The p53 family in differentiation and tumorigenesis

Thorsten Stiewe

Abstract | The role of p53 as a tumour suppressor is generally attributed to its ability to stop the proliferation of precancerous cells by inducing cell-cycle arrest or apoptosis. The relatives and evolutionary predecessors of p53 — p63 and p73 — share the tumour-suppressor activity of p53 to some extent, but also have essential functions in embryonic development and differentiation control. Recent evidence indicates that these ancestral functions in differentiation control contribute to the tumour-suppressor activity that the p53 family is famous for.

The tumour suppressor p53 is known as the 'guardian of the genome' owing to its ability to integrate many signals that control life or death[1]. Activated by various types of cellular stress, including DNA damage and oncogenic stress, p53 initiates several programmes that ultimately arrest proliferation and prevent the generation of genetically-altered cells. The spectrum of p53-based cell fate decisions ranges from a transient cell-cycle arrest enabling damage repair to an irreversible block of proliferation through senescence or apoptosis. This integrative function is assisted by p63 and p73 (BOX 1), relatives of p53 which are thought to be required for p53 to transactivate pro-apoptotic target genes in some experimental settings[2]. Recent studies inspired by the developmental functions of p63 and p73 indicate that members of the p53 family also cooperate in differentiation control. In contrast to apoptosis, differentiation does not eliminate cells from the organism, but rather removes cells from the proliferative compartment at the same time as preserving cellular integrity and function. By preventing the proliferation of damaged cells, differentiation further helps to maintain the genetic stability of the organism. Differentiation therefore adds to the spectrum of p53-based cell fate decisions and could contribute to the tumour-suppressor activity of the p53 family (FIG. 1). Here, the experimental evidence that members of the p53 family function in differentiation and development is reviewed and discussed in the context of tumour suppression and cancer therapy.

Differentiation and development

Several *in vitro* and *in vivo* assays have shown that wild-type p53 expressed in undifferentiated cells can result in progression to a more differentiated state[3]. For example, endogenous p53 was shown to induce the differentiation of mouse embryonic stem cells by suppressing NANOG expression[4]. Similarly, p63 has been implicated in the specification and differentiation of squamous epithelial cells[5,6], and p73 expression has been shown to induce the expression of neuronal differentiation markers in neuroblastoma cell lines[7].

Despite a substantial body of evidence, the concept that p53 can regulate differentiation has largely been ignored owing to the lack of strong developmental defects in p53 knockout mice (BOX 2). Normal development of p53-null mice is in striking contrast to the severe gastrulation defects observed in p53 depleted *Xenopus laevis* embryos[8]. One possible reason for this species difference is that several p53 family members are expressed in early mouse embryos and potentially compensate for the loss of p53, whereas in frogs p53 is solely responsible for early embryogenesis. Functional compensation by p63 and p73 seems likely, as we know from mouse knockouts that both proteins are crucial for development (BOX 2). Although the knockout phenotypes indicate tissue-specific functions of individual p53 family members, it is conceivable that more fundamental aspects of embryogenesis are functionally redundant so that an early embryonic phenotype only becomes apparent in compound mutant mice. Consistent with this hypothesis, viable double or triple knockout mice have not been observed. Further support comes from recent studies showing that the deregulated transgenic expression of the antagonistic p53 family protein ΔNp73 results in early embryonic lethality around the time of gastrulation[9,10].

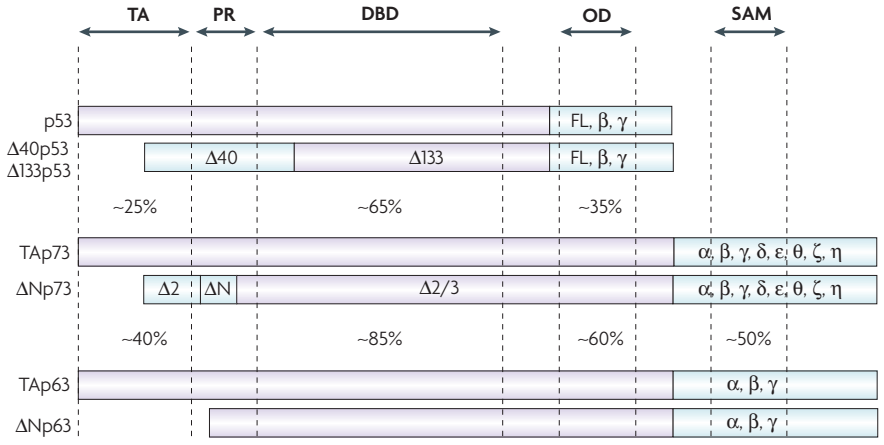
Although the developmental defects of compound mutant and ΔNp73 transgenic mice still need to be characterized in detail to fully understand the role of the p53 family in embryogenesis, the recent animal models highlight the contribution of the p53 family to aspects of cellular differentiation and embryonic development that can no longer be disputed. However, the question remains: how are developmental processes coordinated by p53-family proteins on a molecular level? Experiments on the role of the p53 family in muscle differentiation showed that individual family members are specifically regulated by muscle-regulatory factors, and have distinct but cooperating functions during myogenesis[11,12]. Whereas p53 is a transcriptional activator of the retinoblastoma susceptibility gene *RB1*, p63 and p73 induce the expression of the cyclin-dependent kinase inhibitor p57. Both activities contribute to the maintainance of high levels of active, hypophosphorylated RB1, which is known to be essential for both permanent exit from the cell cycle and the transactivation of muscle-specific genes (FIG. 2a).

Tumour suppression

Even in the modern era of cancer genomics, the classic histopathological differentiation grade of a tumour remains one of the most important prognostic factors for predicting patient survival. A correlation between a poor grade of differentiation and the presence of p53 mutations is often seen in human cancers. For example, the progression of chronic myelogenous leukaemia (CML) into blast crisis is characterized by the rapid expansion of a population of

Box 1 | **The p53 family**

p53 belongs to a multigene family that also includes p63 and p73. The overall protein architecture is highly conserved from *Drosophila melanogaster* to man, and consists of a central sequence-specific DNA binding domain (DBD), an N-terminal transactivation domain (TA) and a C-terminal oligomerization domain (OD). Whereas the p53 tail is a basic domain that has been shown to possess sequence-nonspecific nucleic acid binding ability, both p63 and p73 have a sterile alpha motif (SAM) domain implicated in protein–protein interactions. The highest degree of homology is seen within the DBD, where >97% of all tumour-associated p53 mutations are located. p63 and p73 share ~65% amino-acid identity with the DBD of p53, and even higher identity with each other. All three genes express many differently spliced isoforms — a feature that was thought to be unique for p63 and p73 but has recently been shown to also be true of p53 (REF. 31). All three genes are now known to contain a second intronic promoter that generates N-terminally truncated ΔN proteins (Δ133p53, ΔNp63 and ΔNp73). Further ΔN isoforms are generated by alternative splicing events and alternative initiation of translation (Δ40p53, Δex2p73, Δex2/3p73 and ΔN'p73). As the N terminus is crucial for the transactivation of target genes, transactivating full-length isoforms (FLp53, TAp63 and TAp73) can be functionally distinguished from the transactivation-compromised ΔN isoforms that show anti-apoptotic and dominant–negative properties. Finally, additional variation caused by the alternative splicing of C-terminal exons and the use of cryptic splice sites yields a plethora of different isoforms (α, β, γ, δ, ε, θ, ζ and η) with fundamentally different and still incompletely understood DNA-binding properties, transcriptional activities and biological functions.



differentiation-arrested blast cells that have acquired p53 mutations in 25–30% of all patients[13]. Similarly, the progression of well-differentiated papillary thyroid carcinoma to undifferentiated anaplastic thyroid cancer is frequently associated with p53 mutations[14].

The most compelling evidence for a link between tumorigenesis and loss of p53 family members in the context of deregulated differentiation comes from studies on muscle cells and rhabdomyosarcomas. Loss of p53 significantly impairs the differentiation of muscle progenitor cells and promotes rhabdomyosarcoma development in several oncogene-driven mouse tumour models[15–18]. Furthermore, the loss of a single *Trp53* allele results in the development of rhabdomyosarcoma in approximately 20% of all mice. The same frequency of rhabdomyosarcoma is seen in *Trp63*$^{+/-}$;*Trp73*$^{+/-}$ double heterozygous but not in *Trp63*$^{+/-}$ or *Trp73*$^{+/-}$ single heterozygous mice, indicating that p63 and p73 have redundant, p53-like tumour-suppressor activities in the muscle lineage[19]. In the same study, the loss of *Trp63* or *Trp73* alleles on a *Trp53*$^{+/-}$ background was shown to increase the tumour burden and lead to a more aggressive, highly metastatic rhabdomyosarcoma phenotype[19]. However, the role of p63 in tumour suppression remains controversial, as another study indicated that p63 heterozygous mutant mice are not tumour prone, and mice heterozygous for both *Trp53* and *Trp63* had fewer tumours than *Trp53*$^{+/-}$ mice[20]. In human tumours p53 is typically inactivated by mutation or overexpression of the p53 ubiquitin ligase MDM2, whereas p63 and p73 are more commonly inactivated by sequestration in transcriptionally inert complexes with mutant p53 proteins or the antagonistic p53 family members ΔNp63 or ΔNp73 (REFS 21–24) (FIG. 2b). For example, ΔNp63 has been shown to bind to and inactivate p73 in squamous-cell carcinomas[25]. An increased expression of ΔNp73 is seen in many tumour types, including a high percentage of rhabdomyosarcomas[12,22]. The enforced expression of ΔNp73 in myoblasts completely blocks the myogenic differentiation programme, but is not sufficient for malignant transformation on its own[9,12]. However, ΔNp73 increases the oncogenic potential of rhabdomyosarcoma oncogenes such as the genes that encode insulin-like growth factor 2 (IGF2) and the fusion protein PAX3–FKHR. Whereas PAX3–FKHR-expressing myoblasts differentiate into mature muscle fibres when transplanted into immunodeficient mice, the co-expression of ΔNp73 prevents this differentiation process and results in tumour growth[12].

Given that ΔNp73 is also expressed in other tumour types, it is tempting to speculate that the disruption of p53-regulated differentiation pathways by ΔNp73 also enables tumorigenesis in other tissues. High expression levels of ΔNp73 in neuroblastoma samples serve as an independent prognostic marker for poor patient survival[26]. Highly aggressive neuroblastomas have a less mature differentiation state, and ΔNp73 has been shown to inhibit the neuronal differentiation of neuroblastoma cells, strongly suggesting that ΔNp73 predicts poor survival because it prevents maturation[9,27].

Interestingly, both rhabdomyosarcomas and neuroblastomas are childhood solid tumours (CSTs) that originate in immature tissues where strong mitogenic signals drive expansion and limit the ability of cells to terminally differentiate[28]. The onset of differentiation therefore requires key regulators that promote cell-cycle exit.The p53 family contains both agonistic and antagonistic proteins, making it ideally equipped to fine-tune the balance between proliferation and differentiation. In line with this idea, there is a spatiotemporal switch from ΔNp73 in proliferating nephron precursors to TAp73 in the differentiation domain of the renal cortex[29]. Disruption of this balance through the inactivation of differentiation-promoting isoforms of the p53 family (for example, p53 and TAp73) or the persistent expression of antagonistic isoforms (such as ΔNp73) can prevent progenitor cells from exiting this proliferative phase and therefore drive tumour formation. Although differentiation pathways seem to be most relevant for tumour suppression in the developing organism, it is reasonable to assume that in light of continuous tissue regeneration from adult stem cells similar mechanisms operate in adults, and make differentiation control a tumour-suppressor function of the p53 family in general.

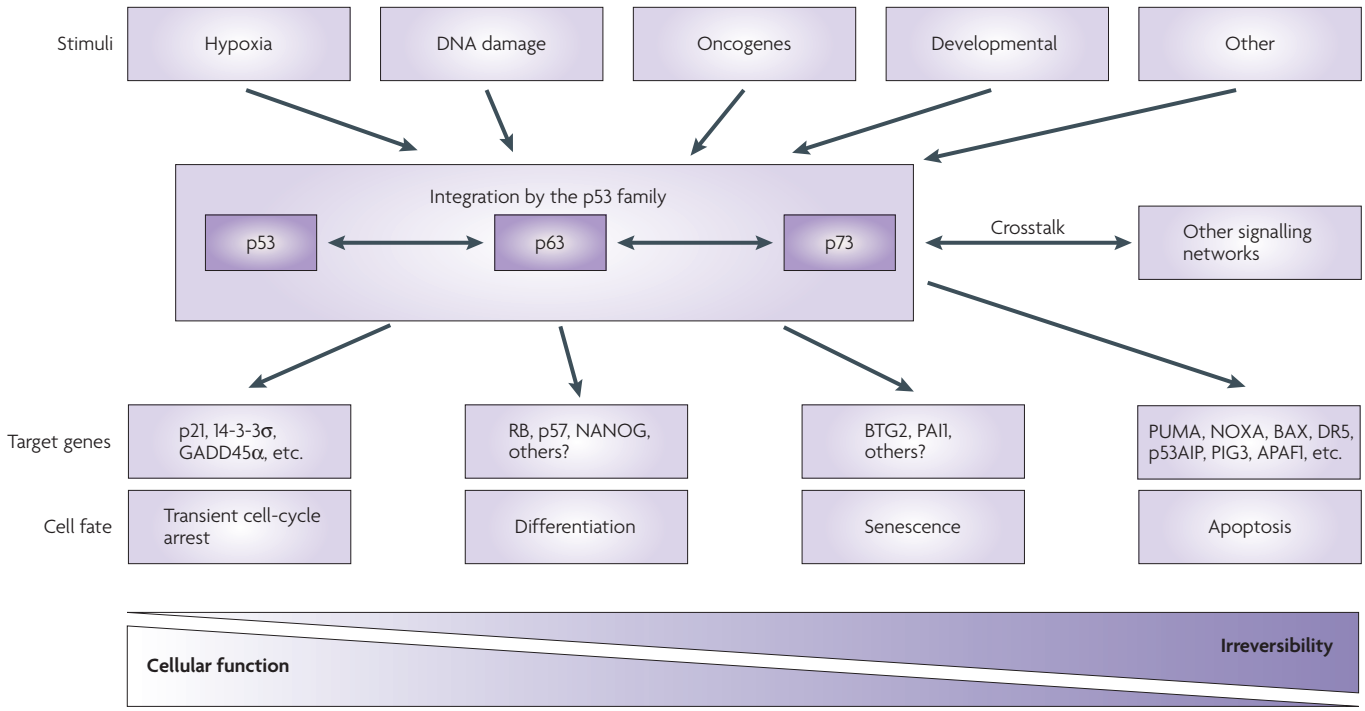


Figure 1 | **p53-family-based cell-fate decisions.** The p53 family is activated by cellular stress, hyperproliferative signals and developmental stimuli. These inputs are integrated within the p53 family in crosstalk with other cellular signalling networks to reach an appropriate cell fate decision that is executed by the selective transactivation of distinct transcriptional programmes. The most common outcomes are reversible cell-cycle arrest, irreversible cell-cycle exit (differentiation or senescence) or apoptotic cell death. APAF1, apoptotic peptidase activating factor 1; GADD45α, growth arrest and DNA-damage-inducible-α; RB1, retinoblastoma susceptibility protein; PAI1, plasminogen activator inhibitor type I; PIG3, p53-induced gene 3 protein.

Perspective: differentiation therapy

The ultimate goal of cancer therapy is the long-lasting inhibition of tumour cell proliferation, preferably by killing tumour cells. As many tumour cells have several defects in the apoptotic pathway, the induction of differentiation is a therapeutically attractive means of inducing an irreversible arrest in proliferation. The function of the p53 family in differentiation control is blocked in tumours by several mechanisms that include p53 mutation, the expression of p53 inhibitors (such as MDM2) and/or by the expression of ΔNp73 (FIG. 2b). Reactivation of the p53 family using drugs that target these aberrations would be expected to repair defective differentiation programmes and stop tumour cell proliferation permanently, even in the presence of apoptosis defects (FIG. 2c). In fact, recent data show that the MDM2-inhibitor Nutlin-3a activates a p53-dependent neuronal differentiation programme in certain neuroblastoma cell lines[30]. Therefore, existing data predict that drugs designed to target the p53 family will also be therapeutically useful as mediators of differentiation.

Despite considerable progress on the role of the p53 family in differentiation control, many questions still need to be answered. Although single knockout mice identify tissue-specific functions of individual p53-family proteins, shared developmental functions remain to be elucidated through the detailed analysis of compound mutant mice. Also unknown is how p53-family members are activated and regulated under physiological conditions in non-stressed cells in a tissue-specific manner. Are the differentiation functions exclusively activated in response to developmental cues or is differentiation also an alternative response to genotoxic stress or hyperproliferative signals? Which are the crucial downstream targets of p53-family-regulated differentiation pathways? And last but not least, can these differentiation pathways be therapeutically exploited to improve cancer treatment?

Box 2 | **Developmental phenotypes of p53, p63 and p73 knockout mice.**

- p53-null mice are viable and show developmental abnormalities only on close examination[32,33]. A small fraction of female null embryos show exencephaly, a condition resulting from the failure of the neural tube to close properly during embryogenesis[34]. p53-null animals have a reduced fertility, and p53-null males of 129/Sv background show a high frequency of multinucleated giant cells within the testicular seminiferous tubules, believed to be a result of an inability to complete meiosis[35].
- p73-null mice are born viable, but show a runting phenotype and a high mortality rate within the first 2 months[36]. The animals suffer from hydrocephalus, indicative of abnormal cerebrospinal fluid dynamics, immunological problems characterized by chronic infections and inflammation, and nervous system abnormalities related to hippocampal dysgenesis, olfactory neuron defects and the loss of sympathetic neurons. Moreover, the animals show abnormal reproductive and social behaviour, which is presumably due to defects in pheromone detection in the vomeronasal organ.
- p63-null mice are born alive but show the most severe developmental phenotype of all p53 family members[37,38]. The limbs are absent or truncated owing to a malfunction of the apical ectodermal ridge. They fail to develop a stratified epidermis and most epithelial tissues (for example, hair follicles, teeth, prostate, lacrimal and salivary glands, and mammary glands), and eventually die from dehydration within hours of birth.

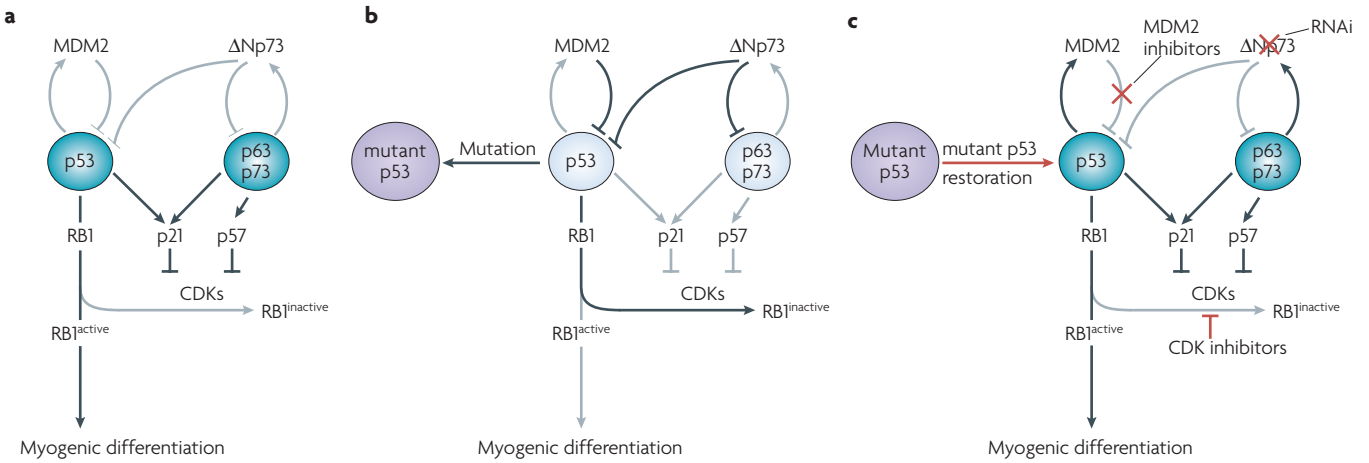


Figure 2 | **p53 family functions in myogenic differentiation and in rhabdomyosarcoma. a** |p53 is a transcriptional activator of the retinoblastoma susceptibility gene *RB1*, which facilitates myogenic differentiation, in concert with the expression of p21 and p57, targets of p63 and p73. **b** | When p53 is mutated, or the regulation of p53 is disrupted by MDM2 and/or the function of p53, p63 and p73 is disrupted by the expression of ΔNp73, p21 and p57 are not expessed, RB1 is inactive and the cells remain in cycle. **c** | There are several potential targets for therapeutic intervention. MDM2 inhibitors, mutant-p53-reactivating compounds, ΔNp73-directed RNA interference (RNAi) and cyclin-dependent kinase (CDK) inhibitors target common aberrations of the p53 family in rhabdomyosarcomas and are predicted to repair the defective myogenic-differentiation pathways. RB1, retinoblastoma susceptibility protein.

Thorsten Stiewe is at the Molecular Tumour Biology Group, Rudolf-Virchow-Center (DFG Research Center for Experimental Biomedicine), University of Würzburg, Versbacher Str. 9, 97078 Würzburg, Germany.
e-mail: thorsten.stiewe@virchow.uni-wuerzburg.de

doi:10.1038/nrc2072
Published online 1 February 2007

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Acknowledgements
I thank the many colleagues who have contributed to these ideas, the members of my lab, and M. Schön for critical reading of the manuscript. I apologize to all colleagues whose work, although relevant, could not be cited owing to space constraints. The work was supported by grants from the Deutsche Forschungsgemeinschaft and the Deutsche Krebshilfe.

Competing interests statement
The author declares no competing financial interests.

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