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REVIEW

The paradigm of mutant p53-expressing cancer stem cells and drug resistance

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It is well accepted that expression of mutant p53 involves the gain of oncogenic-specific activities accentuating the malignant phenotype. Depending on the specific cancer type, mutant p53 can contribute to either the early or the late events of the multiphase process underlying the transformation of a normal cell into a cancerous one. This multifactorial system is evident in ~50% of human cancers. Mutant p53 was shown to interfere with a variety of cellular functions that lead to augmented cell survival, cellular plasticity, aberration of DNA repair machinery and other effects. All these effects culminate in the acquisition of drug resistance often seen in cancer cells. Interestingly, drug resistance has also been suggested to be associated with cancer stem cells (CSCs), which reside within growing tumors. The notion that p53 plays a regulatory role in the life of stem cells, coupled with the observations that p53 mutations may contribute to the evolvement of CSCs makes it challenging to speculate that drug resistance and cancer recurrence are mediated by CSCs expressing mutant p53.

Introduction

Years of intensive research have yielded important clues regarding the nature of cancer. Various experimental models have shown that a normal cell undergoes malignant transformation following deregulation of major cellular signaling pathways (1). This usually occurs by accumulation of mutations in pivotal genes, epigenetic changes and environmental insults. Both acquired mutations and genetic predisposition have been shown to account for the onset and progression of cancer. Currently, full recovery from most cancer types is still an unsolved enigma. Indeed, frequently following therapy, where an apparent regression of tumor is observed, tumors often relapse and acquire a drug-resistant phenotype. Considering this observation, the development of efficient cancer therapy is closely dependent on the unraveling of drug resistance mechanisms operating in cancer cells. Conventional cancer therapy strategy aims to eliminate transformed somatic cells; however, the possibility of converting transformed cancer cells into normal ones should also be considered because it might restore cells with drug sensitivity.

It is well accepted today that cancer development is a multistep process that involves the accumulation of mutations in a given cell (2). Most of the acquired mutations are silent and do not affect the normal homeostasis of the cell. However, it is well established that modifications in oncogenes and tumor suppressor genes are central for tumor development. Both have profound effects on pivotal pathways, such as the cell cycle, programmed cell death, DNA repair, cellular energy metabolism, angiogenesis, cell attachment, immune surveillance and replicative mortality (1). Although oncogenes have been shown to be overactivated in cancer cells, tumor suppressor genes are inactivated, leading to the loss of their normal function.

A frequent event in human cancer development is the impairment of the wild-type (WT) p53 tumor suppressor pathway, most frequently due to a point missense mutation in the *TP53* gene. It is well accepted that mutant p53 exhibits oncogenic gain of function (GOF) that, among others, confers cancer cells with drug resistance. Recent studies suggest that the cancer stem cell (CSC) subpopulation within tumors accounts for the drug resistance of cancer cells. Given the fact that compromised p53 expression may lead to the generation of CSCs, it is of interest to study the mutant p53-expressing CSCs and drug resistance paradigm.

The guardian of the genome and beyond—normal functions of WT p53

The WT p53 is a pivotal tumor suppressor, termed 'guardian of the genome', because it ensures genomic stability and thus prevents cancer onset (3). Under normal conditions, WT p53 is maintained at low levels due to its constant proteasomal degradation, mediated mainly by the E3 ubiquitin ligase, MDM2 (4). Subsequent to cellular insults such as DNA damage, oncogene activation, telomere erosion, hypoxia and ribosomal stress, WT p53 is stabilized and activated. Following its activation, WT p53 may induce a variety of processes, depending on damage severity and specific cell type. These include cell cycle arrest, programmed cell death (apoptosis), DNA repair, differentiation, autophagy, senescence and other processes (4,5).

The role of p53 in animal development. In addition to its tumor suppressive activity, p53 has also been found to be associated with normal development. One of the major obstacles in resolving the question of whether p53 is indeed involved in development was the initial observation that p53 knockout (KO) in mice was not lethal, which initially suggested that p53 is dispensable for proper development. Nevertheless, and in agreement with the notion that p53 is a tumor suppressor, p53 KO mice frequently develop tumors later in life (6–8). Moreover, an in-depth analysis indicated that p53 KO mice exhibit a lower fertility and that some of the newborns display a variety of developmental defects (reviewed in refs 9–11). These include exencephaly, impaired early neural crest development, ocular abnormalities, polydactyly of the hind limbs and defects in upper incisor tooth formation (12–14). Further examination of p53 null mice revealed abnormalities in reproduction. This is manifested by both defects in spermatogenesis in males (15–17) and impaired embryonic implantation in females (18,19), due to abrogated leukemic inhibitory factor activation, which is required for implantation of blastocysts (18). Additionally, we have recently demonstrated that p53 is required for proper brown fat development and function (20). These findings indicate the critical role of p53 during various developmental processes. The existence of viable p53-deficient mice might suggest that there is an incomplete penetrance of the p53 null phenotype, indicating a compensatory mechanism that may involve the interaction between alternative genetic and environmental factors.

The notion that p53 plays a role in development is substantiated by more directed studies demonstrating, in both mouse and chicken models, that the transcription of p53 is tightly regulated during embryonic development (reviewed in refs 21,22). Analysis of early-stage mouse embryos revealed that the expression of p53 mRNA in all tissues declines during the process of organogenesis and is barely detected in terminally differentiated tissues (23).

The role of p53 in differentiation. A growing body of evidence derived from *in vitro* models suggests that p53 plays a major regulatory role in cell differentiation. Interestingly, it was noticed that p53 seems to be a specific regulator in a variety of differentiation programs. Although

Abbreviations: ABC, adenosine triphosphate (ATP)-binding cassette; ALDH, aldehyde dehydrogenase; ASC, adult stem cell; BM, bone marrow; CSC, cancer stem cell; EMT, epithelial-to-mesenchymal transition; ESC, embryonic stem cell; GOF, gain of function; GSH, glutathione; IFNβ, interferon β; iPSC, induced pluripotent stem cell; KO, knockout; miR, microRNA; MSC, mesenchymal stem cell; NF-κB, nuclear factor-kappa B; ROS, reactive oxygen species; SC, stem cell; TNFα, tumor necrosis factor α; WT, wild-type.

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it facilitates some differentiation programs, others are attenuated (10,24,25). Initial studies have shown that reconstitution of WT p53 in a pre-B cell line, deficient in p53, accelerated cell differentiation and reduced the capacity to form tumors following injection into syngeneic mice (26,27). p53 has also been suggested to exert a positive effect on neural cell differentiation (28–30). Indeed, neural differentiation-relevant target genes are transactivated by p53 in the process of PC12 cell differentiation (31). During myogenic differentiation, p53 upregulates transcription of pRb, which is essential for the induction of the muscle differentiation program (32–34). Interestingly, it has been demonstrated that p53 plays contradictory molecular roles in osteogenic differentiation during normal development and tumorigenesis. Whereas p53 decreases differentiation of early osteogenic precursors (35–37), it facilitates terminal differentiation of tumor-forming osteogenic cells and thereby attenuates the cancerous outcome (38). Additionally, p53 was found to differentially regulate adipogenic differentiation. Although it inhibits white adipogenic differentiation (37,39,40), p53 is crucial for proper brown adipogenesis (20). Thus, p53 functions as a homeostatic protein, which promotes proper differentiation in accordance with a given cellular state, thereby avoiding malignant transformation. This is mediated either via its well-established role as an inducer of cell cycle arrest and apoptosis or by regulating the expression of specific differentiation-related factors required for various differentiation programs. In all, the well-established role of p53 in development and differentiation challenges the notion that p53 plays a role in the life of stem cells (SCs).

p53 and SCs. Proper embryonic development and adult tissue homeostasis rely on the capacity of SCs for self-renewal and differentiation into various cell types. Increasing evidence supports the notion that deregulation of the functions of embryonic stem cells (ESCs) and adult stem cells (ASCs) may lead to developmental aberrations, alterations in adult tissue maintenance and generation of CSCs, which may lead to tumor development.

It is well accepted that there is a wide repertoire of SC types. ESCs are pluripotent cells that are able to self-renew and maintain their cellular identity and they can differentiate into the endoderm, mesoderm and ectoderm cell lineages (41). The tissue-specific multipotent ASCs residing within adult organisms are capable of self-renewal and differentiation into the tissue-specific cells. The ASCs are necessary for normal homoeostasis of tissues and are vital for regeneration after damage (42,43). WT p53 has been implicated in the proper regulation of self-renewal and differentiation of ESCs (11,44,45). p53 is also implicated as a major regulator of the ASC compartment through control of cell differentiation (37,39,46), quiescence and asymmetrical division (47). Interestingly, compromised p53 expression in both ESCs and ASCs seems to confer SCs with accentuated oncogenic activity (46,48–50).

The reprogramming technology, which allows the generation of induced pluripotent stem cells (iPSCs) by dedifferentiation of somatic cells (51,52), opened an interesting platform to study the potential contribution of various factors central to the establishment of SCs *in vitro* (53). Of note, iPSCs and cancer cells have similarities between them with respect to overall gene expression patterns and epigenetic status (54,55), which suggests that tumorigenesis and reprogramming processes may share overlapping pathways. Thus, one of the risks of using iPSCs in cell transplantation therapy is cancer development from iPSC-derived cells (56). Numerous studies have implicated p53 as an important regulator of the reprogramming process. In agreement with others, we found that p53-compromised cells exhibit an accelerated rate of iPSC generation (50,57–64). Thus, p53 has an important role in the maintenance of a fine balance among SC generation, self-renewal and differentiation capacity. Interestingly, we found that reprogramming of mouse embryonic fibroblasts harboring a mutated p53 gene led to generation of CSCs that were capable of forming aggressive tumors in mice (50). This suggests that disruption of p53 function may lead to a burst of accentuated levels of SC proliferation in addition to diversion of SCs toward CSCs.

p53 and aging. Aging is defined as the process whereby life span is reduced with age, accompanied by common changes in phenotype occurring over time (65). It is accepted that as a tumor suppressor, activated p53 prevents cancer development, thus increasing longevity; however, overactivated p53 has been shown to promote premature aging (66,67). The most compelling evidences are obtained from mouse models displaying p53-dependent accelerated aging. For example, mice deficient in the DNA-repair-related gene Ku80 (68), telomerase-deficient mice (69) or mice lacking functional lamin A (70) showed enhanced aging phenotypes that could be rescued by reducing the levels of p53. In addition to the well-accepted mechanisms that mediate p53-dependent aging (71,72), it was shown recently that different p53 isoforms and the balance in their expression regulate aging and life span (66,73). One of the hallmarks of aging is the exhaustion of the ASC reservoir within the tissue (74). Although the role of WT p53 in regulating SCs is still to be completely elucidated, it seems that WT p53 prevents cellular transformation of damaged SCs by inducing either differentiation or cell death (46,50,75). As a result, the renewable cells of the tissue might be depleted, leading to premature aging.

p53 in human cancer

In most human tumors, the p53 pathway is altered, with high incidence of missense mutations, reaching to ~50% of all human tumors (76–78). Unlike other tumor suppressor genes, p53 not only loses its tumor-suppressive function (loss of function or LOF) but also gains novel oncogenic features in some of its mutated forms, independently of normal WT p53 roles, a phenomenon that was termed GOF. Furthermore, p53 is initially mutated in a single allele, leading to the concomitant expression of both WT and mutant proteins. Interestingly, in a heterozygous state, it was shown that some of the mutated forms can override the WT p53 in a dominant-negative manner. Mutant p53 GOF notion was first demonstrated in 1984, whereby introduction of mutant p53 was shown to transform cells lacking p53 (79), and this was followed up by vast research in the field (80). The most compelling evidence for mutant p53 GOF was shown in a mutant p53 knockin mouse model, which exhibited high incidence of metastatic tumors compared with KO mice (81,82). In addition to p53 mutations in somatic cells, p53 germ-line mutations were found to be highly associated (~95% of cases) (83) with a rare cancer predisposition called Li–Fraumeni syndrome (84), which is associated with the development of distinct tumor types, including sarcoma, breast cancer, brain tumor and adrenocortical tumors (85).

The mutation patterns of p53. More than 2000 different mutations have been reported in *TP53*, with several hot-spot mutations being frequently found in human cancers (86). p53 mutations can be categorized into two subgroups, according to their effect on p53 stability: 'DNA-contact mutations', which include mutations in residues essential for DNA binding, and 'conformational mutations', which include mutations that affect the conformational folding of the DNA-binding domain. The expression 'mutant p53' is frequently misused because the variety in both mutations and genotype–phenotype relations is a complex issue. Recent studies comparing the function of the different p53 hot-spot mutations suggest that the various p53 mutations exert different activities (87). When different p53 mutations were introduced into immortalized human fibroblast cells, in conjunction with the H-Ras oncogene, we found that different mutations regulated different signaling pathways [e.g. nuclear factor-kappaB (NF-κB) and H-Ras) to induce the expression of cancer-related genes and to promote transformation. Interestingly, the mutant $p53^{G245S}$ barely induced cellular transformation and expression of the cancer-related gene signature (88,89). This observation was further supported by two recent studies that analyzed different mutant p53 knockin mice models. Examination of two different humanized mutant p53 knockin mice revealed that although $p53^{R248Q/-}$ mice showed accelerated tumorigenesis with expended hematopoietic and mesenchymal stem cells (MSCs), compared with KO mice, $p53^{G245S/-}$ mice did not exert oncogenic GOF activities (90). An additional study suggested that unlike mutant $p53^{R172H}$ (human mutant $p53^{R175H}$ equivalent), mutant $p53^{R246S}$ (human mutant $p53^{R249S}$ equivalent) did not show higher levels of the transformed phenotype and did not promote tumorigenesis (91). With the rise of personalized

medicine in cancer therapy, understanding the exact p53 mutation type expressed in a patient's tumors is of great interest.

Normal WT p53 exerts its function in two main ways: activating/repressing transcription through binding to the promoter of a target gene at a specific sequence, namely, the responsive element; and via protein–protein interactions. In the past 2 decades, accumulating data have shed light on the effect of each p53 mutation on the possible properties of p53. Several mechanisms have been implicated in mutant p53 GOF. A key aspect by which mutant p53 exerts its GOF involves accumulation of the protein in the cell (92). Despite the observation of exceptionally high protein levels in tumor tissue, it was initially considered to be merely a side effect. Nevertheless, in both mouse models and Li–Fraumeni syndrome patients, the protein levels of mutant p53 in normal cells are kept at low levels (80,93). This indicates that p53 mutations by themselves are not sufficient for the high expression levels of p53 found in tumors and that mutant p53 accumulation is required for its GOF properties (78).

Mutant p53 GOF activities

The fact that p53 shows a wide range of hot-spot mutations that generate a highly accumulated aberrant p53 protein level in tumor cells suggests that cells expressing mutant p53 acquire selective growth advantage and tumorigenic potential. Indeed, mutant p53 was found to promote most of the events involved in the malignancy process (1), as discussed below.

Mutant p53 disrupts cell cycle control and enhances proliferation. One of the early observations pertaining to the function of WT p53 was that this tumor suppressor is a cell cycle regulator. Following genotoxic stress, intact WT p53 prevents damaged cells from undergoing malignant transformation by promoting either cell cycle arrest or cell death (4). However, when p53 is mutated, this important cell cycle control is disrupted, leading to enhanced proliferation, one of the typical hallmarks of cancer cells. In agreement with this observation, it was shown that expression of mutant p53 in conjunction with nuclear transcription factor Y (NF-Y) augments the expression of cell cycle-promoting genes and increases DNA synthesis (94). Interestingly, these highly expressed genes are clustered with other cell cycle-controlling genes in a gene signature annotated as the 'proliferation cluster'. This cluster of genes is upregulated in various tumor cells and was found to be positively correlated with high-grade breast tumor and with the expression of mutant p53 (95). Accordingly, mutant p53 was found to facilitate the transcription of genes that underlie the increased proliferation of cancer cells (80,86). In addition to regulation of gene expression, mutant p53 was found to regulate the expression of various microRNAs (miRs) that mediate several mutant p53 GOF activities. For example, mutant p53 was found to suppress the expression of miR-27a, which leads to enhanced epidermal growth factor signaling and extracellular signal-regulated kinase activation, which in turn were shown to enhance cell proliferation and augment the tumorigenic phenotype of cells (96).

Mutant p53 mediates genomic instability. By losing their 'guardian of genome' nature, mutant p53 proteins eventually lead to the collapse of the mechanism dominating genome stability and integrity. Cells that have lost their WT p53 and express instead mutant p53 exhibit extensive chromosomal aberrations and high mutation rates. This phenotype was observed in humanized mutant p53 knockin mice that express the chimeric human/murine mutant p53 gene. Genomic analysis of these mice indicated interchromosomal translocations, which were rarely observed in p53 KO cells, and these were accompanied by impaired G_2–M checkpoint, caused by inactivation of ataxia telangiectasia mutated gene (97). These phenomena, initially observed in embryonic fibroblasts and thymocytes, were further confirmed in mammary epithelial cells, in which it was suggested that the impairment of ataxia telangiectasia mutated gene by mutant p53 leads to the expansion of mammary CSCs and to tumor development (98). These observations are in line with chromosomal instability and aneuploidy demonstrated in mutant p53 transgenic mice (99–101). Furthermore, it was recently shown that mutant p53 expression correlated with massive chromosomal rearrangements observed in Sonic-Hedgehog medulloblastoma of Li–Fraumeni syndrome patients and in patients with acute myeloid leukemia. This phenomenon is manifested in cells as chromothripsis, a one-step catastrophic event, further reiterating the notion that mutant p53 exerts an oncogenic GOF activity in deteriorating genomic stability in cells (102).

Mutant p53 drives epithelial-to-mesenchymal transition, cell motility, tumor invasion and metastasis abilities. The abilities of tumor cells to invade the surrounding tissue and to metastasize are crucial for local carcinoma to evolve to a higher grade of malignancy. Interestingly, when mutant p53 knockin mice were initially generated and examined, the spectrum of spontaneously developing tumors was similar to that of p53 KO mice; however, a more in-depth analysis indicated that mutant p53 knockin mice also showed a high incidence of metastases that were not found in their p53 KO counterpart mice (81,82). This points to yet another defined tumor-promoting activity that underlies invasion and metastasis that are solely attributed to the oncogenic GOF activity mediated by mutant p53. Changes in the expression of cell adhesion molecules such as E-cadherin and N-cadherin are central to EMT, a process that allows cell detachment and migration (1). Recently, we found that mutant p53 enhances EMT in prostate tumor cells by elevating the expression of Twist1, a key regulator of EMT (103). This notion is further supported by a recent study suggesting that mutant p53 enhances EMT by modulating the miR-130b–Zeb1 (zinc finger E-box binding homeobox 1) axis in endometrial cancer (104). Additional processes that are enhanced by mutant p53 include cell motility (105) and cell migration that is mediated by overactivation of epidermal growth factor receptor/integrin signaling pathway (106) and by augmented chemokine expression (107). These findings agree with our previous studies showing that mutant p53 cooperates with oncogenic Ras to highly induce cancer-related genes, including chemokines, cytokines and extracellular matrix modulators (88,89).

Mutant p53 regulates nutrient supply by modulating angiogenesis and glycolysis. In order to support the continually accelerated growth, tumors acquire abilities to supply nutrients and oxygen to cells. This is manifested by enhancement of blood supply in the tumors by the generation of blood vessels through angiogenesis. This would occur when endothelial cells are reprogrammed to construct new blood vessels within the tumor mass (1). By binding to E2F1, mutant p53 was found to induce the expression of ID4, which promotes angiogenesis by stabilizing the proangiogenic factors IL8 and GROα (108). Additionally, mutant p53 expression was demonstrated to correlate with the expression of the key angiogenesis factor, vascular endothelial growth factor (109–111). Another mechanism that tumor cells adopt to control nutrient supply is to turn on aerobic glycolysis, whereby cells undergo glycolysis even under normal oxygen conditions, coined in the past as the 'Warburg effect'. This mechanism, although restricting adenosine triphosphate molecules, allows cells to gain metabolites that are incorporated into biosynthetic pathways, including generation of nucleotides and amino acids that are essential for growth (1,112). In accordance with the mutant p53 oncogenic GOF notion, presence of mutant p53 in cells was also associated with the 'Warburg effect'. In this case, mutant p53 was suggested to be involved in the translocation of the glucose transporter, GLUT1, to the plasma membrane, which is essential for high glucose uptake, by enhancing Rho/Rock signaling pathway (113).

Mutant p53 promotes inflammation. The notion that inflammation plays a critical role in promoting cancer is already well established (114) and thus was recently included as a bona fide oncogenic characteristic of cancer (1). The general notion is that inflammation serves as an important factor in tumor microenvironment that provides the developing tumor with growth and survival factors that limit cell death. Furthermore, inflammation involves proangiogenic factors, extracellular matrix-modifying enzymes that facilitate invasion and metastasis, and reactive oxygen species (ROS). Several studies have indicated that mutant p53 GOF supports processes associated with inflammation. We have previously found that mutant p53 enhances the response of cancer cells to the proinflammatory cytokine tumor

necrosis factor α (TNFα) (115). This was further reinforced by a study using a mouse model for chronic inflammation of the colon, in which mutant p53 was found to promote chronic inflammation associated with colorectal cancer (116). Although the mechanisms underlying the oncogenicity-enhancing inflammatory response are not entirely elucidated, several studies suggest possible molecular links. For example, we showed that mutant p53 enhances the expression of proinflammatory genes by activating Ras oncogene, ERK-MAPK, phosphoinositide 3-kinase and NF-κB signaling (88,89). This is supported by the observations that the proinflammatory genes signature that includes chemokines, cytokines and extracellular matrix modulators was also found to be synergistically elevated by mutant p53 and Ras oncogene in adult murine colon cells (117). These factors were shown to be highly elevated by mutant p53 in tumor-derived cell lines, mediating tumor cell migration (107). Mutant p53 was found to enhance the activity of the NF-κB effector, p65 (also termed RelA), as observed by higher p65 nuclear localization (115), and mutant p53 was suggested to promote the transcriptional activation of NF-κB by facilitating its binding to chromatin (116). Additionally, mutant p53 was found to induce the expression of another NF-κB family member, NF-κB2, which leads to chemoresistance (118). We have recently found an interesting cross talk between interferon β (IFNβ) and mutant p53. When cancer-associated fibroblasts and mutant p53-expressing tumor cells are cocultured, cancer-associated fibroblasts significantly promote the IFNβ pathway, which attenuates the migration of tumor cells and reduces mutant p53 mRNA levels. In turn, mutant p53 moderates the response to IFNβ in cancer cells by inhibiting the IFNβ downstream effector, signal transducers and activators of transcription 1 (STAT1), in a negative feedback loop (119).

Mutant p53 attenuates cell death and mediates drug resistance. The observation that mutant p53 confers cells with drug resistance and thus avoids apoptosis can be seen as the first milestone in the suggested mutant p53 GOF notion. Indeed, early studies have shown that M1/2 cells, expressing temperature-sensitive mutant p53, under conditions allowing mutant p53 conformation, avoid apoptosis induced by serum starvation (120). Attenuation of apoptosis in these cells was also observed following γ-irradiation and chemotherapy treatment (e.g. doxorubicin and cisplatin) (121), in addition to being seen in other tumor-derived cellular systems (122). Apparently, the attenuation of cell death conferred by mutant p53 is not restricted to chemotherapy. Indeed, over the years, independent studies have indicated that mutant p53 protects cells from additional death inducers such as 12-*O*-tetradecanoylphorbol 13-acetate (123), TNFα (115) and vitamin D (124). Figure 1 presents an example for mutant p53-dependent attenuation of TNFα-induced cell death. Although H1299 cells that were treated with high doses of TNFα underwent cell death, mutant p53-overexpressing cells were barely affected (Figure 1, unpublished data). Finally, it was recently suggested that mutant $p53^{P151S}$ displays Anoikis resistance (125), which was found to be essential for survival of metastatic cells (126). Several mechanisms can be attributed to the resistance to death effected by mutant p53, as illustrated in Figure 2. For example, it was suggested that the integrity of N′ terminus of p53, containing the transactivation domain, is essential for mutant p53-dependent chemoresistance, suggesting that mutant p53 attenuates drug-dependent death by transactivation activity (127). Indeed, mutant p53 was found to transcriptionally induce the expression of the multidrug resistance gene MDR1 by stimulating its promoter. As an adenosine triphosphate-dependent efflux pump, MDR1 transports foreign substances out of cells and clears drug accumulation in cells. Thus, by elevating its expression, mutant p53 confers tumor cells with drug resistance (128). In addition, mutant p53 was found to modulate the expression of genes involved in cell death regulation, such as the elevation in the antiapoptotic gene Bcl-xL (129), augmentation in EGR1 expression (130) and the downregulation of the proapoptotic gene Fas (131). More information regarding the modulation of expression of other death-related genes is reviewed in references 80,86. A recent study has suggested that tumors with high levels of p53, as observed in mutant p53-expressing cells, may evade apoptosis through the inhibition of caspase 9 activity (132). A well-studied mechanism for mutant p53 GOF is its interaction with other p53 family members, e.g. p63 and p73 (133). These interactions may explain mutant p53-mediated chemoresistance in colon adenocarcinoma-derived cell line, SW480 (134). Finally, regulation of miRs by mutant p53 is an additional mechanism explaining mutant p53-dependent drug resistance and death protection. Indeed, mutant p53 was found to induce the expression of miR-128 in lung cancer (135) and to downregulate the expression of miR-223 in breast and colon cancers (136), conferring resistance to chemotherapy.

To conclude, the various oncogenic GOF activities mediated by the mutant p53 protein greatly support tumor cell survival and can be attributed to the common cancer recurrence frequently seen following the standard therapy accepted today. In the past decades, relevant research was mostly focused on understanding the role of mutant p53 GOF in somatic cells because these cells were seen as the main target for cancer therapy. However, ample data accumulated recently indicate that CSCs residing within tumors are of great significance in conferring tumor aggressiveness. Accordingly, the role of mutant p53 in conferring CSCs drug resistance becomes a central issue in tumor recurrence at large.

Cancer stem cells

It appears that as in normal tissues, tumors show population hierarchy, whereby a subpopulation of CSCs has the most pronounced tumorigenic potential compared with the general cancer cell population (137). CSCs are characterized as rare, chemotherapy-resistant, malignant cells within the tumor, which are able to self-renew and differentiate and thus can recreate a tumor with the entire original complex cell pool when injected into immunosuppressed mice (138). Human and mouse CSCs were first isolated from hematological malignancies (139,140) and later have been identified in a wide range of solid human cancers, such as cancers of the breast (141), brain (142), pancreas (143), colon (144,145), ovaries (146–148) and other organs. To date, the accepted method for CSC isolation from tumors takes advantage of cell sorting by specific surface markers. For example, CD44 and CD133 are common for a variety of tumors; however, tissue-specific markers have also been reported (149). Other strategies for CSC isolation and enrichment include spheroid formation assay, which is based on accentuated self-renewal capacity of the CSCs (150), side-population assay that relies on the capacity of CSCs to cause the efflux of certain chemicals (151) and an assay based on selection of cells displaying high aldehyde dehydrogenase (ALDH) activity (48). Although the notion that CSCs are central for tumor development is quite accepted, exceptions exist. For example, melanoma was proposed to obey the CSC model (152); however, an in-depth analysis of a permissive *in vivo* model indicated that most of the cancerous cells are capable of initiating new tumors, thus arguing against the CSC model in this tumor type (153).

Evolvement of CSCs. One of the important unsolved issues pertains to the understanding of the evolution of CSCs. The cells of origin for CSCs are assumed to be normal SCs that underwent oncogenic genetic modifications—inherent as germ-line mutations or induced by environmental agents—that lead to cancer initiation (11,154). Another approach would suggest that unlike the rigid hierarchy between normal SCs and differentiated cells, tumors could acquire plasticity, which allows progenitor or somatic cells to undergo dedifferentiation and gain SC characteristics to become CSCs. Once these cells acquire the adaptive ability to become CSCs, tumors can exploit this capacity in order to gain drug resistance and escape cancer therapy (46,149). The first theory is supported by the observation that SCs reside in the human body for a prolonged period of time—some dormant and others constantly dividing—and this raises the probability of undergoing malignant cell transformation. In addition, SCs possess intrinsic properties of self-renewal and ability to migrate inside or outside of their organ; such dynamic cellular processes may facilitate malignant transformation. The second theory is substantiated by the fact that the incidence of SCs is rather rare, with orders of magnitude less in comparison with the incidence of differentiated cells. Thus, the

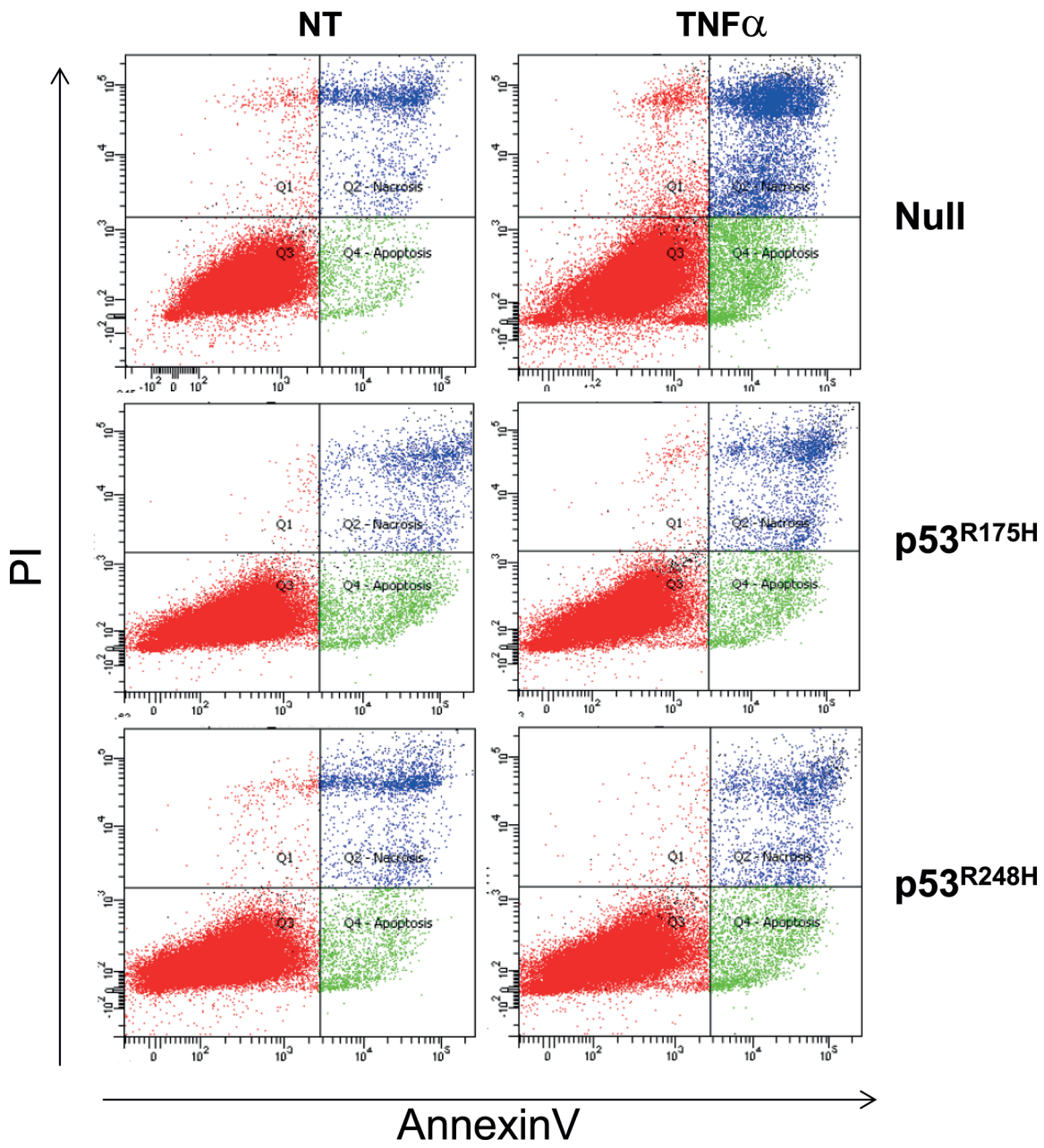

Fig. 1. Mutant p53 attenuates TNFα-induced cell death. H1299, non-small cell lung carcinoma, overexpressing mutant $p53^{R175H}$ and mutant $p53^{R248Q}$ were treated with 50 ng/ml TNFα for 72 h, followed by annexin V and propidium iodide staining. Analysis of cell death was performed by flow cytometry (fluorescence-activated cell sorting). Green dots represent the early apoptotic cells (Q4), and blue dots represent late apoptotic and necrotic cells (Q2). Cell death is presented as the sum of cells in Q2 and Q4.

occurrence of mutations in the resident SCs is at a very low frequency to affect the entire population. Furthermore, SCs were shown to have high genomic fidelity, lowering the chances of spontaneous transformation (155). Following the discovery of reprogramming, it became clear that dedifferentiation of transformed/normal somatic cells can explain the generation of CSCs. For example, ablation of respiratory SCs led luminal secretory cells to dedifferentiate into basal SCs (156), a normally occurring physiological process that, when uncontrolled, can contribute to a malignant phenotype. The state of the cell can be altered, similarly to cellular processes such as EMT and mesenchymal-to-epithelial transition, which under physiological conditions are involved in development and tissue repair (157). When these processes are out of control, they allow invasion and metastasis of cancer cells and generation of CSCs (158). In all, it seems that both SC transformation and dedifferentiation mostly depend on tumor type and context (46).

Functional perturbation in p53 leads to CSC generation. The emerging notion that mutations in p53 play a major role in CSC formation is greatly supported by the correlation between tumors with mutations in p53 and their undifferentiated phenotype. In thyroid gland carcinoma, for instance, p53 mutations were restricted to poorly differentiated tumors (159,160). Furthermore, one particular tumor showed different degrees of differentiation within regions, whereas overexpression of p53 was constrained to a less-differentiated area of the tumor (159). In addition, these studies suggested a link between mutations in p53, CSC formation and poor prognosis (159–162). Interestingly, it was shown that ESCs and undifferentiated tumors, such as breast, brain and bladder malignancies, express common specific gene signatures

and that similar transcription factors are shared among them (54). Moreover, mutations in p53 were shown to allow stem-like phenotype in breast and lung cancers (163). As mentioned above, several laboratories concomitantly suggested that WT p53 serves as a barrier in the reprogramming process by negatively regulating the rate of reprogramming (50,164). Such a regulatory activity agrees with the notion that self-renewal of SCs should be tightly controlled to attenuate the burst of accentuated excessive SC proliferation.

Accordingly, when mutant p53 knocked-in mouse embryonic fibroblasts were induced to reprogram, we found a GOF in the facilitated rate of reprogramming compared with that seen in the KO p53 mouse embryonic fibroblasts. Mutant p53 iPSCs exhibited the typical ESC markers, such as Nanog and Oct4, and underwent cell differentiation under *in vitro* conditions. Nevertheless, unlike WT p53 iPSCs that induced benign teratomas, the mutant p53 iPSCs induced the development of aggressive tumors *in vivo*. Global gene expression analysis indicated that mutant p53 iPSCs share expression patterns with pre-iPSCs (165). Interestingly, we found that p53-compromised iPSCs express gene members that are regarded as CSC markers and confer cells with drug resistance (unpublished data). This suggests that mutant p53 not only facilitates the reprogramming process but also affects the nature of the generated iPSCs. In all, p53 has control of the quality and quantity during the course of iPSC formation (50).

Interestingly, we have recently shown that heterozygous p53 cells have similar reprogramming efficiencies as WT p53, implying that WT p53 dominates over the mutant in the reprogramming process (75).

Another example that further connects the expression of mutant p53 and the malignant phenotype of CSCs is derived from glioma tumors. It was found that a p53 deletion is insufficient to make CSCs acquire their malignant phenotype. Rather, expression of mutant p53 (frequency of 26% in these tumor types) (76) is critical for the manifestation of the full malignant potential of these CSCs. In addition, this study provided an elegant proof that gliomas originate from neuronal stem cells in the subventricular zone (166). Recently, it was shown that not all neuronal stem cells are capable of initiating a neoplasm but that, specifically, the oligodendrocyte precursor cells alone could do so (167). In addition, MSCs were suggested to be the cell of origin in soft tissue sarcomas. Several mouse models have shown that MSCs lacking p53 formed malignant sarcomas (49,168–170). Furthermore, we have recently demonstrated that MSCs derived from heterozygous-mutant $p53^{R172H}$ adolescent mice that underwent p53 loss of heterozygosity do not form tumors *in vivo*, in comparison with cells derived from older mice, which induced malignant sarcoma when injected to immunodeficient mice (75). This implies that p53 loss of heterozygosity is an initiating event in the process of transforming MSCs, allowing other age-dependent perturbations to occur. Strikingly, we found that between 4 and 10% of the adherent bone marrow (BM) progenitors underwent p53 loss of heterozygosity *in vivo* in adult mice (75), suggesting that BM-derived MSCs are the origin of sarcomas. Nevertheless, Choi *et al.* (171) demonstrated that local MSCs, which reside within the tissue and not at the BM, are the cells that yield soft tissue sarcomas in an inactivated p53/Rb mouse model; yet, the lack of strictly defined markers of MSCs makes it difficult to rule out BM–tissue migration and the source of cells remains an open question (172).

Some pathways have been suggested to explain how p53, or its absence, exerts its effects on CSCs. It was shown that p53 represses the expression of CD44, which is commonly reported as a CSC marker and is involved in the metastasizing ability of CSCs (173,174). CD44 repression by p53 hampered the tumorigenic potential of CSCs in breast, lung and prostate tumors (175,176). Moreover, p53 was recently shown to repress the expression of c-KIT, another common CSC marker (146,177,178), through the p53 target miR-34a family. This downregulation resulted in reduction of sphere formation ability, chemoresistance and stemness phenotype in colorectal cancer (179). p53 was also shown to repress the expression of other SC genes Nanog and Oct4 (180). These two genes were shown to be crucial for the CSCs population in various tumors (181–183). Moreover, the repression of Nanog by p53 activation inhibits gliomagenesis *in vivo* (184,185). The EMT was recently suggested to be linked with the gain of SC properties by epithelial cells (158). p53 is known to negatively regulate the EMT process through transcriptional activation of miRs. For example, the miR-34 family targets the EMT activator Snail (186). Moreover, another p53 target, the miR-200 family, was shown to negatively regulate the expression of Zeb1 and Zeb2, EMT transcriptional activators (187,188). The attenuation of the EMT process by p53 may fulfill its role in restricting the SC pool. Finally, we have shown that WT p53 exerts a negative effect on reprogramming, which is mediated by the suppression of Klf4 that in turn suppresses mesenchymal-to-epithelial transition (189). Taken together, p53 is interwoven in the cellular circuits governing CSCs (Figure 3).

Several characteristics have been offered to describe CSCs. We have discussed the capability of CSCs to initiate new tumors; this is a crucial criterion for defining a cell as a CSC. Other characters are

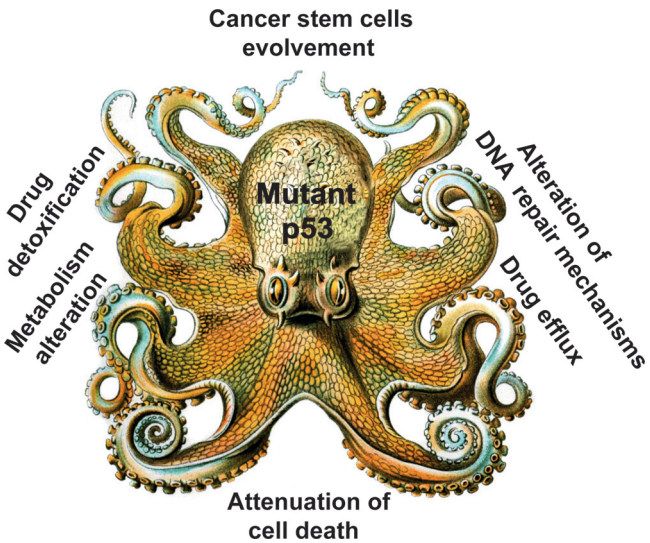

Fig. 2. Mutant p53 oncogenic activities pertaining to cell drug resistance. Mutant p53, as a 'multiarm' protein, confers cancer cells with drug resistance in several ways: enabling the evolvement of CSCs from differentiated cells and SCs and maintaining the CSC pool; elevating certain DNA repair mechanisms allowing the cells to survive; elevating expression of ABC transporters allowing efflux of drugs out of the cells; attenuating cell death by elevating the expression of antiapoptotic proteins and reducing expression of proapoptotic proteins; modulating expression of metabolic scavengers; and elevating expression of detoxifying enzymes.

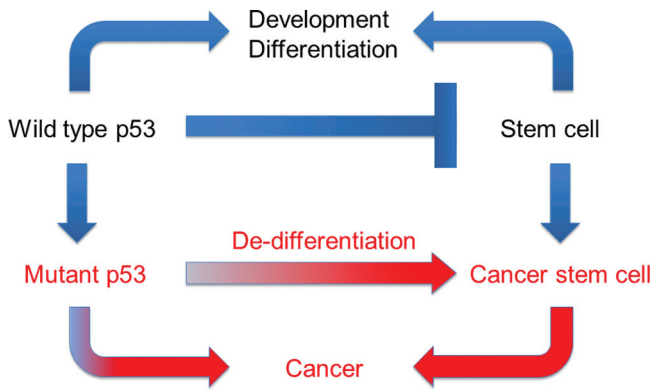

Fig. 3. The proposed p53–SCs circuit. WT p53 plays a regulatory role in the controlled development and differentiation of SCs. When p53 is mutated, it gains various oncogenic functions supporting tumorigenesis, including dedifferentiation of somatic cells into CSCs and transformation of SCs into CSCs.

heterogeneity, due to acquired changes in each cycle of the parental cell; asymmetric division, maintaining the CSC pool while contributing to the tumor bulk; quiescence, despite being transformed, it is believed that, to some extent, they remain in a slow cycling state and thus are resistant to agents that affect proliferating cells (190). However, the most potent hallmark of CSCs on cancer progression and relapse is drug resistance.

Drug resistance mechanisms of CSCs

It is well accepted that chemoresistance of cancer cells can be divided into two major categories: *de novo* and acquired chemoresistance (191). *De novo* chemoresistance is defined as the preexisting ability of cancer cells to resist chemotherapy, whereas acquired chemoresistance is defined as acquisition of drug resistance, which arises during chemotherapy treatment. The latter develops due to drug-induced selection pressure leading to clonal expansion based on survival advantage. There are several mechanisms that allow cancer cells to elude chemotherapy. This includes limiting drug influx, excessive drug efflux, alterations in apoptosis and survival signaling pathways, expression of detoxification enzymes and alterations in DNA repair mechanisms.

Drug efflux. The adenosine triphosphate (ATP)-binding cassette (ABC) transporter family is the most notable group executing the function of expelling anticancer drugs across the plasma membrane. There are three central members that have been extensively studied in relation to multidrug resistancein cancer: ABCB1 (multidrug resistance, MDR1), ABCC1 (MRP1) and ABCG2 (BCRP), which were shown to act on a broad range of conventional chemotherapy drugs (192,193) and to account for chemotherapy failure in various cancers (193,194). This phenotype of drug resistance is attributed to the CSC population that is contained within growing tumors (195). The ability of CSCs to expel drugs enabled their isolation in a method termed side population sorting method (151), which relies on the observation that somatic cancer cells, when stained, retain the dyes; however, CSCs expel the dyes, which is mediated by the ABC transporter proteins. In many primary tumors and cell lines, including breast cancer, lung cancer and brain tumors, side populations were detected (151). The expression of ABC transporters in CSCs is another trait shared with normal SCs. For example, hematopoietic stem cells express high levels of ABCG2 and/or ABCB1, in contrast to further differentiated cells of the hematopoietic system (195).

Drug detoxification. ALDH enzymes are also thought to be involved in chemoresistance of cancer cells. These proteins are members of the nicotinamide adenine dinucleotide (phosphate) -dependent enzymes that have a role in detoxifying a broad variety of endogenous aldehydes and xenobiotic aldehydes by oxidizing and converting them into carboxylic acids (196). Indeed, studies have shown that cells highly expressing ALDH genes, especially ALDH1A1 and ALDH3A1, exert drug resistance (197–199), whereas inhibition of activity of the ALDH enzymes leads to effective chemotherapy (200,201). However, the exact mode of action underlying this pathway is yet to be elucidated. High ALDH1 activity was found in several types of cancers, including head-and-neck, lung, liver, pancreas, cervix, ovaries, breast, prostate, colon and bladder cancers (202). Because ALDH is also expressed at variable levels in normal ASCs, it has been suggested to be a reliable marker for CSCs only in tissues that harbor ASCs expressing limited ALDH levels, such as breast, lung and colon tissues, and not in liver and pancreatic tissues wherein the residing ASCs express high ALDH levels (203).

Alterations in metabolism. In addition, cancer cells were shown to have the ability to inactivate different drugs, e.g. platinum drugs such as cisplatin and oxaliplatin, by covalently conjugating them with the thiol glutathione (GSH). The generated complex is a substrate for ABC transporter protein, resulting in inactivation of the drug (204). Accordingly, GSH was shown to be highly expressed in various cancers, providing them with chemoresistance ability (204–208). GSH was shown to be a critical cellular reducing agent and antioxidant that is responsible for reducing ROS levels. ROS are found at high levels in many cancer cells, contributing to the vicious cycle of aggravating damage to the DNA and other parts of the cell (209). In normal SCs, such as hematopoietic stem cells and mammary SCs, ROS are found at low levels, mainly due to elimination by scavengers such as GSH (210,211). Interestingly, it was shown that CSCs share a ROS-level phenotype similar to that in their normal SC counterparts. Several studies have shown that the CSC population contains low levels of ROS and higher capacity to synthesize ROS-scavenging molecules compared with somatic cancer cells. These low levels of ROS were shown to confer CSCs with resistance to radiotherapy (211).

DNA repair mechanisms. The ability of cancer cells to repair DNA damage significantly affects their response to chemotherapy. Several studies support the notion that alterations in DNA repair mechanisms confer chemoresistance to cancer cells. For example, excision repair cross-complementing 1, a crucial component of the nucleotide-excision repair pathway, was shown to be elevated in various tumors, thereby increasing chemoresistance of several cancer types, including non-small cell lung carcinoma and ovarian, colorectal and gastric cancers (212–216). However, mismatch repair deficiency has also been implicated in chemoresistance in a variety of cancers (217–219). This is due to the involvement of mismatch repair proteins in mediating cell cycle arrest and apoptosis in response to DNA damage (220,221). Interestingly, in CSCs contained within a given tumor, the DNA damage response is tilted toward enhanced DNA repair, as opposed to the situation in somatic cancer cells. For instance, glioma SCs expressing CD133 were shown to be resistant to γ-irradiation by elevation of the checkpoint activation in response to DNA damage. The phosphorylation of ataxia telangiectasia mutated, Rad17, Chk1 and Chk2 was higher in $CD133^+$ cells compared with the same in $CD133^-$ cells. Moreover, alkaline comet assay showed greater DNA repair efficiency in $CD133^+$ cells compared with that in $CD133^-$ cells (222). Elevated phosphorylation of Chk1 was also observed in colon and lung CSCs in response to chemotherapy (223,224). In addition, MCF-7-derived CSCs showed higher activation of the DNA single-strand break repair mechanism compared with the mechanism in the parental cells. This higher single-strand break repair activation was indicated by higher expression of single-strand break repair-associated protein APE1 (225).

Alterations in apoptosis and survival signaling pathways. Cancer therapy is aimed at the eradication of cancer cells, thereby challenging a central cancer hallmark, resistance to cell death (1). Indeed, cancer cells have developed multiple mechanisms to prevent cell death, e.g. regulation of the expression of Bcl-2 family members either by inducing antiapoptotic regulators including Bcl-2 and Bcl-x_L or by downregulating proapoptotic regulators such as Bax, Bim and Puma. Another important player in acquiring drug resistance is NF-κB, which promotes cell survival and exerts resistance to chemotherapy (194,226). In all, the alterations in cell death and survival signaling pathways mentioned above may hinder chemotherapy. Similarly, in glioma SCs, antiapoptotic Bcl-2 and Bcl-x_L, in addition to the inhibitor of apoptosis (IAP) family members X-linked inhibitor of apoptosis, cIAP1, cIAP2, neuronal apoptosis inhibitor protein, and survivin, were found at significantly higher levels in CD133-positive drug-resistant cells in contrast to their counterparts (227). In both colon (228) and hepatic CSCs (229), Bcl-2 contributes to chemoresistance, in addition to preferential activation of Akt/protein kinase B, in the CSCs of the liver (229), which is absent the activation of Akt/protein kinase B is absent in somatic cancer cells.

In all, CSCs adopt a variety of pathways to escape therapy. Indeed, much of the research made before the CSC hypothesis can now be explained in retrospect and can be shown to be attributable to the small CSC population.

Cross talk of mutant p53 and CSCs underlying drug resistance

Mutant p53 GOF. The fact that mutant p53 is frequently expressed in a variety of human tumors makes it an important target for cancer therapy (230). Mutant p53, the well-characterized genomic guardian, is known for its oncogenic GOF. Depending on the tumor-type specificity, p53 can be associated with various steps along the process

of malignant transformation. Mutant p53 was shown to modify the cell cycle checkpoints, accelerating proliferation, conferring genomic instability, affecting cell plasticity and inducing invasiveness and metastasis, in addition to being known for its non-cell autonomous affects, such as inflammation and angiogenesis (78,231). Mutant p53 acts as a multitask protein that simultaneously affects a number of pivotal pathways, all of which culminate in the acquisition of resistance to chemotherapeutic drugs (Figure 2). This feature of mutant p53 has long been known in the field of oncology, yet no approved therapy targeting mutant p53 in the Western world is available to date (232).

The p53 and SC connection. The initial observations that WT p53 plays a role in cell differentiation and development paved the way toward the understanding that WT p53, a cell cycle controller, plays an important role in restraining the normal repertoire of SCs. In recent experiments, taking advantage of cell reprogramming, it was shown that p53 acts as a barrier to the reprogramming of differentiated cells into the pluripotent state (50,59,61–63,233). This is in agreement with the notion that the reprogramming process shares some resemblance to malignant transformation. Furthermore, data derived from a variety of experimental models suggested that expression of mutant p53 in SCs might lead the way toward the evolvement of CSCs (166,169).

Mutant p53-CSCs share gene pathways. Of note, mutant p53 seems to affect specific pathways, which are central to the drug-resistant capacity of CSCs (Figure 2). For example, ABC transporters that are an important machinery in acquiring drug resistance by exporting drugs out of cells are often expressed in CSCs. Interestingly, MDR1, an important member of the ABC family, was shown to be upregulated by mutant p53 (128). Moreover, mutant p53 was shown not only to lose the capacity of WT p53 to induce apoptosis but also to gain function in augmenting the expression of antiapoptosis proteins (Bcl-2 and Bcl-x_L) and in reducing proapoptosis signals (Bax, Bad and Bid). Similarly to mutant p53, CSCs modify the Bcl-2 family to attenuate drug-induced death. WT p53 plays a major role in DNA repair mechanisms, such as nucleotide-excision repair, base-excision repair, mismatch repair, homologous recombination and non-homologous end joining (234). These repair mechanisms are impaired in somatic cancer cells; however, recently, we have found that murine p53-mutant-expressing MSCs that form malignant sarcomas exhibited elevated homologous recombination and non-homologous end-joining genes (75). As mentioned above, CSCs were shown in various models to express high levels of DNA-repair-related genes and to efficiently repair DNA damage, compared with somatic cancer cells.

Despite these similarities, not many studies of the role of p53 in drug resistance of CSCs were performed. It was shown that in colorectal cancer cells, attenuation of the SC marker c-Kit by the p53 target miR-34a sensitizes the cells to 5-fluorouracil (179). In addition, the anticancer phytochemical resveratrol reduces the tumor-initiating capacity of

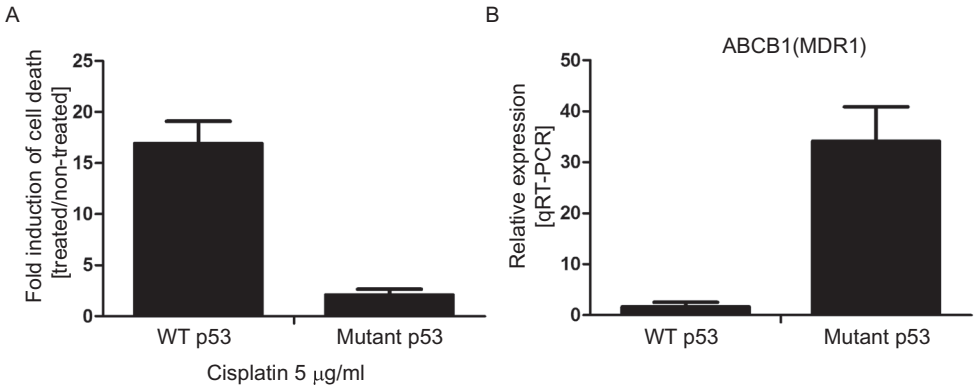

Fig. 4. Mutant p53-expressing MSCs exhibit resistance to cisplatin and express high levels of MDR1. MSCs were extracted from the BM of WT p53- and mutant p53-containing mice. (**A**) Cells were treated with cisplatin (5 μg/μl) for 24 h, followed by propidium iodide (PI) staining. Cell death was assessed according to PI exclusion by flow cytometry (fluorescence-activated cell sorting). (**B**) Relative mRNA expression of ABCB1A (MDR1) in WT p53- and mutant p53-containing MSCs, as measured by quantitative reverse transcriptase–polymerase chain reaction.

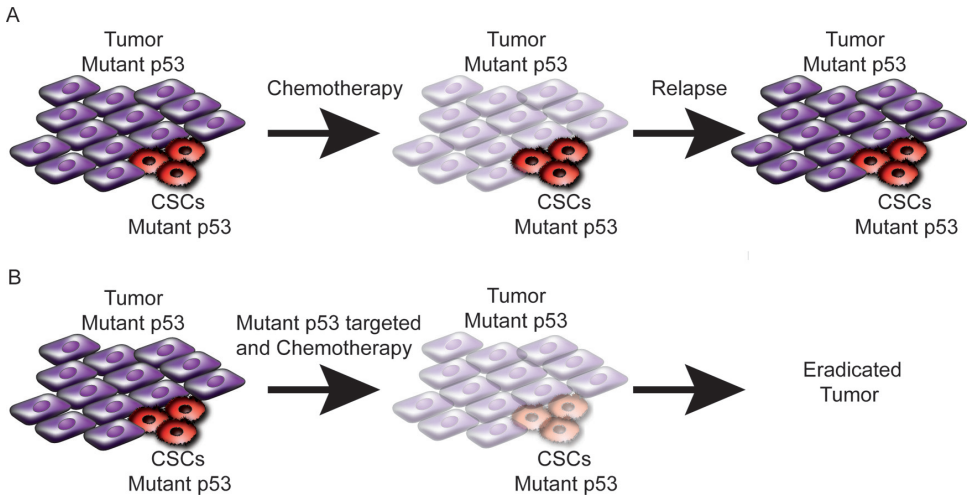

Fig. 5. Suggested model for combining mutant p53-targeted cancer therapy and conventional chemotherapy. (**A**) Tumor expressing mutant p53 when treated with chemotherapy will show regression due to elimination of the bulk tumor cells. However, the CSC compartment is resistant to chemotherapy-induced death, thus allowing tumor relapse. (**B**) Treatment with mutant p53-targeted therapy will convert the mutated p53 into intact p53 and sensitize CSCs to chemotherapy. Hence, both the bulk tumor cells and the CSCs will be eliminated and full eradication is expected.

glioma SCs by promoting the degradation of Nanog in a p53-dependent manner (184). A similar phenotype involving stemness-attenuating features was observed in the CSCs of nasopharyngeal carcinoma (235). Recently, it was shown that only in the absence of p53, colon CSCs are resistant to paclitaxel due to higher levels of autophagy and lower levels of apoptosis (236). All of these studies emphasize that much of the CSC resistance to chemotherapy is evident in conjunction with a cellular compromised-p53 status. In our recent studies, we found that iPSCs that express the mutant p53 and induce aggressive tumors in mice were found to highly express detoxifying enzymes associated with drug resistance. Furthermore, MSCs expressing mutant p53, which form aggressive tumors, exhibited drug resistance to cisplatin that correlated with the expression of MDR1, a central gene in acquiring drug resistance (Figure 4, unpublished data). This indicates that mutant p53 expression is important in inducing iPSCs and MSCs to acquire a transformed phenotype and drug resistance.

Therapeutic approach. In all, the notion that p53 plays a regulatory role in the life of SCs, coupled with the observations that p53 mutations may contribute to the evolvement of CSCs, makes it challenging to speculate that drug resistance and cancer recurrence are mediated by CSCs that express mutant p53. Accordingly, it may suggest that efficient cancer therapy in mutant p53-expressing tumors should be based on a combination of chemotherapy and a p53-based therapy. The chemotherapy will target the tumor bulk, whereas only the conversion of mutant p53 protein into WT p53 form will allow CSC eradication (Figure 5). We speculate that reverting mutant p53 in CSCs will render them sensitive to chemotherapy.

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References

1. Hanahan,D. *et al.* (2011) Hallmarks of cancer: the next generation. *Cell*, **144**, 646–674.
2. Weinberg,R. (2013) *The Biology of Cancer*. Garland Science, New York. NY.
3. Lane,D.P. (1992) Cancer. p53, guardian of the genome. *Nature*, **358**, 15–16.
4. Levine,A.J. *et al.* (2009) The first 30 years of p53: growing ever more complex. *Nat. Rev. Cancer*, **9**, 749–758.
5. Vousden,K.H. *et al.* (2002) Live or let die: the cell's response to p53. *Nat. Rev. Cancer*, **2**, 594–604.
6. Donehower,L.A. *et al.* (1992) Mice deficient for p53 are developmentally normal but susceptible to spontaneous tumours. *Nature*, **356**, 215–221.
7. Jacks,T. *et al.* (1994) Tumor spectrum analysis in p53-mutant mice. *Curr. Biol.*, **4**, 1–7.
8. Purdie,C.A. *et al.* (1994) Tumour incidence, spectrum and ploidy in mice with a large deletion in the p53 gene. *Oncogene*, **9**, 603–609.
9. Danilova,N. *et al.* (2008) p53 family in development. *Mech. Dev.*, **125**, 919–931.
10. Molchadsky,A. *et al.* (2010) p53 is balancing development, differentiation and de-differentiation to assure cancer prevention. *Carcinogenesis*, **31**, 1501–1508.
11. Rivlin,N. *et al.* (2014) p53 orchestrates between normal differentiation and cancer. *Semin Cancer Biol*.
12. Armstrong,J.F. *et al.* (1995) High-frequency developmental abnormalities in p53-deficient mice. *Curr. Biol.*, **5**, 931–936.
13. Sah,V.P. *et al.* (1995) A subset of p53-deficient embryos exhibit exencephaly. *Nat. Genet.*, **10**, 175–180.
14. Rinon,A. *et al.* (2011) p53 coordinates cranial neural crest cell growth and epithelial-mesenchymal transition/delamination processes. *Development*, **138**, 1827–1838.
15. Rotter,V. *et al.* (1993) Mice with reduced levels of p53 protein exhibit the testicular giant-cell degenerative syndrome. *Proc. Natl Acad. Sci. USA*, **90**, 9075–9079.
16. Beumer,T.L. *et al.* (1998) The role of the tumor suppressor p53 in spermatogenesis. *Cell Death Differ.*, **5**, 669–677.
17. Bornstein,C. *et al.* (2011) SPATA18, a spermatogenesis-associated gene, is a novel transcriptional target of p53 and p63. *Mol. Cell. Biol.*, **31**, 1679–1689.
18. Hu,W. *et al.* (2007) p53 regulates maternal reproduction through LIF. *Nature*, **450**, 721–724.
19. Levine,A.J. *et al.* (2011) The p53 family: guardians of maternal reproduction. *Nat. Rev. Mol. Cell Biol.*, **12**, 259–265.
20. Molchadsky,A. *et al.* (2013) p53 is required for brown adipogenic differentiation and has a protective role against diet-induced obesity. *Cell Death Differ.*, **20**, 774–783.
21. Choi,J. *et al.* (1999) p53 in embryonic development: maintaining a fine balance. *Cell. Mol. Life Sci.*, **55**, 38–47.
22. el-Deiry,W.S. (1998) Regulation of p53 downstream genes. *Semin. Cancer Biol.*, **8**, 345–357.
23. Schmid,P. *et al.* (1991) Expression of p53 during mouse embryogenesis. *Development*, **113**, 857–865.
24. Almog,N. *et al.* (1997) Involvement of p53 in cell differentiation and development. *Biochim. Biophys. Acta*, **1333**, F1–F27.
25. Zambetti,G.P. *et al.* (2006) Skeletons in the p53 tumor suppressor closet: genetic evidence that p53 blocks bone differentiation and development. *J. Cell Biol.*, **172**, 795–797.
26. Shaulsky,G. *et al.* (1991) Alterations in tumor development *in vivo* mediated by expression of wild type or mutant p53 proteins. *Cancer Res.*, **51**, 5232–5237.
27. Aloni-Grinstein,R. *et al.* (1993) Wild type p53 functions as a control protein in the differentiation pathway of the B-cell lineage. *Oncogene*, **8**, 3297–3305.
28. Montano,X. (1997) P53 associates with trk tyrosine kinase. *Oncogene*, **15**, 245–256.
29. Zhang,J. *et al.* (2006) p53 is required for nerve growth factor-mediated differentiation of PC12 cells via regulation of TrkA levels. *Cell Death Differ.*, **13**, 2118–2128.
30. Hughes,A.L. *et al.* (2000) Mediation of nerve growth factor-driven cell cycle arrest in PC12 cells by p53. Simultaneous differentiation and proliferation subsequent to p53 functional inactivation. *J. Biol. Chem.*, **275**, 37829–37837.
31. Brynczka,C. *et al.* (2007) NGF-mediated transcriptional targets of p53 in PC12 neuronal differentiation. *BMC Genomics*, **8**, 139.
32. Tamir,Y. *et al.* (1998) p53 protein is activated during muscle differentiation and participates with MyoD in the transcription of muscle creatine kinase gene. *Oncogene*, **17**, 347–356.
33. Porrello,A. *et al.* (2000) p53 regulates myogenesis by triggering the differentiation activity of pRb. *J. Cell Biol.*, **151**, 1295–1304.
34. Cam,H. *et al.* (2006) p53 family members in myogenic differentiation and rhabdomyosarcoma development. *Cancer Cell*, **10**, 281–293.
35. Wang,X. *et al.* (2006) p53 functions as a negative regulator of osteoblastogenesis, osteoblast-dependent osteoclastogenesis, and bone remodeling. *J. Cell Biol.*, **172**, 115–125.
36. Lengner,C.J. *et al.* (2006) Osteoblast differentiation and skeletal development are regulated by Mdm2-p53 signaling. *J. Cell Biol.*, **172**, 909–921.
37. Molchadsky,A. *et al.* (2008) p53 plays a role in mesenchymal differentiation programs, in a cell fate dependent manner. *PLoS One*, **3**, e3707.
38. Radinsky,R. *et al.* (1994) Terminal differentiation and apoptosis in experimental lung metastases of human osteogenic sarcoma cells by wild type p53. *Oncogene*, **9**, 1877–1883.
39. Armesilla-Diaz,A. *et al.* (2009) p53 regulates the proliferation, differentiation and spontaneous transformation of mesenchymal stem cells. *Exp. Cell Res.*, **315**, 3598–3610.
40. Hallenborg,P. *et al.* (2009) The tumor suppressors pRB and p53 as regulators of adipocyte differentiation and function. *Expert Opin. Ther. Targets*, **13**, 235–246.
41. Ng,H.H. *et al.* (2011) The transcriptional and signalling networks of pluripotency. *Nat. Cell Biol.*, **13**, 490–496.
42. Simons,B.D. *et al.* (2011) Strategies for homeostatic stem cell self-renewal in adult tissues. *Cell*, **145**, 851–862.
43. Moore,K.A. *et al.* (2006) Stem cells and their niches. *Science*, **311**, 1880–1885.

44. Lin,T. *et al.* (2005) p53 induces differentiation of mouse embryonic stem cells by suppressing Nanog expression. *Nat. Cell Biol*., **7**, 165–171.
45. Solozobova,V. *et al.* (2010) Regulation of p53 in embryonic stem cells. *Exp. Cell Res*., **316**, 2434–2446.
46. Aloni-Grinstein,R. *et al.* (2014) p53: the barrier to cancer stem cell formation. *FEBS Lett*.
47. Cicalese,A. *et al.* (2009) The tumor suppressor p53 regulates polarity of self-renewing divisions in mammary stem cells. *Cell*, **138**, 1083–1095.
48. Flesken-Nikitin,A. *et al.* (2013) Ovarian surface epithelium at the junction area contains a cancer-prone stem cell niche. *Nature*, **495**, 241–245.
49. Rubio,R. *et al.* (2010) Deficiency in p53 but not retinoblastoma induces the transformation of mesenchymal stem cells *in vitro* and initiates leiomyosarcoma *in vivo*. *Cancer Res*., **70**, 4185–4194.
50. Sarig,R. *et al.* (2010) Mutant p53 facilitates somatic cell reprogramming and augments the malignant potential of reprogrammed cells. *J. Exp. Med*., **207**, 2127–2140.
51. Takahashi,K. *et al.* (2006) Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. *Cell*, **126**, 663–676.
52. Chambers,S.M. *et al.* (2011) Cell fate plug and play: direct reprogramming and induced pluripotency. *Cell*, **145**, 827–830.
53. Graf,T. *et al.* (2009) Forcing cells to change lineages. *Nature*, **462**, 587–594.
54. Ben-Porath,I. *et al.* (2008) An embryonic stem cell-like gene expression signature in poorly differentiated aggressive human tumors. *Nat. Genet*., **40**, 499–507.
55. Calvanese,V. *et al.* (2008) Cancer genes hypermethylated in human embryonic stem cells. *PLoS One*, **3**, e3294.
56. Semi,K. *et al.* (2013) Cellular reprogramming and cancer development. *Int. J. Cancer*, **132**, 1240–1248.
57. Zhao,Y. *et al.* (2008) Two supporting factors greatly improve the efficiency of human iPSC generation. *Cell Stem Cell*, **3**, 475–479.
58. Banito,A. *et al.* (2009) Senescence impairs successful reprogramming to pluripotent stem cells. *Genes Dev*., **23**, 2134–2139.
59. Hong,H. *et al.* (2009) Suppression of induced pluripotent stem cell generation by the p53-p21 pathway. *Nature*, **460**, 1132–1135.
60. Kawamura,T. *et al.* (2009) Linking the p53 tumour suppressor pathway to somatic cell reprogramming. *Nature*, **460**, 1140–1144.
61. Li,H. *et al.* (2009) The Ink4/Arf locus is a barrier for iPS cell reprogramming. *Nature*, **460**, 1136–1139.
62. Marión,R.M. *et al.* (2009) A p53-mediated DNA damage response limits reprogramming to ensure iPS cell genomic integrity. *Nature*, **460**, 1149–1153.
63. Utikal,J. *et al.* (2009) Immortalization eliminates a roadblock during cellular reprogramming into iPS cells. *Nature*, **460**, 1145–1148.
64. Takenaka,C. *et al.* (2010) Effective generation of iPS cells from CD34(+) cord blood cells by inhibition of p53. *Exp. Hematol*, **38**, 154–162.
65. Johnson,F.B. *et al.* (1999) Molecular biology of aging. *Cell*, **96**, 291–302.
66. Mondal,A.M. *et al.* (2013) p53 isoforms regulate aging- and tumor-associated replicative senescence in T lymphocytes. *J. Clin. Invest*., **123**, 5247–5257.
67. Rodier,F. *et al.* (2007) Two faces of p53: aging and tumor suppression. *Nucleic Acids Res*., **35**, 7475–7484.
68. Lim,D.S. *et al.* (2000) Analysis of ku80-mutant mice and cells with deficient levels of p53. *Mol. Cell. Biol*., **20**, 3772–3780.
69. Chin,L. *et al.* (1999) p53 deficiency rescues the adverse effects of telomere loss and cooperates with telomere dysfunction to accelerate carcinogenesis. *Cell*, **97**, 527–538.
70. Varela,I. *et al.* (2005) Accelerated ageing in mice deficient in Zmpste24 protease is linked to p53 signalling activation. *Nature*, **437**, 564–568.
71. Collado,M. *et al.* (2007) Cellular senescence in cancer and aging. *Cell*, **130**, 223–233.
72. Poyurovsky,M.V. *et al.* (2010) P53 and aging: a fresh look at an old paradigm. *Aging (Albany NY)*, **2**, 380–382.
73. Maier,B. *et al.* (2004) Modulation of mammalian life span by the short isoform of p53. *Genes Dev*., **18**, 306–319.
74. López-Otín,C. *et al.* (2013) The hallmarks of aging. *Cell*, **153**, 1194–1217.
75. Shetzer,Y. *et al.* (2014, in press) The onset of p53 loss of heterozygosity is differentially induced in various stem cell types and may involve the loss of either allele. *Cell Death and Differentiation*.
76. Petitjean,A. *et al.* (2007) Impact of mutant p53 functional properties on TP53 mutation patterns and tumor phenotype: lessons from recent developments in the IARC TP53 database. *Hum. Mutat*., **28**, 622–629.
77. Levine,A.J. *et al.* (1991) The p53 tumour suppressor gene. *Nature*, **351**, 453–456.
78. Brosh,R. *et al.* (2009) When mutants gain new powers: news from the mutant p53 field. *Nat. Rev. Cancer*, **9**, 701–713.
79. Wolf,D. *et al.* (1984) Reconstitution of p53 expression in a nonproducer Ab-MuLV-transformed cell line by transfection of a functional p53 gene. *Cell*, **38**, 119–126.
80. Brosh,R. *et al.* (2009) When mutants gain new powers: news from the mutant p53 field. *Nat. Rev. Cancer*, **9**, 701–713.
81. Olive,K.P. *et al.* (2004) Mutant p53 gain of function in two mouse models of Li-Fraumeni syndrome. *Cell*, **119**, 847–860.
82. Lang,G.A. *et al.* (2004) Gain of function of a p53 hot spot mutation in a mouse model of Li-Fraumeni syndrome. *Cell*, **119**, 861–872.
83. Gonzalez,K.D. *et al.* (2009) Beyond Li Fraumeni Syndrome: clinical characteristics of families with p53 germline mutations. *J. Clin. Oncol*., **27**, 1250–1256.
84. Malkin,D. *et al.* (1990) Germ line p53 mutations in a familial syndrome of breast cancer, sarcomas, and other neoplasms. *Science*, **250**, 1233–1238.
85. Malkin,D. (2011) Li-Fraumeni syndrome. Genes Cancer **2**, 475–484.
86. Freed-Pastor,W.A. *et al.* (2012) Mutant p53: one name, many proteins. *Genes Dev*., **26**, 1268–1286.
87. Bisio,A. *et al.* (2014) TP53 mutants in the tower of babel of cancer progression. *Hum. Mutat*.
88. Buganim,Y. *et al.* (2010) p53 regulates the Ras circuit to inhibit the expression of a cancer-related gene signature by various molecular pathways. *Cancer Res*., **70**, 2274–2284.
89. Solomon,H. *et al.* (2012) Various p53 mutant proteins differently regulate the Ras circuit to induce a cancer-related gene signature. *J. Cell Sci*., **125**, 3144–3152.
90. Hanel,W. *et al.* (2013) Two hot spot mutant p53 mouse models display differential gain of function in tumorigenesis. *Cell Death Differ*., **20**, 898–909.
91. Lee,M.K. *et al.* (2012) Cell-type, dose, and mutation-type specificity dictate mutant p53 functions *in vivo*. *Cancer Cell*, **22**, 751–764.
92. Rotter,V. (1983) p53, a transformation-related cellular-encoded protein, can be used as a biochemical marker for the detection of primary mouse tumor cells. *Proc. Natl Acad. Sci. USA*, **80**, 2613–2617.
93. Terzian,T. *et al.* (2008) The inherent instability of mutant p53 is alleviated by Mdm2 or p16INK4a loss. *Genes Dev*., **22**, 1337–1344.
94. Di Agostino,S. *et al.* (2006) Gain of function of mutant p53: the mutant p53/NF-Y protein complex reveals an aberrant transcriptional mechanism of cell cycle regulation. *Cancer Cell*, **10**, 191–202.
95. Brosh,R. *et al.* (2010) Transcriptional control of the proliferation cluster by the tumor suppressor p53. *Mol. Biosyst*., **6**, 17–29.
96. Wang,W. *et al.* (2013) Mutant p53-R273H gains new function in sustained activation of EGFR signaling via suppressing miR-27a expression. *Cell Death Dis*., **4**, e574.
97. Song,H. *et al.* (2007) p53 gain-of-function cancer mutants induce genetic instability by inactivating ATM. *Nat. Cell Biol*., **9**, 573–580.
98. Lu,X. *et al.* (2013) The gain of function of p53 cancer mutant in promoting mammary tumorigenesis. *Oncogene*, **32**, 2900–2906.
99. Caulin,C. *et al.* (2007) An inducible mouse model for skin cancer reveals distinct roles for gain- and loss-of-function p53 mutations. *J. Clin. Invest*., **117**, 1893–1901.
100. Hingorani,S.R. *et al.* (2005) Trp53R172H and KrasG12D cooperate to promote chromosomal instability and widely metastatic pancreatic ductal adenocarcinoma in mice. *Cancer Cell*, **7**, 469–483.
101. Murphy,K.L. *et al.* (2000) Mutant p53 and genomic instability in a transgenic mouse model of breast cancer. *Oncogene*, **19**, 1045–1051.
102. Stephens,P.J. *et al.* (2011) Massive genomic rearrangement acquired in a single catastrophic event during cancer development. *Cell*, **144**, 27–40.
103. Kogan-Sakin,I. *et al.* (2011) Mutant p53(R175H) upregulates Twist1 expression and promotes epithelial-mesenchymal transition in immortalized prostate cells. *Cell Death Differ*., **18**, 271–281.
104. Dong,P. *et al.* (2013) Mutant p53 gain-of-function induces epithelial-mesenchymal transition through modulation of the miR-130b-ZEB1 axis. *Oncogene*, **32**, 3286–3295.
105. Xia,M. *et al.* (2007) Tumor suppressor p53 restricts Ras stimulation of RhoA and cancer cell motility. *Nat. Struct. Mol. Biol*., **14**, 215–223.
106. Muller,P.A. *et al.* (2009) Mutant p53 drives invasion by promoting integrin recycling. *Cell*, **139**, 1327–1341.
107. Yeudall,W.A. *et al.* (2012) Gain-of-function mutant p53 upregulates CXC chemokines and enhances cell migration. *Carcinogenesis*, **33**, 442–451.
108. Fontemaggi,G. *et al.* (2009) The execution of the transcriptional axis mutant p53, E2F1 and ID4 promotes tumor neo-angiogenesis. *Nat. Struct. Mol. Biol*., **16**, 1086–1093.

109. Famulski,W. *et al.* (2006) P53 correlates positively with VEGF in preoperative sera of colorectal cancer patients. *Neoplasma*, **53**, 43–48.
110. Khromova,N.V. *et al.* (2009) p53 hot-spot mutants increase tumor vascularization via ROS-mediated activation of the HIF1/VEGF-A pathway. *Cancer Lett*., **276**, 143–151.
111. Tian,Y. *et al.* (2006) Analysis of p53 and vascular endothelial growth factor expression in human gallbladder carcinoma for the determination of tumor vascularity. *World J. Gastroenterol*., **12**, 415–419.
112. Vander Heiden,M.G. *et al.* (2009) Understanding the Warburg effect: the metabolic requirements of cell proliferation. *Science*, **324**, 1029–1033.
113. Zhang,C. *et al.* (2013) Tumour-associated mutant p53 drives the Warburg effect. *Nat. Commun*., **4**, 2935.
114. Cooks,T. *et al.* (2014) Caught in the crossfire: p53 in inflammation. *Carcinogenesis*.
115. Weisz,L. *et al.* (2007) Mutant p53 enhances nuclear factor kappaB activation by tumor necrosis factor alpha in cancer cells. *Cancer Res*., **67**, 2396–2401.
116. Cooks,T. *et al.* (2013) Mutant p53 prolongs NF-κB activation and promotes chronic inflammation and inflammation-associated colorectal cancer. *Cancer Cell*, **23**, 634–646.
117. McMurray,H.R. *et al.* (2008) Synergistic response to oncogenic mutations defines gene class critical to cancer phenotype. *Nature*, **453**, 1112–1116.
118. Scian,M.J. *et al.* (2005) Tumor-derived p53 mutants induce NF-kappaB2 gene expression. *Mol. Cell. Biol*., **25**, 10097–10110.
119. Madar,S. *et al.* (2013) Mutant p53 attenuates the anti-tumorigenic activity of fibroblasts-secreted interferon beta. *PLoS One*, **8**, e61353.
120. Peled,A. *et al.* (1996) Cooperation between p53-dependent and p53-independent apoptotic pathways in myeloid cells. *Cancer Res*., **56**, 2148–2156.
121. Li,R. *et al.* (1998) Mutant p53 protein expression interferes with p53-independent apoptotic pathways. *Oncogene*, **16**, 3269–3277.
122. Blandino,G. *et al.* (1999) Mutant p53 gain of function: differential effects of different p53 mutants on resistance of cultured cells to chemotherapy. *Oncogene*, **18**, 477–485.
123. Buganim,Y. *et al.* (2006) Mutant p53 protects cells from 12-O-tetradecanoylphorbol-13-acetate-induced death by attenuating activating transcription factor 3 induction. *Cancer Res*., **66**, 10750–10759.
124. Stambolsky,P. *et al.* (2010) Modulation of the vitamin D3 response by cancer-associated mutant p53. *Cancer Cell*, **17**, 273–285.
125. Xie,T.X. *et al.* (2013) Serine substitution of proline at codon 151 of TP53 confers gain of function activity leading to anoikis resistance and tumor progression of head and neck cancer cells. *Laryngoscope*, **123**, 1416–1423.
126. Frisch,S.M. *et al.* (2001) Anoikis mechanisms. *Curr. Opin. Cell Biol*., **13**, 555–562.
127. Matas,D. *et al.* (2001) Integrity of the N-terminal transcription domain of p53 is required for mutant p53 interference with drug-induced apoptosis. *EMBO J*., **20**, 4163–4172.
128. Chin,K.V. *et al.* (1992) Modulation of activity of the promoter of the human MDR1 gene by Ras and p53. *Science*, **255**, 459–462.
129. Bossi,G. *et al.* (2008) Conditional RNA interference *in vivo* to study mutant p53 oncogenic gain of function on tumor malignancy. *Cell Cycle*, **7**, 1870–1879.
130. Weisz,L. *et al.* (2004) Transactivation of the EGR1 gene contributes to mutant p53 gain of function. *Cancer Res*., **64**, 8318–8327.
131. Zalcenstein,A. *et al.* (2003) Mutant p53 gain of function: repression of CD95(Fas/APO-1) gene expression by tumor-associated p53 mutants. *Oncogene*, **22**, 5667–5676.
132. Chee,J.L. *et al.* (2013) Wild-type and mutant p53 mediate cisplatin resistance through interaction and inhibition of active caspase-9. *Cell Cycle*, **12**, 278–288.
133. Oren,M. *et al.* (2010) Mutant p53 gain-of-function in cancer. *Cold Spring Harb. Perspect. Biol*., **2**, a001107.
134. Irwin,M.S. *et al.* (2003) Chemosensitivity linked to p73 function. *Cancer Cell*, **3**, 403–410.
135. Donzelli,S. *et al.* (2012) MicroRNA-128-2 targets the transcriptional repressor E2F5 enhancing mutant p53 gain of function. *Cell Death Differ*., **19**, 1038–1048.
136. Masciarelli,S. *et al.* (2014) Gain-of-function mutant p53 downregulates miR-223 contributing to chemoresistance of cultured tumor cells. *Oncogene*, **33**, 1601–1608.
137. Dick,J.E. (2008) Stem cell concepts renew cancer research. *Blood*, **112**, 4793–4807.
138. Vermeulen,L. *et al.* (2008) Cancer stem cells—old concepts, new insights. *Cell Death Differ*., **15**, 947–958.
139. Lapidot,T. *et al.* (1994) A cell initiating human acute myeloid leukaemia after transplantation into SCID mice. *Nature*, **367**, 645–648.
140. Bonnet,D. *et al.* (1997) Human acute myeloid leukemia is organized as a hierarchy that originates from a primitive hematopoietic cell. *Nat. Med*., **3**, 730–737.
141. Al-Hajj,M. *et al.* (2003) Prospective identification of tumorigenic breast cancer cells. *Proc. Natl Acad. Sci. USA*, **100**, 3983–3988.
142. Singh,S.K. *et al.* (2003) Identification of a cancer stem cell in human brain tumors. *Cancer Res*., **63**, 5821–5828.
143. Li,C. *et al.* (2007) Identification of pancreatic cancer stem cells. *Cancer Res*., **67**, 1030–1037.
144. Ricci-Vitiani,L. *et al.* (2007) Identification and expansion of human colon-cancer-initiating cells. *Nature*, **445**, 111–115.
145. O'Brien,C.A. *et al.* (2007) A human colon cancer cell capable of initiating tumour growth in immunodeficient mice. *Nature*, **445**, 106–110.
146. Zhang,S. *et al.* (2008) Identification and characterization of ovarian cancer-initiating cells from primary human tumors. *Cancer Res*., **68**, 4311–4320.
147. Curley,M.D. *et al.* (2009) CD133 expression defines a tumor initiating cell population in primary human ovarian cancer. *Stem Cells*, **27**, 2875–2883.
148. Alvero,A.B. *et al.* (2009) Molecular phenotyping of human ovarian cancer stem cells unravels the mechanisms for repair and chemoresistance. *Cell Cycle*, **8**, 158–166.
149. Sugihara,E. *et al.* (2013) Complexity of cancer stem cells. *Int. J. Cancer*, **132**, 1249–1259.
150. Yang,L. *et al.* (2013) Ovarian cancer stem cells enrichment. *Methods Mol. Biol*., **1049**, 337–345.
151. Hirschmann-Jax,C. *et al.* (2004) A distinct "side population" of cells with high drug efflux capacity in human tumor cells. *Proc. Natl Acad. Sci. USA*, **101**, 14228–14233.
152. Schatton,T. *et al.* (2008) Identification of cells initiating human melanomas. *Nature*, **451**, 345–349.
153. Quintana,E. *et al.* (2010) Phenotypic heterogeneity among tumorigenic melanoma cells from patients that is reversible and not hierarchically organized. *Cancer Cell*, **18**, 510–523.
154. Valent,P. *et al.* (2012) Cancer stem cell definitions and terminology: the devil is in the details. *Nat. Rev. Cancer*, **12**, 767–775.
155. Mandal,P.K. *et al.* (2011) DNA damage response in adult stem cells: pathways and consequences. *Nat. Rev. Mol. Cell Biol*., **12**, 198–202.
156. Tata,P.R. *et al.* (2013) Dedifferentiation of committed epithelial cells into stem cells *in vivo*. *Nature*, **503**, 218–223.
157. Wagers,A.J. *et al.* (2004) Plasticity of adult stem cells. *Cell*, **116**, 639–648.
158. Mani,S.A. *et al.* (2008) The epithelial-mesenchymal transition generates cells with properties of stem cells. *Cell*, **133**, 704–715.
159. Donghi,R. *et al.* (1993) Gene p53 mutations are restricted to poorly differentiated and undifferentiated carcinomas of the thyroid gland. *J. Clin. Invest*., **91**, 1753–1760.
160. Fagin,J.A. *et al.* (1993) High prevalence of mutations of the p53 gene in poorly differentiated human thyroid carcinomas. *J. Clin. Invest*., **91**, 179–184.
161. Yamaguchi,T. *et al.* (1996) Loss of heterozygosity and tumor suppressor gene mutations in chondrosarcomas. *Anticancer Res*., **16**, 2009–2015.
162. Kemp,C.J. *et al.* (1993) Reduction of p53 gene dosage does not increase initiation or promotion but enhances malignant progression of chemically induced skin tumors. *Cell*, **74**, 813–822.
163. Mizuno,H. *et al.* (2010) Inactivation of p53 in breast cancers correlates with stem cell transcriptional signatures. *Proc. Natl Acad. Sci. USA*, **107**, 22745–22750.
164. Krizhanovsky,V. *et al.* (2009) Stem cells: the promises and perils of p53. *Nature*, **460**, 1085–1086.
165. Sridharan,R. *et al.* (2009) Role of the murine reprogramming factors in the induction of pluripotency. *Cell*, **136**, 364–377.
166. Wang,Y. *et al.* (2009) Expression of mutant p53 proteins implicates a lineage relationship between neural stem cells and malignant astrocytic glioma in a murine model. *Cancer Cell*, **15**, 514–526.
167. Liu,C. *et al.* (2011) Mosaic analysis with double markers reveals tumor cell of origin in glioma. *Cell*, **146**, 209–221.
168. Rubio,R. *et al.* (2013) The differentiation stage of p53-Rb-deficient bone marrow mesenchymal stem cells imposes the phenotype of in vivo sarcoma development. *Oncogene*, **32**, 4970–4980.
169. Rodriguez,R. *et al.* (2012) Modeling sarcomagenesis using multipotent mesenchymal stem cells. *Cell Res*., **22**, 62–77.
170. Rodriguez,R. *et al.* (2009) Loss of p53 induces tumorigenesis in p21-deficient mesenchymal stem cells. *Neoplasia*, **11**, 397–407.

171. Choi,J. *et al.* (2010) Local mesenchymal stem/progenitor cells are a preferential target for initiation of adult soft tissue sarcomas associated with p53 and Rb deficiency. *Am. J. Pathol*., **177**, 2645–2658.
172. Mutsaers,A.J. *et al.* (2014) Cells of origin in osteosarcoma: mesenchymal stem cells or osteoblast committed cells? *Bone*, **62C**, 56–63.
173. Hiraga,T. *et al.* (2013) Cancer stem-like cell marker CD44 promotes bone metastases by enhancing tumorigenicity, cell motility, and hyaluronan production. *Cancer Res*., **73**, 4112–4122.
174. Hermann,P.C. *et al.* (2010) Cancer stem cells in solid tumors. *Semin. Cancer Biol*., **20**, 77–84.
175. Liu,C. *et al.* (2011) The microRNA miR-34a inhibits prostate cancer stem cells and metastasis by directly repressing CD44. *Nat. Med*., **17**, 211–215.
176. Godar,S. *et al.* (2008) Growth-inhibitory and tumor- suppressive functions of p53 depend on its repression of CD44 expression. *Cell*, **134**, 62–73.
177. Adhikari,A.S. *et al.* (2010) CD117 and Stro-1 identify osteosarcoma tumor-initiating cells associated with metastasis and drug resistance. *Cancer Res*., **70**, 4602–4612.
178. Kang,M.K. *et al.* (2008) Potential identity of multi-potential cancer stem-like subpopulation after radiation of cultured brain glioma. *BMC Neurosci*., **9**, 15.
179. Siemens,H. *et al.* (2013) Repression of c-Kit by p53 is mediated by miR-34 and is associated with reduced chemoresistance, migration and stemness. *Oncotarget*, **4**, 1399–1415.
180. Qin,H. *et al.* (2007) Regulation of apoptosis and differentiation by p53 in human embryonic stem cells. *J. Biol. Chem*., **282**, 5842–5852.
181. Wang,M.L. *et al.* (2013) Targeting cancer stem cells: emerging role of Nanog transcription factor. *Onco Targets Ther*., **6**, 1207–1220.
182. Jeter,C.R. *et al.* (2011) NANOG promotes cancer stem cell characteristics and prostate cancer resistance to androgen deprivation. *Oncogene*, **30**, 3833–3845.
183. Chiou,S.H. *et al.* (2010) Coexpression of Oct4 and Nanog enhances malignancy in lung adenocarcinoma by inducing cancer stem cell-like properties and epithelial-mesenchymal transdifferentiation. *Cancer Res*., **70**, 10433–10444.
184. Sato,A. *et al.* (2013) Resveratrol promotes proteasome-dependent degradation of Nanog via p53 activation and induces differentiation of glioma stem cells. *Stem Cell Res*., **11**, 601–610.
185. Zbinden,M. *et al.* (2010) NANOG regulates glioma stem cells and is essential *in vivo* acting in a cross-functional network with GLI1 and p53. *EMBO J*., **29**, 2659–2674.
186. Siemens,H. *et al.* (2011) miR-34 and SNAIL form a double-negative feedback loop to regulate epithelial-mesenchymal transitions. *Cell Cycle*, **10**, 4256–4271.
187. Korpal,M. *et al.* (2008) The miR-200 family inhibits epithelial-mesenchymal transition and cancer cell migration by direct targeting of E-cadherin transcriptional repressors ZEB1 and ZEB2. *J. Biol. Chem*., **283**, 14910–14914.
188. Chang,C.J. *et al.* (2011) p53 regulates epithelial-mesenchymal transition and stem cell properties through modulating miRNAs. *Nat. Cell Biol*., **13**, 317–323.
189. Brosh,R. *et al.* (2013) p53 counteracts reprogramming by inhibiting mesenchymal-to-epithelial transition. *Cell Death Differ*., **20**, 312–320.
190. Li,L. *et al.* (2006) Normal stem cells and cancer stem cells: the niche matters. *Cancer Res*., **66**, 4553–4557.
191. Kerbel,R.S. *et al.* (1994) Intrinsic or acquired drug resistance and metastasis: are they linked phenotypes? *J. Cell. Biochem*., **56**, 37–47.
192. Thomas,H. *et al.* (2003) Overcoming multidrug resistance in cancer: an update on the clinical strategy of inhibiting p-glycoprotein. *Cancer Control*, **10**, 159–165.
193. Fletcher,J.I. *et al.* (2010) ABC transporters in cancer: more than just drug efflux pumps. *Nat. Rev. Cancer*, **10**, 147–156.
194. Holohan,C. *et al.* (2013) Cancer drug resistance: an evolving paradigm. *Nat. Rev. Cancer*, **13**, 714–726.
195. Dean,M. (2009) ABC transporters, drug resistance, and cancer stem cells. *J. Mammary Gland Biol. Neoplasia*, **14**, 3–9.
196. Sophos,N.A. *et al.* (2003) Aldehyde dehydrogenase gene superfamily: the 2002 update. *Chem. Biol. Interact*., **143–144**, 5–22.
197. Magni,M. *et al.* (1996) Induction of cyclophosphamide-resistance by aldehyde-dehydrogenase gene transfer. *Blood*, **87**, 1097–1103.
198. Moreb,J. *et al.* (1996) Overexpression of the human aldehyde dehydrogenase class I results in increased resistance to 4-hydroperoxycyclophosphamide. *Cancer Gene Ther*., **3**, 24–30.
199. Tanei,T. *et al.* (2009) Association of breast cancer stem cells identified by aldehyde dehydrogenase 1 expression with resistance to sequential paclitaxel and epirubicin-based chemotherapy for breast cancers. *Clin. Cancer Res*., **15**, 4234–4241.
200. Moreb,J.S. *et al.* (2000) Expression of antisense RNA to aldehyde dehydrogenase class-1 sensitizes tumor cells to 4-hydroperoxycyclophosphamide *in vitro*. *J. Pharmacol. Exp. Ther*., **293**, 390–396.
201. Croker,A.K. *et al.* (2012) Inhibition of aldehyde dehydrogenase (ALDH) activity reduces chemotherapy and radiation resistance of stem-like ALDHhiCD44$^+$ human breast cancer cells. *Breast Cancer Res. Treat*., **133**, 75–87.
202. Ma,I. *et al.* (2011) The role of human aldehyde dehydrogenase in normal and cancer stem cells. *Stem Cell Rev*., **7**, 292–306.
203. Deng,S. *et al.* (2010) Distinct expression levels and patterns of stem cell marker, aldehyde dehydrogenase isoform 1 (ALDH1), in human epithelial cancers. *PLoS One*, **5**, e10277.
204. Ishikawa,T. *et al.* (1993) Glutathione-associated cis-diamminedichloroplatinum(II) metabolism and ATP-dependent efflux from leukemia cells. Molecular characterization of glutathione-platinum complex and its biological significance. *J. Biol. Chem*., **268**, 20116–20125.
205. Estrela,J.M. *et al.* (2006) Glutathione in cancer biology and therapy. *Crit. Rev. Clin. Lab. Sci*., **43**, 143–181.
206. Ishikawa,T. (1992) The ATP-dependent glutathione S-conjugate export pump. *Trends Biochem. Sci*., **17**, 463–468.
207. Perry,R.R. *et al.* (1993) Glutathione levels and variability in breast tumors and normal tissue. *Cancer*, **72**, 783–787.
208. Tatebe,S. *et al.* (2002) Expression of heavy subunit of gamma-glutamylcysteine synthetase (gamma-GCSh) in human colorectal carcinoma. *Int. J. Cancer*, **97**, 21–27.
209. Trachootham,D. *et al.* (2009) Targeting cancer cells by ROS-mediated mechanisms: a radical therapeutic approach? *Nat. Rev. Drug Discov*., **8**, 579–591.
210. Ito,K. *et al.* (2004) Regulation of oxidative stress by ATM is required for self-renewal of haematopoietic stem cells. *Nature*, **431**, 997–1002.
211. Diehn,M. *et al.* (2009) Association of reactive oxygen species levels and radioresistance in cancer stem cells. *Nature*, **458**, 780–783.
212. Boyer,J. *et al.* (2004) Characterization of p53 wild-type and null isogenic colorectal cancer cell lines resistant to 5-fluorouracil, oxaliplatin, and irinotecan. *Clin. Cancer Res*., **10**, 2158–2167.
213. Hector,S. *et al.* (2001) *In vitro* studies on the mechanisms of oxaliplatin resistance. *Cancer Chemother. Pharmacol*., **48**, 398–406.
214. Metzger,R. *et al.* (1998) ERCC1 mRNA levels complement thymidylate synthase mRNA levels in predicting response and survival for gastric cancer patients receiving combination cisplatin and fluorouracil chemotherapy. *J. Clin. Oncol*., **16**, 309–316.
215. Dabholkar,M. *et al.* (1994) Messenger RNA levels of XPAC and ERCC1 in ovarian cancer tissue correlate with response to platinum-based chemotherapy. *J. Clin. Invest*., **94**, 703–708.
216. Lord,R.V. *et al.* (2002) Low ERCC1 expression correlates with prolonged survival after cisplatin plus gemcitabine chemotherapy in non-small cell lung cancer. *Clin. Cancer Res*., **8**, 2286–2291.
217. Sarkaria,J.N. *et al.* (2008) Mechanisms of chemoresistance to alkylating agents in malignant glioma. *Clin. Cancer Res*., **14**, 2900–2908.
218. Fink,D. *et al.* (1998) The role of DNA mismatch repair in drug resistance. *Clin. Cancer Res*., **4**, 1–6.
219. Fink,D. *et al.* (1996) The role of DNA mismatch repair in platinum drug resistance. *Cancer Res*., **56**, 4881–4886.
220. Hawn,M.T. *et al.* (1995) Evidence for a connection between the mismatch repair system and the G2 cell cycle checkpoint. *Cancer Res*., **55**, 3721–3725.
221. D'Atri,S. *et al.* (1998) Involvement of the mismatch repair system in temozolomide-induced apoptosis. *Mol. Pharmacol*., **54**, 334–341.
222. Bao,S. *et al.* (2006) Glioma stem cells promote radioresistance by preferential activation of the DNA damage response. *Nature*, **444**, 756–760.
223. Gallmeier,E. *et al.* (2011) Inhibition of ataxia telangiectasia- and Rad3-related function abrogates the *in vitro* and *in vivo* tumorigenicity of human colon cancer cells through depletion of the CD133(+) tumor-initiating cell fraction. *Stem Cells*, **29**, 418–429.
224. Bartucci,M. *et al.* (2012) Therapeutic targeting of Chk1 in NSCLC stem cells during chemotherapy. *Cell Death Differ*., **19**, 768–778.
225. Karimi-Busheri,F. *et al.* (2010) Senescence evasion by MCF-7 human breast tumor-initiating cells. *Breast Cancer Res*., **12**, R31.
226. Chuang,S.E. *et al.* (2002) Basal levels and patterns of anticancer drug-induced activation of nuclear factor-kappaB (NF-kappaB), and its attenuation by tamoxifen, dexamethasone, and curcumin in carcinoma cells. *Biochem. Pharmacol*., **63**, 1709–1716.

227. Liu,G. *et al.* (2006) Analysis of gene expression and chemoresistance of CD133+ cancer stem cells in glioblastoma. *Mol. Cancer*, **5**, 67.
228. Di Franco,S. *et al.* (2011) Colon cancer stem cells: bench-to-bedside-new therapeutical approaches in clinical oncology for disease breakdown. *Cancers (Basel)*, **3**, 1957–1974.
229. Ma,S. *et al.* (2008) CD133+ HCC cancer stem cells confer chemoresistance by preferential expression of the Akt/PKB survival pathway. *Oncogene*, **27**, 1749–1758.
230. Oren,M. *et al.* (2010) Mutant p53 gain-of-function in cancer. *Cold Spring Harb. Perspect. Biol*., **2**, a001107.
231. Lane,D. *et al.* (2010) p53 research: the past thirty years and the next thirty years. *Cold Spring Harb. Perspect. Biol*., **2**, a001107.
232. Cheok,C.F. *et al.* (2011) Translating p53 into the clinic. *Nat. Rev. Clin. Oncol*., **8**, 25–37.
233. Kawamura,T. *et al.* (2009) Linking the p53 tumour suppressor pathway to somatic cell reprogramming. *Nature*, **460**, 1140–1144.
234. Sengupta,S. *et al.* (2005) p53: traffic cop at the crossroads of DNA repair and recombination. *Nat. Rev. Mol. Cell Biol*., **6**, 44–55.
235. Shen,Y.A. *et al.* (2013) Resveratrol impedes the stemness, epithelial-mesenchymal transition, and metabolic reprogramming of cancer stem cells in nasopharyngeal carcinoma through p53 activation. *Evid. Based Complement. Alternat. Med*., **2013**, 590393.
236. Wu,S. *et al.* (2013) Autophagy of cancer stem cells is involved with chemoresistance of colon cancer cells. *Biochem. Biophys. Res. Commun*., **434**, 898–903.

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