New Plays in the p53 Theater

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Summary

The p53 tumor suppressor and its paralogs p63 and p73 are at the crux of a network modulating cellular responses against potentially tumorigenic events. p53 acts primarily as a transcription factor, regulating the expression of both coding and non-coding RNAs, as well as the activity of RNA processing complexes. In line with their anti-tumorigenic function, p53 and p63 have recently been implicated in restricting tumor cell invasion. In parallel, a growing number of non-canonical target genes have been added to the p53 repertoire. These include genes encoding for proteins that impinge on a broad spectrum of cellular functions, from cell metabolism to stem cell renewal. The p53 story is still far from being fully told.

Introduction

Humans with germline *p53* mutations are affected by the Li-Fraumeni syndrome, characterized by very high cancer susceptibility[1–2]. *p53* knockout mice develop tumors with short latency and 100% penetrance [3]. In approximately 50% of human cancers p53 is mutated; in many of the remaining 50%, the function of the retained wild type (wt) p53 protein is compromised by deregulation of upstream or downstream components of the p53 pathway [4]. En masse, these observations demonstrate the critical role of p53 in tumor prevention.

In unstressed cells, p53 is constitutively restrained by Mdm2, an E3 ubiquitin ligase that promotes p53 degradation; the *Mdm2* gene is positively regulated by p53, defining a negative feedback loop that controls p53 activity. Cellular stress relieves Mdm2's inhibitory effects, triggering p53 stabilization and activation. Once activated, p53 facilitates DNA repair and inhibits the proliferation of potentially tumorigenic cells, chiefly through instigating cell cycle arrest, senescence or apoptosis.

The p53 response is elicited by a wide variety of stress signals conducive to or associated with malignant transformation, such as DNA damage, oncogene activation, abnormal mitosis, loss of cell-cell contact and hypoxia [5]. Although seemingly dissimilar, many of these signals may actually converge on one another. Biochemically, p53 is a potent transcriptional regulator capable of controlling the expression of hundreds of genes [4–5]. Within this context, it interacts with numerous cofactors and binding partners that modulate its transcriptional output. The p53 gene family includes two additional members, p63 and p73, also acting as transcriptional modulators.

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The great interest in p53 has spawned numerous excellent reviews. Therefore, we will focus only on a limited set of recent studies, pertaining particularly to new functions of p53 and family.

p53 and metabolism

Recent years have seen a renaissance of interest in the links between cancer and metabolism; p53 research is no exception.

p53 engages in an intricate interplay with reactive oxygen species (ROS). Under conditions of mild, physiological oxidative stress, p53 preferentially induces expression of antioxidant genes; when ROS production is aberrantly high, p53 instead activates pro-oxidant genes that may facilitate apoptosis, along with overt proapoptotic genes such as PUMA, Bax and Pig3 [6]*. Antioxidant genes upregulated by p53 include glutathione peroxidase 1 (GPX1), mitochondrial superoxide dismutase 2 (SOD2), aldehyde dehydrogenase 4 family member A1 (ALDH4A1) and sestrin 1 and 2 (SESN1 and SESN2). The induction of antioxidant genes by p53 is likely aimed to minimize the genotoxic danger that even basal levels of ROS pose to DNA. Strikingly, dietary supplementation with the antioxidant N-acetylcysteine (NAC) completely abolished the incidence of lymphoma in p53−/− mice [6], implicating p53's antioxidant function as essential for its tumor suppressing action.

Even under normal physiological conditions, p53 may participate in homeostatic regulation of ROS formation and metabolic processes by maintaining the optimal mode of glucose metabolism and energy boost in response to dips in ATP levels. Reliance on aerobic glycolysis (the Warburg Effect) is a trademark of tumor cells.

The reprogramming of metabolic pathways endows cancer cells with multiple growth advantages. These include better growth in low oxygen conditions and mobilization of pathways that promote nucleotide biosynthesis and production of fatty acids for lipid biosynthesis, necessary for intensive proliferation [7]. p53 antagonizes the Warburg Effect and inhibits glycolysis by decreasing glucose uptake [8], inducing glycolysis-repressing genes [9] and enhancing mitochondrial respiration [10] (Figure 1A).

p53 also indirectly impinges on metabolism via the mTOR pathway (Figure 1B). Besides responding to glucose levels, mTOR senses changes in availability of amino acids, ATP/ AMP and growth factors [11]. AMP-activated kinase (AMPK) is one of the major upstream inhibitors of mTOR activity. Perhaps not surprisingly, AMPK both activates and is activated by p53 in response to energetic stress [12–13]. Other p53 response genes negatively affecting the mTOR pathway include IGF-BP3, PTEN, TSC2, Sestrin1/2 and REDD1 (reviewed in [11]). Thus, p53 leads a multifaceted campaign against the Warburg Effect, both under normal and metabolically challenged conditions. The involvement of p53 in maintaining metabolic homeostasis raises the intriguing possibility that loss of p53 might make cancers more susceptible to drugs that target metabolic pathways.

mTOR is also a negative regulator of autophagy, a process affording cell survival during nutrient starvation by catabolic breakdown of cellular components. Autophagy also contributes to genome stability by destroying potentially harmful cytoplasmic organelles, such as defective mitochondria which otherwise would emit genotoxic ROS. Accordingly, p53 can positively affect autophagy, both by inhibiting mTOR activity and by transactivating pro-autophagic genes such as DRAM [14]*[15]*. Yet, p53 was also reported to inhibit autophagy, particularly under conditions where p53 is cytoplasmic [16]*. To make the picture even more complex, autophagy can promote tumor cell survival under stress, including chemotherapy. Indeed, tumor cells retaining wtp53 may reap a survival advantage from the improved autophagic response endowed by their p53, thereby ingeniously

distorting the anti-cancer apparatus into a pro-cancer machinery. Additional forays into the links between p53 and autophagy will likely be rewarding.

p53 and non-coding RNAs

MicroRNAs (miRNAs) are short non-coding RNA molecules that regulate protein levels by binding to specific mRNAs, inhibiting their translation and often also accelerating their degradation. As is the case for protein-coding mRNAs, miRNA expression patterns are also grossly altered in cancer. p53 modulates the expression of numerous miRNA species, including miR-34a,b and c [17–18]. It may not be coincidental that some of the mRNA species targeted by p53-responsive miRNAs are also directly transcriptionally modulated by p53. In this way, p53 governs either the amplification or fine-tuning of signals that impinge on cell fate. For example, miR-34a contributes to p53-dependent apoptosis, as well as cell cycle arrest and senescence [17–19]. Validated targets of miR-34a include CDK4, Cyclin E2, Bcl2 and c-Met, all of which are also transcriptionally repressed by p53 (Figure 2A). An additional interesting target of miR-34a is the deacetylase Sirt1. Since Sirt1 is a negative regulator of p53, its downregulation by miR-34a defines a positive feedback loop that amplifies p53 activity [20].

Other miRs transactivated by p53 include miR-192, miR-215 [21] and miR-145 [22]. MiRs-192 and -215 both upregulate p21, a canonical p53 target gene product, defining a feed-forward cycle that restricts cell proliferation (Figure 2B). On the other hand, miR-145 downregulates c-Myc [22]*, a proto-oncogene that is also transcriptionally repressed by p53 (Figure 2A).

A cluster of cancer-associated miRNAs, including miR-106b/93/25 and others, is repressed by p53 in an E2F1-mediated manner [23]*. These miRNAs target antiproliferative genes that are themselves E2F1 targets (Figure 2C); accordingly, overexpression of these miRNAs promotes cell proliferation [23]*. By repressing them, p53 tilts the balance towards growth arrest and senescence.

Other classes of non-coding RNA such as large intervening non-coding RNA (lincRNA) are also regulated by p53 [24]. lincRNAs may guide chromatin remodeling factors to target loci or else act together with transcription factors (perhaps also p53?) to modulate pre-existing transcriptional programs. For instance, the p53-induced lincRNA TUG1 facilitates repression of cell cycle-related genes through binding the polycomb repression complex PRC2 [25].

A new twist in the regulation of miRNA expression by p53 was revealed by showing that the DNA-binding domain of p53 binds to the Drosha complex in response to DNA damage. Drosha cleaves primary miRNA transcripts into hairpin structures (pre-miRs) that are subsequently processed into mature, functional miRNAs by another endonuclease complex, Dicer. p53 binding enhances recruitment of Drosha to target precursor miRNA and its processing activity towards a subset of miRNAs [26]**. By modulating Drosha activity, p53 might alter the inventory of pre-miRs available for Dicer operation. Interestingly, one of the miRNAs whose processing is altered in such manner is miR-145, a transcriptional target of p53.

The p53 family and tumor cell invasion

Increased invasiveness of cancer cells is a major driver of metastasis and malignancy. This has not escaped the attention of p53 and its family member p63 (Figure 3). Loss of p53 augments cancer cell invasion [27]; conversely, p53 activation suppresses migration and invasion [28]. This inhibitory effect of p53 is partly mediated by Mdm2, which promotes the

ubiquitination and degradation of Slug and Snail, pivotal transcription factors that drive tumor cell invasiveness [29–30]. The anti-invasive role of Mdm2, a well-established oncogene, is rather intriguing. Or perhaps not; hyperproliferation and invasiveness are emerging as two uncoupled and often opposing properties of cancer cells, underpinning distinct stages in tumor progression. In addition, p53 inhibits invasion by modulation of cell adhesion proteins such as EpCAM [31]. Furthermore, p53-regulated miRNAs such as miR-34a, which downregulates c-Met, Notch1 and Jagged1, also contribute to p53's ability to repress migration and invasion [32–33]. The critical importance of p53's anti-invasive action for cancer suppression is reinforced by recent experiments employing a mouse model of Wnt-driven intestinal carcinogenesis (Y. Ben-Neriah, personal communication).

Like its cousin p53, p63 also modulates adhesion, migration and invasion [34–37]. The picture is confounded by the existence of multiple p63 isoforms, either possessing or lacking the N-terminal transactivation domain (TAp63 and Delta-Np63, respectively), whose transcriptional effects and biological impact are often opposite. It remains to be firmly established how each distinct p63 isoform impinges on tumor cell invasion. Yet, recent work has highlighted a new interesting aspect of this story. About half of all human tumors harbor p53 mutations, often with excessive accumulation of mutant p53 (mutp53) protein. Mounting evidence indicates that such mutp53 proteins acquire cancer-promoting gain of function activities, including promotion of metastasis [38]. It now emerges that the latter may be largely due to inhibition of anti-invasive and anti-migratory effects of p63 by mutp53 [39–40]*. The outcome of this activity of mutp53 is repression of anti-invasion genes [39], enhancement of integrin and EGF receptor (EGFR) recycling [40] driving activation of the EGFR pathway [41], and eventual promotion of metastasis. This might offer an appealing explanation to the observation that p53 mutations often correlate with advanced, invasive stages of tumor progression.

p53 in stem cells and aging- two sides of the same coin?

Recently, there has been a flourish of publications demonstrating that p53 deficiency facilitates reprogramming of differentiated cells into induced pluripotent stem (iPS) cells, closely resembling embryonic stem (ES) cells [42–46]*. The exact nature of the antagonism between p53 and reprogramming pathways is still debated. One possibility is that the iPS procedure indirectly causes DNA damage, driving p53 to activate a barrier of anti-proliferative senescence [47]. Alternatively, explicit pro-differentiation effects of p53 [48] might actively inhibit the iPS program. Indeed, in frog development, p53 interacts with Smad transcriptional regulators to direct embryonic germ layer specification [49]. Interestingly, p53 was reported to protect mouse ES cells from DNA damage not by exerting cell cycle arrest or apoptosis, but by inducing differentiation via suppression of the stem cell (SC)-specific genes Nanog [50] and Oct4 [51].

Adult SCs share with ES cells pluripotency and capacity to self-renew. Yet, these long-lived renewable reservoirs may provide a cellular compartment with increased neoplastic potential. Germline deletion of p53 in mice with critically short telomeres spares damaged SCs from apoptosis and protracts their survival [52–53]. The skin of such p53-deleted mice displays improved wound healing and hair growth, apparently due to increased numbers of epidermal SCs [54]. Similarly, elegant in vivo competition experiments show that p53-deficient mouse hematopoietic stem cells (HSCs) have improved repopulation capacity in transplantation assays, while the outcompeted p53-proficient SCs acquire senescence-like features [55]. Surprisingly, recent analysis of human HSCs [56]* reveals a strikingly different picture. As expected, p53-deficient HSCs better resist radiation-induced apoptosis. However, upon repeated in vivo expansion without acute genotoxic insult, they actually display reduced self-renewal capacity, apparently due to persistent accumulation of

unrepaired DNA damage. Thus, in human HSCs, p53 serves as a positive regulator of self-renewal, by maintaining rigorous genome-integrity quality-control. Beyond illustrating the complexity of the links between p53 and SCs, these findings also raise the alarming possibility that mechanisms of p53-mediated tumor suppression may differ between mouse and human.

Tumors represent rare perturbed clonal outgrowths, whose continuous propagation may rely on a subset of tumor-initiating cells with SC-like properties. In a mouse model of ErbB2-driven breast cancer, cultured p53−/− mammospheres were found enriched for self-renewing "SCs" due to loss of p53 control over asymmetric cell division [57]**. In the hematopoietic system, K-Ras activation instigates a burst of hyperproliferation, but subsequent p53-driven terminal differentiation of stem and progenitor cells provides a p53-dependent barrier against limitless proliferation of undifferentiated leukemia-initiating cells [58]. Prevention of expansion of the cancer-initiating cell pool thus emerges as an important tumor suppressor activity of p53.

The anti-proliferative effect of p53 might not be all advantageous. In fact, mice with hyperactive p53 amass fewer HSCs due to decreased self-renewal capacity, probably accounting for their accelerated aging phenotype [59]; the same may hold for SCs of other tissues. That being said, other studies suggest an anti-aging effect of p53: transgenic mice with only a mild increase in p53 exhibit a reduced rate of age-related oxidative damage, more efficient clearing of DNA-damaged cells and enhanced longevity [60]. Interestingly, in mice and humans, p53 activity declines with age [61], probably contributing to the concomitant increase in cancer frequency. It remains to be determined whether the diminished p53 function promotes aging or, contrarily, the reduced p53 function is a consequence of the aging process.

Conclusion

Since its discovery more than 30 years ago, a massive amount of data has accumulated that attests to the tumor suppressing role of p53. However, as we keep exploring the intricacies of p53 activity, more and more of its diverse functions are cropping up. The field of tumor suppression is experiencing a growing interest in « esoteric » subjects such as metabolism and stem cells. Perhaps, with knowledge from this broader picture, we will also better understand the workings of cancer cells.

Acknowledgments

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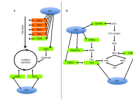
Figure 1. Metabolic regulation by p53
(A) p53 transcriptionally induces numerous inhibitors of mTOR, thus negatively affecting cell growth; (B) p53 inhibits glycolysis and facilitates oxidative respiration via transcriptional regulation of relevant genes. p53-induced genes are colored green; p53-repressed genes are red.
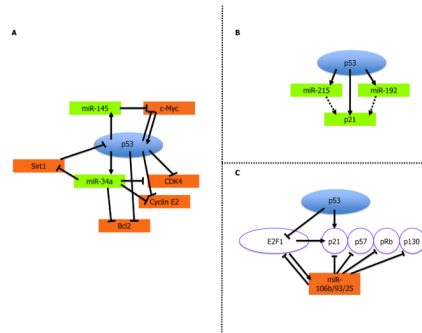
Figure 2. miRNAs in the service of p53
(A) p53 induces the expression of miRNAs that target p53-repressed genes; (B) p53 induces the expression of miRNAs that enhance p53 activity output by augmenting p21 levels; (C) p53 represses miRNAs that target E2F1-induced antiproliferative genes, thereby restricting cell proliferation. See text for further details.

Figure 3. p53 and p63 inhibit cell migration, invasion and metastasis
p53 and p63 transcriptionally activate inhibitors of invasion and repress enhancers of invasion. Mutp53 inhibits p63, thereby abrogating its anti-invasion activity.