Review

p53 balances between tissue hierarchy and anarchy

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Normal tissues are organized in a hierarchical model, whereas at the apex of these hierarchies reside stem cells (SCs) capable of self-renewal and of producing differentiated cellular progenies, leading to normal development and homeostasis. Alike, tumors are organized in a hierarchical manner, with cancer SCs residing at the apex, contributing to the development and nourishment of tumors. p53, the well-known 'guardian of the genome', possesses various roles in embryonic development as well as in adult SC life and serves as the 'guardian of tissue hierarchy'. Moreover, p53 serves as a barrier for dedifferentiation and reprogramming by constraining the cells to a somatic state and preventing their conversion to SCs. On the contrary, the mutant forms of p53 that lost their tumor suppressor activity and gain oncogenic functions serve as 'inducers of tissue anarchy' and promote cancer development. In this review, we discuss these two sides of the p53 token that sentence a tissue either to an ordered hierarchy and life or to anarchy and death. A better understanding of these processes may open new horizons for the development of new cancer therapies.

Keywords: p53, stem cell, gain of function, cancer stem cells, adult stem cells, embryonic development, tissue hierarchy

Introduction

Balancing the equilibrium between self-renewal and differentiation of stem cells (SCs) is central to proper development and homeostasis maintenance of tissues. Alteration of this balance can result in tissue malfunction and tumor development (Clarke and Fuller, 2006). Tumor initiation and development were shown to be ascribed to a subpopulation of cells contained within a tumor, known as the 'cancer SCs' (CSCs) population (Visvader and Lindeman, 2008). These CSCs were shown to have similar capacities as SCs including the ability to self-renew and differentiate. Furthermore, these CSCs resist chemotherapy, which eventually leads to treatment failure and cancer recurrence (Shackleton et al., 2009).

The pivotal tumor suppressor, p53, acts as a key regulator of several major signaling pathways and cell fate decisions. Following different types of stress, including DNA damage and oncogene activation, p53 undergoes posttranslational modifications that lead to its activation, stabilization, and accumulation in the cell. The tumor suppressor activity of p53 is mainly attributed to its ability to modulate transcription of genes that are involved in numerous cellular processes, such as cell cycle arrest, apoptosis, senescence, DNA repair, and differentiation (Chipuk and Green, 2006; Levine and Oren, 2009; Aylon and Oren, 2011; Rivlin et al., 2011). p53 mutations are the most frequent alterations in human tumors (Brosh and Rotter, 2009). Most of the p53 mutations are missense mutations that produce full-length mutant p53 proteins, which not only lost the wild-type (WT) p53 tumor suppressor activity but also gained oncogenic functions (GOF), that facilitate carcinogenic features (Brosh and Rotter, 2009; Muller and Vousden, 2014).

Ample data obtained from various studies indicate a role of p53 in regulating embryonic development and maintaining adult tissue homeostasis by monitoring the self-renewal and differentiation of SCs. p53 mutations were shown to impair the fine equilibrium between self-renewal and differentiation of SCs, toward self-renewal, that results in CSC formation and tumor development (Rivlin et al., 2015). In this review, we discuss the roles of the WT p53 tumor suppressor and the mutant p53 GOF in the biology of SCs, as well as in CSCs. On the one hand, we will focus on the role of WT p53 as the 'guardian of tissue hierarchy' and on the other hand, the role of mutant p53 GOF in inducing tissue anarchy resulting in tumor development.

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The role of p53 in early development

Embryonic development from a single zygote is a complex multistage process that is tightly regulated. p53 levels were found to be altered during embryogenesis; high levels of p53 are expressed in the early embryogenesis stage whereas expression of p53 is downregulated in later stages of organogenesis, suggesting a role for p53 in embryonic development (Choi and Donehower, 1999; Goh et al., 2012). Indeed, p53-null mice exhibit developmental defects including neural tube defects, upper incisor tooth formation defects, and ocular abnormalities. Moreover, low fertility and spermatogenesis defects were reported in female and male p53-null mice, respectively (Rotter et al., 1993; Hu et al., 2007). Apparently, in the uterus of female mice, p53 upregulates the leukemia inhibitory factor, a crucial factor for blastocyst implantation and continuous embryo development (Hu et al., 2007). Altogether, these studies suggest that p53 does play a role in early development. Nevertheless, it is surprising that p53-null mice, as well as mutant p53 knock-in mice, develop normally into adult mice, which suggest that normal embryonic development can be selected toward p53 independency. Nevertheless, it should be born in mind that p53-null mice, as well as mutant p53 knock-in mice, develop tumors in their adult life. This may suggest that in the embryonic stage there is a stringent mechanism to assure for genetic fidelity whereas, in adult life, this genetic stringency is less pronounced.

Embryonic SCs (ESCs) are derived from the undifferentiated inner cell mass of a developing blastocyst before implantation into the uterus. These ESCs possess a pluripotency capacity that enables the cells to give rise to all tissues of the body by differentiating into progenies that comprise all three germ layers, including ectoderm, mesoderm, and endoderm. Although there are contradicting reports (Spike and Wahl, 2011; Rivlin et al., 2015), many studies demonstrate that p53 has a role in maintaining genome integrity in ESCs. The lack of p53 in ESCs led to higher proliferation potential concomitantly with less susceptibility to apoptosis upon differentiation, indicating a p53 tumor suppressor activity in ESCs (Sabapathy et al., 1997). Based on the prominent role of p53 in the DNA damage response of somatic cells, it is reasonable to presume that p53 has a similar role in the DNA damage response of ESCs. p53 was shown to be highly expressed in murine ESCs and localized mainly in the cytoplasm. Different DNA damage insults trigger p53 translocation into the nucleus, where it can execute its tumor suppressor activities by the transactivation of target genes and by promoting apoptosis (Grandela et al., 2007; Solozobova et al., 2009; He et al., 2016). In somatic cells, p53 was shown to have functions unrelated to its transcription activity by interacting with different mitochondrial proteins and inducing mitochondria-dependent apoptosis (Chipuk and Green, 2006). Similarly, p53 was shown to induce apoptosis of ESCs, upon DNA damage, not only through transcription activity but also through the mitochondrial pathway (Qin et al., 2007; Li et al., 2015). Nevertheless, one should bear in mind that due to the critical requirement for genome integrity in ESCs, the DNA damage response in ESCs is not only p53-dependent (Nagaria et al., 2013).

Two essential characteristics of SCs that distinguish them from somatic cells are their abilities to self-renew and to differentiate. p53 was shown to play a role in maintaining ESCs genome integrity by inducing apoptosis, as mentioned above, or by inducing ESCs differentiation. Upon UV irradiation and doxorubicin treatment, p53 was shown to bind to the promoter of *Nanog*, an essential transcription factor that maintains the self-renewal capacity of ESCs and inhibit its expression. Interestingly, *Nanog* expression was also reduced by p53 following retinoic acid-induced differentiation (Lin et al., 2005), indicating a pivotal role of p53 in balancing self-renewal and differentiation of ESCs, regardless of DNA damage. This is further supported by the demonstration that p53 activation led to both a decrease in the expression of pluripotent genes, including the pivotal ESCs transcription factor, *Oct-4*, as well as to an increase in the expression of different differentiation markers (Qin et al., 2007; Maimets et al., 2008; Li et al., 2012). p53 was also shown to indirectly repress the expression of different pluripotent genes through the induction of the expression of different microRNA that antagonized pluripotent genes in ESCs including *Nanog*, *Sox2*, *Oct-4*, *Klf-4*, and *N-Myc* (Rivlin et al., 2015). Treatment of ESCs with doxorubicin led to the identification of five members of the Wnt ligand family as new p53 target genes (Lee et al., 2010). The notion that the Wnt signaling pathway has been associated with the self-renewal capacity of ESCs and has antidifferentiation activity led to the assumption that p53 has a role in maintaining the self-renewal property of ESCs by inhibiting their differentiation. Interestingly, a later in-depth study demonstrated that Wnt signaling pathway components, Wnt3 and its receptor Fzd1, are p53 target genes that serve as critical factors driving the mesendodermal differentiation of ESCs (Wang et al., 2017b).

Recent studies have suggested that epigenetic modulation plays an important role in embryonic development and ESCs differentiation. This is associated with chromatin changes, mainly by DNA methylation that regulates the transcriptional programs during lineage commitment and cell fate specification (Atlasi and Stunnenberg, 2017). It is accepted that the DNA methylation machinery consists of DNA methyltransferases (DNMTs) and DNA-demethylating proteins, which govern DNA methylation dynamic in the stem state as well as during differentiation. p53 deficiency led to global hypermethylation in the thymus and liver through the overexpression of Dnmt1 and Dnmt3b, encoding for two centrals DNMTs (Park et al., 2005). A recent study demonstrated a more direct connection between p53 and DNA methylation homeostasis. Accordingly, p53 maintains the balance between the different component of the DNA methylation machinery where, on the one hand, it directly inhibits the expression of DNMTs Dnmt3a and Dnmt3b, and, on the other, it induces the expression of Tet1 and Tet2, which are DNA demethylases. This function of p53 was shown to be central for maintaining the methylation landscape homogeneity within the ESCs population, which most likely is essential for proper differentiation and for preventing tumorigenesis (Tovy et al., 2017).

p53 was shown to be a dynamic and an unstable protein, which can balance between a WT or a mutant conformation

(Cañadillas et al., 2006). The pivotal role of p53 in ESCs described above is also substantiated by the observation that in ESCs that are derived from mutant knock-in mice, the mutant p53 protein can be tilted toward a WT p53 conformation. Interestingly, protein networks influencing protein conformation and stabilization such as chaperones, ubiquitin-related proteins, and posttranslation modification regulators were found to bind the p53 protein and induce its folding into a WT p53 conformation (Rivlin et al., 2014). Overall, the WT form of the p53 tumor suppressor plays a key role in maintaining the ESCs genome and epigenomic integrity that permits proper embryonic development and ESCs differentiation. Accordingly, great effort is made to retain the WT conformation and diminish the lethal effects of the mutant p53.

The role of p53 in adult tissue hierarchies

Proper development of a multicellular organism is based on the evolvement of hierarchically organized tissues. At the apex of the hierarchy is positioned an adult SC (ASC) that dominates the balance between self-renewal and differentiation. In normal developing tissue, the ASCs remain quiescent within the niche, until new cells are required. Following tissue injury, ASCs are triggered to undergo asymmetric cell cycle divisions that concomitantly permit SC renewal and the generation of committed progenitor daughter cells, which will eventually replenish the damaged cells. Thus, ASCs fulfill an important role in the maintenance of tissue and organ homeostasis.

The mammary epithelium exhibits a typical hierarchy that, in this case, consists of bipotent mammary SCs that can give rise to the two cellular entities of the mammary tissue, luminal and basal myoepithelial cells that form the two layers of the mammary epithelium, the basal and luminal layers (Van Keymeulen et al., 2011; Rios et al., 2014). The lower platform of the hierarchy consists of two types of unipotent SCs that can differentiate into one cell type, accordingly to the epithelium layer which they were derived from (Van Keymeulen et al., 2011; Blaas et al., 2016). As mentioned above, SCs undergo asymmetric division, which serves to maintain the SC pool. In order to prevent uncontrolled self-renewal of damaged SCs that may lead to tumorigenesis, this process must be tightly regulated. Indeed, p53 depletion in mammary SCs led to a disruption of this tight balance and induced a shift of the equilibrium toward self-renewal and increment of unfit SC frequency that eventually promoted breast cancer development (Cicalese et al., 2009; Tao et al., 2011). Mechanistically, it was shown that inhibition of the development pathway, Notch signaling pathway, inhibits the expansion of p53 knock-out (KO) mammary SCs but not of p53 WT mammary SCs, suggesting that p53 might restrict mammary SC self-renewal through Notch signaling inhibition (Tao et al., 2011). This notion is supported by another study suggesting a negative regulation of the Notch signaling pathway by p53 during T-cell development (Laws and Osborne, 2004). An additional in-depth study suggests that upon asymmetric division, Numb, an asymmetrical cell division regulator during development, is accumulated in the daughter cell, which eventually adopts an SC phenotype. In the daughter SC, Numb increased p53 activity, which restricted the daughter SC to remain in a quiescence state and by that prevented tumorigenesis (Tosoni et al., 2015). These observations might suggest that Numb attenuates the Notch signaling pathway, not only directly as previously shown (Spana and Doe, 1996) but also in an indirect manner through p53 activation. Overall, although there are still open questions regarding p53 regulation of SCs fate, it is clear that p53 plays an important role in regulating mammary SCs self-renewal and tissue homeostasis.

An additional example where p53 plays a role in maintaining the balance between self-renewal and differentiation is the neuronal SCs (NSCs) of the nervous system. NSCs are the most primordial cellular entity in the nervous system that can self-renew and differentiate and give rise to all cell types of the neuronal lineage. p53 was shown to reduce the self-renewal capacity of NSCs. This was accompanied by a reduction in the expression of the p53 known target gene, p21, previously shown to reduce the self-renewal of NSCs (Kippin et al., 2005; Meletis et al., 2006). Importantly, olfactory bulb-derived progenitors, originated from KO p53 mice, exhibited an increase in self-renewal capacity associated with a modification in the neuronal differentiation patterns, suggesting a role of p53 in neuronal differentiation (Armesilla-Diaz et al., 2009a). Consistently, the transformation of p53-null NSCs required additional mutagenesis processes leading to impaired differentiation and to a shift of the system toward self-renewal of subventricular zone-derived NSCs (Gil-Perotin et al., 2006). A more mechanistic study revealed a dual function of p53 and Pten in inhibiting c-Myc expression and its associated gene signature expression that was associated with proper NSCs differentiation, preventing gliomagenesis (Zheng et al., 2008). Interestingly, an additional study showed that *Pten*$^{-/-}$*Ink4a/Arf*$^{-/-}$ mouse NSCs, although expressing low c-Myc levels, could induce gliomagenesis. Isolation of the glioma SCs from the *Pten*$^{-/-}$*Ink4a/Arf*$^{-/-}$ NSC-derived primary tumors revealed that they highly express c-Myc. The elevated expression of c-Myc was the consequence of the downregulation of its negative regulator, Fbxw7, that occurred as a result of spontaneous p53 mutations (Kim et al., 2012). In sum, p53 regulates the self-renewal and differentiation equilibrium of NSCs by inhibiting c-Myc expression in a direct as well as in an indirect manner. An additional pathway controlling the self-renewal capacity of NSCs is the hedgehog–Gli pathway that promotes NSCs proliferation, self-renewal, and brain tumorigenesis. p53 was shown to negatively regulate Gli expression, activity, and nuclear localization in SC-derived neurospheres (Stecca and Ruiz i Altaba, 2009). Moreover, p53 was shown to regulate the self-renewal and differentiation of NSCs by repressing the expression of smad1 and Id1. These two factors accelerated differentiation toward neural rather than glial lineage, while Id1 also augmented the proliferation of NSCs (Liu et al., 2013). These ample reports indicate a pivotal role of p53 in maintaining the homeostasis of NSCs, which is controlled by multiple p53 mechanisms.

The hematopoietic developmental hierarchy in the bone marrow is the best-characterized tissue hierarchy found in the

adult body. At the apex of the hierarchy stands a hematopoietic SC (HSC) that can produce multipotent progenitors that finally dictate restricted lineages that lead to the production of all mature blood cell types (Bryder et al., 2006). Maintaining the SC pool is central for retaining tissue homeostasis and lifelong hematopoietic cell production. The latter can be achieved by cellular quiescence of HSCs. The first link between p53 activity and HSC self-renewal was demonstrated by an early study showing that p21, the pivotal p53 target gene, regulated HSC pool by restricting HSC self-renewal and controlled the entry into the cell cycle (Cheng et al., 2000). The observation that p53 is highly expressed in long-term reconstituting HSCs led to the discovery that p53 maintains the quiescent state of HSCs and its absence results in enhancing HSCs self-renewal and a rapid entering of the HSCs into the cell cycle. This p53 activity is controlled by two p53 target genes that regulate the quiescent state of HSCs, *Gfi-1* and *Nedcin* (Liu et al., 2009). Further studies supported the notion that p53 negatively regulates the proliferation and self-renewal of HSCs (Pant et al., 2012). Furthermore, p53, as in somatic cells, was shown to be an important regulator promoting apoptosis of HSCs upon genotoxic stressors (Bonizzi et al., 2012). Although p53 KO mice initially displayed normal hematopoiesis (Lotem and Sachs, 1993), in-depth studies indicated a role of p53 in HSC self-renewal and quiescence, as well as in hematopoietic cells differentiation (Aloni-Grinstein et al., 1993; Molchadsky et al., 2010). At large, the observations that p53 plays an important role in controlling HSC self-renewal, HSC integrity and proper hematopoiesis suggest that p53 prevents malignant transformation of HSCs.

Friedenstein et al. (1970) demonstrated for the first time that mesenchymal SCs (MSCs), which reside in the bone marrow, are multipotential stromal cells that are able to form colonies. These colonies-derived cells have the ability to support the hematopoietic niche and to differentiate into mesoderm lineages including adipocytes, chondrocytes, and osteocytes (Williams and Hare, 2011). It is well accepted that the MSC population is very heterogeneous, mainly due to divergent differentiation potential. In an intra-population of a single MSC isolate, variable differentiation capacity can be found, including clones that possess trilineage (multipotent) differentiation ability (adipocytes, chondrocytes, and osteoblast) and other clones that are more restricted and are able to differentiate only to two or one lineages (bi and monopotent) (Pevsner-Fischer et al., 2011). Accumulating experimental evidence indicate a role of p53 in MSC differentiation. Several reports demonstrate that p53 negatively regulates MSC differentiation, and its inhibition can lead to accelerated differentiation and cancer development (Lengner et al., 2006; Molchadsky et al., 2008; Armesilla-Diaz et al., 2009b). On the other hand, p53 expression in an osteosarcoma cell line led to increased differentiation, apoptosis, and inhibition of metastases development *in vivo* (Radinsky et al., 1994). Interestingly, an in-depth later study indicated that p53 inactivation sentence the cells to osteogenic fate at the expense of adipogenesis and this is due to downregulation of the genes *PPARG* and *TWIST2* (Boregowda et al., 2018). One possibility that underlines the negative regulation of mesodermal differentiation by p53 might be a mesodermal lineage restriction, permitting only monopotent differentiation capacity, regulated by p53. When this restriction is unleashed, upon p53 inactivation, the multipotency differentiation capacity is restored. This notion is supported by a study analyzing single cell clones derived from WT p53 MSCs and p53 KO MSCs. This analysis indicated that WT p53-derived clones either underwent senescence or lost their trilineage differentiation capacity compared to single cell clones derived from p53 KO MSCs that retained their multipotency differentiation ability (He et al., 2015). p53 was shown to regulate not only differentiation but also the self-renewal of MSCs. We and others have shown that abolishment of p53 led to a higher incidence of colonies, indicating higher self-renewal capacity and the acquirement of the ability to form sarcomas *in vivo* (Shetzer et al., 2014; Boregowda et al., 2018; Lonetto et al., 2018). Altogether, in MSCs, as with other adult tissue-derived SC, p53 plays a role in balancing self-renewal and differentiation, which is a crucial equilibrium to prevent tumorigenesis.

During aging, tissues lose the capacity to maintain homeostasis and repair. Accumulating data suggest that this process involves loss of SC function that impairs the ability to replenish the differentiated cells that maintain tissue functionality (Rando, 2006). While the role of p53 as a tumor suppressor is well established, its role in aging remains a matter of debate. Results from mouse models suggest that p53 may function as a pro-aging factor (Tyner et al., 2002; Maier et al., 2004), though others have shown a function of an anti-aging regulator (Armata et al., 2007; Matheu et al., 2007). Bearing in mind that p53 prevents the transformation of embryonic and ASCs, it is tempting to speculate that the elimination of aged DNA-damaged SCs is also the outcome of p53 function. This notion is further supported by a study showing that a mouse model with a hyperactive p53 activity exhibited premature aging phenotypes including reduced cellularity and organ mass accompanied by a reduction in HSC number and in their ability to be engrafted when transplanted (Dumble et al., 2007). Similarly, aged epidermis SCs of a mouse model with a specific epidermis Mdm2 deletion exhibited an increase in p53 levels and its target genes, resulting in aging phenotypes such as epidermal layer thinning, reduction in skin wound healing, and a widespread of hair loss. These phenotypes also correlated with premature cell senescence of the epidermal SCs and a reduction in epidermal SC frequency (Gannon et al., 2011). Importantly, p53 activation must be tightly regulated to balance between premature reductions of tissue SC number but yet to avoid the survival of damaged SCs that emerge with age. Indeed, p53 deletion in aging telomere-dysfunction intestinal SCs compromised their integrity, through chromosomal instability, ending in premature tissue failure and destruction (Begus-Nahrmann et al., 2009).

Recently, a new role of p53 in muscle SC activation, leading to muscle generation, was described. The p53–Notch axis was found to be an essential pathway for muscle SC activation, and self-renewal activation of the p53–Notch pathway involves the upregulation of p53 that serves as a protector from muscle SC

death by preserving their self-renewal and regenerative capacity. This is achieved by the upregulation of Hey1, a canonical Notch target that inhibits the expression of Mdm2, a p53-negative regulator. Upon aging, this axis is unfunctional leading to impaired muscle regeneration. Indeed, in aged mice, p53 stabilization resulted in muscle SC survival, and muscle tissue generation was restored (Liu et al., 2018). Altogether, while p53 plays a protective role against tissue deterioration and preserve tissue integrity, its levels must be tightly regulated to prevent SC loss and premature tissue aging.

The role of p53 in dedifferentiation and reprogramming

Studies carried >20 years ago have linked the differentiation state of tumors to p53 status showing that high grade/dedifferentiated sarcomas and carcinomas correlate with p53 loss and increased malignancy (Aloni-Grinstein et al., 2014). The burst of the reprogramming era, 10 years later, highlightened the notion that cells have the potential to dedifferentiate and with the appropriate conditions to reprogram. Indeed, the loss of p53 was shown to be associated with dedifferentiation of adult tissues and the acquisition of an SC phenotype. For example, pancreatic acinar cells derived from p53KO mice exhibited a higher proliferation rate and expression of epithelial to mesenchymal transition (EMT)-associated genes and pancreatic progenitor cell markers (Pinho et al., 2011). In accordance, a later study demonstrated that the NFATC1–Sox2 axis, which promotes dedifferentiation of pancreatic cancer cells into an SC-like state, was counteracted by p53 through the elevation of its target gene, miR-200, that negatively regulates Sox2 (Singh et al., 2015). Furthermore, p53 loss was shown to permit the dedifferentiation of mature hepatocytes into malignant reprogrammed progenitor cells that promoted the development of liver cancers. This was achieved by inducing the expression of the SC marker *Nestin,* which is negatively regulated by p53, that antagonizes Sp1/3 binding to the *Nestin* promoter (Tschaharganeh et al., 2016).

Somatic cell reprogramming was shown to be induced *in vitro* by conjunctional overexpression of four transcription factors, involving specific epigenetic changes, that permit the reversion of differentiated somatic cells into induced pluripotent SCs (iPSCs; Plath and Lowry, 2011). Interestingly, p53 functions as a barrier of cellular reprogramming processes (Hong et al., 2009; Kawamura et al., 2009; Utikal et al., 2009) through several p53 target genes that counteract with the reprogramming process. Expression of p21, the *bona fide* target of p53, in *p53*-null mouse embryonic fibroblasts (MEFs) was shown to decrease iPSC generation, indicating that p21 mediates the p53 suppression of iPSC generation (Hong et al., 2009). p21 was shown to counteract the reprogramming process not only through inhibition of cell proliferation (Hanna et al., 2009) but also through inhibition of mesenchymal to epithelial transition accounting for the reprogramming of fibroblasts (Brosh et al., 2013). Likewise, miR-34, another well-known p53 target gene, was shown to be a downstream regulator that reduces reprogramming efficiency, mainly by suppressing the expression of pluripotency regulators, including *Nanog*, *Sox2*, and *Mycn* (Choi et al., 2011). Furthermore, a recent study demonstrated that another known p53 target, PHLDA3, hampered the reprogramming process by regulating the AKT–GSK3β pathway (Kawase et al., 2009; Qiao et al., 2017). Additionally, an elegant study demonstrated that p53 functions as a barrier of reprogramming by inducing the expression of large intergenic noncoding RNA p21 (lincRNA-p21) that preserves the repressive epigenetic hallmark, H3K9me3, and CpG methylation at the promoters of pluripotency genes. Knockdown of lincRNA-p21 resulted in the upregulation of pluripotency genes. The function of p53 as a barrier of reprogramming was shown to be augmented in cells harboring damaged DNA (Marion et al., 2009). Accordingly, p53 suppression resulted in the generation of iPSCs displaying genomic instability and with an ability to form malignant tumors instead of benign teratomas (Sarig et al., 2010; Tapia and Scholer, 2010). Overall, p53 inhibits the backslide of somatic cells to a more SC state by counteracting reprogramming through several mechanisms. Additionally, p53 conserves genomic fidelity of the reprogramed cells and prevents malignant processes.

In all, p53 has a role not only in maintaining proper equilibrium between the self-renewal and the differentiation of SCs but also in preventing the dynamic of cellular hierarchy by counteracting the climbing back of somatic cells to the apex of the hierarchy and reverting into SCs.

The role of p53 in tumor hierarchy

Cancer development and progression are explained by the two well-accepted models, the 'clonal evolution' and the 'cancer SC (CSC)' models (Marjanovic et al., 2013). According to the Darwinian evolutionary theory, the clonal evolution model suggests that accumulation of genetic and epigenetic alterations, accompanied by microenvironment stress, imposes clonal selection that is based on proliferation and survival advantage. This model supports the notion that most cells within a tumor have an equal potential to generate a tumor. On the other hand, the CSC model suggests that tumors are hierarchically organized according to the tumorigenic and metastatic potential of the cancer cells. Tumor hierarchy, similar to normal tissue hierarchy, is attributed to the existence of a subpopulation of CSCs. Alike SCs, CSCs have the ability to self-renew and possess long-term repopulation potential. They reside in the apex of the hierarchy of the tumor due to their ability to recapitulate the tumor of origin by differentiating into multiple tumor cell types. Moreover, these CSCs are believed to resist chemotherapy and metastasize (Visvader and Lindeman, 2008).

Although mutations in the p53 gene are frequent in most human cancers, they appear to be more associated with undifferentiated high-grade tumors (Fujimoto et al., 1992; Ahmed et al., 2010; Junttila et al., 2010), which implies a role of p53 in impeding cancer stemness and plasticity. Furthermore, analysis of breast tumors revealed that p53 inactivation correlated with the expression of an SC signature (Mizuno et al., 2010).

Accordingly, it is not surprising that p53 plays a prominent role in CSCs. Interestingly, p53 was shown to bind directly to CSC marker promoters and repress their expression. For example, p53 was shown to transcriptionally repress CD44, a known CSC marker in various tumor types that was shown to be implicated in cancer cell survival, migration, invasion, and metastasis (Zoller, 2011; Rivlin et al., 2015). This p53-mediated repression reduced the tumorigenic capacity of a human lung adenocarcinoma line (Godar et al., 2008). A more recent study demonstrated that p53 can bind to the promoter and inhibit the expression of CD133, another known CSC marker, and by that reduce cellular proliferation and tumor formation (Park et al., 2015). Recently, CD51 was identified as a functional marker for colorectal CSCs (Wang et al., 2017a). Indeed, CD51 is important for the self-renewal and drug resistance of CSCs and metastatic potential (Sui et al., 2018). Similarly to p53 suppression of *Nestin* expression by antagonizing Sp1/3, thus preventing dedifferentiation and liver cancer development (Tschaharganeh et al., 2016), it was shown that p53 inhibits the expression of CD51 by also by antagonizing its transcription activator Sp1/3 (Sui et al., 2018). Moreover, p53 regulates CSCs through another indirect mechanism by elevating the expression of its target gene miR-34a that inhibits CSC marker expression, CSC expansion, and tumorigenesis. For example, *CD44* was shown to be a direct target of miR-34a in prostate CSCs. Reduced expression of miR-34a contributed to prostate cancer development and enhanced CSCs properties (Liu et al., 2011). Furthermore, in colorectal cancer, miR-34a was shown to negatively regulate the expression of *c-Kit*, a known CSC marker in numerous cancer types (Kang et al., 2008; Zhang et al., 2008; Adhikari et al., 2010). This regulation was p53-dependent and led to a reduction in chemotherapy resistance, migration, invasion, and spheres formation abilities of the cells that was accompanied by a reduction in the expression of additional stemness markers including CD44, Lgr5, and BMI-1 (Siemens et al., 2013). In a mesotheliomas mouse model, p53 and miR-34a were shown to downregulate the expression of c-Met that was shown to have a role in inducing mesothelioma cell migration, invasion, and maintaining mesothelioma CSC population. Furthermore, a study on multidrug-resistant breast cancer MCF-7/ADR cells revealed that miR-34a targeted NOTCH1 and by that reduced the ability to form mammospheres, lowered the breast CSC frequency, sensitized the cells to doxorubicin treatment, and reduced tumor formation (Park et al., 2014). Breast cancer cells overexpressing the Δ133p53β isoform, which lacks the transactivation domain, had enhanced ability to form mammospheres, induced the expression of pluripotency and stemness regulators, and possessed enhanced CSC and metastatic potential. Thus, it is plausible that the enhanced CSC potential in cells expressing the Δ133p53β isoform is due to the negative dominant effect that hampered the full-length WT p53 function (Arsic et al., 2015). Loss of p53 activation in a hepatocarcinoma cell line prompted a c-MYC-induced reprogramming of the liver cancer cells into cancer stem-like cells with enhanced expression of CSC markers, including EpCAM, NANOG, and BMI1. p53 inactivation also led to the increased self-renewal potential of the liver cancer cells that was dependent on c-MYC activation (Akita et al., 2014). Furthermore, p53 knockdown led to the expansion of EpCAM-expressing stem-like cells that possess an ability to form ovarian tumors (Motohara et al., 2011). Altogether, p53 regulates tumor hierarchy by negatively regulating the self-renewal and expansion of CSCs.

CSCs possess different mechanisms in order to inhibit the function of WT p53. BMI1, a core component of the polycomb repressive complex 1, was shown to have a role in CSC proliferation and maintenance and their chemoresistance (Siddique and Saleem, 2012). Interestingly, BMI1 negatively regulates p53 by serving, together with Ring1A and Ring1B, as an E3 ubiquitin ligase that directly binds to p53 and induces its polyubiquitination and subsequent degradation in embryonic cancer precursors (Calao et al., 2013). The high-mobility group A1 (HMGA1) protein was shown to be a master regulator of ESCs, cellular reprogramming, and also of CSCs, thus suggesting to be a promising target to specifically eradicate CSCs in various tumors (Yanagisawa and Resar, 2014). HMGA1 was also shown to be overexpressed in colon tumor SCs and to possess the ability to bind directly to the promoter of p53 and inhibit its transcription. Knocking down of HMGA1 resulted in a decrease of proliferation and self-renewal of the colon CSCs, implying that HMGA1 might play a role in regulating self-renewal by inhibiting p53 transcription (Puca et al., 2014).

Autophagy is a multistep process of self-degradation of cellular components in which double-membrane autophagosomes sequester organelles or portions of cytosol are fuse with lysosomes or vacuoles for breakdown by resident hydrolases. This process is executed at basal levels in every cell and promotes cellular homeostasis by regulating organelle and protein turnover (Levine and Kroemer, 2008). Various studies support the notion that autophagy is an active pathway in CSCs, which supports the maintenance of CSCs and tumor development (Rausch et al., 2012; Gong et al., 2013; Vitale et al., 2015). Autophagy was shown to inhibit p53 function in hepatic CSCs. PINK1, a kinase associated with mitophagy (a mitochondrial-specific form of autophagy), phosphorylates p53 at serine-392 leading to its association with the mitochondria. Upon mitophagy activation, the active form of p53 (pS392) is degraded together with the mitochondria. When mitophagy is inhibited, the active form of p53 can translocate to the nucleus and suppress the expression of NANOG, which is essential for CSC maintenance, resulting in a reduction of stemness and hepatocarcinogenesis (Liu et al., 2017).

Mutant p53 GOF, tissue anarchy, and tumorigenesis

TP53 is the most frequently mutated gene in human cancers. Even though alterations in the p53 protein were found in almost every region of the protein, the most common mutations found in human patients are missense mutations that frequently occur in six 'hot-spot' amino-acids. These six 'hot-spot' mutations were

shown not only to cause the loss of the tumor suppressor activity of the protein but also to gain oncogenic characteristics that promote the tumorigenic process. Together, these properties are known as mutant p53 GOF activities. The Li–Fraumeni syndrome is an inherited cancer predisposition syndrome, which is commonly caused by germ line p53 mutations (Malkin et al., 1990). This syndrome is characterized by an early onset of multiple tumor types within an individual and high-cancer incidence in the affected family. The mutant p53 GOF concept is further supported by the fact that patients with Li–Fraumeni syndrome that harbor missense p53 mutations, which lead to the production of a full-length mutant p53 proteins, have an early onset of cancer development compared with patients harboring loss of function p53 mutations (Bougeard et al., 2008). Furthermore, generation of *in vivo* mice models revealed an observation in which mice that are knock-in with frequent p53 missense mutations, which produce full-length mutant p53 proteins, displayed higher metastatic phenotype compared to p53KO mice (Lang et al., 2004; Olive et al., 2004). Accumulated data during the past 25 years have demonstrated that mutant p53 GOF activity regulates almost every feature in cancer development such as the promotion of cancer cell proliferation, survival, invasion, migration, and drug resistance (Brosh and Rotter, 2009; Muller and Vousden, 2014). Additionally, different mutant p53 proteins were shown to interfere with DNA repair mechanisms and exert genome instability (Shetzer et al., 2016). Several studies have shown a role of mutant p53 GOF activity in disrupting tissue hierarchy by enhancing self-renewal of CSCs. Introduction of the mutant p53 (R175H) into mouse mammary tumor virus (MMTV)-Wnt-1 transgenic mice led to augmented cancer initiation and development that were associated with an expansion of the mammary epithelial SC pool (Lu et al., 2013). Next, it was reported that introduction of R172H, a p53 mutation, which is analogous to the p53 mutation R175H in human tumors, into MMTV-ErbB2/Neu mouse model led to a more aggressive phenotype that included early tumor onset, increased mammary tumor multiplicity resulting of the expansion of mammary SCs, and reduced survival (Yallowitz et al., 2015). Comparison of mouse mutant p53 SCs with their p53 null counterparts indicated a significant GOF activity of mutant p53 in enhancing self-renewal and the tumorigenic phenotype.

EMT is a process that was found to be essential for embryonic development and was also shown to be exploited by cancer cells in order to acquire invasion and metastasis capacities (Thiery, 2002; Hanahan and Weinberg, 2011). EMT was shown to be associated with the acquisition of CSC phenotype. EMT induction, by ectopic expression of either Twist or Snail, in immortalized human mammary epithelial cells, resulted in the acquisition of a CSC phenotype exemplified by a higher percentage of $CD44^{HIGH}/CD24^{LOW}$ population that was previously shown to present the breast CSC population in mice (Al-Hajj et al., 2003). This phenotype was associated with a higher capacity to generate mammospheres, an additional CSC feature, and higher tumorigenicity (Mani et al., 2008). Interestingly, we and others have previously shown a GOF activity in promoting the EMT program (Kogan-Sakin et al., 2011; Dong et al., 2013). These observations might suggest a role of mutant p53 in acquiring a plasticity and stemness phenotype. This was further supported by the observation that mutant p53 GOF induces the dedifferentiation of human sarcoma cells into CSCs (Di Fiore et al., 2014). We have recently shown that the mechanism by which mutant p53 regulates the expression of CSC markers directly involves the binding of mutant p53 to the CSC marker promoter sequences. This positive regulation was associated with the augmentation of mutant p53-dependent tumorigenicity of colon cancer cells (Solomon et al., 2018). Another proposed mechanism by which mutant p53 enhances the proliferation and survival of breast and glioblastoma CSCs suggests that mutant p53 controls the recycling of different membranal receptors. This is mediated by the activation of AKT2, leading to the activation of the WIP pathway that resulted in YAP/TAZ stabilization (Escoll et al., 2017).

Additional evidence demonstrating the acquisition of a CSC state by mutant p53 GOF activity is greatly supported by *in vitro* iPSCs. Whereas the WT p53 seems to negatively control the incidence of iPSCs induction (Hong et al., 2009; Kawamura et al., 2009; Utikal et al., 2009), mutant p53 facilitates the establishment of iPSCs that possess a transformed phenotype similar to CSCs. Indeed, we have shown that mutant p53 has a GOF activity in enhancing the efficiency of the reprogramming process of MEFs. These iPSCs are capable of inducing undifferentiated malignant tumors instead of benign teratomas that were shown to be induced by WT p53-expressing iPSCs (Sarig et al., 2010). Recently, we have established an *in vitro* MSC p53-based system, which permitted the tracing of a cancer multistep process of ASCs and their conversion into CSCs. Eventually, we established aggressive mutant p53-expressing CSC-like cell lines that allowed the identification of a gene signature entailing embryonic specific genes in conjunction with cancer-associated genes. The identified ESC gene signature-derived genes correlated with poor patient survival and human tumors harboring p53 hotspot mutations (Lonetto et al., 2018). Interestingly, a recent study from our laboratory showed that the established CSC-like cell lines exhibited a mutant-dependent metabolic profile that included the increment of mitochondrial mass and oxidative metabolism (Lonetto et al., 2018). Moreover, mutant p53 was shown to upregulate the mevalonate pathway genes that were shown to be important for the self-renewal and survival of breast CSCs (Ginestier et al., 2012). It should be noted that the mevalonate pathway is considered a very important metabolic pathway in cancer cells, thus these data might suggest that mutant p53 may contribute to a CSC phenotype by enhancing different metabolic pathways that support the self-renewal of SCs and CSCs. In all, mutant p53 GOF is associated with the disruption of tissue hierarchy by enhancing SC self-renewal and transformation, CSC expansion, and by facilitating reprogramming and dedifferentiation into an embryonic-like CSC state.

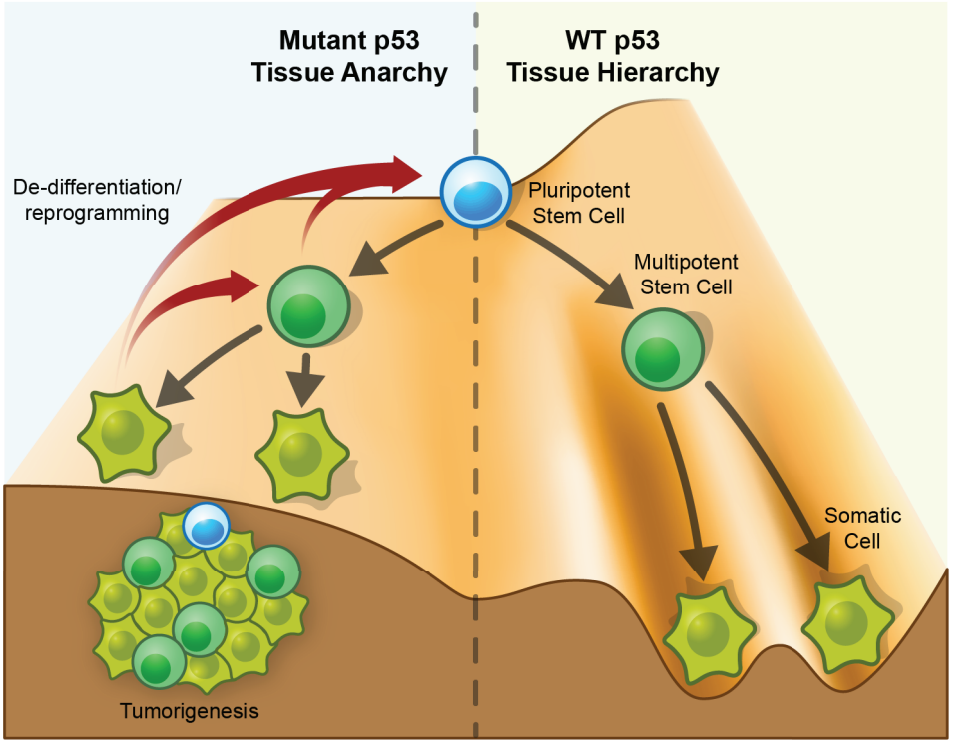

Figure 1 p53 balances between tissue hierarchy and anarchy. The Waddington landscape of development is adapted here to present the differences between WT and mutant p53 in the outcome of tissue organization as a function of p53 status within the cells. While the WT p53 protein underlies normal development through the sliding of cells in a gradient manner from a high stemness state to an established somatic state, mutant p53 induces deformation of the tissue hierarchy resulting in plasticity and eventually tumorigenesis. The mild sliding of the cells down the moderate slop is attributed to a GOF activity of mutant p53, which preserves the cells in a high stemness state, facilitating cell reprograming and inducing tissue anarchy and tumorigenesis.

Perspective and conclusions

The capacities of self-renewal and differentiation of resident SCs to all cell types of the tissue are fundamental for tissue development as well as for maintaining tissue homeostasis and function. Accumulated data indicate that p53 plays a role in tissue-specific SCs of many adult tissues including breast, neuronal, hematopoietic, and mesenchyme. p53, aside of its classical functions as the 'guardian of the genome', also assures proper regulation of tissue development and homeostasis and prevents the generation of abnormal SCs that can lead to tumor development. p53 was shown also to serve as a barrier for dedifferentiation and reprogramming by constraining the cells to a somatic state and preventing their conversion to SCs. Similar to the observed in normal tissues, p53 fulfills its tumor suppressor role by preventing the acquisition of a cancer stemness phenotype and cancer plasticity by inhibiting the expansion of CSCs and negatively regulating CSC-associated genes. Overall, WT p53 serves as the guardian of normal tissue hierarchies. Moreover, WT p53 preserves cancer cells rolling downward the tumor hierarchy by inhibiting the self-renewal of CSCs. On the other hand, mutant p53 proteins, harboring an oncogenic GOF activity, unleash the tissue hierarchy boundaries by inducing SC self-renewal and transformation. Furthermore, mutant p53 GOF activity facilitated reprogramming and dedifferentiation, CSC formation, cancer plasticity and expansion, and the expression of the embryonic SC 'gene signature'.

In sum, WT p53 activity serves as a cornerstone of a proper developmental lineage hierarchy and organismal development, while mutant p53 disrupts tissue hierarchy and promotes tissue anarchy that leads to tumor development (Figure 1). A deeper understanding of the mechanisms in which mutant p53 exerts tissue anarchy will shed a light on important regulators that may be targeted to prevent tissue anarchy and tumor development. Moreover, restoration of the WT p53 conformation may tilt anarchy toward proper hierarchy and prevent mutant p53-dependent cancer plasticity. Indeed, we and others are in the process of developing various chemical and biological approaches aiming at converting the mutant conformation into the WT one with hope to restore proper development and homeostasis (Blanden et al., 2015; Bykov et al., 2016; Tal et al., 2016).

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