p53 in stem cells

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Abstract

p53 is well known as a “guardian of the genome” for differentiated cells, in which it induces cell cycle arrest and cell death after DNA damage and thus contributes to the maintenance of genomic stability. In addition to this tumor suppressor function for differentiated cells, p53 also plays an important role in stem cells. In this cell type, p53 not only ensures genomic integrity after genotoxic insults but also controls their proliferation and differentiation. Additionally, p53 provides an effective barrier for the generation of pluripotent stem cell-like cells from terminally differentiated cells. In this review, we summarize our current knowledge about p53 activities in embryonic, adult and induced pluripotent stem cells.

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Key words: p53; Embryonic stem cells; Adult stem cells; Induced pluripotent stem cells; Cell differentiation

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INTRODUCTION

p53 is one of the most well-known and most intensively investigated tumor suppressor proteins. p53 is not a critical protein for survival, as mice and men can develop in the absence of p53 or when the tumor suppressor protein is mutated. The benefit merely comes into play when cells are exposed to conditions that bear an elevated risk of acquiring mutations, such as irradiation or nucleotide deprivation. Then, p53 halts the cell cycle to allow time for repair of damaged DNA, or it initiates a cell death program to eliminate cells with damaged or mutated DNA from the cell population. Functionally, p53 is a transcription factor. After activation, it binds to the promoters of target genes and stimulates transcription of certain genes, while repressing others

Due to its antiproliferative activity, p53 is under tight control. The rapid degradation of p53 in 26S proteasomes ensures that its abundance in non-stressed cells is low. However, when its activity is required, p53 is protected from degradation and accumulates to high levels, while an array of post-translational modifications fine-tunes its activity. The major regulator of p53 abundance is the oncoprotein Mdm2. Mdm2 mediates the polyubiquitination of p53 and its association with 26S proteasomes, resulting in p53 degradation. Nonetheless, p53 can also be degraded by other ubiquitin ligases and by ubiquitin-independent pathways. The p53 tumor suppressor protein is part of a multigene family that also includes p63 (TAp63) and p73


Differentiation

Stem cells are present throughout embryonic development and in adult organs. Basically, there are two types of stem cells: embryonic stem cells (ESCs) that can be isolated from the inner cell mass of blastocysts, and adult stem cells (ASCs) that are found in various tissues and organs. ESCs are pluripotent and can differentiate into all tissues of an embryo, whereas ASCs are more restricted in their differentiation potential. ASCs are, however, vital for the normal turnover of regenerative organs, such as blood, skin or intestine, and they are necessary for replenishing specialized cells when they are lost, for example, after tissue damage[9]. Since extensive proliferation and differentiation of stem cells can contribute to hyperproliferative disorders, a coordinated control of stem cell self-renewal and differentiation is fundamental for maintaining tissue and organ homeostasis. p53 appears to contribute to this restraint by controlling the proliferation, self-renewal and differentiation of embryonic and ASCs. With the increasing interest in stem cell biology in the past few years, these activities of p53 have gained significantly more attention. In this review, we provide an overview of the current knowledge on p53 regulation and activity in embryonic, adult and induced pluripotent stem cells.

In the following sections, we distinguish the observations made with murine ESCs (mESCs) or human ESCs (hESCs).

**p53 IN ESCs**

The first observation about a potential role of p53 in ESCs dates back to 1980 when Mora et al[10] observed that p53 was highly expressed in primary cell cultures obtained from 12-14-d old mouse embryos but not in cells from 16-d old embryos. One year later, they observed that the amount of p53 protein decreased significantly during embryogenesis[11]. This observation was further supported in 1985 when Rogel et al[12] noticed a considerable reduction in p53 mRNA during embryogenesis from day 11 onwards. Six years later, Schmid et al[13] reported the tissue-specific expression of the p53 gene during development and confirmed the strong decline of p53 mRNA in cells undergoing terminal differentiation. Subsequent publications further substantiated the finding that p53 is highly abundant in mESCs[14,15]. Despite its high abundance and the fact that the tumor suppressor protein was more strongly acetylated at lysine 383 in hESCs compared to differentiated cells, p53 was found to be inactive in stem cells[16,17].

During differentiation, p53 protein and RNA decrease significantly[12,13,14,15]. Whether the reduction in p53 protein levels also corresponds to reduced activity is an open question. Although Lin et al[14] observed an increase in p53 activity during differentiation and transcription of its target genes p21, mdm2 and killer/DR5, Sabapathy et al[16] observed a conformational change in the tumor suppressor protein, and concomitantly, a decrease in its ability to bind DNA, which would imply a decrease in the transcriptional activity of p53 instead.

In ESCs, p53 is predominantly found in the cytoplasm[18,20]. This mainly cytoplasmic localization may also account for the weak activity of this tumor suppressor protein in differentiated cells.

**p53 and the proliferation of ESCs**

In differentiated cells, p53 is an important regulator of cell proliferation. By controlling expression of the p21 gene, which encodes a prominent inhibitor of cyclin-dependent kinases, p53 influences transition from G1 to S-phase of the cell cycle[21]. In addition, p53 is able to initiate apoptosis by both the extrinsic and intrinsic pathways[22,23]. In concordance with these activities in differentiated cells, p53 also controls proliferation and cell death in ESCs. Its absence leads to increased proliferation and reduced levels of spontaneous apoptosis of mESCs[11]. Treatment of hESCs with nutlin, an inhibitor of p53 degradation, leads to the rapid accumulation of p21 and to cell cycle arrest at the G1/S boundary[23,24] (Figure 1A). Although removal of the drug after short-term exposure re-establishes normal hESC morphology, extended treatment results in extensive cell death[24].

**Figure 1** Differentiation of embryonic stem cells by p53. A: Treatment of embryonic stem cells (ESCs) with nutlin leads to p53 accumulation and transcriptional activation. Activated p53 stimulates transcription of p21, whose gene product initiates cell cycle arrest at the G1/S border and differentiation; B: Treatment of ESCs with retinoic acid (RA) leads to phosphorylation of p53 at serine 315 and repression of nanog followed by differentiation; C: Irradiation of ESCs with UV light or treatment with doxorubicin leads to activation of p53. Activated p53 represses transcription of nanog and oct4, resulting in the differentiation of ESCs.

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**Image**

A: Nutlin → p53 ↑ → p21 ↑ → G1/S arrest → Differentiation

B: RA → p53-Ser315↑ → nanog repression → Differentiation

C: DNA damage → p53 activation → nanog/oct4 repression → Differentiation
Conversely, the inhibition of the transcriptional activity of p53 by pifithrin-α, a small molecule inhibitor of p53, or shifting a temperature-sensitive mutant of p53 to the non-permissive temperature reduces apoptosis in mESCs [27,28]. Surprisingly, although pifithrin-α reduces both DNA-damage-induced as well as spontaneous apoptosis in mESCs, treatment of hESCs fails to inhibit apoptosis induction by p53 [17]. The reason for this discrepancy is unclear. However, since pifithrin-α only inhibits the transcriptional activities of p53 and not its non-transcriptional proapoptotic activities in the cytoplasm [29], this result may indicate that the mitochondrial pathway of apoptosis induction is more important for p53-dependent apoptosis in hESCs than is the “classical” transcription-dependent pathway.

Role of p53 in the differentiation of ESCs

p53 is a major driving force for the differentiation of ESCs. Spontaneous differentiation of hESCs is significantly reduced when p53 abundance is decreased [17]. The connection between p53 and differentiation became particularly evident when Lin et al. [30] found that p53 binds to the promoter of nanog and suppresses its transcription in mESCs (Figure 1B, Table 1). The homeodomain protein Nanog is highly abundant in ESCs and is required for self-renewal and maintenance of an undifferentiated state [31,32]. Suppression of nanog transcription decreases the amount of Nanog protein, and thus, supports ESC differentiation [33]. In addition to the nanog promoter, p53 binds to the oct4 promoter where it also reduces gene transcription [17]. Like Nanog, Oct4 belongs to the group of pluripotency factors that are necessary for maintaining ESCs in an undifferentiated state [34,35]. Treatment of hESCs with nutlin, which is a drug that leads to the strong accumulation of p53, results in decreased nanog and oct4 expression and induction of the differentiation markers gata4 and gata6 [17].

Further evidence for the importance of p53 for ESC differentiation has come from the analysis of retinoic acid (RA)-mediated differentiation. Treatment with RA is a widely used method for differentiating ESCs in culture. This treatment of ESCs with RA also leads to suppression of nanog transcription. Downregulation of nanog after treatment with RA is, however, greatly attenuated in ESCs when p53 is genetically deleted, indicating that p53 plays an important role in RA-mediated suppression of nanog [17]. It is yet unclear how RA is linked to p53, although the phosphorylation of p53 at serine 315 appears to be particularly important for the suppression of nanog transcription (Figure 1B). This phosphorylation enables the recruitment of the corepressor mSin3a to the nanog promoter, which is essential for the full suppression of nanog transcription [17].

In addition to favoring the differentiation of ESCs, p53 also has antidifferentiation activity. The Wnt signaling pathway is extremely important for the maintenance of self-renewal and pluripotency of murine and human ESCs [31,32]. Wnt signaling is activated by binding of Wnt-ligands to their cognate Frizzled receptor, which culminates in the activation of the Lef1/Tcf transcription complex [33]. Activation of p53 also counteracts differentiation by leading to the induction of Wnt ligands and receptors and Lef1 [34] (Table 1).

### Table 1 p53 target genes regulating stem cell behavior

| Gene | Regulation | Cell type | Effect | Ref |
|------|------------|-----------|--------|-----|
| nanog | Repression | mESCs | Pro-differentiation | [2] |
| 4-Oct | Repression | mESC | Pro-differentiation | [27] |
| wnt13, wnt3A, wnt8a, wnt8b, wnt9A, fzd1, fzd 2, fzd6, fzd8, fzd10, lefl | Activation | mESCs | Anti-differentiation | [24] |
| mRNA-200c | Activation | Mammary epithelial cells | Inhibition of epithelial-mesenchymal transition | [26] |
| onexit | Repression | Mesenchymal stem cells | Inhibition of osteogenic differentiation | [26] |
| runx2 | Repression | Mesenchymal stem cells | Inhibition of osteogenic differentiation | [26] |
| ppyary | Repression | Mesenchymal stem cells | Inhibition of adipogenic differentiation | [26] |
| duox2; duox1 | Activation | Neural stem cells | Activation of neurogenesis | [17] |
| gfi1 | Activation | Hematopoietic stem cells | Maintenance of quiescence | [17] |
| neddin | Activation | Hematopoietic stem cells | Maintenance of quiescence | [17] |
| p21 | Activation | Hematopoietic stem cells | Regulation of HSC amount | [17] |

### p53 activities in ESCs in response to DNA damage

In differentiated cells, DNA damage leads to the accumulation of p53 in the nucleus and mitochondria and to the transcription of its target genes, including mdm2, p21, bax, puma and noxa, followed by cell cycle arrest at the G1/S boundary and initiation of cell death [36]. Although the elevated abundance of p53 in ESCs is generally accepted, there are contradictory reports about its activity in ESCs in response to DNA damage. Some studies have reported that p53 is only weakly or not activated in response to γ-irradiation of mESCs, or after treatment with n-phosphonacetyl-L-aspartate, and that both its protein level and expression of its target genes p21 and mdm2 should remain unchanged. Other studies, including our own, have reported p53 accumulation in the nucleus of mESCs in response to UV light or γ irradiation, or after treatment with doxorubicin, as well as transcriptional activation of its target genes p21, mdm2, puma and noxa [2,16,18,36].

Despite transcription of the p21 gene after p53 activation, no p21 protein is produced in mESCs [18,26,36]. For hESCs, there are conflicting data regarding p53 activity in response to DNA damage. Qin et al. [17] failed to observe...
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an increase in p21 mRNA in response to UV irradiation despite accumulation and phosphorylation of p53, whereas Filion et al. reported a significant induction of p21 mRNA in response to ionizing radiation. However, as reported in mESCs, the p21 protein was hardly detectable in hESCs.

Similar to differentiated cells in which p53 halts the cell cycle and drives cells with damaged DNA into apoptosis, p53 is regarded as being responsible for the high sensitivity of mESCs to DNA damage. Following its accumulation in response to UV irradiation, p53 rapidly induces apoptosis in mESCs, leading to the death of a majority of the cells. Treatment of hESCs with etoposide leads to association of p53 with mitochondria and to increased expression of puma. Subsequently, Bax and Mecl1 are co-localized in perinuclear structures that resemble mitochondrial aggregates, followed by rapid and extensive induction of apoptosis. hESCs stably transduced with an shRNA that is targeted against p53 show significant reduction of bax and puma expression and apoptosis after treatment with etoposide, indicating a requirement of p53 for the induction of cell death in hESCs in response to DNA damage. In mESCs, the colony forming ability after UV irradiation is more strongly reduced in p53-positive mESCs than in those that lack the tumor suppressor protein. Most interestingly, although ESCs rapidly undergo apoptosis in response to UV-irradiation or treatment with etoposide, ionizing radiation is less efficient in inducing cell death. Conflicting observations have, however, been made regarding the regulation of the clonogenic potential by p53 after ionizing irradiation. Corbet et al. have observed a stronger reduction in the colony forming ability of p53-positive mESCs after γ irradiation in comparison to p53-deficient mESCs, although we failed to observe this difference after ionizing radiation.

Apart from the increase in abundance, the p53 protein is also post-translationally modified in response to DNA damage. Of note, these post-translational modifications differ between ESCs and differentiated cells and between hESCs and mESCs, both in quality and in intensity. In response to UV- or ionizing radiation, p53 from hESCs is barely phosphorylated at serine 9, which is an amino acid that is phosphorylated in response to cellular stress in differentiated cells. Also, phosphorylation of serine 20 (serine 23 of murine p53) is rather weak in mESCs. One explanation for this weak phosphorylation of p53 in mESCs is that Chk2, the kinase that usually phosphorylates this site, is hyperphosphorylated and tethered in aggregates in mESCs, thus limiting its availability for phosphorylating its cellular targets. Conversely, phosphorylation on serine 15 (serine 18 of murine p53) of p53 is stronger in hESCs than in differentiated human cells, while its intensity is similar in mESCs and mouse embryonic fibroblasts (MEFs).

In contrast to differentiated cells in which damage-induced p53 activities are mostly restricted to the induction of cell cycle arrest and apoptosis, p53 activation also affects differentiation in ESCs as a response to DNA damage. By binding to the promoters of oct4 and nanog, p53 represses the transcription of these pluripotency factors and facilitates the differentiation of damaged ESCs (Figure 1C). However, p53 can also induce transcription of Wnt ligands and receptors in response to DNA damage, which has antidifferentiation properties.

Overall, by enhancing the sensitivity to DNA damaging agents, induction of cell death and by encouraging differentiation, p53 acts as a guardian of the genome for ESCs despite its failure to induce G1 arrest in this cell type.

### p53 IN ASCs

In addition to the developing embryo, stem cells have also been identified in somatic tissues of adults, including the nervous system, bone marrow, epidermis, skeletal muscle, mammary gland and liver. Stem cells in somatic tissue are usually called tissue or ASCs in order to distinguish them from ESCs that are derived from the inner cell mass of blastocysts. Even in adult organs, tissue stem cells retain the potential for self-renewal and differentiation into different cell types, but they have lost pluripotency as well as the capacity to form a complete new organism. In organs, ASCs reside in specific niches where they remain in a quiescent state during most of the host’s lifetime. However, when new cells are required, these tissue stem cells divide postnatally, frequently in an asymmetric way, by which they generate another stem cell as well as a committed progenitor daughter cell. The progenitor cell then proliferates further and produces a pool of differentiated cells. These differentiated cells replenish cells as they die, due to natural wearing away or after injury. Therefore, stem cells are intimately involved in maintaining tissue homeostasis.

Similar to ESCs, proliferation, self-renewal and genomic stability are tightly controlled in ASCs. Defects in these parameters contribute to premature aging, to failure to repair tissue injury and to the development of cancer. Detailed knowledge about the processes that regulate proliferation, self-renewal and transformation of tissue stem cells is therefore crucial to enable safe usage of these cells for stem-cell-based therapies. The tumor suppressor protein p53 is a key regulator of these processes. In the following sections, we highlight the most important results regarding p53 regulation and function in various types of tissue stem cells.

### Mammary gland stem cells

Mammary gland stem cells (MGSCs) direct mammary gland development and functionality, and alterations in the proliferation of these cells result in defects of the mammary gland. p53 appears to be a critical control component for the development of mammary glands. This appearance became particularly evident when mammary glands of mice that expressed one wild-type and one C-terminally deleted allele of p53 were
In addition to controlling proliferation and symmetry of MGSC division, p53 regulates epithelial-mesenchymal transition (EMT) in the mammary gland. EMT and the reverse process, mesenchymal-epithelial transition (MET), are key processes for the regulation of embryogenesis. EMT and MET are a series of events during which epithelial or mesenchymal cells lose many of their characteristics and take on properties that are typical of the other cell type. This transition occurs as a result of a number of intercellular and intracellular adjustments. p53 suppresses EMT by binding to the promoter of the microRNA miR-200c and activating its expression (Table 1). miR-200c is a microRNA that regulates EMT by inhibiting ZEB1/2, a transcriptional repressor of E-cadherin. Re-expression of miR-200c in stem cells with deleted p53 reduces formation of mammospheres, indicating that the control of proliferation and asymmetric cell division of MGSCs by p53 may primarily occur by regulating this microRNA.

**Mesenchymal stem cells**

Mesenchymal stem cells (MSCs) reside in the bone marrow and can differentiate into different types of cells of mesodermal origin, such as osteoblasts, adipocytes and chondrocytes. Multipotent bone marrow MSCs express low levels of adipogenic and osteogenic factors. When this balance of adipogenic and osteogenic factors is tipped, cells become committed toward one of these lineages, and lineage-specific transcription factors that promote one particular cell fate are transcribed. Lineage-specific transcription factor activation usually occurs concomitantly with repression of the other cells’ fate. Loss of p53 in MSCs results in severe alterations of tissue homeostasis. The complete absence of p53 promotes a higher proliferation rate of bone-marrow-derived MSCs, which acquire the typical MSC surface phenotype earlier than wild-type MSCs do. In addition, more precursors are generated that are able to form colonies.

MSCs can be isolated and expanded in vitro, which predestines these cells for tissue engineering and therapeutic applications. However, MSCs that have been extensively propagated in vitro frequently acquire mutations that may lead to spontaneous transformation. Therefore, extensively propagated MSCs should be taken with care with regard to patient therapy. Moreover, the expression profiles and mutation spectra of p53 of these extensively propagated MSCs are similar to those found in tumors.
in human tumors. Therefore, these findings raise the conjecture that mesenchymal tumors may originate from aged MSCs[86]. The coincidence of p53 mutations and the development of tumors in aged MSCs suggests that p53 activity might be required for maintaining genomic stability in this cell type, and for suppression of spontaneous transformation in long-term MSC cultures[77]. This notion is further supported by the observation that MSCs from mice with a genetic deletion of both p53 alleles are capable of forming tumors when they are injected into immunodeficient mice[79]. Conversely, when both alleles of the p21 gene, a target gene of p53 that is mainly responsible for p53-mediated cell cycle arrest, are deleted, MSCs do not show any signs of tumoral transformation[80,81]. Only when at least one allele of p53 is mutated in addition to p21 does p53 expression become lost after long-term culture, and a significant increase in growth rate and genomic instability is observed[82]. Loss of p53 expression is further accompanied by the loss of expression of the cdk inhibitor p16, upregulation of p19[90] and c-myc, and by the complete loss of any senescence phenotype[82].

In addition to controlling proliferation and transformation, p53 also influences the differentiation of MSCs (Figure 2B). MSCs that lack p53 generally differentiate into adipocytes or osteocytes more rapidly than wild-type MSCs. This increased differentiation occurs along with enhanced expression of osteoblast differentiation factors osteonectin and runx2, which are normally repressed by p53, and by increased expression of runx2 and pparγ[83-85] (Table 1). Conversely, osteoblast progenitor cells with elevated p53 activity show reduced proliferation and reduced differentiation, indicating that p53 may negatively regulate the differentiation process[84]. Accelerated differentiation in combination with an increase in macrophage colony-stimulating factor is thought to be the reason for the high bone mass that is observed in the absence of p53[86].

**Neural stem cells**

The nervous system develops from neural stem cells (NSCs), which have the capacity to self-renew and differentiate into neurons, oligodendrocytes and astrocytes[86,87]. Proliferation and differentiation of the nervous system of mammals is limited after birth, although certain areas in the brain retain multipotent precursor cells with the ability to self-renew and differentiate along neural lineages. Neurogenesis in the adult brain has been particularly associated with the ability to self-renew and differentiate along neural lineages[86]. The nervous system develops from neural stem cells (NSCs). Enforced expression of c-myc in NSCs and allow differentiation towards neuronal and glial lineages[80,81]. An increased proliferation of p53-negative NSCs becomes apparent when NSCs from p53 knockout mice are taken into culture. These NSCs form an increased number of clonal aggregates called neurospheres in comparison to those from wild-type mice. In addition, neurospheres from p53-null NSCs are larger in size, due to increased cell content[90,91]. When NSCs are recovered from these neurospheres, a greater proportion of cells are capable of initiating new neurospheres compared to NSCs from wild-type mice[90,91]. These observations strongly suggest that p53 is a negative regulator of NSC self-renewal.

Proliferation of NSCs lacking p53 is further enhanced when cells are also null for phosphatase and tensin homolog (PTEN). Moreover, although NSCs that lack p53 still respond to differentiation cues, this property is not the case when PTEN and p53 are both genetically deleted. Instead, the cells retain their stem-cell-like morphology and continue to express NSC lineage markers, even after experiencing differentiation cues[86]. The reason for this increased proliferation and inhibition of differentiation is probably a substantial increase in c-myc expression in the absence of p53 and PTEN, whereas c-myc expression is only marginally elevated in p53−/ or PTEN−/− NSCs. Enforced expression of c-myc in p53−/− NSCs also enhances proliferation and represses differentiation in the absence of p53 alone, whereas genetic deletion of c-myc in p53/PTEN-deficient NSCs restores their differentiation potential[86]. Thus, p53 and PTEN may both suppress c-myc expression in NSCs and allow differentiation to take place. Loss of one of these proteins may be compensated by the other factor or other components in the cell, while loss of both leads to snapping through of the differentiation-blocked phenotype.

Alterations in cell number can be achieved by modulating proliferation or by enhancing or reducing cell death. p53 can affect both possibilities; it influences cell proliferation by controlling expression of the edk-

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inhibitor p21, and cell death by activating transcription of proapoptotic genes such as bax, puma or noxa, as well as by binding to pro- and antiapoptotic members of the Bel-2 family[5]. Yet, the ability of p53 to induce cell death appears to be negligible for constraining proliferation of NSCs. The smaller neurospheres that are formed in the presence of p53 show no indication of cell death. Also, overexpression of an isofrom of p53 (∆Np53) that is shorter and more stable, and therefore, more active, reduces the size of neurospheres but does not increase the level of apoptosis[61-63]. Consistent with these in vitro results, mice overexpressing ΔNp53 show a clear age-dependent decline in the number of proliferating cells in the SVZ, and a reduction in the supply of new olfactory bulb neurons compared to mice expressing normal p53. Olfactory bulb neuron exhaustion is not apparent in younger mice, indicating that it is caused by premature NSC exhaustion[59]. In line with the reduction in NSC proliferation and the absence of increased cell death, overexpression of ΔNp53 leads to constitutive expression of the cdk-inhibitor p21, compromises the re-entry of dormant NSCs into the cell cycle and to extended durations of cell-cycle passages[99].

Although there is unanimity about the regulatory role of p53 in NSC proliferation, there are differing reports regarding the impact of p53 for differentiation. Jonas Meletis et al[84] have reported that neurospheres from p53-null and wild-type NSCs contain a similar number of neurons, astrocytes and oligodendrocytes, whereas Armellina-Diaz et al[98] and Masato Nagao et al[99] have found that differentiation of neurospheres from p53-null mice is biased towards neuronal precursors. Regardless of the differing reports regarding the role of p53 in NSC differentiation, p53 activity increases during development and is at the time when neuronal differentiation takes place at its maximum[100,102]. Also in cultured NSCs, p53 expression increases during differentiation[103], which might be caused by a gradual upregulation of p19Arf. p19Arf binds to the central domain of the Mdm2, which is the most important negative regulator of p53, and inhibits Mdm2-mediated degradation of the tumor suppressor protein[5]. As a result of this inhibition, p53 accumulates in the cell in an Arf-dose-dependent manner. The amount of p19Arf increases up to 20-fold from E13.5 to postnatal day 2, which could be responsible for the increase in p53 expression[100]. Consistent with a regulation of p53 by p19Arf and a putative role of p53 in suppressing self-renewal of NSCs and supporting differentiation towards glia cells, early-stage NSCs, which express little p19Arf, retain a high self-renewal capacity and differentiate towards the neurogenic lineage, whereas late-stage NSCs, which possess more p19Arf, have a lower self-renewal capacity and predominantly generate glia cells[100]. In line with this notion, the enhanced downregulation of p19Arf or genetic deletion of p53 enhances the self-renewal potential of NSCs as well as the production of neurons, whereas overexpression of p19Arf reduces NSC proliferation and promotes differentiation into glia cells[100] (Figure 3). Furthermore, induction of NSC differentiation leads to phosphorylation of p53 at serine 15 and enhances its DNA binding activity. It is, therefore, not surprising that differentiation of NSCs is concurrent with alterations in the expression of p53 target genes[104]. Two possible target genes of p53 during neuronal differentiation are dual oxidase (DUOX1) and its maturation factor DUOXAI (Table 1). Expression of p53 in P19 pluripotent embryonal carcinoma cells increases the level of both DUOX1 and DUOX1A1 and allows their physical interaction[103]. Both proteins, DUOX1 and DUOX1A1, play an important role in the regulation of neuronal differentiation through DUOX1-mediated reactive oxygen species production and modulation of intermediate filaments[105]. Transcriptional activation of these two proteins may, therefore, contribute to the differentiation-inducing activity of p53 in NSCs.

About 50% of human tumors express a mutated p53 gene, including brain tumors, where p53 mutations are found frequently. Mutations of p53 are associated with both tumor initiation and expansion in the brain, supporting the importance of this tumor suppressor protein for maintaining tissue homeostasis[106]. In differentiated cells, the activity of p53 comes mainly into play when the DNA of a cell has been damaged, at which point p53 initiates cell cycle arrest and cell death[107]. More recently, it has been shown that when cell cycle regulation or DNA integrity is perturbed in neural precursors, p53 is a major proapoptotic protein[108]. Telencephalic cells grown to neurospheres are arrested at the G1/S boundary of the cell cycle after irradiation, and show enhanced induction of apoptosis along with enhanced abundance and nuclear localization of p53 and its downstream targets p21 and Bax. At the same time, Rad51 and Rrm2 are transcriptionally repressed by p53, demonstrating that in response to genotoxic stress, p53 regulates transcription and repres-
sion of its target genes in neural progenitors. Cultures from p53−/− neural progenitors are not arrested in G1/S phase and exhibit a significantly lower level of spontaneous and DNA-damage-induced apoptosis than their wild-type counterparts, thereby showing that DNA damage-induced G1 arrest and apoptosis in neural precursor cells are critically dependent on p53. Also, immortalized human NSCs show upregulation and phosphorylation of p53 in response to ionizing radiation, indicating that this is a general response of NSCs to DNA damage. Importantly, although neurospheres derived from wild-type animals exhibit only slight karyotypic modifications, neurospheres derived from p53-null animals show major structural chromosomal aberrations and aneuploidy, particularly with higher passage numbers, demonstrating that p53 is required for the maintenance of chromosomal stability in NSCs.

**Hematopoietic stem cells**

Most adult hematopoietic stem cells (HSCs) exist in a relatively quiescent state in the microenvironment of the bone marrow. Once activated, they start to proliferate and differentiate along the different hematopoietic cell lineages. As shown for stem cells in other tissues, p53 activity also affects cell number, proliferation potential and differentiation of HSCs. Accordingly, HSCs of mice with a hyperactive mutant form of p53 show a reduced number of proliferating HSCs. Conversely, a reduction in p53 increases proliferation and self-renewal of HSCs.

Absence of the p53 target gene p21 results in an increased number of HSCs, whereas high levels of p21 mRNA in the quiescent stem cell fraction restrict stem cells from entry into the cell cycle. Nevertheless, it should be noted that p21 abundance is also regulated by mechanisms independent of p53.

In addition to controlling proliferation of HSCs, p53 is an essential component for maintaining quiescence of HSCs. This activity of p53 may also be regulated by p21, although others have found that the role of p21 in maintaining HSC function is limited. Other p53 target genes that might be involved in mediating quiescence are gfi-1 and neclin (Figure 2C). Both genes have been identified as direct p53 targets by transcript profiling. Gfi-1 is a zinc-finger-containing repressor protein that has been shown in the past to restrict HSC proliferation. Neclin is a growth-inhibitory protein that interacts with multiple proliferation-promoting proteins, such as simian virus 40 large T antigen or E2F-1. Downregulation of Neclin diminishes HSC quiescence, whereas quiescence is increased when neclin is overexpressed.

Furthermore, p53 is an important component of the DNA damage response of HSCs. HSCs from mice lacking p53 are more resistant to irradiation-induced apoptosis compared to HSCs from wild-type mice, indicating that p53 induces cell death in HSCs in response to DNA damage. p53 also controls a process called cell competence in HSCs in response to DNA damage (Figure 2C). Cell competition is the active elimination of suboptimal cells, basically enabling the selection of the fittest. This process has initially been described for Drosophila, but it probably plays a wider role in tissue homeostasis of all metazoans. Cell competition is activated in HSCs after DNA damage and selects for the least-damaged cells. Despite being controlled by p53, cell competition is distinct from the classical p53-mediated DNA damage response. It persists for several months, appears to be specific for HSCs and progenitor cells, and depends on relative rather than absolute p53 levels in competing cells. Cell competition in response to DNA damage is probably mediated by a non-cell-autonomous induction of proliferation arrest and senescence-related gene expression in those cells that possess higher p53 activity.

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**p53 IN INDUCED PLURIPOTENT STEM CELLS**

Somatic cells can be reprogrammed into pluripotent stem cell-like cells by overexpression of combinations of different pluripotency factors. The reprogramming of differentiated cells is a possible source for autologous pluripotent cells that can be transplanted into patients.

Since 2006, when the first induced pluripotent stem cells (iPSCs) were generated by overexpression of Oct4, Sox2, Klf4 and c-Myc in adult and embryonic fibroblasts, iPSCs have been generated from cells of multiple origins, including embryonic fibroblasts, adult fibroblasts, neural progenitor cells, hepatocytes, epithelial cells and keratinocytes, by combinations of different reprogramming factors. However, the low frequency and the tendency to induce malignant transformation by overexpression of proto-oncogenes raises doubts about the clinical applicability of this approach.

Reprogramming only occurs in a very small percentage of transfected cells, suggesting the existence of reprogramming barriers. Since decreased protein levels of p53 and p21 were observed in iPSCs derived from MEFs, it was assumed that p53 might play a role in the reprogramming process of somatic cells. One of the first such reports came from Zhao et al. who have observed that downregulation of p53 by siRNA dramatically enhances the efficiency of iPSC generation from human adult fibroblasts. This result has been confirmed by Kawamura et al. and Li et al., who have observed that reducing p53 levels by p53 shRNA dramatically enhances the efficiency of reprogramming, whereas re-expression of p53 in p53-null MEFs markedly reduces it. Downregulation of p19, a prominent regulator of p53 activity, also enhances reprogramming efficiency of MEFs and murine keratinocytes. The reprogramming efficiency of mouse fibroblasts lacking p19 is similar to that of MEFs lacking p53, suggesting that the reprogramming barrier that is controlled by p19 is mediated by p53 (Figure 4). However, despite the leading role of p53 for reprogramming of human cells, p19 regulates...
Figure 4  p53 provides a barrier for the reprogramming of somatic cells to induced pluripotent stem cells. Somatic cells, such as mouse embryonic fibroblasts (MEFs), can be reprogrammed to induced pluripotent stem cells (iPSCs) after overexpression of a combination of reprogramming factors, such as Klf4, Sox2, Myc and Oct4. Klf4 and Myc induce p19\(^{\text{ARF}}\) expression, which prevents p53 degradation, resulting in its accumulation and expression of its target genes. Among the target genes of p53 is p21, which causes senescence, and bax, puma and noxa, which lead to apoptosis.

appears to be more important for murine cells\(^{[132,134]}\). It is presently unclear how and why p53 imposes a reprogramming barrier. One possible explanation is that the reprogramming factors c-Myc, Oct4 and Sox2 induce p53 activity, thus, eliciting a stress response that finally leads to cell cycle arrest and/or cell death (Figure 4). This assumption is further supported by the finding that overexpression of the antipapoptotic gene bel2 increases the efficiency of reprogramming, indicating that p53 implements the reprogramming barrier by eliminating cells that express the reprogramming factors by apoptosis\(^{[133]}\). Most interestingly, when p53 levels are reduced, MEFs can be reprogrammed to pluripotency in the absence of oncogenes, such as c-myc or klf4, simply by the overexpression of oct4 and sox2\(^{[133]}\). Moreover, in the absence of p53, up to 10% of transduced cells became iPSCs, and iPSCs can even be generated from terminally differentiated lymphocytes\(^{[135]}\). p53 may also implement a barrier for reprogramming by inducing senescence. Expression of the four reprogramming factors oct4, sox2, klf4 and c-myc triggers a senescence-like phenotype in human fibroblasts\(^{[136]}\) (Figure 4). Senescence is an irreversible cell cycle arrest in the G1 phase of the cell cycle caused, for example, in response to cellular stress, such as DNA damage, treatment with chemotherapeutic drugs, or aberrant expression of oncogenes. This arrest occurs mainly through the activation of p53 and upregulation of p16\(^{\text{INK4a}}\) and p21\(^{[137]}\). In fact, p21 has been shown to be an important target of p53 in the context of implementing a reprogramming barrier\(^{[138]}\). Nevertheless, other target genes of p53 also appear to contribute, as the absence of p21 enhances reprogramming efficiency by only about fourfold, whereas the absence of p53 does so by 7-10-fold\(^{[134,135]}\). Transfection of somatic cells with the four reprogramming factors significantly increases the percentage of cells arrested in G1 phase without inducing apoptosis, indicating a possible involvement of p21. Moreover, cells expressing the reprogramming factors display an enlarged cytoplasm, a senescence-associated \(\beta\)-galactosidase activity and heterochromatin foci. Ablation of the senescence effectors, p16\(^{\text{INK4a}}\), p53 and p21 improves the efficiency of reprogramming significantly\(^{[136]}\).

Although suppression of p53 appears to be a feasible way of improving reprogramming efficiency, permanent suppression of p53 may lower the quality of iPSCs, by allowing the outgrowth of iPSCs with permanent DNA lesions and chromosomal aberrations. p53 appears to be critically involved in preventing the reprogramming of cells with damaged DNA, short telomeres or reduced DNA repair deficiency. MEFs with critically short telomeres or MEFs treated with low doses of \(\gamma\)-irradiation or UV light show low reprogramming efficiency. Abrogation of p53 in these cells restores reprogramming efficiency to the level of undamaged p53-null cells\(^{[138]}\).

**CONCLUSION**

Although mice with genetic deletions of both p53 alleles develop normally, there is increasing evidence for a role of p53 in the regulation of stem cell proliferation, differentiation and the maintenance of stem cell genetic stability. At present, we are only beginning to understand the different actions that p53 exerts in stem cells, and the underlying mechanisms are even less clear. It is of particular interest to establish how p53 avoids the acquisition of mutations and genomic alterations after prolonged propagation of ASCs in vitro. Another important question is why p53 implements a barrier for the generation of iPSCs and how to circumvent this barrier for the generation of isotypic stem cells from patients without instigating the risk of expanding stem cells with genetic alterations. Transient suppression of p53 might be a useful compromise, however, it is presently unclear whether cells that survive the reprogramming process are truly free of mutations and aberrations when p53 has been inactivated for some duration. Therefore, more research is required to allow the safe use of stem cells for therapy.

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