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Review

p53: The barrier to cancer stem cell formation

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ABSTRACT

The role of p53 as the "guardian of the genome" in differentiated somatic cells, triggering various biological processes, is well established. Recent studies in the stem cell field have highlighted a profound role of p53 in stem cell biology as well. These studies, combined with basic data obtained 20 years ago, provide insight into how p53 governs the quantity and quality of various stem cells, ensuring a sufficient repertoire of normal stem cells to enable proper development, tissue regeneration and a cancer free life. In this review we address the role of p53 in genomically stable embryonic stem cells, a unique predisposed cancer stem cell model and adult stem cells, its role in the generation of induced pluripotent stem cells, as well as its role as the barrier to cancer stem cell formation.

1. Introduction

The balance between genome stability and plasticity is crucial in determining cell fate, yet this balance varies between somatic and stem cells (SCs). In a somatic cell, p53 has a major role in translating stress signals into classic processes such as apoptosis, cell cycle arrest, DNA repair and senescence, contributing to its main role as the "guardian of the genome" [1]. However, p53's function in SCs varies in a context-dependent manner. Imbalance between genome stability and plasticity may lead to intensive senescence or apoptosis, which can result in a severe depletion of the functional SC reservoir and to improper development or early aging. This dilemma emphasizes the important balance between the quantity and quality of SCs [2]. In recent years, p53 was found to have great impact in processes such as cellular differentiation [3–7], self-renewal [8,9] and plasticity [10,11], ensuring a balance between genome stability and plasticity in normal SCs.

SCs have a profound impact on embryonic development and are central for organ renewal during adult life [12]. As such, SC genomes must be guarded to minimize genetic lesions that may occur during their expansion and may lead to premature aging, failure to repair tissue injury and to cancer [13–15]. Genomic stability and fidelity are a hallmark of pluripotent Embryonic Stem Cells (ESCs). ESCs can differentiate into three lineages in the embryo, including germ cells [16]; thus genome stability is crucial for avoiding tumorigenesis as well as preventing mutations from being passed onto progeny. Indeed, ESCs have a low rate of spontaneous mutations compared to somatic cells [17]. Adult Stem Cells (ASCs), which reside in many tissues of the body, also hold the potential for self-renewal and differentiation into specific cell lineages – although they do not have the capacity to form an embryo. ASCs proliferate through asymmetric cell division, giving rise to one daughter SC and one transit-amplifying cell. Their activation occurs during particular developmental stages or after external injury, and their regulation is strictly controlled in their niches [18].

Dedifferentiation of somatic cells holds promise as a source for patient-specific transplantation therapies. Conversion of differentiated cells into a pluripotent state has been achieved by three methods: nuclear transfer – first achieved by transferring the nuclei of differentiated intestinal epithelium cells of feeding tadpoles into enucleated recipient eggs [19,20]. The second method used fusion of human amniocytes with differentiated mouse muscle cells, which provided valuable insights but not as a source of cells for regenerative medicine [21,22]. However, the major breakthrough in the field was provided by Takahashi and Yamanaka, who demonstrated the induction of pluripotent SCs from mouse embryonic fibroblasts (MEFs) by introducing four defined factors, Oct3/4, Sox2, Klf4 and c-Myc (OSKM) under Embryonic Stem (ES) cell culture conditions [23]. This development of induced pluripotent embryonic stem cells (iPSCs) provides insights into the biology of ESCs. Since then iPSCs have been generated from multiple tissues by various combinations of factors or techniques [24]. These iPSCs

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hold ES-like features, i.e. cells that retain the potential to differentiate into all three germ layers in vitro, form teratomas (a differentiated and non-malignant tumor) when injected into immunodeficient mice, and produce chimeric live pups when injected into blastocyst or germ cells. In fact, germ-line transmission is the most convincing demonstration of true pluripotency. Recently, it was shown that removing epigenetic barriers can improve reprogramming efficiency and induce pluripotency in nearly all the cells in a deterministic manner [25]. Yet the major concern in the use of iPSCs for therapeutic means – their tumorigenic potential – still remains. Thus, elucidation of the specific master regulators of pluripotency may enable efficient induction of safer cells to be used in regenerative medicine in numerous diseases. Indeed, studies by Buganim et al. have shed some light on the phases of transcriptional and epigenetic changes that occur during reprogramming and on the hierarchy of the regulators involved [26,27]. These studies may provide criteria that will allow assessment of iPSCs quality.

Much attention in the SCs field is drawn to the Cancer Stem Cell (CSC) theory. The CSC theory is based on the developmental hierarchy seen in normal tissue, wherein the undifferentiated SCs reside at the top, followed by a gradient of various degrees of differentiated cells. Similarly, tumors are organized in a hierarchical order that sustains a distinct subpopulation of CSCs. CSCs can divide asymmetrically, giving rise to a bulk tumor cell and a CSC, keeping the CSC reservoir small in numbers. Only the CSCs have the capability to initiate new tumors. These CSCs were found in a number of human hematological and solid tumors and have been defined experimentally by their ability to seed new tumors [28]. Just as normally proliferating tissues such as wounds are nourished and regenerated by SCs, so is a tumor – which may be considered as a "wound that never heals" [29] – nourished by tumor cells with an unlimited renewal potential. Indeed, CSCs and SCs share functions, such as self-renewal asymmetric cell division, the ability to generate a large number of differentiated cells, and the expression of specific markers [12,30,31]. Moreover, just as normal SCs have the ability to migrate to distinct parts of the body where they exert their functions, CSCs also seem to have the potential to migrate and establish metastasis [32]. Taken together, it is not surprising that SCs and CSCs share similar regulatory factors that modulate these biological functions [33]. However, SC function remains under physiological control, whereas the division and differentiation of CSCs are decidedly not [34,35]. These uncontrolled pathways include those regulated by WNT/β-catenin, PTEN, TGF-β, Hedgehog, Notch and Bmi-1 [36]. Moreover, CSCs are also resistant to chemotherapy and radiation and may be, as normal SCs are, protected against various insults, likely by mechanisms such as quiescence, expression of ATP binding cassette (ABC) pumps which may lead to multidrug resistance, high expression of anti-apoptotic proteins and resistance to DNA damage [37–39]. Unfortunately, CSC-rich tumors are associated with aggressive disease and poor prognosis [40] emphasizing the importance of unraveling their biology and the need to develop means to combat them.

CSC may arise from the transformation of a normal ASC or progenitor cell. Although the number of SCs is very small, they can undergo continuous division for a long time and are thus more likely to accumulate the molecular mutations that cause tumorigenesis. Indeed, Dick and colleges showed that only the transfer of a small population of human leukemia cells, displaying the cell surface markers of HSC, into immunodeficient mice, rather than more differentiated cells gave rise to new tumors. This suggests that normal primitive SCs, rather than committed progenitor cells, are the target of leukemic transformation [41,42].

Other studies favor the option that CSCs may have taken advantage of cellular plasticity and originate from differentiated cells through a process of dedifferentiation [43]. Regulated dedifferentiation may be regarded as a cellular homoeostasis mechanism through which tissues can regenerate after SCs are lost. For example, single secretory cells from the epithelium of the mouse trachea were able to dedifferentiate into multipotent SCs. This dedifferentiation process was triggered upon SC ablation and was prevented by direct contact of SCs with the committed cells, ensuring epithelial architecture. The authors suggest that the reciprocal interaction of stem and committed cells may have been designed to ensure robust self-organizing properties in diverse tissue types [44]. Recently, a role for p53 during salamander limb regeneration was published. It was shown that the activity of p53 initially decreases and then returns to baseline. The down-regulation is required for formation of the blastema and is critical for cell cycle reentry of post-mitotic differentiated cells, and the up-regulation is necessary for the redifferentiation phase to muscle. The authors suggest that the regulation of p53 activity is a pivotal mechanism that controls the plasticity of the differentiated state during regeneration [45]. These studies indicate that dedifferentiation is a regulated process in homeostasis and regeneration. Unfortunately, uncontrolled dedifferentiation may have cancerous consequences. Although much knowledge on CSCs has been obtained in the past years, how and when a CSC is formed in a particular tumor are still open questions. The two ways to obtain CSC do not exclude each other, but rather depend on the cancer type and context (Tables 1 and 2). Regardless of whether it is transformation of a progeni-

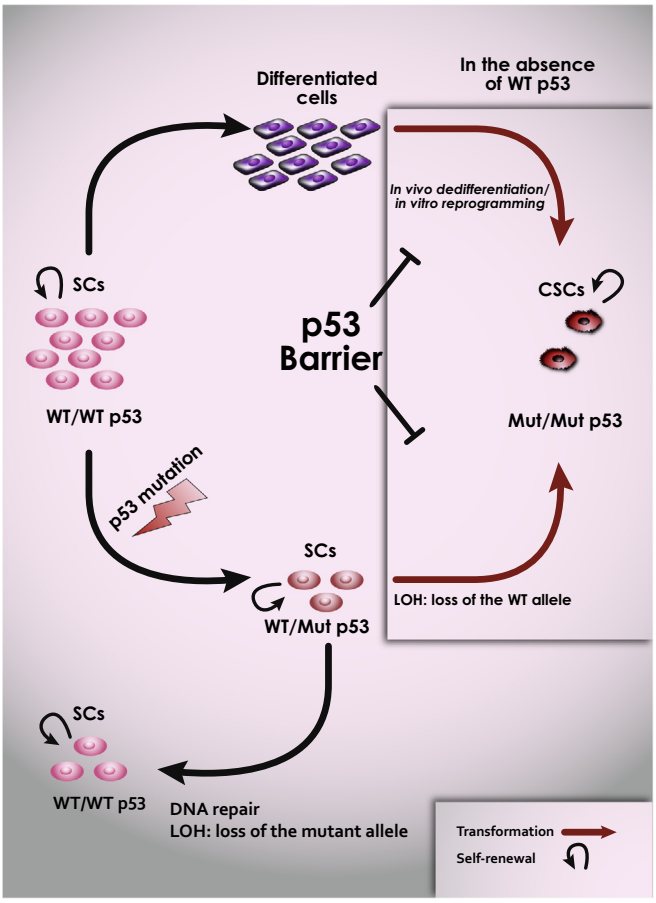

Fig. 1. p53 the barrier to cancer stem cells formation. p53 maintains a pool of normal SCs by controlling the quantity and quality of SCs. p53 restricts processes of in vivo dedifferentiation and in vitro reprogramming, preventing the transformation and dedifferentiation of differentiated cells into CSCs. SCs have the potential to undergo mutation in p53. In heterozygous p53 SCs LOH can occur as a DNA repair process, leading to the loss of the mutant allele and ensuring the quality of the SCs. In the case where the WT allele is lost CSCs will be formed.

Table 1
Cancer types in which p53 aberrant ASCs has been shown to be involved in their initiation and progression.

Cancer	Stem cell	Refs.
Multiple myeloma	Hematopoietic stem/progenitor cells	[136]
Leiomyosarcoma	Fat-derived MSCs	[92]
Fibrosarcoma (mouse model)	Aged MSC	[87]
Osteosarcoma	MSC of the limb bud	[137]
Glioma -glioblastoma	NSCs	[138]
Glioma- astrocytoma	NSCs	[94]
Ovarian cancer	Ovarian stem-like cells	[139]

tor/stem cell or dedifferentiation, p53 stands as a barrier to both routes of transformation.

During the last several years the stem cell field has expanded, providing more questions than answers. The findings that induced pluripotency and induced tumorigenesis are related processes, as judged by gene expression profiles [46], and that CSC hierarchy mimics normal SC hierarchy, emphasize the need for regulatory proteins that will guard and maintain a cancer-free repertoire of normal SCs. In this review we address the role of p53 in normal SCs as well as CSC prevention. The fate of an intermediate phase of SCs, namely those that harbor both wild type and mutant p53, presenting a state predisposed to CSCs, will also be discussed.

2. p53 in the life of a normal stem cell

2.1. The role of p53 in ESCs

Over 30 years ago, a set of studies described the expression of p53 in primary cell cultures obtained from embryos. High expression of p53 was observed in cell cultures of 12–14 day old mouse embryos, which declined in cells of 16 day old embryos [47–49]. These studies, among others, highlighted that although p53 is highly abundant in mouse ESCs [50,51], it was localized mainly in the cytoplasm [52,53] and was found to be inactive [54,55]. In contrast to mouse ESCs, in human ESCs p53 is localized in the nucleus, in a deacetylated inactive state and at low levels [56]. Indeed, whereas in somatic cells p53 classical response to DNA damage is G1/S cell cycle arrest, apoptosis or cellular senescence, this is not the case in mouse ESCs [55]. Although these observations are in line with the requirement of ESCs for rapid cell division and self-renewal, they also present a paradox; how do ESCs manage to maintain a stable genome without the classical functions of the "guardian of the genome"? Does p53 exert its guardian functions through other biological pathways? Moreover, the observations from the early nineties that p53 knockout mouse embryos developed normally, suggesting that p53 is redundant in embryogenesis [57], prompted more questions on the role of p53 in embryogenesis. Since then many studies have shed light on the important roles played by p53 in embryonic development. Indeed, a role in regulating suppression of self-renewal and induction of differentiation after DNA damage was assigned to p53. p53 binds and suppress the promoter of the master transcription factor Nanog and the pluripotency factor Oct4, which are highly abundant in mouse ESCs and drive self-renewal and the maintenance of an undifferentiated state [58,59]. Thus, suppression of these two genes in DNA damaged mouse ESCs will force differentiation [6] into cell types that can be subjected to classical p53 processes such as cell-cycle arrest or apoptosis. Recently, it was reported that silencing of Oct4 in human ESCs leads to the activation of p53, through the reduction in the expression of Sirt1, a deacetylase known to inhibit p53 activity, leading to increased acetylation of p53 at lysine 120 and 164 and promotion of differentiation [60]. Moreover, p53 was found to activate the expression of miR-34a and miR-145, which in turn repress stem cell factors Oct4, KLF4, LIN28A and Sox2 and prevent backsliding to pluripotency [56]. Furthermore, it was reported that a single aurora kinase A (Aurka)-mediated phosphorylation event is largely responsible for inactivating p53 and that in the absence of Aurka, increased p53 signaling promotes mouse ESC differentiation [61]. Recently an in-depth study of the genes regulated by p53 in human ESCs in response to early differentiation, induced by retinoic acid, revealed that p53 promotes differentiation of human ESCs by activating expression of developmental transcription factor genes involved in patterning, morphogenesis and organ development. Differentiation-specific p53 gene targets in human ESCs include several members of the homeodomain family (HOX, LHX, DLX, PAX), the forkhead family of FOX genes, the SOX gene family, and members of the TBX family of genes, all of which regulate a wide variety of developmental processes [62]. In addition, p53 targets members of the CBX family, specifically CBX2 and CBX4, which are part of the Polycomb complex and are crucial for cell-fate determination [63]. Moreover it was found that several p53 gene targets are down regulated during RA-mediated differentiation, including genes that direct mesodermal differentiation (FOXO3, KLF6, HDAC5, HDAC6) and telomere repeat binding factor TERF1, associated with pluripotency [64]. In all, in ESCs p53 seems to be a homeostatic protein ensuring proper development by governing pluripotency potential. In ESCs with damaged DNA p53 will force differentiation by harnessing many developmental pathways.

2.2. The role of p53 in iPSCs

Many studies have addressed the role of p53 in the biology of iPSCs. p53 was found to have a major role in the generation of iPSCs both in attenuating reprogramming as well as in quality control of the reprogramming. Indeed, in agreement with others, we found that WT p53 constrains iPSC generation in vitro [65–73]. It was

Table 2
Cancers and tumor lines in which p53 aberrations resulted in dedifferentiated phenotype.

Cancer	Phenotype	Refs.
Chondrosarcoma	High grade/dedifferentiated zones of chondrosarcoma	[140,95]
Liposarcoma	Dedifferentiated liposarcoma	[141]
Adenoid Cystic Carcinoma (AdCC)	Dedifferentiated AdCC	[142,143]
Thyroid carcinoma	Poorly differentiated and undifferentiated thyroid tumors	[144,145,97]
Carcinoma	Carcinomas from the p53 null and hemizygotes are more frequently undifferentiated than those from wild-type mice	[96]
Glioma	Dedifferentiation of astrocyte during tumorigenesis	[138]
Wilms tumor	Strong association between the appearance of anaplastic clones and TP53 mutations	[146]
Undifferentiated-Gastric Carcinoma (UGC)	The inactivation of wild-type TP53 is an earlier event before dedifferentiation to mixed-type UGC	[147]
Medulloblastoma	TP53-ARF pathway is disrupt in anaplastic medulloblastoma	[148,149]
Hepatocellular carcinoma	Mutant p53 may have contributed to dedifferentiation during the development of HCC	[150,151]

found that fibroblasts with compromised p53 exhibit a higher frequency of iPSC generation. Furthermore, it was suggested that p53 may induce cell cycle arrest and apoptosis and thus function as a barrier to select exclusively perfect reprogrammed SCs [74]. A p53 mediated DNA damage response was shown to limit reprograming to ensure iPSC genomic integrity [70]. An additional role of p53 during reprogramming may be an indirect effect on cell proliferation [75]. One scenario suggests that p53 up regulates miR-199a-3p, which imposes G1 cell cycle arrest [76]. Another study demonstrated that p53 exerts its suppression of iPSC generation through the axis of p53-upregulated modulator of apoptosis (PUMA) [77]. We showed that p53 restricts mesenchymal-to-epithelial transition (MET) during the early phases of reprogramming and that this effect is primarily mediated by the ability of p53 to inhibit Klf4-dependent activation of epithelial genes [11]. Recently we have reported that iPSCs generated from homozygous mutant p53 MEFs, using only 2 transcription factors (Oct4 and Sox2), exhibited fully reprogrammed iPSC phenotype in vitro yet formed malignant terato-carcinomas in vivo, instead of the benign teratomas induced by the WT p53 iPSCs [73]. It is conceivable that these are pre-iPSCs [78] that may represent cancer iPSCs. Latest studies in the field suggest that the reprogramming process is comprised of an early stochastic phase and a late hierarchical one [26]. Reactivation of p53 at any of the stages hampers the formation of iPSC clones [79]. This suggests that p53 is not a transient roadblock, but rather a full-time monitoring agent. Recently, homologous recombination (HR) pathway genes were found to be necessary for the reprogramming process. Interestingly, in the absence of p53, cells with a defective HR pathway could undergo reprogramming, allowing the generation of iPSCs with genetic aberrations, emphasizing the role of p53 in the quality control of this process [80]. In all, this suggests that in addition to the rate-limiting role p53 plays in reprogramming it also has a quality control role, ensuring the generation of proper cancer-free iPSCs.

2.3. The role of p53 in ASCs

Under physiological conditions, an optimal balance exists between the maintenance of a sufficient ASC pool for tissue regeneration and the elimination of severely damaged SCs, thus ensuring maximal longevity. However, when encountering severe DNA damage programmed cell death or, alternatively, temporary or permanent cell cycle arrest is induced. The latter, which prevents cancer development, may tilt this fine balance and by the same token cause depletion in the SC reservoirs leading to long-term negative effects [81]. Although damage can be repaired in cells through one or more of the many sophisticated genome maintenance pathways, DNA repair and incomplete restoration of chromatin after substantiate damage may produce sequence mutations and epi-mutations, both of which have been shown to accumulate with age. The accumulation of faulty DNA containing mutations and/or epi-mutations in aged tissues increases cancer risk [2]. As p53 is regarded as the "guardian of the genome" [1] it is not surprising that dysfunction of p53 will affect processes critically dependent on genomic fidelity such as proliferation, differentiation and transformation of various ASCs.

The term ASCs includes many types of SCs, the more familiar of which are mammary gland SCs, neural SCs, hematopoietic SCs and mesenchymal SCs (MSCs). In this review we will address only the role of p53 in MSCs. MSCs represent a population of adult heterogeneous multipotent stem cells, which can be isolated from many adult tissues throughout the body and are able to self-renew and differentiate into various cell types of mesodermal origin [82,83]. p53 was shown to control differentiation of MSCs [4,84]. We and others have demonstrated that the absence of WT p53 [85] or the presence of a mutant p53 (unpublished results) confers selective advantages in the acquisition of typical MSC markers along with an increased proliferation of BM-derived MSC progenitors. Both knockout p53 [85] and mutant p53 mice (unpublished result) contained a larger number of colony forming precursors compared to WT progenitors. Furthermore, knockout p53 MSCs presented genomic instability with an increased expression of c-MYC [85]. MSC strains derived from mutant p53 also exhibited genome instability as judged by spectral karyotyping analysis (unpublished results). Interestingly, chromosome 11, where the p53 gene resides, exhibited major alterations that increased with age. A role for p53 in MSC aging may be suggested by the specific decrease in p53 RNA and protein in MSCs during the aging process, which does not occur in heart or spleen and may explain how MSCs avoid age-related senescence [86]. Moreover, aged MSCs were shown to exhibit spontaneous expression of embryonic factors and p53 point mutations, suggesting that mesenchymal tumors may have originated from aged MSCs [87]. Interestingly, MSCs also have a tumor promoting effect as supportive cells. p53 status in tumor stromal cells has a key role in tumor development by modulating immune responses. The tumor-promoting effect of p53-deficient MSCs was not observed in immune-compromised mice, indicating that the immune response has a critical role [88]. Altogether, p53 plays an essential role in MSC proliferation, maintaining their quantity as well as assuring their quality by preventing their transformation. The decrease in p53 levels upon aging or the acquisition of a mutation in the p53 gene may contribute to the high risk of MSC sarcomagenesis and to the role of MSCs in supporting carcinogenesis.

3. p53 as the barrier to formation of CSCs

CSCs could arise from accumulation of genetic insults in normal stem or progenitor cells or by dedifferentiation of existing differentiated cells. One example of the transformation of stem/progenitor cells into CSCs is provided by MSCs, which were proposed as candidate cells of origin for several sarcoma types [89]. Increasing evidence suggests that MSCs that acquire mutations in oncogenes or tumor suppressors may function as tumor initiating cells (TICs) leading to *de novo* tumor formation. In this regard MSCs might be the TICs capable of initiating sarcomagensis [90] as was shown for hematopoietic SCs, which may serve as TICs for hematopoietic malignancies [41]. Several studies in mouse models have indicated that p53 deficient MSCs may lead to sarcomagenesis. Transformation of MSCs seems to be highly dependent on alterations in the p21/p53 pathway, mainly by the abolishment of WT p53, but not on the retinoblastoma pathway [90–93]. Moreover, analysis of fibrosarcomas derived from aged mice showed that these tumors may have originated from MSCs harboring mutated p53. Furthermore, MSCs isolated from young mice and then aged in culture revealed the acquisition of clinically significant p53 mutations [87]. Another example of tumors originating from SCs was provided by mouse models based on conditional inactivation of p53, NF1 and Pten. This study showed that brain tumors originate from neural stem/progenitor cells while more mature cells cannot form tumors [94], identifying SCs as the cell of origin of CSCs. Table 1 provides examples of cancer types in which p53 aberration in ASCs has been shown to promote initiation and progression.

Reports on the link between p53 loss and the differentiation state of tumors were first published about 20 years ago [95–98]. Those studies showed that the high grade/de-differentiated phenotype of some sarcomas and carcinomas correlates with p53 loss and increased malignancy. Although these reports were consistent, they received little attention. Only after the burst of the reprogramming era came the understanding that all cell types have the potential to

dedifferentiate. In addition, reprogramming only occurs in a very small percentage of the transfected cells, suggesting the existence of reprogramming barriers. Indeed, we and others showed that down regulation of p53 enhances the efficiency of iPSC generation, whereas re-expression of p53 in p53 null MEFs markedly impedes this [65–73]. In addition, we have shown a new gain-of-function property of mutant p53 that enhances reprograming efficiency beyond that of p53 null MEFs. However, homozygous mutant p53 iPSCs formed malignant terato-carcinomas in vivo, perhaps recapitulating the transition of a differentiated p53 mutant cell to a dedifferentiated CSC. Others have extended our observation, demonstrating that the Myc pathway cooperates with the p53-R175H human mutant protein to disrupt the efficiency of reprogramming and that different mutant alleles of p53 have diverse efficiencies in enhancing iPSC colonies formation [79]. Thus, it is conceivable that a differentiated cell in the body gains mutations that drive the first phase of the cancer phenotype. Following a second hit of a p53 mutation, the barrier of dedifferentiation and formation of CSCs is removed. Indeed, an analysis of human tumors revealed that poorly differentiated aggressive tumor express an ESC transcription signature as observed in SCs [99]. Interestingly, breast, lung and prostate tumors with an ESC signature were found to contain a p53 mutation. In contrast, well-differentiated tumors contained a WT p53 [100,101]. One mechanism by which p53 prevents dedifferentiation is by binding to the promoter of CD44, one of the better known CSC markers, repressing its expression. Interestingly, constitutive expression of CD44 blocks p53 dependent apoptosis leading to cells resistant to doxorubicin [102]. Moreover, loss of p53 may lead to increased expression of the multidrug-resistance genes (ABCB1 or MDR1) and to chemotherapy resistance. Table 2 provides examples of cancers and tumor lines in which p53 aberrations resulted in a dedifferentiated phenotype.

4. Facing a chronic DNA insult – the story of the p53 heterozygous stem cells

At the junction between normal SCs and CSCs lay the heterozygous p53 SCs, namely SCs which concomitantly express a functional WT p53 and a mutant p53. Such a genotype is presented in Li-Fraumeni syndrome (LFS) patients. LFS is a rare type of cancer predisposition syndrome associated with germ line p53 mutations [103]. It appears that in LFS patients, as well as corresponding mouse models [104], the WT p53 is dominant over the mutated p53 allele, and they apparently develop normally. Only later in adult life do they acquire a wide spectrum of tumors, including bone and soft-tissue sarcomas, acute leukemia, early onset of breast cancer, brain cancers such as glioblastoma, and adrenocortical tumors occurring over a wide age range [105]. Approximately 60% of the initially analyzed tumors exhibited loss of heterozygosity (LOH) in the p53 locus. The remaining 40% bypass the suppressive effect of the WT allele by diverse mechanisms such as promoter hypermethylation [106], increased activity of Mdm2, the E3 ligase responsible for p53 ubiquitination [107], by impairing other components of the p53 pathway [108] or by the enhanced oncogenic potential of missense p53 mutations that are common in both LFS and sporadically mutation somatic cells [109]. Gain of function mutants or those showing dominant negative features may be sufficient to induce tumor formation in the presence of the WT gene, especially in context of other genetic or environmental insults [105,110,111].

The mouse model of LFS (R172H which is homologous to human R175H hot-spot mutation) holds great promise to unravel questions regarding the role of p53 in SCs of various origins and functions. As SCs harboring exclusively either WT or mutant p53 represent an end-point of either a normal or a mutated SC, the p53 heterozygous SC may give a "snap shot" on the process of tumorigenesis in SCs, as manifested by the LOH process. Importantly, this mouse model reflects the majority of p53 aberrations in human malignancies, which are missense mutations (75%) [112]. Moreover, it is tempting to speculate that the presence of the mutant p53 in these heterozygous SCs endows them with CSC characteristics. This speculation is based on the fact that although $p53^{+/-}$ and $p53^{+/R172H}$ tumors show similarities, only osteosarcomas and carcinomas from $p53^{+/R172H}$ mice metastasize to various organs [104].

We have established ESCs and MSCs derived from heterozygous p53 LFS mice and generated iPSCs from MEFs of these mice. This panel of cells enables us to evaluate the impact of p53 LOH on tumorigenesis as a function of cell origin. Heterozygous p53 MEFs, an example of somatic cells, undergo in vitro p53 LOH in a robust manner. In contrast, the frequency of p53 LOH varied among the various SCs as a function of their genome stability. It is well accepted that ESCs have a high genome stability and fidelity mainly due specialized mechanisms aimed at preserving their genome [17]. Indeed, no p53 LOH was observed in heterozygous p53 ES cells that exhibited stemness characteristics typical of WT p53 ESCs (unpublished results). With iPSCs heterozygous for p53 the situation is less defined. iPSCs, on the one hand, resemble ESCs and are considered fairly genomically stable. On the other hand, iPSCs are generated from MEFs, which were shown to be less stable. Although both WT and mutant p53 iPSCs present normal SC markers, mutant p53 iPSCs appear earlier with greater reprogramming efficiency. Moreover, when injected in vivo the mutant iPSCs give rise to malignant tumors [73]. Heterozygous p53 iPSCs resemble WT p53 iPSCs- both exhibit similar rates of iPSC formation. However, about 20% of the heterozygous p53 iPSC clones did undergo LOH, giving rise to iPSCs that resemble p53 mutant iPSCs, which induce malignant tumors in mice. The observation that all heterozygous p53 MEFs undergo p53 LOH but the majority of heterozygous p53 iPSCs do not, suggests that reprograming from a less stable somatic cell into a more stable SC triggers mechanisms that guard genome fidelity. It seems that in ESCs and iPSCs the presence of a functional WT p53 is sufficient to maintain genome stability. Thus, ESCs and iPSCs employ mechanisms, yet to be defined, to prevent p53 LOH. Moreover, an in-depth examination of single cell sub-clones of iPSCs revealed that a small fraction of cells lose their mutant allele rather than the WT p53 allele (unpublished results). This phenomenon of bi-directional p53 LOH emphasizes the great efforts made by iPSCs to maintain a stable genome. Since emerging data suggests that dedifferentiation is a natural homeostasis process [44], it is conceivable that p53, as a first line of defense, regulates and controls the processes of dedifferentiation in vivo and reprogramming in vitro. In the event that this control checkpoint is compromised, a second line of defense will be triggered. This line of defense includes the attenuation of p53 LOH, which may otherwise lead to the loss of the WT p53, or the activation of a DNA repair LOH process leading to the loss of the mutant p53 allele. Taken together, it appears that p53 functions to maintain a balance between somatic cells and SCs. Moreover, great efforts are made to sustain a functional WT p53 in SCs and to ablate the mutant p53, ensuring genome stability.

LFS patients and LFS mouse models predominantly develop sarcoma of mesenchymal origin [103,104]. As mentioned above, sarcomas may arise from damaged MSCs. Although sarcomas are one of the most dominant tumor types in LFS patients, as well as in the mouse and rat LFS models [103,104,113], no data so far has pointed to a p53 LOH process occurring in SCs of mesenchymal origin. The availability of heterozygous p53 mice at various ages makes it possible to address the above question, both in vitro and in vivo, with regard to aging. Interestingly, the in vitro p53 LOH process is more pronounced in MSC isolates established from bone marrow of adult

mice than adolescent mice, reflecting the higher p53 LOH rates as a function of aging. Only the heterozygous p53 MSC isolates which were established from adult mice induced sarcomas upon injection into immunocompromised mice, suggesting that while p53 may be a barrier to sarcomagenesis, its removal is not sufficient to induce cancer and further mutations are needed. Genotyping of single cell clones revealed that, as in iPSCs, an attempt to lose the mutant allele also occurs in MSCs but to a lesser extent. In contrast to heterozygous p53 iPSCs, in heterozygous p53 MSCs most p53 LOH events involved the loss of the WT allele, as expected from a less stable SC. Similarly, *ex-vivo* examination of bone marrow progenitors has revealed that p53 LOH is non-existent or very rare in bone marrow of adolescent mice, reflecting the normal development and the lack of tumors in patients and mice. However, the p53 LOH process was accelerated with age, reaching up to 10% of the progenitor SCs in adult mice, pointing to a tight connection between p53 LOH and aging in vivo (unpublished results). This observation raises the question of whether LOH, as a marker of genomic instability, leads to aging or whether aging leads to increased LOH. In agreement with these results, studies in yeast have revealed an increase in LOH as the mother cell ages [114]. Analysis of the colony forming units derived from adult mouse bone marrow indicated that in addition to the well-documented WT p53 LOH, which endows cells with growth advantage, loss of the mutant allele may also take place (unpublished results). It seems that in cells that are assumed to be genomically stable, such as BM progenitors and iPSCs, the loss of the mutant p53 allele is detected more frequently than the loss of the WT allele. Thus it is tempting to speculate that p53 LOH can be a physiological DNA repair mechanism that helps maintain genomic integrity. Unfortunately, when this DNA repair mechanism fails and the WT allele is lost, the final outcome will be takeover by the homozygous mutant p53 cells, leading to accumulation of other mutations and tumor formation.

5. Facing the future – eliminating CSCs using p53

Conventional anti-cancer therapies kill proliferating cells and often lead to shrinkage of the tumor. These therapies do not eliminate quiescent tumor stem cells that may, with time, arise and cause relapse of the disease. Thus, while targeting the proliferating tumor cells is the first step in combating cancer, targeting CSCs may be crucial to finally eradicating various tumor types. This goal may be achieved by either differentiation therapy or elimination therapy. Differentiation therapy is based on the induction of differentiation of CSCs. This process will lead to the loss of their self-renewal properties and to susceptibility to DNA damage responses. A proof of concept was achieved and adopted in clinical practice with the treatment of acute promyleocytic leukaemia (APL) patients with all-trans retinoic acid (ATRA). The amazing effect of ATRA as a differentiation inducer has flipped APL from the most-difficult-to-treat into the most-easy-to-treat acute leukemia [115]. Similarly, the differentiating agent 13-cis-retinoic acid (RA) is used as a standard treatment for high-risk neuroblastoma, improving survival by 35% in children with metastatic neuroblastoma [116]. In glioblastomas, induction of astrocytic differentiation with bone morphogenetic proteins (BMPs) reduces the frequency of $CD133^+$ CSCs [117]. Recently, data has been published providing proof-of-concept that inhibitors targeting mutant isocitrate dehydrogenases 1 and 2 (IDH1 and IDH2) could have potential applications as a differentiation therapy for cancer. Treatment with such an inhibitor (AGI-6780) induced differentiation of TF-1 erythroleukemia and primary human acute myelogenous leukemia cells in vitro [118]. Another inhibitor of mutant IDH1 was shown to delay growth and promote differentiation of glioma cells [119]. Other approaches towards differentiation therapy are based on mediating gene expression through histone deacetylases [120] and miRNAs [121]. For example, in glioblastoma, miR-34a targets Notch1 and Notch2 mRNAs, resulting in CSCs differentiation [122], while medulloblastoma CSCs undergo neural differentiation by virtue of miR-34a targeting the Notch ligand Delta-like ligand 1 (DLL1) [123]. Transfection of either miR-124 or miR-137 into glioblastoma multiforme CSCs ($CD133^+$) also induces cell cycle arrest and differentiation [124]. The profound role of p53 as a differentiation inducer in various cell types, together with its restricting activity in processes of dedifferentiation and reprogramming, places p53 as an attractive candidate for differentiation therapy. Initial data supporting this notion was obtained twenty years ago. Stable and regulated expression of WT p53 in a pancreatic carcinoma tumor model was shown to have multiple phenotypic consequences: the majority of the tumor cells (60–70%) underwent G1 growth arrest and apoptosis while the rest of the cells exhibited irreversible growth-arrest with morphologic and antigenic properties of a differentiated neuroendocrine-like phenotype in vitro [125]. Injection of lung metastases of human osteogenic sarcoma cells with WT p53 is associated with in vivo induction of terminal differentiation and apoptosis, inhibiting progressive growth of metastases [126]. SCs with target mutation in p53 possess the same self-renewal properties as CSCs and their number increases progressively in p53 null premalignant mammary glands [127]. Pharmacological reactivation of p53 correlates with restoration of asymmetric division of CSCs and tumor growth reduction [127]. In a model of squamous cell carcinomas (SCCs), one of the most aggressive and heterogeneous skin cancers, p53 restoration induces skin tumor cell differentiation and suppression with no apparent effect on apoptosis, proliferation, or senescence [128].

Another way to combat CSCs is to eliminate them. This could be achieved by targeting signaling pathway of self-renewal. For example, Hedgehog pathway inhibition is emerging as a feasible and promising therapeutic approach in several cancers and some inhibitors that directly target the positive Hedgehog signal transducer Smoothened (SMO) have entered clinical trials [129]. Attempts to target CSCs via surface markers were also suggested, although the expression of these surface markers may vary in different stages of the disease and may even vary between patients with the same disease [130]. Another strategy takes advantage of old chemotherapy drugs and combines them with a CSC targeting strategy. For example, treating gastric tumor cells, which express CD90, with trastuzumab (humanized anti-ERBB2 antibody) combined with traditional chemotherapy reduced the CD90+ population in tumor mass and suppressed tumor growth [131]. The same strategy has provided encouraging data in primary ovarian cancer cell lines and patient-derived xenograft models [132], non-small cell lung cancer cells [133] and primary colon cancer cells [134]. Similarly, it was shown that combining a p53 pathway-restoring agent such as ellipticine with a classical chemotherapy agent (5-fluoruracil) is associated with depletion of putative colon CSCs [135]. The mechanism leading to this phenomenon has yet to be defined, but it is conceivable that restoration of a functional WT p53 might reduce the expression of the ABC transporters, leading to an increase in the concentration and efficacy of some anticancer drugs.

6. Concluding remarks

SCs are essential for normal development and are crucial for organ regeneration. Damaged SCs may result in improper development, early aging and tumorigenesis. Thus, it is not surprising that p53 plays a major role in various processes ensuring that SCs will remain in sufficient quantity and quality. p53 serves as a barrier between normal SCs and CSCs by preventing processes such

as dedifferentiation and the formation of damaged SCs. Furthermore, p53 LOH is under tight control in genomically stable SCs. Moreover, in these SCs, the p53 LOH process is targeted towards the loss of the mutant allele, ensuring quality-controlled functional SCs (Fig. 1). Further studies aimed at understanding the mechanisms ensuring genomically stabled SCs and the pathways that lead to CSC formation may contribute to the development of means to combat cancer.

Conflict of interest statement

We state no competing interest.

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