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Review Article

Roles of p53 in Various Biological Aspects of Hematopoietic Stem Cells

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Hematopoietic stem cells (HSCs) have the capacity to self-renew as well as to differentiate into all blood cell types, and they can reconstitute hematopoiesis in recipients with bone marrow ablation. In addition, transplantation therapy using HSCs is widely performed for the treatment of various incurable diseases such as hematopoietic malignancies and congenital immunodeficiency disorders. For the safe and successful transplantation of HSCs, their genetic and epigenetic integrities need to be maintained properly. Therefore, understanding the molecular mechanisms that respond to various cellular stresses in HSCs is important. The tumor suppressor protein, p53, has been shown to play critical roles in maintenance of "cell integrity" under stress conditions by controlling its target genes that regulate cell cycle arrest, apoptosis, senescence, DNA repair, or changes in metabolism. In this paper, we summarize recent reports that describe various biological functions of HSCs and discuss the roles of p53 associated with them.

1. Introduction

Adult stem cells have recently attracted significant public attention, mostly because they can be a source of donor cells for replacing cells in transplantation therapies to treat various incurable diseases. In fact, hematopoietic stem cell (HSC) transplantation is now routinely performed to treat patients with hematopoietic malignancies and other disorders of the blood and immune systems. Thus, understanding the regulatory molecular networks that regulate stem cells is very important to develop new strategies of treatment for intractable diseases.

Among adult stem cell types, HSCs are the most extensively studied because they are relatively easy to obtain from both healthy and diseased persons, compared with isolation of adult stem cells from other tissues. HSCs are considered a very important cell population capable of self-renewal and differentiating into and supplying all blood cell types for life, whereas other hematopoietic cells such as hematopoietic progenitor cells (HPCs) and more differentiated cells undergo transient proliferation and die within a limited time period. Moreover, HSCs are of interest because of their plasticity to become cell types of other tissues [1, 2].

Under steady-state conditions, most HSCs are in quiescence, a period in the G0 phase of the cell cycle, and proliferate very slowly [3]. Thus, elucidation of the regulatory molecular mechanisms that execute self-renewal as well as entry into and exit from the quiescence of HSCs is essential to understand the biology of HSCs.

Additionally, understanding the molecular pathways of HSCs under the stress conditions of DNA damage is also critical to better deal with suppression of the hematopoietic system by irradiation or the cytotoxic effects of anticancer drugs including arsenic trioxide, anthracycline, cisplatin, and bleomycin that are currently used for the treatment of cancer.

Recent reports have suggested that HSCs are controlled by various cell cycle regulators such as p53, $p16^{Ink4a}$, and

$p19^{Arf}$ under both steady and stress conditions [4, 5]. Among these regulators, p53 is extensively studied and well known as a major tumor suppressor involved in various critical cellular functions such as proliferation, cell cycle arrest, apoptosis, and DNA repair mechanisms [6, 7].

In this paper, we describe the molecular mechanisms for regulation of HSCs under both steady and stress conditions, and particularly the roles of p53 associated with HSC functions such as responses to cellular stresses, apoptosis, self-renewal, senescence, and plasticity in addition to leukemia stem cells (LSCs).

2. p53 as a DNA Damage Checkpoint Molecule

Somatic cells, including immature tissue stem cells, constantly receive intrinsic and extrinsic DNA damage caused by various stresses. To maintain the genomic integrity of stem cells as well as tissue homeostasis, checkpoint mechanisms that activate DNA damage repair are crucial [8]. Among the types of DNA damage, double strand breaks (DSBs), which can be caused by current therapeutic approaches such as ionizing radiation and chemotherapy, are the most cytotoxic type of DNA lesion [9].

To minimize adverse effects caused by DSBs, cells rapidly activate the DNA damage checkpoint pathway after exposure to such stresses. Upon DNA damage, the sensor protein ataxia-telangiectasia mutated (ATM) is activated, which phosphorylates various downstream target proteins and induces the cell cycle checkpoint response [10, 11]. After sensing DNA damage, activated ATM directly phosphorylates the tumor suppressor p53 at serine 15 within its amino-terminal transactivation domain. ATM also activates CHK2, a serine threonine kinase, which phosphorylates p53 at threonine 18 and serine 20. MDM2, an E3 ubiquitin ligase that targets p53, is also phosphorylated by ATM. These phosphorylations that modify p53 and MDM2 directly or indirectly by ATM lead to transcriptional activation and stabilization of p53 [11]. Accumulation of p53 following low or repairable levels of DNA damage leads to activation of $p21^{Cip1}$ transcription, which inhibits cyclin-dependent kinases (CDKs) and induces a delay or arrest of the cell cycle [12, 13]. During this delay or arrest of cell cycle progression induced by the checkpoint mechanism, cells have an opportunity to repair DNA damage. When DNA damage is high or irreparable, p53 induces transcription of proapoptotic genes such as BAX, NOXA, and PUMA that eliminate damaged cells [14, 15].

Tumor suppressors $p16^{Ink4a}$ and $p19^{Arf}$ are also CDK inhibitors. $p16^{Ink4a}$ and $p19^{Arf}$ are distinct proteins translated from alternative reading frames of the same genomic locus called Cdkn2a. Whereas $p16^{Ink4a}$ binds to CDKs and prevents CDKs binding to cyclin D, which results in the inhibition of phosphorylation of the retinoblastoma (pRb) protein, $p19^{Arf}$ binds to Mdm2 and inhibits its ubiquitin ligase activity toward p53, resulting in promotion of p53 stabilization. These genes are negatively regulated by Bmi-1, which is strongly associated with HSC function as described below in the "p53 apoptosis pathway" section.

3. p53 Signaling against Reactive-Oxygen-Species- (ROS-) Induced Stresses

In living cells including HSCs, ROS such as superoxide anion ($\bullet O_2^-$), singlet oxygen ($^1O_2$), hydrogen peroxide (H_2O_2), and hydroxyl radicals ($\bullet$OH) are continuously generated owing to metabolic and other biochemical reactions as well as stresses caused by various extrinsic factors [16, 17]. While ROS play physiological roles as secondary messengers in intracellular signaling cascades, they also induce oxidative stress that can cause damage to cellular structures, including lipids, membranes, proteins, and DNA, which is thought to contribute toward cancer development [16–18].

Ito et al. reported that $CD34^-$ $lineage^-$ $Sca1^+$ $c\text{-}kit^+$ ($CD34^-$ LSK) HSCs can be separated into two fractions, cells with high and low ROS levels (ROS^{high} and ROS^{low} HSCs, resp.) and showed that ROS^{low} HSCs retain their long-term repopulating ability throughout serial transplantation assays, whereas this capacity decreases in serially transplanted ROS^{high} HSCs [19]. This decreased functional activity of ROS^{high} HSCs can be restored by treatment with the antioxidant N-acetyl-L-cysteine (NAC), which acts as an antioxidative agent by scavenging ROS, indicating that the ROS level affects HSC functions [19]. Indeed, it has been reported that ROS is an inducer of cell cycling by disrupting the maintenance of HSC quiescence [20].

DNA damage induced by ROS results in p53 upregulation, but p53 expression has not been detected in ROS^{high} HSCs and only slightly in ROS^{low} HSCs [21]. The reason for this low level of p53 in ROS^{low} HSCs is probably because a higher level of p53 decreases the intracellular ROS level to protect the genome from ROS-induced genomic damage by upregulating several antioxidant genes in ROS^{low} HSCs [22]. This role of p53 in downregulation of the ROS level in HSCs is enhanced by several molecules such as hypoxia inducible factor 1α (HIF-1α), an intrinsic transcription factor activated under a hypoxic condition [23]. HIF-1α stabilizes p53 in a hypoxic area of the bone marrow in vivo [24]. Indeed, HIF-1α deficiency causes an increase in ROS levels and a decrease in HSC numbers during various stresses including a serial transplantation assay, suggesting that HSCs maintain their normal functions via downregulation of ROS by the HIF-1α-p53 pathway [24].

Thus, p53 appears to play a central role in repressing ROS-induced stresses by upregulating antioxidant genes, which results in maintaining quiescence for the survival of HSCs.

4. p53 Apoptosis Pathway

As discussed in the above section, p53 functions in the survival and maintenance of HSCs, but it is also a critical regulator of apoptosis to eradicate HSCs in certain situations. It has been shown that the loss of Bmi-1 in HSCs results in impairment of HSC self-renewal owing to accumulation of $p19^{Arf}$, which causes p53-dependent apoptosis [25].

Similarly, conditional deletion of Cbp/p300-interacting transactivator with Glu/Asp-rich carboxy-terminal domain,

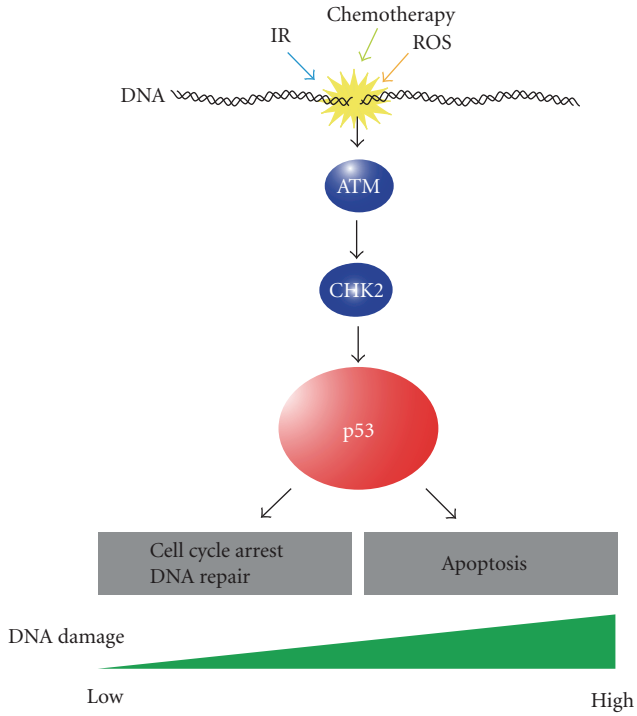

Figure 1: Functions of p53 in response to DNA damage. Genomic stresses caused by ionizing radiation (IR), chemotherapy, and reactive oxygen species (ROS) activate DNA damage check point pathway, which leads to the activation of ATM and CHK2 and subsequent stabilization of p53. Then, stabilized p53 induces cell cycle arrest and DNA repair when DNA damage is moderate, whereas it activates apoptosis pathway when DNA damage is extensive.

2 (Cited2) that controls proliferation of mouse embryonic fibroblasts (MEFs) and highly expressed in long-term (LT)-HSCs [26, 27], results in decreased HSC numbers [26]. This phenotype is rescued by additional deletion of p53, indicating that decreased HSC numbers in mice lacking Cited2 are caused by p53-induced cell death [26]. Moreover, inactivation of F-box and WD-40 domain protein 7 (Fbxw7), a subunit of the SKP1-CUL1-F-box protein (SCF)-type ubiquitin ligase complex, leads to impairment of the HSC repopulating capacity and a reduced HSC pool owing to active cell cycling and p53-dependent apoptosis [28, 29].

As discussed above, p53 has two opposing roles in regulating the fate of HSCs, which might depend on the level of damage to HSCs and the effect on p53 activity in each situation (Figure 1).

5. Roles of the Mdm2-p53 Pathway in HSCs

To examine the significance of the Mdm2-p53 interaction in more detail, p53 mutant protein ($p53^{515C}$ encoding p53R172P) that maintains the ability to induce senescence and cell cycle arrest, but not apoptosis, was analyzed in $Mdm2^{-/-}p53^{515C/515C}$ mice [30]. $Mdm2^{-/-}p53^{515C/515C}$ mice are born at normal Mendelian ratios but die by postnatal day 13 owing to hematopoietic failure [30]. Whereas the HSCs of $Mdm2^{-/-}p53^{515C/515C}$ mice are normal in fetal livers at embryonic day (E)14.5, HSC numbers are significantly decreased in the bone marrow of E18.5 and postnatal mutant mice [30]. Moreover, ROS levels in fetal livers at E14.5 are low, but the bone marrow of E18.5 and postnatal $Mdm2^{-/-}p53^{515C/515C}$ mice has higher ROS levels compared with that of $Mdm2^{+/-}p53^{515C/515C}$ controls [30]. HSCs with a high ROS level in postnatal $Mdm2^{-/-}p53^{515C/515C}$ mice show a reduced repopulating capacity, but this phenotype is rescued by NAC [30]. These results suggest that Mdm2 regulates appropriate hematopoiesis in postnatal mice by repressing ROS levels and regulating p53 activity.

6. Function of p53 in HSC Self-Renewal and Quiescence

It has been shown that hematopoiesis in $p53^{-/-}$ mice is almost normal [31]. However, p53 is preferentially expressed in HSC populations compared with that in various myeloid progenitor cells including common myeloid progenitors (CMPs), granulocyte/monocyte progenitors (GMPs), and megakaryocyte/erythroid progenitors (MEPs) [4]. Lin^- $Sca\text{-}1^+$ cells are thought to contain HSC population in mice, and cells in quiescence are detected as $PyroninY^{low}$ cells [4]. Liu et al. have performed PyroninY staining of Lin^- $Sca\text{-}1^+$ cells from $p53^{-/-}$ mice and found a reduction of $PyroninY^{low}$ cells in the population, indicating that loss of p53 leads to decreased HSCs in quiescence [4]. HSCs in quiescence are also identified as $CD34^-$ $lineage^-$ $Sca1^+$ $c\text{-}kit^+$ side population cells ($CD34^-$ LSK SP cells) based on their ability to efflux the fluorescent dye Hoechst 33342 [3, 32]. Liu et al. also found that $CD34^-$ LSK SP cells are decreased in adult bone marrow lacking p53, and proliferation of $p53^{-/-}$ $CD34^-$ LSK cells is increased significantly [4]. Furthermore, they identified growth factor independent-1 (Gfi1) and Necdin as p53 target genes that maintain HSC quiescence by comparing the expression profiles of wild-type and p53-null HSCs [4]. Therefore, p53 appears to play a role in promoting HSCs into quiescence, and HSCs tend to enter the cell cycle from quiescence in the absence of p53 (Figure 2). Consistent with this notion, it has been reported that loss of p53 function by the chemical p53 inhibitor, pifithrin β, promotes the proliferation of HSCs in vitro and in vivo [33].

HSCs lacking p53 are more successful at reconstituting bone marrow in a competitive repopulation assay, indicating that the repopulation capability of HSCs for the first transplantation of bone marrow is improved by inhibition of p53 [4, 34]. On the other hand, Chen et al. compared bone marrow reconstitution abilities between $SLAM^+$ $p53^{+/+}$ and $SLAM^+$ $p53^{-/-}$ HSCs in a serial transplantation assay and found that lack of p53 reduces the repopulating ability of individual HSCs for serial transplantation, indicating that loss of p53 does not improve the long-term function of HSCs [35]. These results suggest that loss of p53 can improve self-renewal and bone marrow repopulating capabilities over a short term but cannot maintain these capabilities of HSCs over a long term, probably owing to the reduced population of quiescent HSCs that have a long-term repopulating ability.

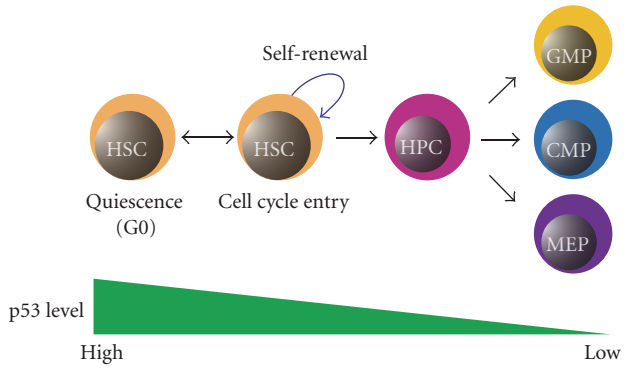

FIGURE 2: Regulation of quiescence and self-renewal in HSCs by p53. Maintenance of quiescence in HSCs needs proper expression of p53. Decrease in p53 expression promotes self-renewal and differentiation of HSCs.

HSC self-renewal is also affected by pathways activated by extracellular signaling molecules. Notch is a cell surface receptor that influences cell fate decisions such as cell differentiation, survival/apoptosis, and proliferation, which is activated by its ligand Jagged1 [36]. Activation of Notch leads to cleavage and release of the intracellular domain of Notch, which enters the nucleus and activates various transcription factors such as C promoter binding factor 1 (CBF1) [36]. Using transgenic Notch reporter mice, Duncan et al. reported that Notch pathway activation increases HSC self-renewal and decreases HSC differentiation in vivo [37]. However, Mancini et al. reported that HSC self-renewal does not require Jagged1-mediated Notch signaling, using conditional Jagged1$^{-/-}$ mice [38]. Thus, the requirement of Notch signaling for HSC self-renewal is still controversial [39, 40]. It should be noted that p53 is downregulated by Notch signaling [41], implying that an increase in HSC self-renewal by the activation of Notch signaling might be partly mediated by the suppression of p53 function.

HSC self-renewal is also regulated by Wnt signaling, which is activated by binding of the extracellular "Wnt" ligand to the cell surface receptors of the Frizzled family. When Wnt binds to a Frizzled family receptor, β-catenin is stabilized by release from its inhibitory complex consisting of axin, glycogen synthase kinase-3β (GSK-3β), and adenomatous polyposis coli (APC) (the APC/Axin/GSK-3β complex). The release of β-catenin from the APC/Axin/GSK-3β complex allows the translocation of β-catenin to the nucleus to interact and activate T cell factor and lymphoid enhancer factor (TCF/LEF) transcription factors [42, 43]. Reya et al. elucidated that β-catenin overexpression or treatment with soluble Wnt3a in culture promotes HSC self-renewal [44, 45]. Furthermore, activation of Wnt signaling in HSCs increases the expression of Notch1 and HoxB4, which strongly promotes HSC self-renewal [44, 45].

Additionally, microRNA-34 (miR-34) has been shown to bind to the untranslated region of β-catenin, leading to its downregulation [46]. Expression of miR-34 is induced by p53, and, therefore, p53 might downregulate Wnt signaling by miR-34-mediated inhibition of β-catenin, resulting in the suppression of self-renewal in HSCs.

Another essential signaling pathway involved in the regulation of stem cell self-renewal is Hedgehog signaling [47]. However, conditional knockout of Smoothened (Smo), an essential regulator of the Hedgehog pathway, does not show any dysfunction of HSCs, indicating that this pathway is dispensable for adult HSC self-renewal and other functions [48, 49].

The ability of HSCs to self-renew is also affected by the epigenetic status of chromatin structure regulated by components of the Polycomb complex, including Mel18, Rae28, and Bmi-1 [50]. Mice lacking Mel18 exhibit increased HoxB4 expression resulting in increased fetal HSC self-renewal [51]. In contrast, Rae28$^{-/-}$ mice exhibit decreased HSC self-renewal without affecting HoxB4 expression [25, 52]. Moreover, deficiency of Bmi-1 increases p16^{Ink4a} and p19^{Arf} levels, resulting in an increased p53 level, which leads to the suppression of HSC self-renewal [25]. Thus, Polycomb genes appear to regulate HSC self-renewal by various mechanisms.

In addition, another epigenetic regulator of chromatin, Mi-2β, a component of the chromatin remodeling nucleosome remodeling deacetylase (NuRD) complex, is involved in the regulation of HSC self-renewal [53]. Conditional inactivation of Mi-2β in bone marrow promotes HSC differentiation and inhibits HSC self-renewal [53].

The significance of the p53 pathway in regulation of HSC self-renewal by Polycomb or NuRD complexes is poorly understood, except for the molecular mechanism responsible for the inhibition of HSC self-renewal in Bmi-1$^{-/-}$ mice as described above.

CDK inhibitors involved in the G1 checkpoint of the cell cycle have also been shown to regulate HSC self-renewal [47]. Based on their sequence homology and specificity of action, CDK inhibitors are divided into two families: the Cip/Kip family including p21^{Cip1}, p27^{Kip1}, and p57^{Kip2}, and the Ink4 family including p16^{Ink4a}, p15^{Ink4b}, p18^{Ink4c}, and p19^{Ink4d} [54].

Among the Cip/Kip family, p21^{Cip1} is upregulated by p53 in response to DNA damage, which induces cell cycle arrest by inhibiting CDKs as discussed above in Section 2 [12, 13, 55]. Cheng et al. showed that p21^{Cip1} plays an essential role in HSC quiescence and self-renewal by analyzing cells from B6/129 mixed background mice [56]. However, van Os et al. showed that HSC self-renewal is not impaired by analyzing pure B6 mice lacking p21^{Cip1} under normal conditions, whereas a deficiency of p21^{Cip1} decreases self-renewal in a competitive repopulation assay only when HSCs undergo irradiation stress [57]. The discrepancy regarding the importance of p21^{Cip1} for HSC self-renewal in a steady state between these two studies might be due to the difference in the genetic background of the mice used for analysis.

p57^{Kip2}, another member of the Cip/Kip family, has been shown to be predominantly expressed in the LT-HSC population [58, 59]. Matsumoto et al. generated conditional p57^{Kip2}-knockout mice and showed that p57^{Kip2} deficiency decreases HSC quiescence and self-renewal. In addition, loss of p57^{Kip2} results in upregulation of p53, leading to

activation of the p53-dependent apoptosis pathway in HSCs, suggesting that $p57^{Kip2}$ is required for the maintenance of both quiescence and self-renewal of the HSC pool in adult mice [59].

On the other hand, Zou et al. isolated HSCs from $p57^{Kip2-/-}$ embryos, which are neonatal lethal [60, 61], and showed that loss of $p57^{Kip2}$ results in a substantial reduction in the repopulating capacity of embryonic HSCs, but does not affect the number of HSCs in quiescence [58]. Moreover, deletion of $p57^{Kip2}$ results in upregulation of $p27^{Kip1}$ in embryonic HSCs, and loss of both $p57^{Kip2}$ and $p27^{Kip1}$ impairs maintenance of the quiescence and self-renewal of HSCs, which is more obvious compared with those of HSCs in mice lacking $p57^{Kip2}$ alone, suggesting that $p57^{Kip2}$ and $p27^{Kip1}$ cooperate to maintain embryonic HSC quiescence and self-renewal, and $p27^{Kip1}$ can partially compensate for the function of $p57^{Kip2}$ [58]. The slight difference regarding the roles of $p57^{Kip2}$ in the maintenance of quiescence in HSCs might be due to their origin.

Furthermore, loss of $p27^{Kip1}$ alone in adult mice does not affect HSC self-renewal and quiescence, suggesting that $p27^{Kip1}$ is not essential for HSC function under normal conditions [58, 62].

It has been shown that loss of other CDK inhibitors, such as $p16^{Ink4a}$, $p15^{Ink4b}$, or $p18^{Ink4c}$ in the Ink4 family, results in an increase in HSC self-renewal, although the function of $p19^{Ink4d}$ for HSC self-renewal remains unknown [5, 63–65], indicating that they are independent negative regulators of HSC self-renewal. Interestingly, mice with triple knockout of $p16^{Ink4a}$, $p19^{Arf}$, and p53 show a remarkable increase in HSC self-renewal. $p19^{Arf}$ binds to Mdm2 and inhibits the degradation of p53, thus $p16^{Ink4a}$ and the $p19^{Arf}$-p53 pathway synergistically downregulate the self-renewal capacity of HSCs [5].

Interestingly, among CDK inhibitors, loss of Cip/Kip family members such as $p21^{Cip1}$, $p27^{Kip1}$, and $p57^{Kip2}$ results in reduced HSC self-renewal, whereas loss of Ink4 family members such as $p16^{Ink4a}$, $p15^{Ink4b}$, and $p18^{Ink4c}$ leads to increased HSC self-renewal [5, 56–59, 62, 65, 66]. Although this difference regarding the roles of the two families in HSC self-renewal is interesting, its significance remains unknown.

7. Cellular Senescence, Organismal Aging, and p53 in HSCs

Although senescence and quiescence can be considered as analogous phenomena, they are different from each other. While senescence is programmed and essentially irreversible, quiescence is dependent upon environmental stimuli and is reversible [67]. Both are initiated by failure to progress through the G1 phase. Campisi have revealed that cellular senescence plays a critical role in tumor suppression in vivo [68].

Mutation and shortening of telomeres, a region of repeated nucleotide sequences at both ends of a chromosome, are important factors in cellular senescence [69, 70]. During DNA replication, DNA polymerase that synthesizes new DNA cannot completely replicate the telomere. Telomerase extends the telomere region to prevent telomere shortening. Telomerase consists of two essential components: a telomerase RNA component (Terc) and telomerase reverse transcriptase (Tert) [71]. Both components have been shown to be essential for telomerase activity. $Terc^{-/-}$ mice exhibit significantly shortened telomeres in HSCs, reduced regenerative capacity, and impaired hematopoiesis [71, 72]. These hematopoietic failures in $Terc^{-/-}$ mice are caused by activation of p53-dependent senescence in response to DNA damage caused by telomere shortening [71, 73, 74].

Mice expressing a truncated mutant of p53 lacking the first six exons ($p53^{+/m}$ mice) show hyperactive p53 activity, compared with that of wild-type mice, and exhibit an organismal aging phenotype such as reduced longevity, osteoporosis, generalized organ atrophy, and diminished stress tolerance [75]. Moreover, in older mice (18–20 months), the number of LT-HSCs in $p53^{+/-}$ mice increases compared with that in $p53^{+/+}$ mice, and the number of LT-HSCs in $p53^{+/m}$ mice decreases compared with that in $p53^{+/+}$ mice, suggesting that an increase or activation of p53 leads to cellular senescence and organismal aging in HSCs [76], although the contribution of cellular senescence to organismal aging is still controversial [77].

8. LSCs and p53

Cancer stem cells (CSCs) are defined as cells that can self-renew, produce various types of progeny cells with more differentiated characteristics, and have a strong ability to drive continued expansion of malignant cells [78–80]. These properties of CSCs have similarities with those that define normal tissue stem cells.

CSCs in leukemia are called LSCs [81], and LSCs in acute myeloid leukemia (AML) have been well characterized [82–84]. Bonnet and Dick have shown that a $CD34^{+}$ $CD38^{-}$ rare subpopulation of leukemic cells is capable of initiating leukemia in nonobese-diabetic severe combined immunodeficient (NOD-SCID) mice, which is histologically very similar to the original AML [82]. Thus, LSCs share phenotypical similarities with normal HSCs, such as self-renewal and expression of the surface marker, CD34, although there are some differences such as the expression of the interleukin-3 receptor α (IL-3Rα) [85, 86].

The proliferation of LSCs and normal HSCs/HPCs has been shown to be regulated by the Polycomb group (PcG) gene Bmi-1 [87]. Lessard and Sauvageau have shown that Hoxa9 and Meis1a (AML-associated oncogenes) transduced fetal liver cells can form AML in sublethally irradiated syngeneic mice regardless of the presence of Bmi-1. However, Hoxa9-Meis1a transduced leukemic bone marrow cells lacking Bmi-1 cannot induce AML in secondary recipient mice, whereas control Hoxa9-Meis1a transduced leukemic bone marrow cells having Bmi-1 can induce AML, suggesting that Bmi-1 is important for LSCs to retain their capacity to initiate leukemias in vivo [87].

Additionally, in human acute promyelocytic leukemia (APL), expression of PML-RAR, a fusion type of oncogene, induces deacetylation and degradation of p53, leading to

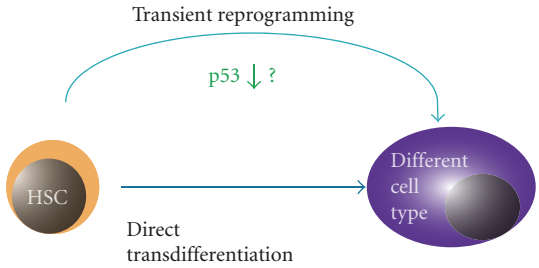

Figure 3: Possible mechanisms of HSC transdifferentiation. HSCs might directly transdifferentiate into another cell type (lower arrow) or through transient reprogramming in certain conditions. Loss of p53 might promote transient reprogramming for HSC transdifferentiation.

repressed p53 transcriptional activity and allowing APL cells to overcome p53-mediated stress responses that induce their eradication [88]. Interestingly, Viale et al. reported that PML-RAR expression in HSCs causes DNA damage and results in upregulation of p21 [89], leading to the cell cycle restriction and repair of damaged DNA [89]. The authors also suggested that the presence of moderate DNA damage, caused by oncogenes, and DNA repair activity enhanced by upregulated p21 increase the chance of mutagenesis in HSCs [89]. Thus, p21 and the associated DNA repair mechanisms appear to play critical roles in initiation and maintenance of LSCs in APL and may be appropriate targets for the treatment of this disease.

Interestingly, the behavior of LSCs is suggested to be associated with the drug resistance of certain types of leukemia [90]. For instance, $CD34^+$ LSCs in chronic myelogenous leukemia (CML) in the G0 phase of the cell cycle (quiescence) are highly insensitive to Imatinib methylate (Gleevec or Glivec; previously known as STI-571 or CGP57148B) that targets the tyrosine kinase activity of BCR-ABL oncogene, whereas most dividing cells are eradicated by the drug [91–102]. Therefore, reduction of an LSC population in quiescence by the inhibition of senescence-inducing proteins such as p53 might be an effective strategy to negate the drug resistance of LSCs [103, 104].

9. Plasticity of HSCs

The notion that tissue-specific stem cells can only differentiate into cells of their tissue origin has been widely accepted, but several recent reports indicate that tissue-specific stem cells, including HSCs, can differentiate into cell types of various lineages [105, 106]. In 1998, Ferrari et al. described that unfractionated normal bone marrow cells transplanted into SCID mice with chemically induced muscle damage can contribute to muscle regeneration [107]. Similarly, Bittner et al. have performed transplantation of normal bone marrow cells into mice with experimental muscular dystrophy, a genetic disease with progressive weakness of skeletal muscles, and found that bone-marrow-derived cells are recruited to skeletal and cardiac muscles and differentiate into muscle cells, although the bone marrow subpopulation that engrafted in muscles was not clearly shown [108]. Orlic et al. reported that transplanted lineage-negative bone marrow cells expressing c-kit (Lin^- $c\text{-}kit^+$ cells) can contribute to myocardial regeneration in a mouse model of experimental myocardial infarction [109]. Other groups also indicate that bone marrow contains stem cells capable of differentiating into functional muscle cells [1, 2].

Bone marrow cells have also been suggested to contribute to the regeneration of liver cells. Lagasse et al. injected $c\text{-}kit^{high}$ Thy^{low} Lin^- $Sca\text{-}1^+$ (KTLS) HSCs intravenously into lethally irradiated mice with progressive liver failure and renal tubular damage owing to a lack of fumarylacetoacetate hydrolase (FAH) and found that KTLS HSCs can give rise to functional hepatocytes [110]. Such differentiation of a bone marrow population enriched with HSCs into mature hepatocytes in rodents has also been described by other studies [111, 112]. Moreover, the differentiation of bone-marrow-derived cells into mature hepatocytes has also been found in humans [113, 114].

Lin et al. showed that a small population of circulating endothelial progenitor cells (CEPs) derived from bone marrows exist in peripheral blood and contribute to postnatal neovascularization [115]. Asahara et al. performed transplantation of bone marrow mononuclear cells derived from transgenic mice expressing β-galactosidase (lacZ) driven by the endothelial cell-specific promoter (Flk1/LZ or Tie2/LZ) into lethally irradiated immunodeficient mice and examined neovascularization under various conditions by observing lacZ-positive cells. They concluded that bone-marrow-derived CEPs incorporate into and contribute to postnatal physiological and pathological neovascularization [116]. Krause et al. reported that adult bone marrow cells can differentiate into epithelial cells in the liver, lung, gastrointestinal tract, and skin [117].

As discussed above, although the plasticity of HSCs or cells in bone marrow is intriguing, the molecular mechanisms responsible for the transdifferentiation of HSCs or cells in bone marrow into cells of other tissue lineages remain unknown. One possibility is that the transdifferentiation of HSCs might occur by direct conversion of the epigenetic status of genes. Another possibility is that it might be induced by transient reprogramming of the genome (Figure 3). It has recently been shown that suppression of the p53-p21 pathway promotes the reprogramming efficiency of somatic cells by transfection of reprogramming factors such as Oct3/4 (also known as Pou5f1), Sox2, Klf4, and c-Myc in mice [118]. Thus, inhibition of p53 might facilitate the plasticity of HSCs or bone marrow cells.

10. Conclusions

In this paper, we focused on recent advances in research regarding the roles of p53 associated with the regulation of HSCs and LSCs. It is surprising that one molecule plays roles in the various aspects of important normal cells as well as malignant cells (Figure 4). The importance of HSCs for various transplantation therapies of incurable diseases such as leukemias is obvious, and continuous efforts to elucidate

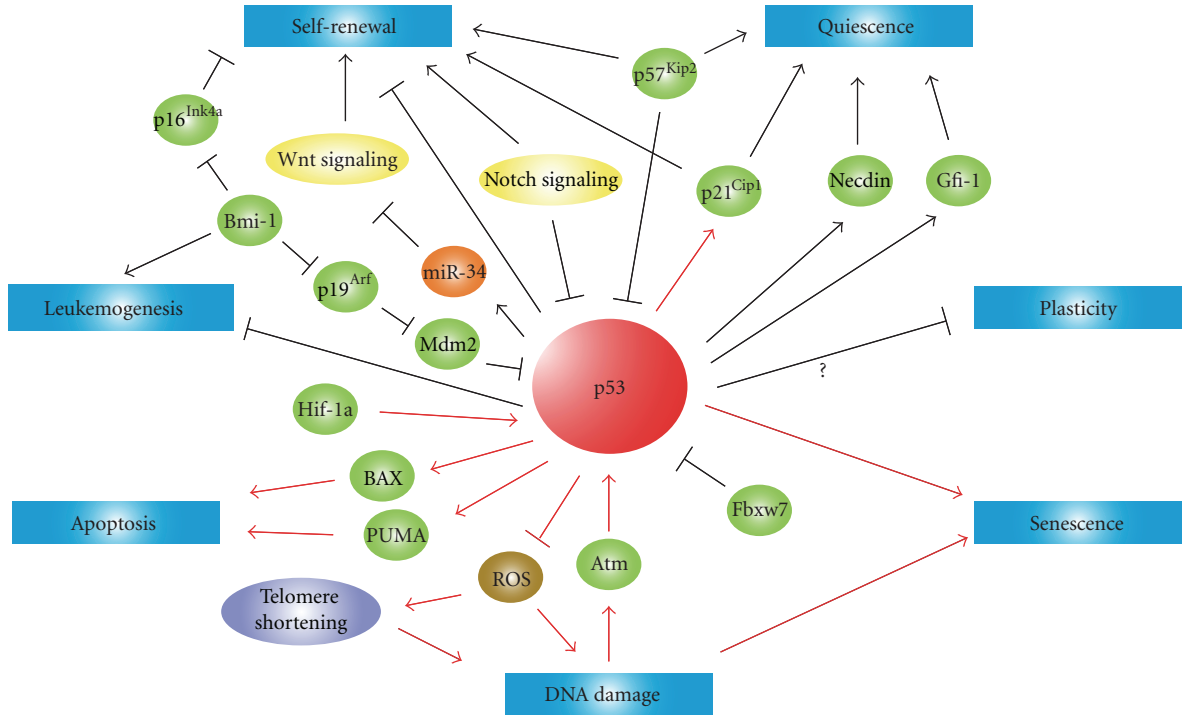

Figure 4: Schematic diagram of p53 roles in HSCs. p53 is involved in the control of response to DNA damage, self-renewal, quiescence, apoptosis, senescence, leukemogenesis, and plasticity in HSCs. Lines with an arrowhead indicate promotion and lines with a bar inhibition in steady state (black) or stress conditions (red).

the precise functions of p53 as a main regulator of HSCs will remain crucial and provide an insight into new strategies for treating various disorders.

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