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REVIEW

p53 is balancing development, differentiation and de-differentiation to assure cancer prevention

Alina Molchadsky, Noa Rivlin, Ran Brosh, Varda Rotter* and Rachel Sarig

Department of Molecular Cell Biology, Weizmann Institute of Science, Rehovot 76100, Israel

*To whom correspondence should be addressed. Tel: +972 8 9344070; Fax: +972 8 9465265;
Email: varda.rotter@weizmann.ac.il

Many of the roles played by the tumor suppressor p53 in restraining cancer initiation and progression are well established. These include the ability of p53 to induce cell-cycle arrest, DNA repair, senescence and apoptosis. In addition, during the 30 years of p53 research, numerous studies have implicated p53 in the regulation of differentiation and developmental pathways. Here, we summarize the data on these relatively less-characterized functions of p53, including its involvement in embryogenesis and various differentiation programs, as well as its function in restraining de-differentiation of mature somatic cells. Besides the well-known functions of p53 as a cell-cycle regulator and a mediator of apoptosis, both coincide with differentiation processes, p53 was shown to exert its effects on various differentiation programs via direct regulation of specific key factors controlling these programs. The complex regulation by p53, which acts to suppress or to induce differentiation, is mainly the result of the specific cell type and fate. We argue that regulation of differentiation is pivotal for the tumor-suppressive activity of p53, which act to maintain the proper cellular state, preventing improper maturation or reprogramming. This conclusion is further supporting the notion that aberrant differentiation is associated with malignant transformation.

p53 plays a regulatory role in development

The tumor suppressive functions of p53 (encoded by TP53 in human and Trp53 in mice), namely, the regulation of cell proliferation and apoptosis (1,2), are also tightly associated with the regulation of normal development. Indeed, several lines of data implicate p53 in embryonic development. For instance, in mouse and chicken models, the messenger RNA (mRNA) and protein levels of p53 were found to be significantly downregulated during embryogenesis (3–5). Specifically, *in situ* hybridization studies of mouse embryos demonstrated high expression of p53 mRNA in all tissues until midgestation. During the process of organogenesis, p53 levels decrease, and it is hardly detected in terminally differentiated tissues (5). In order to investigate in detail the regulation of p53 transcription during mouse embryogenesis, several transgenic mouse models expressing a reporter gene under the control of the p53 promoter were generated (6–8). These demonstrated a differential expression pattern of p53; while in early embryos, strong reporter activity was observed in most tissues, in later developmental stages, the activity became heterogenic and restricted to specific tissues, at distinct differentiation stages. Notably, in late stages of embryogenesis and in newborn mice, high reporter activity was found in the nervous system.

Despite the tight regulation of p53, and its known fundamental roles, the generation of apparently normal developed vital p53-knockout (KO) mice (9), strongly suggested that p53 is dispensable for proper development. Nevertheless, in agreement with the fact that p53 is a tumor suppressor; p53-KO mice developed a wide range of tumors at an early age (9–11). However, as research progressed, it became clear that p53-KO mice exhibit a significant frequency of developmental defects. This is perhaps most clearly manifested as a reduction in p53-null female progeny, which tend to develop exencephaly (12,13) In addition, other developmental defects including ocular abnormalities, polydactily of the hind limbs and defects in upper incisor tooth formation were reported to occur at a higher incidence in p53-KO mice (12). Additional studies examining p53-KO mice revealed that both males and females exhibit lower fertility due to either defects in spermatogenesis (14,15) or impaired embryonic implantation (16), respectively. The fact that p53-KO mice do develop and are born alive, indicates that there is an incomplete penetrance of the p53-null phenotype, suggesting that a compensatory mechanism which is dependent on an interaction between genetic and environmental factors may exist.

The importance of p53 during development seems more pronounced in other species, as p53-deficient *Xenopus laevis* embryos exhibit inhibition of mesodermal differentiation and severe gastrulation defects (17). This difference may be explained by the fact that the other p53 family members, p63 and p73, which are expressed during mouse embryogenesis and may compensate for the absence of p53, are not expressed during early developmental stages in frogs (18,19). Similarly to the frog's embryonic development, p53 was also shown to be involved in embryogenesis of other vertebrates, such as zebrafish (20,21). In addition, inhibiting p53 expression in salamander results in inhibition of limb regeneration (22).

The role of p53 in development was also demonstrated in mouse models that exhibit increased p53 protein levels due to disruption of the *Mdm2* or *Mdm4* genes. Mdm2 functions as an E3 ubiquitin ligase that targets p53 for degradation (23), whereas Mdm4 inhibits p53 transcriptional activity (24). Both *Mdm2*- and *Mdm4*-KO mice die during early embryogenesis. This death is attributed to a failure of p53 inhibition during gestation that results in accelerated cell-cycle arrest and apoptosis during a stage in which rapid cell divisions are required. The direct contribution of p53 to this phenotype was evident by concomitant deletion of p53, which completely rescued the embryonic lethality of *Mdm2*- and *Mdm4*-deficient mice (25–27). These results clearly indicate that reduction in p53 activity mediated by either Mdm2 or Mdm4 is essential for normal development.

Thus, the tight regulation of p53 levels and its activity along distinct developmental stages is required for proper development. Whether p53 exerts its role in developmental regulation through its abilities to induce cell-cycle arrest and apoptosis or whether other activities are at play is still uncertain. Nevertheless, its participation in embryonic development may also point at its involvement in differentiation programs that occur in adult tissues. In the following sections, we will elaborate on key studies that implicated p53 along regulatory networks of various differentiation programs.

p53 and neural differentiation

During neural development, neuronal death is a fundamental process whereby approximately half of the neurons produced in the nervous system die to ensure the establishment of appropriate neural connections (28). The most prominent effect of p53 deficiency is evident as deranged neuronal development resulting in exencephaly in approximately a quarter of p53-KO embryos. This neural tube malformation is a result of either extensive cell outgrowth or reduced apoptosis in the neural tissue (12,13). The partial penetrance of the exencephalic

Abbreviations: ESC, embryonic stem cell; HSC, hematopoietic stem cell; iPS, induced pluripotent stem; KO, knockout; mRNA, messenger RNA; PPARγ, proliferator-activared-receptor-γ; pRb, retinoblastoma protein.

phenotype suggests the existence of a compensatory mechanism, probably mediated by the other p53 family members, p63 or p73 (28). At the cellular level, p53 was shown to be involved in the regulation of proliferation and differentiation of neural progenitor cells, promotion of neuronal maturation and axonal growth and regeneration following neuronal injury (29).

The induction of neuronal differentiation involves two interrelated cellular processes; progression through the stages of neurite outgrowth and cell-cycle arrest (30). Neuronal precursors derived from p53-KO mice display an enhanced proliferative potential, supporting a specific role for p53 in mediating an antiproliferative signal to neurons (31). Using primary cultures of rat oligodendrocytes and neurons, as well as of the neuronal pheochromocytoma cell line, PC12, it was demonstrated that during differentiation, p53 translocates into the nucleus, whereas in mature differentiated cells, it is present mainly in the cytoplasm. Inhibition of p53 activity by introduction of a dominant-negative p53 protein inhibited the differentiation of oligodendrocytes and of PC12 cells and protected neurons from spontaneous apoptotic death. These findings suggest that p53 plays a regulatory role in directing primary neurons and oligodendrocytes toward differentiation or apoptosis *in vitro* (32). In PC12 cells, p53-dependent activation of nerve growth factor receptor is required for the transduction of the nerve growth factor signaling, which leads to growth arrest and differentiation (33–35). By utilizing a genome-wide chromatin immunoprecipitation cloning technique of nerve growth factor-treated PC12 cells, Brynzka *et al.* unraveled novel p53-regulated genes. The most prominent differentiation-relevant target genes included *Wnt7b*, which is involved in dendritic extension, and *Grhl3*, which is implicated in ectodermal development. These authors concluded that p53 transcriptional activity is involved in PC12 differentiation and suggested a direct contributory role for p53 in neuronal development (36).

p53 was shown to control the neural stem/progenitor cells self renewal, differentiation and tumorigenic potential. Concomitant deletion of p53 and *Pten* in mouse central nervous system led to the development of glioma. The dual inactivation of p53 and *Pten* promoted a high self-renewal of neural stem cells and their undifferentiated state. The double-KO neural stem cells exhibited increased Myc levels and activity, which was shown to contribute to their impaired differentiation and enhanced renewal capacities, as well as to the formation of tumorigenic neurospheres (37). Interestingly, Wang *et al.* revealed that p53 deficiency provides no significant growth advantage to adult brain cells but appears to induce pleiotropic accumulation of cooperative oncogenic alterations driving gliomagenesis. Accumulation of mutant p53 proteins occurs first in neural stem cells in the subventricular zone. These cells start to proliferate, giving rise to transit-amplifying progenitor-like cells expressing an aberrant pattern of neural progenitor markers, which initiate glioma formation (38). Thus, the expression of mutant p53 abrogates proper maturation of neural stem cells, leading to their malignant transformation.

Involvement of p53 in osteogenic differentiation

The differentiation of osteoblasts from mesenchymal precursors requires a series of cell-fate decisions controlled by a hierarchy of transcription factors. In particular, RunX2 and Osterix are key differentiation regulators that function together to commit progenitor cells toward the osteoblast lineage (39,40). The involvement of p53 in osteogenic differentiation and bone formation is intriguing since it plays opposite molecular roles during normal development compared with tumorigenesis, i.e. whereas p53 attenuates the course of bone formation and differentiation of early osteogenic precursors; it promotes terminal differentiation of tumor-forming osteogenic cells and by this attenuates the cancerous outcome (41).

Induction of differentiation is usually considered as one of p53 tumor-suppressive activities. Therefore, it was surprising to reveal that p53 functions as a negative regulator of osteoblast differentiation, skeletal development and bone remodeling. Wang *et al.* and Legner *et al.* showed that osteoblasts from p53-deficient mice exhibit accelerated differentiation, which results in a higher rate of bone formation and bone density. This was manifested by downregulation of the key osteogenic transcription factors Ostreix or RunX2 (42,43). In agreement with this, two additional independent studies provided further evidence of osteogenesis acceleration in p53-null mesenchymal stem cells (44,45), albeit the terminal differentiation of these cells was impaired (44). In addition to their aberrant differentiation, osteoprogenitor cells of p53-null mice also demonstrate a higher proliferation rate and may contribute to osteosarcoma formation, which is known to be abundant in p53-null mice. (43). Notably, in contrast to the inhibitory role of p53 in mesenchymal stem cells differentiation, p53 plays a positive regulatory role during osteogenic reprogramming of muscle-committed cells (46,47). Thereby, p53 acts as a regulator that may either induce or inhibit osteogenic differentiation, depending on the specific cellular type and its cancerous potential.

Development of osteosarcoma may be a result of genetic and epigenetic changes that interrupt normal osteogenic differentiation of mesenchymal stem cells (48). Interestingly, p53 was found to be frequently inactivated in human and mouse osteosarcoma cell lines (49–52). Furthermore, patients with the Li-Fraumeni syndrome, which usually harbor a germ line p53 mutation, are at high risk to develop osteosarcomas (53). The study of Radinsky *et al.* shows that reintroduction of wild-type p53, but not mutant p53, into p53-null human osteogenic sarcoma cells results in terminal differentiation and apoptosis and in inhibition of lung metastases upon the injection of these cells to mice. Thus, linking p53 deficiency to aberrant differentiation, which leads to tumor formation (41).

p53 and myogenic differentiation

The skeletal muscle differentiation program involves expression of defined myogenic regulatory transcription factors and is coupled with a permanent withdrawal from the cell-cycle (54). Skeletal muscles of p53-KO mice develop normally (9), and an excellent formation of myotubes was observed during the process of muscle regeneration in p53-KO mice (55). In contrast, several *in vitro* studies have reported that p53 is required for myogenic differentiation. This discrepancy might be a result of the different triggers required for inducing differentiation under *in vitro* and *in vivo* conditions.

Initially, it was shown that p53 mRNA levels are upregulated during myogenic differentiation (56). It was further demonstrated that during myogenic differentiation of immortal and primary murine myoblasts, endogenous wild-type p53 protein becomes transcriptionally active. Introduction of a dominant-negative p53 peptide into these cells resulted in inhibition of terminal differentiation into myotubes. This p53 inactivation did not alter the cell-cycle withdrawal typical of terminal differentiation, indicating that interference with endogenous p53 directly affects cell differentiation, independently of its ability to induce cell growth arrest (57,58). Later on, the works of Tamir and Bengal and Porrello *et al.* shed light on the mechanism by which p53 regulates myogenic differentiation. While Tamir and Bengal suggested that p53 is involved in the activation of muscle creatine kinase, Porrello *et al.* reported that p53 is responsible for upregulation of retinoblastoma protein (pRb) at the transcriptional level, which is essential for induction of the muscle differentiation program, together with the MyoD regulatory factor (59,60). Finally, Cam *et al.* showed that all three p53 family members (p53, p63 and p73) cooperate to promote skeletal muscle differentiation. It appears that while p53 transactivates the *pRb* gene; p63 and p73 induce the cyclin-dependent kinase inhibitor p57 to maintain pRb in an active, hypophosphorylated state. The activation of the pRb protein is important for permanent cell-cycle withdrawal and transactivation of muscle-specific genes (18,61).

The inactivation of all three p53 family members by a dominant-negative peptide represses myogenic differentiation and, in cooperation with other oncogenes, contributes to malignant transformation of the myoblasts (61). Additionally, alteration of the p53 pathway has been implicated in disruption of muscle progenitor cell differentiation and promotion of rhabdomyosarcoma formation (62–64), which is a family of soft tissue tumors associated with the myogenic lineage

(65). The fact that p53 contributes to rhabdomyosarcoma development is substantiated by the observation that both p53 heterozygous mice (66) and Li-Fraumeni patients (53) exhibit a high incidence of rhabdomyosarcoma.

Thus, myogenic differentiation provides additional evidence linking the regulatory function of p53 during in differentiation and its critical role as safeguard of proper cell maturation and tumor formation.

p53 in differentiation of hematopoietic cells

During the process of blood production, hematopoietic stem cells (HSCs) give rise to a hierarchy of differentiating progenitor cell populations that can constantly repopulate the blood system (67). Although abnormality of hematopoiesis was not initially observed in p53-KO mice (9), in-depth studies demonstrated that p53 do play a role in the differentiation of hematopoietic cells (68). This was evident in the process of B-cell maturation, where it was found that reconstitution of wild-type p53 in an early pre-B cell line that lacks p53 expression (L12) resulted in the maturation of these cells, as manifested by expression of the μ immunoglobulin heavy chain and the B-cell-specific surface marker, B220. Furthermore, when these cells were injected into syngeneic mice, they induced a lower incidence of tumors and these tumors were less aggressive compared with the p53-deficient parental cell line (69). This may suggest that the differentiation block in the p53-deficient cells enhances their tumorigenic potential. Treatment of another pre-B cell line (70Z/3), which expresses wild-type p53, with the differentiation inducer lipopolysaccharide or with γ irradiation resulted in increased levels of p53 mRNA in these cells. This was accompanied by the induction of κ light chain. Accordingly, it was found that p53 trans-activates the promoter of the κ light chain gene. In contrast, however, overexpression of mutant p53 in these cells interfered with their ability to differentiate (70,71). In addition, lipopolysaccharide treatment of the wild-type p53 expressing pre-B cells, 13A60, led to increased p53 mRNA levels and to secretion of IgA antibodies. These results suggested that p53 is involved in regulation of B-cell differentiation, a pathway requiring genomic rearrangements that may be accompanied by generation of faulty DNA (70). Recently, Slatter *et al.* generated a transgenic mouse model (mDeltapro) lacking the proline-rich domain of p53. mDeltapro mice develop a late-onset B-cell lymphoma comprised of incorrectly differentiated B cells, leading the authors to suggest that by keeping B-cell populations in check, p53-dependent apoptosis prevents development of lymphomas from irregular B cells (72).

p53 expression was also shown to positively regulate myeloid differentiation. Soddu *et al.* (73) and Banerjee *et al.* (74) reported that introduction of wild-type p53 into the p53-deficient HL-60 promyelocytic leukemia cells induced their differentiation through the granulocytic or monocytic pathways. Careful examination of the HL-60 cells revealed that induction of differentiation or apoptosis in these cells depends on differential expression levels of wild-type p53 protein, i.e. high levels of wild-type p53 induce HL-60 cells to undergo apoptosis, whereas differentiation is mediated by low levels of p53 (75). In agreement with the above-mentioned findings, granulocytic differentiation of myeloid precursor cells and primary bone marrow cells was inhibited by p53 dominant-negative peptides interfering with the endogenous wild-type p53 expressed in these cells (57,58).

p53 overexpression induced the differentiation of the leukemic monoblastic U-937 cells, as well as facilitated their differentiation following Vitamin D3 treatment (76). Notably, this p53-mediated differentiation induction was shown to depend on its transcriptional activity (77).

A positive role of p53 in erythropoietic differentiation was demonstrated in K562 cells, an erythroid acute-phase chronic myeloid leukemia cell line (57,76,78) and in Friend erythroleukemia cells (79). In addition, it was demonstrated that p53-dependent apoptosis is required for the final stages of normoblast differentiation, resulting in nuclear condensation and expulsion without cell death (80). Mechanistically, it appears that in the maintenance of erythropoietic homeostasis, glucocorticoid receptor and p53 function as opposing forces; the former favoring proliferation of erythrocytes under stress conditions (81), whereas the latter counteracts its proliferative effects, thereby favoring differentiation (82).

p53 is also activated during megakaryocytic differentiation, and its role is to control polyploidization and the transition to endomitosis by impeding cell cycling and promoting apoptosis (83,84).

The earliest stages of blood development begin with the long-term repopulating HSCs that then differentiate into short-term repopulating HSCs and non-self renewing multipotent progenitors. These cell populations are capable of differentiating into a spectrum of mature blood cells but differ in their self-renewal and proliferative capacity. Long-term HSCs express high levels of p53 transcripts. The relative quiescence of long-term HSCs probably protects these cells from exposure to reactive oxygen species and toxic metabolites that could lead to DNA damage. Thus, the upregulation of p53 in these cells may play an important role in maintaining their integrity (85). Moreover, by using a variety of *in vivo* and *in vitro* assays, Liu *et al.* have shown that HSCs quiescence is impaired in the absence of p53 and that p53 function is essential for the enhanced stem cell quiescence observed in *Mef*-null mice (86).

Thus, the dual role played by p53 in hematopoesis, inducing proper cellular maturation as well as maintaining the quiescence of the stem cell population contributes to the homeostasis of the hematopoietic system, assuring the prevention of malignant transformation.

p53 and adipogenic differentiation

Adipocytes arise from mesenchymal stem cells by a sequential pathway of distinct differentiation stages. The principle regulators, which are indispensable for white fat formation, are proliferator-activared-receptor-γ (PPARγ) and CCAAT/enhancer-binding protein (C/EBP) (87). Early studies demonstrated that p53 is downregulated during adipogenic differentiation of 3T3-L1 preadipocytes (88) and exhibits a reduction in its DNA-binding activity (89), suggesting a negative role in regulating adipogenesis. In contrast, it was recently shown that the protein levels of p53 remain constant during adipogenic differentiation of 3T3-L1 cells. Moreover, in late stages of this differentiation, p53 is phosphorylated on two N-teminal residues, which may indicate its activation (90). We and others have observed an increased adipogenic differentiation potential in p53-null mesenchymal stem cell populations (45,47,91). In part, these results could stem from increased proliferation rate of the p53-KO cells (45). However, our data suggest that this p53-dependent inhibition of adipogenesis is mediated by repression of the key adipogenic transcription factor *PPARγ*, e.g. treatment of wild-type p53 cells with a drug that activates p53 results in downregulation of *PPARγ*. Furthermore, application of a PPARγ inhibitor to p53-deficient mouse embryonic fibroblasts resulted in complete inhibition of adipocyte differentiation, suggesting that upregulation of other important adipogenic transcription factors downstream to PPARγ in p53-deficient cells is unlikely (47).

The notion that p53 negatively regulates adipogenesis was also supported by *in vivo* studies. p53 is highly induced in adipocytes of the genetically obese ob/ob mice in a fed state. This induction leads to a suppression of sterol regulatory element-binding protein-1 (SREBP-1), a key transcriptional regulator of triglyceride synthesis and the concomitant downregulation of lipogenic enzymes. This data suggest that p53 activation might constitute a negative feedback loop against excess fat accumulation in adipocytes (92). In addition, transgenic mice overexpressing an active p53 form exhibit a reduction in body mass, adipose tissue deposition and subcutaneous adipose tissue (93).

The negative effects exerted by p53 on adipogenesis and body fat accumulation may be linked to alterations in metabolism, which are considered one of the hallmarks of cancer (94). Accordingly, p53 was reported to regulate both oxidative phosphorylation and glycolysis, an important feature for its ability to suppress tumorigenesis (95). p53 was shown to regulate energy metabolism by tilting the balance between the glycolitic and respiratory pathways. This effect is mediated, at least partially, by p53-dependent trans-activation of

Cytochrome-C-Oxidase-2 (CCO2), which is essential for mitochondrial respiration (96), and TP53-induced-glycolysis and apoptosis regulator that inhibits glycolysis (97). Thus, in p53-deficient cells there is a shift from oxidative phosphorylation toward glycolysis (96). This shift may increase the availability of acetyl-CoA molecules as substrates for fatty acid synthesis and may contribute to the increased accumulation of fat.

Recently, p53 was shown to play a crucial role in the regulation of insulin resistance in adipose tissue (98). The adipose tissue of genetically obese mice suffering from insulin resistance exhibited features of premature aging and inflammation. These senescence-like changes were manifested by increased expression of senescence-associated β-galactosidase, elevated p53 levels and high expression of pro-inflammatory cytokines. Inhibition of p53 in the adipose tissue decreased the inflammation and improved insulin sensitivity (98,99).

The adipose tissue serves not only as an organ for an energy storage in the form of triglycerides but also as an endocrine and a metabolic organ. In response to endocrine and metabolic signals from other organs, the adipose tissue may secrete free fatty acids, hormones and cytokines that can affect the function of other tissues (100). Impaired adipogenic differentiation may eventually result in obesity. Obesity, in turn, induces a variety of pathological conditions such as type 2 diabetes, fatty liver and cardiovascular pathology, which are in large part a result of insulin resistance (87). In addition, epidemiological studies indicate that obesity is associated with increased risk of cancers such as colorectal, breast, endometrial, kidney, liver and others. However, the biological mechanisms that link obesity to cancer and the role of p53 in this pathway are still poorly understood (101).

Overall, the abovementioned studies demonstrate an important role for p53 in maintaining proper differentiation and function of the adipose tissue, which may provide a link between obesity, aging, abnormal metabolism and cancer.

A novel suggested role for p53 in guarding the genomic integrity of induced pluripotent cells

The role of p53 in maintaining proper differentiation and developmental processes may imply on its involvement also in the reverse process of de-differentiation. Indeed, several seminal papers were recently published implicating p53 in restraining reprogramming of somatic cells into induced pluripotent stem (iPS) cells (102–109). Collectively, these studies showed that reducing p53 activity resulted in increased reprogramming efficiency of various mouse and human cells. Thus, it appears that the p53 pathway serves as a barrier not only for tumorigenesis but also for somatic cell reprogramming.

p53 is well known as the 'guardian of the genome' (1). Safeguarding the genome may be even more significant in stem cells than in somatic cells because the former can give rise to various cell lineages and can self-renew. Since various somatic cells are continuously replaced by maturation of stem cells, it is reasonable to speculate that similar mechanisms such as those underlying embryonic stem cells (ESCs) properties operate in adult stem cells as well. Indeed, a recent work revealed that p53 is a regulator of polarity in the divisions of mammary stem cells and in its absence, cells acquired self-renewal properties as those typical for cancer stem cells, rather than normal stem cells (110). Other reports show that p53 negatively regulates self-renewal and drives hematopoietic stem cells toward quiescence, through two p53 target genes, *Gfi-1* and *Necdin* (86,111,112). Similar properties were also shown in adult neural stem cells, where p53 was shown to negatively regulate proliferation and self-renewal (37,113–115).

As p53 was already found to be involved in the regulation of various stem cell properties, its involvement in the regulation of cellular reprogramming is not surprising. In mouse ESCs, p53 was shown to bind the promoter of *Nanog*, a key gene required for ESCs self-renewal and to suppress its expression following DNA damage (116). The reduced expression of *Nanog* led to ESCs differentiation, enabling their cell-cycle arrest, and a subsequent repair of the damaged DNA or alternatively, in case of a persistent damage, to programmed cell death. Thereby, p53 probably serves to maintain the genomic integrity of the ESCs. Similarly, activation of p53 by Nutlin, an inhibitor of p53–Mdm2 interaction (117), in human ESCs, prevents S-phase entry and subsequently leads to cell-cycle arrest (118). Finally, germ cells were demonstrated to be spontaneously reprogrammed in the absence of p53 (119).

In addition to the roles of p53 in regulating the DNA damage response, proliferation and self-renewal in stem and progenitor cells, it was also shown to be implicated in the core regulatory circuit of the factors used for the reprogramming process. As mentioned above, p53 represses *Nanog* in ESCs following DNA damage (116). It was also shown that in certain contexts, *Klf4*, one of the factors required for reprogramming, can directly repress p53 transcription in mouse embryonic fibroblasts. This study showed that when *Klf4* is overexpressed alone, the expression of the cell-cycle regulator, p21, was increased, leading to cell-cycle arrest. However, in conjunction with the Ras^{V12} oncogene, p53 was repressed, which eventually resulted in cell transformation (120). A reciprocal trans-activation of *Klf4* by p53 was also demonstrated (121,122). The regulatory circuit between *Klf4*, p53 and Nanog raised the possibility that p53-deficiency may substitute for the role played by *Klf4* in the reprogramming process. This hypothesis was tested by several groups; while Zhao *et al.* failed to reprogram p53-deficient human cells in the absence of *Klf4*, Kawamura *et al.* showed that using p53-deficient mouse embryonic fibroblasts, iPS cells can be produced with only *Oct4* and *Sox2*, although at a very low yield. This demonstrates that p53 reduction does not completely substitute for the role of *Klf4* in the process of iPS cells generation.

As for the mechanisms by which p53 inhibition enhances reprogramming efficiency; the different studies mentioned above point to several possibilities. Zhao *et al.* speculated that reprogramming factors such as c-Myc could lead to p53-dependent induction of senescence and apoptosis (102). Indeed, Banito *et al.* demonstrated that activation of either the quartet of factors together (*c-Myc*, *Oct4*, *Sox2* and *Klf4*) or each of them separately induces senescence in both mouse and human cells (108). Moreover, the *Ink4a/Arf* locus, which encodes both the p16 and ARF tumor suppressors, was shown to act as a barrier to reprogramming, and is silenced during the process (105,107,108). These findings also implicate p53 as a regulator of reprogramming because the primary function of ARF is to activate p53 by inhibiting its Mdm2-dependent degradation (123). Banito *et al.* (108) further showed that during reprogramming, p16, p21 and p53 are activated. This is in agreement with Kawamura *et al.* (104), which demonstrated an increase in p53 levels by different combinations of the four reprogramming factors. Moreover, Hong *et al.* (103) highlight the importance of p21 as a p53 target in the reprogramming process. Finally, Marion *et al.* (106) emphasizes the role of p53-dependent apoptosis in preventing suboptimal cells, carrying DNA damage, from becoming iPS cells.

In contrast to the suggested role of p53 in restraining de-differentiation of somatic cells into pluripotent stem cells, Hanna *et al.* claimed that p53 does not directly play a role in this process, and its effect is only due to its function as a regulator of the proliferation rate of the cells. This was supported by inhibition of the p53–p21 pathway or, alternatively, overexpression of *Lin28*, which promoted proliferation and enhanced the efficiency of iPS formation in direct proportion to the increase in proliferation rate (124).

Overall, it seems that p53 inhibition enhances the reprogramming process, increasing both the yield of the generated iPS cells and the rate of their formation. However, in spite of the scientific worldwide race toward achieving better and more efficient techniques to reprogram cells, the use of p53 elimination in this process should be considered with great caution since reduction in the levels of this tumor suppressor protein may be detrimental for the integrity of the reprogrammed cells. One can speculate that in the absence of an appropriate defense mechanism, the reprogrammed cells will be transformed and give rise to cancer initiating cells (125,126). Indeed, we have found that reprogramming of cells in which the p53 pathway is

abrogated give rise to cells with pluripotential capacity *in vitro*, but upon injection to nude mice, these cells induced the formation of malignant tumors (Sarig et al., unpublished data). This concern is supported by the demonstration that mouse fibroblasts lacking the pRb tumor suppressor function undergo aberrant reprogramming, yielding transformed cells capable to initiate tumor formation (127). Also, several of the recently published studies show preliminary evidence for the risks of eliminating p53 in the reprogramming process. For instance, Marion *et al.* reported that iPS cells deficient of p53 exhibit foci of DNA damage and chromosomal aberrations, as well as loss of their typical round morphology after expansion (106). Moreover, mice generated from p53-null iPS cells died of tumors originating from these cells (103). Trying to circumvent the tumorigenic potential of p53-null iPS cells, it was suggested that a transient suppression of p53 during reprogramming may be useful for future iPS cell production for medical use. However, if p53 is required for maintaining the genomic integrity of the cells throughout the reprogramming process, this may still give rise to abnormal iPS cells.

The connection between reprogramming and cancer is further supported by the observation that while c-Myc is a well-known oncogene,

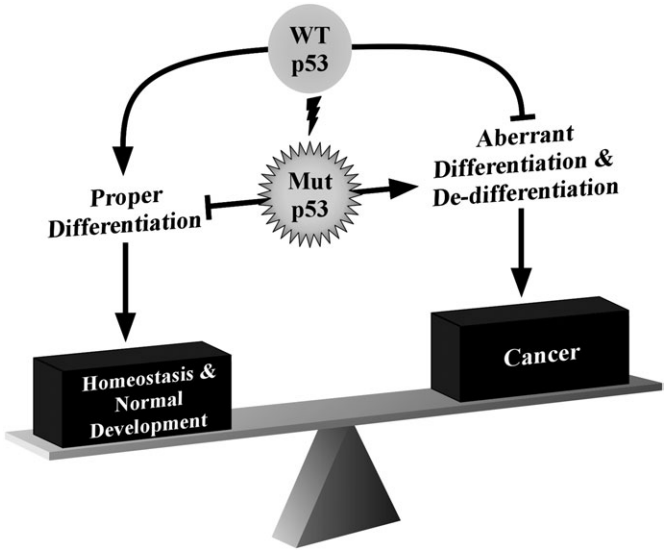

Fig. 1. A model depicting the role of p53 as a mediator of differentiation and de-differentiation processes. Wild-type p53 is a homeostatic gene promoting proper differentiation and development in order to prevent tumorigenesis. This is mediated by its well-established roles as an inducer of the cell-cycle arrest and apoptosis, to prevent abnormal maturation of stem and progenitor cells. p53 also regulates de-differentiation by restraining somatic cells reprogramming, thus preventing the formation of abnormal stem-cells, which may lead to tumor development. In contrast to the tumor suppressive activity of the wild-type protein, mutant p53 abrogates p53-dependent activities in controlling differentiation and de-differentiation processes, blocking differentiation and promoting tumorigenesis.

Klf4 appears to display both growth inhibitory and growth promoting abilities, depending on the cell type (120). Oct4 was shown to act as a dose-dependent oncogenic fate determinant, and ectopic expression of Oct4 was reported to promote dysplastic growth in epithelial tissues (128, 129). Nanog expression in NIH3T3 cells drives the cells toward transformation (130) and leads to a differentiation block of myoblasts (131). Lin28 was also shown to promote transformation and to play a role in germ cell malignancy (132,133). These data are not surprising considering the fact that part of their role as stemness factors is to maintain cells in a self-renewal, proliferative state. This concept is further highlighted by studies demonstrating the tight link between cancer and ESCs. It was shown that aggressive poorly differentiated human tumors have an ESC-like gene expression pattern (134) and that activation of an ESC-like transcriptional program can induce epithelial tumor initiating cells (135).

As cancer cells and ESCs share common features in general, and more specifically, cancer cells and iPS cells, it is plausible to assume that p53 may be involved not only in protecting ESCs and perhaps adult stem cells from malignant transformation but also play a similar role in the reprogramming process, assuring the stability of the reprogrammed cells. The low efficiency of the reprogramming process may indicate on a stringent selection of the reprogrammed cells, raising the possibility that as a result of p53 activity, cells that carry mutations in their genome or with aberrant DNA repair do not survive the reprogramming process, thus explaining the suppressive effect of p53 on the reprogramming process. If this is indeed the case, the removal of p53 might allow these abnormalities to be passed on to the reprogrammed cells and their descendents.

In sum, these recent discoveries suggest that in agreement with the well-accepted notion that p53 is the genome guardian at large, it plays a pivotal role in maintaining the genomic stability of ESCs and reprogrammed cells, restraining de-differentiation, transformation and further proliferation of abnormal cells.

p53 as a regulator of differentiation and de-differentiation—implications for cancer development

The broad involvement of p53 in numerous differentiation programs and in restraining de-differentiation of mature somatic cells imply on its fundamental role as a homeostatic gene that regulates proper maintenance of the cellular state (Figure 1). Its multifaceted functions in differentiation are dependent on the cell type and fate, i.e. it can either inhibit differentiation in stem cell populations, whereas induce differentiation in more committed, progenitor cells (47).

The tight link between stem cells, cancer and cancer stem cells is gaining more evidence and interest (136,137). The major property indicating stem cells as the best candidates to initiate tumor formation is their self-renewal capacity. However, whether tumors initiate from more mature progenitor cells or even from terminally differentiated cells, still remains unknown. It was believed for many years that external agents, such as chemicals and viruses can facilitate transformation by inducing anaplasia, a phase that allows cancerous growth by de-differentiation of mature differentiated cells. Another

Table I. Differentiation-associated genes regulated by p53

Differentiation program	p53 effect	Target genes[a]	References
Neural	Facilitates neural differentiation	*Nerve growth factor receptor* (*NGFR*), *Wnt7b*, *Grhl3* and *Myc*	(33–37)
Osteogenic	Inhibits differentiation of non-transformed cells	*Osterix* and *Runx2*	(42,43)
Myogenic	Promotes myogenic differentiation	*Mck* and *pRb*	(59–61)
B-cells	Induces cell maturation	*κ light chain immunoglobulin*	(70)
Adipogeneic	Negatively regulates adipogenic differentiation	*Pparγ* and *Srebp-1*	(47,92)

[a]In addition to the established roles of p53 as a cell-cycle regulator and mediator of apoptosis during differentiation, p53 was shown to affect differentiation by regulating specific genes required for proper differentiation programs. The table summarizes the examples of such genes mentioned throughout the review.

school supported the 'embryonal rest' model, which was first suggested by Wirchow in 1855 (138). Based on histological similarities between tumors and embryonic tissues, it was suggested that tumors in adults develop from embryonal rudiments that remained in matured organs. This theory was later re-examined by Conheim, who suggested that tumors develop from residual embryonic remnants that were 'lost' during developmental organogenesis (139). This theory was further revisited in 2004 when it was postulated that tissue stem cells are the modern-day equivalent of embryonal rest and that most tumors arise from the maturational arrest of a cellular lineage derived from a tissue stem cell (140). Recent evidence demonstrating the existence of cells expressing Oct4 and Nanog in adult tissues (141), and the fact that human tumors were shown to have an ESC-like gene expression pattern (134) support Virchow's postulation. This is further supported by a number of studies showing that specific gene signatures expressed in adult stem cell are evident in various tumors (142–144). Nonetheless, there is still no direct evidence, to show that deregulation of stem cells gene expression patterns leads to tumor formation under physiological conditions *in vivo*. In attempts to fate-map the cellular origin of glioblastoma, Wang *et al.* demonstrated that indeed, mouse neuronal stem cells located in the subventricular zone are the cancer initiating cells. As the tumor promoting alteration in this mouse model of gliomagenesis is a brain-specific p53 mutation, it unraveled the fundamental role of p53 in this process. Interestingly, expression of the mutant p53 did not result in increased growth advantage of endogenous neuronal stem cells, but its accumulation in specific cells gave rise to several oncogenic alterations driving tumor formation (38). Additional models mimicking tumor formation *in vivo*, utilizing specific expression of mutant p53 in distinct cell populations may elucidate its role in the cellular origin for cancer formation and its possible contribution along the progression of cancer development.

The major function of p53 as a tumor suppressor is to promote cell-cycle arrest and apoptosis. As described above, via these functions, p53 is involved in various differentiation programs. In addition, its restraining activity along reprogramming was shown to involve its functions in inducing DNA damage repair, apoptosis or cell cycle arrest. However, a more direct role of p53 in differentiation, by regulating the expression of key differentiation proteins was shown for several programs (Table I). Few examples include the regulation of *Runx2* or *Osterix* in osteogenesis (145), *PPARγ* in adipogenesis, *myocardin* in smooth-muscle differentiation (47) and *Wnt7b* and *Tfcp2l4/Grhl3* in neural differentiation (36). Thus, although differentiation coincides with cell-cycle arrest and apoptosis, in addition to these well-known functions of p53, it also plays a direct role in the regulation of specific factors responsible for proper cellular maturation. It will be interesting to determine whether p53 also plays such a direct role during reprogramming, by regulating specific genes required for this complex process.

In sum, the regulation of proper differentiation and de-differentiation processes by p53, either via its established functions or by regulating the expression of specific genes required for the various programs, broaden our knowledge not only on the specific functions played by this multitask protein, but on the process of tumor initiation at large. Since aberrant differentiation or de-differentiation can give rise to transformed cells, abrogation of p53 function by its deficiency or mutation, may result in maturational arrest of stem or progenitor cells, and/or in accumulation of oncogenic events, both of which can induce tumor formation (Figure 1). The complex regulation by p53, which can act to inhibit or to suppress differentiation, is mainly dependent on the specific cell type and fate. Thus, p53 serve to maintain the proper cellular state, preventing improper maturation or reprogramming and may also be referred to as a 'guardian of differentiation'.

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