H2B-GFP.

*Deregulated miRNAs affect similar molecular functions*

To determine which cellular functions are affected by the changes in the miRNAome in aneuploid cells, we performed functional analysis based on the manually curated knowledge base of Ingenuity Pathway Analysis (IPA http://www.ingenuity.com). This analysis revealed that the top five molecular and cellular functions affected by the deregulated miRNAs in response to aneuploidy were largely shared among the seven aneuploid cell lines. In particular, "Cell Cycle" was affected in all sequenced aneuploid cell lines (Fig. 3A). It should be noted, however, that only a few miRNAs (from two in HCT116* 3/3 to seven in HCT116 5/4) were implicated in this biological function. Most miRNAs were involved in "Cellular Movement", "Cellular Growth and Proliferation" and "Cellular Development". The miRNAome of HCT116* 3/3 showed different functional associations than all other cell lines and the number of miRNAs involved was less than seven. HCT116* 3/3 is also the only cell line were the pathway "Cellular Function and Maintenance" was identified as affected. The only pathways in HCT116* 3/3 that were shared with the majority of the other aneuploid cell lines were "Cellular Development" and "Cell Cycle".
The cellular and molecular function categories of IPA are divided into sub-functional annotation terms. We employed IPA to infer an activation state of the biological functions as an activation z-score. The IPA activation z-score is a quantitative measure of the predicted effect of the deregulated miRNAome on a biological function (Kraemer et al., 2014). It is calculated based on the direction of miRNA deregulation and the literature-derived positive or negative effects of these miRNAs on a biological function. Our miRNA dataset was sufficient to retrieve the z-score for the sub-functional annotation terms within the categories "Cell Death and Survival", "Cellular Development, Cellular Growth and Proliferation", "Cellular Growth and Proliferation" and "Cellular Movement" (Fig. 3B). Strikingly, the effect of the deregulated miRNAome on these functions is mostly negative. Almost all sub-functional annotation terms within the four parent categories were predicted to be inhibited or deactivated in HCT116 18/3, HCT116 5/4 and RPE1* 7/3. HCT116 5/4 shows the strongest effect with stringent z-scores below -2. No conclusive z-score could be retrieved for HCT116* 8/3 c7, RPE1 5/3 12/3 and RPE1* 21/3, with the exception of the pathway "Invasion of Tumor Cell Lines" with a z-score of 1.6 in RPE1* 21/3. In concordance with the identity of the five top molecular and cellular functions, HCT116* 3/3 miRNA deregulation does not predict an activation z-score for proliferation or growth associated functions, but only for the category "Cell Death and Survival". Therein, "apoptosis" is predicted to be activated with a z-score of 1.8 and 2 and "cell death" as well as "necrosis" with a z-score of 1.5 and 1.4, respectively. In summary, although only small numbers of commonly deregulated miRNAs were identified in cells with extra chromosomes, the miRNAome affects largely overlapping molecular and cellular functions, which were associated with cellular growth and proliferation. The negative effect on cell proliferation and growth executed either directly by impairing the proliferation pathway or indirectly by activation of cell death and apoptosis was conclusively predicted based on the miRNA changes in four out of seven aneuploid cell lines. We conclude that the miRNA expression changes in response to aneuploidy result in a largely negative effect on cellular proliferation.

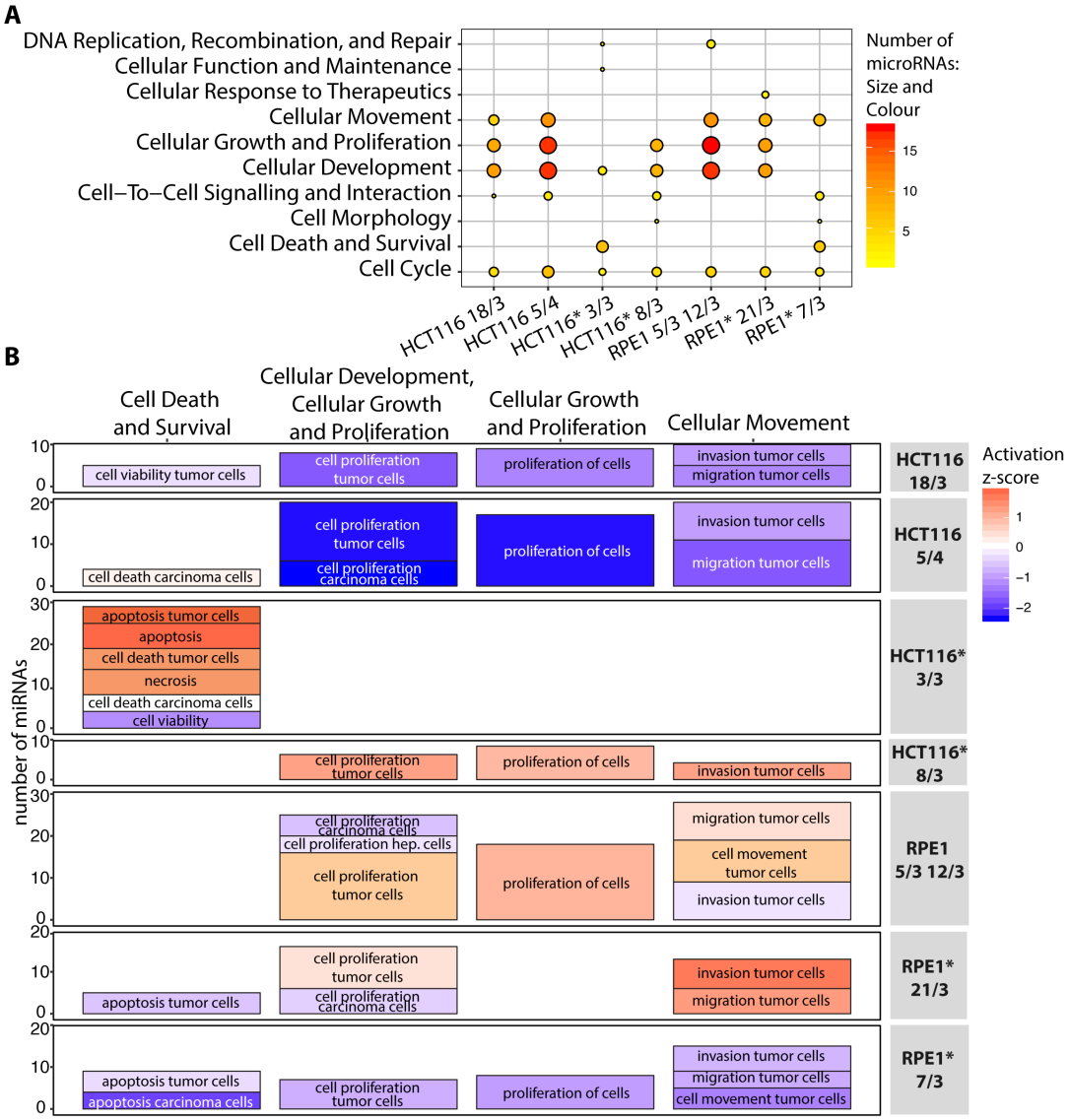


**Figure 3. miRNAome of aneuploid cells affects cellular development, growth and proliferation**
**A** Top five cellular and molecular functions affected by the deregulated miRNAs in individual aneuploid cell lines. Dot size and colour indicates the number of miRNAs that are associated with a specific cellular function. **B** Subfunctional annotation terms for the four cellular and molecular functions with the most miRNAs involved (columns). Each row contains subfunctional annotations for one aneuploid cell line. The size of the boxes indicates the numbers of miRNAs associated (y-axis); the colour indicates the predicted activation z-score. Asterisks indicate cell lines with H2B-GFP. Hep. cells abbreviates hepatoma cell lines.

## *Integration of miRNA, RNA and protein expression data infers an effect on cellular development, cellular growth and proliferation*

To further investigate how the miRNAs execute their negative impact on cellular development, growth and proliferation in aneuploids, we analyzed their targets. This analysis was performed for HCT116 5/4, RPE1 5/3 12/3 and RPE1* 21/3, for which miRNAome, transcriptome and proteome data were available (this work and (Stingele et al., 2012)). In total, 40 miRNAs were found to be associated with the cellular development, growth and proliferation category (Suppl. Fig 3A). The set of miRNAs

affecting cellular development, growth and proliferation was diverse and only partially overlapped within a subset of the analyzed cell lines. For the 20 miRNAs associated with the development, growth and proliferation category in HCT116 5/4, 604 experimentally validated miRNA-target interactions were found, with 325 unique targets (Fig. 4A). In RPE1 5/3 12/3 and RPE1* 21/3, 19 and 13 miRNAs interact with 238 and 208 unique targets, respectively (Suppl. Fig 3B,C). Importantly, chromosome alignment of the miRNAs reveals no bias for the aneuploid chromosome in either of the cell lines.

miRNAs may affect target protein levels only, or mRNA and protein levels simultaneously. To infer the effect of miRNA regulation on previously experimentally validated targets, we matched the transcriptome and proteome data to the targets of miRNAs within the cellular development, growth and proliferation category. Targets were filtered for their expression changes above/below a threshold ratio of +/-0.6 (log2 fold change) and only targets with a potential miRNA relation, that is, targets with inverse expression to the interacting miRNA, were considered for the annotation enrichment analysis. Strikingly, 72% of the filtered targets were downregulated at least on protein level in HCT116 5/4 (Fig. 4B). This is in line with the predicted downregulation of the molecular function term by the activation z-score. Importantly, this holds also true for RPE1 5/3 12/3 and RPE1* 21/3, for which the predicted activation z-scores were below the threshold (Suppl. Fig 3D,E). Thus, the target gene expression substantiates the predicted activation state of the molecular function, which was based solely on the miRNA expression values. This confirms the largely negative effect of the deregulated miRNAs on cell cycle and proliferation in response to aneuploidy.

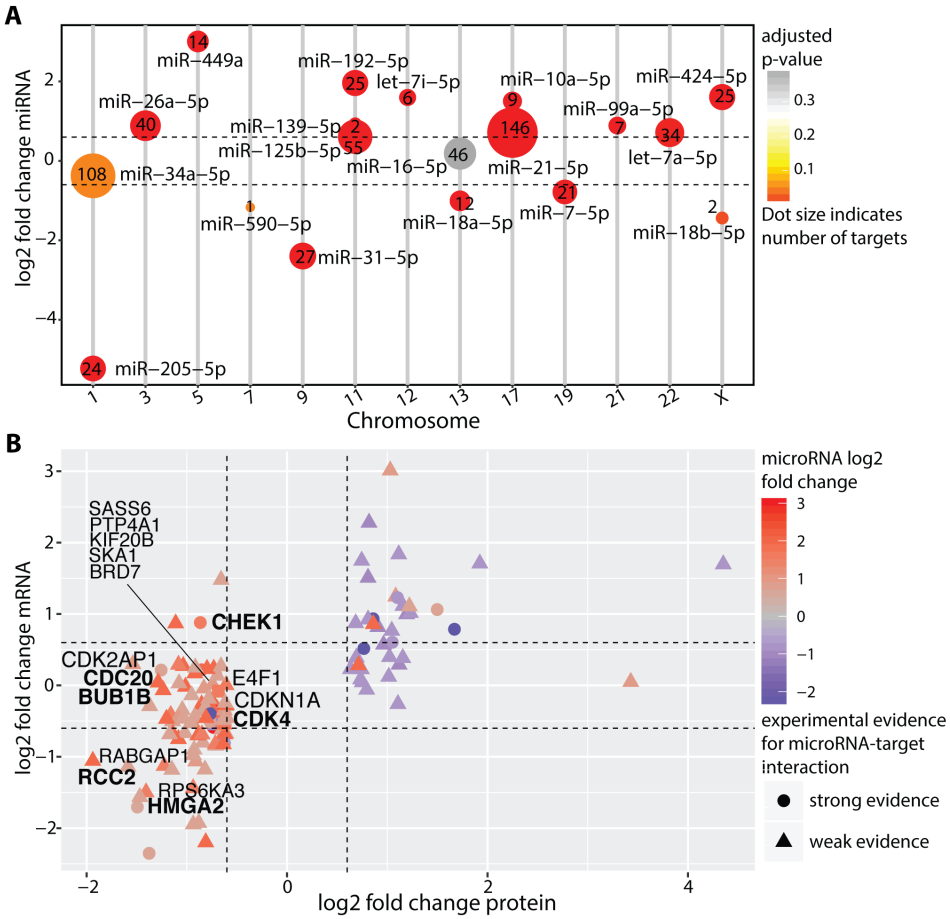


**Figure 4. miRNA target expression analysis infers functional role of miRNAs A** Number of targets with strong evidence for each deregulated miRNA annotated in the "Cellular Development, Cellular Growth and Proliferation" category in HCT116 5/4. The size indicates the number of targets; the colour shows the significance of miRNA deregulation. The miRNAs are presented according to their chromosome location. **B** Target genes within the "Cellular Development, Cellular Growth and Proliferation" category and their mRNA and protein expression. Selected gene labels indicate targets with inverse miRNA-target expression, which are associated with cell cycle processes. Shape indicates the type of experimental evidence for a miRNA-target interaction. The colour indicates log2 fold change miRNA expression.

To analyse whether the filtered miRNA targets were indeed involved in the analyzed molecular functions, we performed functional annotation clustering with the DAVID functional annotation tool. As expected, the top annotation cluster was associated with cell cycle functions in HCT116 5/4 and RPE1 5/3 12/3, respectively (Suppl. Table 2). In RPE1* 21/3, cell cycle associated annotations were enriched in the second top cluster. Among the deregulated targets associated with the cell cycle function in HCT116 5/4 we found key players of proliferation, such as the mitotic checkpoint serine/threonine kinases (BUB1), cell division cycling 20 (CDC20), cyclin-dependent kinase 4 (CDK4) and checkpoint kinase 1 (CHEK1) (Fig. 4B). Two targets, namely the high motility group A2 (HMGA2) and regulator of chromosome condensation (RCC2), show a strong downregulation of both mRNA and protein levels in HCT116 5/4. RCC2 is also downregulated and targeted in RPE1 5/3 12/3. Also in RPE1- derived aneuploid cell lines, RPE1 5/3 12/3 and RPE1* 21/3, cell cycle associated factors were targeted (Suppl. Fig

3D,E). For example, the minichromosome maintenance protein complex members (MCM2, 3 and 6) and Cyclin E1 were found downregulated in RPE1 5/3 12/3. Hence, the deregulated targets of the identified deregulated miRNAs in response to aneuploidy implicated in cellular development, growth and proliferation were indeed involved in these molecular functions and showed a strong association with cell cycle progression. The fact that majority of these targets were found downregulated and the interacting miRNAs upregulated suggests that the miRNAome in aneuploid cells negatively affects cellular proliferation and might contribute to the proliferation defect observed in aneuploid model cell lines.

### *hsa-miR-10a-5p activity is increased in human aneuploid model cell lines*

Remarkably, we found miRNA hsa-miR-10a-5p upregulated in five out of seven aneuploid cell lines. Quantitative real-time PCR of 18 different aneuploid model cell lines, among them different clones with the same chromosome (for details see Material and Methods), revealed that the mean expression of hsa-miR-10a-5p was significantly increased above the levels of the parental cell line in 7 out of 18 cell lines and elevated in additional 8 cell lines (Fig. 5A). The trend towards upregulation were in line with the sequencing data, where a strong upregulation in HCT116 5/4, RPE1 5/3 12/3 and RPE1* 21/3 was observed, whereas HCT116* 8/3 c7 and HCT116 18/3 c2 showed a minor increase (Fig. 1D). The increased expression of hsa-miR-10a-5p might be a secondary effect of the expression of HOXB3 in which the miRNA sequence is located. Comparison of the hsa-miR-10a-5p and HOXB3 expression levels in four cell lines revealed that only in two cell lines, HCT116 5/4 and RPE1* 21/3, both miR-10a-5p and HOXB3 are significantly upregulated (Suppl. Fig 4). The expression levels are remarkably similar in RPE1* 21/3, suggesting that the increased hsa-miR-10a-5p might indeed be a result of elevated HOXB3 expression in this cell line. In HCT116 5/4, however, the levels of hsa-miR-10a-5p are much higher than HOXB3 levels. In the additional two cell lines, the upregulation of hsa-miR-10a-5p does not coincide with HOXB3 overexpression. Thus we conclude that the increased hsa-miR-10a-5p cannot be generally explained by increased expression of the host gene HOXB3.

To determine whether hsa-miR-10a-5p overexpression indeed affects gene expression in aneuploid cells, we used a luciferase reporter assay to determine its translational repression efficiency. The luciferase reporter consists of a miR-10a binding site in a synthetic 3'untranslated region (UTR) of the Renilla luciferase gene (Fig. 5B). The reporter contains a second unmodified Firefly luciferase gene to normalize for the transfection efficiency. Binding of hsa-miR-10a-5p to the luciferase 3'UTR shall result in translational repression of the luciferase mRNA and subsequent decrease in the luciferase signal. Indeed, the luciferase signal was significantly lower in four out of five tested HCT116- derived and RPE1- derived aneuploid cell lines (Fig. 5C). No changes in luciferase signal were detected in the HCT116* 8/3 c7 cell line; this is likely due to a relatively minor increase in hsa-miR-10a-5p expression in this cell lines (see Fig. 5A). Taken together, hsa-miR-10a-5p is overexpressed in aneuploid cell lines and shows higher endogenous translational repression activity of the luciferase reporter gene.

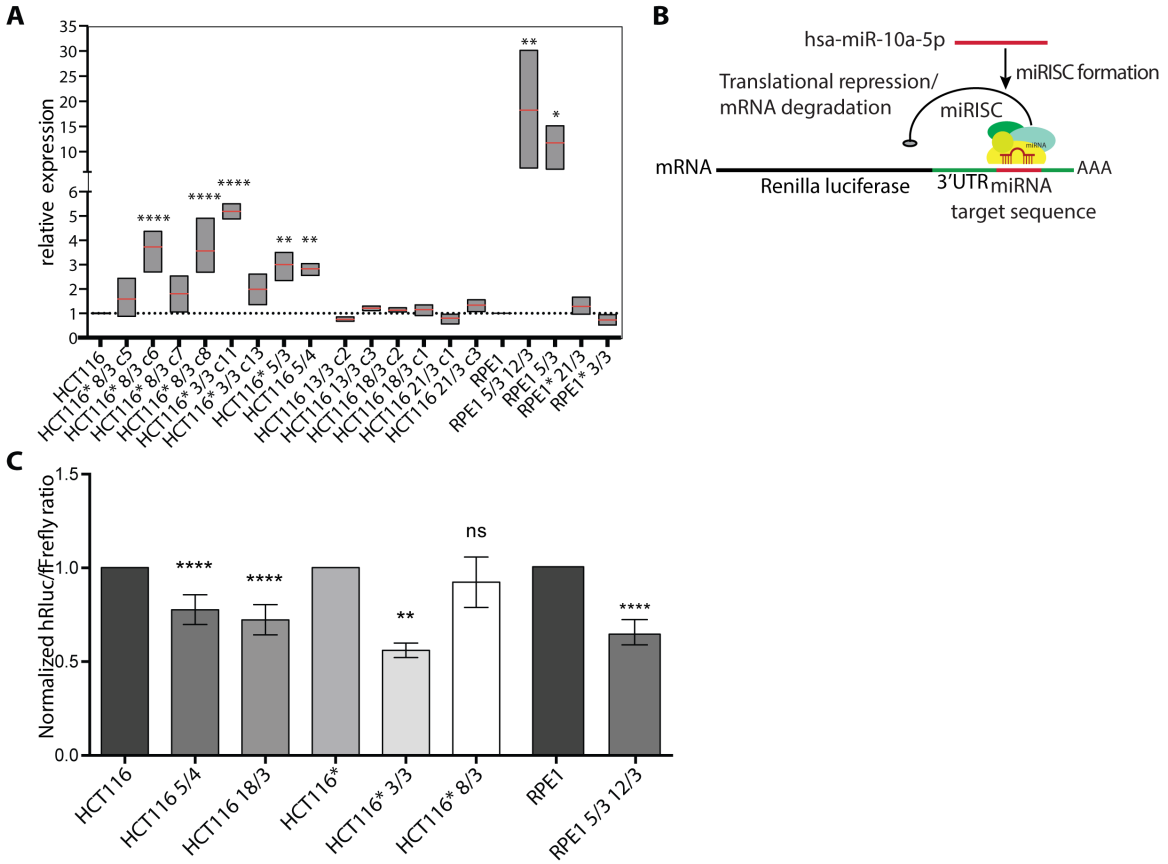


**Figure 5. hsa-miR-10a-5p levels and activity are increased in aneuploid cell lines. A** hsa-miR-10a-5p expression analyzed by quantitative real-time PCR in 18 different aneuploid model cell lines. Boxplots present minimal, maximal and the mean value. Relative expression levels normalized to corresponding parental cell line is shown. **B** Luciferase reporter construct to determine hsa-miR-10a-5p endogenous gene repression activity. hsa-miR-10a-5p targets its binding site in a synthetic 3'UTR of the Renilla luciferase mRNA resulting in translational repression and/or degradation of Renilla luciferase mRNA. Renilla luciferase signal is normalized to internal Firefly luciferase control (not shown). **C** Endogenous hsa-miR-10a-5p activity determined by psiCheck2-10a luciferase reporter assay with hsa-miR-10a-5p binding site in the synthetic 3'UTR. HCT116-derived and RPE1-derived cell lines were transfected with psiCheck2-10a luciferase reporter construct. Luciferase reporter assay was conducted 48hrs post transfection. Data represent the normalized mean values +/- SEM from at least three independent experiments each performed in triplicates. One-way ANOVA, multiple comparison correction with Dunnett test. ns= not significant, *P<0.0332, **P<0.0021, ***P<0.0002, ****P<0.0001. Asterisks indicate cell lines with H2B-GFP.

The target database lists 298 unique target genes for hsa-miR-10a-5p. Majority of the targets have only weak experimental evidence and only nine targets were previously verified by luciferase reporter assays. Functional annotation enrichment of all on transcriptome level deregulated targets in HCT116 5/4 revealed that these were mostly phosphoproteins associated with the membrane and/ or the nucleus (Suppl. Table 3). Only six of the targets were downregulated on both transcriptome and proteome in HCT116 5/4. For example LIM domain and actin-binding protein 1 (LIMA1) that plays a role in cytoskeleton stabilization or metabolic enzymes such as fatty acid synthetase (FAS) and sorbitol dehydrogenase (SORD) (Suppl. Fig 5A). Of the 270 hsa-miR-10a targets with available expression data in RPE1- derived aneuploid cell lines, only two were downregulated on transcriptome and proteome in both RPE1 5/3 12/3 and RPE1* 21/3

(Suppl. Fig 5B,C). Thus, although hsa-miR-10a-5p expression and translational repression activity is increased in majority of the aneuploid model cell lines, only a very few targets of this miRNA show downregulation on mRNA and protein levels. This suggests that the hsa-miR-10a-5p function in regulating gene expression via binding to the 3'UTR of target mRNAs does not strongly contribute to the changes in response to aneuploidy.

### *hsa-miR-10a-5p overexpression governs resistance to starvation stress*

To understand the role of hsa-miR-10a-5p in aneuploid cells, we considered the fact that hsa-miR-10a-5p binds also to the 5'UTR downstream of the 5'TOP motif that is found in mRNAs of ribosomal proteins (RP) and other translation associated protein mRNAs (Ørom et al., 2008). This binding results in enhanced translation of RP mRNAs and alleviates redistribution of RP mRNAs from active polysomes to inactive RNP complexes upon amino acid starvation. We asked whether the observed upregulation of hsa-miR-10a-5p influences the translation of 5'TOP motif mRNAs in aneuploid cells. To analyse the translation efficiency of mRNAs with the 5'TOP motif in aneuploid cells, we employed a luciferase reporter construct containing the transcriptional start site of the ribosomal protein S16 (*Rps16*) and 29nt of the exon 1 including the 5'TOP motif in the 5'UTR of a luciferase gene (pS16-wt-luc) (Fig. 6A, (Ørom et al., 2008)). We first determined the steady state levels of 5'TOP motif mRNA translation with the pS16-wt-luc luciferase reporter. Interestingly, despite the increased levels of endogenous hsa-miR-10-5p in all analyzed aneuploid cell lines, we have not observed any significant increase in the normalized Firefly luciferase signal compared to the wild type (Fig. 6B). In fact, HCT116 5/4 with high endogenous levels showed even significantly decreased luciferase activity.
Next, we asked whether modulating the levels of hsa-miR-10a-5p affects the translation of 5'TOP mRNA in aneuploid cell lines, as it was previously described for Mouse E14 ES cells (Ørom et al., 2008). To this end, we measured the luciferase activity after overexpression of hsa-miR-10a-5p mimic (Suppl. Fig 6A). The functionality of hsa-miR-10a-5p mimic overexpression was validated via its 3'UTR-mediated repression activity (Suppl. Fig 6B). Using the pS16-wt-luc luciferase reporter, we observed that hsa-miR-10a-5p overexpression enhances the 5'UTR-mediated effect on the translation of 5'TOP motif mRNAs in the parental HCT116 cell line as well as in three out of four tested aneuploids (Fig. 6B). A significant induction of luciferase activity was not observed only for HCT116 5/4. Thus, the expression of mRNAs with the 5'TOP motifs is sensitive to hsa-miR-10a-5p levels also in aneuploid cells and the degree of the responsiveness does not correlate with the endogenous amount of hsa-miR-10a-5p.
The translation of 5'TOP motif mRNAs such as RP mRNAs is tightly regulated and cellular stresses such as starvation result in their translational repression and redistribution into inactive ribonucleoprotein complexes (Meyuhas and Kahan, 2015). We hypothesized that upregulation of hsa-miR-10a-5p in aneuploid cell lines might provide resistance to cellular stresses such as starvation. To test this hypothesis, we deprived serum for 3 hours and measured the 5'TOP motif mRNA translation via the luciferase reporter assay (Fig. 6C). Serum starvation led to significant decrease of the luciferase activity in the parental HCT116 cell line. Intriguingly, all aneuploid cell lines were resistant to the reduction of

the 5'TOP motif mRNA translation that is induced by serum starvation in the parental cell line. This suggests that an increase in hsa-miR-10a-5p levels protects aneuploid cell lines from reduction of 5'TOP motif mRNA translation in response to cellular stress.

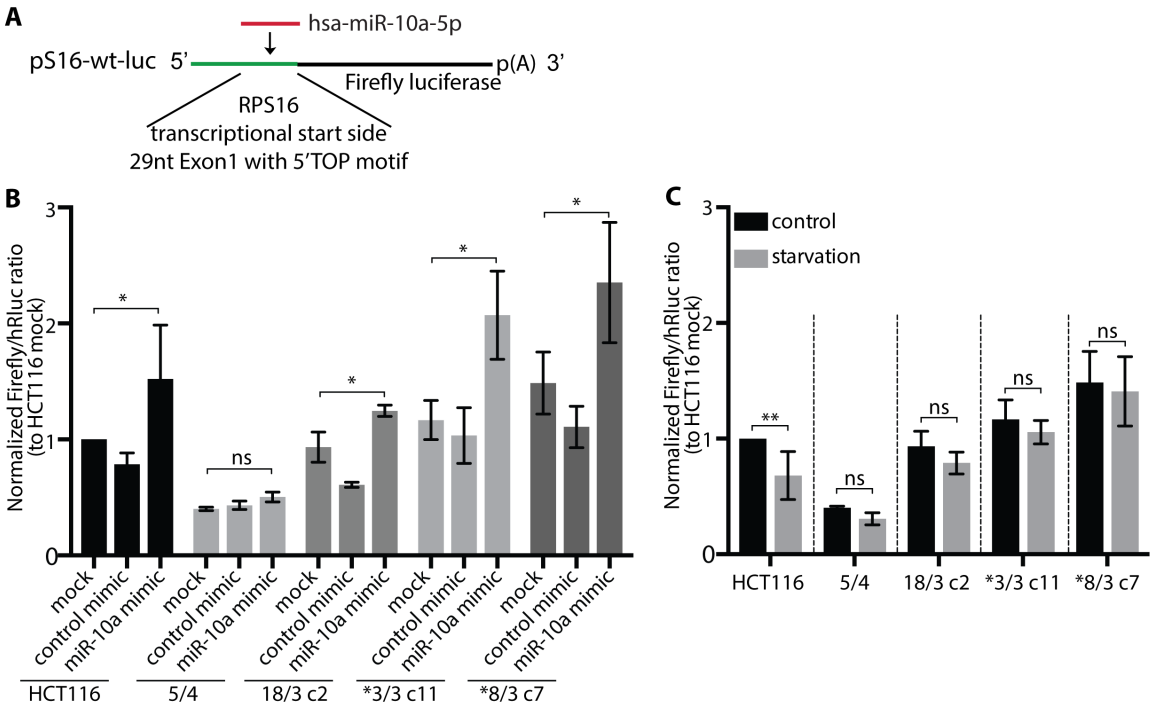


**Figure 6. 5'TOP motif luciferase activity is less sensitive to starvation in aneuploid cells. A** Schematic illustration of the pS-16-wt-luc luciferase reporter construct. RPS16 transcriptional start side and 29nt of Exon 1 including the 5'TOP motif is incorporated into the 5'UTR of luciferase gene. **B** pS16-wt-luc activity after overexpression of hsa-miR-10a-5p. Cells were reverse transfected with hsa-miR-10a-5p mimic or control molecule and forward transfected with pS16-wt-luc and pRL-TK 24hrs post mimic transfection. 72hrs later a luciferase assay was conducted. **C** pS-16-wt-luc activity after starvation. Cells were transfected as in B and 3hrs FCS starved before the luciferase reporter assay was conducted. Data presents Renilla normalized mean +/- SD values. Two-way ANOVA, multiple comparison correction with Sidak test. ns= not significant, *P<0.0332, **P<0.0021, ***P<0.0002, ****P<0.0001.

## Discussion

Using an integrated approach of large-scale miRNA, RNA and protein expression data analysis and cell biology, we document here the deregulation of the miRNAome and its consequences in human aneuploid model cell lines. For the first time, we show that the addition of one or two chromosomes results in extensive genome-wide miRNA expression changes in human cells. We demonstrate that the deregulated miRNAome negatively affects development, growth and proliferation, thereby contributing to the cellular response to aneuploidy. Moreover, our evidence suggests that upregulation of hsa-miR-10a-5p in aneuploid cell lines might present an adaptation to aneuploidy by providing resistance to adverse growth conditions.

Karyotype alterations were previously associated with deregulated miRNA expression in some cancers. In general, miRNA coding regions show high frequency of copy number variations in ovarian, breast, melanoma and lung cancer (Czubak et al., 2015; Zhang et al., 2006). Thus, aneuploidy or genomic instability can be a direct cause for miRNA deregulation. Yet, only in three out of seven analyzed aneuploid cell lines we observed a significant upregulation of the median miRNA expression from the extra chromosomes (Fig. 2, Suppl. Fig. 2). Therefore, the genome-wide deregulation of miRNAs observed in model aneuploid cells rather suggests a response to aneuploidy *per se*.

Aneuploidy results in impaired growth in mammalian and yeast model systems (Stingele et al., 2013; Torres et al., 2007; Williams et al., 2008). This is at least partially reflected in the conserved transcriptional changes in response to aneuploidy that includes the downregulation of DNA and RNA associated pathways (Dürrbaum et al., 2014; Sheltzer et al., 2012). Analysis of the cellular functions affected by the deregulated miRNAs revealed that cellular development, growth and proliferation are commonly targeted. Intriguingly, our integrated data analysis showed that the majority of targets that show an inverse expression to the deregulated miRNAs are indeed downregulated (Fig. 4, Suppl. Fig. 3). Among the downregulated targets are proteins that are crucial for the cell cycle progression such as BUB1, CDC20, CDK4 and CHEK1 in HCT116 5/4 or CyclinE1 and RB1 in RPE1-derived aneuploid cell lines. One of the targets, HMGA2 is strongly downregulated on both mRNA and protein level in HCT116 5/4. HMGA2 is a target of hsa-miR-26a-5p, let-7a-5p and hsa-miR-125b-5p that were found to affect cellular growth and proliferation. Downregulation of HMGA2 by hsa-miR-26a negatively affects cell proliferation and has tumour suppressive effects in gallbladder cancer (Zhou et al., 2014). Another strongly downregulated target is RCC2, which is targeted by hsa-miR-192-5p and hsa-miR-7-5p in HCT1165/4 and RPE1 5/3 12/3. RCC2 is essential for mitotic spindle assembly and recently an important, although poorly characterized function of RCC2 for G1-S transition has been recognized (Mollinari et al., 2003; Rosasco-Nitcher et al., 2008; Yenjerla et al., 2013). Together, these results suggest that the identified deregulation of miRNAs might contribute to the growth defects in aneuploid cells.

Remarkably, the deregulated miRNAome is largely unique for each of the analyzed aneuploid cell lines. Only a few deregulated miRNAs are shared in the majority of the analyzed cell lines. This is similar to the conserved transcriptional response to aneuploidy, where the individual deregulated genes are largely dissimilar, yet the affected pathways are common (Dürrbaum et al., 2014). Among the few commonly upregulated miRNAs is

hsa-miR-139-5p. This is in contrast to cancer where hsa-miR-139-5p is frequently downregulated and associated with poor prognosis (Watanabe et al., 2015; Wong et al., 2011). This opposite expression pattern is similar to the changes of mRNA levels, where the transcriptional response to model aneuploidy is inverse to the cancer transcriptome (Sheltzer, 2013, our unpublished data).

Among the few commonly upregulated miRNAs we found hsa-miR-10a-5p and confirmed its increased endogenous activity using a specific luciferase-based reporter system. The function and expression of this miRNA are largely ambivalent in cancer and up- and downregulation as well as growth inhibiting and promoting functions have been reported (Jia et al., 2014; Ohuchida:2012ju; Long et al., 2012). Only a few hsa-miR-10a-5p targets showed a concordant downregulation on protein or mRNA level in human aneuploid model cell lines. These few affected targets were involved in diverse cellular functions such as cytoskeleton stabilization, transcriptional regulation and metabolic processes (Suppl. Fig 5, Suppl. Table 3).

The observation of only a few inversely expressed hsa-miR-10a-5p targets prompted us to investigate another possible function of hsa-miR-10a-5p. Hsa-miR-10a-5p binds upstream of the mRNA 5'TOP motif, thereby influencing the translation of mRNAs with this motif. The 5'TOP motif is a cis-regulatory motif present in transcripts encoding for the translational machinery, mostly ribosomal proteins ((Levy et al., 1991), reviewed in (Meyuhas and Kahan, 2015)). Assessment of the 5'TOP motif translation with the RPS16 luciferase reporter construct revealed that the higher levels of hsa-miR-10a-5p in aneuploid cells did not coincide with increased steady state 5'TOP motif mRNA translation (Fig. 6). However, overexpression of hsa-miR-10a-5p induced 5`TOP motif luciferase activity in diploid and analyzed aneuploid cell lines, just as previously described (Ørom et al., 2008).

What is the function of the upregulated hsa-miR-10a-5p in aneuploid cells? Upon nutrition deprivation or cell cycle arrest the translation of 5' TOP mRNAs is repressed, which is indicated by their shift to inactive ribonucleoprotein complexes (Ørom et al., 2008). We observed that the 5`TOP motif luciferase activity is not repressed upon starvation in aneuploid cells, whereas in the diploid parental cell line the 5`TOP motif luciferase activity is sensitive to starvation. Thus, we hypothesize that the upregulation of hsa-miR-10a-5p in aneuploid cell lines might be a protective adaption of aneuploid cells to adverse conditions such as starvation.

In conclusion, our systems biology approach demonstrates how the addition of extra chromosomes alters the miRNAome and consequently explains some of the transcriptome and proteome changes that occur in response to aneuploidy. We found that the deregulated miRNAome in response to aneuploidy contributes to the detrimental effects of aneuploidy on cell physiology. Moreover, we show that the miRNA deregulation might also provide an advantage for the cells by granting resistance to stress conditions. Our unique data set describes the aneuploid cells from an "omics" perspective and allows a global, quantitative analysis of the consequences of aneuploidy as well as the comparison of different aneuploid systems. With our analyses we uncovered the complex and multi-layered pathway deregulations in aneuploid cells and described the regulatory relationships that shape the gene expression in response to chromosome copy number changes.

## Materials and Methods

### Cell lines

The retinal pigment epithelial cell line RPE1 hTERT and RPE1 hTERT H2B-GFP were a kind gift from Stefan Taylor (University of Manchester, UK). The human colorectal cell line HCT116 was obtained from American Type Culture Collection (no. CCL-247). HCT116 H2B-GFP was generated previously by lipofection (FugeneHD, Roche) transfection of pBOS-H2B-GFP (BD Pharmingen) according to manufacturer's protocols (Kuffer et al., 2013)The tetrasomic cell line HCT116 5/4 were a kind gift of Minoru Koi (Baylor University Medical Centre, Dallas, Texas, USA). All other trisomic and tetrasomic cell lines were generated by microcell-mediated chromosome transfer as described previously (Donnelly et al., 2014; Passerini et al., 2016; Stingele et al., 2012). Cell lines originating from individual clonal populations arising from a single cell after by microcell-mediated chromosome transfer are indicated with their clone number (c#). The genome sequencing data of all cell lines are available upon request.

**Table 4. List of all cell lines used in the analysis.**

| Cell line name | Origin | Full cell line name | Analysis in this manuscript | Remarks |
|---|---|---|---|---|
| HCT116 | Koi laboratory | | RNA and sRNA sequencing; qPCR | Kindly provided by Minoru Koi |
| HCT116 5/4 | Koi laboratory | | RNA and sRNA sequencing; qPCR | Kindly provided by Minoru Koi |
| HCT116 13/3 c2 | MMTC into HCT116 | HCT116 13/3 clone 2 | qPCR | this work |
| HCT116 13/3 c3 | MMTC into HCT116 | HCT116 13/3 clone 3 | qPCR | This work |
| HCT116 18/3 c1 | MMTC into HCT116 | HCT116 18/3 clone 1 | qPCR | This work |
| HCT116 18/3 c2 | MMTC into HCT116 | HCT116 18/3 clone 2 | sRNA sequencing; qPCR | This work |
| HCT116 21/3 c1 | MMTC into HCT116 | HCT116 21/3 clone 1 | qPCR | This work |
| HCT116 21/3 c3 | MMTC into HCT116 | HCT116 21/3 clone 3 | qPCR | This work |
| HCT116* | HCT116 from AATC introduction of H2B-GFP | HCT116 H2B-GFP | qPCR | (Kuffer et al., 2013) |
| HCT116* 5/3 | MMTC into HCT116 H2B-GFP | HCT116 H2B-GFP 5/3 | qPCR | (Stingele et al., 2012) |
| HCT116* 3/3 c11 | MMTC into HCT116 H2B-GFP | HCT116 H2B-GFP 3/3 clone 11 | sRNA sequencing; qPCR | (Passerini et al., 2016) |
| HCT116* 3/3 c13 | MMTC into HCT116 H2B-GFP | HCT116 H2B-GFP 3/3 clone 13 | qPCR | (Passerini et al., 2016) |
| HCT116* 8/3 c5 | MMTC into HCT116 H2B-GFP | HCT116 H2B-GFP 8/3 clone 5 | qPCR | (Donnelly et al., 2014) |
| HCT116* 8/3 c6 | MMTC into HCT116 H2B-GFP | HCT116 H2B-GFP 8/3 clone 6 | qPCR | (Donnelly et al., 2014) |
| HCT116* 8/3 c7 | MMTC into HCT116 H2B-GFP | HCT116 H2B-GFP 8/3 clone 7 | RNA and sRNA sequencing; qPCR | (Donnelly et al., 2014) |
| HCT116* 8/3 | MMTC into | HCT116 H2B-GFP 8/3 | qPCR | (Donnelly et al., |

| | | | | |
|---|---|---|---|---|
| c8 | HCT116 H2B-GFP | clone 8 | | 2014) |
| RPE1 | Taylor laboratory | RPE1 hTERT | RNA and sRNA sequencing; qPCR | Kindly provided by Steven Taylor |
| RPE1 5/3 12/3 | MMTC into RPE1 | RPE1 hTERT 5/3 12/3 | RNA and sRNA sequencing; qPCR | (Stingele et al., 2012) Spontaneous gain of chromosome 12 |
| RPE1* | Taylor laboratory | RPE1 H2B-GFP hTERT | RNA and sRNA sequencing; qPCR | Kindly provided by Steven Taylor |
| RPE1* 21/3 | MMTC into RPE1 H2B-GFP | RPE1 H2B-GFP hTERT 21/3 | RNA and sRNA sequencing; qPCR | (Stingele et al., 2012) |
| RPE1* 7/3 | MMTC into RPE1 H2B-GFP | RPE1 H2B-GFP hTERT 7/3 | sRNA sequencing; qPCR | This work Spontaneous gain of chromosome 9,12 |
| RPE1* 3/3 | MMTC into RPE1 H2B-GFP | RPE1 H2B-GFP hTERT 3/3 | sRNA sequencing; qPCR | This work |

**RNA sequencing and data processing**

Following aneuploid cell lines and parental diploid cell lines were subjected to RNA sequencing: HCT116, HCT116 H2B-GFP (in the following indicated by *), HCT116 5/4, HCT116* 8/3 clone7, RPE1, RPE*, RPE1 5/3 12/3, RPE1* 21/3.

Total RNA was extracted using RNeasy Mini Kit (QIAGEN). TruSeq RNA library preparation and Illumina HiSeq2500 sequencing with 25 million 100bp single reads per library were performed by the Max Planck-Genome-Center Cologne, Germany (http://mpgc. mpipz.mpg.de/home/). Subsequently, sequencing adapters were removed from the raw sequences with cutadapt and sequencing reads were mapped to the human genome (hg19) using TopHat (v2.0.10) with the following parameters: “tophat2 -g1 -G”. RefSeq information in the GTF file was downloaded from the UCSC genome browser. featureCounts (v1.4.3) was used to generate the count matrix with the same GTF file as for the alignment with the following parameters: “-t exon -g gene_id”. Normalization and differential expression analysis was performed using the R/Bioconductor package DESeq2 (Love et al., 2014). For differential expression analysis, trisomic and tetrasomic cell lines were compared to the parental diploid cell line. The RNA sequencing data of all cell lines are available upon request.

**small RNA sequencing and data processing**

Following aneuploid cell lines and parental diploid cell lines were subjected to small RNA sequencing: HCT116, HCT116*, HCT116 5/4, HCT116 18/3 clone2, HCT116* 8/3 clone7, HCT116* 3/3, RPE1, RPE*, RPE1 5/3 12/3, RPE1* 21/3 and RPE1* 7/3.

Total RNA, including small RNAs from 18 nucleotides upwards, was extracted using miRNeasy Mini Kit (QIAGEN). TruSeq small RNA library preparation and Illumina HiSeq2500 sequencing with 25 million 100bp or 75bp single reads per library were performed by the Max Planck-Genome-Center Cologne, Germany (http://mpgc. mpipz.mpg.de/home/). If necessary, raw sequencing read ends were trimmed for low quality base calls using the FASTQ quality trimmer with the following parameters: “-Q33 -t 20 -l 17“ (Hannon Lab, Cold Spring Harbor Laboratory). Sequencing adapters were removed with cutadapt. Mapping to the human genome hg19 and miRNA identification

was performed using mirdeep2 (v2.0.0.8) (Friedländer et al., 2011) with the following commands: “mapper.pl -e -h -i -j -l 18 -m -p hg19 -q” and “miRDeep2.pl reads.fa hg19.fa genome.arf human_mature_rmspace.fa others_mature_rmspace.fa human_hairpin_rmspace.fa -d -t Human”. The three biological replicates of aneuploid and the corresponding parental cell line were analyzed within the same analysis run. For subsequent normalization and differential expression analysis miRNA_expressed_all_samples.csv was used as an input for DESeq2. Differential expression analysis was performed between aneuploid cell lines and corresponding parental diploid cell line. To account for sequencing batch effects, paired replicate information in addition to condition information was input to the DESeq2 analysis for HCT116 5/4, RPE1 5/3 12/3 and RPE1* 21/3 and corresponding parental cell lines. The sRNA sequencing data of all cell lines are available upon request.

**Integrative mRNA, miRNA and target analysis**

Data analysis, such as integration and data visualization was performed using the computing environment R. Mapping of identifiers as well as genome locations was performed with BioMart using biomaRt package in R (Durinck et al., 2009; Smedley et al., 2015). miRNA (miRNA) identifiers were retrieved from miRBase (v21). miRNA target information was retrieved from miRTarBase (v6.1) (Chou et al., 2016; Vergoulis et al., 2012).

**Functional annotation analysis**

miRNA differential expression datasets were analyzed using the Ingenuity Pathway Analysis Software (IPA http://www.ingenuity.com, QIAGEN). Core analysis was performed with the differential expressed miRNAs (log2 fold change +/-0.6, adjusted p-value <0.05) against the Ingenuity knowledge base considering direct experimentally observed relationships in human species. Functional annotation analysis results were exported and visualized in R.

Functional annotation analysis of target genes was conducted using the Database for Annotation, Visualization and Integrated Discovery (DAVID v6.8) by applying functional annotation clustering.

**Quantitative real-time PCR of miRNAs**

Total RNA, including small RNAs from 18 nucleotides upwards, was extracted using miRNeasy Mini Kit (QIAGEN). Reverse transcription was performed using the universal cDNA synthesis kit miRCURY LNA™ (Exiqon) according to manufacturers protocol. UniSp6 RNA spike-in control was added to each sample in equal amounts. Quantitative PCR was conducted using the Light Cycler 480 System (Roche Diagnostics) using the ExiLENT SYBR® Green master mix miRCURY LNA™ (Exiqon) and miRNA specific LNA™ PCR primer sets (Exiqon) as well as the UniSp6 RNA spike control primer set (Exiqon). Absolute quantification with an external standard was performed and negative non-template controls were included in all experiments. The specificity of the primer product amplification was confirmed in each run by melting curve analysis. miRNA expression was normalized to the control spike RNA and corresponding diploid miRNA expression.

**Luciferase reporter constructs**

hsa-miR-10a-5p 3'UTR luciferase reporter (psiCheck2-10a) was constructed by cloning the complementary miRNA target sequence in the synthetic 3'UTR of psiCHECK™-2 Vector (Promega). PCR cloning was performed with the following primers:
top 5'-*CACAAATTCGGATCTACAGGGTAGTTTAAACCTAGAGCGGCCGCT*-'3 and
bottom 5'-*CTGACCTATGAATTGACAGCCGCGATCGCCTAGAATTACTGC*-'3.
pS16-WT luciferase vector containing the transcriptional start site of the ribosomal protein S16 (Rps16) and 29nt of the exon 1 including the 5'TOP motif was a kind gift of Anders H. Lund (University of Copenhagen, Denmark). pRL-TK Renilla vector was a kind gift of Reinhard Fässler (Max Planck Institute of Biochemistry, Martinsried).

**Endogenous hsa-miR-10a-5p mediated 3'UTR repression assay**

Cell lines were forward transfected with psiCheck2-10a using Lipofectamine 200 (Thermo Fisher Scientific) according to manufacturer's protocol. Cells were reseeded into 96 well plates 24 hours (hrs) post transfection at 20.000/ well HCT116- derived cell lines and 10.000/ well RPE1 derived cell lines. Luciferase activity was monitored 48hrs post transfection using the Dual-Glo® Luciferase Assay System (Promega). Renilla luciferase activity values were normalized to the Firefly luciferase activity and subsequently to the parental cell line. Statistical testing and data plotting was performed in GraphPad Prism 6.

**hsa-miR-10a-5p mediated 5`TOP motif mRNA translation assay**

Cell lines were reverse transfected with miRCURY LNA™ miRNA Mimic or miRCURY LNA™ miRNA Mimic Negative Control (Exiqon) at 50nM using Lipofectamine 200 (Thermo Fisher Scientific) according to manufacturers protocol. Cells were forward transfected with the pS16-wt-luc Firefly luciferase reporter construct and pRL-TK Renilla luciferase control vector 24hrs post mimic transfection. 48hrs post mimic transfection, cells were reseeded into 96 well plates at 20.000/ well HCT116- derived cell lines and 10.000/ well RPE1- derived cell lines. Luciferase activity was monitored 72hrs post transfection using Dual-Glo® Luciferase Assay System (Promega). Starvation was performed by replacing the Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum and 1% penicillin/ streptomycin with DMEM without supplements for 3hrs prior measurement of luciferase activity. Firefly luciferase activity values were normalized to Renilla luciferase activity and subsequently to the parental cell line. Statistical testing and data plotting was performed in GraphPad Prism 6.

## Supplementary Information (upon request)

Supplementary Table 1
Total number and number of deregulated miRNAs per chromosome (log2 fold change >0.6 or <(-0.6).
Supplementary Table 2
DAVID functional annotation clustering of "Cellular Development, Cellular Growth and Proliferation" miRNA target genes that are significantly deregulated (log2 fold change >0.6 or <-0.6) and have an inverse expression to the miRNA expression of HCT116 5/4, RPE1 5/3 12/3, RPE1* 21/3.
Supplementary Table 3

DAVID functional annotation clustering and functional annotation chart of hsa-miR-10a-5p targets, which are downregulated in HCT116 5/4.

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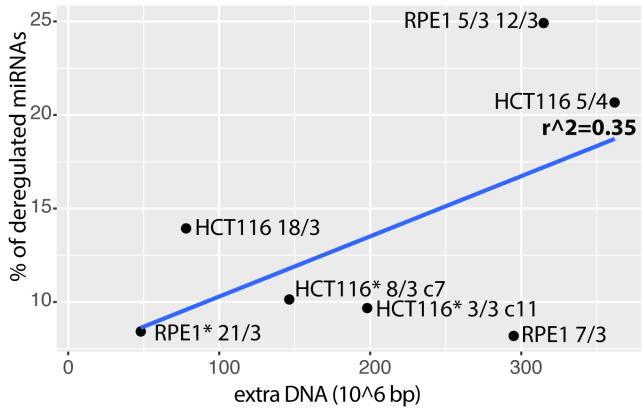
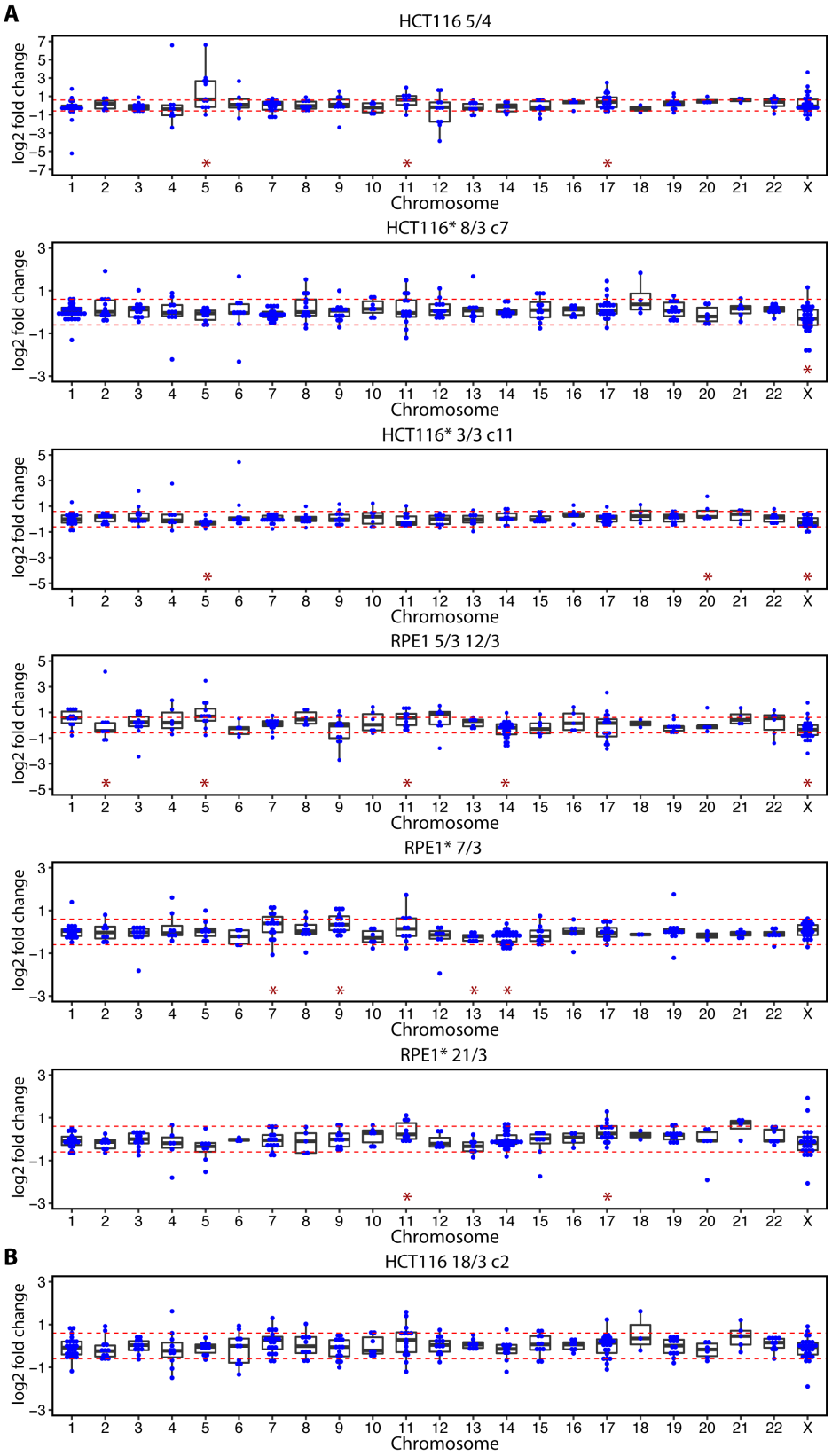
## Supplementary Figures

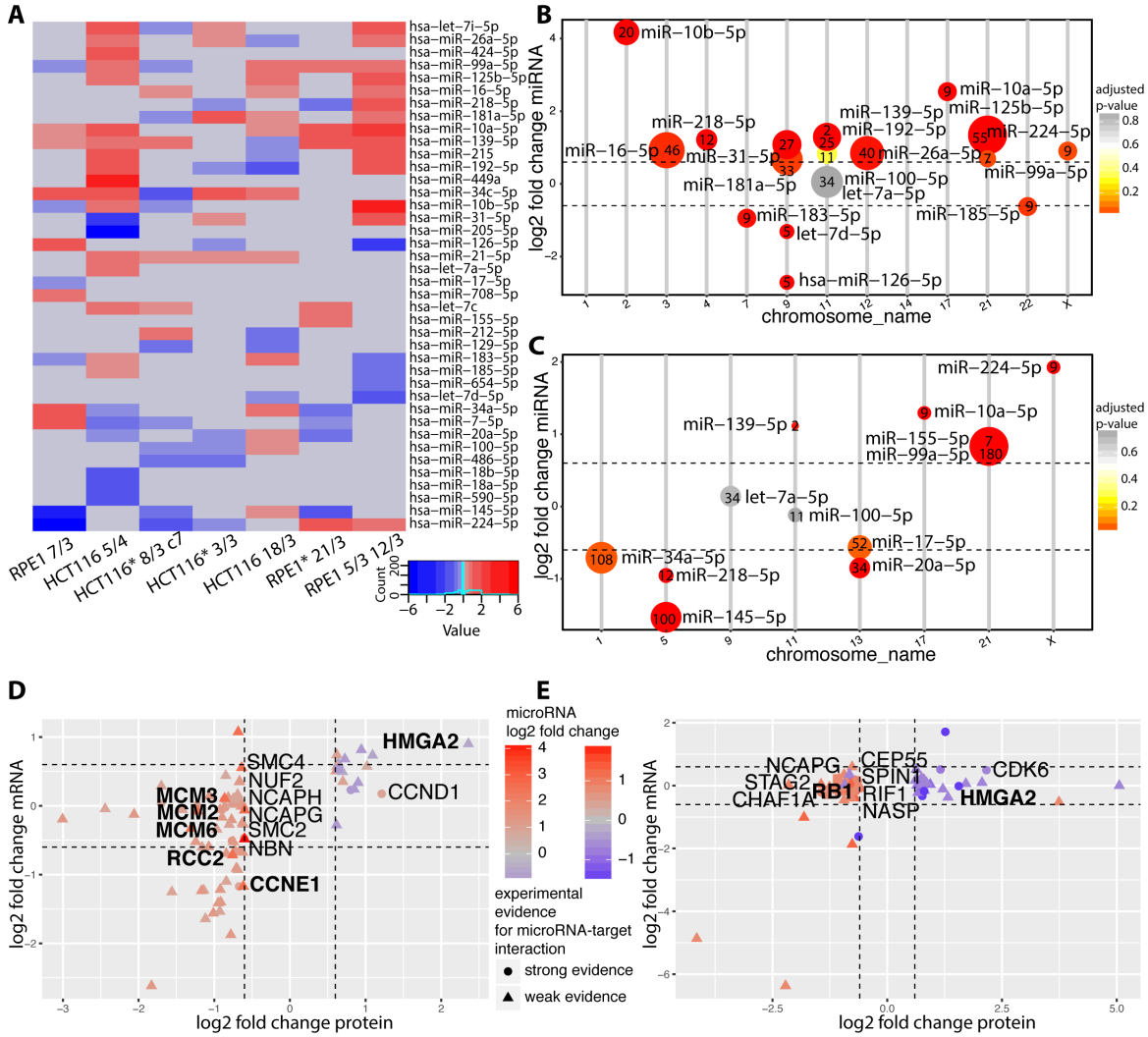



**Supplementary Figure 1.** Correlation of extra DNA and the percentage of deregulated microRNAs in aneuploid cell lines. Extra DNA was estimated as the bp length of the aneuploid chromosome. Blue line presents fitted linear regression with R squared=0.35.

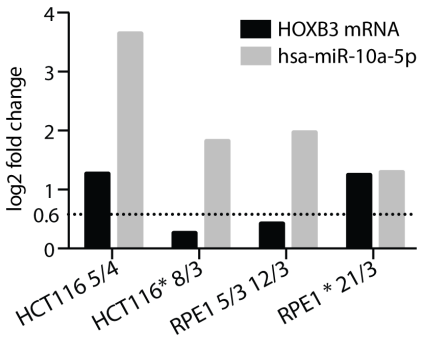



**Supplementary Figure 2.** microRNA expression aligned according to their chromosome position **A** HCT116 5/4, HCT116* 8/3 c7, HCT116* 3/3 c11, RPE1 5/3 12/3, RPE1* 7/3 and RPE1* 21/3 with significantly increased or decreased median expression of chromosomes labelled with a red asterisks (Mann-Whitney-Wilcoxon test, p-value <0.05). **B** HCT116 18/3 c2 does not show altered median miRNA expression of any disomic chromosomes. Each blue dot represents a microRNA with

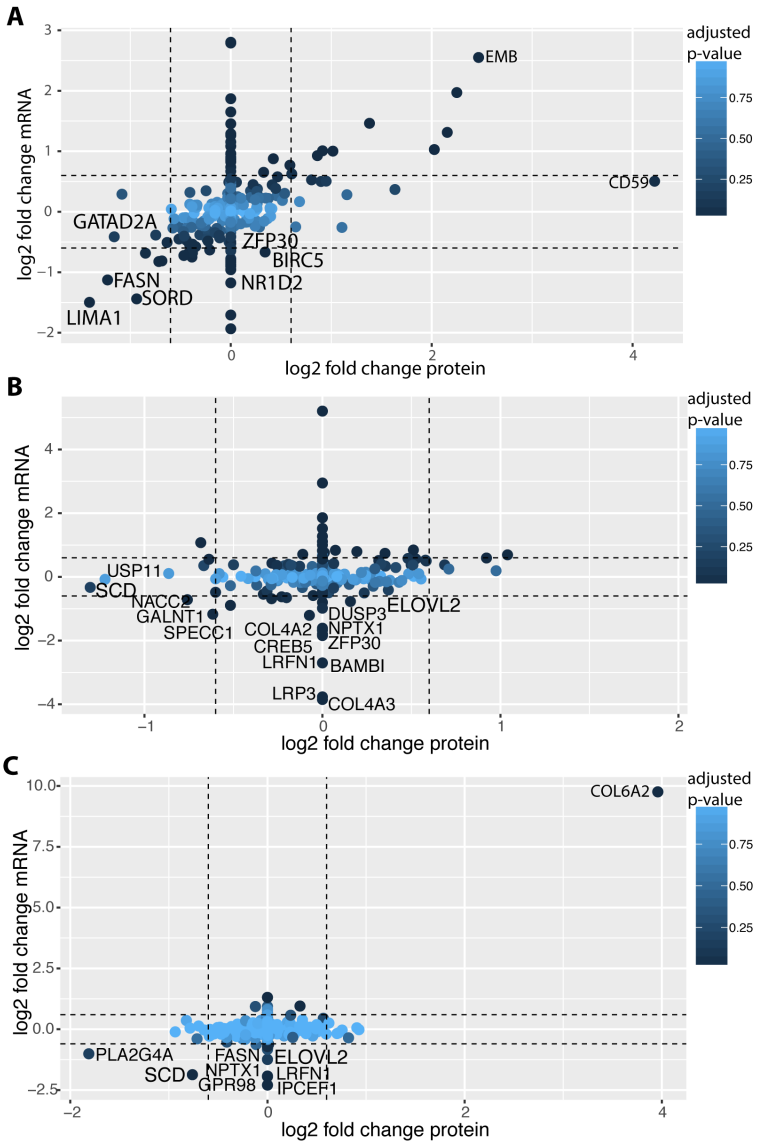
its chromosome location (x-axis) and log2 fold change normalized to corresponding parental cell line (y-axis). Red lines indicate log2 fold change >0.6/ <-0.6. Boxplots present 75%, 50% and 25% quantile of the microRNA expression per chromosome. Asterisks indicate cell lines with H2B-GFP.



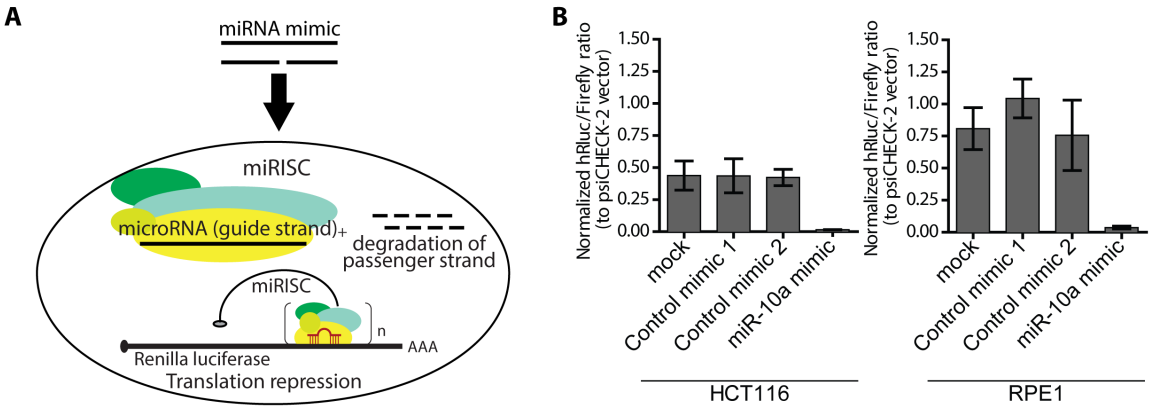

**Supplementary Figure 3.** **A** Heatmap of microRNAs associated with molecular function term "Cellular Development, Cellular Growth and Proliferation". Blue indicates downregulation, red upregulation. **B** Number of targets with strong evidence for each deregulated microRNA annotated in the "Cellular Development, Cellular Growth and Proliferation" category in RPE1 5/3 12/3. The size indicates the number of targets; the colour shows the significance of microRNA deregulation. The microRNAs are presented according to their chromosome location. **C** same as B in RPE1* 21/3. **D** Target genes within the "Cellular Development, Cellular Growth and Proliferation" category and their mRNA and protein expression. Selected gene labels indicate targets with inverse miRNA-target expression, which are associated with cell cycle processes. Shape indicates the type of experimental evidence for a microRNA-target interaction. The colour indicates log2 fold change microRNA expression in RPE1 5/3 12/3 and **E** in RPE1 H2B-GFP 21/3.




**Supplementary Figure 4.** Comparison of hsa-miR-10a-5p expression and HOXB3 mRNA expression. Bars represent log2 fold change expression ratio (aneuploid/ parental cell line) derived from RNA and sRNA sequencing.




**Supplementary Figure 5.** hsa-miR-10a targets mRNA and protein expression levels. **A** hsa-miR-10a-5p target expression in HCT116 5/4 on protein level (x-axis) and mRNA level (y-axis). **B** hsa-miR-10a-5p target expression in RPE1 5/3 12/3. **C** hsa-miR-10a-5p target expression in RPE1* 21/3. Each dot represents one target, colour indicates the adjusted p-value for the mRNA levels. Dotted lines indicate log2 fold change of 0.6 and -0.6. Asterisks indicate H2B-GFP.




**Supplementary Figure 6.** Overexpression of hsa-miR-10a-5p leads to repression of luciferase activity. **A** Schematic illustration of the mechanism of overexpression via miRNA mimic molecule transfection. Mimic transfection leads to enhanced translational repression of target molecules by incorporation of the guide strand into the miRISC. **B** hsa-miR-10a-5p mimic transfection results in repression of Renilla luciferase activity in HCT116 and RPE1 cell lines. Cell lines were transfected with hsa-miR-10a-5p and control mimic molecules. 24hrs post transfection, cell lines were forward transfected with psiCheck2 luciferase reporter construct. Luciferase reporter assay was conducted 72hrs post mimic transfection. Data present Firefly normalized mean values +/- SD.

## 6.4 Chromosomal instability, tolerance of mitotic errors and multidrug resistance are promoted by tetraploidization in human cells

Kuznetsova AY, Seget K, Moeller GK, de Pagter MS, de Roos JA, Dürrbaum M, Kuffer C, Müller S, Zaman GJ, Kloosterman WP, Storchová Z. Chromosomal instability, tolerance of mitotic errors and multidrug resistance are promoted by tetraploidization in human cells. Cell Cycle 2015 Jul 07;14(17):2810–2820.

In this study we investigated the consequences of whole-genome doubling in transformed and non-transformed cell lines. Whole-genome doubling events that occur frequently in cancer are associated with numerical and structural chromosome aberrations. This suggests a link between tetraploidy, GIN and aneuploidy. However, the long-term effects of tetraploidy and the underlying molecular mechanisms of how tetraploid cells survive are not well understood.

To evaluate the long-term effect of tetraploidy, whole-genome doubling was induced and rare surviving cell populations were isolated and analyzed. These post-tetraploid cell lines show mostly a near-tetraploid karyotype with complex numerical and structural chromosomal aberrations. We showed that this is the result of a CIN phenotype with an increased frequency of pre-mitotic and mitotic errors such as anaphase bridges, unattached and lagging chromosomes. The frequency of cell cycle arrest following chromosome missegregation is reduced in post-tetraploid cells, thereby indicating elevated tolerance to mitotic errors. Reduced nuclear p53 activation and expression abnormalities of p53 pathway components suggest an impaired activation of the p53 pathway as the underlying molecular cause of this tolerance. Finally, we demonstrate that post-tetraploid cells confer multi-drug resistance and increased capacity of *in vivo* transformation of some cell lines.

The fate of tetraploid cells is governed by higher tolerance of GIN that results in highly complex aneuploid progenitor cells. The mechanistic cue most likely lies in the perturbed p53 pathway, which might serve as a novel target in preventing GIN and multi-drug resistance in tetraploid-derived tumors.

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REPORT

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# Chromosomal instability, tolerance of mitotic errors and multidrug resistance are promoted by tetraploidization in human cells

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**Keywords:** aneuploidy, cancer, CIN, drug resistance, p53, tetraploidy, whole genome doubling



Up to 80% of human cancers, in particular solid tumors, contain cells with abnormal chromosomal numbers, or aneuploidy, which is often linked with marked chromosomal instability. Whereas in some tumors the aneuploidy occurs by missegregation of one or a few chromosomes, aneuploidy can also arise during proliferation of inherently unstable tetraploid cells generated by whole genome doubling from diploid cells. Recent findings from cancer genome sequencing projects suggest that nearly 40% of tumors underwent whole genome doubling at some point of tumorigenesis, yet its contribution to cancer phenotypes and benefits for malignant growth remain unclear. Here, we investigated the consequences of a whole genome doubling in both cancerous and non-transformed p53 positive human cells. SNP array analysis and multicolor karyotyping revealed that induced whole-genome doubling led to variable aneuploidy. We found that chromosomal instability (CIN) is a frequent, but not a default outcome of whole genome doubling. The CIN phenotypes were accompanied by increased tolerance to mitotic errors that was mediated by suppression of the p53 signaling. Additionally, the expression of pro-apoptotic factors, such as iASPP and cIAP2, was downregulated. Furthermore, we found that whole genome doubling promotes resistance to a broad spectrum of chemotherapeutic drugs and stimulates anchorage-independent growth even in non-transformed p53-positive human cells. Taken together, whole genome doubling provides multifaceted benefits for malignant growth. Our findings provide new insight why genome-doubling promotes tumorigenesis and correlates with poor survival in cancer.

## Introduction

Many malignant tumors contain cells with aberrant chromosome numbers. These changes vary from aneuploidy (imbalanced chromosome numbers) of one or multiple chromosomes, to numbers approaching triploidy or tetraploidy.[1] Aneuploidy is often accompanied by chromosomal instability (CIN), which manifests as ongoing gains and losses of chromosomes during mitosis[2] CIN contributes to tumor heterogeneity and is associated with increased resistance to drug treatment and poor patient prognosis.[3] Studies revealed that CIN might be triggered by mutations in genes that control chromosome segregation.[4-6] Whole-genome doubling that leads to tetraploidy provides another route by which aneuploidy can arise in tumors independently of mutations in mitotic genes.[7,8] In this model, tetraploid cells formed by cytokinesis failure, endoreduplication or cell-cell fusion divide in a highly unstable manner due to supernumerary centrosomes and increased chromosome numbers. The compromised maintenance of genomic stability of tetraploids facilitates CIN and tumorigenesis. Indeed, injection of p53-deficient tetraploid cells into nude mice triggers tumor formation, whereas isogenic diploid cells show no effect.[9] Similarly, tetraploids arising from mouse ovarian surface embryonic cells develop aneuploidy, CIN and promote tumorigenesis when injected into mice.[10]

Remarkably, tetraploid cells were found in early stages of several tumors and in total tetraploidy was documented in 37% of cancers.[11] This frequent occurrence suggests that passage through tetraploidy provides advantages that facilitate tumor growth. However, experimentally generated tetraploid cells often fail to propagate, as these cells arrest in a p53-dependent manner immediately after whole genome doubling or after the first tetraploid mitosis, which is often severely erroneous.[9,12-15] This further impairs the proliferation after tetraploidization, as chromosome missegregation of even a single chromosome triggers a p53-dependent arrest.[14,16,17] Also, aneuploid cells arising due to chromosome missegregation suffer from proliferation delays and other physiological defects.[18,19] So far, only little is known how human cells survive tetraploidy and what the long-term effects of whole genome doubling are. Previous data suggest that

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tetraploidy increases CIN to generate karyotypic heterogeneity, while also providing tolerance to potentially deleterious genetic changes.[20,21] However, little experimental data is available to document the long-term effects of whole genome doubling in human cells.

## Results

### Whole genome doubling triggers aneuploidy and chromosome instability

To determine the long-term consequences of tetraploidization in human cells, we analyzed the fate of tetraploids generated from 2 p53-positive cell lines, HCT116 and hTERT-RPE1. HCT116 is a near-diploid cell line derived from human hereditary non-polyposis colon cancer and is chromosomally stable with characteristic microsatellite instability.[2,22] hTERT-RPE1 (hereafter RPE1) is a chromosomally stable diploid retinal pigment epithelium cell line immortalized by constitutive expression of human telomerase. Both cell lines stably express H2B-GFP for visualization of chromatin by fluorescence microscopy. Tetraploid cells were generated by inhibition of the actomyosin ring with the actin depolymerizing drug cytochalasin D (DCD), which lead to cytokinesis failure in approximately 60% of cells.[9,14] 600 DCD-treated cells were plated by limiting dilution into 96-well plates and allowed to expand for 6 weeks (**Fig. 1A**). All surviving cell populations were analyzed by flow cytometry. This analysis revealed near-tetraploid DNA content in 8 out of the 64 surviving HCT116-derived cell lines and in 7 out of 58 RPE1-derived cell lines (**Fig. S1A**), hereafter referred to as PostTetraploid (PT) cell lines (HPT – derived from HCT116, RPT – derived from RPE1). To minimize the effect of further evolution, all PTs underwent minimal passages before the analysis and were compared at the same "early" passage, unless otherwise stated. The posttetraploid cell lines showed only a mild proliferation delay and the duration of mitosis as well as the robustness of mitotic checkpoint activation were comparable to that of controls (**Fig. S1B-G**).

To determine the copy number changes after tetraploidization, we performed SNP array analysis in 6 HPTs, 3 RPTs cell lines and the respective parental cells. Recurrent chromosome copy number changes were observed in 3 out of 9 analyzed PTs (HPT1, HPT6 and RPT3; **Fig. 1B, C**, and **Fig. S2A, B**). SNP arrays analyze a pool of cells, which only allows detection of common changes that are shared by a majority of cells. To detect chromosomal changes in individual cells, we used multicolor fluorescence *in situ* hybridization (mFISH) karyotyping of HCT116, HPT1 and HPT2 cell lines. This analysis revealed heterogeneity within the PT populations, but not in parental HCT116: all 12 clonal cell lines originating from individual HCT116 cells remained chromosomally stable even after 80 generations, thus excluding that the emergence of karyotypic diversity is a consequence of clonal outgrowth of cells with preexisting karyotypic heterogeneity or an intrinsic characteristic of the parental line (**Fig. 1D**, and **Fig. S2C, S3A-E**). Moreover, the PTs also contained more chromosomal rearrangements than the diploids, although the difference was not statistically significant (**Fig. S3A-E**).

Since all PTs arose from a single cell, we hypothesized that the karyotypic heterogeneity indicates chromosomal instability arising after tetraploidization. We used fluorescence *in situ* hybridization (FISH) utilizing the chromosome enumeration probes to compare "early" karyotypes with karyotypes after additional 36 passages. The distribution of the chromosome copy numbers remained nearly identical in early and late HCT116 cells, whereas in HPT1 and HPT2 the numbers of chromosomes differed markedly (**Fig. 2A** and **Fig. S4A**). Chromosomal instability was also identified in RPT3 after 12 passages; in contrast, RPT1 cell line did not show significant changes in the FISH signal (**Fig. S4B**). Additionally, FISH analysis revealed a loss of the signal of chromosome 7 in 2 out of 4 analyzed posttetraploid cell lines (HPT1 and RPT3) that was present in both early and late passages. Changes in the number or structure of chromosome 7 are common in human cancers: trisomy of chromosome 7 is among the most frequently observed aberrations in cancers of the large intestine, while a loss of part or all of one copy of chromosome 7 is common in leukemia and lymphoma.[23] Taken together, transient tetraploidization can generate aneuploid and chromosomally unstable progeny even in non-transformed p53-proficient parental cell lines.

### Mitotic errors frequently occur in posttetraploid cell lines

To further characterize the chromosomal instability in the posttetraploid progeny, we imaged fixed cells and found that 15.8%, 15.0% and 13.8% of anaphases displayed segregation aberrancies in HPT1, HPT2 and HPT4, respectively, whereas only 3.7% of HCT116 underwent erroneous anaphase (**Fig. 2B**). The frequency of both anaphase bridges as well as the presence of lagging or unattached chromosomes was increased, suggesting that the frequency of both pre-mitotic and mitotic errors was elevated. Among the 3 RPT cell lines, only one displayed increased frequency of abnormal mitoses: 11.6% in RPT3 in comparison to 3.0% in RPE1, 3.1% in RPT1 and 1.4% in RPT4 (**Fig. 2C**). The multipolar mitoses were rare in PTs; the vast majority of cells segregated their chromosomes in a bipolar manner (**Fig. S5A, B**). In addition, a detailed analysis by imaging fixed cells stained with centrosome- and centriole-recognizing antibodies revealed no significant increase in the numbers of centrosomes and centrioles in PTs compared to the respective parental cell lines (**Fig. S5C-G**). This indicates that multipolarity alone cannot explain the high frequency of chromosome segregation errors in PTs.

The frequency of chromosome segregation errors could be elevated simply as a consequence of the increased chromosome numbers under the assumption that the error frequency correlates to chromosome number. However, normalization of the frequency of mitotic errors to the median chromosome number of each cell line revealed that the frequency of abnormal mitoses increases more than expected according to chromosome number in $CIN^+$ PTs (**Fig. S4C**). Specifically, the mitotic error frequency increased 3.7–4.3 fold (from 3.7% to 13.8–15.8%), whereas

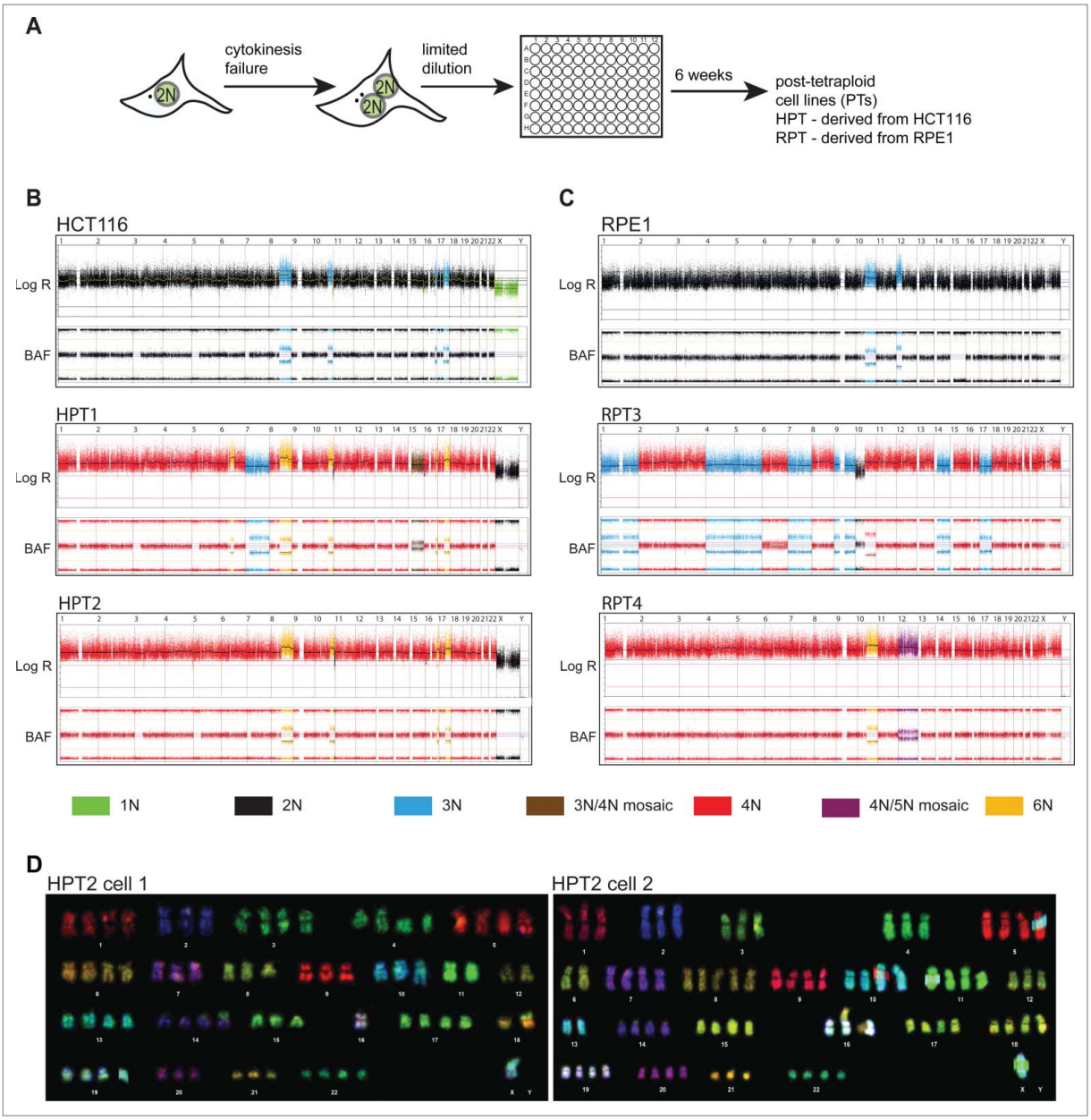


**Figure 1.** Posttetraploid progenies (PTs) are chromosomally unstable. (**A**) Schematic depiction of the experimental strategy. (**B**, **C**) SNP array profiles of HCT116, RPE1 and their posttetraploid derivatives. Copy numbers are indicated by colors. The log R represents the copy number; B-allele frequency (BAF) indicates the allele composition: BAF of 0 or 1 represents genotype of AA / A- / BB / B-, respectively; BAF of 0.5 represents AB. (**D**) Multicolor FISH karyotyping of 2 cells from the HPT2 cell line (number of chromosomes was 72 and 79, respectively). Note the difference in copy number of chromosomes 1, 3, 4, 6, 7, 8, 9, 10, 11, 12, 13, 15, 16, 17, 18 and 20.

modal chromosome numbers increased 1.7–1.8 fold (from 44 to 75–78) in HPTs. Similarly, the increase in chromosome numbers was 1.7 fold in RPT3, whereas the increase in mitotic errors reached 3.9 fold. Thus, the high frequency of mitotic errors in $CIN^+$ PTs does not result from a simple linear increase of mitotic errors due to the higher numbers of chromosomes. Taken together, whole genome doubling supports the emergence of a $CIN^+$ phenotype even in p53-positive non-cancerous cells, but it is not a default consequence.

### Posttetraploid cells show increased tolerance to mitotic errors

The efficient proliferation of PT cell lines despite their aneuploidy and CIN suggests that the cells became more tolerant to mitotic errors. We analyzed the fate of parental diploid and tetraploid cells, as well as PTs after abnormal mitosis by long-term live cell imaging. The analysis revealed that 34.2% of diploid and 54.1% of newly formed tetraploid HCT116 cells that missegregated chromosomes arrested for at least 48 h or died in the

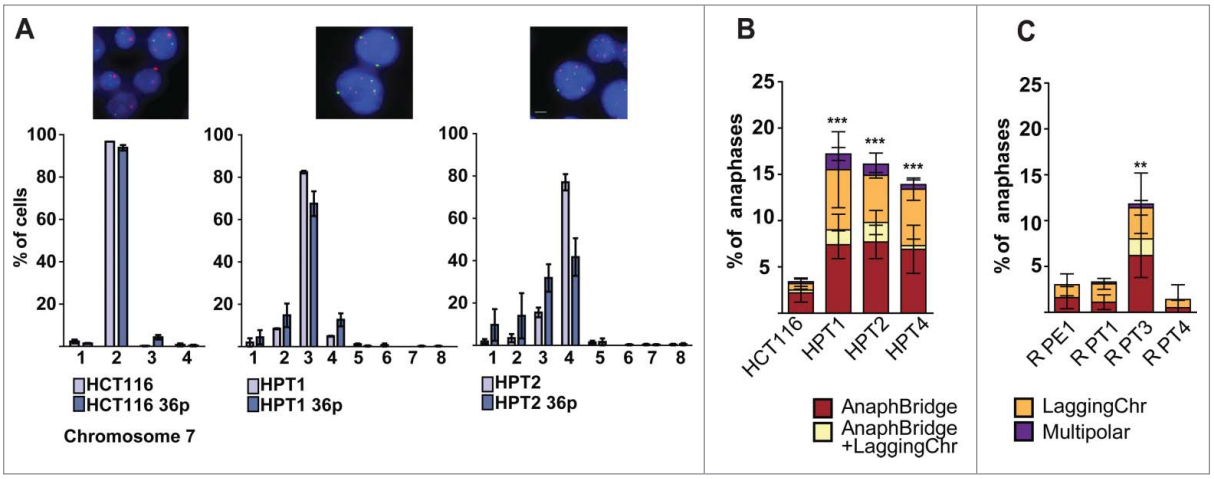


**Figure 2.** Posttetraploid cells display chromosomal instability and an increased frequency of abnormal mitosis. (**A**) Fluorescence *in situ* hybridization (FISH) against centromeric regions of HCT116 and HCT116-derived PTs. Comparison of chromosome number distribution for chromosome 7 in early passages and 36 passages later; mean and SEM of 2 independent FISH experiments. Chromosome 7 – red, chromosome 1- green, DNA was counterstained with DAPI (objective 63x, bar: 10 μm). Percentage of abnormal mitoses evaluated in fixed images of HCT116 (**B**) and RPE1 (**C**) and their respective posttetraploid derivatives; mean and SD of 3 independent experiments. AnaphBridge – cells that contain an anaphase bridge; LaggingChr – cells containing a lagging chromosome; AnaphBridge-LaggingChr – cells containing both an anaphase bridge and a lagging chromosome; Multipolar – cells that underwent multipolar anaphase.

subsequent interphase (**Fig. 3A**). In contrast, only 11.0% of HPT1 and 9.8% of HPT2 cells arrested after chromosome missegregation (**Fig. 3A**, for fate analysis of individual cells see **Fig. S6**). Thus, whole genome doubling increases cell tolerance to errors in chromosome segregation.

### The p53 pathway is deregulated in posttetraploid cells

We hypothesized that the tolerance to abnormal mitosis in PTs is due to changes in activation of the p53 pathway. To test this, we performed a micronucleation assay which allows visualization of missegregated lagging chromosome as a micronucleus in the daughter cells, combined with immunostaining with an antibody against p53. Whereas nearly 42.0% of HCT116 cells with micronuclei accumulated nuclear p53, only 25.0% of HPT1 and 26.9% of HPT2 cells showed nuclear p53 accumulation when a micronucleus was present in the cell (**Fig. 3B, C**). A similar experiment in RPE1 and its posttetraploid derivatives revealed that 66.4% and 50.7% of RPT1 and RPT4 cells that underwent missegregation accumulated nuclear p53, which is similar to parental RPE1 (66.8%). In contrast, we observed that

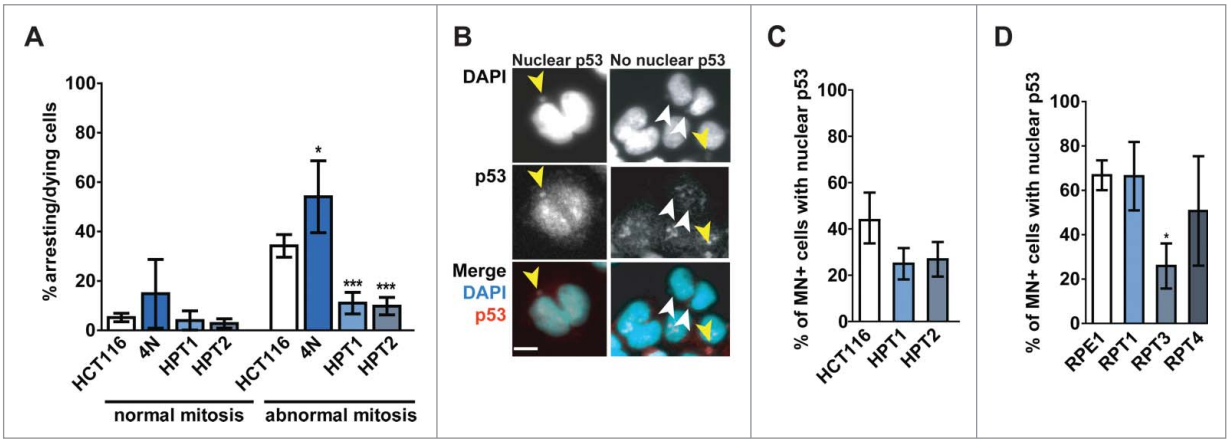


**Figure 3.** Posttetraploid cells are tolerant to mitotic errors. (**A**) Frequency of cell cycle arrest/cell death after bipolar mitosis with no apparent defects (normal mitosis) and with visible chromosome segregation defects (abnormal mitosis). Mean of 4 independent experiments and SD is plotted. Unpaired Student t-test was used to test for statistical significance. (**B**) Examples of p53 accumulation in the nuclei and micronuclei of the micronucleated cells. Yellow and white arrowheads indicate the micronuclei with and without p53 enrichment, respectively. p53-red, DNA was counterstained with DAPI, bar: 10 μm. (**C and D**) Accumulation of p53 in the nuclei of cells forming micronuclei ($MN^+$) in HCT116, RPE1 and their respective posttetraploid derivatives (panels **C**, **D**, respectively). Mean of 4 independent experiments and SEM are plotted.

only 25.9% of RPT3 cells with a micronucleus accumulated p53 in the nucleus (**Fig. 3C, D**). Thus, whereas chromosomally stable PTs accumulated nuclear p53 as the parental cell line, the p53 signaling upon chromosome missegregation is attenuated in the $CIN^+$ posttetraploids (compare **Figs. 2C and 3B-D**).

The activation of the p53 pathway upon chromosome missegregation occurs possibly via phosphorylation of p53 on serine 15 or by activation of the p38/MAPK pathway.[14,16,17] Analysis of the p53 and p38 protein levels revealed no significant changes in parental and PT cell lines during unperturbed growth (**Fig. S7A, B**). Next, we induced chromosome missegregation by treatment with 20 μM VS83, an inhibitor of the kinesin Eg5 that results in the formation of mitotic cells with monopolar spindles. Subsequent wash out of VS83 ensures bipolar spindle formation and progress through mitosis; however, the mitosis is highly erroneous.[16] The frequency and type of errors upon treatment with VS83 was similar in both posttetraploid and parental cell lines (**Fig. S7C, D**). Markedly, we observed that p53 was not stabilized in HPTs and in RPT3 upon VS83 treatment, whereas the levels of p53 increased in HCT116 and RPE1, RPT1 and RPT4 (**Fig. 4A, C**). This finding is in agreement with the diminished nuclear accumulation of p53 that we identified in the $CIN^+$ clones (**Fig. 3C, D**).

Next, we analyzed transcriptome changes in 2 HPTs (HPT1 and HPT2) and 3 RPTs (RPT1, 3 and 4). We focused on 388 previously identified upstream and downstream interactors of p53 (see Material and Methods). Markedly, 23% of the analyzed genes were significantly deregulated with respect to the parental lines with a fold change of 1.5 in at least one of the HPTs or RPTs, but only 6 factors were upregulated in all PTs: LDHA (lactate dehydrogenese A), DGKA (diacylglycerol kinase), HIF1A (hypoxia induced factor 1), 2 inhibitors of apoptosis iASPP (inhibitory member of the ASPP family, encoded by PPP1R13L) and BIRC3 (Baculoviral IAP Repeat Containing 3), transcriptional factor ETS1 and transforming growth factor TGFA (**Fig. 4E**, and **Table S1**). Two genes were downregulated in all PTs: DUSP5, an inhibitor that negatively regulates members of the mitogen-activated protein (MAP) kinase superfamily (MAPK/ERK, SAPK/JNK, p38) and MST1 (macrophage signaling growth factor), a member of the MSP-RON signaling that plays a role in malignant invasive growth. We found 2 genes that were upregulated specifically in $CIN^+$ cells: FOXO1 and NDRG1 that are both involved in response to oxidative and metabolic stress. Taken together, whole genome doubling promotes upregulation of factors promoting cell survival upon stress (HIF1, FOXO1) and alters expression of factors that inhibit apoptosis and MAP kinases (iASPP, BIRC3, DUSP5).

### Posttetraploid cell lines acquire multidrug resistance and transform *in vitro*

To test whether whole genome doubling promotes increased resistance to cancer treatments, we compared the sensitivity of PTs with their respective parental cell lines to a broad range of anti-cancer agents. Effects of compounds on cell proliferation were determined using measurement of intracellular ATP content as an indirect readout of cell number.[24] We profiled 17 different anti-cancer agents at a wide concentration range over 9 points from 32 μM to 0.32 nm on the 2 parental cell lines and in HPT1 and 2, RPT1, 3 and 4 in 3 independent experiments. The inhibitory potency of the compounds was expressed as $pIC_{50}$ ($-^{10}\log IC_{50}$) values. Comparison of the relative drug sensitivity of the parental cells with that of the PTs revealed a general multidrug resistant phenotype in all analyzed posttetraploid lines derived from the non-transformed hTERT-RPE1 cell line (**Table S2**, **Fig. 5A**) as well as in the 3 posttetraploid lines derived from the colon cancer line HCT116 (**Table S2**, **Fig. 5B**). All PTs showed significant resistance to the topoisomerase II inhibitors daunorubicin, doxorubicin and etoposide (**Tables S2 and S3; Fig. 5A, B**). In addition, RPTs were significantly resistant to the pyrimidine antagonist 5-fluoracil, the inhibitor of the p53-MDM2 interaction nutlin3a and the growth factor receptor kinase inhibitor pelitinib. HPT cell lines showed significant resistance to the DNA crosslinker cisplatin, the microtubule-targeting agents docetaxel and paclitaxel, and the inhibitor of histone deacytelases vorinostat (**Tables S2 and S3**). Interestingly, HPTs showed increased sensitivity to the purine antagonist 6-mercaptopurine (**Fig. 5A**). Thus, whole genome doubling provides a general protection against drug treatment in both non-transformed and cancer cells. This marked feature might explain why whole genome doubling correlates with a poor prognosis and resistance to therapy in some cancer types.[21]

Finally, we determined the impact of tetraploidization on transformation capacity by assaying the anchorage independent growth of the posttetraploid progeny in soft agar (**Fig. 5C**). Since HCT116 is a cell line derived from colorectal cancer and therefore proficient in anchorage independent growth, we tested RPE1, a primary p53–positive immortalized cell line and its derivatives. The diploid RPE1 showed no anchorage-independent growth even after initiation/promotion treatment, where cells were exposed to the mutagen 7,12-dimethylbenz[*a*]anthracene (DMBA) alone or followed by exposure to the tumor promoter 12-*O*-tetradecanoylphorbol-13-acetate (TPA). In contrast, RPT cell lines efficiently formed colonies in soft agar even in absence of any treatment, indicating that the selected surviving populations became transformed *in vitro* (**Fig. 5C**). This result was observed in 2 independent experiments in all 3 posttetraploid cells lines derived from RPE1. Thus, whole genome doubling promotes *in vitro* transformation even in p53-positive non-cancerous cell lines.

## Discussion

Whole genome doubling (WGD) is considered to contribute to eukaryotic evolution by facilitating adaptation while simultaneously buffering the possible effects of deleterious mutations.[25] Recent findings support a similar role for WGD in fostering tumor genome evolution in mammalian cells, however, it remains unclear how exactly tetraploidy benefits malignant growth. The cellular fates of tetraploids were previously analyzed in populations isolated by serial FACS sorting.[20,26] These cells were found to be chromosomally stable likely because the serial

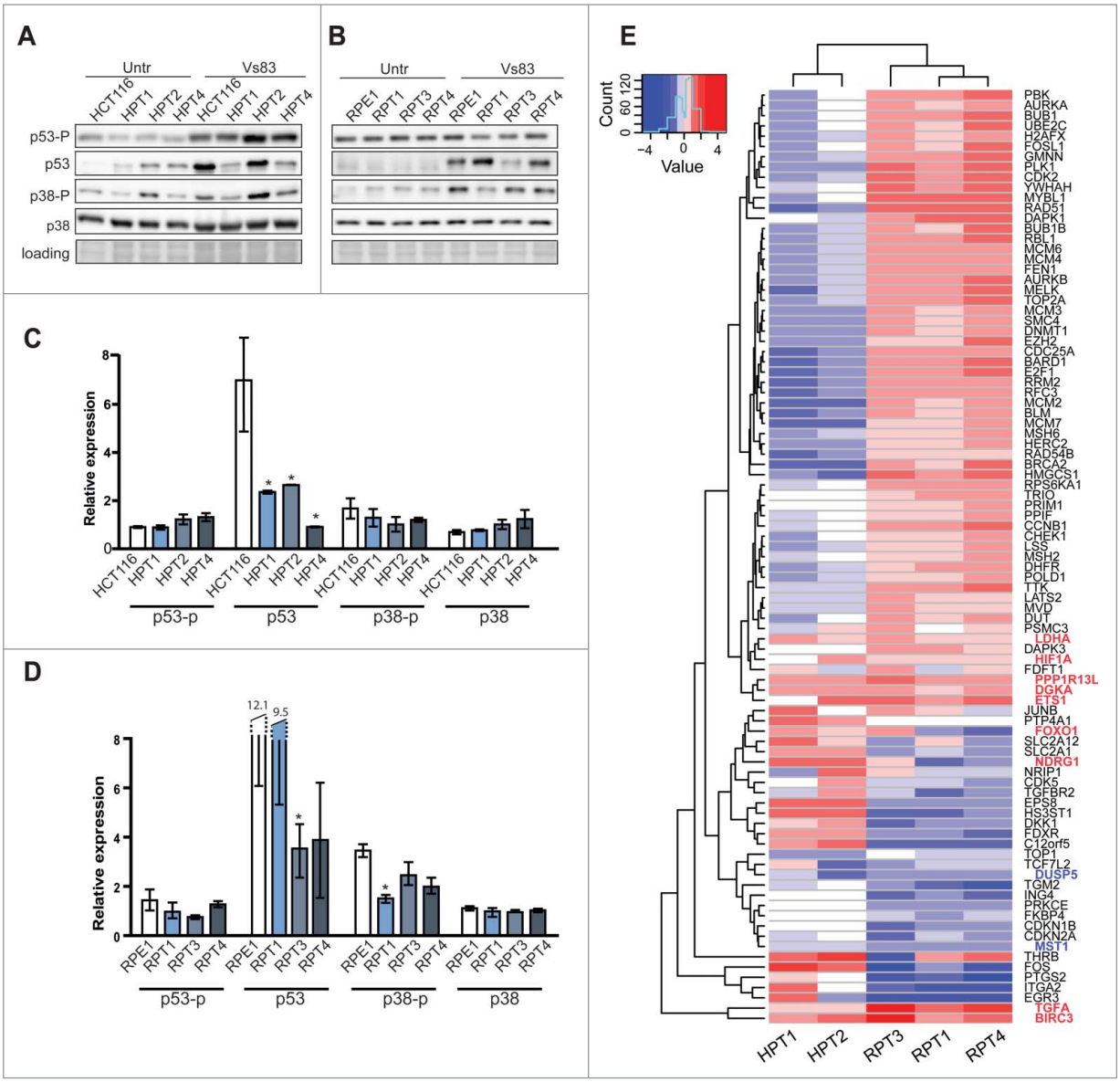


**Figure 4.** The p53 pathway is deregulated in posttetraploids. (**A**, **B**) Changes in abundance in p53, p38 and p21 and phosphorylation of p53 on Ser15 (p53-p) and phosphorylation of p38-p on Threonine180 and Tyrosine 182 (p38-p) in HCT116, RPE1 and respective posttetraploid cell lines with and without VS83 treatment. Four independent experiments were performed, an example of immunoblotting is shown; a Ponceau S stain was used as a loading control. (**C**, **D**) Quantification of the response to the missegregation triggered by release from VS83 treatment. The relative signal levels are presented as fold change of treated-to-untreated cells. Mean of at least 3 independent experiments with SD is shown, * marks statistically significant difference ($P < 0.05$). (**E**) Heat map of transcriptional fold changes of 91 significantly altered p53 interactors in posttetraploid cell lines (normalized to the respective parental cell lines).

sorting to cellular DNA content eliminated aneuploid cells that arise from tetraploids.[26] In another study, spontaneously arising tetraploid cells were isolated from the cancer cell line HCT116.[21] This approach can only be used in cancer cell lines as spontaneous tetraploidization is rare in non-transformed human cells; an additional drawback is that the mechanism of spontaneous tetraploidization and possible underlying mutations remain enigmatic. Here, we isolated clonal cell lines from both cancerous and non-transformed cells that arose from survivors of induced cytokinesis failure. We found that all PTs harbored aneuploid karyotypes, but showed marked differences in the degree of CIN (**Figs. 1, 2**). Our findings demonstrate that tetraploidy leads to aneuploidy and chromosomal instability even in p53-positive non-cancerous cell lines, but also establish that a *bona fide* CIN$^+$ phenotype is not a default outcome of whole genome doubling.

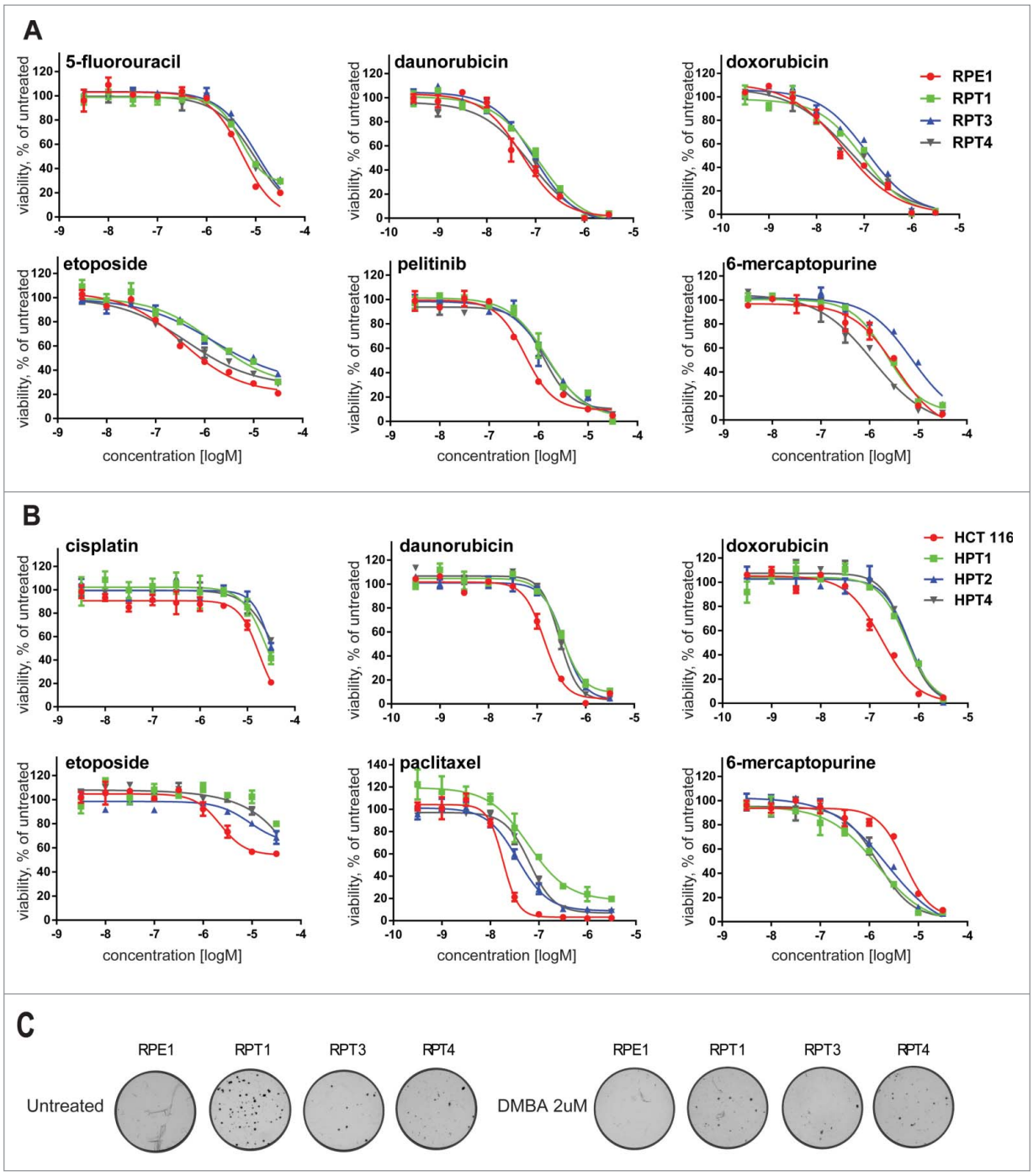


**Figure 5.** Posttetraploid cell lines are resistant to a broad spectrum of chemotherapeutic drugs and transform *in vitro*. (**A**) Dose-response curves of compounds showing different sensitivities in proliferation assays with the hTERT-RPE1 cell line and the posttetraploid cell lines RPT1, RPT3 and RPT4. (**B**) Dose-response curves of compounds showing different sensitivities in proliferation assays with the HCT116 cell line and the posttetraploid cell lines HPT1, HPT2 and HPT4. The posttetraploids lines are resistant to a broad spectrum of anti-cancerous drugs; except HPT1, 2 and 4 that are relatively more sensitive to 6-mercaptopurine. Fitted curve for 2 replicates from one or 2 independent experiments is plotted. Note that no fitted curve was determined for HPT1 upon etoposide treatment. See Material and Methods for details. (**C**) Phase contrast images of anchorage-independent colony growth in soft agar.

What causes the chromosomal instability in posttetraploid cell lines? CIN after whole genome doubling is often explained by a doubling of the centrosome number, however, the centrosome numbers in HPT and RPT cell lines were nearly normal (**Fig. S5**), in line with recent finding that multipolar mitoses are highly detrimental in human cells and thus an early loss of extra

centrosomes is necessary to ensure survival.[26,27] The fact the centrosome numbers and multipolarity are not responsible for the CIN phenotype in PTs is best illustrated by the comparison of the RPT cell lines. Although both RPT1 and RPT3 show similar distribution of centrosome numbers, RPT1 is chromosomally stable, while RPT3 is chromosomally unstable and highly aneuploid (**Fig. S5E** and **Fig. 2C**). This implies that the chromosome composition of the cells may determine whether they are $CIN^+$ or not. For example, imbalanced gene copy numbers due to aneuploidy might affect the functionality of protein complexes required for spindle functions or for the spindle assembly checkpoint.[28] Alternatively, microtubule dynamics might be altered due to changes in expression of microtubule associate proteins and motors, thus interfering with the stability of microtubule-kinetochore attachments. This hypothesis is in line with the previous findings that microtubule dynamics are often altered in $CIN^+$ cancer cell lines and affect the frequency of errors during mitosis.[29,30]

Tetraploidy as well as chromosome segregation errors activate p53 pathway, thereby driving cells into irreversible arrest.[14,16,17] Accordingly, TP53 mutations and p53 pathway alterations are frequently found in CIN tumors.[31] Importantly, we found that the $CIN^+$ posttetraploids were able to overcome the p53 activation as the levels of p53 and its nuclear accumulation were diminished upon chromosome missegregation (**Figs. 3 and 4A**). Additionally, analysis of transcriptome changes of the p53 interactors found 11 factors to be similarly deregulated in all PTs (**Fig. 4D**); all of the identified factors positively affect cell survival. Two genes were downregulated in all PTs: DUSP5, a direct target of p53 and an inhibitor of the mitogen-activated protein (MAP) kinases[32] and MST1 (macrophage signaling growth factor), a member of the MSP-RON signaling; expression of MST1 is repressed in many types of human cancer.[33] Notably, expression of apoptotic inhibitors iASPP and BIRC3 (cIAP2) was upregulated in all PTs. Both iASPP and cIAP2 are upregulated in many cancers and facilitates their survival.[34-36] TGFα, a growth factor, which activates signaling pathways for proliferation, differentiation and development and has been associated with many types of cancers,[37] is upregulated in all posttetraploids, similar to another pro-proliferative factor Ets1 that controls the expression of cytokines and chemokines.[38] Another upregulated transcription factor, Hif-1α, is required for the response to hypoxia as well as for regulation of apoptosis.[39] PTs also upregulate expression of a downstream target of Hif-1α, the lactate dehydrogenase A (LDHA) that catalyzes the conversion of pyruvate to lactate. LDHA is a key factor of anaerobic glycolysis and instrumental for the switch from oxidative phosphorylation to increased glycolysis, so called Warburg effect that is typical for malignant cells.[40]

Two p53 interactors were overexpressed only in $CIN^+$ cells: FOXO1, the main target of insulin signaling and a transcription factor that regulates metabolic homeostasis in response to oxidative stress,[41] and NDRG1 that has a poorly characterized function in stress response.[42] The upregulation of stress-response factors in $CIN^+$ PTs suggests that chromosomal instability imposes an ongoing metabolic and oxidative stress on human cells that might arise in response to the disruption of the protein homeostasis due to ongoing changes in chromosome content.[43]

Aneuploidy and CIN in tumors are often associated with increased resistance to drug treatment and therefore poor prognosis for cancer patients.[44,45] We found that the posttetraploid cell lines gained resistance to a broad spectrum of small inhibitors that are used in chemotherapy. This multidrug resistance profile was found in both cells derived from a cancer cell line HCT116 as well as in posttetraploids originating from RPE1, a non-transformed and p53-positive cell line (**Fig. 5**). Several lines of evidence have been advanced recently demonstrating that aneuploid and tetraploid cancer cells confer resistance to some drugs.[20,46,47] Why whole genome doubling provides increased multidrug resistance remains enigmatic and should be analyzed in future. Finally, we found that whole genome doubling promotes anchorage-independent growth, and hence *in vitro* transformation even in p53-positive cells. This ability was independent of the $CIN^+$ phenotype. We propose that the expression changes that allow efficient proliferation despite abnormal karyotype by inhibiting apoptosis and stimulating pro-proliferative pathways contribute to the multidrug resistance and to *in vitro* transformation of human cells.

Taken together, tetraploidization benefits uncontrolled growth in both cancerous and non-cancerous cells. The molecular mechanisms underlying these effects remain to be addressed in the future. Identification of pathways that promote tetraploidy and its survival will be essential not only to understand the mechanisms leading to tumor formation but also for the development of novel strategies to prevent acquired multidrug resistance during cancer treatment.

## Materials and Methods

### Generation and culturing of posttetraploid cell lines

HCT116 H2B-GFP and RPE1 H2B-GFP (a gift from Dr. Steven Taylor, The University of Manchester, UK) were treated with 0.75 μM of the actomyosin inhibitor dihydrocytochalasin D (DCD, Sigma) for 18 h. The cells were then washed, placed into a drug-free medium and subcloned by limiting dilution in 96-well plates (0.5 cell per well). Tetraploid RPE1 H2B-GFP cells were grown on plates coated with gelatin (Merck). After clone expansion, cells were harvested for flow cytometry to measure the DNA content. All cell lines were cultured in Dulbecco's modified medium (Gibco), supplemented with 10% fetal calf serum (Gibco), 50 IU/ml penicillin, and 50 μg/ml streptomycin (Gibco) at 37°C with 5% $CO_2$.

### Immunoblotting

Total cell lysate was separated by SDS-PAGE and transferred to a polyvinylidene fluoride membrane (Roche) or nitrocellulose membrane (Whatman) as previously described. Following antibodies were used: anti-p53 antibody (1:100,Santa Cruz Biotechnology, Inc.), anti-pSer15-p53 antibody (1:500, Santa Cruz Biotechnology, Inc.), anti- p21 antibody (1:1000, Cell

Signaling), anti-p38 antibody (1:1000, Cell signaling), anti-pThr180/Tyr182-p38 antibody (1:200, Cell signaling). Immunoblot quantification was performed using Image J software.

**Live cell microscopy**

Long term live cell data were recorded on an inverted Zeiss Observer.Z1 microscope (Visitron Systems) equipped with a humidified chamber (EMBLEM) at 37°C, 40% humidity and in the atmosphere of 5% $CO_2$ using CoolSNAP HQ2 camera (Photometrics), Plan Neofluar 20x or 10x air objective NA 1.0, epifluorescent X-Cite 120 Series lamp (EXFO), using GFP filter and differential interference contrast (DIC) in DMEM. Imaging of fixed cells was carried out on Marianas SDC™ system (inverted Zeiss Observer.Z1 microscope, Plan Apochromat 63x magnification oil objective or 20x magnification air objective, equipped with spinning disc head (Yokogawa) and a CoolSNAP-HQ2 and CoolSNAP-EZ CCD Photometrics cameras (Intelligent Imaging Innovations, Inc.).

**Chromosome spreads**

The cells were treated with 50 ng/ml microtubule-depolymerizing drug colchicine (Serva) for 4.5 h, collected and pelleted using table-top centrifuge, swollen in 75 mM KCl in a 37°C water bath for 15 minutes, fixed with Carnoy solution (75% methanol and 25% acetic acid) and spread on a wet glass slide with a glass Pasteur pipette. The slides were dried at 42°C and stained with Giemsa dye (Fluka).

**Fluorescence *in situ* hybridization (FISH)**

FISH was carried out using satellite enumeration probes against centromeric regions of specific chromosomes (1, 3, 7, and 12) conjugated either to a red or a green fluorophore according to manufacturer's protocol (Cytocell, UK). DNA was counterstained with DAPI, and the cover slips were mounted on slides using antifade solution (Cytocell, UK).

**Whole chromosome multicolor FISH (mFISH karyotyping)**

Multicolor FISH was performed as previously described with a DNA probe mixture (24XCyte Human Multicolor FISH Probe Kit, MetaSystems, Altlussheim, Germany). The analysis was carried out using Adobe Photoshop (Adobe Systems, San Jose, USA) for visual inspection of the images; statistical analysis was performed using MS Excel (Microsoft) and Prism. Aberration ratio was calculated as number of derivative chromosomes normalized to a total number of chromosomes identified in analyzed cell spread.

**SNP array profiling**

Human CytoSNP-12 bead chip arrays (Illumina) were used for detection of copy number aberrations (CNAs) in clonal aneuploid and diploid cell lines. Array hybridization was performed according to the manufacturer's recommendations. CNAs were identified using Nexus software (version 7.5.1) with standard settings. To identify unique CNAs in clonal cell lines, we used the Nexus call coordinates and removed all calls of the same type with a reciprocal overlap of at least 60%. All profiles were manually checked.

**Analysis of mitotic abnormalities**

The cells were grown in the glass-bottom 96-black well plates and fixed with 100% methanol for 10 min at -20°C. DNA was stained with SYTOX Green Nucleic Acid dye (Molecular Probes, Invitrogen) with added RNAse. The imaging was carried out on Visitron Systems microscope.

**Micronucleation test followed by anti-p53 immunostaining**

The cells were seeded in the glass-bottomed 96-black well plates 48 h prior the experiment and then treated with DCD for 18 h. Only cells that became binucleated were scored. Cells were fixed with 100% MeOH and stained with DAPI (Carl Roth). Anti-p53 antibody (1:500, Cell Signaling) was used. The acquisition and analysis were performed using Slidebook 5 software with 3I microscope, 20x magnification objective. p53 status in the nuclei was determined by automated measurement of median intensities of p53 in the nucleus normalized by median intensity of p53 in cytoplasm.

**Transcriptome data processing and analysis**

Total RNA was extracted using RNeasy Mini Kit (QIAGEN). For the HPT cell lines microarray data preprocessing, normalization and analysis was conducted as described previously.[48] For next generation RNA sequencing of the RPTs, TruSeq RNA library preparation and Illumina HiSeq2500 sequencing with 25 million 100bp single reads per library were performed by the Max Planck-Genome-Center Cologne, Germany (http://mpgc.mpipz.mpg.de/home/). Subsequently to adapter removal with cutadapt, reads were mapped to the human genome (hg19) using TopHat (v2.0.10) with the following parameters: "tophat2 -g1 -G". RefSeq information in the GTF file was downloaded from the UCSC genome browser. featureCounts (v1.4.3) was used to generate the count matrix with the same GTF file as for the alignment and the following parameters: "-t exon -g gene_id". Normalization and differential expression analysis of the count matrix data was performed using the R/Bioconductor package DESeq2. For differential expression analysis, PTs were compared to the parental diploid cell line. Processed and normalized RNA sequencing data (RPE1 derived cell lines) or microarray data (HCT116 derived cell lines) were analyzed by QIAGEN's Ingenuity® Pathway Analysis and visualized with R. p53 physical and genetic interactors were identified in the IPA knowledge base.

**Cell proliferation assay**

The compounds were obtained from commercial suppliers and dissolved in 100% DMSO. Cells were dispensed in a 384-well plate at 400 cells per well. After 24 h, 5 μl of compound dilution was added and plates were further incubated for another 72 h, after which 25 μl of ATPlite 1Step™ (PerkinElmer, Groningen, The Netherlands) solution was added to each well. Luminescence was recorded on an Envision™ multimode reader (PerkinElmer, Waltham, MA, USA). $IC_{50}$s were fitted by non-linear regression using XLfit™5. A two-tailed Student's t-test was performed

to determine whether differences in sensitivity (ΔpIC50) were statistically significant (i.e., $P < 0.05$).

### Anchorage independent growth assay

Cells were treated with ethanol as a vehicle or DMBA (2μM or 4 μM) for 3 days. Subsequently cells were either seeded on soft agar or treated with 100 ng/ml TPA or with DMSO as a vehicle control for next 10 days before seeding on soft agar.

### Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.


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### Supplemental Material

Supplemental data for this article can be accessed on the publisher's website.



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# 7 Discussion

Aneuploidy has profound effects on cellular physiology. Although there are many variations of the aneuploid karyotype, the phenotypic consequences such as impaired proliferation and sensitivity to certain cellular stresses are similar. During my thesis, I set out to determine the common molecular cues of the response to aneuploidy. I applied large-scale analysis of miRNAome, transcriptome and proteome data and their integration to reveal the common determinants of the effects of aneuploidy.

## 7.1 Proteotoxic stress underlies the response to aneuploidy

The addition of extra chromosomes results in unbalanced and elevated gene expression that bear two major challenges for the cell: first, a higher demand for the protein synthesis as well as protein folding machinery; second, an imbalance of the protein stoichiometry, particularly affecting protein complex members. Cumulative evidence points to a defect in maintenance of proteostasis in aneuploid model systems (Donnelly et al., 2014; Oromendia and Amon, 2014; Pavelka et al., 2010; Tang et al., 2011; Torres et al., 2007), but the underlying molecular mechanisms have not been fully characterized. We show that the proteotoxic stress in aneuploid cells is characterized by impaired HSP90 dependent protein folding. Further, my global analysis of the transcriptome and proteome of aneuploid cells revealed that the HSP90 family is significantly downregulated. To find the underlying causes for impaired protein folding and downregulation of the HSP90 family, we investigated the levels and activity of HSF1. Although steady state levels of HSF1 were not significantly altered, we found an impaired induction of HSF1 activity upon heat stress. Endogenous overexpression of HSF1 protects aneuploid cells from sensitivity to protein folding stress. Why HSF1 function is impaired and fails to transcriptionally activate the heat shock response in aneuploid cells remains an open question. Certain is that the proteotoxic stress and especially compromised HSP90 function inflicts severe consequences on the cells. Our new finding that proteotoxic stress is a hallmark of aneuploid human cells suggests that proteotoxic stress is the underlying cause for the other well-documented phenotypes of aneuploid human cells such as the growth defect, accumulation of protein deposits and activation of autophagy, maintenance of genome stability and the global transcriptional response

### 7.1.1 Protein deposits and elevated autophagy as a consequence of proteotoxic stress in aneuploid cells

Most likely a direct consequence of unbalanced proteostasis in aneuploid cells is the accumulation of protein deposits that show features of protein aggregates observed in aneuploid yeast and human cells (Oromendia et al., 2012; Stingele et al., 2013). Defective HSP90-dependent protein folding might result in accumulation of unfolded or misfolded proteins. In addition, the altered gene expression results in unbalanced levels of subunits of protein complexes. Subunits of protein complexes are particularly aggregation-prone because of their exposed hydrophobic amino acids and are kept soluble by their binding to cellular chaperons (Balchin et al., 2016). Thus, an excess of macromolecular complex subunits results in protein aggregates, a process that might be further enhanced by

reduced chaperone levels that fail to keep unbound protein complex subunits in solution. In both cases, these aggregates might not only be the result of HSP90 disturbances, but may also contribute to it by overburdening the proteostasis network and titrating away chaperons, similar as demonstrated in yeast and *C. elegans* (Geiler-Samerotte et al., 2011; Gidalevitz et al., 2009). To date, it is not known whether the observed deposits are mainly due to unbalanced protein complex subunits or due to defective protein folding in general. Recent advances in quantitative proteomics allow studying the composition of protein aggregates (May et al., 2014). Such an approach could be applied to determine the exact protein aggregate composition, which would give further insights into the main mechanism leading to aggregate formation in aneuploid cells.

The requirement for clearance of these aggregates might connect defects in the maintenance of proteostasis with another feature of aneuploid cells, which is the activated autophagy (Ariyoshi et al., 2016; Ohashi et al., 2015; Stingele et al., 2013). The autophagy-lysosome system is, among other functions, involved in targeted clearance of aggregates that are resistant to degradation by the ubiquitin-proteasome system (Kubota, 2009). Although proteasomal degradation is one way to compensate excess complex subunits (Dephoure et al., 2014), increased autophagic activity has also been detected in yeast and human aneuploid cell lines. Upon acute chromosome missegregation, autophagosomes in lysosomes accumulate and are not efficiently degraded (Santaguida et al., 2015). This leads to translocation of the transcription factor TFEB into the nucleus and subsequent activation of transcription of autophagic and lysosomal genes. Whether similar mechanisms lead to the upregulation of autophagic and lysosomal genes in human model cell lines with chronic autophagy remains to be investigated.

### 7.1.2 Proteotoxic stress might contribute to the proliferation defect of aneuploid cells

The proteostasis network and the central hub protein HSP90 are crucial for functional cellular processes (da Silva and Ramos, 2012). We found that endogenous overexpression of HSF1 mitigates the proliferation defect compared to aneuploid cells without additional copy of HSF1 (Donnelly et al., 2014). This in turn suggests that compromised HSF1 transcriptional activity contributes to the growth defect of aneuploid cells. Reduced HSF1 transcriptional activity might result in slow cell cycle progression since HSF1 target genes include proliferation and mitogenic signaling genes (Santagata et al., 2013). Moreover, reduced HSF1 transcriptional activity and resulting low HSP90 levels might contribute to the impaired growth of aneuploid cells, since several cell cycle regulators are among the HSP90 clients including regulators of the G1/ S phase transition such as CDK 4, 6 and 2 and Cyclin D as well as Cyclin E (Burrows et al., 2004). In addition, the unbalanced proteostasis network and accumulation of aggregates might present a cellular stressor leading to reduced growth. In support, depletion of the deubiquitinating enzyme Ubp6, which results in acceleration of proteosomal substrate processing, increases the fitness of aneuploid yeast cells (Torres et al., 2010). Thus, I hypothesize that the cumulative effects of disturbed proteostasis and the consequential effects on cell physiology lead to growth defects in aneuploid cells (Figure 6).

### 7.1.3 Proteotoxic stress might affect genomic maintentance of aneuploid cells

Proteotoxic stress might also affect genome maintenance in aneuploid cell lines. Recent results demonstrate that human cells with additional chromosomes are sensitive to replication stress and exhibit increased GIN (Passerini et al., 2016). I demonstrated by proteome analysis that the aneuploid proteome landscape overlaps with the proteome of HeLa cells, in which HSP90 was inhibited (Donnelly et al., 2014). In particularly, DNA replication and other DNA-associated pathways were downregulated in both cases. This suggests, that impaired HSP90 function affects clients associated with genome maintenance. Indeed, impaired HSP90 function promotes GIN by various mechanisms (reviewed in (Kaplan and Li, 2012). For instance, HSP90 is important for several DNA repair pathways; for example by stabilization of the Fanconi anemia complex members HSP90 facilitates repair of DNA crosslinks (Oda et al., 2007). Moreover, inhibition of HSP90 impairs DNA double strand repair via its clients and has secondary effects on the RAD51 mediated homologous recombination (Noguchi et al., 2006). In addition, HSP90 inhibition destabilizes CHK1 kinase and sensitizes cells to replication stress (Arlander et al., 2003). Since CHK1 is a key regulator of the cell cycle, DNA replication and origin firing upon DNA damage, depletion of CHK1 is detrimental for genomic stability. Based on these reports disturbed HSP90 function in aneuploid cells might play a role in aneuploidy driven genome instability. Moreover, affected CHK1 kinase might explain the sensitivity to replication stress. An intriguing possibility is that limited HSP90 function not only contributes to GIN in aneuploid cells but also mediates the adaptive potential of the cells. In wild type haploid yeast strains, a growth under HSP90 inhibiting conditions induced aneuploidy at high rates and conferred adaptations to various stress conditions (Chen et al., 2012a). Thus, GIN promoted by hampered HSP90 function may convey adaptation potential to adverse conditions. Future studies will shed light on the relation of impaired HSP90 function and GIN as well as the adaptability of aneuploid cells to stress conditions.

### 7.1.4 Proteotoxic stress shapes the conserved transcriptional response to aneuploidy

It has become clear from various aneuploid model systems that the genome-wide expression is affected by aneuploidy (Upender et al., 2004; Torres et al., 2007; Nawata et al., 2011; Stingele et al., 2012; Hervé et al., 2016). Yet, whether these transcriptional changes are common to different aneuploid cell lines has not been systematically analyzed. My in-depth comparison of multiple different human aneuploid cell lines created in different laboratories and of different cell line origin or type of aneuploidy revealed that the transcriptional response is largely conserved (Dürrbaum et al., 2014). The conservation was found within the identity of deregulated pathway annotations; downregulation of DNA and RNA metabolic pathways and upregulation of endoplasmic reticulum, Golgi apparatus, lysosome and vacuoles as well as membrane metabolism associated pathways. The deregulated pathways overlap with the pathways that were found to be affected by aneuploidy in mammalian cell lines (Sheltzer et al., 2012). In yeast, the transcriptional response to aneuploidy closely resembles an environmental stress response (ESR) that characterizes a gene expression signature commonly observed upon various environmental stresses (Gasch et al., 2000; O'Duibhir et al., 2014). The pathways

affected in the ESR are partially overlapping with the pathways altered in the response to aneuploidy in mammalian cells. Particularly, RNA and ribosomal associated pathways are downregulated in response to aneuploidy in both yeast and mammalian cells (Sheltzer et al., 2012). In contrast, the upregulated pathways differ; for example genes associated with oxidative stress response and protein folding are upregulated in aneuploid yeast strain but not in mammalian aneuploids. Although the upregulated pathways in yeast and mammalian aneuploid cells differ in both cases, the altered pathways in mammalian cells might also reflect a stress response, such as the ESR in yeast.

What are the factors shaping the conserved transcriptional response to aneuploidy? One limitation of the global transcriptional analysis of an adapted aneuploid cell population is that the conserved aneuploid pathway response pattern probably reflects both the primary and secondary effects of aneuploidy. The transcriptome might be directly influenced by the expression of genes encoded in the aneuploid regions. Some deregulated genes of the aneuploid regions, such as transcription factors, may further affect the genome-wide transcription including the expression of genes in disomic regions. As a secondary effect, the altered genome content *per se* affects the cellular physiology, which initiates a feedback to gene expression. We compared the transcriptional response to aneuploidy with the response to various stresses. Only the inhibition of autophagy results in similar pathway deregulations as aneuploidy (Dürrbaum et al., 2014). Given that in aneuploid cells autophagy is activated, these similarities might be rather counterintuitive. Since inhibition of autophagy causes proteotoxic stress (Doerr et al., 2013), one possible explanation is that the common transcriptome changes reflect the proteotoxic stress in both conditions. Further evidence for this comes from my comparative analysis with the transcriptome of human hepatocellular carcinoma cell line depleted for HSF1, that also shows large similarities to the transcriptional response to aneuploidy (Donnelly et al., 2014). Thus, these results suggest that one factor shaping the conserved transcriptional response in aneuploid cell is proteotoxic stress.

One of the complications in finding the determinants of the conserved transcriptional response is that the aneuploid phenotypes are interlinked: for instance proteotoxic stress is probably connected to the growth defect and GIN. This makes it difficult to clearly differentiate between primary and secondary responses. For example, the transcriptome of aneuploid yeast strains correlates with the transcriptome of yeast strains with delayed cell cycle progression due to mutations in regulatory cell cycle factors Cdc28 and Cdc23 (Torres et al., 2007). Moreover, the transcriptome of mammalian aneuploid cells also correlates with the transcriptome of these growth-delayed yeast strains (Sheltzer et al., 2012). Yet, in our analysis of aneuploid cells derived from a tetraploid intermediate that proliferate without any significant growth defect revealed similar pathway deregulations (Dürrbaum et al., 2014). In addition, aneuploid yeasts grown with the same proliferation rate as the diploid yeast strain still show transcriptional changes that are shared by most aneuploid yeast strains, but are not present in growth impaired mutant control strains, such as ribosomal biogenesis and nucleic acid metabolism associated genes (Torres et al., 2007). Thus, impaired proliferation might contribute to the transcriptional profile changes that arise in response to aneuploidy, but does not seem to be the major factor determining the aneuploidy response pattern.

Taken together, I hypothesize that the transcriptional response to aneuploidy is determined by the cumulative physiological consequences of aneuploidy (Figure 6). Most of the physiological consequences can be explained by the impact of proteotoxic stress, in particular compromised HSF1 and HSP90 function, on cellular processes. This shapes directly, via HSF1 transcriptional activity, or indirectly, via transcriptional feedback of the affected pathways, the conserved transcriptional response to aneuploidy.

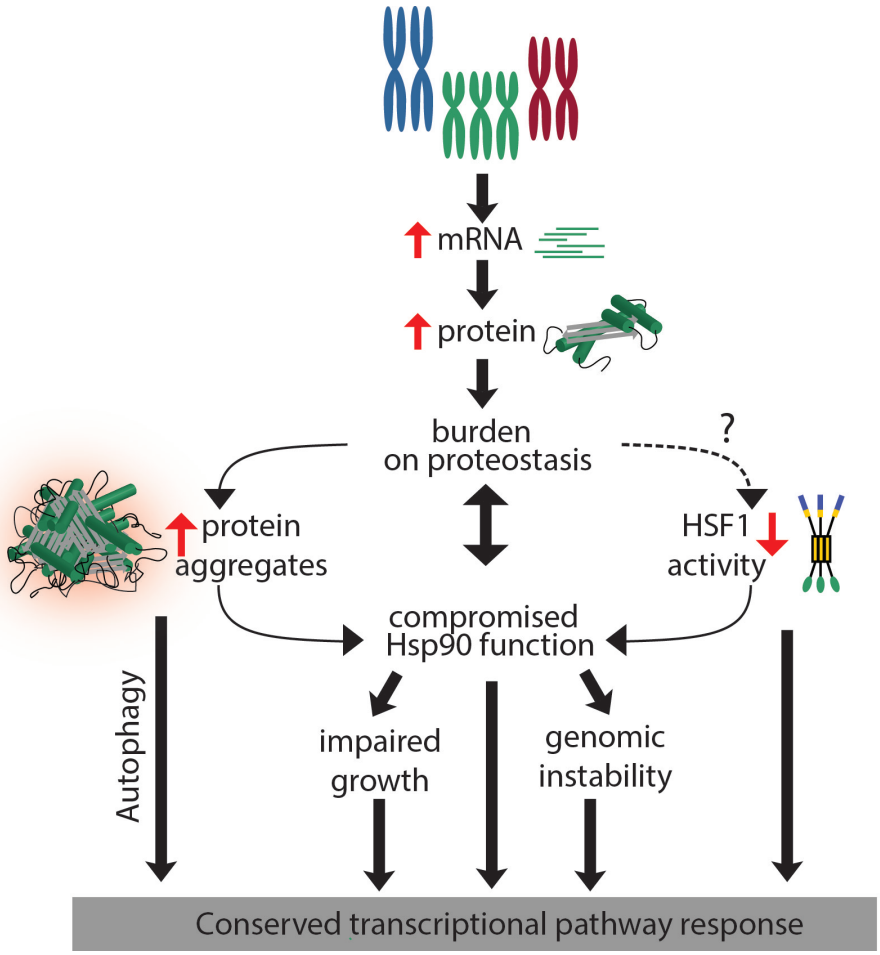


**Figure 6.** The physiological and conserved transcriptional response to aneuploidy is shaped by proteotoxic stress. Addition of chromosomes results in increased mRNA expression and protein levels. This additional unbalanced expression presents a burden for proteostasis that might be the cause of the observed protein deposits and increased autophagy. The activity of the transcription factor HSF1 is reduced by as yet unknown mechanisms. In turn, both protein aggregates and reduced HSF1 activity may lead to compromised HSP90 function. Diminished HSP90 function may impair growth of aneuploid cells and facilitate genomic instability. These altered cellular functions and the reduced activity of HSF1 transcription factor may feed back to transcription, resulting in conserved transcriptional changes.

## 7.2 Implications of the transcriptional response to aneuploidy in cancer

Since many cancer cells are aneuploid, the question arises whether a similar transcriptional profile as detected in model aneuploid cell lines can be identified in aneuploid cancer. In the transcriptional analysis of cancers, so called “gene signatures” are often employed that describe a group of genes for which the expression correlates with certain cancer characteristics (Chibon, 2013). To assess the implication of the transcriptional response to aneuploidy in cancer, we can evaluate the correlation of these signatures with aneuploidy and then compare the pathways for which the gene signatures are enriched with the deregulated pathways in aneuploid cells. Several transcriptional analyses of aneuploid cancers revealed gene expression signatures that correlate with aneuploidy and/or chromosomal instability (reviewed in (Dürrbaum and Storchova, 2015a)). For instance, the previously identified CIN70 signature contains 70 genes, for which high expression correlates with the overall ploidy across diverse tumor types (Carter et al., 2006). Moreover, a signature correlating with karyotype heterogeneity in the NCI-60 cancer cell line panel and high expression of the involved genes was predictive of poor prognosis (Sheltzer, 2013). These signatures are enriched for genes with a function in proliferation and cell cycle regulation, mitosis, replication and DNA damage repair and high expression of these genes predicts poor prognosis. However, genes involved in these processes are largely downregulated in the conserved transcriptional pathway response in aneuploid model systems (Dürrbaum and Storchova, 2015b; Dürrbaum et al., 2014; Sheltzer et al., 2012; Torres et al., 2007). Similarly, the mean expression levels of nine cancer subtypes anticorrelate with gene expression levels from trisomic mouse embryonic fibroblasts (Sheltzer, 2013). As a result, the pathways that are enriched among the upregulated genes in cancer are among the downregulated pathways in aneuploid cells. This includes the cell cycle, DNA repair, transcriptional and chromosome associated pathways, which were found in the conserved transcriptional response in aneuploid model cell lines. Among the pathways that are upregulated in aneuploid cells and downregulated in cancer are mainly membrane-associated pathways. Few pathways are still commonly upregulated, such as ER to Golgi transport vesicle, extracellular matrix binding and protease binding associated pathways (Sheltzer, 2013). Thus, the global gene expression deregulation in aneuploid model cell lines and cancer does not correlate. This is reflected in the inverse deregulation of pathways that were found in the conserved transcriptional response to aneuploidy.

How can we explain this anticorrelation of the transcriptional response? First of all, it reflects the generally opposite phenotypes of aneuploid model cell lines and aneuploid cancers, most prominently the proliferation defect observed in aneuploid model cell lines versus the unlimited proliferation of cancer cells. As discussed above, an imbalanced proteostasis network most likely causes the phenotypes of aneuploid cells. The question arises, whether this is different in aneuploid cancers. Tumorigenesis is associated with multiple stress phenotypes such as DNA damage stress, mitotic stress, metabolic stress, oxidative stress and also proteotoxic stress (Wondrak, 2014). A variety of exogenous and endogenous stimuli such as hypoxia, nutrient deprivation and mutation-driven misfolding of proteins but also aneuploidy result in accumulation of unfolded and misfolded proteins, which in turn challenges proteostasis (Clarke et al., 2012; Dai et al., 2012; Feldman,

2005). Yet, evidence described below suggests that in the course of tumorigenesis, the cancer cells evolve various mechanisms to counteract the stresses and to promote cell survival. The adaptation mechanisms to the various stresses experienced by cancer cells are multifold including mutations and copy number variations. Large-scale analysis of over 8,200 tumors demonstrated that somatic copy number alteration are non-random, but specifically selected for regions with known tumor suppressors or oncogenes (Davoli et al., 2013). Interestingly, the fourth most frequent amplification is the 8q chromosome arm on which HSF1 is encoded. Indeed, HSF1 is frequently upregulated in different human cancers such as in human colorectal cancer, breast cancer and endometrial carcinoma (Jiang et al., 2015; Mendillo et al., 2012). We show that additional copies of HSF1 mitigate the effects of proteotoxic stress in our aneuploid model cell lines (Donnelly and Storchova, 2015). Therefore, one possibility would be that copy number gain of HSF1 in cancer presents a way to counteract proteotoxic stress. Overcoming the proteotoxic stress and the secondary adaptive changes through higher levels of chaperons and their interactors would ultimately lead to a different transcriptional response than in aneuploid cells.

Another possibility how the adaptation to cellular stresses such as proteotoxic stress results in a different transcriptional program in cancer cell lines compared to aneuploidy model cell lines comes from genome-wide analysis. Analysis of the HSF1 occupancy and transcription activity revealed that the HSF1 transcriptional program in malignant cells differs from the heat shock program (Mendillo et al., 2012). Although it includes some heat shock proteins such as HSP90, multiple cellular processes are activated in addition such as translation, cell cycle, DNA repair and chromatin remodeling. Moreover, this HSF1 transcriptional response was conserved across a panel of cancer cell lines. These transcriptionally activated pathways were downregulated in response to aneuploidy. In the context of the cellular stresses cancer cells experience, gain of additional copies of HSF1 may result in a malignancy specific transcriptional response that fundamentally differs from the conserved response to aneuploidy.

In summary, the effects of aneuploidy *per se* in model cell lines result in an anticorrelated transcriptional response compared to aneuploid cancer cell lines. I hypothesize that this is the result of adaptive mechanisms in cancer cells that counteract the proteotoxic stress response. The resulting transcriptome changes differ from the transcriptional response in aneuploid model cell lines that suffer from proteotoxic stress.

## 7.3 microRNA regulation of the response to aneuploidy in human cells

Given the conserved transcriptional changes in response to aneuploidy and diversity of the affected pathways, we hypothesized that multiple different factors may affect the transcriptional consequences of aneuploidy. One recently discovered route of gene expression regulation is via miRNA post-transcriptional regulation of mRNA levels and translation. The deregulation of miRNAs is associated with cancer and altered cancer cell physiology (for example reviewed in (Chen et al., 2012b; Peng and Croce, 2016; Svoronos et al., 2016)). However, the relation between miRNA regulation and the response to aneuploidy *per se* has not been studied so far. In some cancers, the copy

number changes are a direct source of the miRNA deregulation (Calin et al., 2004; Ramsingh et al., 2013; Veigaard and Kjeldsen, 2014). We found that indeed an addition of one or more chromosomes alters the miRNA expression in human cell lines. Interestingly, only in a minority of aneuploid cell lines the extra chromosome directly affects the expression of miRNAs encoded on that chromosome. Thus, our results suggest that miRNA deregulation in aneuploid cells is a general response to the extra chromosomes.

How do the deregulated miRNA contribute or regulate the response to aneuploidy? Based on our analysis, the deregulated miRNAs particularly affect cellular development, growth and proliferation. We show that it is not a single miRNA, but the cumulative effect of the deregulated miRNAome that targets cellular proliferation. Similar synergistic effect of the miRNAome on target repression was demonstrated for example in gastric (Hashimoto et al., 2013) and lung cancer (Kumar et al., 2013). This may be explained by the fact that one miRNA targets multiple transcripts and one transcript is targeted by multiple miRNAs (Zhou et al., 2013). As discussed above, impaired proliferation is one of the cardinal features of the response to aneuploidy. Our target expression analysis of the deregulated miRNAs revealed, that majority of the targets with inverse expression to the corresponding miRNAs are downregulated. Many of these targets are involved in cell cycle regulation such as BUB1, CDC20, CDK4 and Cyclin E1. Our analysis provides the first indications that the deregulated miRNAome might contribute to the reduced proliferation of aneuploid cells. Importantly, the individual deregulated miRNAs and the individual affected targets are different between the aneuploid cell lines. Hence, although similar functions are affected by the deregulated miRNAs, different aneuploid cell lines achieve this by different means. This is remarkably similar to the findings in the global transcriptome analysis where the affected pathways are conserved but the individual genes causing or contributing to the pathway deregulation are different.

We found that the identity of the deregulated miRNAs in the aneuploid cell lines largely differs. One of the commonly deregulated miRNAs is hsa-miR-10a-5p that is upregulated in 5 out of 7 sequenced cell lines. Our data suggests, that the reason for hsa-miR-10a-5p overexpression in response to aneuploidy is due to a 3'UTR independent function of hsa-miR-10a-5p. hsa-miR-10a-5p binds downstream of the 5'TOP motif of ribosomal protein mRNAs (Ørom et al., 2008). The 5'TOP motif is characteristic for mRNAs of proteins involved in translation, mainly ribosomal proteins (Hamilton et al., 2006). Binding of hsa-miR-10a-5p enhances 5'TOP motif mRNA translation and alleviates starvation-induced relocation from active polysomes to inactive sub-polysomal fractions (Ørom et al., 2008). We found that overexpression of hsa-miR-10a-5p enhances 5'TOP motif mRNA translation in the analyzed aneuploid cell lines. Further, we demonstrated that the elevated expression of hsa-miR-10a-5p in aneuploid cells renders them less sensitive to translational downregulation of mRNAs with the 5'TOP motif. Currently, we do not understand the exact reason for the protection against starvation stress by hsa-miR-10a-5p overexpression. One hypothesis is that the extra chromosomes in aneuploid cell lines infer a higher requirement for the translation machinery, in particularly under stress conditions. Common upregulation of hsa-miR-10a-5p expression might be an adaptation mechanism of aneuploid cells. Further studies are required to test whether the overexpression of hsa-miR-10a-5p in cells with extra chromosomes indeed protects them from adverse growth conditions such as starvation or amino acid deprivation.

In summary, we show for the first time that the miRNAome is altered as a response to aneuploidy *per se*. The deregulated miRNAs may affect the response to aneuploidy in two different ways. First, the deregulated miRNAome seems to negatively affect proliferation, thus suggesting a contribution to the growth impairment of aneuploid cells. Second, the conserved upregulation of hsa-miR-10a-5p in aneuploid cell lines may present an adaptation to the aneuploid stress conditions and grants a robust translation under adverse conditions such as starvation. This would suggest upregulation of hsa-miR-10a-5p as a crucial mediator of survival of aneuploid cells. Future studies will reveal its potential in aneuploid cancer research.

## 7.4 Tolerance to genomic instability in post-tetraploid cells and its implications in cancer

Large-scale analysis of 4934 cancers revealed that approximately 37% of cancers underwent tetraploidization during tumorigenesis (Zack et al., 2013). This suggests that tetraploidization might be beneficial for tumorigenesis. Yet, induction of tetraploidy often results in cycle arrest, in majority of the cases by p53 activation (Andreassen et al., 2001; Castedo et al., 2006; Ganem et al., 2009; Kuffer et al., 2013; Lanni and Jacks, 1998). How cancer cells survive genome-doubling and how the survived cells contribute to the tumor development has not been fully understood. To elucidate the consequences of whole-genome doubling, we induced tetraploidization and followed the fate of surviving post-tetraploid cells (Kuznetsova et al., 2015). We found that tetraploid progenies are highly aneuploid and often show elevated rates of chromosome missegregations and abnormal mitosis. Moreover, these post-tetraploid cells are more tolerant to these errors, as they arrest less frequent than diploids or tetraploid cells immediately after whole-genome doubling. Also evolved naturally occurring tetraploids show high CIN (Dewhurst et al., 2014), suggesting that karyotype instability is a characteristic outcome of whole-genome doubling survival. My in-depth transcriptome analysis of the post-tetraploid cells revealed possible molecular mechanisms for the tolerance to mitotic errors. The expression of one fourth of p53 interactors is changed in chromosomally unstable post-tetraploid cell lines (Kuznetsova et al., 2015), among them commonly upregulated expression of the inhibitor of apoptosis-stimulating protein of p53 (iASPP), which is a negative regulator of p53. Moreover, nuclear accumulation of p53 is diminished in these cells. These results suggest that overriding the p53 response is the underlying mechanism of the tolerance to mitotic errors. This is supported by transcriptome analysis after acute tetraploidization by cytokinesis inhibition that revealed activation of the p53 pathway in retinal epithelial cell lines (Potapova et al., 2016). In addition, knock down of p53 or p21 resulted in increased proliferation. Taken together, cells that survived tetraploidization are unstable, probably due to a diminished p53 response. Tumors that have undergone a whole-genome doubling event exhibit a high genome instability score (Dewhurst et al., 2014). Since TP53 mutations and alterations of the p53 pathway are common in cancer, abrogation of the p53 response might present one way to allow karyotype instability also in tetraploid cancers.

Tetraploidy is associated with poor prognosis based on survival analysis of colorectal cancer patients (Dewhurst et al., 2014). Given that genome instability in cancer

correlates with tumor aggressiveness as well (Carter et al., 2006; reviewed in McGranahan et al., 2012), this suggests that the tolerance to genome instability in tetraploid cells confers an advantage for tumorigenesis. We found that chromosomal instable post-tetraploid cells show increased tolerance to a broad spectrum of chemotherapeutic drugs (Kuznetsova et al., 2015). Moreover, the transcriptome analysis revealed upregulation of pro-proliferative genes and downregulation of proapoptotic genes. These results suggest that post-tetraploid cells have a general tolerance to adverse stress conditions. One possibility is that higher rates and tolerance to karyotype instability endows higher adaptive potential to cellular stress as experienced by cancer cells. In the cancer context, surviving post-tetraploid cells might therefore benefit the adaptability of tumors. Thus, the elevated genome instability and the increased tolerance to stresses might bring major advantages to the cells in the changing environment during tumor development. Therefore, tetraploidization observed early in tumor development probably benefits tumor evolution and aggressiveness.

## 7.5 Big data in aneuploidy research – conclusions and perspective

The work presented in this thesis aimed to elucidate the effects of aneuploidy *per se* on the cell physiology in human aneuploid model cell lines with defined whole-chromosome aneuploidy or post-tetraploid cells with complex aneuploidy by applying transcriptome, miRNAome and proteome data analysis. This approach is unique as it allows global quantitative analysis of the response to aneuploidy within a cell line and large-scale comparison between different model systems.

What becomes evident from the transcriptome analysis of aneuploid cells is that the differential expression of individual genes is largely unique for different whole-chromosome and also complex aneuploid cells, but the affected pathways are similar (Dürrbaum et al., 2014; Kuznetsova et al., 2015; Potapova et al., 2016; Sheltzer et al., 2012). This suggests that while the ultimately affected pathways are the same and reflect common adaptation mechanisms, aneuploid cells undertake different evolutionary routes to adapt to the abnormal karyotype. In cancer, the adaptation to aneuploidy results in an inverse transcriptional response compared to aneuploid model systems (Sheltzer, 2013). The landscape of mutations and genomic aberrations is highly diverse between cancers and different combinations of tumor suppressor inactivations or oncogene activations may promote tumor development (Vogelstein et al., 2013). Yet, the ultimate resulting hallmarks of cancer such as unlimited proliferation, resistance to cell death, increased invasion ability and metastasis are common to all cancers. Thus, similar as in the response to aneuploidy, the adaptation routes of cancer cells are diverse, but the resulting affected pathways are the same. This suggests that in both cases, the conserved altered cellular functions are evolutionary favored and selected for.

The aneuploidy research has greatly benefited from the cancer sequencing projects. With the access to public databases of high-throughput genome, transcriptome and proteome data, it is nowadays possible to translate hypotheses drawn from studies in model cell lines to cancer by meta-analysis of cancer genome and transcriptome data. For instance, published transcriptome and corresponding patient outcome data allowed to test

whether significant alterations in chromosomal instability associated genes is of clinical relevance (Sheltzer, 2013). The net expression levels of these genes are predictive for cancer progression and patient outcome. In another study, published survival and SNP-array data from 539 patients was used to verify the association of whole-genome doubling events with poor prognosis (Dewhurst et al., 2014). Thus, whereas the "omics" perspective on aneuploid model cell lines helps us to understand the effects of aneuploidy *per se*, the rapidly growing amount of data in cancer research allows to test the relevance of the observed effects in model cell lines in tumorigenesis.

Besides the new possibilities through the technical advances of big data science, there are two major obstacles in big data driven aneuploidy research as presented in this thesis. First, the cellular heterogeneity within an aneuploid tumor population and also within aneuploid cell lines masks the differences in the aneuploidy response that may occur only in a subset of the cell population. Although single cell sequencing has advanced our understanding of the occurrence of aneuploidy during tumor evolution (Navin et al., 2011), most major cancer genome studies are based on high-throughput analysis of a whole tumor cell population. Moreover, the current state-of-the-art sequencing parameters do not allow to characterize an aneuploid or clonal heterogeneous tumor sample in-depth (Griffith et al., 2015). Single-cell analyses have given insights into the heterogeneity and stochasticity of gene expression in healthy tissue (reviewed in (Yu and Lin, 2016)). Given the additional genome changes and evolutionary adaptation in tumor cells, this complexity probably scales by orders of magnitude. Single cell sequencing of a wide range of tumors, but also of aneuploid model cell line systems, might reveal new aspects of the consequences of aneuploidy in terms of hidden population patterns in aneuploid subpopulations and the connectivity and interactions between the subpopulations. Yet, besides providing researchers new opportunities to understand aneuploidy, single cell sequencing poses additional challenges in respect to data complexity and variety to computational biologists. For example, the analysis of single cells is much more prone to technical artifacts and noise as the analysis of a whole population. In addition, having the information of multiple single cells of multiple tumors multiplies this problem. To account for this, new computational tools and approaches are being developed. Continued improvement of single cell analysis opens up new avenues for capturing the variation within a tumor and based on that a personalized cancer therapy.

A second limitation of the current large-scale aneuploid "omics" paradigms is that these reflect mostly a steady state picture of the aneuploid cell. This is exemplified by the conserved transcriptional response to aneuploidy in diverse model systems, which poses a challenge to dissect primary gene expression changes from secondary transcriptional feedback. Time-resolved "omics" analysis would therefore accelerate the comprehensive understanding of the evolutionary adaptation mechanisms. Since the evolutionary routes might be multifold, only a large-scale study would allow drawing conclusion on the favorable evolutionary paths of the adaptation to aneuploidy. Insights into the key processes important for the adaptation to an abnormal karyotype might provide novel therapeutic strategies to target aneuploidy in cancer.

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To the memory of my mother.

# 10 Curriculum vitae

Milena Dürrbaum

## Education

| | |
|---|---|
| 01/2013-02/2017 | **Max Planck Institute of Biochemistry, PhD student**, **International Max Planck Research School for Molecular and Cellular Life Sciences**, Martinsried<br>Maintenance of Genome Stability and Computational Biology<br>"A systems biology perspective on the consequences of aneuploidy in human cells " |
| 10/2010-11/2012 | **Technical University Munich, M. Sc., Biology**<br>Master Thesis, Max Planck Institute of Biochemistry, Maintenance of Genome Stability: "Physiological response of human cells to aneuploidy- transcriptome studies and cellular assays" |
| 09/2011-01/2012 | **Hong Kong University of Science and Technology**<br>Exchange semester and research internship "Lignocellulosic biomass as a new energy source- a novel cellobiase gene cba5 from Cellulomonas biazotea" |
| 10/2007-09/2010 | **Technical University Munich, B. Sc., Biology**<br>Bachelor Thesis, Chair of Chemistry of Biopolymers: "Sequence specific dynamics and secondary structure of the amyloid precursor protein transmembrane" |
| 07/1998-06/2007 | **Freie Evangelische Schule Lörrach**, Germany, Abitur |

## Awards and Fellowships

| | |
|---|---|
| 03/2013-04/2017 | **International Max Planck Research School for Molecular and Cellular Life Sciences Fellowship** |
| 2/2014 | **CIPSM Munich Center of Excellence** Center for Integrated Protein Science, Award for the best poster |
| 02/2013-02/2017 | **e-fellows online stipend** career and mentoring network |
| 09/2011-12/2011 | **DAAD PROMOS** Scholarship to support the exchange of German university students<br>**TUM Exchange** supported exchange program of Technical University Munich |

## Publications

| | |
|---|---|
| 2015 | Dürrbaum, M. and Storchova Z. Consequences of Aneuploidy in Cancer: Transcriptome and Beyond. *Springer Int. Publishing, Recent Results in Cancer Research pp. 195–224* |
| 2015 | Dürrbaum, M. and Storchova Z. Effects of aneuploidy on gene expression: implications for cancer. *FEBS Journal, 283(5), pp.791–802* |
| 2015 | Kuznetsova, A.Y., Seget, K., Moeller, G.K., de Pagter, M. S, de Roos, J.A.D.M., Dürrbaum, M., Kuffer, C., Müller, S., Zaman G.J.R., Kloosterman, W.P. and Storchova Z. *Cell Cycle, 14(17), pp.2810–2820* |
| 2014 | Donnelly, N., Passerini, V., Dürrbaum, M., Stingele, S., Storchova, Z. *The EMBO Journal, 33(20), pp.2374–2387* |
| 2014 | Dürrbaum, M., Kuznetsova, A.Y., Passerini, V., Stingele, S., Stoehr, G., Storchova, Z. Unique features of the transcriptional response to model aneuploidy in human cells. *BMC Genomics, 15(1), pp.1–14* |

| | |
|---|---|
| 2013 | Pester, O., Barret, P.J., Hornburg, D., Hornburg, P., Pröbstle, R., Widmaier, S., Kutzner, C., Dürrbaum, M., Kapurniotu A., Sanders, C.R., Scharnagl, C., Langosch, D. *Journal of the American Chemical Society, 135(4), pp.1317–1329* |

## Teaching and Supervision

| | |
|---|---|
| 10/2015-10/2016 | **Supervisor** of two master students |
| 23+24/09/2015 | **Instructor of a R data analysis and annotation/ visualization workshop** |
| 04/2015-08/2015 | **Supervisor** of two internship students |
| 04/2011 | **Lab practical supervisor** of 20 undergraduate students |
| 11/2010-02/2011 | **Lecturer microbiology seminar**; weekly lectures for 15 students |

## International Conferences and Training Events

| | |
|---|---|
| 14-16/03/2016 | **International Meeting of the German Society for Cell Biology**, poster |
| 06/2015-07/2015 | **MaxQuant Summer School**, proteomics data analysis conference |
| 03-07/03/2014 | **European Bioinformatics Institute Cambridge**, UK, next-generation sequencing data analysis training |
| 03-06/02/2014 | **CIPSM Munich Center of Excellence conference**, poster |

## Languages

**German** (native) • **English** (fluent, oral and written) • **Chinese** (conversational) • **French** (basic)