Opinion

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The emerging role of p53 in stem cells

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Among the hundreds of oncogenes and tumor suppressors that have been identified in the past 50 years, p53 is probably the best characterized; nevertheless, new functions are constantly being discovered. As a tumor suppressor, p53 regulates cellular responses to different stress stimuli by inducing reversible cell cycle arrest and DNA repair, or triggering senescence or apoptosis. Recent findings on the regulation of stem cell (SC) division and reprogramming suggest the intriguing possibility that p53 also carries out its tumor suppression function by regulating SC homeostasis. Specifically, p53 activation may counteract SC expansion by several emerging mechanisms including restriction of self-renewing divisions, inhibition of symmetric division and block of reprogramming of somatic/progenitor cells into SCs.

Introduction

Since 1992, after Donehower and colleagues published their observations on mice genetically deleted for the p53 gene ($p53^{-/-}$ mice) [1], p53 has been defined as the 'guardian of the genome' [1,2]. More than 50 000 publications have subsequently documented the role of p53 in mediating different cellular responses to stress stimuli, including DNA damage (DD), ranging from transient cell cycle arrest and DNA repair, when damage is at low levels, to apoptosis or permanent cell cycle arrest (senescence) in more severely damaged cells.

p53 is mutated in approximately 50% of human cancers, whereas the remaining tumors often carry alterations of upstream genes that ultimately lead to attenuation of its function [3]. p53 is activated by Chk1/2, through the ATM/ATR kinase cascade, in response to DD, telomere dysfunction or replication stress, as well as by the ARF pathway in response to oncogenes [4]. These stimuli induce p53 upregulation mainly by blocking its MDM2-dependent ubiquitination and degradation [5]. Once activated, p53 acts as a transcription factor, upregulating a series of target genes that are involved in inhibition of the cell cycle, induction of apoptosis or senescence, control of genomic stability and inhibition of blood vessel formation [6]. Recently, p53 has been shown to regulate microRNA (miRNA) processing and also serves as an inducer of miRNA expression [7,8]; as downregulation of miRNAs is often observed in human cancer, this is an important finding that highlights the possibility of novel therapeutic approaches based on modulating the converging p53 and miRNA pathways. Among these p53-regulated miRNAs, the miR-34 family appears to be strongly involved in the control of quiescence, apoptosis and even stemness in various contexts [9]. Notably, loss of p53 correlates with decreased levels of a component of the miR-200c family and increased expression of epithelial–mesenchymal transition (EMT) factors, which have been linked to the stem cell (SC) phenotype [10]. The pivotal role of p53 in cancer, and its potential use as a therapeutic target, is confirmed by recent reports that indicate that re-expression or reactivation of p53 in p53-deficient or p53-defective tumors causes tumor regression in different mouse models of lymphoma, sarcomas, hepatocarcinomas, established osteosarcoma and lymphoma xenografts [11–16].

Most of the observations on the many effects of p53 were generated using established cell lines or relatively differentiated primary cells. In recent years, the increased interest in SCs combined with the idea that these cells and/or early progenitors might be the targets of neoplastic transformation drew attention to the role of p53 and its tumor suppressor activities in SCs. These new studies, while confirming some of the known functions of p53 in SCs, have highlighted novel and unexpected roles for this protein. This opinion article will focus on recent advances in p53 research with regard to the regulation of the adult SC pool in different compartments. In particular, we will first review the role of p53 in SC reprogramming and then we will discuss p53 functions in mammary, hematopoietic and brain SCs, analyzing its role in the regulation of self-renewal and asymmetric versus symmetric division. Finally, we will introduce the possibility of eradicating tumors by targeting p53 in cancer stem cells (CSCs). We will attempt to show that p53 loss can deregulate these mechanisms, ultimately leading to SC increase.

p53 in induced pluripotent stem cell (iPSC) reprogramming

Recently, p53 has been involved in the regulation of the process of dedifferentiation (reprogramming) of somatic cells into a pluripotent state similar to that of embryonic stem (ES) cells [17,18]. These are termed induced pluripotent stem cells (iPSCs) and can be obtained with low efficiency (less than 1%) by the transient expression in somatic cells of four 'reprogramming' genes (the Yamanaka factors: Sox2, Oct4, Klf4 and c-myc) [19]. These cells are virtually indistinguishable from ES cells in gene expression and epigenetic status and are able to differentiate into

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all tissue types, generating teratomas upon transplantation in adult mice and chimeric mice if implanted in blastocysts [19,20]. The low efficiency of reprogramming processes and their long latency [19] have been attributed either to the activation of checkpoint responses leading to senescence or apoptosis, or to the difficulty to convert somatic cells to an epigenetic status similar to ES cells. At least two of the four reprogramming genes (c-myc and klf-4) are very well-known oncogenes that are able to activate a checkpoint response. Expression of the reprogramming genes, inserted either in combination or alone, induces a complex response involving upregulation of γH2AX, p53, p21, p16 and p15 and leads to a senescent phenotype, characterized by low BrdU incorporation, representative of low levels of DNA replication, and high βGal staining, indicating senescence [21,22]. Importantly, one of the first events occurring during reprogramming seems to be the epigenetic silencing of the *ink4a* locus, which encodes p16 and p19arf. These proteins are positive regulators of the pRb and p53 tumor suppressor pathways, respectively. However, the p53 pathway seems more relevant to the reprogramming of mouse cells, whereas pRb is more important in human cells [21,22]. Notably, telomere shortening, which is known to induce a p53 response, is also able to counteract the reprogramming process [23].

These observations indicate that p53 acts as a negative regulator of the reprogramming process. The first evidence for a role of p53 in the control of this process came from studies on human fibroblasts in which either SV40 large T antigen (a repressor of p53 and pRb pathways) [24] or p53 small interfering RNA (siRNA) [25] were used together with the Yamanaka factors to improve the efficiency of iPSC reprogramming. Subsequently, it was demonstrated that inhibition or loss of p53 increases reprogramming efficiency by 3- to 10-fold, depending on the cell system or the manner of deletion or inactivation of p53 [22,23,26–28]. The molecular mechanism(s) underlying the role of p53 in reprogramming is still unclear. Li and colleagues have suggested that the Yamanaka factors repress the *Ink4/Arf* locus as an early event during reprogramming, causing deactivation of both the p53 and pRb pathways [22]. Other studies have proposed that p53 counteracts reprogramming by activating a stress response that in turn induces senescence [26,28] or apoptosis [23,27]. The different responses observed in these studies could reflect a cellular heterogeneity in the expression of reprogramming factors. An elegant way to circumvent this problem came from the work of the Jaenisch group [29]. In their paper, the authors describe the setting-up of a 'secondary system' to obtain iPSCs. Primary iPSCs were obtained by infecting fibroblasts with the Yamanaka factors, under the control of a doxycycline inducible promoter, and then implanted in blastocysts to obtain secondary chimeric mice. Secondary pre-B cells were then isolated and plated, in the presence of doxycycline, as single cells. Using this system, which enabled homogeneous factor expression, Jaenisch and colleagues demonstrated that virtually all the secondary cells were able to reprogram into iPSCs, although with different latency (3–5% of reprogramming after 2 weeks, >92% after 18 weeks). Notably, p53 knockdown accelerated the kinetics of reprogramming (>93% after 8 weeks) by enhancing the cell proliferation rate. This situation is compatible with a model in which reprogramming proceeds through a series of limiting steps and epigenetic changes that occur during cell division/expansion.

Regardless of the specific mechanisms, these studies draw attention to a novel putative role for p53 in SCs: it might act as a barrier to reprogramming, preventing the generation of new SCs from their differentiated progenies. Because SCs are putative targets of cancer transformation, the restraining activity of p53 on the number of SCs and their direct progenies might contribute to its tumor suppressive function (Figure 1). In this scenario, in tumors where p53 is mutated or inactivated, reprogramming could contribute to the expansion of cancer SCs. Interestingly, neonatal (but not mature) mouse testis cells are able to spontaneously reprogram *in vitro*, albeit with a very low frequency (1 cell in 15 million), into ES-like cells [30]. Consistent with previous findings, p53 loss increases the reprogramming frequency of neonatal cells and allows reprogramming of mature cells. Notably, *in vivo*, $p53^{-/-}$ mice show a high frequency of testicular teratoma [30]. Although there is no direct evidence of reprogramming occurring *in vivo*, it is possible that some somatic cells may reprogram into adult SCs, especially the scarcely differentiated progenitor cells that have not yet accumulated many genetic and epigenetic changes.

p53 in mammary stem cells

Adult mammary SCs can be cultured *in vitro* in nonadherent conditions as floating aggregates called mammospheres [31,32]. We have recently characterized both human [33] and mouse [34] mammospheres from normal tissues (wild type, WT) as spherical colonies derived from the clonal expansion of individual mammary SCs. Mammospheres contain an average of 1 SC per sphere and can be propagated over several passages of dissociation and reformation. Despite showing self-renewal capability, mammosphere serial replating leads to functional exhaustion of WT SCs, thus indicating that they possess limited life span in culture. In addition, direct imaging of the initial cell division and analysis of the partitioning of the cell fate determinant Numb has revealed that WT mammary SCs prevalently divide through asymmetric division [34], a specific division strategy that ensures both SC self-renewal and differentiation. Following asymmetric division in fact, one daughter cell retains SC identity, while the other commits towards differentiation [35]. By contrast, mammospheres derived from $p53^{-/-}$ mice contain an average of 5–6 SCs, are immortal and expand geometrically in culture, thus showing unlimited self-renewal potential.

In view of the accepted role of p53 in cell cycle restriction and induction of apoptosis, the 'immortal' behavior or the increased self-renewal of $p53^{-/-}$ SCs is not entirely surprising. However, the geometric expansion of the $p53^{-/-}$ mammospheres and the 5- to 6-fold increase in the number of SCs per sphere together strongly suggest that p53 controls the mode of SC divisions. In addition, we confirmed *in vivo* that $p53^{-/-}$ SCs prevalently adopt a symmetric strategy of self-renewing divisions [34]. Notably, the increased numbers of SCs observed in $p53^{-/-}$ breast

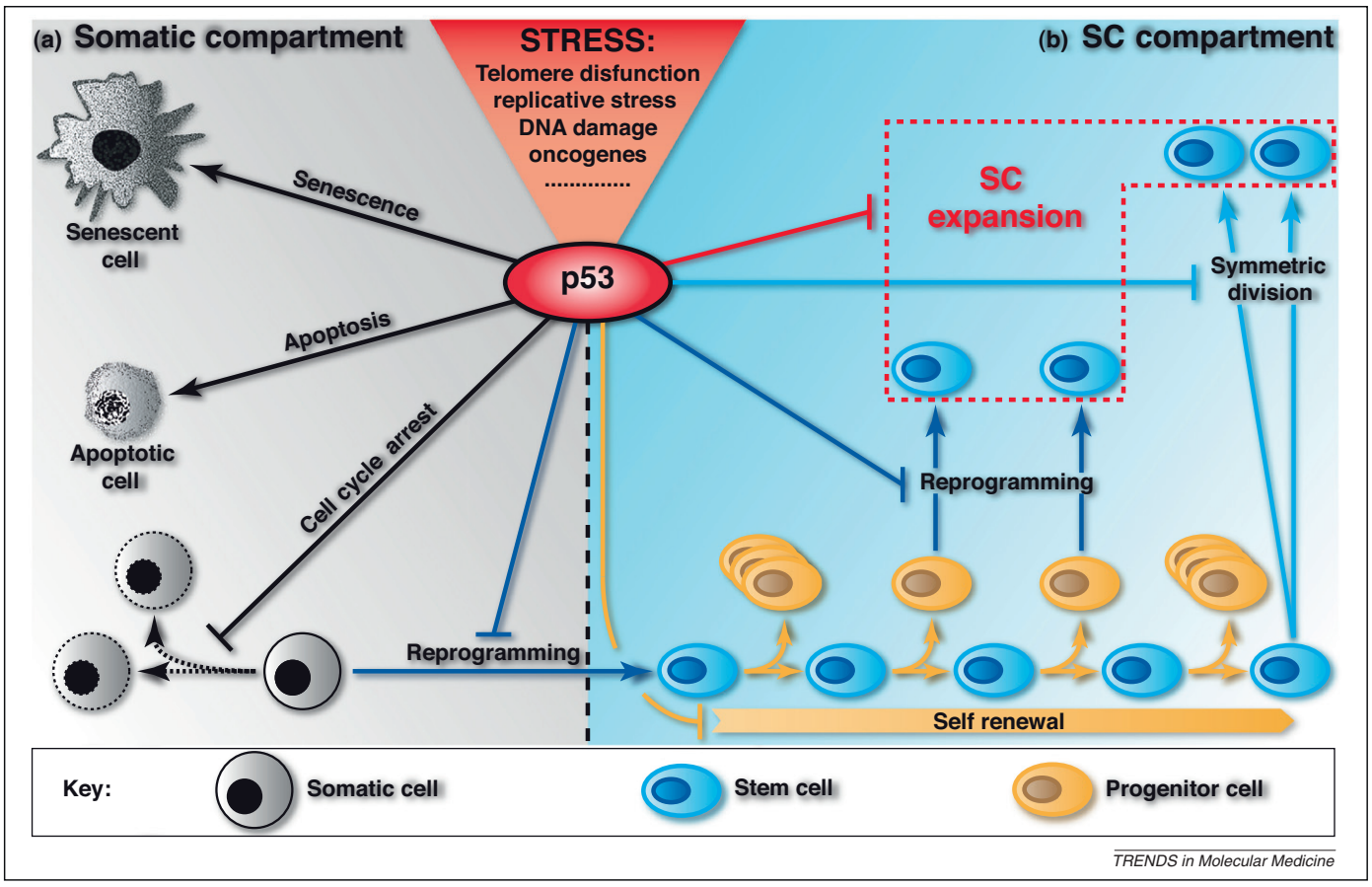

Figure 1. Role of p53 in stem cells (SCs): a possible model. **(a)** Somatic compartment. Different types of stress, as indicated, activate p53 that induces, in turn, cell cycle arrest (gray line) to allow DNA repair, apoptosis or senescence (gray arrows). **(b)** Stem compartment. Stem cells (SCs, cyan oval cells) are able to self-renew by (i) dividing asymmetrically (cyan arrows) to generate both a new SC and a progenitor cell (ochre oval cells), which rapidly divides (represented by overlapping cells) or (ii) by dividing symmetrically, with less frequency (cyan arrows) to generate two SCs. Progenitor cells can become reprogrammed to generate additional SCs (blue arrows) that may further divide symmetrically. Self-renewal, symmetric divisions and reprogramming contribute to maintain numbers of SCs (red box). p53 regulates SC numbers (red line) by restraining self-renewal (ochre line), inhibiting SC symmetric divisions (cyan line) and blocking reprogramming of progenitors (blue lines).

tissues and mammospheres might also be due to reprogramming of progenitor or somatic cells, as a consequence of p53 loss; the relative contribution of reprogramming, however, remains to be determined.

Despite the fact that $p53^{-/-}$ mammary glands have an increased SC pool and $p53^{-/-}$ mammary SCs geometrically expand in culture, transplantation of these cells in recipient mice generates a normal mammary gland. Accordingly, $p53^{-/-}$ mice, at least in the murine genetic background C57/BL6, rarely develop mammary tumors [34,36–39]. Nevertheless, several observations suggest that loss of p53 is involved in the etiopathology of both human and mouse mammary tumors: (i) mutations of p53 are found in many breast cancers and women affected by the Li–Fraumeni syndrome (an inherited predisposition to cancer development linked to germline mutations in the p53 gene) often develop breast tumors [38]; (ii) in the BALB/c background, approximately 75% of $p53^{-/-}$ mice have microscopic lesions in the mammary gland (sarcomas, epithelial hyperplasia and alterations in stromal morphology) [40]; (iii) transplantation of the $p53^{-/-}$ BALB/c epithelium into fat pads of WT syngeneic mice leads to the development of mammary carcinomas in 60–75% of mice [40,41]; (iv) in a conditional mammary tumor model, approximately 70% of mice that carry tissue-specific inactivation of p53 develop mammary carcinomas [42]; and (v) in ErbB2 transgenic mice, which develop mammary carcinomas with high penetrance and short latency, p53 impairment is responsible for the immortal behavior and the geometric expansion of mammary CSCs *in vitro* and for carcinoma growth *in vivo* [34].

In this scenario, an intriguing possibility is that p53 may carry out its tumor-suppressive function in SCs, on the one hand, by activating canonical DNA damage responses and, on the other hand, in the absence of any DNA damage, by preventing the expansion of the SC pool through the induction of an asymmetric mode of division (Figure 1). In this context, it is worth noting that symmetric divisions *per se* may induce aneuploidy, thus further contributing to cancer initiation. This is the case for *Drosophila* neuroblasts, which become aneuploid upon a few rounds of symmetric divisions [43], probably because the same machinery that controls the modality of SC division also regulates centrosome functions, mitotic spindle orientation and chromosome segregation [44,45].

p53 in hematopoietic stem cells

Hematopoietic stem cells (HSCs) are probably the best characterized among somatic SCs, owing to the availability of well-established cell surface markers that facilitate their

purification (~50% enrichment of the lin$^-$ Sca1$^+$ cKit$^+$ CD150$^+$ CD48$^-$ population or SLAM HSC population). Several studies have recently investigated p53 functions in HSCs, both under steady-state conditions and in response to various forms of cellular stress. They have shown that expression of p53 is crucial for the regulation of several aspects of HSC behavior, including quiescence, self-renewal, survival, DNA repair and cell competition.

Most HSCs are noncycling under steady-state conditions and maintenance of quiescence is critical for the preservation of their function. Deletion of p53 in mice leads to increased self-renewal of HSCs, both in culture and *in vivo*, and to expansion of the HSC pool [46]. Interestingly, Chen *et al.* found that transplantation of SLAM HSCs from p53 null mice into lethally irradiated recipients resulted in reduced engraftment in the second serial transplantation, suggesting that the increased self-renewal of p53 null HSCs is not associated with an increased repopulating ability of individual HSCs [47]. It is not known whether this reduction may also happen in the second serial transplantation passage of mammary SCs, although mammosphere serial replating experiments indicate that loss of p53 does not reduce either self-renewal or functionality of mammary SCs *in vitro* [34]. Notably, Chen *et al.* also reported that recipients of p53 null HSCs did not develop lymphomas or other tumors, whereas transplantation of the whole bone marrow (WBM) led to both enhanced engraftment of p53 null HSCs and lymphomas [47]. These findings might reflect the presence of tumor initiating cells in the WBM of p53 null mice. Interestingly, loss of p53 could promote acquisition of self-renewal by early hematopoietic progenitor cells [48], suggesting that progenitor reprogramming might contribute to the increased frequency of reconstitution and to the lymphomas of the p53 null bone marrow.

Abrogation of oncogene-induced antiproliferative responses in HSCs and/or progenitors represents a further putative mechanism by which loss of p53 might contribute to oncogenesis. Recent studies have revealed that in various tumors (cancer of the lung, colon, prostate or bladder, as well as melanomas and lymphomas), during the earliest stages of cancer progression, oncogene expression is associated with accumulation of DNA damage and activation of a DNA damage response involving p53 and leading to cellular senescence [49–55]. This p53-dependent cellular response is considered to be a powerful barrier to tumor development. Indeed, genetic evidence has demonstrated that p53-dependent cellular senescence is able to suppress Pten-deficient tumorigenesis *in vivo* [51]. The Lowe group has recently reported that both short hairpin RNA (shRNA)-mediated suppression and genomic deletion of p53 induce aberrant self-renewal of myeloid progenitor cells. In the absence of p53, expression of oncogenic KRas under its promoter in progenitors was able to initiate and propagate acute myeloid leukemia [56], suggesting that p53 regulates the cellular response to oncogene expression in progenitors. However, p53 suppression could not reverse the Ras-induced depletion of HSCs, suggesting that loss of p53 does not protect HSCs from oncogenic stress [56].

Yet it is not clear how SCs and progenitors respond to DD and oncogene expression, and what is the role of p53 in this response. Three recent papers address the issue of how both mouse and human hematopoietic stem and progenitor cells (HSPCs) respond to radiation-induced DD [57–59]. These studies indicate that hematopoietic cells can adopt different means of handling DD, depending on their differentiation stage. Surprisingly, they also show that DD responses in mouse or human cells follow opposite routes and unravel a different role for p53 in the DD response of HSPCs. Mouse HSPCs (defined as lin$^-$ Sca1$^+$ cKit$^+$ flk2$^-$) have a unique cell intrinsic mechanism that ensures their survival following X-ray treatment and which involves the activation of p53 and its transcriptional target p21 and DNA repair [58,59]. By contrast, human cord blood HSPCs exhibit p53-dependent radiation-induced apoptosis. Here, p53 inactivation reduces apoptosis and preserves *in vivo* HSC repopulating functions but leads to reduced self-renewal of HSCs in secondary transplants (due to accrual of DD) [57]. It is not clear, however, whether the differences between these two studies reflect differences between species, between ontogenic stages or between markers used for the isolation of stem and progenitor populations. Further studies are needed to evaluate how p53 levels are specifically regulated in HSCs following irradiation and what specific molecular mechanisms regulate the different DD responses of stem and progenitor cells.

Recently, three elegant studies have highlighted a role for p53 in regulating the competitive selection of stem and progenitor cells (HSPCs following the stress of ionizing radiation (IR) [59–61]. After IR, cells with lower levels/activity of p53 outgrew those with higher levels/activity. Notably, although cells with lower levels of p53 proliferated more rapidly, this effect was also due to non-cell-autonomous induction of growth arrest and expression of senescence-related genes in the outcompeted cells with higher p53 activity. Thus, p53-dependent cell competition represents a putative mechanism that might contribute to the selective expansion of early tumor cells.

p53 in neural stem cells

Both overexpression of the E6/E7 oncogenes, which leads to inhibition of the p53 and pRb pathways, and deletion of p53 have been reported to increase proliferation and self-renewal of neural SCs in a neurosphere-based system [62–64], suggesting that one of the physiological activities of p53 is to restrain overproliferation of neural SCs by limiting the frequency of self-renewing divisions.

This activity may contribute to the tumor suppressor function of p53 in brain CSCs, as recently shown in glioblastoma multiforme (GBM), a highly invasive type of brain tumor [65]. In this tumor type, Nanog, a homeoprotein required to maintain ES pluripotency [66], appears essential for SC functions as its inactivation reduces neurosphere formation *in vitro* and GBM growth *in vivo*. Importantly, in this system, p53 negatively regulates Nanog [65], suggesting a role for p53 in the control of both CSC self-renewal and GBM growth.

CSC-targeted treatment of tumors

Currently available antiproliferative drugs have been selected for their ability to reduce tumor size in early clinical trials; however, tumor size reduction does not necessarily

imply elimination of the rare CSC population. Several observations suggest that both normal SCs and CSCs are more resistant to chemotherapy than their differentiated progenies. This may reflect the well-known property of SCs and CSCs to remain quiescent or may be due to the increased expression of antiapoptotic proteins in SCs and CSCs, such as members of the BCL-2 family [67,68], or transport proteins such as MDR1 and the ABC transporters [69]. Thus, it has been proposed that CSCs might survive conventional treatments and be responsible for tumor relapse. Accordingly, selective ablation of the rare CSCs, probably in association with conventional antiproliferative drugs, is foreseen as a potentially more effective approach to eradicate tumors.

As mentioned above, p53 is also emerging as a potential target for therapies that could lead to CSC elimination. Three groups have recently reported that re-expression of p53 by different means causes regression of various p53-null tumors. In the first report, hydroxytamoxifen-mediated activation of a p53/estrogen receptor fusion protein caused rapid apoptosis in lymphomas and significantly increased survival of the mice [11]. In the second study, p53 re-expression, mediated by CRE recombinase, induced regression of spontaneous lymphomas and sarcomas via apoptosis and senescence, respectively [15]. Finally, p53 restoration caused tumor regression in a hepatocarcinoma xenograft model by inducing senescence and differentiation [16].

In those tumors in which p53 is not mutated (~50% of human cancers [3]) but only functionally attenuated due to the activation of p53 inhibitory pathways, pharmacological reactivation of p53 (using Nutlin3, which inhibits binding of p53 to Mdm2, a protein that leads to p53 degradation) also results in tumor regression, as in the case of established osteosarcomas and lymphoma xenografts [12–14].

Our group has used Nutlin3 to treat ErbB2 transgenic mouse breast tumors where CSCs express low levels of p53 and the following results were found: (i) Nutlin3 selectively reduces the number of CSCs, (ii) it induces a switch from symmetric to asymmetric CSC division, and (iii) it reduces tumor size by stabilizing p53 [34]. Notably, Nutlin3 exerted little or no effects on all measured properties of WT SCs (life span, frequency of self-renewing divisions and ability to generate proliferating progenitor cells) [34], confirming the generally accepted notion that only tumor cells contain signals, such as oncogenic or replicative stress,

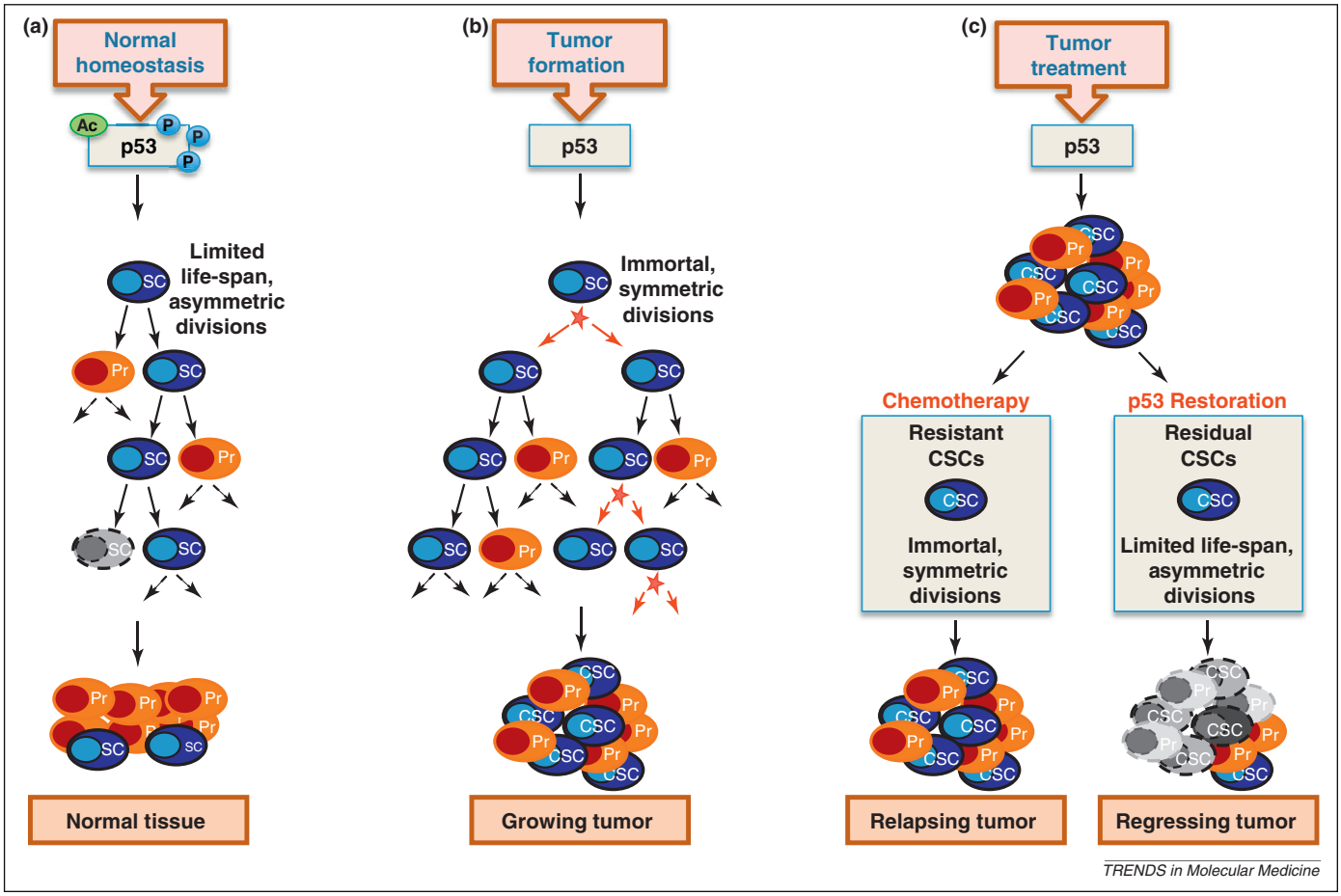

Figure 2. Role of p53 in tissue homeostasis, tumor formation and tumor response to treatment: a possible model. **(a)** Activated p53, indicated as a box with the protein modifications acetylation (Ac) and phosphorylation (P) maintains normal tissue homeostasis by imposing a prevailing asymmetric mode of SC division (black arrows) and restricting SC life span. Stem cells: SCs; Pr: progenitors; gray SCs: pro-senescent SCs. **(b)** Functionally impaired, mutated or deleted p53 (indicated as a box without protein modifications) contributes to tumor formation and growth by increasing the frequency of cancer SCs (CSCs) symmetric divisions (red arrows and star) and conferring on them immortality. Alternate usage of symmetric and asymmetric divisions permits tumor expansion and cancer cell differentiation. **(c)** Treatment of tumors with conventional chemotherapy or radiotherapy induces tumor regression and selects for rare resistant CSCs that are responsible for tumor relapse (left); restoration of p53 activity in tumors using MDM2 inhibitors (such as Nutlin) or by re-expression of wild type p53 reverts the mode of division of CSCs from prevailingly symmetric to asymmetric and restores the CSC senescence program, leading to tumor regression.

that activate p53 after restoration of its expression. Nutlin3 reverted the life span and self-renewal properties of CSCs to those typical of WT SCs, consistent with the observed physiological function of p53 in SCs [34]. Further studies are now focusing on the cellular and molecular targets of p53 restoration in different tumor types.

Concluding remarks

Recent studies designed to highlight p53 functions in SCs, while confirming its central role in the control of cell cycle progression, hint at the possibility that p53 is involved in the regulation of SC homeostasis, as demonstrated by the fact that p53-null mice show an expansion of the SC pool in several compartments, such as blood [46], mammary glands [34] and brain [63,64].

In view of its well-established antiproliferative effect, it is not surprising that p53 loss also increases SC self-renewal in these compartments [34,46,62,63]. However, increased self-renewal alone cannot account for SC expansion, if the modality of SC division remains prevalently asymmetric. Interestingly, it has been shown that p53 controls the modality of cell division in both mouse and human fibroblasts, as well as in adult mammary epithelial cells, and this function might also take place in adult SCs [70–72]. Indeed, we have recently demonstrated [34] *in vitro* that p53 loss allows mammary SC symmetric divisions and that this fact correlates *in vivo* with amplification of the mammary SC pool and geometric expansion of SCs during the life of p53 null mice. Thus, the combination of increased self-renewal and symmetric divisions might explain the amplification of the SC pool observed in p53 null animals. Nonetheless, it remains to be formally shown by direct imaging that p53 loss also increases *in vivo* the frequency of SC symmetric divisions and that this increase also occurs in tissues other than the mammary gland.

The involvement of p53 in iPSC reprogramming suggests that its loss or inactivation can induce reprogramming of somatic cells into adult SCs, thus contributing to SC expansion. However, because the vast majority of studies have focused on the process of de-differentiation into ES cells, direct evidence of reprogramming of progenitor/somatic cells into adult SCs of the same compartment, or that reprogramming can occur *in vivo*, is still lacking.

Regardless, these observations suggest that p53 carries out its tumor suppression role not only via well-accepted functions, such as cell cycle inhibition and induction of apoptosis or senescence (Figure 1a) but also by regulating SC homeostasis. Specifically, p53 appears able to restrain SC self-renewal, impose an asymmetric mode of division and block the reprogramming of somatic/progenitor cells into SCs (Figures 1b and 2a). Thus, suppression of p53 should favor tumor formation due to the expansion of CSCs resulting from increased self-renewal, symmetric divisions and reprogramming (Figures 1b and 2b). On the contrary, restabilization of p53 in CSCs should reduce SC number and block cancer progression and growth (Figure 2c).

This assumption opens new therapeutic opportunities aimed at restoring p53 functions in order to treat cancer by rescuing some of the physiological properties of SCs (Box 1). Re-establishment of p53 functions could be achieved with new drugs able to block p53 degradation, either through the use of mediators of p53 downstream effects, such as specific miRNAs, or by targeting new pathways controlled by p53.

Box 1. Outstanding questions and future perspectives

- Identify reliable membrane markers to recognize and purify SCs and CSCs.
- Identify new membrane markers capable of discriminating symmetric versus asymmetric divisions in SCs and CSCs.
- Develop new drugs capable of restoring asymmetric divisions by stabilizing p53.
- Define assays able to predict the outcome of a CSC targeted therapy in both preclinical models of cancer and patients.

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