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How do I kill thee? Let me count the ways: p53 regulates PARP-1 dependent necrosis

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Abstract

Understanding the impact of the p53 tumor suppressor pathway on the regulation of genome integrity, cancer development, and cancer treatment has intrigued scientists and clinicians for decades. It appears that the p53 pathway is a central node for nearly all cell stress responses, including: gene expression, DNA repair, cell cycle arrest, metabolic adjustments, apoptosis, and senescence. In the past decade, it has become increasingly clear that p53 function is directly regulated by poly(ADP-ribose) polymerase-1 (PARP-1), a nuclear enzyme involved in DNA repair signaling. Here, we will discuss the impact of PARP-1 on p53 function, along with a recently described novel role for the reciprocal regulation of p53 regulated, PARP-1 dependent necrosis following DNA damage.

Keywords

apoptosis; necrosis; p53; PARP-1; ROS

Introduction

The p53 signaling pathway is undoubtedly one of the most relevant and highly studied pathways in cancer biology – as evidence, a PubMed search at time of publication using “p53” as a key term identifies nearly 70,000 publications. When discovered approximately 30 years ago, p53 was identified as a 54 kDa protein that interacted with SV40 virus large T antigen in transformed cells [1, 2]. This discovery was also accompanied by the observation that large T antigen regulated p53 protein levels, sparking suggestions that p53 was an oncogene that positively influenced cellular transformation via unknown mechanisms [3].

A decade later, the notion that p53 functioned as a tumor suppressor, and not an oncogene, surfaced. This novel concept was based on several studies which identified that mutant forms of p53 are present in both human cancer cells and tumors [4–6]. In the years that

followed, the role of p53 in tumorigenesis became a topic of high interest, and the notion that p53 functions within a tumor suppressor pathway was widely accepted.

Another seminal observation in the p53 field that sparked decades of intense research came from a 1990 study that identified Li-Fraumeni Syndrome patients carry a germ line mutation in the p53 gene, *tp53*. These patients develop a wide variety of tumors throughout their lifetimes with nearly 100% penetrance [7]. Studies in the years that followed revealed that similar p53 mutations were present in as high as 50% of all human cancers, further supporting the notion that p53 plays a key role in the development of cancer. While the role of the p53 pathway in preventing tumorigenesis is well established, the mechanisms by which p53 functions as a tumor suppressor remain controversial and highly debated.

At present, the p53 protein is often referred to as “the guardian of the genome” due to its pivotal role in regulating the cellular responses to multiple stress signals, including oncogene activation and DNA damage. Transcriptional induction, along with post-translational modifications of the p53 protein leading to increased stabilization, allow p53 to increase function in both the nucleus and cytoplasm to dictate the cellular phenotype after stress [8]. As the p53 pathway is highly studied, there are numerous cellular phenotypes attributed to its activation, including apoptosis, autophagy, cell cycle arrest, metabolic changes, and senescence. The final outcome of p53 activation depends largely on the initial stress, but also on the cellular milieu and availability of growth factors and/or nutrients at the time of stress. For example, how many DNA damage events have occurred in the recent past? Is the cell competent to induce cell cycle arrest or autophagy? Does the stressed cell over-express anti-apoptotic proteins that prevent cell death leading to the maintenance of dangerous mutations that promote cancer?

While many years of cancer research have focused on understanding the cellular mechanisms of p53-dependent regulation of apoptotic cell death, there is an emerging literature suggesting that p53 can kill a cell in more than one way (Fig. 1). A recent paper by Montero et al., in *Cell Death and Differentiation* describes a novel observation linking a DNA damage response protein, poly(ADP-ribose) polymerase-1 (PARP-1), and the necrotic cell death machinery [9]. Here, we will discuss the PARP-1 and p53 pathways, along with providing context to better understand how PARP-1 signaling and the necrosis pathways may functionally intersect to kill cancer cells. For detailed discussion on the p53 and PARP-1 pathways, please see reviews by Vousden et al., and Poirier et al., respectively [10, 11].

p53 induces multiple cell death pathways

Cell death is a physiological process that is essential for regulating metazoan development, tissue homeostasis, and eliminating cells faced with irreparable damage. Aberrations in the cell death pathways are implicated in many human diseases including cancer, autoimmunity, degenerative disorders, and stroke. There are several distinct pro-death mechanisms that a cell can activate after exposure to irreparable stress. Indeed, it is not infrequent that multiple pro-death pathways are initiated within a population of cells, or even within a single cell, and the final outcome is dependent upon the concerted interplay between these pathways and the cellular environment. Numerous biochemical, cellular, and genetic studies have provided much insight into the mechanisms governing apoptotic and autophagic responses. On the other hand, necrosis has long been thought to simply be a passive cellular downfall in response to excessive stress and damage. While it has become increasingly clear throughout the literature that p53 plays a crucial role in mediating apoptotic and autophagic responses to cell stress, p53's role in regulating necrosis is yet to be fully defined. We will briefly discuss

these pathways below, but it is important to keep in mind that other modes of cell death also exist.

Autophagy and cell death

Autophagy is regulated by signaling pathways that are intimately linked to cellular metabolism, and directly coordinated by the cell survival and growth machinery. In recent years, the role of p53 in autophagy regulation has been a topic of high interest. In response to DNA damage, it is thought that p53 can induce an autophagic response through both transcriptional and non-transcriptional regulation of downstream signaling components of the mTOR and PI3K pathways [13, 14]. Depending on the type of stress and duration, as well as the cell type, p53-dependent autophagy can have both growth promoting and death promoting consequences. In a *c-myc* driven lymphoma model, p53 was shown to promote cellular survival. Inhibition of autophagosome formation in this model resulted in p53-induced apoptosis of tumor cells [15]. A study by Tasdemir et al. [16], added further complexity to the notion of antagonistic pleiotropic functions of the p53 pathway by showing that genetic or pharmacological inhibition of p53 increased autophagy in normal and transformed cells (in the absence of any additional cellular stress). Interestingly, this effect was independent of transcriptional regulation by p53 as the introduction of exogenous nuclear p53 did revert autophagic responses in *trp53*-deficient cells. This work suggested that under unstressed cellular conditions, basal p53 could actively inhibit autophagy; yet when a cell encounters DNA damage or oncogenic stress, the cellular response leading to the p53 pathway would actively promote autophagy. Importantly, these works not only highlight the differences between basal and activated p53, but also reveal that the downstream consequences of p53 activation depended heavily on the nature and extent of upstream stress triggers.

Apoptotic cell death

There is a significant literature describing the role of p53 in mediating cellular sensitivity to apoptosis, along with directly regulating numerous critical components of the extrinsic and intrinsic pathways. In response to cellular stressors such as DNA damage and oncogene activation, p53 is phosphorylated, activated by a host of additional post-translational modifications, and stabilized. Subsequently, the protein accumulates in the nucleus, directly binds p53 response elements within the genome, and recruits transcriptional co-regulators to up-regulate the expression of multiple pro-apoptotic BCL-2 family members (e.g. BAX and PUMA), core components of the apoptosis machinery (e.g. caspases and APAF-1), and several components of the death receptor signaling complexes (e.g. CD95/FAS). While the main function of p53 is to alter gene expression leading to cell cycle arrest, apoptosis, or senescence, several studies have suggested that p53 may also possess cytoplasmic functions; for example, interactions between p53 and the BCL-2 family are well documented. The p53 protein can directly bind and inhibit anti-apoptotic members of the BCL-2 family, as well as directly activate the pro-apoptotic effectors BAK and BAX at the outer mitochondrial membrane, which leads to permeabilization of the membrane and the initiation phases of caspase activation [18].

Necrotic cell death

Many intracellular molecular events can lead to bioenergetic failure and subsequent necrosis. These include increases in mitochondrial ROS production (leading to DNA damage), inhibition of ATP production, and activation of proteases such as the calpains and cathepsins that cleave numerous cellular substrates [19]. In the majority of normal cells, ATP generation occurs due to complementary efforts between glycolysis, the Krebs cycle,

and oxidative phosphorylation. In rapidly proliferating cells or in tumor cells, ATP production becomes highly dependent on glycolysis; and the inhibition of glycolysis can induce necrosis due to marked ATP depletion. From an alternative perspective, there are stress-induced pathways that consume enormous amounts of metabolic precursors that are essential for ATP generation, which causes a precipitous decrease in cellular ATP leading to necrosis. An example of such a pathway is mediated by PARP-1, which is activated by DNA lesions and rapidly consumes nicotinamide adenine dinucleotide (NAD⁺) leading to reduced mitochondrial ATP generation and necrosis. Next, we will discuss the PARP-1 signaling mechanisms and how they functionally intersect with the p53 pathway.

How does PARP-1 activation result in cell death?

The PARP-1 pathway is important for cellular differentiation, proliferation, and genomic homeostasis; and these processes depend upon PARP-1's ability to gain enzymatic activity following the recognition of DNA damage. PARP-1 is a nuclear enzyme that is rapidly recruited to sites of DNA nicks and double stranded breaks, and it functions to post-translationally modify a host of substrates involved in DNA damage repair. Activated PARP-1 produces branched poly(ADP-ribose) polymers that stabilize the surrounding chromatin and allow for the recruitment of DNA repair machinery [20]. Under conditions of severe DNA damage and genomic stress, PARP-1 over-activation can lead to cell death by at least two pathways. In the first pathway, it is thought that poly(ADP-ribose) polymers induce a form of cell death termed *parthanatos* that signals through mitochondrially regulated caspase-independent pathways [21]. The second pathway is the focus in Montero et al., in which the hyper-activation of PARP-1 results in the depletion of cytosolic NAD⁺ reserves resulting in a dramatic reduction of cellular ATP levels (Fig. 2). In parallel to reduced ATP, the nucleotide pools ratios (i.e. AMP:ATP) are subsequently disrupted, which is described to activate the AMP-activated protein kinase contributing to necrotic cell death.

How is p53 regulated by PARP-1?

Both p53 and PARP-1 are named “guardians of the genome” due to their functions in maintaining genomic integrity. In the late 1990s, studies from Vaziri et al., and Kumari et al., were the first to demonstrate that a functional interaction between the p53 and PARP-1 pathways was crucial for maintaining genomic integrity (Fig. 3). In one of these papers, p53 and PARP-1 were found to interact both in vitro and in vivo in aging cells undergoing telomere shortening [22]. Although the precise molecular changes resulting from this interaction were not described, PARP-1 binding was shown to be critical for p53 activation and function in these cells. In the second paper, a molecular interaction between p53 and PARP-1 was demonstrated in cells undergoing apoptosis in response to DNA damage, independent of any poly(ADP-ribosylation) of p53 [23]. More recently, p53 was shown to be poly(ADP-ribosylated) following DNA damage in neurons, which impacted on p53 transcriptional regulation [24]. Furthermore, p53 stabilization and transcriptional activity are mediated by PARP-1 as evidenced by inhibition or genetic deletion of PARP-1; but this may be limited to irradiation-induced DNA damage [25, 26]. It seems that the functional consequences of the p53 · PARP-1 interaction largely depend on the severity of the cellular stress. If a cell is to undergo DNA repair following an acute stress, poly(ADP-ribosylation) of p53 may occur in order to prevent the induction of apoptosis. However, in a situation of extreme genomic stress, the intersections between PARP-1 and p53 may enhance p53-dependent apoptosis.

While these studies establish a role for PARP-1 in regulating p53-mediated functions, a reciprocal relationship for the two proteins has not been described until now. In Montero et al., the authors propose that under certain cellular conditions, p53 can positively regulate

PARP-1 activity leading to PARP-1-dependent necrosis. As discussed above, it is recognized that DNA damage can induce at least two distinct mechanisms of cell death (e.g. p53 induced apoptosis and PARP-1 induced necrosis), and recent evidence suggests that there may be important crosstalk between the critical mediators of these seemingly distinct modes of cell death.

Does the p53 pathway regulate PARP-1 activity and necrosis?

In Montero et al., the authors first observed that p53 null mouse embryonic fibroblasts (*trp53*^{-/-} MEFs) demonstrated resistance to hydrogen peroxide (H₂O₂) induced cell death, which was in contrast to wild type MEFs. The study also examined MEFs derived from animals that cannot induce the mitochondrial pathway of apoptosis (*bak*^{-/-}*bax*^{-/-} MEFs), and these cells remained fully responsive to H₂O₂-induced cell death. These first observations suggested that H₂O₂-induced cell death was directly regulated by p53, but independent of the intrinsic apoptotic pathway. While H₂O₂ treated *trp53*^{-/-} MEFs displayed a decrease in proliferation, they maintained long term, clonogenic survival suggesting a true inhibition of cell death signaling.

How do *trp53*^{-/-} MEFs resist H₂O₂-induced cell death? As a starting point, the authors speculated that H₂O₂ caused DNA lesions via reactive oxygen species (ROS) generation, and indeed all of the MEF lines presented with equivalent levels of DNA damage confirming that survival was not due to a lack of DNA lesions (or sensing the lesions) in the *trp53*^{-/-} MEFs. In response to high levels of DNA damage, as discussed earlier, PARP-1 activation leads to the marked poly(ADP-ribosyl)ation of cellular substrates that results in NAD⁺ depletion and subsequent cell death. The authors confirmed that H₂O₂ treatment resulted in PARP-1 activation followed by subsequent decreases in NAD⁺ and ATP in wild type MEFs. Curiously, while H₂O₂ treatment of all the MEF lines resulted in DNA damage and subsequent NAD⁺ depletion, only the *trp53*^{-/-} MEFs recovered cellular NAD⁺ levels within a few hours, and this observation suggests that the loss of p53 likely regulated the function of PARP-1. Indeed, the inhibition of PARP-1 in wild type and *bak*^{-/-}*bax*^{-/-} MEFs also rescued ATP depletion.

In a panel of MEFs with epigenetic and genetic removal of p53 function, as well as human cancer cell lines with varying *trp53* genotypes, the authors demonstrated that the loss of p53 had no effect on PARP-1 protein expression; however, the absence of p53 decreased overall PARP-1 enzymatic activity thereby likely protecting against PARP-1 mediated necrosis. In these model systems, pharmacological inhibition of PARP-1 activity resulted in a switch to apoptotic cell death in response to H₂O₂ treatment. This switch, however, was not observed in the absence of p53, indicating that both the necrotic and apoptotic responses to ROS-induced DNA damage are p53 regulated in these systems. In summary, the take home messages of this work are: (i) the presence of p53 leads to hyper-activation of PARP-1 following DNA damage, and this leads to rapid NAD⁺ depletion, loss of ATP, and necrosis; (ii) pharmacological inhibition of PARP-1 rescues ATP loss in wild type cells, but leads to apoptotic cell death; and (iii) the loss of p53 renders cells resistant to both DNA-damage induced apoptosis and necrosis.

Conclusions and outlook

While the regulation of PARP-1 activity by p53 described in Montero et al., is novel, a role for p53 in the regulation of necrotic cell death has been previously suggested in the literature. In a 2009 study, Tu et al. [27] demonstrated that in response to ROS-induced DNA damage, p53 activation can promote apoptosis but also trigger a necrotic program through the activation of the lysosomal cysteine protease cathepsin Q. Moreover, a recent

study by Vaseva et al. [28], showed that in response to low levels of H₂O₂ treatment, stabilized p53 accumulated within the mitochondrial matrix, bound to cyclophilin D, and led to the opening of the mitochondrial permeability transition pore to initiate necrosis. As suggested by Montero et al., the above publications also pointed to a novel concept within the p53-regulated cell death literature: a balance exists between apoptotic and necrotic signaling pathways in response to cellular stress, and these pathways likely regulate each other to ensure death of stressed and/or damaged cells. Furthermore, these studies highlight the differential outcomes (i.e. apoptosis vs. necrosis) of p53 activation in response to varying levels of upstream stress and pro-death signaling cascades.

The potential significance of dual regulation of p53 and PARP-1 may be the requirement for p53-induced, PARP-1 mediated, necrosis in tumor suppression pathways *in vivo*. A recent study demonstrated that mice expressing mutant forms of p53 (e.g. p53^{K117R.K161R.K162R}) that are not competent to induce apoptosis remain protected from the early-onset of spontaneous thymic lymphoma that occurs in the complete absence of wild type *trp53*. While the pro-apoptotic function of p53 was ablated in the above study, the authors noted that metabolic regulation and anti-oxidant functions of the mutant p53 remained comparable to wild type p53 [8]. In a complementary study, mice were generated that lacked the major down-stream effectors of p53-mediated cell cycle arrest and apoptosis (i.e. *p21*^{-/-}*puma*^{-/-}*noxa*^{-/-}), and these animals also remained tumor free for almost two years [29]. Again, the authors implicated that a role for p53 in metabolism may be crucial for tumor suppression. Together, these studies could be interpreted as when p53-mediated apoptosis is either suppressed or genetically ablated, an additional tumor suppressor function of p53 may be revealed that is sufficient to protect organisms from cancer. Is this additional tumor suppressive function of p53 related to PARP-1 mediated necrosis, as links to both metabolism and ROS signaling are key?

To address the above question requires a better understanding of how ROS-induced DNA damage signaling directly impacts on p53 activity to regulate PARP-1 and necrosis. In Montero et al., there are no indications of whether transcriptional or non-transcriptional activities of p53 are required (Fig. 3). Is it possible that p53 transcriptionally regulates the expression of an unidentified PARP-1 co-factor that is required for its activity? The use of cell lines with *trans*-activation deficient mutants of p53 (e.g. p53^{QS}) or small molecule inhibitors to p53 (e.g. PFTα) would possibly provide some mechanism for the requirement of p53 in mediating PARP-1 activity. Another aspect to consider is if p53 stabilization after ROS-induced DNA damage results in a post-translational modification of PARP-1 that is required for its activation. Examining all of the above components, pathways, and treatments in genetic model systems of cancer will ultimately evaluate the *in vivo* significance of these observations. Given the marked interest to pharmacologically target p53 and PARP-1 in cancer, these pathways may provide key insights to our understanding of eliminating cancer cells... *all of which we shall love better after their death.*

Abbreviations

AMP	adenosine monophosphate
APAF-1	apoptotic protease activating factor 1
ATP	adenosine triphosphate
BAK	bcl-2 homologous antagonist killer
BAX	bcl-2 associated protein X
BCL-2	B-cell lymphoma 2

DNA	deoxyribonucleic acid
MEF	mouse embryonic fibroblast
mTOR	mammalian target of rapamycin
NAD	nicotinamide adenine dinucleotide
p53	tumor suppressor protein 53
PARP-1	poly(ADP-ribose) polymerase-1
PI3K	phosphoinositide-3-kinase
PUMA	p53 upregulated modulator of apoptosis
ROS	reactive oxygen species
SV40	simian vacuolating virus 40.

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Box 1 So many ways to die!

Autophagy is an evolutionarily conserved catabolic mechanism of lysosomal degradation of cellular contents. In response to cellular stressors such as nutrient deprivation, oxidative stress, ER stress, and hypoxia, autophagic responses allow for the recycling of essential nutrients, as well as the removal of damaged organelles. Autophagy is often described as a cell survival and/or protective mechanism; but if the process is hyperactivated, autophagy will ultimately lead to cellular demise [12]. Characteristic morphological features of autophagic cell death include marked vacuole formation and a continual decrease in cellular volume that eventually leads to death.

Apoptosis is a physiological form of programmed cell death that is essential for both development and organ homeostasis. There are two distinct pathways of apoptosis: (i) the extrinsic pathway, which is initiated by extracellular ligands to the death receptors on the cell surface; (ii) and the intrinsic pathway, which is activated by intracellular stressors such as DNA damage, growth factor withdrawal, and oncogenic activation. While the upstream signaling pathways are distinct, both the intrinsic and extrinsic pathways converge upon the activation of cytosolic proteases known as caspases. These proteases are responsible for inducing the morphological phenotypes associated with apoptosis, such as DNA laddering, cellular blebbing, and the loss of plasma membrane asymmetry. Proteins of the BCL-2 family are the major regulators of apoptosis, and they function to directly control the integrity of the outer mitochondrial membrane (OMM) before and after exposure to stress. Once compromised, the OMM releases pro-apoptotic molecules from the inner mitochondrial space, which are directly responsible for caspase activation and the apoptotic phenotypes listed above [17].

Necrosis is mostly thought of as a passive and/or consequential response that is mechanistically and morphologically distinct from other forms of cell death. While the detailed cellular mechanisms governing apoptotic and autophagic cell death are well characterized, the same is not true for necrosis as it is likely not genetically controlled. Necrosis is most often observed in response to physicochemical stressors such as extreme temperature, extracellular pH changes, and mechanical stress. Cells undergoing necrosis display aggregated DNA, swelling of organelles, and the rapid loss of plasma membrane integrity – morphological features that can be recapitulated by the depletion of ATP.

Figure 1. p53 activation engages multiple cell death pathways. In response to cellular stress such as DNA damage, oncogene activation, or hypoxia, the p53 tumor suppressor is activated and stabilized. Active p53 is able to interact with various cellular signaling pathways. Depending on the cellular context, p53 activation can lead to at least three distinct cell death phenotypes: autophagy, apoptosis, or necrosis.

Figure 2.

DNA damage induced PARP-1 activation and phenotypes. Following DNA damage, PARP-1 is recruited to the sites of DNA lesions. Depending on the level of stress, PARP-1 activation results in cellular survival or necrosis. Following a mild stress, components of DNA repair machinery such as XRCC1 and DNA-PK are ADP-ribosylated and recruited to the sites of damage, thus promoting cell survival. In response to severe DNA damage, PARP-1 activation and the production of poly(ADP-ribose) polymers greatly increases leading to the consumption of NAD⁺ reserves and necrosis.

Figure 3.

Functional relationships between the PARP-1 and p53 pathways. Upon cellular stress, PARP-1 is known to have several effects on p53, including the stabilization and poly(ADP-ribosyl)ation of p53 which regulates its transcriptional activity. In addition, cellular stress can also activate p53, which induces PARP-1 function. The mechanism of p53-induced PARP-1 activation remains unknown, but likely includes a combination of transcriptional and posttranslational events. See text for further information.