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The role of chromosomal instability in therapy response of colorectal cancer

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Hiermit erkläre ich, die Dissertation mit dem Titel " The role of chromosomal instability in therapy response of colorectal cancer " eigenständig angefertigt und keine anderen als die von mir angegebenen Quellen und Hilfsmittel verwendet zu haben.

Göttingen, den 19.06.2017 Xiyang Liu

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Abbreviations

℃	Degree celsius
5-FU	5-Fluoruracil
A-MTs	Astral microtubules
APC/C	Anaphase promotions complex or cyclosome
ATCC	American type culture collection
ATM	Ataxia-telangiectasia mutated
CFSs	Common fragile sites
ch-TOG/CAKP5	Cytoskeleton-associated protein 5
Chk2	Checkpoint kinase 2
CIN	Chromosome instability
CRC	Colorectal cancer
DME	Dimethylenastron
DMSO	Dimethysulphoxide
DNA	Deoxyribonucleic acid
DSB	Double-strand DNA break
EDTA	Ethylenediaminetetraacetic acid
EGFR	Epidermal growth factor receptor
ER	Estrogen receptor
et al.	Et alii
FACS	Fluorescence activated cell sorting
FCS	Fetal calf serum
G_0	Gap-phase 0
G_1	Gap-phase 1
G_2	Gap-phase 2
H_2O	Water
IP-MTs	Inter-polar microtubules
min	Minute
MIN/MSI	Microsatellite instability
Mps1	Monopolar spindle 1

MTs	Microtubules
nM	Nanomolar
ns	Not significant
NSLCL	Non-small-cell lung cancer
PI	Propidium iodide
RNA	Ribonucleic acid
RNase	Ribonuclease
RT	Room temperature
S	Synthesis
S-CIN	Segmental chromosome instability
SAC	Spindle assembly checkpoint
sem.	Standard error of the mean
TS	Thymidylate synthase
VEGF	Endothelial growth factor
W-CIN	Whole chromosome instability
µl	Microliter
µM	Micromolar

Abstract

Chromosomal instability (CIN) represents a major hallmark of cancer and is defined as the perpetual gain or loss of whole chromosomes during mitotic cell division. It is thought that CIN can drive tumor cell evolution by contributing to the generation of genetic heterogeneity in cancer. Importantly, tumor evolution might also fuel therapy resistance, a major problem for cancer patients in the clinic. However, whether CIN contributes directly to the generation of therapy resistance is unclear. So far it was not possible to systematically investigate the role of CIN and perpetual mitotic chromosome missegregation for the development of therapy resistance due to the fact that CIN could not be suppressed in chromosomally instable cancer cells. However, most recently, our lab has established means to correct an important molecular trigger for CIN in colorectal cancer (CRC) cells, namely increased microtubule dynamics during mitosis. In this way, it became possible to suppress chromosome missegregation and the evolvement of aneuploidy in otherwise chromosomally instable CRC cells. This now opens the possibility to investigate the role of CIN in therapy response, which was the aim of this study.

I used various cell systems to compare the therapy response towards commonly used chemotherapeutic drugs in isogenic CIN and non-CIN cells. These include chromosomally instable CRC cells, in which CIN was suppressed by treatment with low doses of Taxol or by partial suppression of the microtubule polymerase chTOG/CKAP5. In addition, CIN was induced in chromosomally stable CRC cells by Aphidicolin treatment mediated replication stress or by inhibition of the mitotic spindle assembly checkpoint kinase Mps1. CIN and non-CIN cells were treated with Oxaliplatin, Cisplatin, 5-FU, Adriamycin, and Irinotecan. I found an increased resistance towards Oxaliplatin only in CIN cells with *CHK2* deficiency. Other CIN cells (e.g. SW620, chromosomally stable HCT116 cells treated by Aphidicolin or MPS1-IN-3) did not show any response differences compared with non-CIN cells. These results might indicate the CIN phenotype *per se* does not confer drug resistance, but loss of *CHK2* function itself might contribute to the drug resistance.

1. Introduction

1.1. The eukaryotic cell cycle

The cell cycle is an accurate and ordered process, during which a parental cell replicates its genome and distributes the copies evenly onto two daughter cells. This process is divided into two stages: interphase and mitosis. Interphase comprises three distinct phases: gap-phase 1 (G_1) phase, synthesis (S) phase, and G_2 phase. Mitosis consists of five distinct phases: prophase, prometaphase, metaphase, anaphase and telophase (Vermeulen et al. 2003) (Figure 1-1).

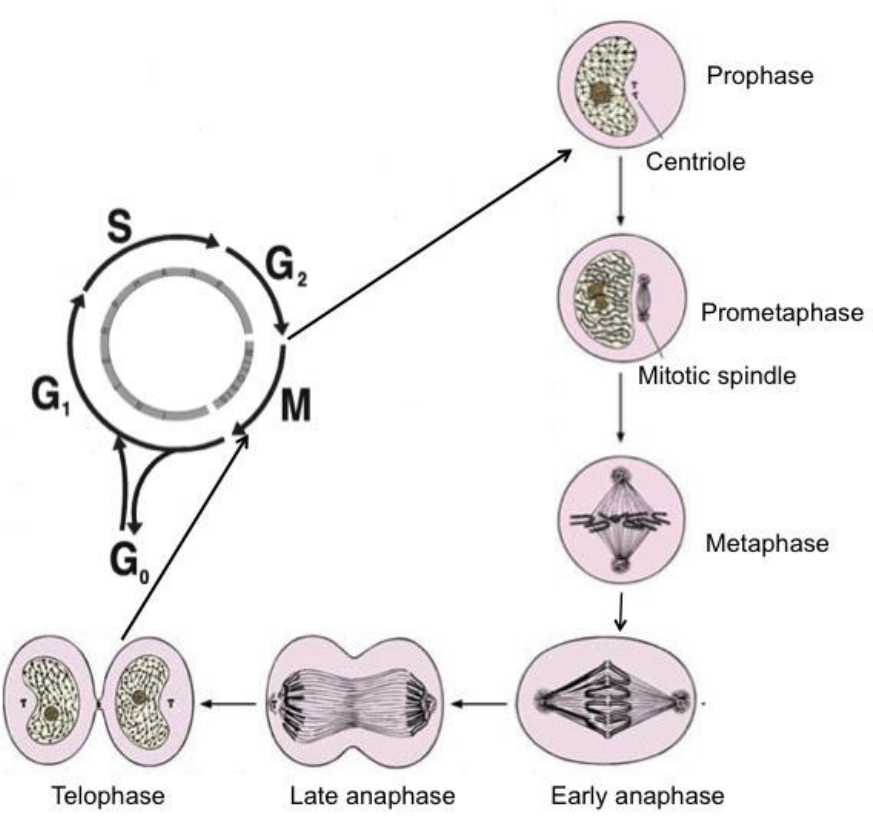

Figure 1-1 The eukaryotic cell cycle.

The eukaryotic cell cycle is divided into G_1-, S-, G_2, and M phase. M phase is further subdivided into prophase, prometaphase, metaphase, anaphase and telophase. Cells can exit the cell cycle and enter a resting state called G_0.（Figure is modified from Vermeulen et al., 2003, p. 132）

The G_1 and G_2 phases of the cell cycle are "gap phases" that occur between the two main events of the cell cycle, DNA synthesis and mitosis. In G_1 phase the cells prepare for DNA replication. When the extracellular environment lack of nutrient supply or during differentiation, the cells can exit the cell cycle to enter the resting G_0 phase. Upon certain proliferative stimuli, the cells enter G_1 and proceed into S phase, during which the DNA as well as the centrosomes are duplicated. Once the DNA replication is achieved, cells enter mitosis. During prophase, chromatin is condensed and chromosomes become visible. At this stage, each chromosome consists of two sister chromatids that are attached to each other at the centromere. The nuclear envelope breaks down, and the mitotic spindle is assembled during prometaphase. In addition, the kinetochores assemble at the centromeric region of the sister chromatids, thereby providing binding sites for the microtubules. Firstly, the chromosomes are randomly attached to microtubules in a process called "search and capture" (Heald et al. 1996). Then they are aligned on the metaphase plate during mitosis (Caspersson et al. 1968). During early anaphase, cohesion protein complexes that link the sister chromatid are cleaved by separase (Abrieu et al. 2001; Nakajima et al. 2007). Due to microtubule shortening, the sister chromatids are separated and move towards the spindle poles. The two spindle poles move apart during late anaphase (Saxton and McIntosh 1987). During telophase, two new daughter nuclei are formed, and during cytokinesis, the separation of the daughter cells is completed by cleavage of the cytoplasm (Terada et al. 1998).

1.2. Mitotic spindle assembly and the mitotic spindle assembly checkpoint

The mitotic spindle is a self-organized and dynamic macromolecular structure, which is composed of microtubules (MTs), MT-associated proteins and motor proteins. Mitotic spindle assembly is dependent on the highly regulated nucleation of MTs (Prosser and Pelletier 2017). Three broad categories of MTs exist within a mitotic spindle: kinetochore MTs (K-MTs), astral MTs (A-MTs) and inter-polar MTs (IP-MTs) (Dumont and Mitchison 2009). K-MTs attach the chromosome to spindle poles via the kinetochores. A-MTs radiate from the spindle poles and interact with the

cell cortex, which is crucial for spindle positioning (Khodjakov et al. 2000; McNally 2013). IP-MTs originate from opposite poles, which helps to separate the poles and stabilizes the spindle (Prosser and Pelletier 2017).

During chromosome alignment MTs act in a highly dynamic manner and constantly change from a growing to a shrinking state. This characteristic of MTs is known as "dynamic instability" (Mitchison and Kirschner 1984). A major regulator of MT growth is the MT polymerase ch-TOG/CKAP5, which resides at the growing plus tips of the MTs and act as a MT stabilizer (Al-Bassam and Chang 2011; Tournebize et al. 2000).

Before cells initiate sister chromatid segregation during anaphase, each chromosome has to be aligned at the metaphase plate. This is ensured by a signaling pathway known as the spindle assembly checkpoint (SAC) (Musacchio and Salmon 2007; Sacristan and Kops 2015). The SAC comprises several kinetochore-based proteins including Mad1, Mad2, Bub1, BubR1 and the Mps1 kinase (Foley and Kapoor 2013). The SAC is activated in response to unattached kinetochores that are present when chromosomes are not properly aligned at the metaphase plate. In turn, the SAC inhibits the ubiquitin ligase activity of the anaphase promotions complex or cyclosome (APC/C) and thereby prevents the degradation of its key substrates including cyclin B and securin (Foley and Kapoor 2013; Musacchio and Salmon 2007). Thus, activation of the SAC in the early phases of mitosis prevents premature sister chromatic separation and exit from mitosis.

1.3. Genome instability and tumor evolution

A major hallmark of human cancer is the presence of profound alterations of genome. Different forms of genome instability can be distinguished:

1. Accumulations of point mutations, which are due to defects in mismatch or nucleotide excision repair (Wood 1997). A particular form of this phenotype in colorectal cancer (CRC) is known as microsatellite instability (MIN/MSI), where defects in mismatch repair genes account for instabilities of microsatellite sequences (Lengauer et al. 1997).
2. Accumulation of segmental or structural chromosome aberrations, which can be due to DNA repair or replication defects (Feuk et al. 2006). This form of

instability is termed as segmental or structural chromosome instability (S-CIN) (Geigl et al. 2008).

3. Accumulation of numerical aneuploidy, which arises upon whole chromosome missegregation during mitosis. This form of instability is called whole chromosome instability (W-CIN) (Geigl et al. 2008). In fact, chromosomally stable cells show less than 1% of cell division missegregation events, whereas in W-CIN cells this rate is increased to 20%-50% (Lengauer et al. 1997; Thompson and Compton 2008).

1.3.1. Causes of W-CIN

Different causes responsible for the induction of W-CIN have been discussed (Bastians 2015; Thompson et al. 2010). For instance, malfunction of the spindle assembly checkpoint (Musacchio and Salmon 2007), the presence of supernumerary centrosomes (Pihan et al. 1998) or defects in the chromatid cohesion (Barber et al. 2008) can account for the mitotic chromosome missegregation and W-CIN. A recent study revealed that DNA replication stress can also cause W-CIN (Burrell et al. 2013). In fact, while induction of replication stress induced aneuploidy, alleviating replication stress in cancer cells reduced the frequency of chromosome missegregation (Burrell et al. 2013).

A major source for W-CIN appears to be the generation of erroneous microtubule-kinetochore interactions (Foley and Kapoor 2013). Three types of erroneous attachments can occur (1) Monotelic attachments, where only one of the sister kinetochores attaches to microtubules. (2) Syntelic attachments, where both sister kinetochores attach to the microtubules from the same spindle pole. (3) Merotelic attachments, where one kinetochore attaches to microtubules from both spindle poles (Figure 1-2) (Tanaka and Hirota 2009, 2016). Monotelic and syntelic attachments can directly or indirectly be sensed by the SAC, which detects the lack of microtubule attachment to the kinetochores or the lack of proper tension generated between the two kinetochores (Tanaka and Hirota 2009). The SAC delays anaphase onset until the errors are corrected. However, merotelic attachments, cannot be sensed by the SAC and thus, can lead to missegregation of chromosomes (Gregan et al. 2011). It is thought that merotelic attachments are a leading cause for W-CIN and are

associated with the generation of lagging chromosomes during anaphase (Figure 1-2b) (Gregan et al. 2011; Nicholson and Cimini 2013).

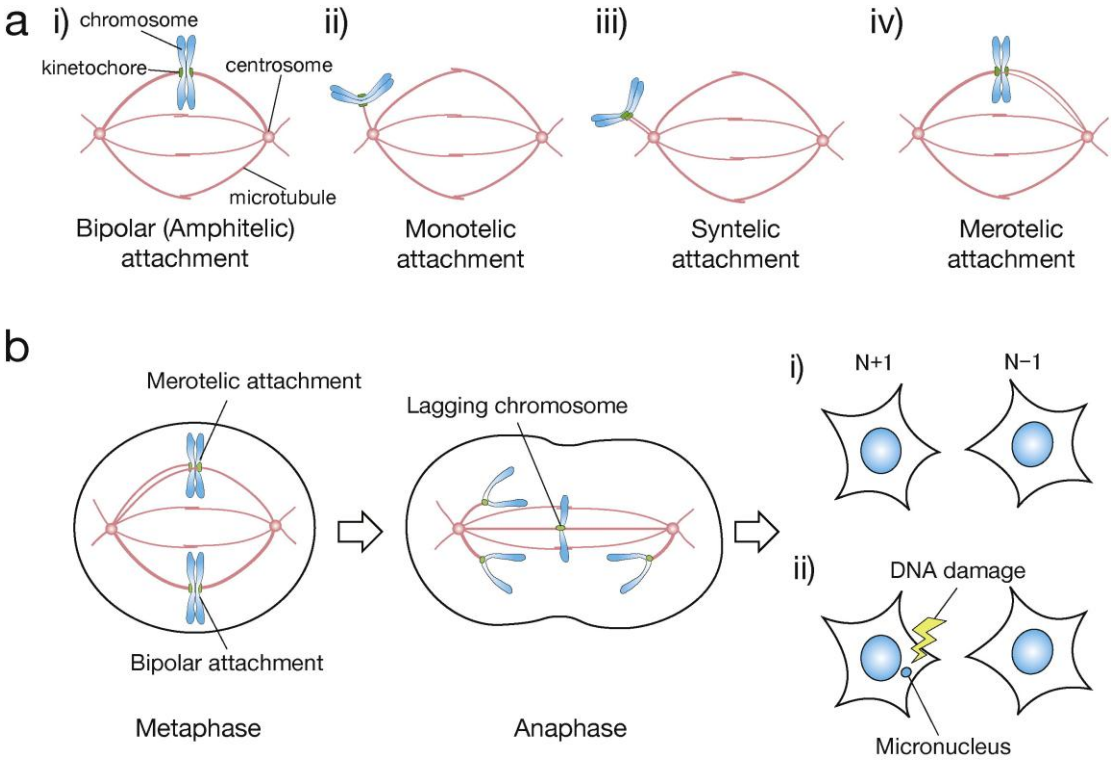

Figure 1-2 Classification of kinetochore-microtubule attachments

(**a**) Four types of kinetochore-microtubule attachments are described. (**i**) Bipolar (amphitelic) attachments describe kinetochores attached to microtubules emanating from opposite spindle poles. (**ii**) Monotelic attachments involve kinetochores attached to the microtubules emanating from the same pole. (**iii**) Syntelic attachments are defined as kinetochores attached to the microtubules emanating from the same spindle pole. (**iv**) Merotelic attachments are conditions where a single kinetochore is attached to microtubules from both spindle poles. (**b**) Merotelic attachments can lead to lagging chromosomes during anaphase. (**i**) The lagging chromosomes can segregate to the wrong side resulting in whole chromosome aneuploidy. (**ii**) Lagging chromosomes can be entrapped in micronuclei and maybe subsequently damaged by the recapture of micronuclei, which can lead to structural aneuploidy. (Figure is modified from Tanaka and Hirota, 2016, p. 67)

Interestingly, the presence of supernumerary centrosomes can lead to the formation of transient multipolar spindle intermediates that support the formation of merotelic kinetochore attachments and thereby, causes chromosome missegregations and W-CIN.

Recently, our lab established that increased microtubule plus-end assembly rates within mitotic spindles can contribute to CIN. By analyzing the plus-end assembly rates during mitosis in MIN/MSI and CIN colorectal cancer (CRC) cell lines, it was revealed that elevated MT growth rates are present only in CIN cells. Moreover, restoration of proper microtubule assembly rates in CIN cells by chemical suppression of microtubule dynamics (by treatment with low-doses of Taxol) or by partial repression of the microtubule polymerase *ch-TOG/CKAP5* was sufficient to suppress W-CIN. *Vice versa*, by genetic means (e.g. by overexpression of *Aurora-A* or by the loss of *CHK2*) or by treatment with low-doses of nocodazole, microtubule plus-end assembly rates were increased, thereby inducing the generation of lagging chromosomes, chromosome missegregation and CIN. These findings revealed that abnormally increased microtubule growth rates during mitosis can act as a trigger for the induction of CIN. Importantly, by the use of Taxol treatment or by repression of *ch-TOG/CKAP5* CIN can be efficiently suppressed in otherwise chromosomally instable cancer cells (Ertych et al. 2014).

1.3.2. Consequences of CIN

CIN is a key characteristic of almost all solid cancers (Ohyashiki et al. 2017). It is thought that CIN can drive tumor cell evolution by altering gene expression that may facilitate rapid cell adaptation (McGranahan et al. 2012). In this way, CIN can contribute to genetic heterogeneity in cancer and thereby might drive therapy resistance, a major problem in the clinic (Chen et al. 2012; Gerlinger et al. 2014; Turner and Reis-Filho 2012). The relationship between cancer prognosis and CIN has been explored across multiple types of cancers.

In non-small-cell lung cancer (NSLCL), several independent studies found that CIN was a poor prognosis factor. It was independent of conventional risk factors such as sex, age, and tumor stage in variate analysis (Choi et al. 2009; Nakamura et al. 2003; Yoo et al. 2010). In breast cancers, however, CIN is a prognostic factor that is

dependent on the estrogen receptor (ER) state. In ER-positive breast cancers, CIN is associated with tumor progression and unfavorable prognosis (Smid et al. 2011). While in ER-negative breast cancer, CIN is associated with an improved prognosis (Birkbak et al. 2011; Roylance et al. 2011). In colorectal cancer (CRC) CIN correlated with a worse prognosis regarding overall survival or progress-free survival compared to the non-CIN CRC (Walther et al. 2008). Studies in other cancers such as oral, ovarian, synovial, and endometrial cancers, together with diffuse large B-cell lymphomas have also demonstrated that CIN is associated with poor prognosis (Bakhoum et al. 2011; Murayama-Hosokawa et al. 2010; Nakagawa et al. 2006; Sato et al. 2010). Hence, these studies have established that CIN is an important prognosis factor in a wide range of cancer types.

1.3.3. Cancer genome evolution

The basic evolution principles of Darwin evolution that the differential fitness variation is heritable can be applied in the context of cancer evolution (Nowell 1976). When a tumor is detected, it has undergone many generations of cell division, and cells have stochastically acquired somatic mutations in each generation (Gerlinger et al. 2014; Rosenthal et al. 2017). Among these mutations, a small subset of mutations called driver events may show evolutionary advantage that allows the cells to outcompete others (Rosenthal et al. 2017). Also, the clonal selection and evolution of the cells may lead to outgrowth of subclones that lead to branched evolutionary phylogenies (Gerlinger et al. 2012; Gundem et al. 2015).

The majority of established driver events are clonal, which indicates that the clonal driver events may occur in the early stage of tumor evolution (McGranahan et al. 2015; McGranahan and Swanton 2015). Whereas subclonal driver events that have been identified across numerous cancers are believed to play a role in tumor maintenance and progression (Yates et al. 2015). Subclonal populations of cancer cells induce a heterogeneous environment within the tumor. However, each subclone is not an isolated entity (Rosenthal et al. 2017). Studies have also revealed that genetically distinct subclones interact with each other to some extent during tumor evolution and this interaction may be competitive or cooperative (Marusyk et al. 2014). For vital resources such as nutrients, oxygen, or space, one subclone may

outcompete another (Marusyk and Polyak 2010). To many aspects, clonal cooperation contributes to tumor growth and progression (Neelakantan et al. 2015; Polyak and Marusyk 2014). For example, through endocrine signaling networks, low-frequency clones may promote resistance to treatment or support the growth of dominant clones (Acharyya et al. 2012; Chung et al. 2013; Hobor et al. 2014). Clonal interference can also result in tumor collapse. When a subclone that outcompetes the subclone that drives tumor growth is dependent on the current microenvironment, a change in the tumor environment may lead to tumor collapse (Marusyk et al. 2014).

In sum, tumors represent a complex dynamic ecological system, in which heterogeneity can promote tumor development and progression but not only a substrate of evolution.

1.3.4. CIN and responses to anti-cancer treatment

Assuming that all cancer cells are sensitive to a given treatment to the same extent, any therapy that kills the cancer cells faster than they divide would eventually result in a cure of the disease. Unfortunately, the tumor heterogeneity prevents this from happening in most cases. Drug resistance is a severe problem for cancer treatment. Numerous examples reveal that tumor heterogeneity can drive drug resistance. Epidermal growth factor receptor (EGFR) gene heterogeneity within non-small-cell lung cancers results in a reduced gefitinib response (Taniguchi et al. 2008). In CRCs, the clonal evolution drives anti-EGFR treatment resistance (Siravegna et al. 2015). To assess the association between CIN and drug response in CRC, Lee and colleagues treated CIN and non-CIN cell lines of CRC individually with a library of kinase inhibitors. They found that CIN cell lines showed significantly increased multidrug resistance compared to non-CIN cells, which was independent of somatic mutation status. Tetraploid isogenic cells showed generally higher resistance compared to their diploid counterparts (Lee et al. 2011).

Taken together, these studies indicate that tumor heterogeneity is related with drug resistance and poor clinical outcome in diversity cancers.

1.4. Colorectal cancer

Colorectal cancer (CRC) is a premier model for studying CIN. It is the third most diagnosed cancer in worldwide (Rao et al. 2016). MIN/MSI and CIN are two main distinct patterns of genomic instability in CRC (Lengauer et al. 1998). 80%-90% of CRC cases are characterized by CIN and this is thought to contribute to progression and recurrence of the disease (Rao et al. 2016). Due to its high incidence, the investigation of CIN in CRCs has a high clinical relevance. CRCs often show nonmucinous histology, moderate differentiation and less tumor-infiltrating lymphocytes (Silver et al. 2012; Sinicrope et al. 2006). Most CRCs exhibit *APC*, *KRAS*, and *TP53* mutation and these genetic alterations are associated with CIN (Rowan et al. 2005). Moreover, CIN is clearly associated with bad prognosis and reduced the patient survival in CRC (Watanabe et al. 2012).

The treatment of CRCs is based on number, size, localization and progression of the tumor, presence or absence of biochemical markers and the health condition of the patients. The treatment strategies for CRCs include surgical resection, chemotherapy, and monoclonal antibodies against EGFR and vascular endothelial growth factor (VEGF) in *KRAS* wild-type tumors combined with traditional chemotherapy. Most patients with metastatic CRC disease are treated with chemotherapy. The first line of chemotherapy with fluoropyrimidines (5-FU or Capecitabine) combined with Leucovorin, and other cytotoxic agents, such as Irinotecan (5-FU/LV/Irinotecan (FOFIRI)), or Oxaliplatin (5-FU/Leucovorin/oxaliplatin (FOLFOX)) and (Capecitabine/Leucovorin/Oxaliplatin (CAPOX)). Leucovorin can increase the reaction rate of fluoropyrimidines. When tumors relapse, and the patients have good tolerance a second line chemotherapy is frequently used. When patients are refractory to Irinotecan, the Oxaliplatin-containing regimens FOLFOX or CAPOX can be used. When refractory to Oxaliplatin, Irinotecan monotherapy or FOLFIRI can be utilized (Benson et al. 2017).

1.5. Scope of the study

This thesis project aimed to address whether ongoing chromosome missegregation constituting a W-CIN phenotype affects therapy response in CRC cells *in vitro*. For this, I used chromosomally instable CRC cells, in which the W-CIN phenotype and

chromosome missegregation could be specifically suppressed. On the other hand, I also induced the W-CIN phenotype in chromosomally stable CRC cells. CRC cells with or without W-CIN were treated with clinical relevant chemotherapeutic drugs including 5-FU, Oxaliplatin, Irinotecan, Cisplatin, and Adriamycin. The drug responses were assessed by colony formation assays and FACS analysis. The goal of the project was to investigate whether W-CIN, perpetual chromosome missegregation or aneuploidy affects therapy responses.

2. Materials and Methods

2.1. Materials

All the working materials including cell culture dishes, pipette tips and reaction tubes were purchased from Sarstedt (Nümbrecht, Germany), Eppendorf (Hamburg, Germany).

2.1.1. Equipment

Equipment used to assist this study is listed in Table 2-1.

Table 2-1 Equipment

Equipment	**Model**	**Company**
CO_2 Incubator	HERAcell 240 CO_2 Incubator	Thermo Fisher Scientific, Karlsruhe, Germany
Cooling Centrifuge	Multifuge X3R	Thermo Fisher Scientific, Karlsruhe, Germany
Electrophoresis Power Supply	Power supply EV231	Peqlab, Erlangen, Germany
Flow Cytometer	BD FACSCanto© II	Becton Dickinson, San Jose, CA, USA
Electroporation Device	GenePulser Xcell©	BioRad Laboratories, München, Germany
Magnetic Mixer	IKAMAG© RCT	IKA Laboratories, München, Germany
Microscope	Zeiss Axio Imager Z1	Zeiss, Göttingen, Germany
Microscope Camera Adaptor	A3474-07	Hamamatsu Photonics, Hamamatsu, Japan
Pipettes	Pipetman©	Gilson International, LimburgOffheim, Germany
Pipettor	Pipetboy acu	Intergra Biosciences, Fernwald, Germany

Equipment	Model	Company
Sterile Workbench	HERAsafeM	Thermo Fisher Scientific, Karlsruhe, Germany
Tabletop Centrifuge	Biofuge pico	Thermo Fisher Scientific, Karlsruhe
Tabletop Centrifuge cooling	Biofuge fresco	Thermo Fisher Scientific, Karlsruhe
Vortex Mixer	VORTEX-GENIE©2	Scientific Industries Inc., Bohemia, NY, USA
Quato IntelliScan	Quato IntelliScan 1600	Quatographic Technology, Schleswig - Holstein, Germany

2.1.2. Software

The software used in the study is listed in Table 2-2

Table 2-2 Software

Software	Company
Hokawo Laucher	Hamamatsu Photonics, Hamamatsu, Japan
ImageJ	NIH Image, Bethesda, MD, USA
BD FACSDivaTM	Becton Dickinson, San Jose, CA, USA
Prism 6	Graphpad, La Jolla, CA, USA

2.1.3. Chemotherapeutic drugs and inhibitors

All chemotherapeutic drugs and inhibitors used in this study were purchased from Sigma-Aldrich (Taufkirchen, Germany), VWR international (West Chester, PA, USA), Th. Geyer (Höxter, Germany), Santa Cruz (Dallas, TX, USA), Calbiochem (La Jolla, CA, USA).

Chemotherapeutic drugs and inhibitors used in this study and their respective working concentrations and effects are listed in Table 2-3.

Table 2-3 Chemicals

Chemical	Used concentration	Effect	Dissolvent	Stock concentration	Company
Adriamycin (Myers et al. 1977)	5, 10, 15, 20, 30, 50, 400, 600 nM	Intercalates into DNA and stabilizes topoisomerase II	H_2O	500 µM	Th. Geyer, Höxter, Germany
Oxaliplatin (Arango et al. 2004)	0.1, 0.2, 0.4, 0.8, 1, 4, 8 µM	Forms DNA adducts, disrupts DNA replication and transcription	DMSO	50 mM	VWR International, West Chester, PA, USA
Cisplatin (Siddik 2003)	0.2, 04, 0.8, 1.6, 2 µM	Forms DNA adducts, disrupts DNA replication and transcription	PBS	5 mM	Santa Cruz, Dallas, TX, USA
5-FU (Longley et al. 2003)	0.2, 0.5, 1, 2, 4, 5, 10 µM	Inhibition thymidylate synthase (TS) and incorporation its metabolites into RNA and DNA	DMSO	50 mM	Sigma-Aldrich Taufkirchen, Germany

Chemical	Used concentration	Effect	Dissolvent	Stock concentration	Company
Irinotecan (Hurwitz et al. 2004)	0.05, 0.1, 0.2, 0.4, 0.6 µM	Inhibition topoisomerase I	DMSO	30 mM	VWR International, West Chester, PA, USA
Dimethylenastron (DME) (Gartner et al. 2005)	2 µM	Inhibition EG5-kinesin	DMSO	10 mM	Calbiochem, La Jolla, CA, USA
Puromycin (Nathans 1964)	1µg/ml	Inhibition of translation	DMSO	1 mg/ml	Sigma-Aldrich Taufkirchen, Germany
Taxol (Schiff et al. 1979)	0.5 nM	Stabilization of microtubules	DMSO	5 µM	Sigma-Aldrich Taufkirchen, Germany
Aphidicolin (Ikegami et al. 1978)	1, 2 µg/ml	Inhibition DNA polymerase δ	DMSO	10 mg/ml	Santa Cruz, Dallas, TX, USA
MPS1-IN-3 (Tannous et al. 2013)	0.5 µM	Inhibition MPS1	DMSO	10 mM	Sigma-Aldrich Taufkirchen, Germany

2.1.4. Human cell lines

Human cell lines used for this study are listed in Table 2-4. The table also provides information about the culture medium, the selection antibiotics, origin and their reference.

Table 2-4 Human cell lines

Cell Line	Medium	Selection	Origin	Reference
HCT116	RPMI 1640	None	Colon carcinoma	(Brattain et al. 1981)
SW620	RPMI 1640	None	Colon carcinoma	(Leibovitz et al. 1976)
HCT116-*CHK2*$^{-/-}$	RPMI 1640	G418	Colon carcinoma	(Jallepalli et al. 2003)
HCT116-*CHK2*$^{-/-}$-sh*ch-TOG*	RPMI 1640	Puromycin	Colon carcinoma	(Ertych et al. 2014)
SW620-sh*ch-TOG*	RPMI 1640	Puromycin	Colon carcinoma	(Ertych et al. 2014)

Colorectal cancer HCT116 and SW620 cell lines were purchased from the American Type Culture Collection (ATCC; Manassas, VA, USA). HCT116-*CHK2*$^{-/-}$ cells were kindly provided by Prof. Bert Vogelstein (John Hopkins University, Baltimore, Maryland, USA).

2.2. Methods

2.2.1. Cultivation of human cell lines

Human cell lines were cultured in RPMI 1640 medium (Sigma-Aldrich, Taufkirchen, Germany) supplemented with 10% (v/v) Fetal Calf Serum (FCS) (GE Healthcare, Chalfont St. Giles, Great Britain), 100 µg/ml streptomycin and 100 U/ml penicillin (Sigma-Aldrich, Taufkirchen, Germany) at 37 °C with 5% CO_2 in a humidified incubator. Cells were splitted every 2-3 days to enable enough space and sufficient nutrient supply for proliferation. To do this, cells were washed with 10 ml PBS once and detached by 1 ml trypsin-EDTA (Sigma-Aldrich, Taufkirchen, Germany) treatment. A defined dilution of cell suspension was transferred in to a new cell culture dish containing RPMI 1640 medium. Stable cell lines expressing shRNA that

targeting *ch-TOG/CKAP5* and the control scrambled shRNAs were growing in the presence of 1 µg/ml puromycin. HCT116-*CHK2*$^{-/-}$ cells were growing in the presence of 300 µg/ml G418.

Cells were stored in liquid nitrogen for long time storage. For this, cells were harvested and centrifuged at 1,000 rpm, then resuspended in 400 µl freezing medium, which containing 70%(v/v) RPMI 1640, 20%(v/v) FCS and 10% (v/v) DMSO. The cell suspensions were slowly cooling down to -80 ℃ by using a cryo freezing box filled with 2-propanol (VWR international, West Chester, PA, USA) and transferred to the vapor phase of liquid nitrogen after 24 h.

2.2.2. Generation of single cell clones in the presence of Aphidicolin or MPS1-IN-3.

1,000 HCT116 cells were seeded into 10 cm cell culture dish 24 h before the treatment. The cells were treated with RPMI 1640 medium containing 1ng/ml or 2 ng/ml Aphidicolin or 0.5 µM MPS1-IN-3 or DMSO. RPMI 1640 medium containing drug was changed every 2-3 days. After 7 days small colonies were transferred into a 24-well plate, and further cultivated in the presence of the drug. After growing for 30 generations, single cell clones were analyzed.

2.2.3. Karyotype analysis by metaphase chromosome counting

Cells were treated with 2 uM DME for 4h to arrest in mitosis. Afterwards the cells were harvested in 15 ml Falcon tube and centrifuged at 1,000 rpm. To swell the cells, the cell pellets were resuspended in 2 ml hypotonic solution (40%(v/v) RPMI 1640 medium in aqua dest) and incubated at room temperature (RT) for 20 min. Subsequently, the swelled cells were fixed with 1 ml ice-cold Carnoy's fixdative (75%(v/v) methanol, 25%(v/v) glacial acetic acid) and centrifuged at 2,000 rpm for 5 min. The fixation step was repeated 3 times. Then the cells were directly processed or stored at -20 ℃ until further usage. Cells were resuspended in 100 - 500 µl 100% glacial acetic acid and dropped onto a pre-cooled, wet objective slide from a vertical height of about 30 cm. The objective slides with cell drops were incubated at 42 ℃ in a wet chamber for 5 min. Afterwards the slides were dried at RT. After the objective

slides had been thoroughly dried, they were stained with 8% (v/v) Giemsa staining solution (Merck, Darmstadt, Germany) for 25 min. Objective slides were then washed and dried at RT. After thoroughly dried, the objective slides were embedded into Euparal (Carl Roth, Karlsruhe, Germany). The stained objective slides were analyzed by a Zeiss Axioscope FS microscope (Zeiss, Oberkochen, Germany) equipped with a Hamamatsu C4742-95 camera and the Hokawo Launcher 2.1 software (Hamamatsu Photonics, Hamatsu, Japan).

2.2.4. Flow cytometry

A BD FACS Canto II (Becton Dickinson, San Jose, CA, USA) was used to perform Fluorescence Activated Cell Sorting (FACS) analysis. BD FACS Diva™ (Becton Dickinson, San Jose, CA, USA) software was used to analyze the data. Cells were harvested in 15 ml Falcon tube and centrifuged 5 min at 1,000 rpm. Afterwards the cells were resuspended in 200 µl PBS. After fixing in 1 ml 70% (v/v) ice-cold ethanol, which was added dropwise while the suspension was continuously vortexing, the cells were subsequently stored at 4 ℃ overnight. DNA intercalating dye propidium iodide (PI) (Sigma-Aldrich, Taufkirchen, Germany) was used to determine the DNA content. Fixed cells were centrifuged at 2,000 rpm for 5 min, the cell pellet was washed with 1 ml PBS once, then resuspended in 100 ul 1µg/ml DNA free RNase A (Applichem, Darmstadt, Germany) and incubated at RT for 15 min. After adding 10 µl 50 µg/ml PI the cells were analyzed.

2.2.5. Colony formation assay

3,000 cells were seeded in 6-well plate 24 h before the treatment. The cells were treated with different concentrations of Oxaliplatin (VWR International, West Chester, PA, USA), Adriamycin (Th. Geyer, Höxter, Germany), 5-FU (Sigma-Aldrich Taufkirchen, Germany), Irinotecan (VWR International, West Chester, PA, USA), Cisplatin (Santa Cruz, Dallas, TX, USA). Cells were incubated at 37 ℃ and 5% CO_2 for 10 days. The RPMI1640 medium containing the drugs was changed every 2-3 days. The growing colonies were fixed with 70% (v/v) ethanol and stained with crystal violet solution (0,1%(w/v) Crystal violet, 20% (v/v) ethanol in H_2O). The growth area was scanned using a Quato IntelliScan 1600 (Quatographic Technology,

Schleswig - Holstein, Germany) and colony area was determined by using ColonyArea plugin of ImageJ (NIH Image, Bethesda, MD, USA) (Guzman et al. 2014).

2.2.6. Statistical analysis

All data are shown as mean ± standard error of the mean (sem.). One tailed Student's *t*-test was performed by Prism software package, version 6 (Graphpad, La Jolla, CA, USA).

3. Results

3.1. Responses towards platinum drugs in cells exhibiting CIN and after suppression of CIN.

3.1.1. Suppression of CIN in HCT116-*CHK2*$^{-/-}$ but not in SW620 by treatment with low-dose of Taxol results in increased sensitivity towards Oxaliplatin treatment.

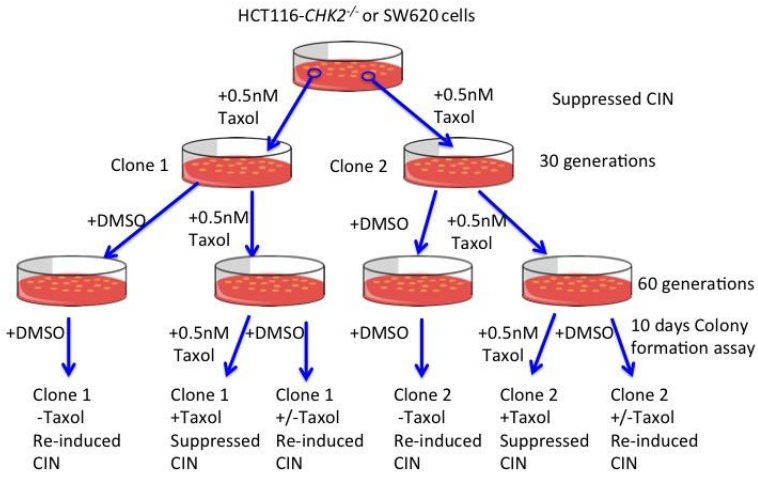

Figure 3-1 Schematic overview of single cell clones in the presence or absence of low-dose of Taxol.

Two independent single cell clones derived from HCT116-*CHK2*$^{-/-}$ or SW620 were treated with 0.5 nM Taxol for 30 generations. These cell clones were cultured either in the presence of 0.5 nM Taxol (+Taxol) or absence of Taxol (-Taxol) for another 30 generations. After 60 generations, these cell clones were treated with different chemotherapeutic drugs for 10 days for colony formation assay. The cells continuously cultured with 0.5 nM Taxol were also treated with different drugs in the absence of 0.5 nM Taxol during the assay of 10 days (+/-Taxol).

Treatment with low-dose of Taxol restores proper mitotic microtubule assembly rates and suppresses CIN in chromosomally unstable colorectal cancer cells (Ertych et al. 2014). I investigated whether suppression of CIN impacted on drug response towards chemotherapeutic drugs. For this, I used two independent single cell clones of HCT116-*CHK2*$^{-/-}$ and SW620 cells, both of which exhibited increased microtubule plus-end assembly rates and CIN, which were treated with Taxol for 30 generations thereby suppressing CIN. Single cell clones generated in the presence of Taxol were used for the subsequent colony formation assays. Each cell clone was either treated with Taxol for another 30 generations (suppressed CIN) or left untreated for another 30 generations (re-induced CIN). I treated these cell clones with increasing concentrations of Oxaliplatin in the presence of Taxol (suppressed CIN) or in the absence of Taxol (CIN) (Figure 3-1).

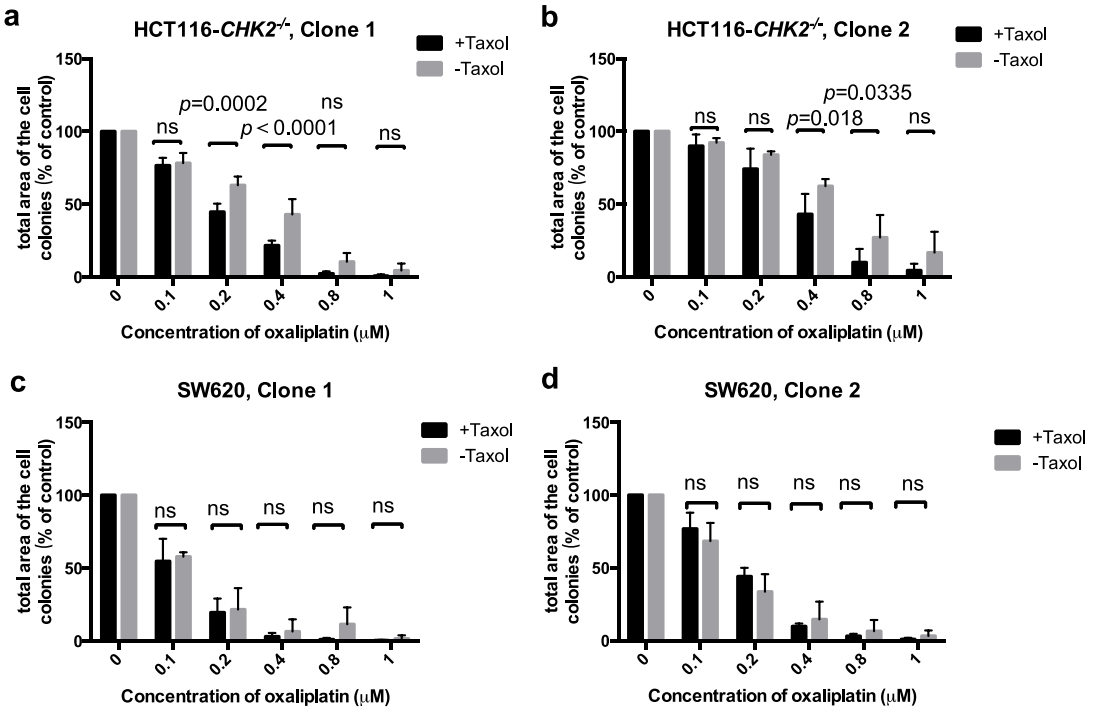

Figure 3-2 Quantification of the area of the cell colonies of HCT116-*CHK2*$^{-/-}$ and SW620 cells treated with different concentration of Oxaliplatin in the presence or absence of low-dose of Taxol.

(a) Cells of HCT116-*CHK2*$^{-/-}$ cell clone 1 were treated with increasing concentrations of Oxaliplatin in the presence or absence of 0.5 nM Taxol and the area of the cell

colonies was determined after ten days. (**b**) Cells of HCT116-*CHK2*$^{-/-}$ cell clone 2 were treated with increasing concentrations of Oxaliplatin in the presence or absence of 0.5 nM Taxol and the area of the cell colonies was determined after ten days. (**c**) Cells of SW620 cell clone 1 were treated with increasing concentrations of Oxaliplatin in the presence or absence of 0.5 nM Taxol and the area of the cell colonies was determined after ten days. (**d**) Cells of SW620 cell clone 2 were treated with increasing concentrations of Oxaliplatin in the presence or absence of 0.5 nM Taxol and the area of the cell colonies was determined after ten days. For the colony formation assays, 3,000 cells were seeded 24 h before treatment, and five different concentrations of oxaliplatin were used as indicated. After ten days of the area of the cell colonies covered by the cells was determined. The areas of the cell colonies were normalized to control treated cells and represented as bar graphs (mean ± sem., *t*-test, n=3 independent experiments, ns = not significant).

Colony formation assays in the continuous presence of Oxaliplatin revealed that HCT116-*CHK2*$^{-/-}$ cells that grown in the absence of Taxol were more resistant towards Oxaliplatin compared to the same clone grew in the presence of low-does Taxol (Figure 3-2a and 3-2b). In HCT116-*CHK2*$^{-/-}$ single clone 1 cells, the area of the cell colonies in the absence of Taxol was 1.41-fold greater than the cells in the presence of Taxol when treated with 0.2 µM Oxaliplatin, and was 1.97-fold greater when treated with 0.4 µM Oxaliplatin (Figure 3-2a). In the second single cell clone of HCT116-*CHK2*$^{-/-}$ cells, the area of the cell colonies in the absence of Taxol was 1.44-fold greater than the cells in the presence of Taxol when treated with 0.4 µM Oxaliplatin, and was 1.71-fold greater when treated with 0.8 µM Oxaliplatin (Figure 3-2b). However, no significant differences in drug sensitivity were detectable in two independent cell clones of SW620 cells that grown in the presence or absence of Taxol (Figure 3-2c and 3-2d).

3.1.2. Short-term re-induction of chromosome missegregation mediated by short-term removal of Taxol results in decreased sensitivity towards Oxaliplatin in HCT116-*CHK2*$^{-/-}$ cells, but not in SW620 cells.

Long-time (30 days) removal Taxol can re-induce CIN and aneuploidy in the same clone generated in the presence of low-dose of Taxol (Ertych et al. 2014). To investigate whether short-term removal of Taxol and thus, acute chromosome missegregation without significant alterations of the karyotype impacted on the drug response, I used two cell clones for HCT116-*CHK2*$^{-/-}$ and two cell clones for SW620 cells that were continuously grown in the presence of Taxol to suppress CIN. These cells were then treated with increasing concentrations of Oxaliplatin in the presence or absence of Taxol during the colony formation assay for ten days only (Figure 3-1).

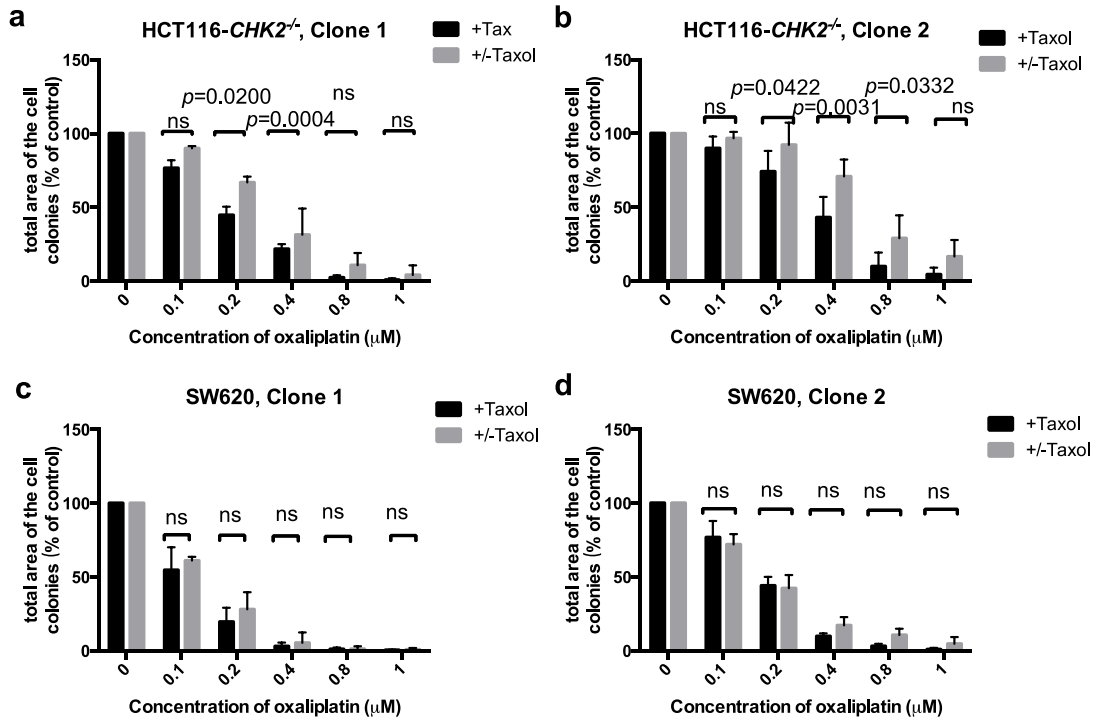

Figure 3-3 Quantification of the area of the cell colonies of HCT116-*CHK2*$^{-/-}$ and SW620 cells treated with different concentration of Oxaliplatin in the presence or short time absence of low-dose of Taxol.

(a) HCT116-*CHK2*$^{-/-}$ cells of Taxol-treated clone 1 were treated with increasing concentrations of Oxaliplatin in the presence or absence of 0.5 nM Taxol during the assay, and the area of the cell colonies was determined after ten days. (**b**) HCT116-*CHK2*$^{-/-}$ cells of Taxol treated clone 2 were treated with increasing

concentrations of Oxaliplatin in the presence or absence of 0.5 nM Taxol during the assay, and the area of the cell colonies was determined after ten days. (**c**) SW620 cells of Taxol treated clone 1 were treated with increasing concentrations of Oxaliplatin in the presence or absence of 0.5 nM Taxol during the assay, and the area of the cell colonies was determined after ten days. (**d**) SW620 cells of Taxol treated clone 2 were treated with increasing concentrations of Oxaliplatin in the presence or absence of 0.5 nM Taxol, and the area of the cell colonies was determined after ten days. The areas of the cell colonies were normalized to control treated cells and represented as bar graphs (mean ±sem., *t*-test, n=3 independent experiments, ns = not significant).

These analyses revealed that HCT116-*CHK2*$^{-/-}$ cells that grew in the absence of Taxol during the assay were more resistant towards Oxaliplatin compared to the same cells cultured in the presence of Taxol (Figure 3-3a and 3-3b). In clone 1 of HCT116-*CHK2*$^{-/-}$ cells, the area of the cell colonies in the absence of Taxol during the assay was 1.18-fold greater than the cells in the presence of the Taxol when treated with 0.2 µM Oxaliplatin, and was 1.49-fold greater when treated with 0.4 µM Oxaliplatin (Figure 3-3a). Whereas the area of the cell colonies in the absence of Taxol was 1.24-fold greater than the cells in the presence of Taxol when treated with 0.2 µM Oxaliplatin, 1.65-fold greater when treated with 0.4 µM Oxaliplatin, and 2.91-fold greater when treated with 0.8 µM Oxaliplatin in clone 2 of HCT116-*CHK2*$^{-/-}$ cells (Figure 3-3b). However, no significant response differences were found in two independent clones of SW620 cells that were growing in the presence or short-term absence of Taxol (Figure 3-3 c and 3-3d).

3.1.3. Suppression of CIN by stable knockdown of *ch-TOG/CKAP5* in HCT116-*CHK2*$^{-/-}$ and SW620 results in increased sensitivity towards Oxaliplatin treatment.

Partial stable knockdown of *ch-TOG/CKAP5* restores proper mitotic plus-end microtubule assembly rates and suppresses CIN in chromosomally unstable colorectal cancer cells (Ertych et al. 2014). I used two independent stable *ch-TOG/CKAP5* knockdown single cell clones of HCT116-*CHK2*$^{-/-}$ and SW620 cells to investigate

whether suppression CIN affects drug response towards Oxaliplatin independently of Taxol treatment. Compared with the control cell clones, *ch-TOG*/*CKAP5* knockdown cells were more sensitive towards Oxaliplatin treatment in HCT-*CHK2*$^{-/-}$ cells (Figure 3-4a). In HCT116-*CHK2*$^{-/-}$ cells, the mean value of the area of the control cell colonies was 1.25-fold greater than *ch-TOG/CKAP5* knockdown cells when treated with 0.1 µM Oxaliplatin, 1.85-fold greater when treated with 0.2 µM Oxaliplatin, and 5.38-fold greater when treated with treated with 0.4 µM Oxaliplatin (Figure 3-4a). SW620-sh*ch-TOG* clones showed increased sensitivity towards Oxaliplatin (0.1 and 0.2 µM) as well, albeit at a lesser extent (Figure 3-4b).

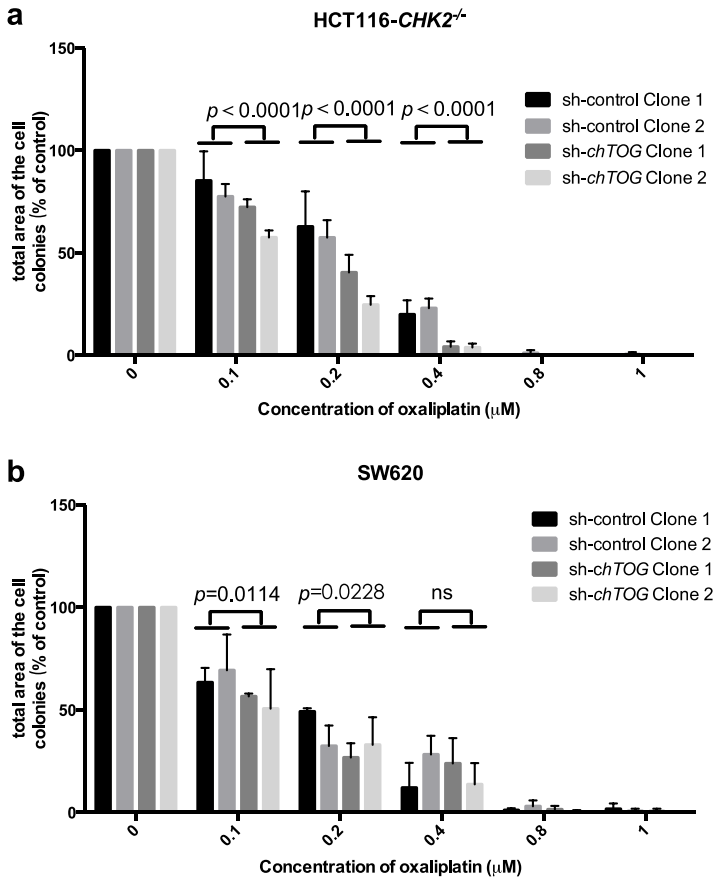

Figure 3-4 Quantification of area of the cell colonies treated with different concentration of Oxaliplatin in HCT116-*CHK2*$^{-/-}$-sh*ch-TOG* and SW620-sh*ch-TOG* cells.

(**a**) Two independent shRNA control single cell clones and two different sh*ch-TOG* single cell clones derived from HCT116-*CHK2*$^{-/-}$ were treated with increasing concentrations of Oxaliplatin, and the area of the cell colonies was determined after

ten days. (**b**) Two independent shRNA control single cell clones and two different sh*ch-TOG* single cell clones derived from SW620 were treated with increasing concentrations of Oxaliplatin, and the area of the cell colonies was determined after ten days. The areas of the cell colonies were normalized to control scrambled cells and represented as bar graphs (mean ± sem., *t*-test, n=3 independent experiments, ns = not significant).

3.1.4. Suppression of CIN in HCT116-*CHK2*$^{-/-}$ cells by low-dose of Taxol does not result in acute response differences towards Oxaliplatin.

To further investigate the acute cellular response towards higher concentrations of Oxaliplatin in HCT116-*CHK2*$^{-/-}$ cells after CIN suppression, I used FACS analyses to analyze the acute cell response in the presence or absence of low-dose of Taxol treated with increasing concentrations (1 μM, 4 μm and 8 μM) of Oxaliplatin in cells of HCT116-*CHK2*$^{-/-}$ clone 1. These cells were cultured in the presence of low-dose Taxol (+Taxol), only in the absence of Taxol for 30 days (-Taxol), or absence of Taxol only during the Oxaliplatin treatment (+/-Taxol). After 48 h of treatment, the proportion of cells with a Sub-G_1 DNA content, which represents apoptotic cells (Kajstura et al. 2007), was determined by FACS.

These analyses overall revealed no drug response differences torwards Oxaliplatin between CIN and after suppression CIN by Taxol. Only after treatment with 8 μM Oxaliplatin, cells that were grown in the absence of Taxol showed more apoptotic cells in response of the drug treatment (Figure 3-5).

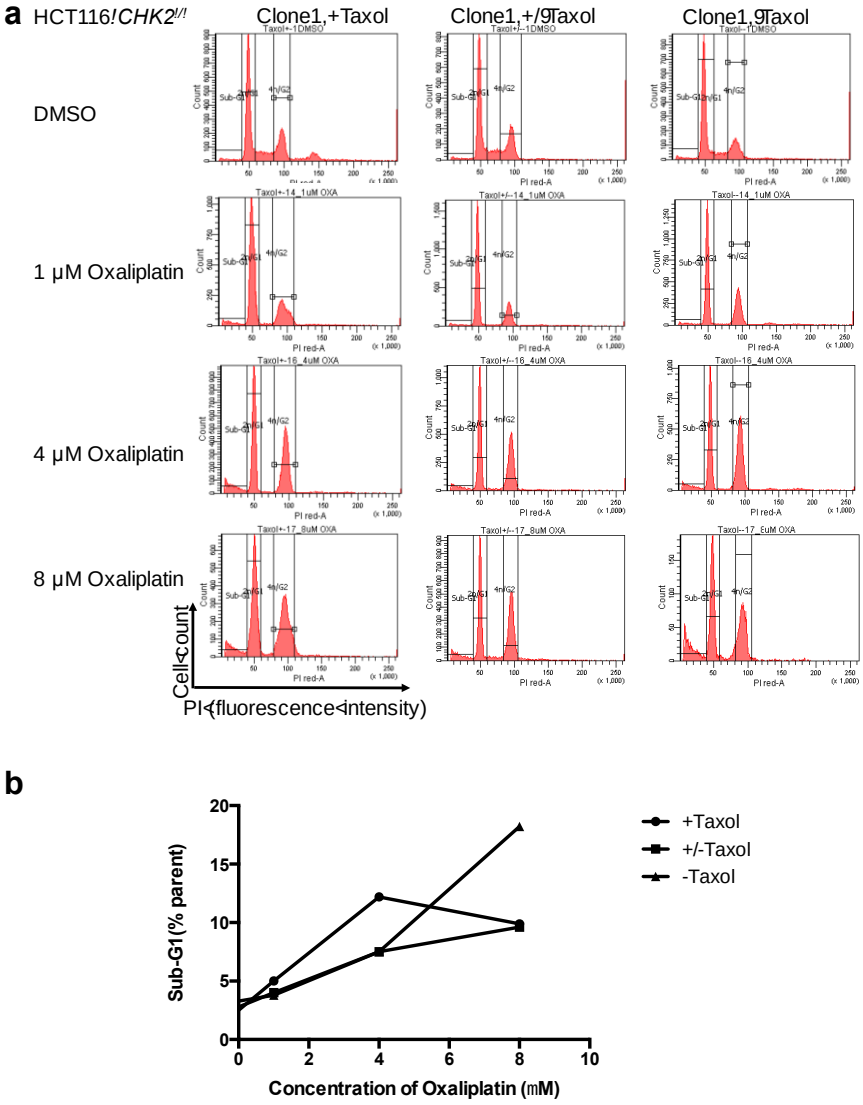

Figure 3-5 Analyses of the acute cellular response towards different concentrations of Oxaliplatin treatment in cells of HCT116-*CHK2*$^{-/-}$ Clone 1 in the presence or absence of Taxol.

(**a**) DNA content profiles of HCT116-*CHK2*$^{-/-}$ in the presence or absence of low-dose Taxol after 48 h of Oxaliplatin treatment. (**b**) The proportion of cells with a Sub-G_1 DNA content after 48 h different concentrations of Oxaliplatin treatment was determined. (n=1 experiment)

3.1.5. Suppression of CIN treated by low-dose of Taxol does not result in response differences towards Cisplatin neither in HCT116-*CHK2*$^{-/-}$ nor in SW620 cells.

Cisplatin is the first generation of platinum compounds. Its cytotoxic mode of action is mediated by its interaction with DNA to form DNA adducts, which is similar to Oxaliplatin (Siddik 2003). I used Cisplatin to test whether it had a similar effect as Oxaliplatin on cell growth after CIN suppression. On a cell growth after CIN suppression, however, unlike Oxaliplatin, colony formation assays revealed that no differences in drug sensitivity in the presence or absence of Taxol in HCT116-*CHK2*$^{-/-}$ cells (Figure 3-6a and 3-6b). Similar results were observed in two independent cell clones of SW620 cells (Figure 3-6c and 3-6d).

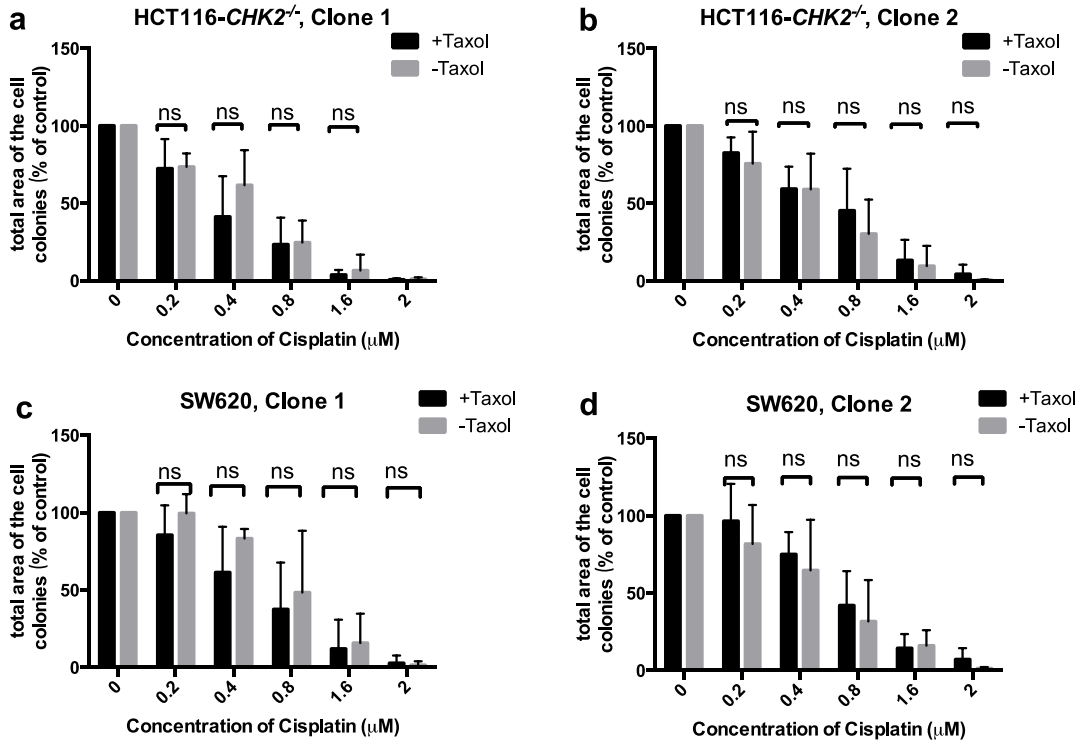

Figure 3-6 Quantification of the area of the cell colonies of HCT116-*CHK2*$^{-/-}$ and SW620 cells after treatment with different concentration of Cisplatin in the presence or absence of low-dose of Taxol.

(**a**) Cells of HCT116-*CHK2*$^{-/-}$ cell clone 1 were treated with increasing concentrations of Cisplatin in the presence or absence of 0.5 nM Taxol and the area of the cell colonies was determined after ten days. (**b**) Cells of HCT116-*CHK2*$^{-/-}$ cell clone 2

were treated with increasing concentrations of Cisplatin in the presence or absence of 0.5 nM Taxol and the area of the cell colonies was determined after ten days. (**c**) Cells of SW620 cell clone 1 were treated with increasing concentrations of Cisplatin in the presence or absence of 0.5 nM Taxol and the area of the cell colonies was determined after ten days. (**d**) Cells of SW620 cell clone 2 were treated with increasing concentrations of Cisplatin in the presence or absence of 0.5 nM Taxol and the area of the cell colonies was determined after ten days. The areas of the cell colonies were normalized to control treated cells and represented as bar graphs (mean ± sem., *t*-test, n=3 independent experiments, ns = not significant).

3.1.6. Short-term re-induction of chromosome missegregation does not result in response differences towards Cisplatin in HCT116-*CHK2*$^{-/-}$ and SW620 cells.

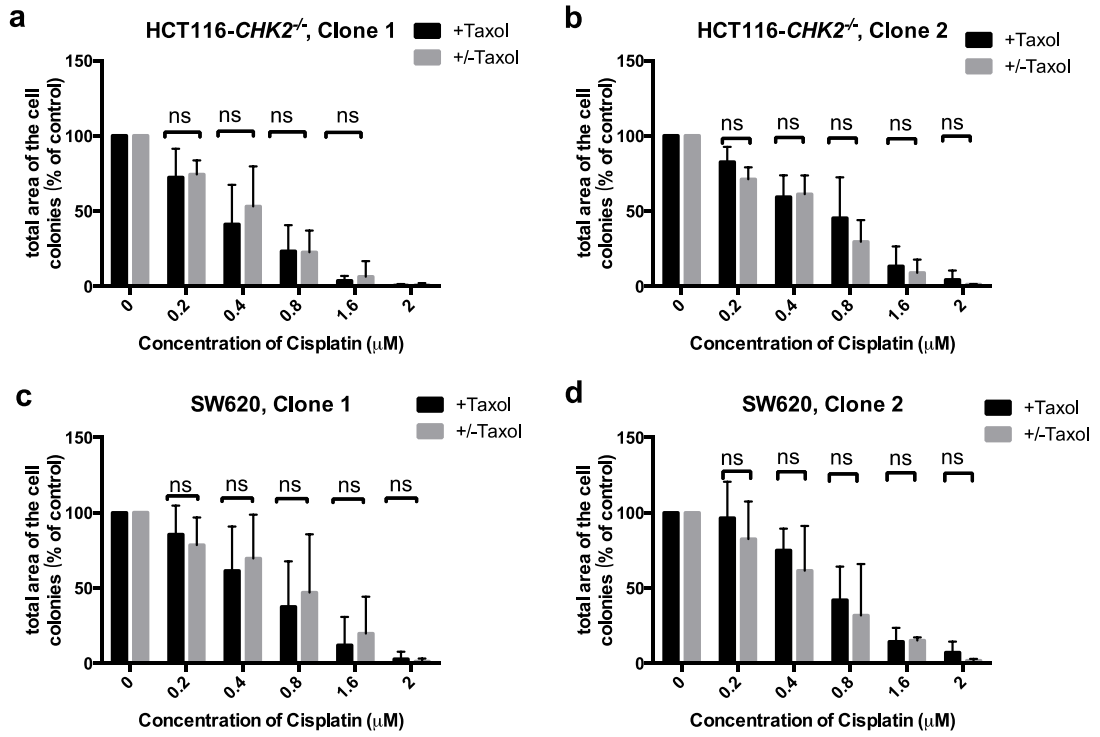

Figure 3-7 Quantification of the area of cell colonies of HCT116-*CHK2*$^{-/-}$ and SW620 cells treated with different concentration of Cisplatin in the presence or short-term absence of low-dose of Taxol.

(**a**) Cells of HCT116-*CHK2*$^{-/-}$ single cell clone 1 were treated with increasing concentrations of Cisplatin in the presence or absence of 0.5 nM Taxol during the

assay, and the area of the cell colonies was determined after ten days. (**b**) Cells of HCT116-*CHK2*$^{-/-}$ single cell clone 2 were treated with increasing concentrations of Cisplatin in the presence or absence of 0.5 nM Taxol during the assay, and the area of the cell colonies was determined after ten days. (**c**) Cells of SW620 single cell clone 1 were treated with increasing concentrations of Cisplatin in the presence or absence of 0.5 nM Taxol during the assay, and the area of the cell colonies was determined after ten days. (**d**) Cells of SW620 cell single cell clone 2 were treated with increasing concentrations of Cisplatin in the presence or absence of 0.5 nM Taxol during the assay, and the area of the cell colonies was determined after ten days. The areas of the cell colonies were normalized to control treated cells and represented as bar graphs (mean ± sem., *t*-test, n=3 independent experiments, ns = not significant).

Removal of Taxol during the colony formation assay was also used during Cisplatin treatment. However, no drug sensitivity differences towards Cisplatin were found in two independent cell clones of HCT116-*CHK2*$^{-/-}$ cells that grown in the absence of Taxol during the colony formation assay (Figure 3-7a and 3-7b). Similar results were obtained for two independent clones of SW620 cells that were grown in the presence or short-term absence of Taxol (Figure 3-7c and 3-7d).

3.1.7. Taxol treatment does not affect drug response towards Oxaliplatin or Cisplatin *per se*.

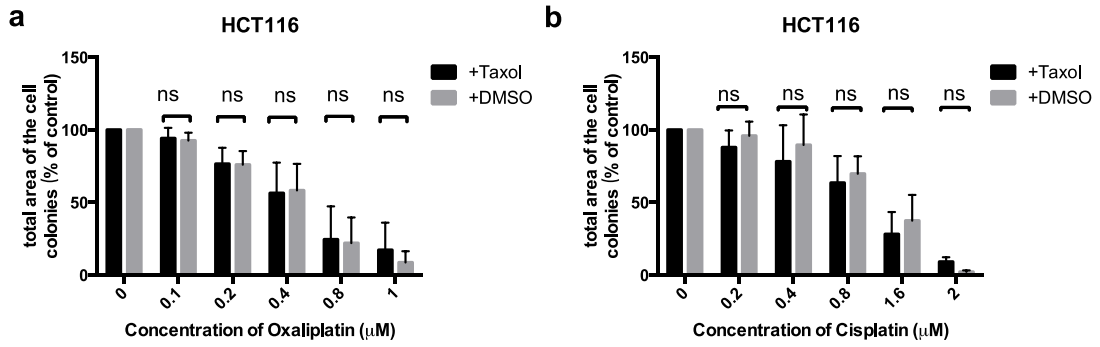

Figure 3-8 Quantification of the area of cell colonies of cell populations of HCT116 treated with different concentrations of Oxaliplatin or Cisplatin.

(**a**) HCT116 cells were treated with increasing concentrations of Oxaliplatin in the presence of 0.5 nM Taxol or DMSO, and the area of the cell colonies was determined

after ten days. (**b**) HCT116 cells were treated with increasing concentrations of Cisplatin in the presence of 0.5 nM Taxol or DMSO, and the area of the cell colonies was determined after ten days. The areas of the cell colonies were normalized to control treated cells and represented as bar graphs (mean ± sem., *t*-test, n=4 independent experiments, ns = not significant).

To investigate whether low-dose treatment of Taxol itself affects drug response, I used chromosomally stable HCT116 cells and treated them with Oxaliplatin or Cisplatin in the presence or absence of Taxol. After ten days of treatment, no drug response differences were detectable (Figure 3-8). Thus, low-dose of Taxol does not affect drug response towards Oxaliplatin or Cisplatin *per se*.

3.1.8. Suppression of CIN by knockdown of *ch-TOG/CKAP5* results in decreased sensitivity in HCT116-*CHK2*$^{-/-}$, but increased sensitivity in SW620 towards Cisplatin treatment.

The *ch-TOG/CKAP5* stable knockdown cells of HCT116-*CHK2*$^{-/-}$ and SW620 showed increased sensitivity towards Oxaliplatin treatment (Figure 3-4), to test whether these cells also exhibit increased sensitivity towards Cisplatin, the cells were treated with increasing concentrations of Cisplatin. Surprisingly, compared with the control cell clones, *ch-TOG/CKAP5* knockdown HCT116-*CHK2*$^{-/-}$ cells were more resistant towards Cisplatin treatment (Figure 3-9a). When treated with 0.2 µM Cisplatin, the mean area of the *ch-TOG/CKAP5* knockdown cell colonies was 1.23-fold greater than the control cells, 1.41-fold greater when treated with 0.4 µM Cisplatin, and 1.60-fold greater when treated with 0.8 µM Cisplatin (Figure 3-9a). In contrast, stable *ch-TOG/CKAP5* knockdown cells derived from SW620 cells showed increased sensitivity towards Cisplatin (Figure 3-9b). The area of *ch-TOG/CKAP5* knockdown cell colonies was 1.46-fold lower than the control cells when treated with 0.4 µM Cisplatin, and 1.33-fold lower when treated with 0.8 µM Cisplatin in SW620 cells (Figure 3.9b). Thus, knockdown of *ch-TOG/CKAP5* shows different effects towards Ciplatin treatment in HCT116-*CHK2*$^{-/-}$ and SW620 cells.

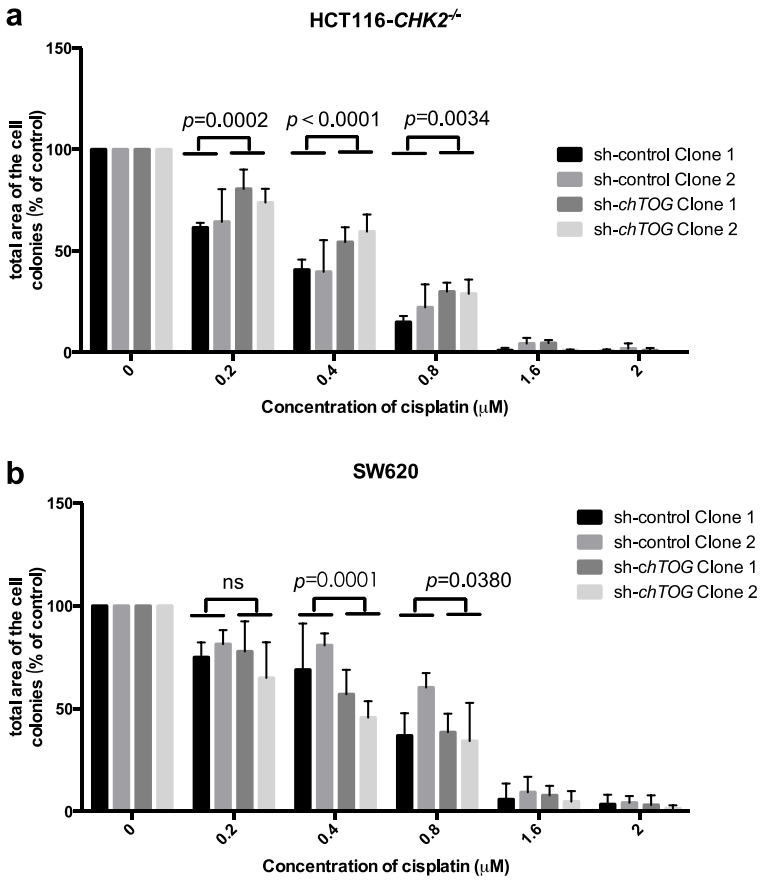

Figure 3-9 Quantification of the area of cell colonies of HCT116-*CHK2*$^{-/-}$-sh*ch-TOG* and SW620-sh*ch-TOG* cell clones treated with different concentrations of Cisplatin.

(**a**) Two independent shRNA control single cell clones and two independent sh-*chTOG* clones from HCT116-*CHK2*$^{-/-}$ were treated with increasing concentrations of Cisplatin, and the area of the cell colonies was determined after ten days. (**b**) Two indenpent shRNA control single cell clones and two independent sh-*chTOG* clones derived from SW620 were treated with increasing concentrations of Cisplatin, and the area of the cell colonies was determined after ten days. The areas of the cell colonies were normalized to control scrambled cells and represented as bar graphs (mean ± sem., *t*-test, n=3 independent experiments, ns = not significant).

3.2. Drug responses towards other chemotherapeutic drugs in cells exhibiting CIN and after suppression of CIN

After evaluating the CIN-dependent drugs responses towards platinum compounds, the cellular response towards various other chemotherapeutic drugs that are used for the treatment of CRC were also investigated.

In fact, 5-FU is used as a single drug treatment or combined with other chemotherapeutic drugs in CRC treatment (Longley et al. 2003). Adriamycin has exhibited a broad spectrum of anti-tumor effect, particular against solid cancers including CRC (Myers et al. 1977). Irinotecan has anti-tumor activity against CRC and is used as the first-line treatment or after the failure of 5-FU or Oxaliplatin as a second-line treatment (Pitot et al. 1997; Rougier et al. 1997; Saltz et al. 2000). Two independent single cell clones of HCT116-*CHK2*$^{-/-}$ and SW620 described in Figure 3-1 were treated with increasing concentrations of 5-FU, Adriamycin, and Irinotecan and the cellular response were evaluated by colony formation assays.

The assays in the continuous presence of 5-FU, Adriamycin, and Irinotecan treatment revealed no differences in drug sensitivity in two independent cell clones of HCT116-*CHK2*$^{-/-}$ or SW620 cells in the presence or absence of Taxol (Figure 3-10, 3-11, and 3-12).

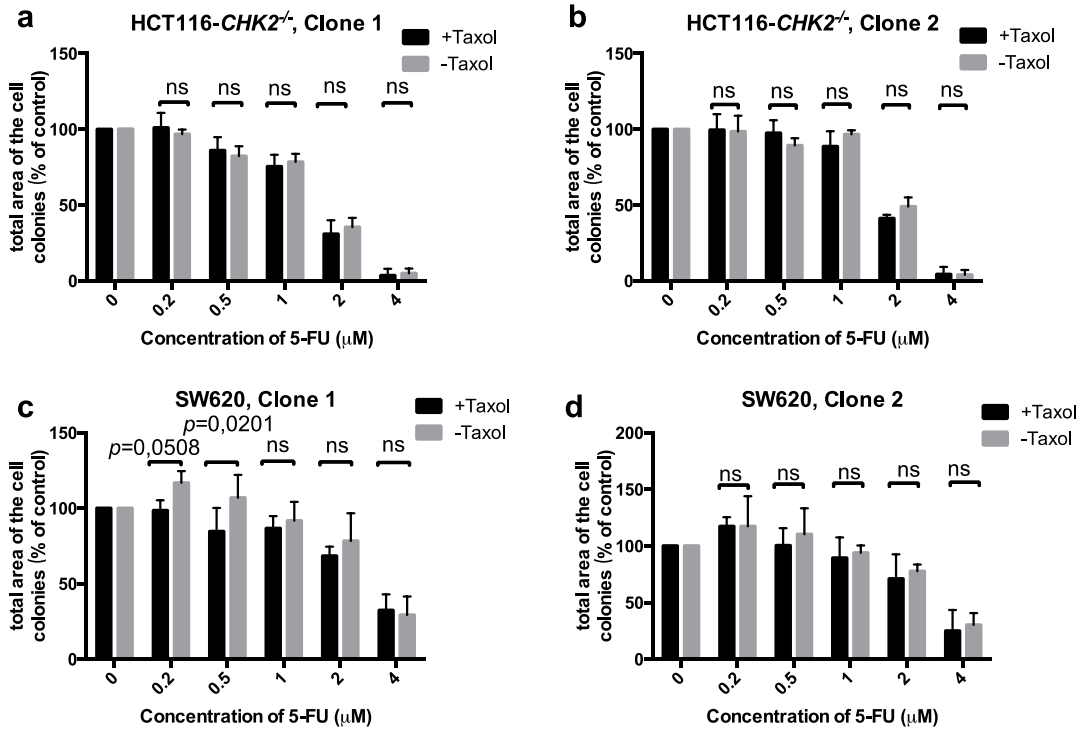

Figure 3-10 Quantification of the area of cell colonies of HCT116-*CHK2*$^{-/-}$ and SW620 cells treated with different concentrations of 5-FU in the presence or absence of low-dose of Taxol.

(**a**) Cells of HCT116-*CHK2*$^{-/-}$ single cell clone 1 were treated with increasing concentrations of 5-FU in the presence or absence of 0.5 nM Taxol and the area of the cell colonies was determined after ten days. (**b**) Cells of HCT116-*CHK2*$^{-/-}$ single cell clone 2 were treated with increasing concentrations of 5-FU in the presence or absence of 0.5 nM Taxol and the area of the cell colonies was determined after ten days. (**c**) Cells of SW620 single cell clone 1 were treated with increasing concentrations of 5-FU in the presence or absence of 0.5 nM Taxol and the area of the cell colonies was determined after ten days. (**d**) Cells of SW620 single cell clone 2 were treated with increasing concentrations of 5-FU in the presence or absence of 0.5 nM Taxol and the area of the cell colonies was determined after ten days. The areas of the cell colonies were normalized to control treated cells and represented as bar graphs (mean ±sem., *t*-test, n=3 independent experiments, ns = not significant).

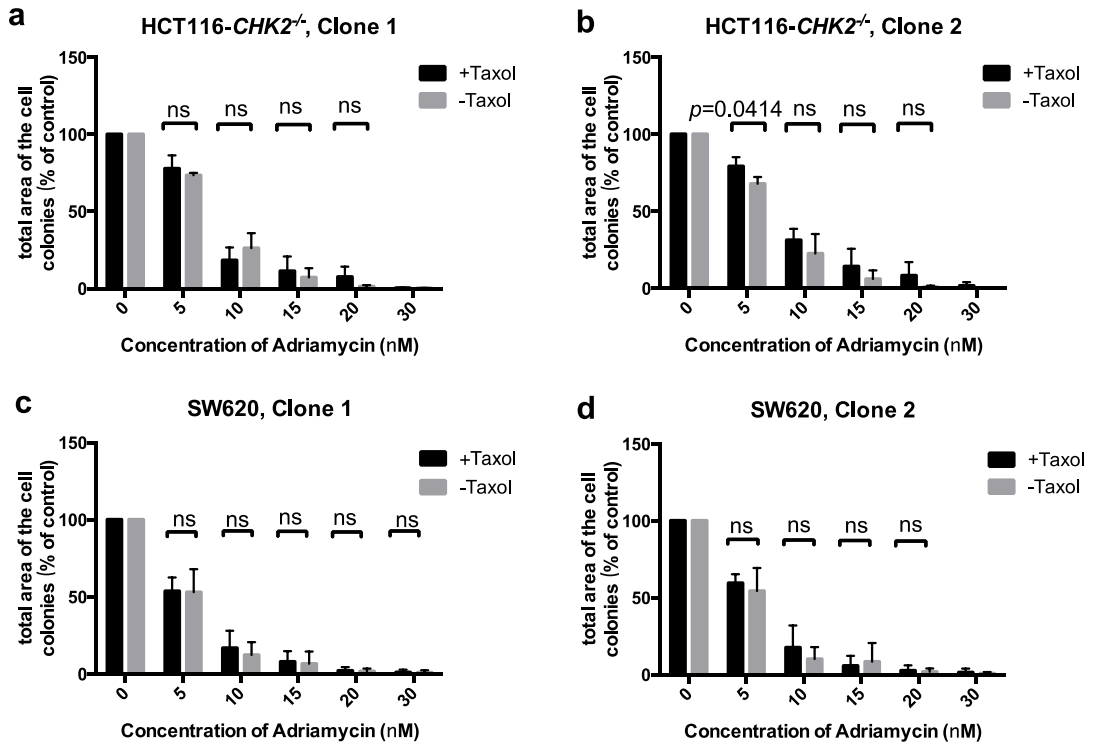

Figure 3-11 Quantification of the area cell colonies of HCT116-*CHK2*$^{-/-}$ and SW620 cells treated with different concentrations of Adriamycin in the presence or absence of low-dose of Taxol.

(**a**) Cells of HCT116-*CHK2*$^{-/-}$ single cell clone 1 were treated with increasing concentrations of Adriamycin in the presence or absence of 0.5 nM Taxol and the area of the cell colonies was determined after ten days. (**b**) Cells of HCT116-*CHK2*$^{-/-}$ single cell clone 2 were treated with increasing concentrations of Adriamycin in the presence or absence of 0.5 nM Taxol and the area of the cell colonies was determined after ten days. (**c**) Cells of SW620 single cell clone 1 were treated with increasing concentrations of Adriamycin in the presence or absence of 0.5 nM Taxol and the area of the cell colonies was determined after ten days. (**d**) Cells of SW620 single cell clone 2 were treated with increasing concentrations of Adriamycin in the presence or absence of 0.5 nM Taxol and the area of the cell colonies was determined after ten days. The areas of the cell colonies were normalized to control treated cells and represented as bar graphs (mean ± sem, *t*-test, n=3 independent experiments, ns = not significant).

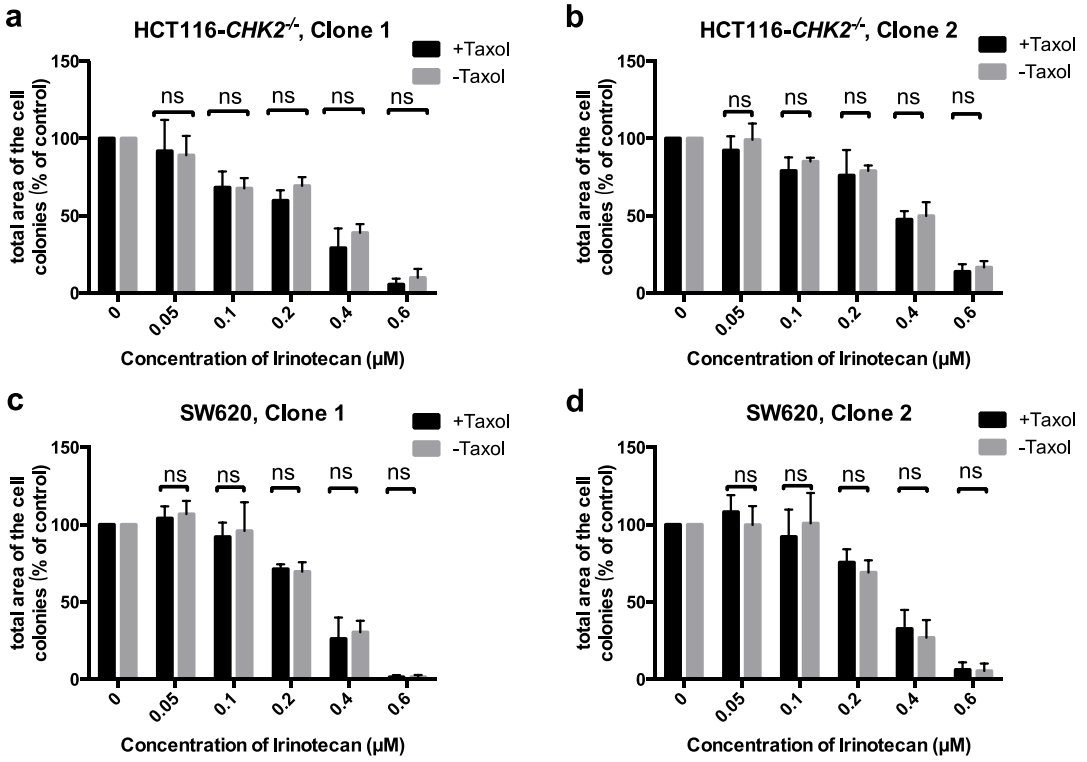

Figure 3-12 Quantification of the area of cell colonies for HCT116-*CHK2*$^{-/-}$ and SW620 cells treated with different concentration of Irinotecan in the presence or absence of low-dose of Taxol.

(a) Cells of HCT116-*CHK2*$^{-/-}$ single cell clone 1 were treated with increasing concentrations of Irinotecan in the presence or absence of 0.5 nM Taxol and the area of the cell colonies was determined after ten days. (**b**) Cells of HCT116-*CHK2*$^{-/-}$ single cell clone 2 were treated with increasing concentrations of Irinotecan in the presence or absence of 0.5 nM Taxol and the area of the cell colonies was determined after ten days. (**c**) Cells of SW620 single cell clone 1 were treated with increasing concentrations of Irinotecan in the presence or absence of 0.5 nM Taxol and the area of the cell colonies was determined after ten days. (**d**) Cells of SW620 single cell clone 2 were treated with increasing concentrations of Irinotecan in the presence or absence of 0.5 nM Taxol and the area of the cell colonies was determined after ten days. The areas of the cell colonies were normalized to control treated cells and represented as bar graphs (mean ± sem., *t*-test, n=3 independent experiments, ns = not significant).

I also investigated the effect of short-time removal of Taxol (Figure 3-1) for 5-FU, Adriamycin, and Irinotecan treatment in HCT116-*CHK2*$^{-/-}$ and SW620 cell clones. Again, no differences were observed (Figure 3-13, 3-14, and 3-15).

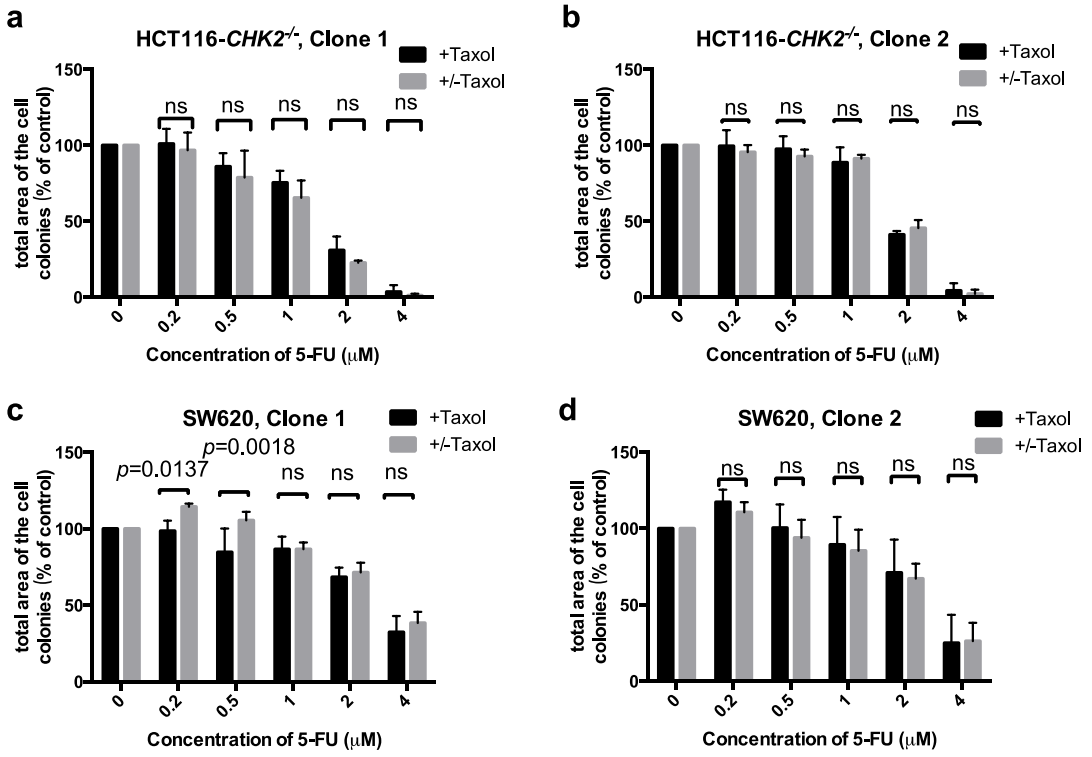

Figure 3-13 Quantification of the area of cell colonies of HCT116-*CHK2*$^{-/-}$ and SW620 cells treated with different concentration of 5-FU in the presence or short-term absence of low-dose of Taxol.

(**a**) Cells of HCT116-*CHK2*$^{-/-}$ single cell clone 1 were treated with increasing concentrations of 5-FU in the presence or absence of 0.5 nM Taxol during the assay, and the area of the cell colonies was determined after ten days. (**b**) Cells of HCT116-*CHK2*$^{-/-}$ single cell clone 2 were treated with increasing concentrations of 5-FU in the presence or absence of 0.5 nM Taxol during the assay and the area of the cell colonies was determined after ten days. (**c**) Cells of SW620 single cell clone 1 were treated with increasing concentrations of 5-FU in the presence or absence of 0.5 nM Taxol during the assay, and the area of the cell colonies was determined after 10 days. (**d**) Cells of SW620 single cell clone 2 was treated with increasing concentrations of 5-FU in the presence or absence of 0.5 nM Taxol during the assay, and the area of the cell colonies was determined after ten days. The areas of the cell

colonies were normalized to control treated cells and represented as bar graphs (mean ± sem., *t*-test, n=3 independent experiments, ns = not significant).

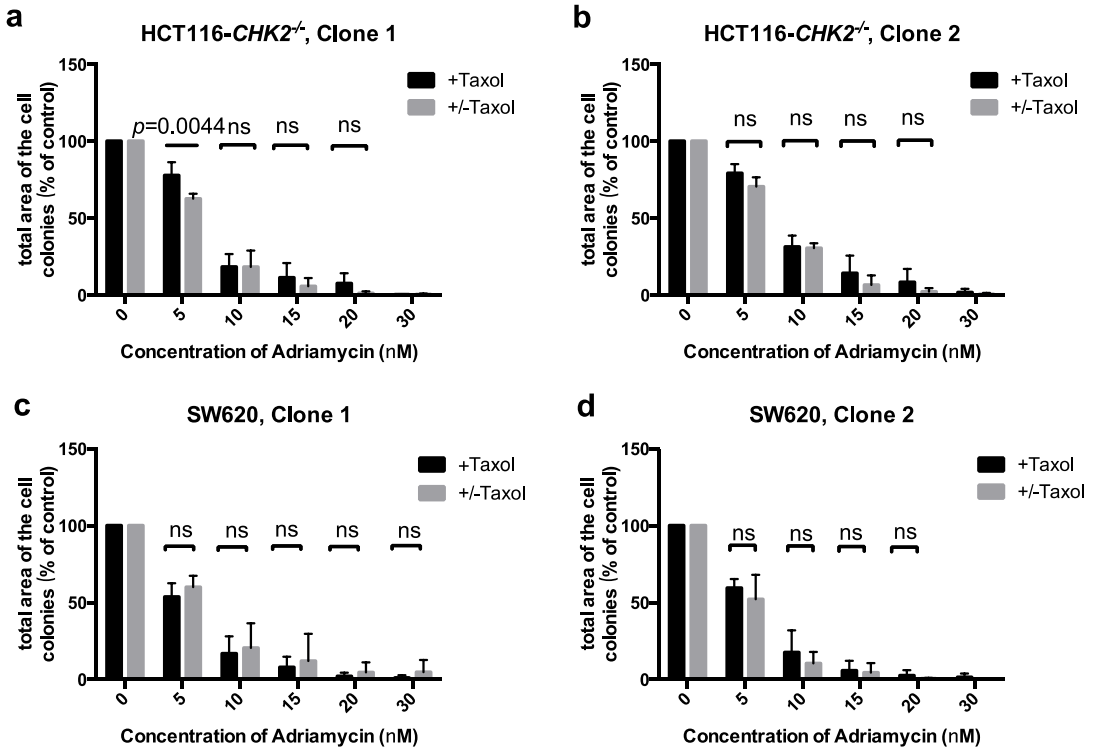

Figure 3-14 Quantification of the area of cell colonies of HCT116-*CHK2*$^{-/-}$ and SW620 cells treated with different concentrations of Adriamycin in the presence or short-time absence of low-dose of Taxol.

(a) Cells of HCT116-*CHK2*$^{-/-}$ single cell clone 1 were treated with increasing concentrations of Adriamycin in the presence or absence of 0.5 nM Taxol during the assay, and the area of the cell colonies was determined after ten days. (**b**) Cells of HCT116-*CHK2*$^{-/-}$ single cell clone 2 were treated with increasing concentrations of Adriamycin in the presence or absence of 0.5 nM Taxol during the assay, and the area of the cell colonies was determined after ten days. (**c**) Cells of SW620 single cell clone 1 were treated with increasing concentrations of Adriamycin in the presence or absence of 0.5 nM Taxol during the assay, and the area of the cell colonies was determined after ten days. (**d**) Cells of SW620 single cell clone 2 were treated with increasing concentrations of Adriamycin in the presence or absence of 0.5 nM Taxol during the assay, and the area of the cell colonies was determined after ten days. The

areas of the cell colonies were normalized to control treated cells and represented as bar graphs (mean ± sem., *t*-test, n=3 independent experiments, ns = not significant).

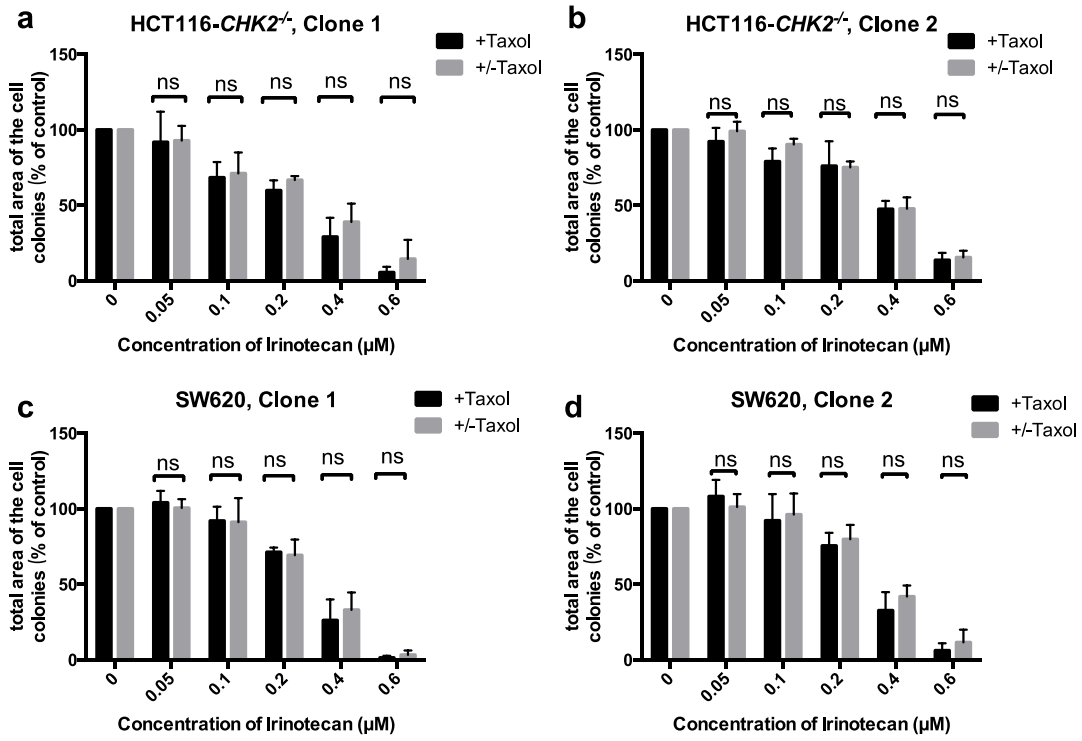

Figure 3-15 Quantification of area of cell colonies of HCT116-*CHK2*$^{-/-}$ and SW620 cells treated with different concentration of Irinotecan in the presence or short-term absence of low-dose of Taxol.

(**a**) Cells of HCT116-*CHK2*$^{-/-}$ single cell clone 1 were treated with different concentrations of Irinotecan in the presence or absence of 0.5 nM Taxol during the assay and the area of the cell colonies was determined after ten days. (**b**) Cells of HCT116-*CHK2*$^{-/-}$ single cell clone 2 were treated with increasing concentrations of Irinotecan in the presence or absence of 0.5 nM Taxol during the assay and the area of the cell colonies was determined after ten days. (**c**) Cells of SW620 single cell clone 1 were treated with increasing concentrations of Irinotecan in the presence or absence of 0.5 nM Taxol during the assay and the area of the cell colonies was determined after ten days. (**d**) Cells of SW620 cell clone 2 were treated with increasing concentrations of Irinotecan in the presence or absence of 0.5 nM Taxol, and the area of the cell colonies was determined after ten days. The areas of the cell colonies were

normalized to control treated cells and represented as bar graphs (mean ± sem., *t*-test, n=3 independent experiments, ns = not significant).

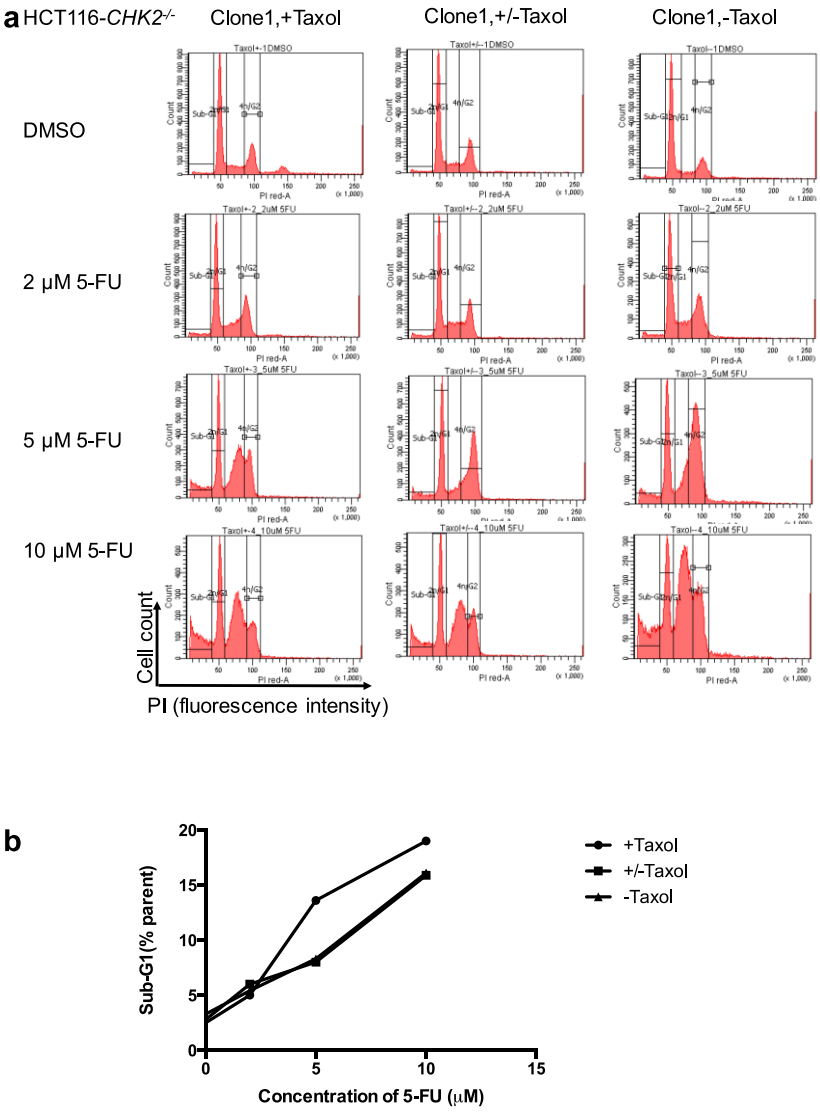

Figure 3-16 Analyses of the acute response towards different high concentrations of 5-FU treatment in cells of HCT116-*CHK2*$^{-/-}$ Clone 1 in the presence or absence of Taxol.

(a) DNA content profiles of HCT116-*CHK2*$^{-/-}$ in the presence or absence of low-does Taxol after 48 h of 5-FU treatment. (**b**) The proportion of cells with a Sub-G_1 DNA content after 48 h different concentrations of 5-FU treatment was determined. (n=1 experiment)

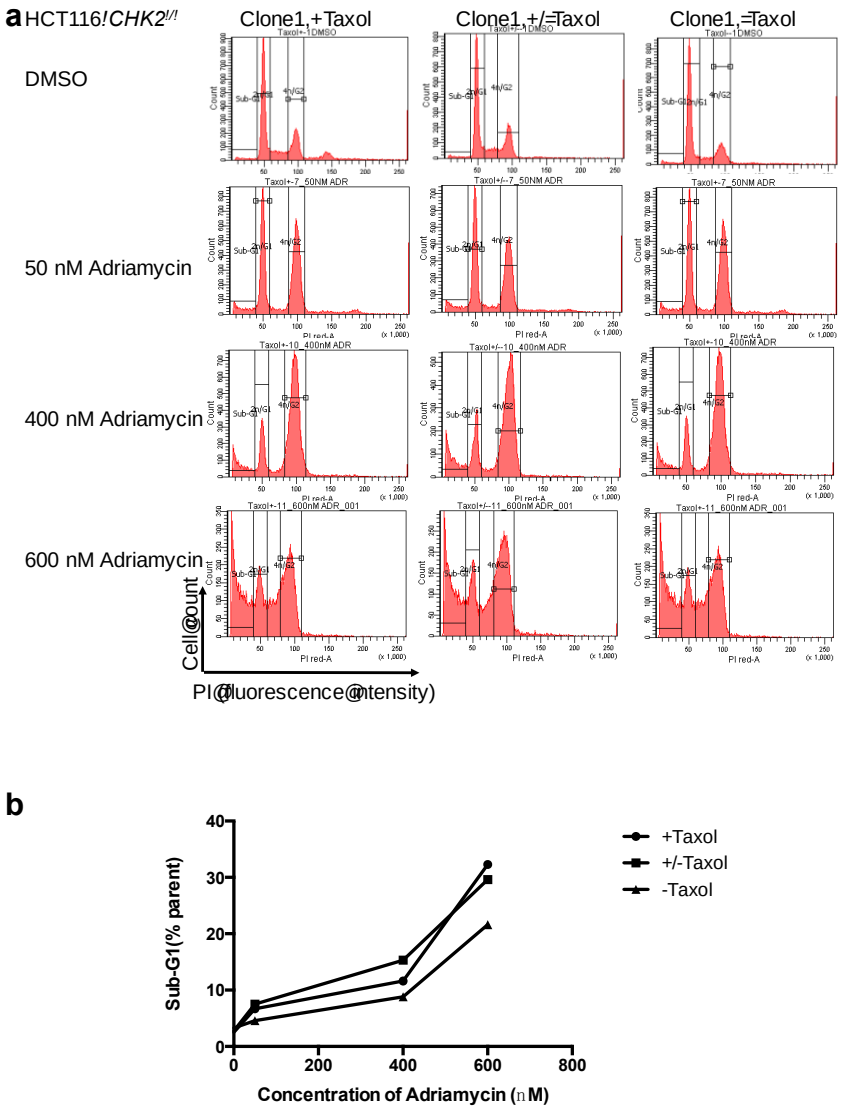

Figure 3-17 Analyses of the acute response towards different high concentrations of Adriamycin in cells of HCT116-*CHK2*$^{-/-}$ clone 1 in the presence or absence of Taxol.

(**a**) DNA content profiles of HCT116-*CHK2*$^{-/-}$ cells in the presence or absence of low-does Taxol after 48 h of Adriamycin treatment. (**b**) The proportion of cells with a Sub-G_1 DNA content after 48 h different concentrations of Adriamycin treatment was determined. (n=1 experiment)

Finally I investigated the acute response towards 5-FU and Adriamycin in HCT116-*CHK2*$^{-/-}$ cells by FACS analyses. Also, these experiments showed no

significant differences in the cellular response towards higher concentrations of 5-FU or Adriamycin treatment (Figure 3-16 and 3-17).

In sum, suppression of CIN does not cause drug response differences towards other chemotherapeutic drugs such as 5-FU, Adriamycin, or Irinotecan.

3.3. Drug responses in chromosomally stable CRC cells and after induction of CIN by DNA replication stress.

3.3.1. Induction of CIN by Aphidicolin treatment in HCT116 cells.

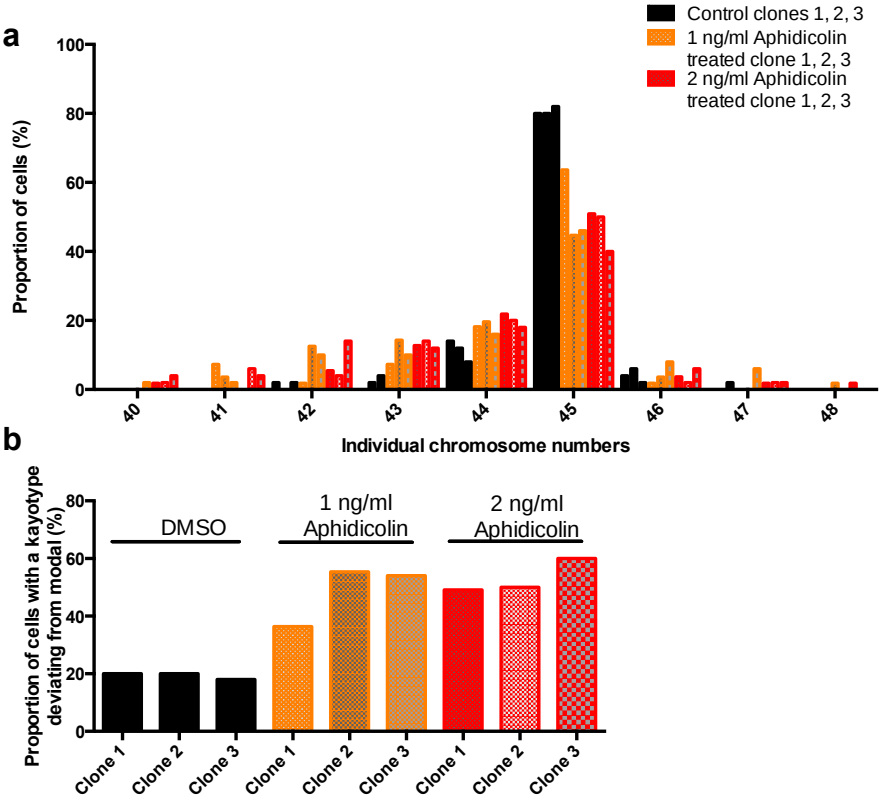

Figure 3-18 Aphidicolin induces CIN in chromosomally stable HCT116 cells.

(**a**) Karyotype analysis by chromosome counting of metaphase spreads of single cell clones derived from HCT116 cells treated with DMSO, 1 or 2 ng/ml Aphidicolin for 30 generations (n=50 cells). (**b**) The percentage of cells with chromosome numbers deviating from the modal were calculated.

Aphidicolin is a reversible inhibitor of the DNA polymerase α and thus, inhibits proper DNA replication during S phase, thereby inducing replication stress (Ikegami et al. 1978). Since replication stress was associated with numerical aneuploidy (Burrell et al. 2013), I tested whether low-dose Aphidicolin treatment is sufficient to induce CIN. For this, HCT116 cells were treated with DMSO (control) or with two concentrations of Aphidicolin (1 and 2 ng/ml). Single cell clones were grown for 30 generations. Subsequently, the number of chromosomes per cell were determined by chromosome counting in metaphase spreads. The proportion of cells with chromosome numbers deviating from the modal (45 chromosomes) was analyzed.

3.3.2. Inducing CIN by Aphidicolin does not cause response differences towards Oxaliplatin or Cisplatin treatment in HCT116 cells.

To investigate whether induction of CIN upon replication stress would affect drug response in CRC cells, I used two HCT116 single cell clones that were either treated with DMSO or 2 ng/ml Aphidicolin. Colony formation assays in the continuous presence of Oxaliplatin or Cisplatin showed no significant response differences between Aphidicolin and DMSO treated HCT116 cells (Figure 3-19). These results indicate that acute induction of CIN in chromosomally stable CRC cell by replication stress is not sufficient to alter drug sensitivities towards Oxaliplatin or Cisplatin.

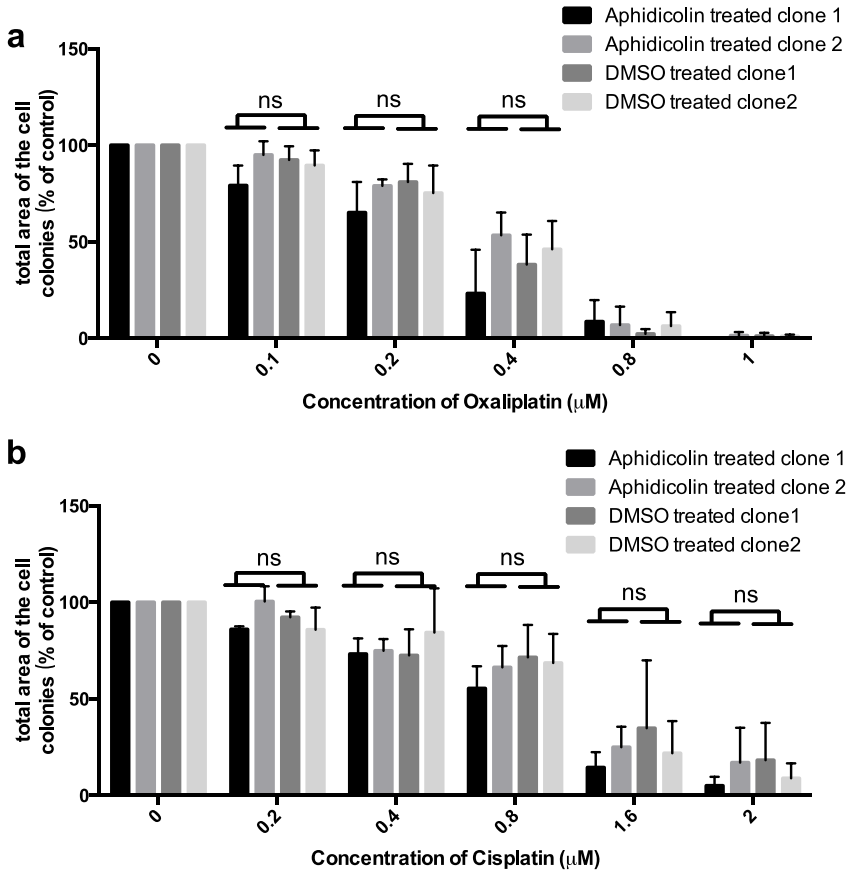

Figure 3-19 Quantification of the area of cell colonies after treatment with different concentration of Oxaliplatin or Cisplatin in Aphidicolin- and DMSO-treated single cell clones.

(**a**) Two independent DMSO-treated control single cell clones and two independent 2 ng/ml Aphidicolin-treated single cell clones of HCT116 cells were treated with increasing concentrations of Oxaliplatin, and the area of the cell colonies was determined after ten days. (**b**) Two independent DMSO-treated control single cell clones and two independent 2 ng/ml Aphidicolin-treated single cell clones of HCT116 cells were treated with increasing concentrations of Cisplatin, and the area of the cell colonies was determined after ten days. The areas of the cell colonies were normalized to control treated cells and represented as bar graphs (mean ± sem., *t*-test, n=3 independent experiments, ns = not significant).

3.3.3. Short-term removal of Aphidicolin does not affect the drug response towards Oxaliplatin treatment, but increases resistance towards Cisplatin treatment in one single cell clone.

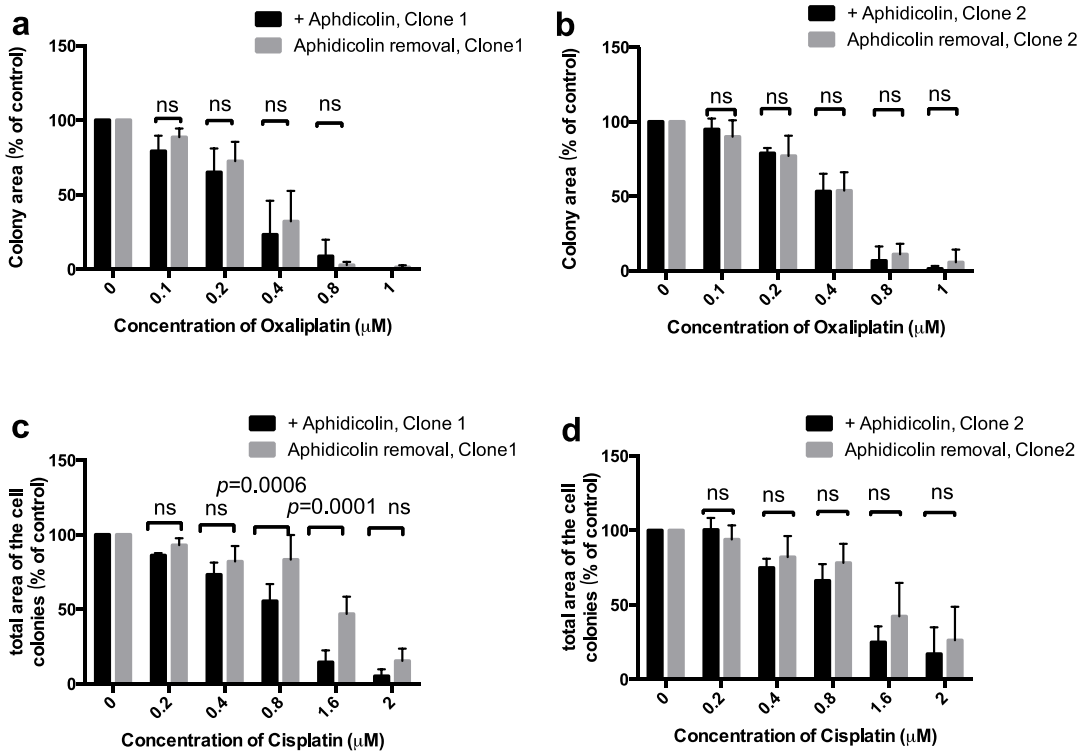

Figure 3-20 Quantification of the area of cell colonies of Aphidicolin treated cells treated with different concentration of Oxaliplatin or Cisplatin in the presence or absence of Aphidicolin.

(**a**) Cells of single cell clone 1 derived from 2 ng/ml Aphidicolin treated HCT116 cells were treated with increasing concentrations of Oxaliplatin in the presence or absence of Aphidicolin during the assay, and the area of the cell colonies was determined after ten days. (**b**) Cells of single cell clone 2 derived from 2 ng/ml Aphidicolin treated HCT116 cells were treated with increasing concentrations of Oxaliplatin in the presence or absence of Aphidicolin during the assay, and the area of the cell colonies was determined after ten days. (**c**) Cells of single cell clone 1 derived from 2 ng/ml Aphidicolin treated HCT116 cells were treated with increasing concentrations of Cisplatin in the presence or absence of Aphidicolin during the assay, and the area of the cell colonies was determined after ten days. (**d**) Cells of single cell clone 2 derived from 2 ng/ml Aphidicolin treated HCT116 cells were treated with

increasing concentrations of Cisplatin in the presence or absence of aphidicolin during the assay, and the area of the cell colonies was determined after ten days. The areas of the cell colonies were normalized to control treated cells and represented as bar graphs (mean ±sem., *t*-test, n=3 independent experiments, ns = not significant).

Removal of Aphidicolin during the colony formation assay is expected to reveal whether ongoing chromosome missegregation or aneuploidy itself would affect drug response. On the other hand, it could also show whether Aphidicolin itself impacts on the drug response. Hence, I used two aneuploid single cell clones of 2 ng/ml Aphidicolin treated HCT116 cells and treated them with increasing concentrations of Oxaliplatin or Cisplatin in the presence or absence of Aphidicolin during the assay.
These colony formation assays showed that upon Oxaliplatin treatment, no response differences were observed in the presence or absence Aphidicolin during the assay (Figure 3-20a and 3-20b). Upon Cisplatin treatment, single cell clone 1 derived from Aphidicolin treated cells showed a slightly higher resistant towards two concentrations of Cisplatin (0.8 and 1.6 µM) in the absence of Aphidicolin during the assay when compared to in the presence of Aphidicolin (Figure 3-20c). However, using a second single clone derived from Aphidicolin treated HCT116 cells, no significant differences were detectable towards Cisplatin treatment in the presence or absence of Aphidicolin during the assay (Figure 3-20d), indicating that the observed slight effect is not representative.
Together, induction of CIN by Aphidicolin-triggered replication stress does not affect the cellular response towards Oxaliplatin or Cisplatin.

3.4. Drug response in chromosomally stable CRC cells and after induction of CIN by treatment with a Mps1 inhibitor.

3.4.1. Pharmacological inhibition of the mitotic spindle assembly checkpoint as a strategy to induce CIN.

The Mps1 kinase plays a critical role in SAC signaling (Abrieu et al. 2001). Inhibition of the Mps1 kinase with the small molecular inhibitor MPS1-IN-3 causes chromosomal misalignment, and inhibition of the spindle checkpoint and thus, leads

to chromosome missegregation and aneuploidy (Tannous et al. 2013). To induce CIN in chromosomally stable HCT116 cells, cells were treated with 0.5 µM MPS1-IN-3 or treated with DMSO as a control. Single cell clones were grown for 30 generations of treatment and the chromosome numbers were analyzed by chromosome counting. Subsequently, the proportion of cells with chromosome numbers deviating from the modal was determined. These analyses showed, as expected, that 0.5 µM MPS1-IN-3 treated cells had a higher karyotype variability than the control treated cells, with a proportion of deviating from the modal from ~20% to 45-52% (Figure 3-21). Thus, treatment of HCT116 cells with the MPS1-IN-3 causes transient induction of CIN during inhibitor treatment.

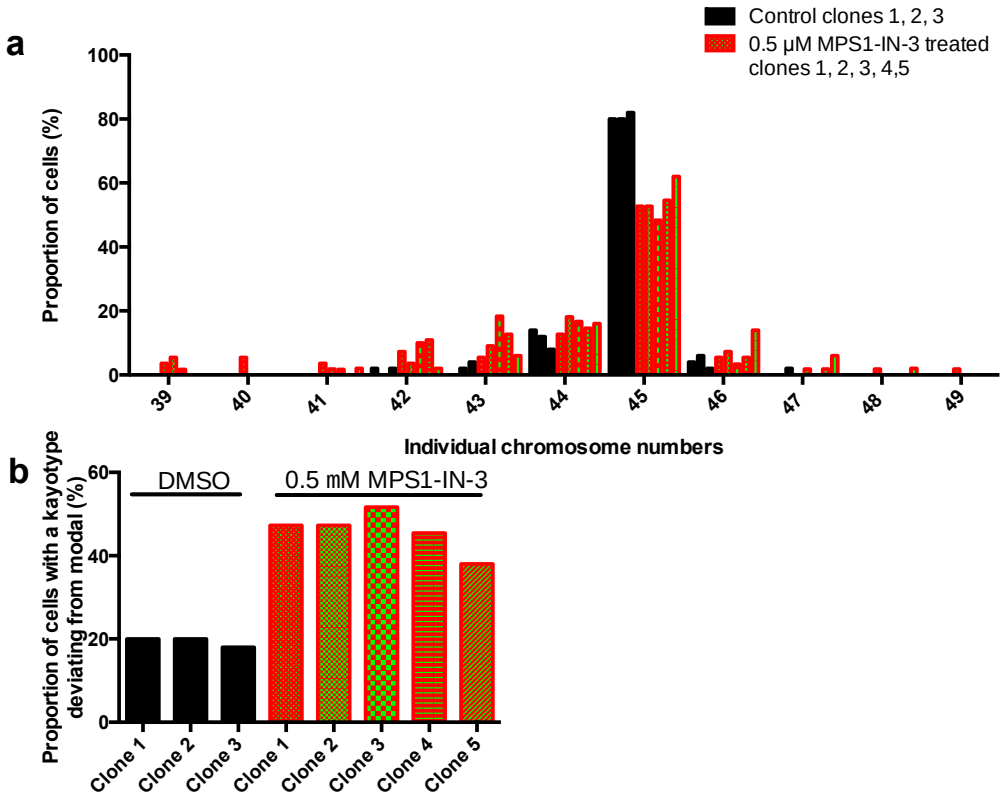

Figure 3-21 Treatment with the MPS1 inhibitor MPS1-IN-3 exhibits CIN.

(**a**) Karyotype analysis by chromosome counting in metaphase spreads of the indicated single cell clones derived from HCT116 cells treated with DMSO or 0.5 µM MPS1-IN-3 for 30 generations (n=50 cells). (**b**) The percentage of cells with chromosome numbers deviating from the modal were calculated.

3.4.2. Induction of CIN mediated by Mps1 inhibitor does not result in drug response differences towards Oxaliplatin or Cisplatin treatment in HCT116 cells.

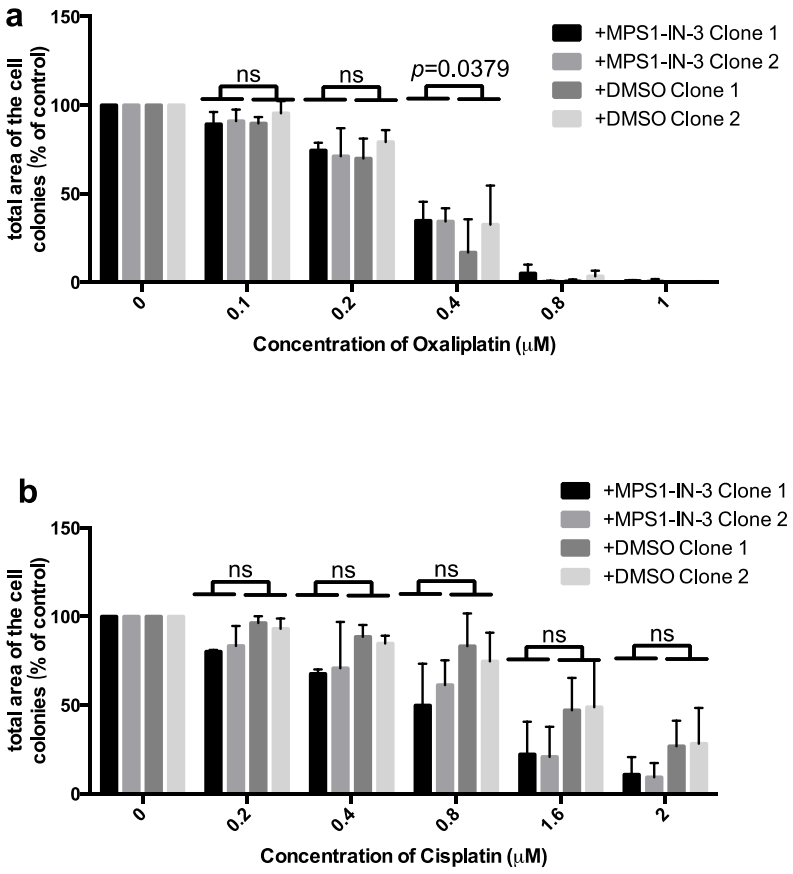

Figure 3-22 Quantification of the area of cell colonies of MPS1-IN-3 and DMSO-treated HCT116 cells treated with different concentration of Oxaliplatin or Cisplatin.

(a) Two independent DMSO-treated control single cell clones and two separate 0.5 μM MPS1-IN-3-treated single cell clones derived from HCT116 cells were treated with increasing concentrations of Oxaliplatin, and the area of the cell colonies was determined after ten days. (**b**) Two independent DMSO-treated control single cell clones and two independent 0.5 μM MPS1-IN-3-treated single cell clones from HCT116 cells were treated with increasing concentrations of Cisplatin, and the area of the cell colonies was determined after ten days. The areas of the cell colonies were normalized to control treated cells and represented as bar graphs (mean ± sem., *t*-test, n=3 independent experiments, ns = not significant).

To investigate whether induction of CIN by inhibition of the Mps1 kinase would affect drug responses in CRC cells, I used two independent single cell clones derived from 0.5 μM MPS1-IN-3 or DMSO treated HCT116 cells. These cell clones were treated with various concentrations of Oxaliplatin or Cisplatin and colony formation assays were performed. These analyses showed no significant differences in drug response towards Oxaliplatin (Figure 3-22a) or Cisplatin (Figure 3-22b)

Thus, induction of CIN by inhibition of the spindle assembly checkpoints in chromosomally stable CRC cells does not affect the drug responses towards Oxaliplatin or Cisplatin.

3.4.3. Short-term removal of MPS1-IN-3 does not result in drug response differences towards Oxaliplatin, but increases the resistance towards Cisplatin in one cell clone.

As treatment with Mps1 inhibitor induces CIN transiently, removal of MPS1-IN-3 is expected to reduce ongoing chromosome missegregation in cells. Hence, the two single cell clones of MPS1-IN-3 treated HCT116 cells were treated with different concentrations Oxaliplatin or Cisplatin in the presence or absence of MPS1-IN-3 and colony formation assays were performed.

These analyses showed that upon Oxaliplatin treatment, no significant response differences were detectable in the presence or absence MPS1-IN-3 during the assay (Figure 3-23a and 3-23b). Upon Cisplatin treatment, two concentrations of Cisplatin (0.8 and 1.6 μM) were slightly more resistant in the absence of MPS1-IN-3 during the assay in the first MPS1-IN-3 treated single cell clone (Figure 3.23c). However, using the second MPS1-IN-3 treated single cell clone of HCT116, no significant differences were found (Figure 3-23d).

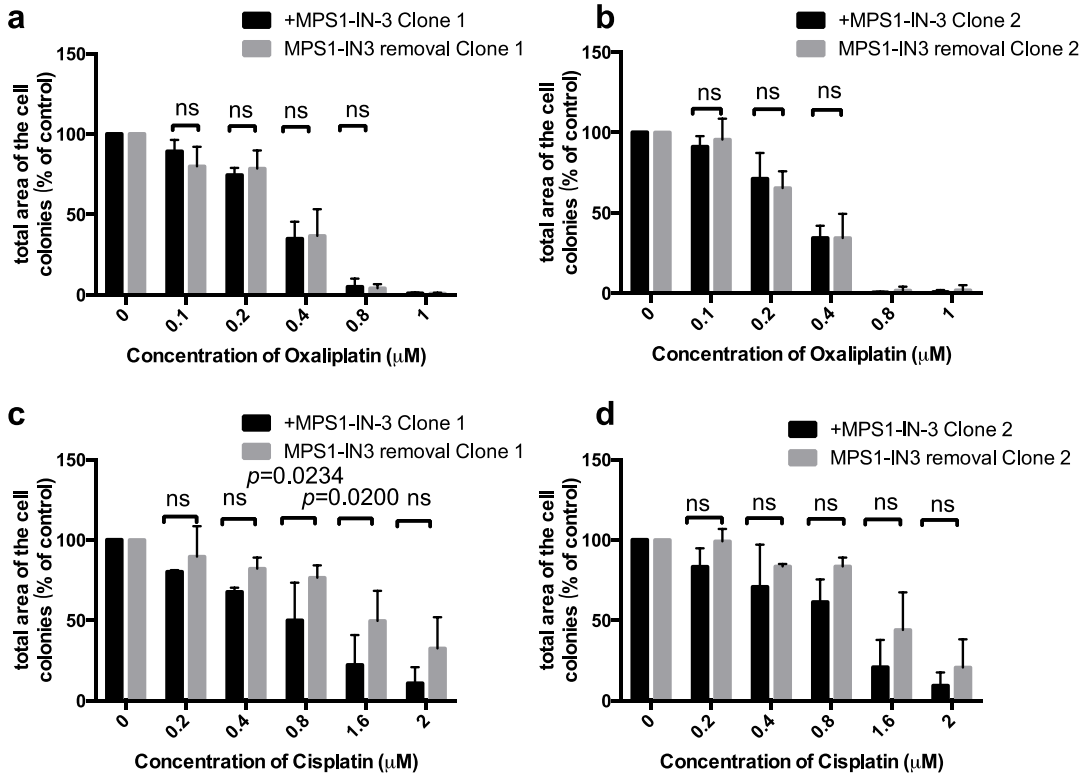

Figure 3-23 Quantification of the area of cell colonies of MPS1-IN-3 treated cells treated with different concentration of Oxaliplatin or Cisplatin in the presence or absence of MPS1-IN-3.

(**a**) Cells of MPS1-IN-3 treated clone 1 from HCT116 cells were treated with increasing concentrations of Oxaliplatin in the presence or absence of MPS1-IN-3, and the area of the cell colonies was determined after ten days. (**b**) Cells of MPS1-IN-3 treated clone 2 from HCT116 cells was treated with increasing concentrations of Oxaliplatin in the presence or absence of MPS1-IN-3, and the area of the cell colonies was determined after ten days. (**c**) Cells of MPS1-IN-3 treated clone 1 from HCT116 cells were treated with increasing concentrations of Cisplatin in the presence or absence of MPS1-IN-3, and the area of the cell colonies was determined after ten days. (**d**) Cells of MPS1-IN-3 treated clone 1 from HCT116 cells were treated with increasing concentrations of Cisplatin in the presence or absence of MPS1-IN-3, and the area of the cell colonies was determined after ten days. The areas of the cell colonies were normalized to control treated cells and represented as bar graphs (mean ±sem., *t*-test, n=3 independent experiments, ns = not significant).

Hence, induction of replication stress by Aphidicolin and inhibition of Mps1 are sufficient to cause CIN in chromosomally stable CRC cells. However, inducing of CIN by these two means does not result in drug response differences towards Oxaliplatin or Cisplatin compared to chromosomally stable cells.

4. Discussion

4.1. Drug responses towards commonly used chemotherapeutic drugs in CRC cells exhibiting CIN and after suppression of CIN.

The aim of my study was to investigate whether chromosomal instability (CIN) is causally related to differential drug responses in colorectal cancer cells. For this, I used two different cell systems based on *CHK2* deficient HCT116 cells and on SW620 cells that are both chromosomally unstable. In both cases, previous work from our lab has established that the CIN phenotype and thus, perpetual chromosome missegregations during mitosis are triggered by an increase in microtubule dynamics. Intriguingly, this defect can be corrected, e.g. by treatment of the cells with low doses of Taxol or by partial repression of the microtubule polymerase *ch-TOG/CKAP5* (Ertych et al. 2014). Therefore, I was able to directly compare the drug responses in individual single cell clones that were grown either in the absence or in the presence of Taxol or upon repression *ch-TOG/CKAP5*.

During the course of my work, I found that HCT116-*CHK2*$^{-/-}$ cells that grew in the absence of Taxol were more resistant toward Oxaliplatin compared to cells growing in the presence of Taxol (Figure 3-2a and 3-2b). Even short-term removal of Taxol had a similar effect (Figure 3-3a, and 3-3b). This might indicate that ongoing chromosome missegregations during mitosis may cause drug resistance, which is not dependent on karyotype evolvement over longer periods of time. Similarly, partially repression of *ch-TOG/CKAP5* caused a higher sensitivity towards Oxaliplatin in HCT116-*CHK2*$^{-/-}$ cells (Figure 3-4a), which might further confirm that ongoing chromosome missegregation during mitosis causes drug resistance.

Regarding the immediate response towards higher concentrations of Oxaliplatin, no response differences were observed in the presence or absence of Taxol (Figure 3-5). Since chromosome missegregation occurs only in 20%-50% CIN cells per cell cycle (Lengauer et al. 1997; Thompson and Compton 2008), this might not be sufficient to result in drug response differences between CIN cells and cells where CIN was suppressed.

In contrast to *CHK2* knockout HCT116 cells, no drug response differences towards Oxaliplatin were observed in two independent clones of SW620 cells that were grown in the presence or absence of Taxol (Figure 3-2c, 3-2d, 3-3c and 3.3d), although also in these cells a suppression of CIN in response to Taxol treatment have been established (Ertych et al. 2014). The SW620 cells with partial repression of *ch-TOG/CKAP5*, however, seemed to be more sensitive towards Oxaliplatin (Figure 3-4b). If we looked carefully into the drug response of the two independent clones of sh*ch-TOG* and sh*control*, the drug response of the two control clones was different. In this case, it is not possible to draw a clear conclusion that partially repression *ch-TOG/CKAP5* in SW620 causes more sensitivity towards Oxaliplatin.

Thus, while HCT116-*CHK2*$^{-/-}$ cells showed a decreased sensitivity towards Oxaliplatin that appears to be dependent on microtubule dynamics and/or CIN, this was not reproducible in SW620 cells. This might indicate that the suppression of chromosome missegregation in *CHK2* deficient cells by low-dose of Taxol is more efficient than in SW620 cells. Since also the short-term removal of Taxol affects drug response, it might be that the acute chromosome missegregation determines the response towards Oxaliplatin. Another possibility is that the observed difference in the response towards Oxaliplatin might be specifically due to the loss of *CHK2* rather than due to the presence of a CIN phenotype. Chk2 is a multifunctional kinase with known functions central to the induction of cell cycle arrest and apoptosis after DNA damage (Ahn et al. 2004; Antoni et al. 2007). Therefore, loss of *CHK2* in the HCT116-*CHK2*$^{-/-}$ cells might directly affect the response towards various DNA damaging drugs including Oxaliplatin. However, previous work showed that the loss of *CHK2* does neither affect p53 activation nor the DNA damage response after 5-FU-induced DNA damage (Jallepalli et al. 2003). It remains also unclear why Taxol treatment and partial repression of *ch-TOG/CKAP5* would affect the drug response in *CHK2* deficient cells. Nevertheless, my studies revealed that cancer cells with a loss of *CHK2* expression can be sensitized towards Oxaliplatin treatment by co-treatment with subnanomolar concentrations of Taxol, which might represent an interesting addition to the current chemotherapeutic treatment scheme for the colorectal cancer.

In contrast to Oxaliplatin, other chemotherapeutic drugs, including 5-FU, Adriamycin, and Irinotecan showed no drug response differences in two independent clones of HCT116-*CHK2*$^{-/-}$ or SW620 before or after CIN-suppression (Figure 3-10 - 3-16). This might indicate that chromosome missegregation caused drug resistance is specific to platinum compounds but the molecular reason for this remains unclear.

To clarify whether the observed resistance is specific to Oxaliplatin, I investigated also the response towards a second platinum compound namely Cisplatin, which is also widely used in the clinic for cancer therapy. Surprisingly, no drug response differences for Cisplatin were detectable in HCT116-*CHK2*$^{-/-}$ or in SW620 in the presence or absence of Taxol (Figure 3-6 and 3-7). On the other hand, partially repression of *ch-TOG/CKAP5*, which suppressed CIN, caused an increasing resistance towards Cisplatin in HCT116-*CHK2*$^{-/-}$ (Figure 3-9a). For SW620 cells, the two independent control clones showed opposite response as exhibited in Oxaliplatin treatment (Figure 3-9b), which represent inconsistent results overall.

Why is there such a difference in response towards two related platinum compounds? Both, Cisplatin and Oxaliplatin form DNA adducts. However, the structure of DNA adducts in response to Cisplatin and Oxaliplatin treatment are different (Figure 4-1) (Chaney et al. 2005).

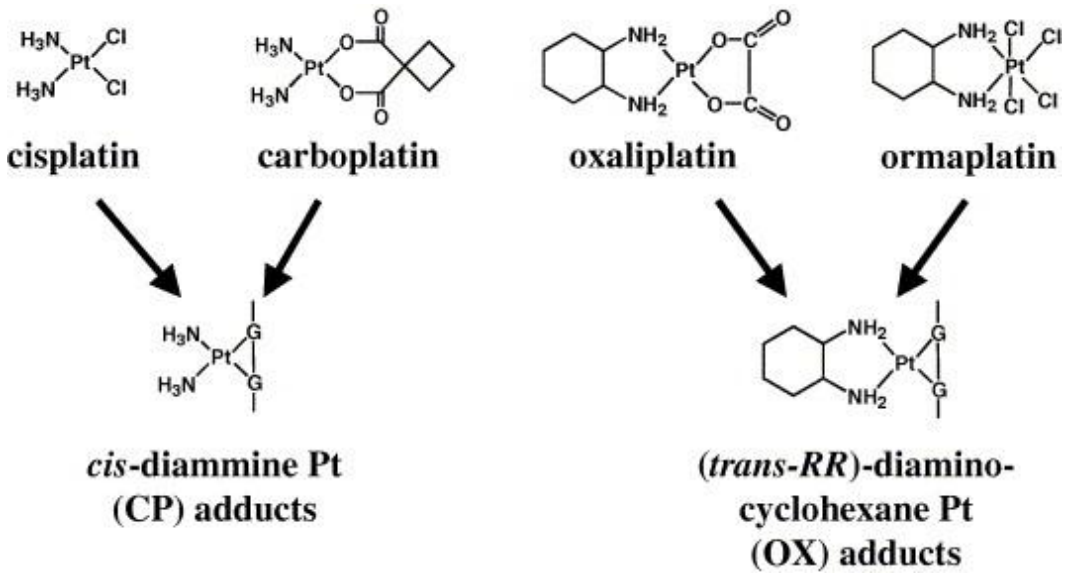

Figure 4-1 Selected platinum compounds and their DNA adducts. (from Chaney et al., 2005, p. 4)

Because of the structural differences of the Platinum-DNA adducts, these two platinum compounds have different affinity to cellular proteins. Binding of the cellular proteins to the Platinum-DNA adducts increases the cytotoxicity of the DNA adducts. Several studies showed that Cisplatin-DNA adducts has higher affinity to mismatch repair proteins and some damage-recognition proteins (McLaughlin et al. 1993; Wei et al. 2001). In contrast, Oxaliplatin-DNA adducts bypass translesion DNA polymerases, which determines whether the translesion synthesis is error-free or error-prone (Chaney et al. 2005). These differential outcomes of Cisplatin- and Oxaliplatin-DNA adducts are thought to contribute to the differences in cytotoxicity of Cisplatin and Oxaliplatin (Chaney et al. 2005), which might also explain why Cisplatin and Oxaliplatin showed entirely different responses regarding *ch-TOG/CKAP5* suppression of HCT116-*CHK2*$^{-/-}$ cells. But it is still unclear why no response difference towards Cisplatin was observed in HCT116-*CHK2*$^{-/-}$ in the presence or absence of Taxol.

4.2. Drug responses in chromosomally stable HCT116 cells and upon induction of CIN by replication stress or inhibition of the mitotic spindle assembly checkpoint.

Aphidicolin is a specific inhibitor of DNA polymerase α, which is primarily involved in DNA replication (Ikegami et al. 1978). Thus, Aphidicolin triggers replication fork instability and leads to instability of particular genomic regions called common fragile sites (CFSs), which are susceptible to replication-stress-induced double-strand breaks (Durkin and Glover 2007; Mazouzi et al. 2016). A recent study revealed that Aphidicolin triggers universal changes in both gene expression and protein phosphorylation patterns (Mazouzi et al. 2016). My work showed that treatment with low-doses of Aphidicolin treatment caused mitotic chromosome missegregation and CIN in chromosomally stable HCT116 cells (Figure 3-18). I used this condition to investigate whether induction of CIN affects drugs responses in CRC cells.

The second condition I used to induce CIN in chromosomally stable CRC cells was pharmacological inhibition of Mps1. Mps1 functions as an essential kinase that activates the spindle assembly checkpoint (SAC) to ensure proper segregation of

sister chromatids onto the daughter cells. It was demonstrated that small molecule inhibitors for the Mps1 kinase cause chromosome missegregation due to disruption of the SAC, thereby inducing CIN (Janssen et al. 2011). By karyotype analysis, I found that the prolonged treatment of HCT116 cells with the MPS1-IN-3 inhibitor indeed caused CIN and whole chromosome aneuploidy (Figure 3-21).

The mechanism of suppression of CIN by low-dose of Taxol or suppression of *ch-TOG/CKAP5* is by restoring proper microtubule assembly rates (Ertych et al. 2014). To compare the drug response between chromosomally stable and instable cells, other mechanisms leading to CIN were desired. Therefore, Aphidicolin and MPS1-IN-3 treated cells, both of which exhibiting CIN were used. However, after treatment with Oxaliplatin and Cisplatin, no drug response differences were observed between Aphidicolin or MPS1-IN-3-treated cells and control cells (Figure 3-19 and 3-22). Removal of Aphidicolin or MPS1-IN-3 resulted in slight resistance towards Cisplatin in one cell clone (Figure 3-20c and 3-23c), but no response differences in the other cell clone (Figure 3-20d and 3-23d), which indicates that the observed effect is not representative. Thus, inducing CIN by replication stress triggered by Aphidicolin or disturbing the spindle assembly checkpoint by treatment with a Mps1 inhibitor did not affect the drug response in HCT116 cells. While it cannot be excluded that the lack of response differences is specific for HCT116 cells, it might be desirable to investigate the induction of CIN in other cancer cell lines.

In summary, my results revealed microtubule dynamics-dependent resistance towards Oxaliplatin only in CIN cells with *CHK2* deficiency. Other CIN cells (e.g. SW620, chromosomally stable HCT116 cells treated by Aphidicolin or Mps1 inhibitor) did not show any response differences compared to non-CIN cells. These results are in contrast to a recent study by Lee et al. who showed that CIN is associated with multidrug resistance (Lee et al. 2011). The main difference between my study and Lee's study is that I used single cell clones with only little cell-to-cell heterogeneity. Several studies have suggested that tumor heterogeneity is associated with drug resistance in patients (Marusyk and Polyak 2010; Turner and Reis-Filho 2012). Clonal analysis of CIN and non-CIN cells are likely responsible for reduced genetic heterogeneity within the cell populations. Therefore, my results might indicate that

the CIN phenotype *per se* does not confer drug resistance, but that a high genetic heterogeneity within the cell population might contribute to altered drug responses.

4.3. *CHK2* deficiency: a key for resistance towards Oxaliplatin treatment?

Since only chromosomally instable *CHK2* deficient HCT116 cells showed a clear tendency of drug resistance towards Oxaliplatin, it seems plausible that the loss of *CHK2* function itself that might contribute to drug resistance.

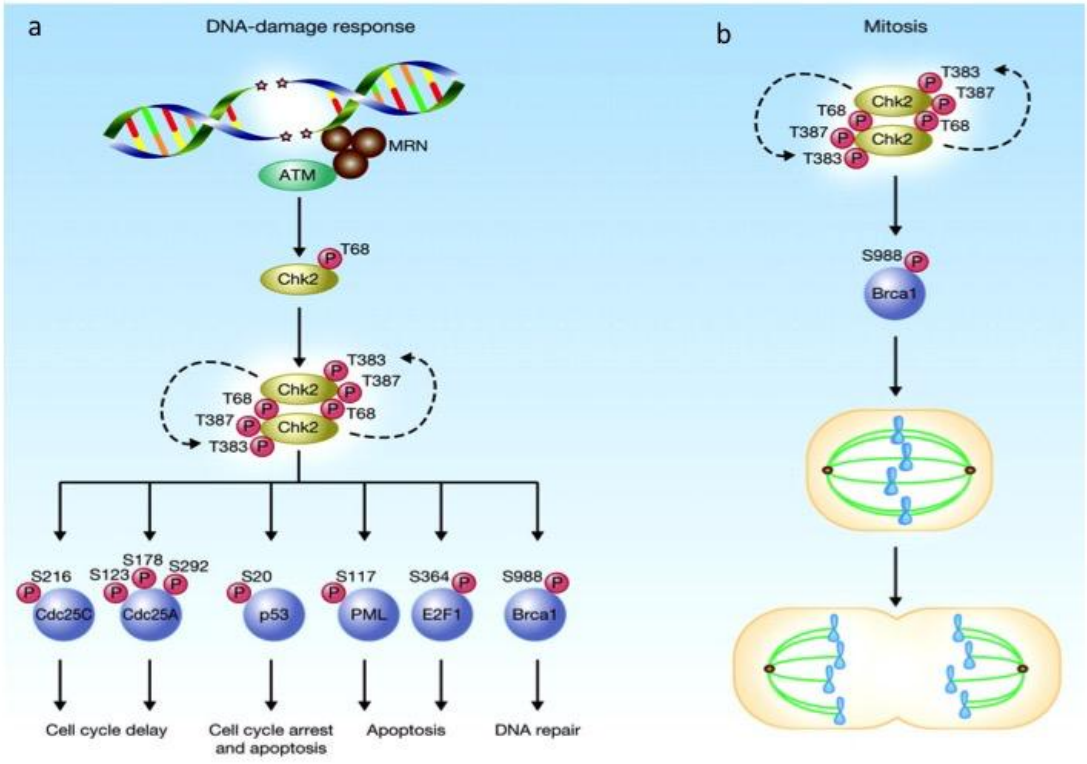

Figure 4-2 The role of Chk2 in DNA damage response and mitosis regulation.

(**a**) Upon DNA double strand breaks, the Chk2 kinase is activated by phosphorylation on threonine-68 by the ATM kinase. After homodimerization and *trans*-phosphorylation of threonine-383 and -387 located within the activation loop, the Chk2 kinase achieves its full activation. Subsequently, Chk2 can phosphorylate critical substrates such as Cdc25C, Cdc25A, p53, PML, E2F1 and Brca1, which is required to mediate cell cycle delay, cell cycle arrest, apoptosis and in response to DNA repair. (**b**) In the absence of DNA damage, the active Chk2 kinase can also

phosphorylate Brca1 on serine-988 during mitosis, which promotes the proper assembly of the normal mitotic spindle. (from Stolz et al., 2011, p. 402)

An important function of the Chk2 kinase is to operate downstream of ATM in response to DNA damage. Chk2 is phosphorylated at threonine-68 by the ATM kinase upon DNA double strand breaks (Ahn et al. 2004). After dimerization and full activation, the Chk2 kinase can mediate cell cycle arrest, apoptosis, and DNA repair through phosphorylating key substrates such as Cdc25C, Cdc25A, p53, PML, E2F1, and Brca1 (Figure 4-2a) (Stolz et al. 2011). In addition to DNA damage response, previous work from our lab also established Chk2 to be required for the maintenance of chromosomal stability during mitosis (Figure 4-2b). In fact, *CHK2* deficiency induces CIN in chromosomally stable cells by increasing microtubule assembly rates (Ertych et al. 2014; Stolz et al. 2010)

Cdc25C is a dual-specificity protein phosphatase that dephosphorylates Cdc2 thereby controlling the entry into mitosis (Peng et al. 1997). Upon DNA damage, Chk2 phosphorylates Cdc25C on serine-216, which causes its inhibition. As a result, the cell cycle arrests at the G_2/M transition (Matsuoka et al. 1998). Cdc25A can activate CDK2 that is needed for entry in S-phase and for DNA synthesis (Hoffmann et al. 1994). However, in response to DNA damage, Chk2 phosphorylates Cdc25A on serine-123, which mediates Cdc25A ubiquitylation and is subsequent degradation, preventing the CDK2 activation at the G_1/S transition (Falck et al. 2001; Jinno et al. 1994).

Previous studies revealed that *CHK2* deficient mice exhibit radioresistance (Falck et al. 2001; Takai et al. 2002), and this resistance is mediated by the ATM-Chk2-Cdc25A checkpoint pathway (Falck et al. 2001). Oxaliplatin forms DNA adducts and thereby also causes DNA damage. Thus, radioresistance and resistance towards Oxaliplatin might be mechanistically related. However, it remains elusive why the differential drug response is alleviated upon restoration of normal microtubule dynamics. It is advised that the future studies will address this important question.

Previous work from our lab also revealed that Chk2 activates Brca1 and thereby ensuring proper microtubule plus-end assembly and chromosomal stability during

mitosis. Loss function of Chk2 results in Aurora-A to act as a negative regulator of Brca1 during mitosis (Ertych et al. 2016). Interestingly, a recent study found that Brca1 inactivation causes resistance towards Oxaliplatin in CRC (Moutinho et al. 2014). Hence, Chk2 might act through Brca1 to regulate Oxaliplatin sensitivity. However, future work needs to address the underlying molecular mechanism leading to resistance towards Oxaliplatin treatment in CRC.

5. References

Abrieu A, Magnaghi-Jaulin L, Kahana JA, Peter M, Castro A, Vigneron S, Lorca T, Cleveland DW, Labbé J-C (2001): Mps1 is a kinetochore-associated kinase essential for the vertebrate mitotic checkpoint. Cell. 106, 83-93

Acharyya S, Oskarsson T, Vanharanta S, Malladi S, Kim J, Morris PG, Manova-Todorova K, Leversha M, Hogg N, Seshan VE, et al. (2012): A CXCL1 paracrine network links cancer chemoresistance and metastasis. Cell. 150, 165-178

Ahn J, Urist M, Prives C (2004): The Chk2 protein kinase. DNA Repair (Amst). 3, 1039-1047

Al-Bassam J, Chang F (2011): Regulation of microtubule dynamics by TOG-domain proteins XMAP215/Dis1 and CLASP. Trends Cell Biol. 21, 604-614

Antoni L, Sodha N, Collins I, Garrett MD (2007): CHK2 kinase: cancer susceptibility and cancer therapy - two sides of the same coin? Nat Rev Cancer. 7, 925-936

Arango D, Wilson AJ, Shi Q, Corner GA, Aranes MJ, Nicholas C, Lesser M, Mariadason JM, Augenlicht LH (2004): Molecular mechanisms of action and prediction of response to oxaliplatin in colorectal cancer cells. Br J Cancer. 91, 1931-1946

Bakhoum SF, Danilova OV, Kaur P, Levy NB, Compton DA (2011): Chromosomal instability substantiates poor prognosis in patients with diffuse large B-cell lymphoma. Clin Cancer Res. 17, 7704-7711

Barber TD, McManus K, Yuen KW, Reis M, Parmigiani G, Shen D, Barrett I, Nouhi Y, Spencer F, Markowitz S, et al. (2008): Chromatid cohesion defects may underlie chromosome instability in human colorectal cancers. Proc Natl Acad Sci U S A. 105, 3443-3448

Bastians H: Causes of chromosomal instability. In: Ghadimi BM, Ried T (Hrsg.): Chromosomal Instability in Cancer Cells. Recent Results in Cancer Research 200. Springer International Publishing, Cham 2015, 95-113

Benson AB, 3rd, Venook AP, Cederquist L, Chan E, Chen YJ, Cooper HS, Deming D, Engstrom PF, Enzinger PC, Fichera A, et al. (2017): Colon Cancer, Version 1.2017, NCCN Clinical Practice Guidelines in Oncology. J Natl Compr Canc Netw. 15, 370-398

Birkbak NJ, Eklund AC, Li QY, McClelland SE, Endesfelder D, Tan P, Tan IB, Richardson AL, Szallasi Z, Swanton C (2011): Paradoxical Relationship between Chromosomal Instability and Survival Outcome in Cancer. Cancer Res. 71, 3447-3452

Brattain MG, Fine WD, Khaled FM, Thompson J, Brattain DE (1981): Heterogeneity of malignant cells from a human colonic carcinoma. Cancer Res. 41, 1751-1756

Burrell RA, McClelland SE, Endesfelder D, Groth P, Weller MC, Shaikh N, Domingo E, Kanu N, Dewhurst SM, Gronroos E, et al. (2013): Replication stress links structural and numerical cancer chromosomal instability. Nature. 494, 492-496

Caspersson T, Farber S, Foley GE, Kudynowski J, Modest EJ, Simonsson E, Wagh U, Zech L (1968): Chemical differentiation along metaphase chromosomes. Exp Cell Res. 49, 219-222

Chaney SG, Campbell SL, Bassett E, Wu Y (2005): Recognition and processing of cisplatin- and oxaliplatin-DNA adducts. Crit Rev Oncol Hematol. 53, 3-11

Chen GB, Bradford WD, Seidel CW, Li R (2012): Hsp90 stress potentiates rapid cellular adaptation through induction of aneuploidy. Nature. 482, 246-250

Choi CM, Seo KW, Jang SJ, Oh YM, Shim TS, Kim WS, Lee DS, Lee SD (2009): Chromosomal instability is a risk factor for poor prognosis of adenocarcinoma of the lung: Fluorescence in situ hybridization analysis of paraffin-embedded tissue from Korean patients. Lung Cancer. 64, 66-70

Chung AS, Wu X, Zhuang G, Ngu H, Kasman I, Zhang J, Vernes JM, Jiang Z, Meng YG, Peale FV, et al. (2013): An interleukin-17-mediated paracrine network promotes tumor resistance to anti-angiogenic therapy. Nat Med. 19, 1114-1123

Dumont S, Mitchison TJ (2009): Force and length in the mitotic spindle. Curr Biol. 19, R749-761

Durkin SG, Glover TW (2007): Chromosome fragile sites. Annu Rev Genet. 41, 169-192

Ertych N, Stolz A, Stenzinger A, Weichert W, Kaulfuss S, Burfeind P, Aigner A, Wordeman L, Bastians H (2014): Increased microtubule assembly rates influence chromosomal instability in colorectal cancer cells. Nat Cell Biol. 16, 779-791

Ertych N, Stolz A, Valerius O, Braus GH, Bastians H (2016): CHK2-BRCA1 tumor-suppressor axis restrains oncogenic Aurora-A kinase to ensure proper mitotic microtubule assembly. Proc Natl Acad Sci U S A. 113, 1817-1822

Falck J, Mailand N, Syljuasen RG, Bartek J, Lukas J (2001): The ATM-Chk2-Cdc25A checkpoint pathway guards against radioresistant DNA synthesis. Nature. 410, 842-847

Feuk L, Carson AR, Scherer SW (2006): Structural variation in the human genome. Nat Rev Genet. 7, 85-97

Foley EA, Kapoor TM (2013): Microtubule attachment and spindle assembly checkpoint signalling at the kinetochore. Nat Rev Mol Cell Biol. 14, 25-37

Gartner M, Sunder-Plassmann N, Seiler J, Utz M, Vernos I, Surrey T, Giannis A (2005): Development and biological evaluation of potent and specific inhibitors of mitotic Kinesin Eg5. Chembiochem. 6, 1173-1177

Geigl JB, Obenauf AC, Schwarzbraun T, Speicher MR (2008): Defining 'chromosomal instability'. Trends Genet. 24, 64-69

Gerlinger M, Rowan AJ, Horswell S, Larkin J, Endesfelder D, Gronroos E, Martinez P, Matthews N, Stewart A, Tarpey P, et al. (2012): Intratumor heterogeneity and branched evolution revealed by multiregion sequencing. N Engl J Med. 366, 883-892

Gerlinger M, McGranahan N, Dewhurst SM, Burrell RA, Tomlinson I, Swanton C (2014): Cancer: evolution within a lifetime. Annu Rev Genet. 48, 215-236

Gregan J, Polakova S, Zhang L, Tolic-Norrelykke IM, Cimini D (2011): Merotelic kinetochore attachment: causes and effects. Trends Cell Biol. 21, 374-381

Gundem G, Van Loo P, Kremeyer B, Alexandrov LB, Tubio JM, Papaemmanuil E, Brewer DS, Kallio HM, Hognas G, Annala M, et al. (2015): The evolutionary history of lethal metastatic prostate cancer. Nature. 520, 353-357

Guzman C, Bagga M, Kaur A, Westermarck J, Abankwa D (2014): ColonyArea: an ImageJ plugin to automatically quantify colony formation in clonogenic assays. PLoS One. 9, e92444

Heald R, Tournebize R, Blank T, Sandaltzopoulos R, Becker P, Hyman A, Karsenti E (1996): Self-organization of microtubules into bipolar spindles around artificial chromosomes in Xenopus egg extracts. Nature. 382, 420-425

Hobor S, Van Emburgh BO, Crowley E, Misale S, Di Nicolantonio F, Bardelli A (2014): TGFalpha and amphiregulin paracrine network promotes resistance to EGFR blockade in colorectal cancer cells. Clin Cancer Res. 20, 6429-6438

Hoffmann I, Draetta G, Karsenti E (1994): Activation of the phosphatase activity of human cdc25A by a cdk2-cyclin E dependent phosphorylation at the G1/S transition. EMBO J. 13, 4302

Hurwitz H, Fehrenbacher L, Novotny W, Cartwright T, Hainsworth J, Heim W, Berlin J, Baron A, Griffing S, Holmgren E, et al. (2004): Bevacizumab plus irinotecan, fluorouracil, and leucovorin for metastatic colorectal cancer. N Engl J Med. 350, 2335-2342

Ikegami S, Taguchi T, Ohashi M, Oguro M, Nagano H, Mano Y (1978): Aphidicolin prevents mitotic cell division by interfering with the activity of DNA polymerase-α. Nature. 275, 458-460

Jallepalli PV, Lengauer C, Vogelstein B, Bunz F (2003): The Chk2 tumor suppressor is not required for p53 responses in human cancer cells. J Biol Chem. 278, 20475-20479

Janssen A, van der Burg M, Szuhai K, Kops GJPL, Medema RH (2011): Chromosome Segregation Errors as a Cause of DNA Damage and Structural Chromosome Aberrations. Science. 333, 1895-1898

Jinno S, Suto K, Nagata A, Igarashi M, Kanaoka Y, Nojima H, Okayama H (1994): Cdc25a Is a Novel Phosphatase Functioning Early in the Cell-Cycle. EMBO J. 13, 1549-1556

Kajstura M, Halicka HD, Pryjma J, Darzynkiewicz Z (2007): Discontinuous fragmentation of nuclear DNA during apoptosis revealed by discrete "sub-G1" peaks on DNA content histograms. Cytometry A. 71, 125-131

Khodjakov A, Cole RW, Oakley BR, Rieder CL (2000): Centrosome-independent mitotic spindle formation in vertebrates. Curr Biol. 10, 59-67

Lee AJ, Endesfelder D, Rowan AJ, Walther A, Birkbak NJ, Futreal PA, Downward J, Szallasi Z, Tomlinson IP, Howell M, et al. (2011): Chromosomal instability confers intrinsic multidrug resistance. Cancer Res. 71, 1858-1870

Leibovitz A, Stinson JC, McCombs WB, 3rd, McCoy CE, Mazur KC, Mabry ND (1976): Classification of human colorectal adenocarcinoma cell lines. Cancer Res. 36, 4562-4569

Lengauer C, Kinzler KW, Vogelstein B (1997): Genetic instability in colorectal cancers. Nature. 386, 623-627

Lengauer C, Kinzler KW, Vogelstein B (1998): Genetic instabilities in human cancers. Nature. 396, 643-649

Longley DB, Harkin DP, Johnston PG (2003): 5-fluorouracil: mechanisms of action and clinical strategies. Nat Rev Cancer. 3, 330-338

Marusyk A, Polyak K (2010): Tumor heterogeneity: causes and consequences. Biochim Biophys Acta. 1805, 105-117

Marusyk A, Tabassum D, Altrock P, Almendro V, Michor F, Polyak K (2014): Non-cell-autonomous driving of tumour growth supports sub-clonal heterogeneity. Nature. 514, 54-58

Matsuoka S, Huang M, Elledge SJ (1998): Linkage of ATM to cell cycle regulation by the Chk2 protein kinase. Science. 282, 1893-1897

Mazouzi A, Stukalov A, Muller AC, Chen D, Wiedner M, Prochazkova J, Chiang SC, Schuster M, Breitwieser FP, Pichlmair A, et al. (2016): A Comprehensive Analysis of the Dynamic Response to Aphidicolin-Mediated Replication Stress Uncovers Targets for ATM and ATMIN. Cell Rep. 15, 893-908

McGranahan N, Swanton C (2015): Biological and therapeutic impact of intratumor heterogeneity in cancer evolution. Cancer Cell. 27, 15-26

McGranahan N, Burrell RA, Endesfelder D, Novelli MR, Swanton C (2012): Cancer chromosomal instability: therapeutic and diagnostic challenges. EMBO Rep. 13, 528-538

McGranahan N, Favero F, de Bruin EC, Birkbak NJ, Szallasi Z, Swanton C (2015): Clonal status of actionable driver events and the timing of mutational processes in cancer evolution. Sci Transl Med. 7, 283ra254

McLaughlin K, Coren G, Masters J, Brown R (1993): Binding activities of cis-platin-damage-recognition proteins in human tumour cell lines. Int J Cancer. 53, 662-666

McNally FJ (2013): Mechanisms of spindle positioning. J Cell Biol. 200, 131-140

Mitchison T, Kirschner M (1984): Dynamic instability of microtubule growth. Nature. 312, 237-242

Moutinho C, Martinez-Cardus A, Santos C, Navarro-Perez V, Martinez-Balibrea E, Musulen E, Carmona FJ, Sartore-Bianchi A, Cassingena A, Siena S, et al. (2014): Epigenetic inactivation of the BRCA1 interactor SRBC and resistance to oxaliplatin in colorectal cancer. J Natl Cancer Inst. 106, djt322

Murayama-Hosokawa S, Oda K, Nakagawa S, Ishikawa S, Yamamoto S, Shoji K, Ikeda Y, Uehara Y, Fukayama M, McCormick F, et al. (2010): Genome-wide

single-nucleotide polymorphism arrays in endometrial carcinomas associate extensive chromosomal instability with poor prognosis and unveil frequent chromosomal imbalances involved in the PI3-kinase pathway. Oncogene. 29, 1897-1908

Musacchio A, Salmon ED (2007): The spindle-assembly checkpoint in space and time. Nat Rev Mol Cell Biol. 8, 379-393

Myers CE, McGuire WP, Liss RH, Ifrim I, Grotzinger K, Young RC (1977): Adriamycin: the role of lipid peroxidation in cardiac toxicity and tumor response. Science. 197, 165-167

Nakagawa Y, Numoto K, Yoshida A, Kunisada T, Ohata H, Takeda K, Wai D, Poremba C, Ozaki T (2006): Chromosomal and genetic imbalances in synovial sarcoma detected by conventional and microarray comparative genomic hybridization. J Cancer Res Clin Oncol. 132, 444-450

Nakajima M, Kumada K, Hatakeyama K, Noda T, Peters JM, Hirota T (2007): The complete removal of cohesin from chromosome arms depends on separase. J Cell Sci. 120, 4188-4196

Nakamura H, Saji H, Idiris A, Kawasaki N, Hosaka M, Ogata A, Saijo T, Kato H (2003): Chromosomal instability detected by fluorescence in situ hybridization in surgical specimens of non-small cell lung cancer is associated with poor survival. Clin Cancer Res. 9, 2294-2299

Nathans D (1964): Puromycin Inhibition of Protein Synthesis - Incorporation of Puromycin into Peptide Chains. Proc Nati Acad Sci U S A. 51, 585-592

Neelakantan D, Drasin DJ, Ford HL (2015): Intratumoral heterogeneity: Clonal cooperation in epithelial-to-mesenchymal transition and metastasis. Cell Adh Migr. 9, 265-276

Nicholson JM, Cimini D (2013): Cancer karyotypes: survival of the fittest. Front Oncol. 3, 148

Nowell PC (1976): The clonal evolution of tumor cell populations. Science. 194, 23-28

Ohyashiki K, Kuroda M, Ohyashiki JH: Chromosomes and Chromosomal Instability in Human Cancer. In: William B.C, Gregory J.T (Hrsg.): The Molecular Basis of Human Cancer. Springer New York, New York, NY 2017, 241-262

Peng CY, Graves PR, Thoma RS, Wu Z, Shaw AS, Piwnica-Worms H (1997): Mitotic and G2 checkpoint control: regulation of 14-3-3 protein binding by phosphorylation of Cdc25C on serine-216. Science. 277, 1501-1505

Pihan GA, Purohit A, Wallace J, Knecht H, Woda B, Quesenberry P, Doxsey SJ (1998): Centrosome defects and genetic instability in malignant tumors. Cancer Res. 58, 3974-3985

Pitot HC, Wender DB, Oconnell MJ, Schroeder G, Goldberg RM, Rubin J, Mailliard JA, Knost JA, Ghosh C, Kirschling RJ, et al. (1997): Phase II trial of irinotecan in patients with metastatic colorectal carcinoma. Journal of Clinical Oncology. 15, 2910-2919

Polyak K, Marusyk A (2014): Cancer: clonal cooperation. Nature. 508, 52-53

Prosser SL, Pelletier L (2017): Mitotic spindle assembly in animal cells: a fine balancing act. Nat Rev Mol Cell Biol. 18, 187-201

Rao CV, Sanghera S, Zhang YT, Biddick L, Reddy A, Lightfoot S, Janakiram NB, Mohammed A, Dai W, Yamada HY (2016): Systemic Chromosome Instability Resulted in Colonic Transcriptomic Changes in Metabolic, Proliferation, and Stem Cell Regulators in Sgo1(-/+) Mice. Cancer Res. 76, 630-642

Rosenthal R, McGranahan N, Herrero J, Swanton C (2017): Deciphering Genetic Intratumor Heterogeneity and Its Impact on Cancer Evolution. Annu Rev Cancer Biol. 1, 223-240
http://www.annualreviews.org/doi/full/10.1146/annurev-cancerbio-042516-011348

Rougier P, Bugat R, Douillard JY, Culine S, Suc E, Brunet P, Becouarn Y, Ychou M, Marty M, Extra JM, et al. (1997): Phase II study of irinotecan in the treatment of advanced colorectal cancer in chemotherapy-naive patients and patients pretreated with fluorouracil-based chemotherapy. J Clin Oncol. 15, 251-260

Rowan A, Halford S, Gaasenbeek M, Kemp Z, Sieber O, Volikos E, Douglas E, Fiegler H, Carter N, Talbot I, et al. (2005): Refining molecular analysis in the pathways of colorectal carcinogenesis. Clin Gastroenterol Hepatol. 3, 1115-1123

Roylance R, Endesfelder D, Gorman P, Burrell RA, Sander J, Tomlinson I, Hanby AM, Speirs V, Richardson AL, Birkbak NJ, et al. (2011): Relationship of extreme chromosomal instability with long-term survival in a retrospective analysis of primary breast cancer. Cancer Epidemiol Biomarkers Prev. 20, 2183-2194

Sacristan C, Kops GJPL (2015): Joined at the hip: kinetochores, microtubules, and spindle assembly checkpoint signaling. Trends Cell Biol. 25, 21-28

Saltz LB, Cox JV, Blanke C, Rosen LS, Fehrenbacher L, Moore MJ, Maroun JA, Ackland SP, Locker PK, Pirotta N, et al. (2000): Irinotecan plus fluorouracil and leucovorin for metastatic colorectal cancer. Irinotecan Study Group. N Engl J Med. 343, 905-914

Sato H, Uzawa N, Takahashi K, Myo K, Ohyama Y, Amagasa T (2010): Prognostic utility of chromosomal instability detected by fluorescence in situ hybridization in fine-needle aspirates from oral squamous cell carcinomas. BMC Cancer. 10, 182

Saxton WM, McIntosh JR (1987): Interzone microtubule behavior in late anaphase and telophase spindles. J Cell Biol. 105, 875-886

Schiff PB, Fant J, Horwitz SB (1979): Promotion of microtubule assembly in vitro by taxol. Nature. 277, 665-667

Siddik ZH (2003): Cisplatin: mode of cytotoxic action and molecular basis of resistance. Oncogene. 22, 7265-7279

Silver A, Sengupta N, Propper D, Wilson P, Hagemann T, Patel A, Parker A, Ghosh A, Feakins R, Dorudi S, et al. (2012): A distinct DNA methylation profile associated with microsatellite and chromosomal stable sporadic colorectal cancers. Int J Cancer. 130, 1082-1092

Sinicrope FA, Rego RL, Foster N, Sargent DJ, Windschitl HE, Burgart LJ, Witzig TE, Thibodeau SN (2006): Microsatellite instability accounts for tumor site-related differences in clinicopathologic variables and prognosis in human colon cancers. Am J Gastroenterol. 101, 2818-2825

Siravegna G, Mussolin B, Buscarino M, Corti G, Cassingena A, Crisafulli G, Ponzetti A, Cremolini C, Amatu A, Lauricella C (2015): Clonal evolution and resistance to EGFR blockade in the blood of colorectal cancer patients. Nat Med. 21, 795-801

Smid M, Hoes M, Sieuwerts AM, Sleijfer S, Zhang Y, Wang YX, Foekens JA, Martens JWM (2011): Patterns and incidence of chromosomal instability and their prognostic relevance in breast cancer subtypes. Breast Cancer Res Treat. 128, 23-30

Stolz A, Ertych N, Kienitz A, Vogel C, Schneider V, Fritz B, Jacob R, Dittmar G, Weichert W, Petersen I, et al. (2010): The CHK2-BRCA1 tumour suppressor pathway ensures chromosomal stability in human somatic cells. Nat Cell Biol. 12, 492-499

Stolz A, Ertych N, Bastians H (2011): Tumor suppressor CHK2: regulator of DNA damage response and mediator of chromosomal stability. Clin Cancer Res. 17, 401-405

Takai H, Naka K, Okada Y, Watanabe M, Harada N, Saito S, Anderson CW, Appella E, Nakanishi M, Suzuki H, et al. (2002): Chk2-deficient mice exhibit radioresistance and defective p53-mediated transcription. EMBO J. 21, 5195-5205

Tanaka K, Hirota T (2009): Chromosome segregation machinery and cancer. Cancer Sci. 100, 1158-1165

Tanaka K, Hirota T (2016): Chromosomal instability: A common feature and a therapeutic target of cancer. Biochim Biophys Acta. 1866, 64-75

Taniguchi K, Okami J, Kodama K, Higashiyama M, Kato K (2008): Intratumor heterogeneity of epidermal growth factor receptor mutations in lung cancer and its correlation to the response to gefitinib. Cancer Sci. 99, 929-935

Tannous BA, Kerami M, Van der Stoop PM, Kwiatkowski N, Wang J, Zhou W, Kessler AF, Lewandrowski G, Hiddingh L, Sol N, et al. (2013): Effects of the selective MPS1 inhibitor MPS1-IN-3 on glioblastoma sensitivity to antimitotic drugs. J Natl Cancer Inst. 105, 1322-1331

Terada Y, Tatsuka M, Suzuki F, Yasuda Y, Fujita S, Otsu M (1998): AIM-1: a mammalian midbody-associated protein required for cytokinesis. EMBO J. 17, 667-676

Thompson SL, Bakhoum SF, Compton DA (2010): Mechanisms of chromosomal instability. Curr Biol. 20, R285-295

Thompson SL, Compton DA (2008): Examining the link between chromosomal instability and aneuploidy in human cells. J Cell Biol. 180, 665-672

Tournebize R, Popov A, Kinoshita K, Ashford AJ, Rybina S, Pozniakovsky A, Mayer TU, Walczak CE, Karsenti E, Hyman AA (2000): Control of microtubule dynamics by the antagonistic activities of XMAP215 and XKCM1 in Xenopus egg extracts. Nat Cell Biol. 2, 13-19

Turner NC, Reis-Filho JS (2012): Genetic heterogeneity and cancer drug resistance. Lancet Oncol. 13, e178-185

Vermeulen K, Van Bockstaele DR, Berneman ZN (2003): The cell cycle: a review of regulation, deregulation and therapeutic targets in cancer. Cell Prolif. 36, 131-149

Walther A, Houlston R, Tomlinson I (2008): Association between chromosomal instability and prognosis in colorectal cancer: a meta-analysis. Gut. 57, 941-950

Watanabe T, Kobunai T, Yamamoto Y, Matsuda K, Ishihara S, Nozawa K, Yamada H, Hayama T, Inoue E, Tamura J, et al. (2012): Chromosomal instability (CIN) phenotype, CIN high or CIN low, predicts survival for colorectal cancer. J Clin Oncol. 30, 2256-2264

Wei M, Cohen SM, Silverman AP, Lippard SJ (2001): Effects of spectator ligands on the specific recognition of intrastrand platinum-DNA cross-links by high mobility group box and TATA-binding proteins. J Biol Chem. 276, 38774-38780

Wood RD (1997): Nucleotide excision repair in mammalian cells. J Biol Chem. 272, 23465-23468

Yates LR, Gerstung M, Knappskog S, Desmedt C, Gundem G, Van Loo P, Aas T, Alexandrov LB, Larsimont D, Davies H, et al. (2015): Subclonal diversification of primary breast cancer revealed by multiregion sequencing. Nat Med. 21, 751-759

Yoo JW, Seo KW, Jang SJ, Oh YM, Shim TS, Kim WS, Lee DS, Lee SD, Choi CM (2010): The Relationship between the Presence of Chromosomal Instability and Prognosis of Squamous Cell Carcinoma of the Lung: Fluorescence in situ Hybridization Analysis of Paraffin-embedded Tissue from 47 Korean Patients. J Korean Med Sci. 25, 863-867

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Curriculum Vitae

My name is Xiyang Liu, I was born on the 28th of November 1985 in Shaodong, Hunan, China. I was enrolled in Shuangquanpu Primary school, Hunan, China in September 1992. After 12-semester studies I completed my elementary study in July 1998. In September of this year, I began my studies in Shuangquanpu Junior High School, Hunan, China. I finished my junior high school in July 2001. After then I started my high school education in Shaodong First High School, Hunan, China in September 2001, and finished it in June 2004.

After school education, I started to study Clinical Medicine in September 2005 in Zhengzhou University, Henan, China. After 10-semester of studies I got my bachelor degree in July 2010. I continued my master studies in the same university, and specialized on oncology studies in September 2010. During the master study period, I studied the topic "Exploring the relationship between CD5 expression and the clinical pathologic features and therapeutic effect of diffuse large B-cell lymphoma" and was awarded with a Master degree in Oncology in July 2013. In addition, I passed the National Medical Practitioner's Qualification Exam and got the certificate in 2011.

In 2013, I got the scholarship from the China Scholarship Council for four years to pursue the doctoral studies in medicine in Germany. I was enrolled in Heidelberg University and started the project in the group of Prof. Dr. Thorsten Zenz in the institute of German Cancer Research Center (DKFZ) and National Center For Tumor Disease (NCT) in September 2013. However, after two years of studies in Heidelberg, I found the project I was working would not work out and I decided to move. I was luckily to meet Prof. Dr. Holger Bastians who offered me a position as a candidate of Dr. med. in his group. I started my M.D. thesis in his lab in Göttingen, and was enrolled in the Medical Faculty of Göttingen University in June 2016, and I expect to finish my M.D. studies in summer 2017.