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Deciphering p53 Signaling in Tumor Suppression

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Abstract

The p53 transcription factor is mutated in over half of human cancers, and *p53*-null mice are highly predisposed to cancer, highlighting p53's essential role in tumor suppression. Studies in mouse models have revealed that p53 cell cycle arrest and apoptosis responses to acute DNA damage signals are dispensable for tumor suppression, prompting a search for new mechanisms underlying p53-mediated cancer suppression. p53 responds to other types of stress signals and regulates a host other cellular processes, including maintenance of genomic stability, metabolism, stemness, non-apoptotic cell death, migration/invasion, and cell signaling, any or all of which could be fundamental for suppressing carcinogenesis. The ability of p53 to govern numerous transcriptional programs and cellular functions likely explains its potent tumor suppressor activity.

Introduction

The gene encoding the p53 transcription factor is mutated in over half of all human cancers, reflecting its crucial role in preventing cancer [1]. This key role as a tumor suppressor is supported by observations from mouse models, where p53 inactivation results in a rapid, fully-penetrant tumor predisposition [2]. Early studies on p53 revealed that it plays a fundamental role in stress responses, especially in triggering cell cycle arrest or apoptosis in response to acute DNA damage signals [1,3]. The p53-mediated cell cycle arrest response was envisaged to allow cells an opportunity to arrest to repair DNA damage before proceeding through the cell cycle and to thereby prevent the propagation of oncogenic mutations, while the apoptotic response was proposed to eliminate damaged or neoplastic cells. p53 was shown to trigger these responses by inducing specific downstream target genes including the CDK inhibitor *p21*, and the pro-apoptotic Bcl-2 family members *Puma* and *Noxa*, which are important for DNA-damage-induced cell cycle arrest and apoptosis, respectively [1,3]. These responses provided reasonable initial explanations for the mechanisms underlying p53-mediated tumor suppression. In support of such mechanisms, evidence from various mouse tumor models has suggested that p53 restricts proliferation and

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triggers apoptosis in developing tumors [reviewed in 3]. As will be described in this review, however, the picture of p53-mediated tumor suppression is much more complex than envisaged originally.

p53 Acute DNA Damage Responses Are Dispensable for Tumor Suppression

In recent years, a series of studies interrogating the requirement of p53 acute DNA damage programs for cancer suppression has challenged the importance of these responses for p53-mediated tumor suppression. The first set of such studies used mice expressing temporally-regulatable versions of p53 to demonstrate that the presence of p53 during exposure to acute DNA damage is dispensable for inhibiting tumorigenesis, and that instead the ability of p53 to respond to oncogenic signals as tumors are developing is most fundamental for tumor suppression [4–6]. This idea was elaborated upon by analyses of *p53* knock-in mouse strains expressing mutants in the first, second or both of two p53 transcriptional activation domains (TADs). Analysis of the TAD1/2 $p53^{25,26,53,54}$ mutant showed first that transcriptional activation potential is critical for p53-mediated tumor suppression [7]. Moreover, these studies revealed a selective activity of the $p53^{25,26}$ TAD1 mutant, which cannot mount responses to acute DNA damage yet is completely effective as a tumor suppressor, indicating that the capacity of p53 to drive cell cycle arrest or apoptosis in response to acute genotoxic stress is dispensable for tumor suppression and that the transcriptional programs responsible for acute DNA damage responses and tumor suppression are distinct. This notion was supported by the generation of another mouse knock-in strain expressing the $p53^{3KR}$ mutant, in which 3 acetylation sites in the p53 DNA binding domain were mutated, rendering the protein unable to induce classical p53 target genes and responses to DNA damage [8]. This protein was nonetheless able to suppress spontaneous tumor development in mice. Finally, an additional study focused on the key effectors for DNA-damage-induced cell cycle arrest and apoptosis – *p21, Noxa,* and *Puma* [9]. Mice lacking these three proteins displayed defective DNA damage responses yet again were not prone to spontaneous tumor development. Collectively, these studies prompted a paradigm shift in the field, as they questioned the significance of the p53 responses to acute DNA damage for tumor suppression, and opened the door to the search for new mechanisms underlying p53-mediated tumor suppression.

If Not Acute DNA Damage Responses, Then What?

If the p53 pathways important for acute DNA damage responses are nonessential for tumor suppression, then how does p53 work? As mentioned, there is ample evidence from various mouse tumor models that p53 inhibits cell division and induces apoptosis in tumors *in vivo*, yet the aforementioned studies suggest that p53-dependent acute DNA damage responses and tumor suppression can be uncoupled. How can these discrepancies be reconciled? We will consider several potential explanations, which are not necessarily mutually exclusive (Figure 1):

1. ***Plasticity in p53 pathways.*** It may be that when p53 DNA damage response pathways are perturbed, compensatory pathways allow p53 to still function to

suppress cancer. Thus, in such a scenario, the DNA damage response pathways are not unimportant, but there is sufficient robustness in the system to compensate for their disruption. Importantly, cell cycle arrest and apoptosis responses to acute DNA damage are effectively perturbed in the models described above, thus any compensation in developing tumors would have to rely on other pathways.

2. ***Cell-type or context-specific differences.*** It may be that classical p53 DNA damage response pathways are unimportant in some contexts, but are essential for the suppression of some tumor types driven by specific initiating events or originating in particular tissues. Indeed, *p21* and *Puma* are required for tumor suppression in certain instances, although even in these cases, they only account for a fraction of p53 activity [10,11].

3. ***Importance of other non-canonical p53 activities for tumor suppression.*** It may be that acute DNA damage response pathways are truly dispensable for tumor suppression *per se*, and that instead, other p53 signaling pathways or p53 cellular functions are more critical for tumor suppression (Figure 2). For example, activation of p53 by stress signals more relevant to the tumor microenvironment, such as chronic low-dose DNA damage or hypoxia could be relevant to tumor suppression (Box 1). It could also be that the ability of p53 to regulate other cellular processes, such as genomic stability, metabolism, and stemness is most critical for tumor suppression (see below). However, it is important to note that p53-mediated regulation of cellular processes must ultimately impact tumor growth, and therefore would be expected to have an effect on proliferation and survival, but presumably through transcriptional networks distinct from those used during acute DNA damage responses. This concept is consistent with the observed effects of p53 on proliferation index and cell viability in tumor models *in vivo* [reviewed in 3]. In addition, the fact that *p53,* rather than individual p53 target genes, is so commonly mutated in cancer suggests that p53 likely controls a multitude of cellular programs that coordinately suppress cancer. We will briefly summarize what is known about these other non-canonical functions next, to highlight the variety of cellular processes p53 can regulate.

An Array of Cellular Functions Regulated by p53

Beyond inducing cell cycle arrest, senescence, and apoptosis in response to acute DNA damage, p53 also regulates various other aspects of cellular behavior (Figure 2). Given the plethora of target genes that p53 regulates, the ability of p53 to control many cellular processes is anticipated, and the coordinate regulation of many different gene expression programs presumably underlies p53's potent tumor suppressor activity. Specifically, p53 has been implicated in the following processes:

Maintaining Genomic Stability

p53 has long been known as the "guardian of the genome" based on its role in acute DNA damage responses. However, beyond this role, an explosion of recent studies has shown that

p53 can maintain genomic integrity through additional mechanisms. First, p53 transactivates various DNA repair genes and directly controls different forms of DNA repair, including mismatch repair, base excision repair, and nucleotide excision repair [12]. Second, p53 responds to abnormal ploidy by driving cell cycle arrest, which unlike DNA damage-induced arrest, is triggered by the Hippo pathway, yet is still mediated at least in part via p21 [13]. Without p53, tetraploid cells continue to divide, giving rise to cells with whole-chromosome aneuploidies and structural rearrangements, fueling carcinogenesis. Third, recent reports suggest that p53 promotes genomic integrity preemptively by enhancing DNA replication fork progression, as p53 deficiency provokes replication fork collapse and genomic instability [14,15]. Different mechanisms have been proposed for this p53 function, one linked to transactivation of *Mdm2*, which itself promotes replication fork progression [14], and the other to p53 preventing DNA topological stress caused by transcription, which perturbs fork progression [15]. Finally, a number of studies have suggested that p53 preserves genomic integrity by restricting the movement of transposons and repetitive elements, which might reflect an evolutionary function of p53 [16,17]. Accordingly, it was shown recently that in Drosophila or zebrafish lacking p53, as well as in p53-deficient cancers, retrotransposon expression is de-repressed. While not well-understood at the molecular level, one experiment suggested that p53 induces H3K9me3 marks to dampen retrotransposon expression. Notably, the inability of p53 human tumor-derived mutants to restrain mobile element expression bolsters the idea that this activity could represent a key tumor suppression mechanism. Important *in vivo* support for the idea that loss of p53-enforced genome stabilization promotes cancer comes from studies of *p53-null* mice. These mice primarily develop thymic lymphomas, which display a high frequency of copy number variations that drive specific oncogenic events important for tumorigenesis [18].

Maintaining Epigenetic Stability

p53 has also been described as a guardian of the epigenome [19,20]. Recent studies in embryonic stem (ES) cells showed that p53 represses the *de novo* DNA methyltransferases Dnmt3a and 3b and activates Tet1 and Tet2, enzymes that promote DNA demethylation. p53 contributes to DNA methylation homeostasis and preserves clonal homogeneity, as p53-deficient ES cells exhibited globally elevated DNA methylation as well as enhanced intra-clonal heterogeneity. Similar global hypermethylation in thymii of thymic lymphoma-prone *p53*-null mice supports the idea that such destabilization of the epigenome could contribute to tumorigenesis [20].

Dampening Metabolic Reprogramming

Cancer cells typically undergo metabolic reprogramming to ensure adequate production of essential building blocks fundamental for rapid growth [21]. p53 dampens this cellular rewiring by inhibiting glycolysis through repression of genes such as the *Glut1* and *Glut4* glucose transporters and by promoting mitochondrial oxidative phosphorylation via activation of genes such as *Sco2* [22]. p53 also helps to limit a damaging oxidative environment, by inducing genes involved in antioxidant programs, including sestrins. Notably, the aforementioned tumor suppression-competent $p53^{3KR}$ mutant retains the capacity to activate certain metabolic and antioxidant target genes, supporting the idea that these functions could contribute to p53-mediated tumor suppression [8]. p53 further

promotes cellular homeostasis by inducing autophagy, which results in degradation of damaged organelles and proteins, and has been associated with transformation suppression [23]. Importantly, the ability of p53 to regulate metabolism also helps to enhance cell survival, thus the full complexity of the p53 metabolic role in cancer remains to be elucidated [22].

Restraining Stemness/Promoting Differentiation

p53 limits a variety of aspects of stem cell behavior, including proliferation and self-renewal, and enhances differentiation [24,25]. The significance of this program in cancer is underscored by the observation that *p53* mutation promotes a stem cell signature in breast and lung cancers [26]. In addition, p53 limits cellular reprogramming, as in the generation of induced pluripotent stem cells (iPSCs) from differentiated cells, again supporting the idea that an aspect of p53 tumor suppressor function is to restrain plasticity and promote differentiation [27]. Indeed, *p53* mutations similar to those arising in cancer are observed during the generation of human iPSCs in culture [28]. Beyond acting through the target genes *p21* and *miR34a* [29,30], p53 can also restrict cellular plasticity through another target gene, the non-coding RNA *Neat1*. Consistent with observations that *Neat1* is induced during differentiation in various cell types [31], recent studies have revealed that *Neat1* safeguards the proper expression of differentiation programs and blocks transformation of pancreatic acinar and ductal cells into premalignant lesions in a mouse pancreatic cancer model [32].

Inducing Non-apoptotic Cell Death

Efforts to elucidate how the tumor suppression-competent $p53^{3KR}$ mutant might suppress cancer led to the identification of ferroptosis, an iron-dependent, non-apoptotic form of cell death as a potential mechanism for p53-mediated tumor suppression [33]. Like wild-type p53, $p53^{3KR}$ suppresses the expression of SLC7A11, a cystine/glutamate antiporter whose inhibition triggers ferroptosis by causing reduced glutathione production and a consequent accumulation of detrimental lipid ROS. While $p53^{3KR}$ can induce ferroptosis and suppress cancer, $p53^{4KR}$, a mouse p53 mutant bearing an additional lysine mutation in residue 98, cannot induce ferroptosis or suppress xenograft tumor growth, correlating ferroptosis and tumor suppression [34]. Moreover, a p53 polymorphic variant, $p53^{S47}$, is deficient in regulating ferroptosis genes, inducing ferroptosis, and suppressing tumorigenesis, again linking these processes [35]. However, other studies suggest that p53 may delay, rather than promote, ferroptosis, suggesting a potential context-dependency to this pathway [36].

Inhibiting Motility and Invasiveness

p53 restricts migration and invasion in *in vitro* systems, suggesting a potential role for p53 in impeding malignant progression [37]. One mechanism through which p53 inhibits migration is by attenuating epithelial-mesenchymal transition (EMT) by transactivating *miR-200c* and *miR34a*, microRNAs capable of silencing EMT drivers, including Zeb1 and Snail [38,39]. In addition, p53 restrains RhoA/ROCK signaling, which is critical for migration and invasion, through transcriptional activation of *RhoE* and *Notch* [37]. p53 also hinders the formation of invadopodia, structures that degrade the extracellular matrix to permit invasion, by transactivating *miR-143* and *Cald1* [37]. Interestingly, mouse models suggest that lung tumors are more metastatic without p53, but that additional stochastic events beyond p53

loss must occur to enable metastasis [40]. Thus, additional studies are needed to better understand p53 function in these processes in different *in vivo* contexts.

Promoting TME Signaling

p53 stimulates the secretion of molecules that direct changes in the tumor microenvironment. For example, p53 signals to immune cells to infiltrate tumors and assist with tumor suppression by attacking and clearing tumor cells [41,42]. In addition, p53 inhibits angiogenesis by such mechanisms as transactivating *Thrombospondin1*, thus attenuating tumor growth and metastasis [43].

Repressing Oncogenic Signaling

One potential mechanism through which p53 might exert pleiotropic effects on cell behavior is through regulation of pathways that themselves exert widespread effects. For example, p53 has been shown to restrain the oncoprotein c-myc, through activation of *miR-145* [44]. Recent studies have also demonstrated that p53 transactivates *Ptpn14* to inhibit the oncoprotein Yap, a Hippo pathway effector that promotes various pro-tumorigenic phenotypes such as proliferation, migration and invasion [45].

Integrating the Pieces of the Puzzle

The aforementioned studies demonstrate that p53 regulates many processes that could in principle contribute to tumor suppression (Figure 3). Understanding the relative contributions of these different effects of p53 to tumor suppression requires the identification of specific p53 target genes involved in each of these pathways and a genetic interrogation of such components for cancer suppression. Adding to the complexity of deciphering tumor suppression pathways is the notion that p53 governs a coordinated program in which multiple functions are co-regulated to enforce tumor suppression, a compelling hypothesis but one that is difficult to test from a technical point of view. If p53 does indeed activate various cooperating pathways to impede tumorigenesis, then its loss would have highly pleiotropic effects and explain why *p53* is so commonly mutated in cancer, while mutations in p53 target genes are less frequent. It is also possible that there may be tissue-specificity in terms of which p53-regulated responses are most crucial for tumor suppression in a given setting, requiring analyses to be performed in a diversity of tumor contexts. Deconvoluting the molecular basis for p53-mediated tumor suppression thus remains a complex but fascinating endeavor which will ultimately fuel new therapeutic development.

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Box 1

p53 Responses to Chronic Stress Signals

Many studies have focused on understanding p53 responses to acute DNA damage and the short-term effects on cell cycle progression or survival in the face of such potent stress signals. Such studies have defined *p21* as a key mediator of cell cycle arrest and *Puma* and *Noxa* as critical mediators of apoptosis. While these approaches may be very relevant for addressing mechanisms of therapeutic responses, they may not be the best strategy for understanding p53 function in tumor suppression, where chronic, lower-dose stresses may be more relevant for eliciting p53 responses in nascent tumors. These include cell-intrinsic stresses such as low-dose chronic DNA damage resulting from telomere attrition, accumulation of ROS, and replication fork collapse, all of which can emerge as incipient cancers proliferate. Indeed, the idea that p53 does respond to some type of DNA damage during tumor suppression is consistent with studies detailing the activation of DNA damage cascades during the genesis of human tumors [46]. p53 may also be activated by cell-extrinsic microenvironmental stresses such as hypoxia and nutrient starvation, which can be triggered by the abnormal vascularization of tumors.

Some evidence supports the idea that in conditions of chronic stress, p53 may act through pathways distinct from those mapped in studies of responses to acute genotoxic stress. Specifically, comparative analyses of the mechanisms of p53-induced cell cycle arrest in response to acute and chronic DNA damage revealed that the $p19^{Arf}$ protein is critical only in the latter case, highlighting mechanistic differences in the pathways [47]. In addition, hypoxia-induced, p53-dependent apoptosis has been proposed to rely predominantly on p53 repression function [48]. While p53 target genes involved in chronic responses remain to be interrogated, these studies underscore the idea that acute and chronic responses have disparate aspects. In future studies, it will be crucial to map pathways of p53 action in contexts most closely recapitulating p53 tumor suppressor function *in vivo*.

Highlights

- p53 responses to acute DNA damage signals are dispensable for tumor suppression.
- p53 transcriptional activity is critical for tumor suppression.
- p53 responds to diverse stresses and regulates a variety of cellular processes.
- Tumor suppression likely relies on p53 coordinately regulating various processes.

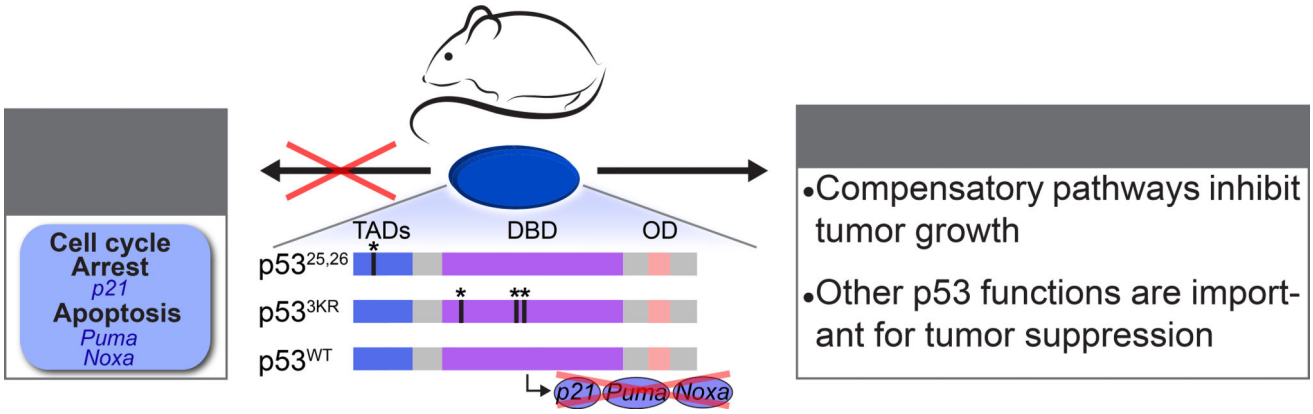

Figure 1. p53 acute DNA damage responses and tumor suppression

Analysis of different p53 mouse strains, including the p53 transactivation domain 1 *Trp53*^{25,26} mutant strain, the acetylation site *Trp53*^{3KR} mutant strain, and the triple knockout *p21*$^{-/-}$;*uma*$^{--}$;*Noxa*$^{-/-}$ strain, demonstrated that p53-induced cell cycle arrest and apoptosis responses to acute DNA damage are dispensable for tumor suppression. Instead, there may be compensatory pathways that are engaged in the absence of acute DNA damage response pathways to allow tumor suppression. Alternatively, acute DNA damage response pathways may be truly dispensable for tumor suppression, and other p53 signaling pathways and downstream p53 functions are responsible for p53 tumor suppressor activity. TAD- transactivation domain, DBD- DNA binding domain, OD- oligomerization domain.

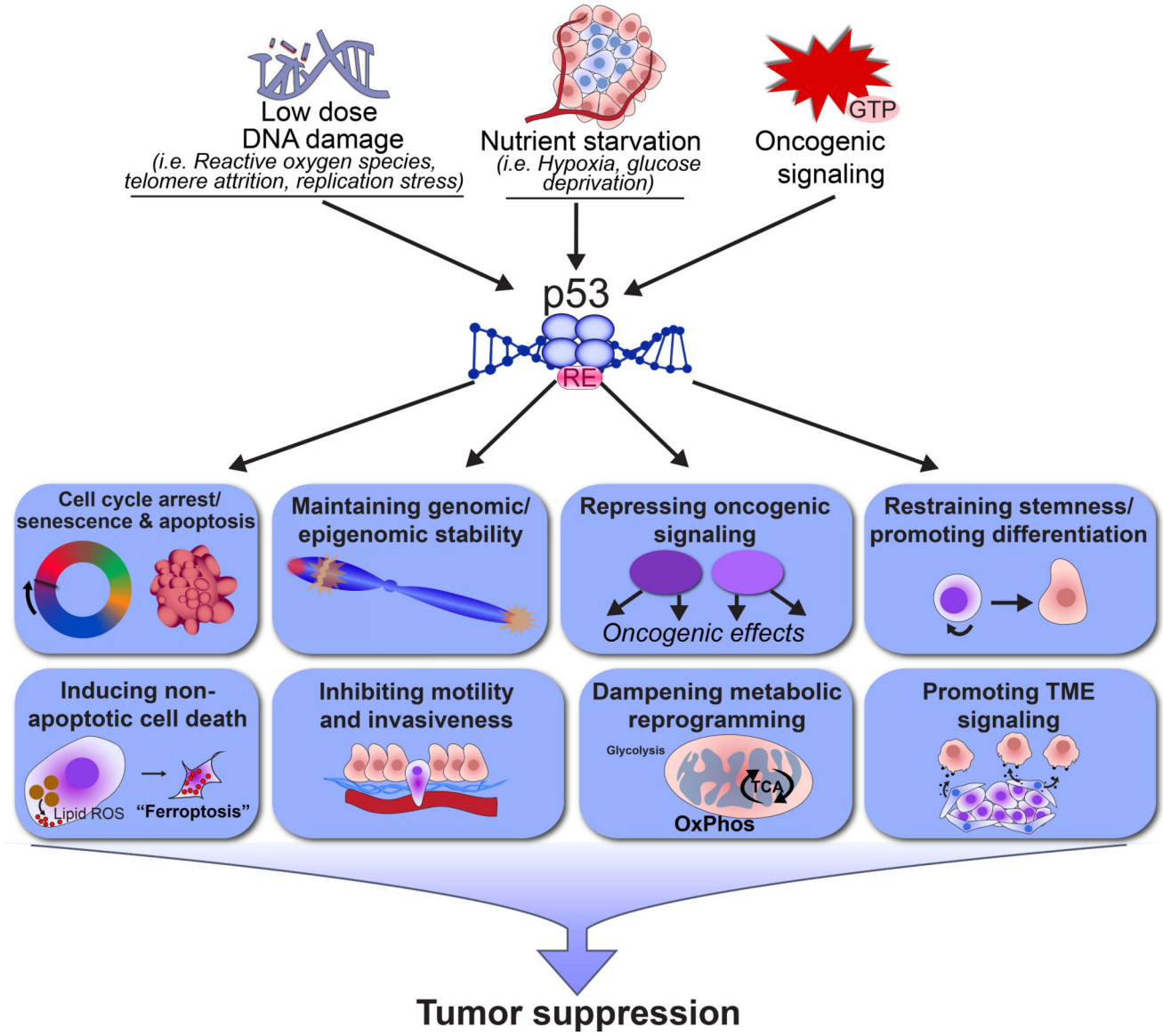

Figure 2. Overview of p53 signaling pathways in tumor suppression

Different stress stimuli relevant to tumor development *in vivo*, such as chronic low-dose DNA damage, nutrient starvation, and oncogenic signaling activate p53. In response to such signals, p53 binds to specific DNA response elements (REs) and regulates gene expression programs to modulate different cellular processes, thereby leading to tumor suppression.

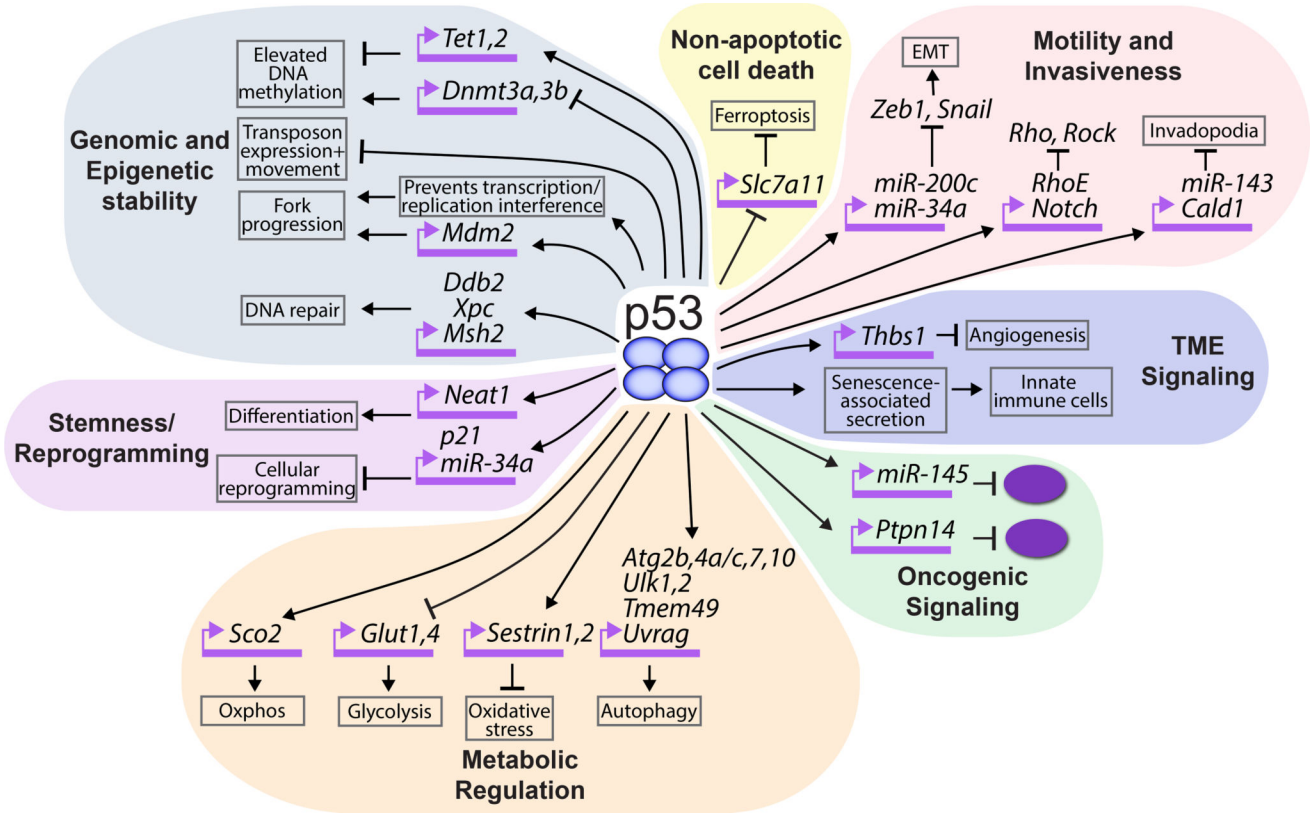

Figure 3. Detailed schematic of non-canonical p53-regulated cellular processes and p53 target genes implicated in these processes

The various non-canonical p53-modulated cell biological processes that may contribute to tumor suppression are depicted. The figure focuses on p53-regulated pathways described in the text and some direct p53 target genes involved in these pathways; it is not meant to depict every reported gene that may participate in each pathway.