We are IntechOpen, the world's leading publisher of Open Access books Built by scientists, for scientists

4,800
Open access books available

122,000
International authors and editors

135M
Downloads

Our authors are among the

154
Countries delivered to

TOP 1%
most cited scientists

12.2%
Contributors from top 500 universities

WEB OF SCIENCE™

Selection of our books indexed in the Book Citation Index in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?
Contact book.department@intechopen.com

Numbers displayed above are based on latest data collected.
For more information visit www.intechopen.com

Chapter 6

Genomic Instability of Pluripotent Stem Cells: Origin and Consequences

Elena Lo Furno, Siem van der Laan and Domenico Maiorano

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/62906

Abstract

Maintenance of genomic stability is crucial in ensuring cellular homeostasis and perpetuation of life. Perpetuation of the genetic information relies upon faithful replication of the genome. Mutations, generated during DNA synthesis and/or cell division and induced by exposure to external chemical agents, are drivers of genetic and associated genomic instability believed to fuel malignant transformation. Curiously, pluripotent stem cells (PSCs) are characterized by a high degree of genomic instability of unknown origin, which resembles that observed in cancer cells. This peculiar feature of PSCs raises the questions of the reasons responsible for this apparent aberrant regulation and of how genome integrity is kept under control. Genomic instability of PSCs also raises important concerns about their use in regenerative medicine, which sets severe limitations in clinical applications. The aim of this chapter is to review current knowledge about the molecular grounds of genomic instability of PSCs of diverse origin, such as embryonic (ESCs), induced pluripotent (iPSCs), and adult (ASCs) stem cells. We will also review how these features undermine the use of PSCs in clinical applications and discuss new emerging perspectives aimed at reducing genomic instability so to improve their use in clinical applications.

Keywords: DNA damage, checkpoints, replication stress, oncogenesis, nucleus, chromatin

1. Introduction

Maintenance of genome stability is primordial for stem cells, given their potential to generate multiple distinct cell lineages. Mutations may lead to the inheritance of DNA discontinuities in

differentiated cells with potentially catastrophic consequences such as chromosomal rearrangements and deletions [1]. Genetic aberrancies can affect the stem cell pool or increase the chances of malignant transformation since these can lead to oncogenes activation and/or tumor suppressors silencing [2, 3]. Paradoxically, embryonic stem cells (ESCs) and induced pluripotent stem cells (iPSCs) display signs of genomic instability, to a level comparable to that observed in cancer cells [4]. In contrast, adult stem cells (ASCs), which have a strongly reduced pluripotency, appear to have more stable genomes. The occurrence of genomic instability also undermines the use of PSCs in regenerative medicine since these cells are known to induce tumors once injected in the organism.

2. Molecular basis of genomic instability

Genomic instability consists in the tendency of cells to accumulate mutations that directly or indirectly affect the structure of the genome, such as deletions, translocations, variation in the chromosomes copy number (CNVs) [5]. Maintenance of genome stability depends upon cellular processes that regulate DNA metabolism, such as DNA replication, transcription, repair, chromatin remodeling and their coordination with the cell cycle. Such coordination is orchestrated by cell cycle checkpoints [6]. Once activated, these signaling pathways slow down the cell cycle, activate DNA repair, and promote recovery of proliferation so to ensure that genetic information is faithfully transmitted to the daughter cells. For instance, the S-phase checkpoint restrains the onset of M-phase so to ensure that all DNA has been replicated before cells enter division. On the other hand, M-phase checkpoint delays anaphase so to ensure that condensed chromosomes are faithfully transmitted to the daughter cell. Importantly, checkpoints also preserve tissues homeostasis, since they can trigger cell death to avoid propagation of cells with unstable and/or highly damaged genomes [5, 7] (see also **Figure 1**).

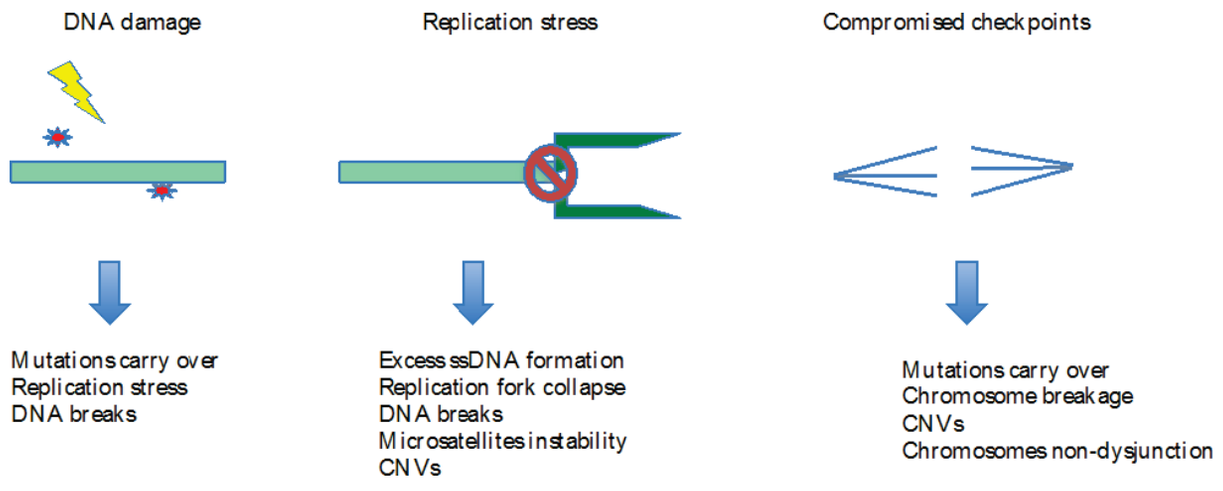

Figure 1. Main causes of genomic instability. Schematic representation of the main causes of genomic instability observed in cells. See text for more details.

http://dx.doi.org/10.5772/62906

2.1. DNA damage

Exogenous cues, such as chemical and radiations, for instance, but equally the metabolism of the DNA itself, generate DNA damage that threatens genome integrity. DNA damage elicits a DNA damage response (DDR) by activating cell cycle checkpoints [8]. Efficient DNA repair mechanisms ensure that DNA lesions are fixed to minimize loss or modification of the genetic information; among these are nucleotide excision repair (NER), base excision repair (BER), mismatch repair (MMR), post-replication repair (PRR), interstrand crosslinks (ICL) repair, homologous recombination (HR), and non-homologous end joining (NHEJ). A defect in any of these DNA repair pathways can compromise genome stability directly, by affecting the structure of the chromosome, or indirectly by generating mutations in genes important for the maintenance of genomic stability. Hence, occurrence of mutations in genes controlling cell cycle checkpoints, promote strong chromosome imbalance [9]. Notwithstanding, not all of these pathways allow faithfully repair of DNA lesions. For instance, repair of double-strand breaks (DSBs) by NHEJ, which involves fusion of broken DNA ends after processing without template assistance, is error-prone [10], in contrast to HR which requires a DNA template for repair and is more error-free. Equally, ICL repair and PRR, both involving DNA translesion synthesis, are also error-prone.

2.2. DDR

The DDR involves the activation of apical PI3KK protein kinases DNA–PK*cs*, ATR, and ATM. ATR is most closely related to ATM, a protein kinase encoded by the gene mutated in the ataxia telangiectasia syndrome. This disorder is characterized by a greatly reduced ability to repair radiation-induced DSBs and increased risk of developing cancer [11]. Activation of PI3KK follows a phosphorylation cascade that leads to the activation of a large number of substrates [12], among which the tumor suppressor proteins p53, BRCA1, and CHK1. These proteins also gather at sites of DNA damage and inhibit DNA replication and cell division apart from promoting DNA repair, recombination, or apoptosis. For example, activated CHK1 (its phosphorylated form) delays cell cycle progression, stabilizes stalled replication forks, and induces the S-phase checkpoint [13]. ATR is activated following several forms of DNA damage, including damaged nucleotides, stalled replication forks, and indirectly by DSBs [14]. ATM instead is more specialized in the response to DSBs and in sensing modifications of the chromatin state. DNA–PK*cs* is involved in the repair of DSBs by non-homologous recombination, and more recently, it has also been implicated in signaling DNA damage synergistically with ATR [15–17]. CHK1 and/or CHK2 phosphorylation mediates cell cycle slow down or arrest by affecting the stability and post-translational state of master cell cycle regulators, such as CDC25 proteins (A, B, and C) and CDKs.

In S-phase, ATR is chromatin-bound to monitor replication fork progression [18] and is activated following generation of excess single-stranded (ss) DNA as a result of replication forks delay or stalling at damaged sites [19]. ATR activation requires synthesis of replication intermediates onto ssDNA followed by the recruitment of specific proteins that recognize this substrate, such as Rad17 and the 9-1-1 checkpoint clamp [20–24]. ATR-dependent phosphorylation of the histone variant H2AX (γH2AX) constitutes a widely used marker of replication

stress (RS) and genomic instability [25]. ATR can be also recruited and activated at DSBs following generation of ssDNA by resection [14].

ATM assembles at DSBs immediately after their formation. Its recruitment depends upon the MRN trimeric complex (made of Mre11, Rad50, and Nbs1 proteins), which holds two DNA ends together, by interacting with Nbs1. DNA damage results in ATM conversion from an inactive homodimer into an active monomer with protein kinase activity [26], which phosphorylates effector molecules that carry out the DDR including H2AX, p53, BRCA1, CHK2, RAD17, RAD9, NBS1 to form repair foci. The MDC1 protein is recruited by γH2AX *via* its BRCT domains and is phosphorylated by ATM, mediating the localization of ubiquitin ligase RNF8 that triggers monoubiquitination of H2AX. RNF168, a second ubiquitin ligase, is recruited and amplifies the ubiquitination response resulting in γH2AX polyubiquitination, which leads to the recruitment of Rad18, p53-binding protein 1 (53BP1), and BRCA1, among other proteins thus promoting DBSs repair by either HR or NHEJ [27].

2.3. RS

RS, defined as a more or less pronounced slow down or arrest of the DNA replication process, is a major source of genomic instability in proliferating cells [28]. Many obstacles can interfere with DNA synthesis. These can be specialized DNA or chromatin structures, or DNA damage (see below). The metabolism of the cell can also induce RS by affecting the availability of nucleotides and/or proteins required for DNA synthesis [29], as well as by production of reactive oxygen species (ROS) that generate a large amount of DNA lesions (about 100,000 per cell per day in an organism) [30]. RS is also generated by interference between DNA synthesis and DNA transcription induced, for instance, by unscheduled re-entry into the cell cycle, a situation observed during malignant transformation [31] and during reprogramming of somatic cells into iPSCs (see Section 3.2). Conflicts between DNA replication and transcription may lead to under replication of the genome [32, 33] as a result of DNA synthesis arrest, or to over replication as a result of aberrant reinitiation of DNA synthesis induced by certain oncogenes [32]. RS induces DNA damage (whose molecular bases are not completely understood) and thus generates a cellular response similar to that observed when cells are challenged with DNA damaging agents.

At the molecular level, the consequence of RS can be: (a) generation of excess ssDNA if the progression of the DNA polymerases and not that of the replicative DNA helicase is perturbed. In this situation, the ssDNA binding protein RPA is recruited and the replication fork can undergo remodeling in a process known as fork regression, dependent upon the Rad51 protein, to limit the extent of ssDNA; (*b*) a pause or a permanent arrest of the replication fork with no excess ssDNA formation due to an impediment to both DNA polymerases and helicase activities ([34] for review). In this situation, stalled replication forks can restart through generation of DSBs followed by resection and HR mediated by the PARP-1 enzyme [28]. (c) Generation of extra copies of the DNA as a result of over replication of the genome leading to collision between replication forks [32]. Recent evidence highlights the presence of regressed replication forks in G_2/M phases generated by unscheduled activation of Cyclin E and CDC25A [35]. At this stage, the endonuclease Mus81 can cleave the DNA and replication can occur to

minimize the lost of genetic information. Nevertheless, DNA damage that could not be fixed before entering mitosis persists in the next cell cycle leading to the formation of nuclear bodies containing 53BP1 in G_1 phase [36]. These nuclear bodies appear symmetrical in the two daughter cells suggesting that they probably mark sister loci from the previous S-phase, where unresolved replication intermediates are still present. This ATM-dependent process hints to the possibility that ATM activation by RS is necessary to preserve genome integrity into following cell cycles.

RS-induced genome instability is a feature of almost all human cancers which can arise from mutations in DNA repair genes as stated by the mutator hypothesis [37]. According to this model, genomic instability is present in precancerous lesions and causes tumor development by increasing the spontaneous mutation rate. Then, mutations occurring in genes controlling the DNA damage checkpoint would allow anarchic proliferation of cells having collapsed replication forks and unstable genomes [38, 39]. The main instability found in cancer cells is chromosomal instability (CIN) or CNVs, where chromosome structure and number varies significantly in comparison with normal cells. Cancer cells can show other forms of genomic instability, including microsatellite instability (MSI or MIN), in which the number of DNA repeats present in microsatellite sequences increases or decreases [40, 41] in addition to increased frequencies of base pair mutations [42, 43].

3. Genomic instability of stem cells

Genomic instability has been extensively reported for ESCs, while ASCs appear to have a much more stable genome. Chromosomes 8 and 11 trisomy in ESCs [44, 45] and trisomy of chromosomes 12 and 17 in hESCs [46] with the amplification of the chromosome arm 20p in these latter have been observed [47]. These changes were reported to confer proliferation advantage. In addition, hESCs were reported to have the tendency to become aneuploid [48]. Very recent data now suggest that aneuploidy in hESCs arises as consequence of RS and chromosome condensation defects [49]. Detection of several markers of RS has been reported in ESCs (though without full activating the DDR) compared with somatic cells, suggesting that ESCs have a strong predisposition to genetic instability. One explanation for this feature may be that ESCs exhibit a contracted cell cycle structure, consisting of a short G_1- and G_2-phase and a high proportion of cells in S-phase [50–53]. These cells are also marked by open heterochromatic structure and an abundance of chromatin-remodeling factors [54, 55].

3.1. ESCs

Due to a highly contracted cell cycle, mESCs have an inefficient G_1/S checkpoint which does not allow them to arrest in G_1 in the presence of DNA damage [56–58], while the S-phase checkpoint is normally activated [53]. The consequence of this regulation is that lesions generated in G_1 are not sensed and therefore cannot be efficiently repaired, so they will persist in S-phase. For instance, unrepaired ssDNA breaks generated in G_1 may be replicated during S-phase, thus generating DSBs that in turn can induce genomic rearrangements. Curiously,

the situation seems to be inversed in hESCs, where the G_1/S checkpoint has been suggested to be functional [59], while the S-phase checkpoint appears to be inefficient [60]. This difference can be explained as possible differences in the molecular circuits that regulate pluripotency between mouse and human stem cells. Absence of a G_1/S checkpoint in mESCs was originally suggested to be due to inefficient p53 function [57]. However, it has been shown that p53 can transactivate target genes in these cells [53, 56, 61]. The molecular grounds of inefficient G1/S checkpoint in mESCs have been more recently explained by the presence of high levels of the CDC25A phosphatase [58] due to its stabilization by the ubiquitin hydrolase DUB3 which is expressed at high levels in mESCs [53]. This results in constitutive dephosphorylation of the CDK2 kinase, which pushes cells into S-phase even in the presence of DNA damage, similar to the phenotype observed in cancer cells overexpressing CDC25A [62]. Interestingly, DUB3 has been shown to regulate the ubiquitination of both H2AX and γH2AX in somatic cells [63]. If this is also the case in mESCs, then it may explain why these cells repair inefficiently DSBs [64], aside from expressing low levels of DNA–PK*cs* [65]. Suppression of the G_1/S checkpoint is untimely linked to pluripotency. The expression of the DUB3 gene in mESCs [53] and that of the CDC25A gene in hESCs [66] are under control of pluripotency factors. Indeed, down-regulation of CDC25A induces a G_1/S delay upon DNA damage and cells spontaneously differentiate [53]. Consistent with this observation, DUB3 is more rapidly downregulated than OCT4 upon onset of differentiation (starting from day 1), making this gene a novel and highly specific marker of pluripotency in mESCs. Another work has shown that the contraction of the G_1 phase is crucial to suppress differentiation of mESCs [67]. Collectively these observations suggest that cell cycle contraction is an essential feature of pluripotency in mESCs.

mESCs exhibit spontaneous formation of γH2AX, RPA, and Rad51 foci but do not appear to display DSBs accumulation consistent with the absence of 53BP1 foci [68, 69], although activation of downstream DDR transducers (CHK1/2, CDC25A) does not seem to be affected [53]. It is possible to envisage the presence of multiple levels of regulation of the S-phase checkpoint by various factors, such as effectors of signaling pathways, unique to stem cells. One example is provided by the observation that the CHK2 kinase appears to be sequestered at the centrosome in mESCs so that it is not activated following induction of DSBs [70]. New evidence suggests that H2AX phosphorylation in cultured ESCs is neither DNA–PK*cs*- nor ATM-dependent but is in part ATR-dependent. This is associated with ssDNA gaps accumulation, reduced fork speed, and frequent fork reversal. All these features are lost upon onset of differentiation [71]. Why is ATR spontaneously activated in mESCs? Ahuja et al. [71] show that hypoxia, DNA methylation, and transcription do not seem to be the main cause of RS in mESCs. RS appears to be linked to the maintenance of self-renewal of embryonic stem cells. Turinetto et al. [68] demonstrated that γH2AX level decrease during mESCs differentiation, while it increases upon treatment with self-renewal-enhancing small molecules such as GSK and MEK inhibitor, which correlates with increased OCT4 and NANOG expression. Further, a pluripotent state-specific gene, named FILIA, has been recently shown to be important for genomic stability in mESCs [72]. This protein is constitutively localized to the centrosomes, is recruited to DNA damage sites, where it stimulates PARP1 enzymatic activity, and contributes to CHK2 activation independently of ATM.

http://dx.doi.org/10.5772/62906

The main kind of spontaneous mutations observed in mESCs are loss of heterozygosity as a consequence of chromosome loss/reduplication. However, the mutation rate of mESCs has been found be to 100 times lower than that of isogenic somatic cells [73]. This is surprising given the high level of RS observed in mESCs and may suggest that these cells could counter-balance genetic instability by increased DNA repair efficiency [74]. One of this could be reduced efficiency of mitotic recombination in ESCs compared to somatic cells as observed ([73] and references therein). In addition, because mESCs have an inefficient G_1/S checkpoint, they activate DNA damage- and p53-dependent differentiation if injured, as a way to enter apoptosis more easily since this checkpoint is restored in differentiated cells [75]. This latter control mechanism then avoids that damaged cells would be part of the pool of differentiated cells.

3.2. iPSCs

Somatic cells expressing defined pluripotency factors can be reprogrammed into iPSCs [76]. These cells share several similarities with ESCs such as a similar contracted cell cycle [77], the ability to undergo self-renewal and differentiation, as well as expression of pluripotency markers such as NANOG, OCT4, SOX2, and SSE-4 amongst others. Reprogramming increases γH2AX levels [78–80] and induces accumulation of genomic aberrancies ranging from whole chromosome aneuploidies, CNVs to point mutations [81], as well as epigenetic abnormalities [82]. The mutation frequency of iPSCs is also increased and has been estimated to be 10 times higher than that of ESCs [83–84]. This is in some way not surprising since the reprogramming protocol involves overexpression of oncogenes, such as *c-myc*, that introduces RS. Decreased genomic instability can be achieved by overexpression of the CHK1 kinase or by nucleosides supplementation during reprogramming [80]. ATM is also important for reprogramming. It has been reported that iPSCs deleted of ATM reprogram less efficiently and have increased genomic instability. Interestingly, these cells display gene expression profiles similar to wild-type ESCs and maintain the ability to differentiate into all three germ layers [85]. In line with this data, iPSCs exhibit G2/M cell cycle arrest and efficient DSB repair if ATM-dependent checkpoint activation signaling cascade is activated by ionizing radiation. iPSCs arrest the cell cycle in G2-phase and repair DSB by HR probably by overexpressing DNA repair genes [86]. Altogether these observations point out to a general requirement for the DNA damage checkpoint in sustaining reprogramming, suggesting that forced induction of proliferation induces RS and cells need a functional DDR to cope with this.

3.3. ASCs

ASCs are characterized by a narrower differentiation potential than ESCs. These cells self-renew to preserve both specific tissue and organ homeostasis throughout the life of an individual. Although ASCs show much less signs of genomic instability than ESCs, they deteriorate with age [87]. It is likely that the accumulation of lesions and mutations observed during ageing of stem cells is caused by acquired defects in DNA repair pathways that reduce stem cell potential. Interestingly, defective DNA repair is tightly linked to regeneration failure in certain tissues. Fanconi anemia patients, who are deficient in ICL repair, are characterized

by a premature failure of bone marrow hematopoiesis. This event is triggered by the accumulation of DNA lesions leading to excessive DDR activation in hematopoietic stem cells (HSCs) and their progenitors [88]. In addition, NER is required for the maintenance of HSCs and prevention of premature ageing [89]. NHEJ is critical for the maintenance of skeletal muscle and muscle stem cells, since decreased Ku80 expression (a subunit of the heterodimeric complex made of Ku70 and 80 proteins that that functions with DNA–PK*cs* in NHEJ) causes accelerated exhaustion of stem cell pool and ageing [90]. In HSCs and their progenitors, ROS accumulation can be provoked by loss of ATM, affecting cell cycle progression. Conditional depletion of ATR or its downstream effector CHK1 is responsible for premature ageing phenotypes in skin, bones, small intestine, and the hematopoietic system [91, 92], resulting in apoptosis and cell cycle arrest because of rapid accumulation of DNA lesions [93, 94].

Interestingly, it has been reported that aging HSCs have a higher rate of genomic instability than young HSCs, fuelled by a high level of RS generated by the reduced expression of components of the MCM2-7 replicative helicase [95]. Reduced expression of the MCM3 gene was also recently shown to be sufficient to impair hematopoietic progenitor cells due to RS [94]. A recently identified protein, NUCLEOSTEMIN, rules a primary function in maintaining the genomic stability of neural stem cells. This protein promotes recruitment of RAD51 to replication-induced DNA damage foci and activates growth arrest independently of p53 [96]. Analysis of the transcriptional program of ESCs compared to ASCs (i.e., neural and hematopoietic) showed unexpected high similarities of gene expression profiles and identified a core set of about 200 genes expressed in all three cell types, accordingly coined as the "*stemness*" factors [97].

Cell type	Causes of genomic instability of stem cells
ESCs	Short cell cycle
	Inefficient checkpoints
	RS
iPSCs	Short cell cycle
ASCs	Inefficient checkpoints
	RS
	Reprogramming-induced DNA damage
	Mutations carry over
	High mutation frequency
	Aging-induced RS*

* Observed in HSCs [100].

Table 1. Summary of main causes of genomic instability of pluripotent stem cells.

http://dx.doi.org/10.5772/62906

The cell cycle of ASCs is remarkably different from that of ESCs. ASCs are mostly quiescent (being mostly in the G_0 state) and display a very slow cell cycle. For example, about 75% of HSCs reside in G_0 [98], whereas ESCs grown in culture display less than 20% of cells in G_1. It has been suggested that HSCs may divide once every 145 days (about five times during a mouse lifetime [99]), while ESCs divide every 11–12 h. These features make ASCs able to activate checkpoints and allow efficient repair. Since NHEJ can also act during G_0/G_1 because of its template independency, HSCs make an attempt to avoid DNA lesions by maintaining a hypoxic status [100] and decreasing the generation of ROS. In these conditions, ATP is generated mainly through glycolysis rather than mitochondrial respiration. Since this latter is activated only following cell cycle entry [101, 102], it explains why the first process is mainly employed by HSCs that are usually quiescent (**Table 1**).

4. Implications of PSCs genomic instability in regenerative medicine

PSCs are of great interest for their use in cell-based therapy. Current protocols involve PSCs differentiation into a specific cell type and then injection into an organ in the aim of replacing existing faulty cells. From a clinical point of view, this is a major concern due to the threat of transplanting immature cells with instable genome. Indeed, when injected in mice, immature PSCs induce teratoma. Hence, a greater understanding of the factors that regulate genomic stability in PSCs is critical to address this issue.

4.1. Hurdles in translating iPSCs technology into the clinic: problems and perspectives

Genetic instability and a high mutation rate constitute the dark side of iPSCs when taken into the clinic [4]. Hence, current efforts are made to generate iPSCs with reduced mutation load and having more stable genomes. Ji et al. [103] have shown that antioxydants reduce the level of γH2AX and *de novo* formation of CNVs in iPSCs suggesting that excessive ROS production in iPSCs increases their genetic instability. Indeed, a very recent report that analyzed the "metabolome" of naïve ESCs compared to that of primed ESCs show significant differences between these two cell types, which in turn may impinge on the level of ROS [104]. Further, recent work suggests that the use of non-integrative vectors to induce reprogramming significantly reduces the number of CNVs in the resulting iPSCs [105, 106]. Furthermore, a recent report that analyzed the mutational load of three distinct pluripotency induction methods shows that a non-integrative approach results in lower mutation load than either retrovirus or Sendai virus-based reprogramming methods [104]. Because integrative vectors induce DNA damage by generating DSBs, this suggests that the manipulation of the DDR can be a useful tool to reduce the genetic instability of iPSCs. It is then conceivable to think that DNA damage generated during reprogramming may be not well taken care, one reason being that iPSCs have inefficient checkpoints [86]. Indeed, a recent report indicates that manipulating the DDR can decrease the genomic instability of iPSCs [80]. This work shows that increasing the cellular levels of the CHK1 protein kinase decreases the level of γH2AX in these cells. In sum, in order to reduce undesired genetic burden arising during reprogramming of somatic

cells, supplementing medium with both antioxidants and nucleoside should be combined to significantly reduce RS and CNVs in iPSCs.

In an effort to reduce genetic manipulation and consequent DNA damage, it has been shown that several transcription factors needed for iPSCs generation (except OCT4) can be replaced with a cocktail of chemical compounds [107]. More recently, generating iPSCs with a mix of small molecules inhibitors that can also replace OCT4 appears to strongly suppress the level of γH2AX, suggesting a reduction in spontaneous DNA damage, while keeping these cells pluripotent [108, 109]. As an example for the downside of genetic manipulation, NANOG expression has been shown to be cell cycle-regulated in human and mouse ESCs [53, 66, 110], whereas during reprogramming, this transcription factor is under constitutive expression. Altogether these data suggest that the use of an optimized set of chemical compounds may not alter natural gene expression during reprogramming and therefore would likely reduce unwanted off-target effects otherwise generated using genetic manipulation for reprogramming. Hence, chemical reprogramming remains a potentially more appropriate method since standardization of the approach is foreseeable and paves a new way of keeping genomic instability of iPSCs under control using pharmacological inhibitors.

Interestingly, mutation in the cell surface protein Glypican4 (Gpc4), implicated in the control of the Wnt/ß-catenin signaling pathway, has been reported to strongly reduce formation of teratoma upon implantation of mESCs in nude mice without affecting pluripotency [111]. Gpc4 mutant ESCs appear to be able to differentiate in all three embryonic layers when injected into developing blastocysts, although with faster kinetics compared to wild-type ESCs. These data propose Gpc4 as a promising target to modulate the teratogenic potential of ESCs. Indeed, more recent data show that ESCs bearing a hypomorphic Gpc4 allele improve recovery of motoneuron defects in a rat model for Parkinson disease without generating teratoma [112]. It is not yet known whether Gpc4 mutations have a similar effect also on human ESCs or iPSCs, and whether spontaneous DNA damage and/or genomic instability are affected.

5. Conclusions and perspectives

Differentiation of iPSCs has been successfully achieved to generate hematopoietic cells, neurons, pancreatic β-islet, and cardiomyocytes; however, production of other cell types is still challenging. One major hurdle is the efficiency of differentiation that still remains very low. In addition, PSCs show several signs of genetic instability, not only in culture but also *in vivo* [71, 106], yet embryos manage to keep this instability under control by generating viable and healthy organisms. Hence, the question arises of how this control is achieved. First, cells with unstable genomes can be eliminated by apoptosis during differentiation, which is actually what it is observed during *in vitro* differentiation. However, γH2AX detection in blastocysts shows that most of the cells stain positive for this marker [71, 106], which makes unlikely that most of them bear indeed highly unstable genomes. Another possibility is that the γH2AX observed in these cells is not only a mark of genetic instability but perhaps also a marker of other DNA transactions, including chromatin remodeling. Chromatin remodeling is known

to change dramatically during differentiation; hence, the decrease of γH2AX observed upon differentiation onset may be also due to changes in chromatin structure. If this is the case, the chromatin structure and epigenetic marks responsible for constitutive γH2AX in ESCs remain to be discovered. Furthermore, recent data show the presence of a high proportion of ssDNA gaps in mESCs; however, the link between these gaps and the level of γH2AX is unclear. Despite the high level of genetic instability, a highly contracted cell cycle and an inefficient G1/S checkpoint, the mutation rate of mESCs has been surprisingly reported to be lower than that of isogenic somatic cells. The significance of this discrepancy needs to be further understood.

How our current knowledge on PSCs can be translated into improving their genetic stability so to foster the development of PSCs with lower mutation load that can be used with success in regenerative medicine? In principle, identifying the molecular basis of genomic instability of PSCs opens the perspective of manipulating the genes implicated, in the aim to decrease their tendency to introduce mutations and so doing, reduce their teratogenicity. For instance, being able to manipulate the structure of the cell cycle of PSCs so to decrease RS and still maintain pluripotency may be of value. Further work in this direction is expected to generate novel insights and hopes into this rather difficult though exciting task. Clinically-compatible quantitative methods to comprehensively analyze the genetic stability of iPSCs would greatly facilitate the selection process of most appropriate iPSCs clones. Recent efforts have shifted the practice and proposed pathway signaling as readout to compare for functionality [113]. Ease of application and selection would guarantee large-scale testing in clinics.

Author details

Elena Lo Furno[1], Siem van der Laan[2] and Domenico Maiorano[1*]

*Address all correspondence to: domenico.maiorano@igh.cnrs.fr

1 Institute of Human Genetics, CNRS-UPR1142, Genome Surveillance and Stability Laboratory, Department of Molecular Bases of Human Diseases, University of Montpellier, Montpellier, France

2 CNRS FRE3690, Parc Euromedicine Cap Delta, Montpellier, France

References

[1] Wood RD (1996) DNA repair in eukaryotes. Annual Review of Biochemistry 65: 135–167.

[2] Kenyon J, Gerson SL (2007) The role of DNA damage repair in aging of adult stem cells. Nucleic Acids Research 35: 7557–7565.

[3] Hakem R (2008) DNA-damage repair; the good, the bad, and the ugly. The EMBO Journal 27: 589–605.

[4] Pera MF (2011) Stem cells: The dark side of induced pluripotency. Nature 471: 46–47.

[5] Shen Z (2011) Genomic instability and cancer: an introduction. Journal of Molecular Cell Biology 3: 1–3.

[6] Hartwell L (1992) Defects in a cell cycle checkpoint may be responsible for the genomic instability of cancer cells. Cell 71: 543–546.

[7] Matt S, Hofmann TG (2016) The DNA damage-induced cell death response: a roadmap to kill cancer cells. Cellular and molecular life sciences: CMLS.

[8] Goldstein M, Kastan MB (2015) The DNA damage response: implications for tumor responses to radiation and chemotherapy. Annual Review of Medicine 66: 129–143.

[9] Schuyler SC, Wu YF, Kuan VJ (2012) The Mad1-Mad2 balancing act—a damaged spindle checkpoint in chromosome instability and cancer. Journal of Cell Science 125: 4197–4206.

[10] Waters CA, Strande NT, Pryor JM, Strom CN, Mieczkowski P, et al. (2014) The fidelity of the ligation step determines how ends are resolved during nonhomologous end joining. Nature Communications 5: 4286.

[11] Savitsky K, Bar-Shira A, Gilad S, Rotman G, Ziv Y, et al. (1995) A single ataxia telangiectasia gene with a product similar to PI-3 kinase. Science 268: 1749–1753.

[12] Matsuoka S, Ballif BA, Smogorzewska A, McDonald ER, 3rd, Hurov KE, et al. (2007) ATM and ATR substrate analysis reveals extensive protein networks responsive to DNA damage. Science 316: 1160–1166.

[13] Gonzalez Besteiro MA, Gottifredi V (2015) The fork and the kinase: a DNA replication tale from a CHK1 perspective. Mutation Research Reviews in Mutation Research 763: 168–180.

[14] Cimprich KA, Cortez D (2008) ATR: an essential regulator of genome integrity. Nature Reviews Molecular Cell Biology 9: 616–627.

[15] Vidal-Eychenie S, Decaillet C, Basbous J, Constantinou A (2013) DNA structure-specific priming of ATR activation by DNA–PKcs. The Journal of Cell Biology 202: 421–429.

[16] Lin YF, Shih HY, Shang Z, Matsunaga S, Chen BP (2014) DNA–PKcs is required to maintain stability of Chk1 and Claspin for optimal replication stress response. Nucleic Acids Research 42: 4463–4473.

[17] Ashley AK, Shrivastav M, Nie J, Amerin C, Troksa K, et al. (2014) DNA–PK phosphorylation of RPA32 Ser4/Ser8 regulates replication stress checkpoint activation, fork restart, homologous recombination and mitotic catastrophe. DNA Repair 21: 131–139.

[18] Hekmat-Nejad M, You Z, Yee MC, Newport JW, Cimprich KA (2000) Xenopus ATR is a replication-dependent chromatin-binding protein required for the DNA replication checkpoint. Current Biology 10: 1565–1573.

[19] Byun TS, Pacek M, Yee MC, Walter JC, Cimprich KA (2005) Functional uncoupling of MCM helicase and DNA polymerase activities activates the ATR-dependent checkpoint. Genes & Development 19: 1040–1052.

[20] Van C, Yan S, Michael WM, Waga S, Cimprich KA (2010) Continued primer synthesis at stalled replication forks contributes to checkpoint activation. The Journal of Cell Biology 189: 233–246.

[21] Recolin B, Van der Laan S, Maiorano D (2012) Role of replication protein A as sensor in activation of the S-phase checkpoint in Xenopus egg extracts. Nucleic Acids Research 40: 3431–3442.

[22] Betous R, Pillaire MJ, Pierini L, van der Laan S, Recolin B, et al. (2013) DNA polymerase kappa-dependent DNA synthesis at stalled replication forks is important for CHK1 activation. The EMBO Journal 32: 2172–2185.

[23] Duursma AM, Driscoll R, Elias JE, Cimprich KA (2013) A role for the MRN complex in ATR activation via TOPBP1 recruitment. Molecular Cell 50: 116–122.

[24] Lee J, Dunphy WG (2013) The Mre11-Rad50-Nbs1 (MRN) complex has a specific role in the activation of Chk1 in response to stalled replication forks. Molecular Biology of the Cell 24: 1343–1353.

[25] Ward IM, Chen J (2001) Histone H2AX is phosphorylated in an ATR-dependent manner in response to replicational stress. Journal of Biological Chemistry 276: 47759–47762.

[26] Bakkenist CJ, Kastan MB (2003) DNA damage activates ATM through intermolecular autophosphorylation and dimer dissociation. Nature 421: 499–506.

[27] Lukas J, Lukas C, Bartek J (2011) More than just a focus: The chromatin response to DNA damage and its role in genome integrity maintenance. Nature Cell Biology 13: 1161–1169.

[28] Lambert S, Carr AM (2013) Impediments to replication fork movement: stabilisation, reactivation and genome instability. Chromosoma 122: 33–45.

[29] Zeman MK, Cimprich KA (2014) Causes and consequences of replication stress. Nature Cell Biology 16: 2–9.

[30] Fraga CG, Shigenaga MK, Park JW, Degan P, Ames BN (1990) Oxidative damage to DNA during aging: 8-hydroxy-2′-deoxyguanosine in rat organ DNA and urine. Proceedings of the National Academy of Sciences of the United States of America 87: 4533–4537.

[31] Gorgoulis VG, Vassiliou LV, Karakaidos P, Zacharatos P, Kotsinas A, et al. (2005) Activation of the DNA damage checkpoint and genomic instability in human precancerous lesions. Nature 434: 907–913.

[32] Neelsen KJ, Zanini IM, Mijic S, Herrador R, Zellweger R, et al. (2013) Deregulated origin licensing leads to chromosomal breaks by rereplication of a gapped DNA template. Genes & Development 27: 2537–2542.

[33] Jones RM, Mortusewicz O, Afzal I, Lorvellec M, Garcia P, et al. (2012) Increased replication initiation and conflicts with transcription underlie Cyclin E-induced replication stress. Oncogene.

[34] Recolin B, van der Laan S, Tsanov N, Maiorano D (2014) Molecular mechanisms of DNA replication checkpoint activation. Genes 5: 147–175.

[35] Neelsen KJ, Zanini IM, Herrador R, Lopes M (2013) Oncogenes induce genotoxic stress by mitotic processing of unusual replication intermediates. The Journal of Cell Biology 200: 699–708.

[36] Lukas C, Savic V, Bekker-Jensen S, Doil C, Neumann B, et al. (2011) 53BP1 nuclear bodies form around DNA lesions generated by mitotic transmission of chromosomes under replication stress. Nature Cell Biology 13: 243–253.

[37] Loeb LA (1991) Mutator phenotype may be required for multistage carcinogenesis. Cancer Research 51: 3075–3079.

[38] Halazonetis TD, Gorgoulis VG, Bartek J (2008) An oncogene-induced DNA damage model for cancer development. Science 319: 1352–1355.

[39] Kinzler KW, Vogelstein B (1997) Cancer-susceptibility genes. Gatekeepers and caretakers. Nature 386: 761, 763.

[40] Fishel R, Lescoe MK, Rao MR, Copeland NG, Jenkins NA, et al. (1993) The human mutator gene homolog MSH2 and its association with hereditary nonpolyposis colon cancer. Cell 75: 1027–1038.

[41] Leach FS, Nicolaides NC, Papadopoulos N, Liu B, Jen J, et al. (1993) Mutations of a mutS homolog in hereditary nonpolyposis colorectal cancer. Cell 75: 1215–1225.

[42] Al-Tassan N, Chmiel NH, Maynard J, Fleming N, Livingston AL, et al. (2002) Inherited variants of MYH associated with somatic G:C→T:A mutations in colorectal tumors. Nature Genetics 30: 227–232.

[43] Nowell PC (1976) The clonal evolution of tumor cell populations. Science 194: 23–28.

[44] Longo L, Bygrave A, Grosveld FG, Pandolfi PP (1997) The chromosome make-up of mouse embryonic stem cells is predictive of somatic and germ cell chimaerism. Transgenic Research 6: 321–328.

[45] Liu X, Wu H, Loring J, Hormuzdi S, Disteche CM, et al. (1997) Trisomy eight in ES cells is a common potential problem in gene targeting and interferes with germ line transmission. Developmental Dynamics 209: 85–91.

[46] Maitra A, Arking DE, Shivapurkar N, Ikeda M, Stastny V, et al. (2005) Genomic alterations in cultured human embryonic stem cells. Nature Genetics 37: 1099–1103.

[47] Lefort N, Feyeux M, Bas C, Feraud O, Bennaceur-Griscelli A, et al. (2008) Human embryonic stem cells reveal recurrent genomic instability at 20q11.21. Nature Biotechnology 26: 1364–1366.

[48] Baker DE, Harrison NJ, Maltby E, Smith K, Moore HD, et al. (2007) Adaptation to culture of human embryonic stem cells and oncogenesis in vivo. Nature Biotechnology 25: 207–215.

[49] Lamm N, Ben-David U, Golan-Lev T, Storchova Z, Benvenisty N, et al. (2016) Genomic instability in human pluripotent stem cells arises from replicative stress and chromosome condensation defects. Cell Stem Cell 18: 253–261.

[50] Becker KA, Ghule PN, Therrien JA, Lian JB, Stein JL, et al. (2006) Self-renewal of human embryonic stem cells is supported by a shortened G1 cell cycle phase. Journal of Cellular Physiology 209: 883–893.

[51] Savatier P, Lapillonne H, Jirmanova L, Vitelli L, Samarut J (2002) Analysis of the cell cycle in mouse embryonic stem cells. Methods in Molecular Biology 185: 27–33.

[52] Ballabeni A, Park IH, Zhao R, Wang W, Lerou PH, et al. (2011) Cell cycle adaptations of embryonic stem cells. Proceedings of the National Academy of Sciences of the United States of America 108: 19252–19257.

[53] van der Laan S, Tsanov N, Crozet C, Maiorano D (2013) High Dub3 expression in mouse ESCs couples the G1/S checkpoint to pluripotency. Molecular Cell 52: 366–379.

[54] Meshorer E, Misteli T (2006) Chromatin in pluripotent embryonic stem cells and differentiation. Nature Reviews Molecular Cell biology 7: 540–546.

[55] Pajerowski JD, Dahl KN, Zhong FL, Sammak PJ, Discher DE (2007) Physical plasticity of the nucleus in stem cell differentiation. Proceedings of the National Academy of Sciences of the United States of America 104: 15619–15624.

[56] Prost S, Bellamy CO, Clarke AR, Wyllie AH, Harrison DJ (1998) p53-independent DNA repair and cell cycle arrest in embryonic stem cells. FEBS Letters 425: 499–504.

[57] Aladjem MI, Spike BT, Rodewald LW, Hope TJ, Klemm M, et al. (1998) ES cells do not activate p53-dependent stress responses and undergo p53-independent apoptosis in response to DNA damage. Current Biology 8: 145–155.

[58] Koledova Z, Kafkova LR, Kramer A, Divoky V (2010) DNA damage-induced degradation of Cdc25A does not lead to inhibition of Cdk2 activity in mouse embryonic stem cells. Stem Cells 28: 450–461.

[59] Barta T, Vinarsky V, Holubcova Z, Dolezalova D, Verner J, et al. (2010) Human embryonic stem cells are capable of executing G1/S checkpoint activation. Stem Cells 28: 1143–1152.

[60] Desmarais JA, Hoffmann MJ, Bingham G, Gagou ME, Meuth M, et al. (2012) Human embryonic stem cells fail to activate CHK1 and commit to apoptosis in response to DNA replication stress. Stem Cells 30: 1385–1393.

[61] Sabapathy K, Klemm M, Jaenisch R, Wagner EF (1997) Regulation of ES cell differentiation by functional and conformational modulation of p53. EMBO Journal 16: 6217–6229.

[62] 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.

[63] Delgado-Diaz MR, Martin Y, Berg A, Freire R, Smits VA (2014) Dub3 controls DNA damage signalling by direct deubiquitination of H2AX. Molecular oncology 8: 884–893.

[64] Momcilovic O, Navara C, Schatten G (2011) Cell cycle adaptations and maintenance of genomic integrity in embryonic stem cells and induced pluripotent stem cells. Results and Problems in Cell Differentiation 53: 415–458.

[65] Banuelos CA, Banath JP, MacPhail SH, Zhao J, Eaves CA, et al. (2008) Mouse but not human embryonic stem cells are deficient in rejoining of ionizing radiation-induced DNA double-strand breaks. DNA Repair 7: 1471–1483.

[66] Zhang X, Neganova I, Przyborski S, Yang C, Cooke M, et al. (2009) A role for NANOG in G1 to S transition in human embryonic stem cells through direct binding of CDK6 and CDC25A. Journal of Cell Biology 184: 67–82.

[67] Pauklin S, Vallier L (2013) The cell-cycle state of stem cells determines cell fate propensity. Cell 155: 135–147.

[68] Turinetto V, Orlando L, Sanchez-Ripoll Y, Kumpfmueller B, Storm MP, et al. (2012) High basal gammaH2AX levels sustain self-renewal of mouse embryonic and induced pluripotent stem cells. Stem Cells 30: 1414–1423.

[69] Banath JP, Banuelos CA, Klokov D, MacPhail SM, Lansdorp PM, et al. (2009) Explanation for excessive DNA single-strand breaks and endogenous repair foci in pluripotent mouse embryonic stem cells. Experimental Cell Research 315: 1505–1520.

[70] Hong Y, Stambrook PJ (2004) Restoration of an absent G1 arrest and protection from apoptosis in embryonic stem cells after ionizing radiation. Proceedings of the National Academy of Sciences of the United States of America 101: 14443–14448.

[71] Ahuja AK, Jodkowska K, Teloni F, Bizard AH, Zellweger R, et al. (2016) A short G1 phase imposes constitutive replication stress and fork remodelling in mouse embryonic stem cells. Nature Communications 7: 10660.

[72] Zhao B, Zhang WD, Duan YL, Lu YQ, Cun YX, et al. (2015) Filia is an ESC-specific regulator of DNA damage response and safeguards genomic stability. Cell Stem Cell 16: 684–698.

[73] Hong Y, Cervantes RB, Tichy E, Tischfield JA, Stambrook PJ (2007) Protecting genomic integrity in somatic cells and embryonic stem cells. Mutation Research 614: 48–55.

[74] Maynard S, Swistowska AM, Lee JW, Liu Y, Liu ST, et al. (2008) Human embryonic stem cells have enhanced repair of multiple forms of DNA damage. Stem Cells 26: 2266–2274.

[75] Lin T, Chao C, Saito S, Mazur SJ, Murphy ME, et al. (2005) p53 induces differentiation of mouse embryonic stem cells by suppressing Nanog expression. Nature Cell Biology 7: 165–171.

[76] Yamanaka S (2008) Induction of pluripotent stem cells from mouse fibroblasts by four transcription factors. Cell Proliferation 41(Suppl 1): 51–56.

[77] Ruiz S, Panopoulos AD, Herrerias A, Bissig KD, Lutz M, et al. (2011) A high proliferation rate is required for cell reprogramming and maintenance of human embryonic stem cell identity. Current Biology: CB 21: 45–52.

[78] Blasco MA, Serrano M, Fernandez-Capetillo O (2011) Genomic instability in iPS: time for a break. The EMBO Journal 30: 991–993.

[79] Marion RM, Strati K, Li H, Murga M, Blanco R, et al. (2009) A p53-mediated DNA damage response limits reprogramming to ensure iPS cell genomic integrity. Nature 460: 1149–1153.

[80] Ruiz S, Lopez-Contreras AJ, Gabut M, Marion RM, Gutierrez-Martinez P, et al. (2015) Limiting replication stress during somatic cell reprogramming reduces genomic instability in induced pluripotent stem cells. Nature Communications 6: 8036.

[81] Weissbein U, Benvenisty N, Ben-David U (2014) Quality control: Genome maintenance in pluripotent stem cells. The Journal of Cell Biology 204: 153–163.

[82] Lister R, Pelizzola M, Kida YS, Hawkins RD, Nery JR, et al. (2011) Hotspots of aberrant epigenomic reprogramming in human induced pluripotent stem cells. Nature 471: 68–73.

[83] Ji J, Ng SH, Sharma V, Neculai D, Hussein S, et al. (2012) Elevated coding mutation rate during the reprogramming of human somatic cells into induced pluripotent stem cells. Stem Cells 30: 435–440.

[84] Gore A, Li Z, Fung HL, Young JE, Agarwal S, et al. (2011) Somatic coding mutations in human induced pluripotent stem cells. Nature 471: 63–67.

[85] Kinoshita T, Nagamatsu G, Kosaka T, Takubo K, Hotta A, et al. (2011) Ataxia-telangiectasia mutated (ATM) deficiency decreases reprogramming efficiency and leads to

genomic instability in iPS cells. Biochemical and Biophysical Research Communications 407: 321–326.

[86] Momcilovic O, Knobloch L, Fornsaglio J, Varum S, Easley C, et al. (2010) DNA damage responses in human induced pluripotent stem cells and embryonic stem cells. PLos One 5: e13410.

[87] Liu L, Rando TA (2011) Manifestations and mechanisms of stem cell aging. Journal Cell Biology 193: 257–266.

[88] Ceccaldi R, Parmar K, Mouly E, Delord M, Kim JM, et al. (2012) Bone marrow failure in Fanconi anemia is triggered by an exacerbated p53/p21 DNA damage response that impairs hematopoietic stem and progenitor cells. Cell Stem Cell 11: 36–49.

[89] Rossi DJ, Bryder D, Seita J, Nussenzweig A, Hoeijmakers J, et al. (2007) Deficiencies in DNA damage repair limit the function of haematopoietic stem cells with age. Nature 447: 725–729.

[90] Didier N, Hourde C, Amthor H, Marazzi G, Sassoon D (2012) Loss of a single allele for Ku80 leads to progenitor dysfunction and accelerated aging in skeletal muscle. EMBO Molecular Medicine 4: 910–923.

[91] Ruzankina Y, Pinzon-Guzman C, Asare A, Ong T, Pontano L, et al. (2007) Deletion of the developmentally essential gene ATR in adult mice leads to age-related phenotypes and stem cell loss. Cell Stem Cell 1: 113–126.

[92] Greenow KR, Clarke AR, Jones RH (2009) Chk1 deficiency in the mouse small intestine results in p53-independent crypt death and subsequent intestinal compensation. Oncogene 28: 1443–1453.

[93] Ruzankina Y, Schoppy DW, Asare A, Clark CE, Vonderheide RH, et al. (2009) Tissue regenerative delays and synthetic lethality in adult mice after combined deletion of Atr and Trp53. Nature Genetics 41: 1144–1149.

[94] Alvarez S, Diaz M, Flach J, Rodriguez-Acebes S, Lopez-Contreras AJ, et al. (2015) Replication stress caused by low MCM expression limits fetal erythropoiesis and hematopoietic stem cell functionality. Nature Communications 6: 8548.

[95] Flach J, Bakker ST, Mohrin M, Conroy PC, Pietras EM, et al. (2014) Replication stress is a potent driver of functional decline in ageing haematopoietic stem cells. Nature 512: 198–202.

[96] Meng L, Lin T, Peng G, Hsu JK, Lee S, et al. (2013) Nucleostemin deletion reveals an essential mechanism that maintains the genomic stability of stem and progenitor cells. Proceedings of the National Academy of Sciences of the United States of America 110: 11415–11420.

[97] Ramalho-Santos M, Yoon S, Matsuzaki Y, Mulligan RC, Melton DA (2002) "Stemness": transcriptional profiling of embryonic and adult stem cells. Science 298: 597–600.

[98] Cheshier SH, Morrison SJ, Liao X, Weissman IL (1999) In vivo proliferation and cell cycle kinetics of long-term self-renewing hematopoietic stem cells. Proceedings of the National Academy of Sciences of the United States of America 96: 3120–3125.

[99] Wilson A, Laurenti E, Oser G, van der Wath RC, Blanco-Bose W, et al. (2008) Hematopoietic stem cells reversibly switch from dormancy to self-renewal during homeostasis and repair. Cell 135: 1118–1129.

[100] Nombela-Arrieta C, Pivarnik G, Winkel B, Canty KJ, Harley B, et al. (2013) Quantitative imaging of haematopoietic stem and progenitor cell localization and hypoxic status in the bone marrow microenvironment. Nature Cell Biology 15: 533–543.

[101] Takubo K, Nagamatsu G, Kobayashi CI, Nakamura-Ishizu A, Kobayashi H, et al. (2013) Regulation of glycolysis by Pdk functions as a metabolic checkpoint for cell cycle quiescence in hematopoietic stem cells. Cell Stem Cell 12: 49–61.

[102] Yu WM, Liu X, Shen J, Jovanovic O, Pohl EE, et al. (2013) Metabolic regulation by the mitochondrial phosphatase PTPMT1 is required for hematopoietic stem cell differentiation. Cell Stem Cell 12: 62–74.

[103] Ji J, Sharma V, Qi S, Guarch ME, Zhao P, et al. (2014) Antioxidant supplementation reduces genomic aberrations in human induced pluripotent stem cells. Stem Cell Reports 2: 44–51.

[104] Sperber H, Mathieu J, Wang Y, Ferreccio A, Hesson J, et al. (2015) The metabolome regulates the epigenetic landscape during naive-to-primed human embryonic stem cell transition. Nature Cell Biology 17: 1523–1535.

[105] Kang X, Yu Q, Huang Y, Song B, Chen Y, et al. (2015) Effects of integrating and non-integrating reprogramming methods on copy number variation and genomic stability of human induced pluripotent stem cells. PLos One 10: e0131128.

[106] Sobol M, Raykova D, Cavelier L, Khalfallah A, Schuster J, et al. (2015) Methods of reprogramming to induced pluripotent stem cell associated with chromosomal integrity and delineation of a chromosome 5q candidate region for growth advantage. Stem Cells and Development 24: 2032–2040.

[107] Zhu S, Li W, Zhou H, Wei W, Ambasudhan R, et al. (2010) Reprogramming of human primary somatic cells by OCT4 and chemical compounds. Cell Stem Cell 7: 651–655.

[108] Xu Y, Shi Y, Ding S (2008) A chemical approach to stem-cell biology and regenerative medicine. Nature 453: 338–344.

[109] Park HS, Hwang I, Choi KA, Jeong H, Lee JY, et al. (2015) Generation of induced pluripotent stem cells without genetic defects by small molecules. Biomaterials 39: 47–58.

[110] Singh AM, Chappell J, Trost R, Lin L, Wang T, et al. (2013) Cell-cycle control of developmentally regulated transcription factors accounts for heterogeneity in human pluripotent cells. Stem Cell Reports 1: 532–544.

[111] Fico A, De Chevigny A, Egea J, Bosl MR, Cremer H, et al. (2012) Modulating Glypican4 suppresses tumorigenicity of embryonic stem cells while preserving self-renewal and pluripotency. Stem Cells 30: 1863–1874.

[112] Fico A, de Chevigny A, Melon C, Bohic M, Kerkerian-Le Goff L, et al. (2014) Reducing glypican-4 in ES cells improves recovery in a rat model of Parkinson's disease by increasing the production of dopaminergic neurons and decreasing teratoma formation. The Journal of Neuroscience 34: 8318–8323.

[113] Makarev E, Fortney K, Litovchenko M, Braunewell KH, Zhavoronkov A, et al. (2015) Quantifying signaling pathway activation to monitor the quality of induced pluripotent stem cells. Oncotarget 6: 23204–23212.