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Tumor-Suppressor Functions of the TP53 Pathway

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The fundamental biological importance of the *Tp53* gene family is highlighted by its evolutionary conservation for more than one billion years dating back to the earliest multicellular organisms. The TP53 protein provides essential functions in the cellular response to diverse stresses and safeguards maintenance of genomic integrity, and this is manifest in its critical role in tumor suppression. The importance of *Tp53* in tumor prevention is exemplified in human cancer where it is the most frequently detected genetic alteration. This is confirmed in animal models, in which a defective *Tp53* gene leads inexorably to cancer development, whereas reinstatement of TP53 function results in regression of established tumors that had been initiated by loss of TP53. Remarkably, despite extensive investigation, the specific mechanisms by which TP53 acts as a tumor suppressor are yet to be fully defined. We review the history and current standing of efforts to understand these mechanisms and how they complement each other in tumor suppression.

The TP53 protein is a critical tumor suppressor that plays a fundamental and multifaceted role in the development of cancer and cancer therapy. Despite more than 30 years of vigorous research and an expansive body of literature, the precise molecular mechanism underlying TP53's tumor-suppressor function has not been defined and remains the focus of active investigation. Understanding the tumor-suppressor function of the *Tp53* gene will not only have profound importance to the understanding of cancer biology but will likely have an impact on cancer therapy and prevention through improved exploitation of wild-type *Tp53* functions as well as gained insight into specific vulnerabilities imposed on tumors by loss of TP53 function. The TP53 protein exerts effector functions that impact on virtually all of the hallmark features of cancer (Hanahan and Weinberg 2011); however, it is still not clear which of these functions is essential to its potent tumor-suppressor function and how these functions interact. Indeed, it is becoming increasingly apparent that multiple pathways are likely to collaborate in exerting this tumor-suppression function and that the TP53 protein has context-specific roles. Here we discuss the functioning of the TP53 protein as a tumor suppres-

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sor and review efforts to understand the underlying mechanisms.

THE TUMOR-SUPPRESSOR TP53 PROTEIN

The TP53 protein was first discovered in 1979 through its association with simian virus 40 (SV-40) large T antigen in virally transformed cancer cells (DeLeo et al. 1979; Lane and Crawford 1979; Linzer and Levine 1979). For the first decade following its discovery, the TP53 protein was considered to be encoded by a proto-oncogene because of its effect on increasing cell growth and survival when forcibly expressed in cell lines. It is now known that this initial research describing TP53 function was inadvertently performed on mutant *Tp53* genes rather than the wild-type form (Levine and Oren 2009). The realization of its role as a tumor suppressor came from a number of important observations. In 1989, the *Tp53* gene was identified as the target of the frequently re-occurring 17p chromosomal deletion observed in human colorectal carcinoma (Baker et al. 1989) with >50% of these tumors harboring missense mutations in the remaining *Tp53* allele. The high frequency of *Tp53* inactivation strongly suggested its tumor-suppressor function. Moreover, in the same year, it was shown that enforced expression of the wild-type TP53 protein could block oncogene-mediated transformation of primary rat embryonic fibroblasts in culture (Eliyahu et al. 1989; Finlay et al. 1989).

The role of the TP53 protein in tumor suppression has been experimentally proven and further examined using mouse models generated by gene targeting. Confirming the tumor-suppressor function of the *Tp53* gene, *Tp53* knockout ($Tp53^{-/-}$) mice and mice with loss-of-function mutations in *Tp53* develop spontaneous tumors with 100% incidence by 9 mo of age (Donehower et al. 1992; Jacks et al. 1994; Lang et al. 2004; Olive et al. 2004). Interestingly, the genetic background influences the tumor spectrum: $Tp53^{-/-}$ mice on a C57BL/6 background mostly develop thymic lymphoma, whereas sarcomas, hemangiomas, B-cell lymphomas, and breast cancers can arise on 129SV, BALB/c, or mixed genetic backgrounds (Harvey et al. 1993a; Jacks et al. 1994; Nacht et al. 1996). The $Tp53^{-/-}$ mice also have an increased susceptibility to carcinogen and γ-irradiation-induced tumor development (Harvey et al. 1993b; Kemp et al. 1994), consistent with the critical role of the TP53 protein in the cellular response to DNA damage. Inactivation of the TP53 pathway can also markedly accelerate oncogene-driven tumor development (Eischen et al. 1999; Schmitt et al. 1999; Michalak et al. 2009). In addition to preventing spontaneous tumor formation, the TP53 protein exerts a strong tumor-suppressive effect in established TP53-deficient tumors. Inducible restoration of the wild-type TP53 protein in established tumors that had been elicited by loss of TP53 function leads to tumor regression and prolonged survival of tumor-burdened mice (Martins et al. 2006; Ventura et al. 2007; Xue et al. 2007). Interestingly, functional TP53 restoration in such tumors in vivo shows dramatic context dependence, with induction of apoptosis in lymphomas but cellular senescence in sarcomas. This may relate to the type of transformed cells or the nature of the oncogenic lesions that drove their transformation (in addition to loss of TP53) (Junttila et al. 2010). Regardless, these studies affirmed the TP53 tumor-suppressor function in vivo.

The importance of the *Tp53* gene as a tumor suppressor is highlighted in human cancer where it is the most commonly mutated gene, with mutations found in a broad variety of cancer types (Vogelstein et al. 2000; Petitjean et al. 2007). Furthermore, in cancers in which the *Tp53* gene remains intact, TP53 function is often impaired, for example, by interference from viral proteins or up-regulation of negative regulators, such as the E3 ubiquitin ligase, MDM2 (called HDM2 in humans) (Vogelstein et al. 2000). Thus, most human cancers contain a genetic or epigenetic alteration that impairs the TP53 pathway.

The requirement for normal TP53 function in tumor suppression is evident in families with the Li–Fraumeni syndrome, which are prone to spontaneous tumor formation (Li and Fraumeni 1969) owing to the inheritance of a germline loss-of-function mutation in one *Tp53*

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allele (Malkin et al. 1990; Srivastava et al. 1990). Li–Fraumeni syndrome patients typically develop cancer before the age of 45 yr, which most often presents as a soft tissue or bony sarcoma, breast cancer, brain tumor, adrenal cortical carcinoma, or leukemia. However, with larger epidemiological studies, it is now apparent that affected families may have a much broader range of malignancies and age of onset, with rare individuals even remaining tumor free and experiencing longevity, highlighting the complexity of the TP53 tumor-suppressor network (Kamihara et al. 2014). In an informative example, a cluster of cases of childhood adrenal cortical carcinoma observed in Southern Brazil (Ribeiro et al. 2001; Achatz et al. 2007) led to the discovery of a mutation, R337H, that results in pH-dependent instability of the TP53 tetramer (DiGiammarino et al. 2002) and tissue-restricted tumor development. The study of human disease continues to provide important insight into the function of the TP53 protein.

The accumulated knowledge of the TP53 tumor-suppressor function from more than 30 yr of research has culminated in its exploitation for the treatment of human cancer. Targeted therapies aimed at specifically increasing, or restoring, TP53 function have proven effective in eliciting tumor regression in preclinical models, for example, by using small molecule inhibitors that block the E3 ubiquitin ligase, MDM2 (HDM2), which is the major negative regulator of TP53 (Vassilev 2005; Brown et al. 2009).

REQUIREMENTS FOR TP53-MEDIATED TUMOR SUPPRESSION

Detection and Response to Oncogenic Stress

The TP53 tumor suppressor can be activated by diverse cellular stresses, including oncogene expression, DNA damage, hypoxia, metabolic dysfunction, and replicative stress, following which it implements appropriate responses to oppose cancer initiation. Activation of the TP53 protein may result in a variety of cellular responses, including apoptosis, cell senescence, cell-cycle arrest, DNA repair, metabolic adaptations, and changes to cellular characteristics, such as differentiation state. The fate of the cell following TP53 activation is determined by the type, duration, and amplitude of the stress signal as well as the context in which it occurs, such as the cell type. The outcome is modulated by the interplay with other signaling pathways that are active. In addition, the TP53 protein exerts substantial control over cellular homeostasis in the steady state, even before "activation" by stress signals. Control of TP53 activity is achieved through an elaborate system of posttranslational modifications, including phosphorylation, acetylation, and ubiquitination, which impact TP53 protein binding to specific sites in the DNA, protein turnover, and interaction with other proteins that affect TP53 protein transcriptional function (Kruse and Gu 2009). Furthermore, there may be an additional role for the regulation of TP53 protein activity according to the levels and sites of *Tp53* gene expression. The TP53 protein, therefore, lies at the convergence of a diverse range of signaling processes that communicate the cell state (Fig. 1). These signals are then integrated to elicit a protective TP53-mediated response; the dynamic regulation and activation of TP53 protein function is critical for effective tumor suppression.

Tumor Suppression and Transcriptional Regulation

Following activation, the TP53 protein functions predominantly as a transcription factor (Riley et al. 2008). The TP53 protein forms a homotetramer (Friedman et al. 1993) that binds to specific *Tp53* response elements in genomic DNA (el-Deiry et al. 1992; Cho et al. 1994) to direct the transcription of a large number of protein-coding genes (Riley et al. 2008). The requirement for TP53 transcriptional activity in tumor suppression has been examined by systematically mutating the transactivation domains of the TP53 protein, rendering it either partially or wholly transcriptionally defective (Brady et al. 2011; Jiang et al. 2011). Importantly, mutations resulting in complete loss of TP53 transcriptional activity ablate its ability to prevent tumor formation, supporting the con-

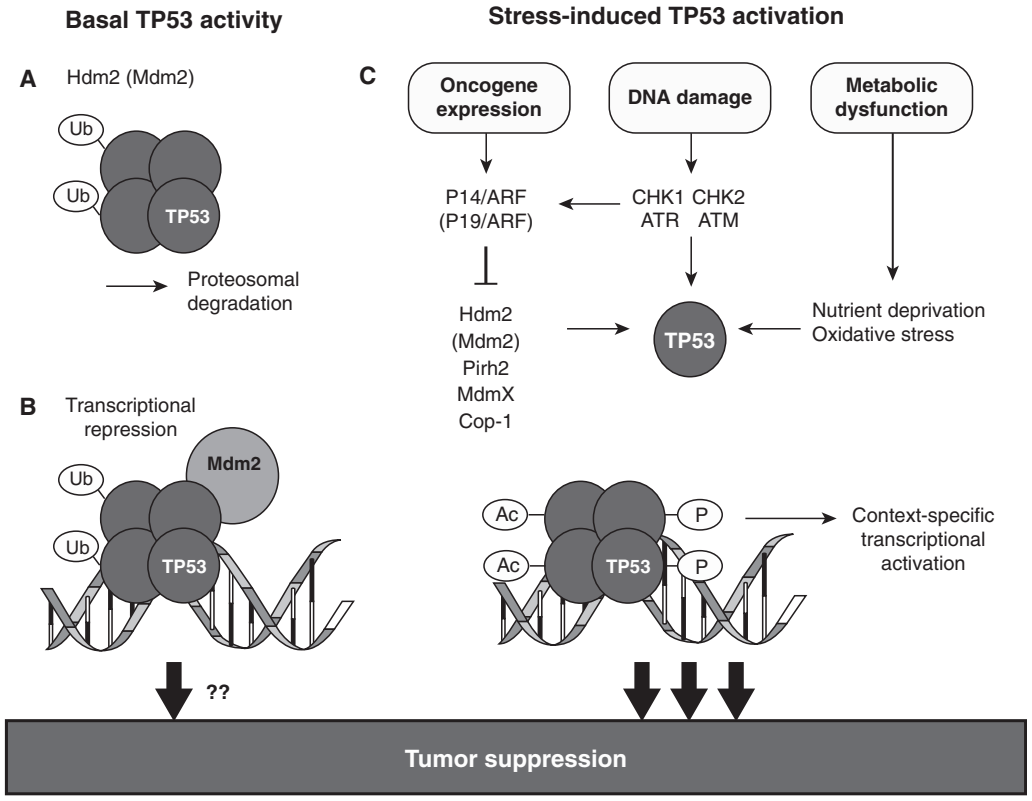

Figure 1. Appropriate activation and feedback control of TP53 activity is critical to effective tumor suppression. (*A*) In the absence of a TP53-activating signal, TP53 protein levels are maintained at low levels in most cell types by the E3 ubiquitin ligase, MDM2 (HDM2), which ubiquitinates (Ub) TP53 and targets it for degradation by the proteosome. (*B*) The TP53 protein may also control gene expression in the absence of an activating stimulus, for example, by transcriptional repression. The extent to which basal TP53 activities contribute to the tumor-suppressor function is not known. (*C*) Stress stimuli, such as oncogene expression, DNA damage, and metabolic dysfunction, rapidly lead to TP53 protein accumulation and activation; this is in part owing to inhibition of MDM2 (HDM2), thus preventing TP53 ubiquitination and proteosomal degradation. Following activation, the TP53 protein acts as a sequence-specific transcription factor directing the expression of a large number of target genes, which are considered the primary determinants of the tumor-suppressor response. The specific mode of the TP53 response is influenced by extensive posttranslational modification, including acetylation (Ac) and phosphorylation (P).

cept that transcriptional regulation is central to the tumor-suppressor function. Nontranscriptional functions for the TP53 protein have been proposed; however, their biological importance remains uncertain (Vousden and Lane 2007). The majority of evidence suggests that TP53-mediated tumor suppression is governed by transcriptional regulation; therefore, understanding the critical TP53 gene targets and mechanisms of their transcriptional regulation is a key objective.

TP53-mediated transcriptional regulation varies according to the type of stress stimulus and type of cell, so that appropriate corrective processes can be implemented. For example, minor DNA damage may institute cell-cycle arrest and activate DNA-repair mechanisms, whereas stronger TP53-activating signals induce senescence or apoptosis. Accordingly, the TP53 transcriptional response varies depending on the nature of the activating signal and the type of cell; the detailed mechanisms underly-

ing these differences in transcriptional induction of target genes remain unknown. However, unprecedented insight into this process has been gained through the analysis of TP53 protein DNA binding and transcriptional regulation using next-generation techniques, such as chromatin immunoprecipitation (ChIP) DNA and RNA sequencing. The number of known or suspected TP53 target genes has increased into the thousands with dramatic differences in transcriptional responses observed among different cell types, different TP53-inducing stress stimuli, and varying time points following TP53 activation (Allen et al. 2014). These studies paint an increasingly complex picture of the modes by which TP53 can regulate gene expression. For example, before TP53 activation, a subset of target genes is transcriptionally repressed by the TP53 protein (Allen et al. 2014). More recently appreciated functions of the TP53 protein include widespread binding and modulation of enhancer regions throughout the genome and transcriptional activation of noncoding RNAs (He et al. 2007; Younger et al. 2015). Interestingly, the TP53-activated long noncoding RNA, *lincRNA-p21*, exerts widespread suppression of gene expression (Huarte et al. 2010). The list of proposed TP53 target genes is vast and they are known to influence diverse cellular processes, including apoptosis, cell-cycle arrest, senescence, DNA-damage repair, metabolism, and global regulation of gene expression, each of which could potentially contribute to its tumor-suppressor function (Fig. 2).

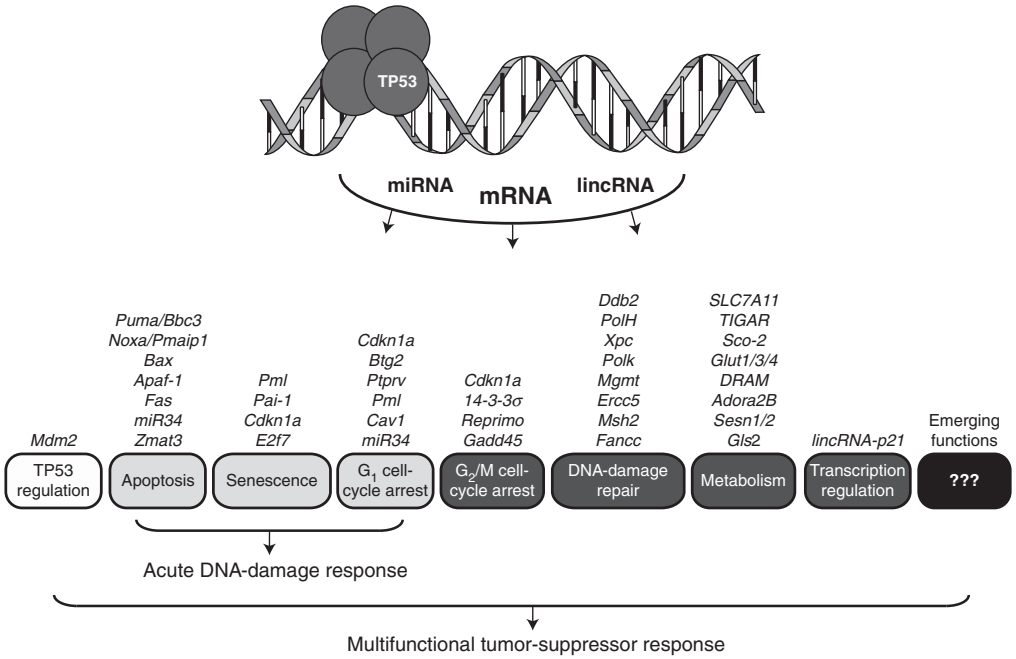

Figure 2. The TP53 protein exerts its tumor-suppressor function as a sequence-specific transcription factor. Following activation, the TP53 protein directs the expression of a large number of genes encoding mRNA, miRNA, and lincRNAs that orchestrate a variety of cellular processes. In addition, TP53 may have as yet undetermined effector functions that are important for tumor suppression. It is increasingly apparent that a single effector function is inadequate to explain the potency and complexity of TP53's tumor-suppressor function. In contrast, specific effector functions may be more or less important depending on the context and multiple effector pathways are likely to collaborate and synergize in the prevention and suppression of tumor formation. Selected TP53-regulated murine genes are shown with their associated cellular processes (some genes may impact on various pathways, e.g., CDKN1a [p21], which is critical for G_1 cell-cycle arrest and cell senescence).

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Insight into the critical TP53 transcriptional targets has been gained from genetic mouse models expressing transcriptionally defective mutant TP53 proteins. Interestingly, a partially transactivation defective TP53 protein, denoted $TP53^{25,26}$ (Brady et al. 2011), can only activate a limited number of TP53 target genes and is unable to induce apoptosis, cell-cycle arrest, or senescence, yet it retains the ability to suppress tumor formation. Complementary findings have been observed in a different mouse model, in which key lysine residues of the TP53 protein, which are modified by acetylation during posttranslational activation, have been mutated to arginine, denoted $TP53^{3KR}$ (Li et al. 2012). Similar to the $TP53^{25,26}$ mutant, the mutant $TP53^{3KR}$ protein is unable to activate target genes that mediate apoptosis, cell-cycle arrest, and cell senescence, yet it still retains the ability to suppress tumor development. Examination of these mutant strains of mice revealed preserved regulation of several TP53 response genes involved in DNA-damage repair and metabolism, implicating a potentially critical role for these processes in tumor suppression. At present, the search for the critical TP53 tumor-suppressor transcriptional targets is underway in earnest.

KEY EFFECTOR FUNCTIONS FOR TUMOR SUPPRESSION

Apoptosis

Apoptosis was one of the earliest identified components of the TP53-mediated tumor-suppressor response (Yonish-Rouach et al. 1991). Induction of apoptosis is among the most extensively studied cellular processes activated by the TP53 protein and has been the focus of much of the investigation into its tumor-suppressor effect. Impaired apoptosis is a cardinal feature of malignancy and genetic alterations that result in evasion from apoptotic cell death markedly accelerate tumor development (Vaux et al. 1988; Strasser et al. 1990; Czabotar et al. 2014). Multiple TP53 target genes have been implicated in TP53-mediated induction of apoptosis: *Puma*, *Noxa*, *Bax*, *Apaf1*, *Fas*, *Tnfrsf10B/DR5*, *miR34*, *TP53AIP1*, *Pidd*, *Pig3*, *Zmat3*, and *Siva*. Among these target genes, *Puma*, *Noxa*, *Bax*, and *Apaf-1* play critical roles in the intrinsic (also called BCL-2-regulated or mitochondrial) apoptotic pathway (Youle and Strasser 2008; Strasser et al. 2011), whereas *Fas* and *Tnfrsf10B/DR5* encode for members of the tumor necrosis factor receptor (TNFR) family (FAS/APO-1/CD95 and TRAIL-R/DR5) that can trigger the death receptor (also called extrinsic) apoptotic pathway (Strasser et al. 2009). Of all these TP53 target genes, only the proapoptotic BH3-only BCL-2 family members PUMA and NOXA have been validated by studies of gene-targeted mice to be essential for TP53-mediated apoptosis (Jeffers et al. 2003; Shibue et al. 2003; Villunger et al. 2003; Michalak et al. 2008). Although the *Bax* and *Apaf-1* genes are also direct TP53 targets (Riley et al. 2008), TP53-deficient cells still express these effectors of apoptosis. Therefore, their induction likely serves to amplify TP53-mediated apoptosis signaling. The miR34 family of microRNAs is a TP53 target (He et al. 2007) predicted to exert broad antioncogenic effects through the post-transcriptional regulation of a variety of genes that not only sensitize to apoptosis, for example, by down-regulation of BCL-2 (Bommer et al. 2007), but also through regulation of cell-cycle progression and differentiation. Surprisingly, however, mice that are deficient of all miR34 family members are not susceptible to spontaneous or oncogene-induced tumor development (Concepcion et al. 2012). Importantly, the death receptor apoptotic pathway is dispensable for TP53-induced apoptosis (Newton and Strasser 2000). However, TP53-mediated induction of *Fas* and *Tnfrsf10B/DR5* expression may serve to sensitize stressed cells to the death receptor ligands, FASL and TRAIL, and it has been proposed that this could be exploited for cancer therapy (Ashkenazi 2008).

The role of TP53-mediated apoptosis in preventing oncogene-driven cancer development has been defined using the *Eμ-Myc* transgenic mouse model (Adams et al. 1985). Here the immunoglobulin heavy chain gene enhancer (Eμ) has been juxtaposed to the *c-Myc* oncogene, resulting in deregulated c-MYC expression and, consequently, the rapid development of

pre-B and B-cell lymphomas. In the *Eμ-Myc* mouse model, spontaneous inactivation of the TP53 pathway, most frequently through mutations in *Tp53* itself, is seen in ~20% of lymphomas (Eischen et al. 1999; Schmitt et al. 1999; Michalak et al. 2009), indicative of its critical role in this setting. Accordingly, inactivation of TP53 function markedly accelerates MYC-driven lymphoma development (Schmitt et al. 1999; Michalak et al. 2009). Strikingly, complete deletion of the *Tp53* gene using CRISPR/Cas9 targeting in *Eμ-Myc* hematopoietic stem/progenitor cells (HSPC) results in the rapid development of lymphoma with a median latency of only 29 d (Aubrey et al. 2015) as compared with nontargeted *Eμ-Myc* HSPC that give rise to lymphoma with a mean latency of >110 d. The specific requirement for individual TP53 apoptotic transcriptional targets in the tumor-suppressor function has been dissected using the *Eμ-Myc* mouse model where loss of BAX (Eischen et al. 2001; Dansen et al. 2006), PUMA, and NOXA (Hemann et al. 2004; Michalak et al. 2009) can each accelerate lymphoma development, although not to the same extent as complete loss of TP53 function. This suggests critical roles for additional pathways in tumor suppression during MYC-driven lymphoma development.

Interestingly, mice engineered to harbor only specific gene knockout of the TP53 apoptotic targets *Puma* (Jeffers et al. 2003), *Noxa* (Villunger et al. 2003), *Bax* (Knudson et al. 2001), or even combined loss of *Puma*/*Noxa*/*p21* (Valente et al. 2013) do not display a propensity for tumor formation. Thus, in the absence of constitutive oncogenic stress, the combined knockout of the major TP53-dependent mediators of apoptosis (PUMA and NOXA) and the major mediator of G_1/S cell-cycle arrest and cell senescence (p21) does not recapitulate the tumor predisposition observed in $Tp53^{-/-}$ mice. Apoptosis clearly plays a critical role in tumor suppression; however, additional pathways must be disabled to fully recapitulate the effect from complete loss of TP53. Furthermore, these studies show that the animal model in which TP53 functions is examined will likely influence the experimental findings. Although PUMA, NOXA, and BAX are important mediators of TP53-induced apoptosis, there may be additional proapoptotic effector mechanisms that have yet to be fully defined. For example, the proapoptotic BH3-only protein BIM may be induced indirectly by TP53 and contribute to the killing of tumor cells by DNA-damage-inducing chemotherapeutic drugs (Happo et al. 2010). Importantly, there is substantial overlap between the regulation of apoptotic cell death and other pathways, such as DNA-damage repair and metabolism; thus, the role of apoptosis in tumor suppression may be intertwined with other TP53-dependent effectors.

Cell-Cycle Regulation and DNA-Damage Repair

Cancer is a disease that results from the progressive acquisition and accumulation of genetic mutations (Hanahan and Weinberg 2011), and the TP53 protein, as the "guardian of the genome" (Lane 1992), has a salient role in maintaining genomic integrity and opposing this process. The TP53 protein plays an intimate role in the cellular response to DNA damage. It is critical to both the acute phase response involving cell-cycle arrest, senescence, and apoptosis as well as long-term surveillance mechanisms for maintaining multiple DNA-damage-repair mechanisms, such as nucleotide excision repair, base excision repair, and nonhomologous end-joining (Sengupta and Harris 2005).

In many cells, the initial TP53-mediated response to acute DNA damage is the induction of transient G_1 cell-cycle arrest, which allows time for the detection and repair of DNA damage before replication of the genome in S phase and subsequent cell division. The TP53 protein also exerts checkpoint control during the G_2/M transition, at which time DNA replication has already occurred and cells prepare to undergo mitotic cell division (Taylor and Stark 2001), a time when failed detection and repair of damaged DNA may be most catastrophic (e.g., resulting in aneuploidy). Both cell-cycle checkpoints are critical to maintaining genomic integrity and the requirement for TP53-mediated cell-cycle arrest in maintaining genomic

stability has been shown experimentally (Barboza et al. 2006). The key mediator of TP53-induced G_1 cell-cycle arrest is thought to be CDKN1a (p21), as shown by cells from *Cdkn1a*$^{-/-}$ mice that show impaired G_1 cell-cycle arrest in response to DNA damage and TP53 activation (El-Deiry et al. 1993; Brugarolas et al. 1995; Deng et al. 1995). Given the integral role of cell-cycle arrest in DNA repair, CDKN1a has been proposed to contribute to TP53-mediated tumor suppression. However, mice that lack CDKN1a (p21) are not prone to spontaneous tumor formation (Deng et al. 1995; Valente et al. 2013). Additional TP53 transcriptional targets also contribute to G_1 phase cell-cycle arrest including, but not limited to, the promyelocytic gene (*Pml*), protein tyrosine phosphatase receptor type-V gene (*Ptprv*), Caveolin-1 (*Cav1*) (Galbiati et al. 2001), and *Btg2* (Rouault et al. 1996). In addition, other TP53 targets specifically instigate cell-cycle arrest at the G_2/M checkpoint, including the growth-arrest and DNA-damage-inducible 45 α gene (*Gadd45α*), *Reprimo*, and the 14-3-3σ protein (Taylor and Stark 2001). The roles of GADD45α, PTPRV, PML, and CAV1 have been examined individually through the generation of knockout mice but none of these animals spontaneously develop cancer (Hollander et al. 1999; Razani et al. 2001; Rego et al. 2001; Doumont et al. 2005). However, similar to other candidate TP53 tumor-suppressor transcriptional targets, their deficiency can accelerate tumor formation under conditions of specific oncogenic stress (Capozza et al. 2003; Tront et al. 2010).

TP53-induced cell-cycle arrest is thought necessary to allow for appropriate DNA-repair processes to occur (Barboza et al. 2006). Precancerous lesions are characterized by the accumulation of DNA damage and consequent activation of the TP53-mediated DNA-damage response (Bartkova et al. 2005; Gorgoulis et al. 2005). The acquisition of mutations during tumor initiation occurs through a variety of mechanisms including mutations and epigenetic modifications followed by propagation of these alterations owing to defective DNA-damage repair mechanisms. The TP53 protein plays pivotal roles in all of these processes and, in keeping with the importance of TP53 in maintaining genomic stability, cells from *Tp53*$^{-/-}$ mice as well as TP53-defective human cancers are characterized by widespread genomic alterations. In line with this, TP53 has a large number of direct transcriptional targets that mediate DNA-repair pathways, including *Polk*, *Mgmt*, *Fancc*, *Ercc5*, *Xpc*, *Ddb2*, *Gadd45α*, *Msh2*, and *PolH* (Allen et al. 2014; Bieging et al. 2014). The central role of genomic instability during the evolution of thymic lymphomas in *Tp53*$^{-/-}$ mice has been directly observed over time, where defective DNA repair results in a very high rate of gene copy number variations, including chromotrypsis-like events, which drive the accumulation of the cooperating genetic lesions required for malignant transformation (Dudgeon et al. 2014). This supports the notion that genomic instability is a key driver of cancer development in the absence of the TP53 protein.

In certain cell types, activation of the TP53 protein can result in the induction of apoptosis resulting in the elimination of irreversibly damaged cells. However, the acute DNA-damage response has been largely excluded from a role in the TP53 tumor-suppressor function through multiple lines of investigation (Christophorou et al. 2006; Brady et al. 2011; Li et al. 2012). The role of the acute DNA-damage response in tumor suppression was evaluated using timed restoration of TP53 protein in *Tp53*$^{-/-}$ mice following γ-irradiation to induce thymic lymphoma formation. Remarkably, transient TP53 restoration during the acute DNA-damage response did not produce a tumor-suppressor effect (Christophorou et al. 2006). In contrast, transient restoration of TP53 function that was delayed until after the acute DNA-damage response had ended, at a time when there was no discernable cell-cycle arrest or apoptosis, was sufficient for tumor suppression. Furthermore, the delayed tumor-suppressor function observed is dependent on p19/ARF, implicating oncogene-mediated TP53 activation in nascent neoplastic cells in this response. This is further confirmed through studies of transcriptionally defective and acetylation defective mutant TP53 proteins that are unable to activate the acute DNA-damage response yet retain potent tu-

mor-suppressor function (Brady et al. 2011; Li et al. 2012). Therefore, the acute pathological DNA-damage response appears to be dispensable for tumor suppression, a remarkable finding that also has major implications for cancer therapy.

The consequences of oncogene overexpression are twofold in the setting of DNA damage and TP53-mediated tumor suppression. First, acquired mutations may result in the activation of oncogenes, and cells expressing oncogenes can be selected for through enhanced proliferation and cell survival. Second, chronic oncogene activation drives abnormal cell growth, thereby increasing the risk of acquiring additional DNA lesions that may activate further oncogenes or inactivate tumor-suppressor genes (Halazonetis et al. 2008). TP53 may be purposed to eliminate or growth-arrest cells marked by oncogene overexpression, which is intimately connected with DNA damage, deregulated cell proliferation, and metabolic deregulation.

Senescence

Induction of cell senescence was first shown to play a critical role in TP53-mediated tumor suppression in a mouse model of erythroleukemia (Metz et al. 1995). Moreover, restoration of TP53 function in established solid-organ tumors (driven by loss of TP53) in vivo leads to the induction of cellular senescence in association with tumor regression (Ventura et al. 2007; Xue et al. 2007). Cellular senescence is a distinct cell state involving permanent cell-cycle arrest of cells that remain viable and metabolically active, which is characterized by a discrete transcriptional profile (Shay and Roninson 2004). The TP53 protein controls cellular senescence by activating a number of transcriptional targets that include *Cdkn1a*, *Pml, Pai1*, and *E2f7* (Pearson et al. 2000; Kortlever et al. 2006; Aksoy et al. 2012), some of which (e.g., *Cdkn1a*) are notable for additional function in cell-cycle regulation. Senescence is often associated with, and is thought to suppress, premalignant lesions preventing their progression to overt malignancy (Collado et al. 2005; Mooi and Peeper 2006). TP53-mediated induction of cell senescence was shown to be critical to preventing malignant transformation in a mouse model of BRAF-driven pulmonary adenoma (Dankort et al. 2007). Moreover, in a study examining the functional interdependence of defects in PTEN and TP53 in the development of prostate carcinoma, TP53-mediated senescence was required to prevent cancer development in the setting of PTEN deletion (Chen et al. 2005). It remains to be examined whether complete loss of all target genes implicated in TP53-mediated senescence can recapitulate the spontaneous tumor development seen in $Tp53^{-/-}$ mice and whether the strong association between senescence and tumor suppression is causal or whether this is an association with other TP53-mediated effects.

Metabolism

The rapid proliferation of cells, anabolic growth, and metabolic stress that typifies neoplastic disease requires substantial metabolic reprogramming (Hanahan and Weinberg 2011). Furthermore, metabolic deregulation not only impacts on energy production and cell growth but also influences additional processes important to sustained cancer growth, such as macromolecular biosynthesis, epigenetic regulation, and antioxidant pathways (Cairns et al. 2011; Ward and Thompson 2012). The TP53 protein is a critical regulator of cellular metabolism and many of the aforementioned processes affected by metabolic stress (Berkers et al. 2013).

The best-characterized description of cancer-associated metabolic reprogramming is the Warburg effect, whereby glucose is predominantly metabolized by glycolysis rather than oxidative phosphorylation, as normally occurs under aerobic conditions (Warburg 1956). TP53 activation stimulates oxidative phosphorylation and inhibits glycolysis, both of which oppose the Warburg effect. The TP53 protein can regulate the expression of several glucose transporters, including GLUT1, GLUT3, and GLUT4 (Schwartzenberg-Bar-Yoseph et al. 2004; Kawauchi et al. 2008), which diminishes glycolysis through impaired glucose uptake. TP53 also transactivates the TP53-induced glycolysis and apoptosis regulator gene (*TIGAR*), which en-

codes a fructose phosphatase enzyme that inhibits glycolysis and increases production of NADPH (Bensaad et al. 2006). NADPH is important for the scavenging of reactive oxygen species (ROS), and this antioxidant effect of TIGAR confers a prosurvival function in the setting of ROS-mediated cell death. However, TIGAR knockout mice do not show spontaneous tumor formation (Cheung et al. 2013) and, in some contexts, TIGAR deficiency actually impedes tumor development (Bensaad et al. 2006). TP53 also directly stimulates mitochondrial oxidative phosphorylation through transcriptional activation of the gene-encoding synthesis of cytochrome *c* oxidase 2 (*Sco2*) (Matoba et al. 2006). Interestingly, dysregulated oxidative phosphorylation has been observed in cells from patients with Li–Fraumeni syndrome, which is attributed partly to altered *Sco-2* expression (Wang et al. 2013).

The strict regulation of cellular antioxidant mechanisms is critical to maintaining intracellular signaling pathways and avoiding ROS-associated toxicity. Therefore, dysregulation of these processes can contribute to cancer development (Finkel 2003). Genes encoding enzymes with antioxidant functions, including *Gls2*, *Sestrin 1*, and *Sestrin 2*, have been identified as TP53 transcriptional targets (Budanov et al. 2004; Hu et al. 2010), defining a mechanism by which TP53 can regulate oxidant signaling and manage oxidative stress. Interestingly, treatment of *Tp53* knockout mice with antioxidant therapy was reported to delay the onset of tumor formation, implicating a role for limiting the ROS accumulation in TP53-mediated tumor suppression (Sablina et al. 2005).

Cancer-associated metabolic stress and hypoxia can activate distinct cell death pathways through a variety of mechanisms, including the intrinsic apoptotic pathway (Czabotar et al. 2014). TP53 can modulate metabolic stress-induced cell death through a number of mechanisms. For example, the TP53 protein drives expression of the *ADORA2B* gene, which can detect nutrient availability and sensitize cells to PUMA-mediated apoptotic cell death (Long et al. 2013). In addition, a recently described form of iron-dependent, nonapoptotic cell death, denoted ferroptosis, is initiated under conditions of metabolic stress and accumulated ROS (Dixon et al. 2012). It has been proposed that TP53 mediates tumor suppression through transcriptional repression of the *SLC7A11* gene, which encodes a cystine/glutamate antiporter that diminishes cellular predisposition to ferroptosis (Jiang et al. 2015).

Finally, autophagy is another TP53-regulated metabolic process that may contribute to tumor suppression (Maiuri et al. 2010; Kenzelmann Broz et al. 2013). Autophagy enables cells to adapt and survive in conditions of limiting nutrient availability by recycling intracellular contents, such as damaged proteins and organelles for the purpose of liberating energy and metabolites to maintain cellular integrity (Mathew and White 2011). Autophagy may further impact on tumor suppression by affecting apoptotic pathways and genomic stability. TP53 regulates autophagy at multiple levels (Feng et al. 2005) by transactivating a large number of genes (Kenzelmann Broz et al. 2013), including the genes encoding damage-associated autophagy mediator (DRAM) (Crighton et al. 2006) and ULK1 (Gao et al. 2011). Interestingly, siRNA-mediated knockdown of DRAM was shown to reduce TP53-dependent apoptosis (Crighton et al. 2006).

EMERGING TP53 FUNCTIONS AND TUMOR SUPPRESSION

New components of the TP53 response continue to emerge and many of these have been implicated in tumor suppression (Bieging et al. 2014; Hager and Gu 2014). These include roles for TP53 in stem-cell function, differentiation, cellular invasion, and metastasis, as well as regulation of the immune response and the tumor microenvironment. For example, in a mouse model of hepatocellular carcinoma, TP53 was shown to influence the microenvironment and immune response via a non-tumor-cell-autonomous mechanism, which impacted the rate of tumor expansion and aggressiveness (Lujambio et al. 2013). These emerging functions have as yet undetermined roles in tumor suppression; however, they highlight the increasingly

complex picture of the role of TP53 in cancer biology.

In addition to newly identified functions for the TP53 protein, novel approaches to understanding the mechanisms of tumor suppression are emerging. Cell competition has recently been established as a bona fide mechanism for tumor suppression (Martins et al. 2014) when it was shown that disruption of normal cell competition in the thymus leads to the formation of acute T-lymphoblastic leukemia. The concept of cell competition provides a view of the overall fitness of cells as they compete within a larger population of cells, accounting for various cellular attributes as well as context-dependent factors. In studies of hematopoietic progenitor cells, the TP53 protein was shown to mediate cell competition (Bondar and Medzhitov 2010), raising the possibility that cell competition may be an important framework within which to approach the question of TP53-mediated tumor suppression.

DISTINCTIONS BETWEEN HUMAN CANCER AND MOUSE MODELS

It is important to recognize a number of key distinctions between findings from mouse models and human disease. In human cancer, inactivation of the *TP53* gene almost always occurs by acquisition of a missense mutation rather than deletion of the *TP53* gene. Furthermore, these mutations frequently result in a single amino acid substitution and the production of a stable, overexpressed TP53 protein that can actively contribute to tumor development and growth over and above the consequences of losing wild-type TP53 function alone (Freed-Pastor and Prives 2012). In addition, the initial acquisition of *Tp53* mutations is usually followed by loss of heterozygosity, which typically involves large deletions of the short arm of chromosome 17 (17p). The large chromosomal deletion results in the loss of several additional genes and this raises the possibility that cooperating lesions on 17p may contribute to human cancers. For example, a gene encoding a component of the RNA polymerase II complex, *POLR2A*, is almost always codeleted in human cancers with the *TP53* gene, and this has functional impact on the resulting tumor (Liu et al. 2015). These are important features of human cancer that are inextricable from the question of understanding how loss or mutation of *TP53* leads to the development of cancer.

CONCLUDING REMARKS

The tumor-suppressor function of the TP53 protein is likely to be mediated through a number of collaborating effector functions rather than through a single pathway or single transcriptional target. Understanding how these mechanisms work together will require creative approaches to investigation that take into account the combinatorial nature and complexity of the TP53 response as well as consideration of its functioning in normal cellular processes. It is an intriguing question as to whether the primary purpose of the TP53 protein is tumor suppression or whether this is a secondary manifestation of its many important roles in normal biology. For example, the importance of the TP53 protein in maintaining genomic stability extends beyond the prevention of cancer to being a basic requirement for sustainable life that ensures an organism's genetic material is transmitted faithfully to subsequent generations (Jackson and Bartek 2009; Kerr et al. 2012) and speaks to the evolutionary conservation of the *Tp53* family of genes from the earliest multicellular organisms to humans (Belyi et al. 2010; Lane et al. 2010). Another important consideration is that TP53 contributes to normal cellular processes that may also be important in established tumors. For example, TP53 facilitates adaptation to some forms of metabolic stress, such as serine deprivation, whereby $Tp53^{-/-}$ cells are actually disadvantaged (Maddocks et al. 2013). As such, the complete loss of TP53 function during cancer initiation may not be entirely advantageous and such vulnerabilities may be exploited for treatment of TP53-deficient cancers. Understanding the role of TP53 in tumor suppression remains one of the most exciting and important biological questions that promises exciting advances for the next 30 years of TP53 research.

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