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p53 in the CNS: Perspectives on Development, Stem Cells, and Cancer

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Abstract

The p53 tumor suppressor potently limits the growth of immature and mature neurons under conditions of cellular stress. Although loss of p53 function contributes to the pathogenesis of central nervous system (CNS) tumors, excessive p53 function is implicated in neural tube defects, embryonic lethality, and neuronal degeneration. Thus, p53 function must be tightly controlled. The anti-proliferative properties of p53 are mediated, in part, through the induction of apoptosis, cell cycle arrest, and senescence. Although there is still much to be learned about the role of p53 in these processes, recent evidence supports exciting new roles for p53 in a wide range of processes, including neural precursor cell self-renewal, differentiation, and cell fate decisions. Understanding the full repertoire of p53 function in CNS development and tumorigenesis may provide us with novel points of therapeutic intervention for human diseases of the CNS.

Keywords: p53, MDM2, MDM4, apoptosis, nervous system, neural stem cell, cancer stem cell, medulloblastoma, glioblastoma, birth defects

Introduction

The p53 family (p53, p63, p73) of transcription factors occupies an important role in the cellular response to stress in developing and homeostatic tissues. In greater than 50% of human cancers, the p53 protein is mutated or functionally inactivated. The ability of p53 to suppress tumorigenesis is fundamentally linked to its central role in the cellular response to stress. Although normally low or undetectable, p53 levels and activity are rapidly increased in response to a wide range of stresses, including DNA damage, hypoxia, oncogene activation, microtubule disruption, and oxidative damage.[1] Activation of p53 induces cellular protective processes such as apoptosis, cell cycle arrest, and senescence largely through the transcriptional regulation of p53 target genes.[2] p53 potently limits the growth of damaged, potentially neoplastic cells in both embryonic and adult tissues, thus earning its moniker "guardian of the genome."[3] The importance of p53 in the apoptosis of mature neurons in response to DNA damage, excitotoxicity, or ischemia has been well established and is the topic of several recent reviews.[4,5] This review will focus predominantly on the role of p53 in regulating neural precursor cell apoptosis during central nervous system (CNS) development and discuss emerging evidence that p53 also plays an important role in the regulation of neural precursor cell self-renewal, differentiation, and cell fate decisions. We also discuss how perturbations in the p53 pathway may contribute to neural tube defects and to the pathogenesis of the CNS tumors, medulloblastoma, and glioblastoma.

p53 in CNS Development

p53 mRNA is ubiquitously expressed throughout the rodent brain in early embryogenesis up to embryonic (E) day 10.5.[6,7] At mid-gestation, coincident with increased cell specification and differentiation that accompanies organogenesis, p53 expression becomes more heterogeneous. Abundant in the highly proliferative neuroepithelium of the ventricular zones of the cerebellum, telencephalon, and mesencephalon, p53 mRNA expression is reduced in the postmitotic mantle zone and cortical plate. Analyses of transgenic mice expressing *lacZ* under control of a p53-responsive promoter reflect robust p53 transactivation function at E10.5,[8,9] at the mid–hind brain boundary, a region from which the midbrain and cerebellum originate. In prenatal and newborn mice, p53 transactivation function is limited to the surface of the thalamus and superficial regions of the cerebral cortex, cerebellum, and hypothalamus, with only sporadic p53 activity in the adult brain.[8-10] Thus, the majority of p53 protein in the developing and adult CNS is likely to be inactive.[8-10]

As p53 potently inhibits cell growth, it was widely anticipated that p53 would occupy an essential role in development. Therefore, it was somewhat unexpected when $p53^{-/-}$ mice, albeit highly tumor prone, were initially described as developmentally normal, suggesting that p53 was not required in embryogenesis. It was later established that in fact a variable percentage (8%-16%) of $p53^{-/-}$ females exhibit exencephaly, a neural tube defect (NTD) in which the neural tube fails to close in the developing

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brain.[11,12] Additional malformations observed in *p53*$^{-/-}$ mice include spina bifida, in which the lower spinal neural tube fails to close,[13] and retinal dysplasia, in which the neuroepithelium of the optic vesicle, a structure formed from the out-pouching of the neural tube, is aberrant folded.[14] Together, these observations suggest that p53 has a physiological role in neural tube closure, a multifactorial process in which cell proliferation, death, and migration must be tightly coordinated. Although the pattern of apoptosis in the developing neural tube appears to be unaltered by the lack of p53,[12] subtle disturbances in any of these processes can lead to NTDs. If p53 has a physiological role in neural tube closure, a compensatory factor such as p63 or p73, both of which are expressed in the developing CNS, must be activated in the majority of p53-deficient embryos that develop normally. Alternatively, the incomplete penetrance of embryonic lethality in *p53*$^{-/-}$ mice may reflect the requirement for p53 in a developmental checkpoint that is activated in only a subset of cells under specific conditions.

Consistent with this latter idea, mouse models deficient in key components of DNA repair pathways support spontaneous DNA damage as a potent p53-activating signal in CNS development. Mice deficient in DNA ligase 4 (Lig4) or XRCC4, two components of the nonhomologous end-joining (NHEJ) pathway that repairs double-stranded DNA breaks, exhibit defects in lymphocyte development, growth retardation, and neuronal apoptosis and die in late gestation.[15,16] In both *Lig4*$^{-/-}$ and *XRCC4*$^{-/-}$ mice, a high level of p53-dependent apoptosis is found in newly formed postmitotic neurons throughout the developing CNS. The onset of apoptosis in *Lig4*$^{-/-}$ and *XRCC4*$^{-/-}$ mice parallels neurogenesis, occurring in the ventral spinal cord at E10 and dorsally at E11, suggesting that apoptosis is triggered upon the cessation of neurogenesis. Nascent postmitotic neurons in the developing embryo are also susceptible to p53-dependent apoptosis in the absence of DNA polymerase-β (Pol-β),[17] which functions in the base excision repair (BER) pathway that repairs DNA lesions such as apurinic/apyrimidinic sites and DNA base modifications. The neuronal apoptotic phenotype of *Pol*-β$^{-/-}$ embryos is less severe than that observed in *Lig4*$^{-/-}$ and *XRCC4*$^{-/-}$ mice, perhaps reflecting the relative level of single- versus double-strand breaks that occur spontaneously.

Cycle dysregulation has also been proposed to signal p53 activation in the developing CNS. Germline retinoblastoma (*Rb*) deficiency in mice elicits p53-dependent apoptosis in postmitotic regions of the CNS, peripheral nervous system (PNS), and ocular lens.[18-20] Rb binds to and inactivates the E2F family of transcription factors that function to promote the G1-S transition. Loss of E2F1 or E2F3 in *Rb*$^{-/-}$ embryos rescues inappropriate S-phase entry and apoptosis,[21,22] thereby suggesting that the apoptotic function of p53 is activated in *Rb*$^{-/-}$ postmitotic neurons in response to the inappropriate exit from the cell cycle. Overexpression of E2F can activate p53 through the transcriptional regulation of ARF (p19^{arf} in mice, p14^{arf} in humans), a negative regulator of the p53 inhibitor, MDM2. However, apoptosis in the *Rb*$^{-/-}$ CNS is not rescued by the loss of ARF,[23] indicating that Rb deficiency activates p53 through an alternative pathway. Interestingly, expression of the hypoxia-related genes, VEGF and LDH-A, is increased in the developing CNS of germline *Rb*$^{-/-}$ mice.[24] However, in mice in which *Rb* is conditionally inactivated in the CNS, inappropriate S-phase entry persists, but the level of apoptosis and hypoxia-inducible gene expression is normal.[24] As *Rb*$^{-/-}$ mice exhibit impaired erythropoeisis, it has been proposed that the pro-apoptotic signal in *Rb*$^{-/-}$ postmitotic neurons originates not from cell cycle dysregulation but rather from hypoxia due to defective erythroid cells. Aberrant proliferation may not therefore be sufficient to activate p53 in nascent postmitotic neurons but may predispose these cells to collaborative pro-apoptotic signals such as hypoxia.

Keeping p53 under Control: The MDM2/MDM4/p53 Axis

The exquisite sensitivity of the developing CNS to p53-mediated apoptosis dictates that strict controls must be operational to prevent p53 activation in the absence of cellular stress. Foremost among the gatekeepers to p53 activation are MDM2 and the related protein, MDM4.

MDM2 binds to and inhibits the ability of p53 to stimulate transcription.[25,26] In addition, MDM2 regulates p53 protein stability. MDM2 targets p53 for destruction by the ubiquitin-proteasome pathway by functioning as an E3 ubiquitin ligase and facilitating the covalent attachment of ubiquitin to p53,[27-29] thus regulating the overall level of p53 protein in a cell. The *Mdm2* gene is a target of p53 transcriptional activation, thereby forming an autoregulatory loop in which p53 regulates expression of it own inhibitor. MDM4 also inactivates p53 transcriptional activity[30] but, unlike MDM2, lacks ubiquitin ligase activity,[31] and *Mdm4* expression is not regulated by p53. Both *Mdm2*$^{-/-}$ and *Mdm4*$^{-/-}$ mice die early in embryogenesis but survive to adulthood when p53 is deleted,[32-35] thereby highlighting the critical roles of MDM2 and MDM4 in negatively regulating p53 during development.

MDM2 and MDM4 are expressed in the CNS.[35,36] Conditional mouse models using nestin-driven Cre recombinase demonstrate that loss of MDM2 in the developing CNS elicits massive p53-dependent apoptosis in the ventricular zone and intermediate zones of the cerebral cortex, leading to the degeneration of the neuroepithelium and hydrocephalus and to perinatal lethality.[37,38] Studies using a novel hypomorphic allele, *Mdm2*^{puro}, support a threshold requirement for MDM2 in the developing CNS. *Mdm2*$^{puro/\Delta 7-9}$ mice express ~30% of the wild-type level of MDM2 and are viable

but have small brains and an elevated level of p53-mediated apoptosis in granular neuronal precursors (GNPs) of the cerebellum.[39,40] As the restoration of p53 function in the mature brain of adult MDM2/p53-deficient mice fails to induce neuronal apoptosis,[41] there appears to be an increased need for MDM2 to buffer p53 from endogenous p53 activating signals in the developing CNS.

The effects of MDM4 loss in the CNS are less severe than those observed for MDM2. Mice with CNS-specific ablation of MDM4 are viable but manifest significant growth retardation and cerebellar ataxia.[37] Loss of MDM4 impairs cell proliferation in the embryonic neuroepithelium, lateral ventricular zone of the cerebral cortex, and external granular layer of the cerebellum. However, in contrast to its anti-proliferative effect in neural precursors, MDM4 deficiency promotes apoptosis in postmitotic neurons.[37,38] As with MDM2, the effects of MDM4 loss on cell proliferation and survival are dependent on the presence of p53. Thus, MDM2 and MDM4 have nonredundant roles in regulating p53 in the developing CNS.

Although MDM2 and MDM4 independently control p53 function, their activities are likely to synergize to maintain p53 at low or undetectable levels. Deletion of one *Mdm4* allele exacerbates the CNS phenotype of *Nes-Cre*; *Mdm2*–null embryos, as does the deletion of one *Mdm2* allele in *Nes-Cre*; *Mdm4*–null mice. On a *Nes-Cre* background, loss of MDM2 did not elicit p53-dependent apoptosis in postmitotic neurons. However, studies with *Nex-cre* mice that specifically express Cre in differentiated neurons reveal that deletion of even one *Mdm2* allele in this cell type potentiates apoptosis when *Mdm4* is co-deleted.[37] Analyses of *Nes-Cre* and *Nex-cre Mdm2*/*Mdm4* compound mutants support a dose-dependent, synergistic effect of these two critical regulators on the inhibition of p53 function. Therefore, MDM2 and MDM4 each have an important role in limiting p53 function in both proliferating neuronal precursors and differentiated postmitotic neurons of developing CNS.[37,38]

Multiple proteins, in addition to MDM2 and MDM4, have been shown to interact with and influence p53 function through either direct regulation of p53 stability and/or activity or through modulation of MDM2 and/or MDM4 activity. A detailed list of proteins known to interact with p53, MDM2, and/or MDM4 can be found in Table 3 of Toledo and Wahl.[42] A discussion of how all these factors may contribute to the regulation of p53 function is beyond the scope of this review. However, as will be explored in the following sections, both the activation and loss of p53 contribute to diseases of the CNS. It will be important to gain an improved understanding of how known regulators of p53 function specifically impinge upon the MDM2/MDM4/p53 axis in the developing and adult CNS.

p53 in Neural Stem and Progenitor Cells

Neural stem cells (NSCs) are self-renewing cells in the nervous system that can generate both neurons and glia.[43] Early in nervous system development, NSCs can be found throughout the embryonic brain. Initial symmetrical division of NSCs serves to expand the stem cell pool, whereas subsequent asymmetric divisions give rise to self-renewing neural stem cells and neuronal progenitors and, later, glial progenitors.[44] Adult neural stem cells have also been found within two well-described neurogenic niches: the subgranular zone (SGZ) in the dentate gyrus of hippocampus and the subventricular zone (SVZ) lining the wall of the lateral ventricles.[45-47] More recently, adult neural stem cells have been identified in non-neurogenic regions of the mature brain, including the cerebellum,[48] substantia nigra,[49] and retina.[50]

In contrast to the hematopoietic system wherein hemapoietic stem cells (HSCs) are required to fulfill a relatively similar function throughout the life of the organism, embryonic and adult neural stem and lineage-restricted progenitors (collectively precursor cells) differ in their self-renewal and differentiation characteristics. NSCs isolated from early stage embryonic rat forebrains, for example, exhibit a high self-renewal capacity compared to late-stage embryonic and adult NSC from the same tissue. Moreover, NSC cell fate determination is temporally regulated during development as the differentiation of NSC from early and late-stage forebrains occurs predominantly toward the neuronal and glial lineages, respectively.[51] Spatial and temporal specification of neural precursors is achieved through a combination of intrinsic and extrinsic cues. Growth factor concentration is one environmental cue predicted to play an important role in establishing neural precursor cell potential and phenotype. Genetic mutations acquired by neural precursor cells that alter properties, such as their sensitivity to different growth factors, may therefore promote cellular transformation.

Recent studies have begun to shed light onto the important role(s) of p53 in the regulation of NSCs. p53 is expressed within the neurogenic niche of the lateral ventricles.[52,53] Abundant nuclear p53 is evident in the ependymal cell lining of the lateral ventricle wall as well as most cells of the SVZ, including astrocytes and progenitors. In agreement with the down-regulation of p53 in differentiating cells observed during embryogenesis, nuclear p53 immunoreactivity is absent or found at low levels in the majority of the mature brain, including differentiating cells in the rostral migratory stream,[52] suggesting that p53 is preferentially expressed in neural precursors.

In the absence of markers to definitively identify NSCs *in vivo*, NSC behavior, including self-renewal and multipotentiality, can be assessed using neurospheres, floating aggregates of heterogeneous cell types, including neural stem and progenitor cells.[45] Neurospheres established from either the embryonic olfactory bulb (OB) or adult SVZ of $p53^{-/-}$ mice proliferate more rapidly, are less apoptotic, and have an

increased capacity for self-renewal than their wild-type counterparts.[52,54-56] Within the SVZ, loss of p53 promotes cell division in slow-dividing astrocyte-like cells ($Nestin^+/GFAP^+$ type B), residing between the ependymal lining and striatal parenchyma,[57,58] without a concomitant increase in cell proliferation,[54] consistent with an enhancement of the self-renewal properties of this putative NSC population.

NSC proliferation, survival, and self-renewal are tightly coordinated with differentiation. When cultured in differentiation medium (2% FCS, EGF/bFGF-free), p53-deficient neurospheres generate an increased number of $Tuj1^+$ neuronal progenitors and decreased number of glial fibrillary acid protein $(GFAP)^+$ astrocytes as compared to neurospheres with wild-type p53.[54,55] The altered differentiation pattern in $p53^{-/-}$ neurospheres suggests that p53 may play a direct role in the negative regulation of neurogenesis. Within the SVZ, type B cells give rise to highly proliferative transit-amplifying precursor cells (type C cells) that can generate migratory neuroblasts (type A cells) and oligodendrycte progenitors,[59] which migrate to the OB along the rostral migratory stream where they differentiate to form olfactory granule cells and periglomerular interneurons.[43] In the absence of p53, the proliferation rate of type C cells is enhanced, although the number of C cells in the $p53^{-/-}$ SVZ is not increased. In contrast, A cells are more abundant in the $p53^{-/-}$ SVZ without an apparent increase in their proliferation rate,[54] suggesting that p53 deficiency promotes that proliferation and rapid differentiation of type C cells into type A cells. Together, these findings suggest p53 may selectively regulate NSC self-renewal, differentiation, and cell fate determination depending on cellular and developmental context.

p53 and Brain Tumor Stem Cells

A growing number of studies have revealed that a small fraction of cells in tumors is capable of tumor initiation. Similar to stem cells found in normal tissues, these select tumors cells have the capacity for self-renewal and for producing a diverse repertoire of differentiated progeny, suggesting that they may be cancer stem cells. Initially identified in hematopoietic malignancies,[60] cancer stem cells have now been found in solid tumors, including those of the breast,[61] pancreas,[62] colon,[63] and brain.[64,65] For brain tumors, it has been demonstrated that as few as a hundred glioblastoma or medulloblastoma (MB) tumor cells expressing the stem cell marker CD133 (prominin-1) can give rise to primary and secondary tumors upon serial xenograft transplantation,[64] thereby supporting the identity of a subset of these cells as brain tumor stem cells (BTSCs). The origin of a BTSC is unclear. It may arise from the transformation of an NSC or from a lineage-restricted precursor cell or mature differentiated cell that, through oncogenic mutations, has acquired stem cell–like properties. Many of the pathways known to regulate NSC stemness are active in brain cancers, suggesting that these same pathways control the self-renewal and cell fate potential of BTSCs.[66] Importantly, a BTSC within a tumor may not necessarily be the cell of origin for that tumor but may arise through the accumulation of de-differentiating mutations.[67] Slowly proliferating and highly resistant to most therapeutic regimens, BTSCs may contribute to tumor regrowth and patient relapse.

The role of p53 in BTSCs has not been well established. Based on our current limited understanding of p53 function in neural precursors, several hypotheses may be put forth. First, loss of p53 may enhance the proliferation and self-renewal of neural stem and/or lineage-restricted progenitors, thereby expanding the pool of cells available for additional oncogenic mutations. Second, as p53 can promote or inhibit cell differentiation depending on cellular context, as well as influence cell fate decisions, p53 mutation may alter the differentiation program of neural precursors. Last, although much focus on p53 is directed at its growth inhibitory properties, accumulating evidence supports a role for p53 in the suppression of cell migration.[68] The neurogenic niche has been shown to be important for the maintenance of stem cells in an undifferentiated state,[69] and in the absence of p53, the premature exit of NSCs from the neurogenic niche may alter their differentiation program or capacity for tissue invasion. A greater understanding of the molecular, cellular, and environmental factors that regulate p53 function in NSCs is predicted to provide new insights into the role of p53 in BTSCs.

p53 in CNS Tumors

The p53 tumor suppressor protein is a potent inhibitor of cell proliferation. Activated in response to a wide range of cellular stresses (e.g., DNA damage, hypoxia, oncogene-induced hyperproliferation, and impaired ribosome biogenesis),[70-73] p53 induces anti-proliferative processes, including cell cycle arrest[73] and apoptosis,[74] thereby potently limiting the survival of potentially pre-neoplastic cells. p53 function is lost in the majority of human tumors due to either mutation of the p53 gene[75] or inactivation of the p53 protein through alternative mechanisms, including mislocalization of the p53 protein[76] and inhibition of p53 by viral (e.g., human papillomavirus E6 protein)[77,78] or cellular (e.g., MDM2)[79,80] proteins, thus underscoring the importance of p53 function for tumor suppression. Here we discuss the role of p53 in two tumors of the CNS, medulloblastoma and glioblastoma.

p53 in Medulloblastoma

Medulloblastoma, a primitive neuroectodermal tumor of the cerebellum, is the most common malignant brain tumor of childhood. It has long been the prevailing view that MB pathogenesis reflects the dysregulation of the normal developmental program of the cerebellum. MB tumors are highly heterogenous with regard to histopathology, age of patient,

Table 1. Schematic Overview of the 4 Subgroups of Medulloblastoma (MB) and the Putative Mechanisms for p53 Inactivation Discussed in This Review

	MB Subgroup			
	Wnt	Shh	C	D
Cell of origin	NSC?	GNP?	?	?
Frequent molecular characteristics	CTNNB1 mutation; elevated MYC	Ptch mutation	Elevated MYC	17q gain
p53 inactivation	p53 mutation	Enhanced MDM2 function?	?	Elevated WIP1
Predominant histology	Classic	Desmoplastic	Classic	Classic

GNP, granular neuronal precursor; NSC, neural stem cell.

and stage of metastasis at time of diagnosis. Current treatments for MB are not very specific for tumor cells, resulting in devastating side effects, including reduced intellect. Despite aggressive treatment with surgical resection, radiation, and chemotherapy, only 60% of children are cured of MB.[81]

The role of p53 in MB pathogenesis has not yet been conclusively defined. Persons with Li-Fraumeni syndrome that is caused by a germline mutation in *p53* develop MB at a higher incidence than the general population.[82,83] Similarly, p53 deficiency in mice in combination with mutations in other genes, including poly (ADP-ribose) polymerase (*PARP*), the cell cycle regulatory protein retinoblastoma (*Rb*), or the Sonic hedgehog (Shh) receptor Patched1 (*Ptch1*), greatly increases tumor incidence,[84-87] indicating that loss of p53 can promote MB tumorigenesis. However, despite the high incidence of *p53* mutations in most human tumors, the *p53* gene is altered in <10% of sporadic human MB.[88-95] Chromosome 17p, where *p53* is located, is lost in 40% to 50% of sporadic MB tumors. It has since been found that loss of 17p in MB and p53 status is unrelated.[88,96]

New support for a role for p53 in MB tumorigenesis comes from a greater understanding of heterogeneity underlying MB tumors. Through the integration of molecular and clinical markers, including genetic abnormalities, patient age at time of diagnosis, tumor histology, and overall and progression-free survival, 4 MB subgroups with distinct signaling pathway alterations and clinicopathological features have been identified (see Table 1). These subgroups are characterized, in part, by deregulated Wnt (A) or Sonic hedgehog (B) signaling or by distinct gene expression profiles (C and D).[97] Examination of *p53* gene status in 310 primary human MB tumors representing all 4 MB subgroups confirmed that *p53* gene mutations in MB are rare and failed to correlate with chromosome 17p deletions.[96] In addition, this study yielded several novel insights. First, *p53* gene mutations are overrepresented in MB tumors that have a mutation in the *CTNNB1* gene (encoding β-catenin), indicative of Wnt activation, or that have a high level of *NMYC* amplification. By comparison, *p53* mutations did not correlate with dysregulation of the Shh pathway or with subgroup D and were not present in MB tumors from subgroup C. Second, MB tumors harboring *p53* mutations typically had a classic or large cell/anaplastic histology, whereas desmoplastic MB tumors that typically exhibit Shh pathway activation did not, consistent with the lack of *p53* mutations in the Shh subgroup of MB. Last, in contrast to an earlier study with a smaller cohort of MB tumors,[98] *p53* gene status did not correlate with unfavorable prognosis.

From the association of p53 mutations with particular subgroups of MB, we may begin to infer how loss of p53 mechanistically contributes to MB tumorigenesis. *CTNNB1* gene mutation and *NMYC* amplification are predicted to impose a strong selective pressure for tumor cells lacking wild-type p53. p53 and β-catenin have been proposed to form a negative feedback loop in which overexpression of β-catenin activates p53 transactivation function and, in turn, p53 down-regulates β-catenin.[99-101] The proliferation of MB tumor cells with aberrant levels of β-catenin resulting from *CTNNB1* gene mutation may be initially restrained by heightened levels of p53. Similarly, deregulated *NMYC* expression can induce apoptosis concomitant with the promotion of cell proliferation,[102] and cooperative mutations that abrogate apoptotic pathways such as p53 are predicted to augment tumorigenesis. In addition, p53 limits genomic instability in NMYC-overexpressing tumor cells by suppressing NMyc-mediated centrosome amplification.[103] For MB tumor cells with *CTNNB1* gene mutation or *NMYC* amplification, p53 mutations may be a necessary event for MB tumor progression. Consistent with the idea that loss of p53 is a late event in this subset of MB, *p53* mutations were more frequently present in MB tumors from children >7 years of age and only rarely present in MB tumors from infants.[96]

Given the prevalence of p53 inactivation in human tumors, p53 function in MB tumors lacking *p53* gene mutations is likely to be abrogated through alternative mechanisms. Recent work in our lab, for example, supports MDM2 as an important contributor to the inhibition of p53 in Shh-driven MB tumorigenesis. In cerebellar development, MDM2 is required to inhibit p53-mediated apoptosis in GNPs,[39] the presumed cell of origin for MB tumors of the Shh subgroup, and MDM2 deficiency potently restricts cerebellar tumorigenesis in *Ptch1*$^{+/-}$ mice, a model of human Shh-induced MB.[39,104] Importantly, we find that Shh stimulation

in GNPs promotes MDM2 protein accumulation and phosphorylation at Ser[166],[39] a modification known to promote MDM2-mediated degradation of p53,[105-107] suggesting that Shh signaling limits p53 activity by increasing the anti-p53 function of MDM2. Accumulating evidence supports this idea. In cultured mesenchymal cells and mouse embryonic fibroblasts (MEFs), Shh signaling enhances the level and p53 binding affinity of MDM2, thereby abrogating p53-mediated growth arrest and apoptosis in response to oncogene-induced DNA damage.[108] In transgenic mice in which the Shh effector Gli1 is overexpressed in the CNS, MDM2 is found highly expressed in the neonatal brain, and overexpression of Gli1 enhances cell proliferation concomitant with a decrease in p53 protein levels in these mice.[56] Conversely, pharmacological inhibition of Shh signaling increases p53-mediated growth inhibition *in vitro* in multiple breast cancer cell lines,[108] and knockdown of Gli1 has stabilized p53 in a glioblastoma cell line.[56] Likewise, Shh deficiency in zebrafish elicits heightened levels of p53-dependent apoptosis in the retina and developing CNS.[109] A hypothesis drawn from these results is that in human MB tumors in which Shh signaling is the initiating event, p53 function will be automatically limited through the same mechanisms that serve to restrict p53 activity in the developing cerebellum. Furthermore, Shh-mediated enhancement of the anti-p53 function of MDM2 is predicted to obviate the selective pressure for p53 mutations as well as for *Mdm2* gene amplification events, which, although present in ~7% of human tumors with wild-type p53,[91] are rare in MB.[88,91,110]

Intriguingly, when the level of MDM2 is reduced in GNPs, Shh signaling is attenuated concomitant with p53 activation,[39] suggesting a complex interplay between the p53 and Shh pathways. Recently, p53 has been shown to negatively regulate the level and subcellular localization of Gli1 in NSCs,[56] and both Gli1 and Gli2 expression is reduced in $Mdm2^{puro/\Delta 7\text{-}9}$ GNPs. Knockdown of p53 promotes NSC renewal synergistically with Gli1 overexpression, suggesting that p53 constitutively attenuates Gli1 function.[56] The negative regulation of Gli activity by p53 would provide a plausible explanation to account for the earlier observation that Gli1 is highly expressed in MB tumors developing in mice lacking functional p53,[86] in which known components of the Shh pathway are wild-type. The coordinate regulation of the p53 and Shh pathways may therefore provide a mechanism to allow Shh to drive high levels of proliferation during important periods of development without necessarily activating the growth inhibitory properties of p53 and, intriguingly, to perhaps also maintain a constant level of Shh signaling.

Several p53-negative regulatory proteins, in addition to MDM2, have also been implicated in MB tumorigenesis. The gene encoding the serine/threonine phosphatase Wip1 (also known as PPM1D) maps to chromosomal region 17q22-q23 and is highly expressed in MB with gain of chromosome 17q or i17q,[111] genetic alterations associated with MB subgroups C and D.[97] Wip1-mediated dephosphorylation of MDM2 enhances MDM2 stability and p53 binding affinity, thereby promoting p53 degradation.[112] Recently, the MAGE-A family of cancer/testis antigens was reported to be overexpressed in 60% of MB cell lines and tumors.[113] These metastasis-associated transcriptional regulators have been proposed to limit the transactivation function of p53 by blocking the binding of p53 to chromatin.[114] Interestingly, MAGE-A is one of the targets down-regulated by miR-34, a microRNA that is deleted in a subset of MB tumors.[115]

The integration of molecular and clinical features of MB has greatly advanced our understanding of the heterogeneity of MB tumors. Rare gene mutations in MB such as those in *p53* gain new significance when analyzed in conjunction with tumor-specific gene expression profiles and clinicopathological features. New knowledge about the gene and signaling pathways involved in different subgroups of MB is predicted to provide insight into MB pathogenesis and foster the development of new, more selective therapies for the treatment of MB.

p53 in Glioblastoma

Gliomas, a large class of tumors that includes low-grade diffuse astrocytoma, oligodendroglioma, and oligoastrocytoma (mixed glioma), are the most common malignant brain tumors in adults, accounting for more than 70% of primary tumors of the CNS. Malignant World Health Organization (WHO) grade IV astrocytoma, glioblastoma multiforme (GBM), is the most aggressive of all brain cancers, resulting in diffuse, highly infiltrating tumors with areas of extensive necrosis and vasculature. GBM affects the cerebral hemispheres and, in rare cases, the cerebellum.[116] GBM can be separated into two distinct diseases, which, although histologically similar, differ in their molecular alterations, age of patients at time of clinical presentation, and response to radio- and chemotherapy. Primary or *de novo* GBMs account for >90% of GBM tumors and typically occur in patients with a mean age of 65 years with little evidence of a precursor lesion. In contrast, secondary GBMs (~5%) are associated with younger patients (mean age 45 years) and develop from a previous low-grade diffuse astrocytoma or anaplastic astrocytoma.[117] Despite recent advances in our understanding of GBM biology and treatment, median survival rate in patients presenting with this tumor type is less than 12 months, and treatments are largely palliative.

Common genetic alterations in primary GBM include epidermal growth factor receptor (EGFR) amplifications (~40%) and PTEN (phosphatase and tensin homolog deleted on chromosome 10) mutations (15%-40%), but these are rare events in secondary GBM.[118] p53 pathway mutations, in contrast to MB, are common in GBM.[119-121] *p53* gene mutations are present in 68% of secondary GBM and typically occur in hotspot codons 248 and 272. Loss of p53 function is likely an early event in secondary

GBM as two-thirds of low-grade astrocytomas, a precursor lesion to secondary GBM, exhibit *p53* mutations,[122] and in mouse models, *p53* inactivation promotes astrocytoma tumorigenesis.[123,124] In primary GBM, *p53* mutations occur less frequently (28%) and are spread throughout the gene. It has been hypothesized that *p53* mutations in these tumors may be a late event, resulting from increased genomic instability.[118]

The importance of p53 pathway inactivation in GBM is further illustrated by the alteration of upstream regulators. The MDM2 ubiquitin ligase and related protein, MDM4, inhibits p53 function.[125] An alternatively spliced transcript from the CDKN2A locus encodes the protein ARF. Binding of ARF to the C terminus of MDM2 results in relocalization of MDM2 to the nucleolus, thereby inhibiting the nucleoplasmic shuttling function of MDM2 required for MDM2-mediated degradation of p53.[126-128] Gene amplifications in *Mdm2* and *Mdm4* occur in 10% and 4%, respectively, of GBMs and exclusively in GBMs that lack *p53* mutations.[121,129,130] Expression of ARF is lost in 76% of GBMs due to promoter methylation, homozygous deletion, or mutation.[121,131] Recently, an integrative analysis of copy number and somatic cell alterations showed that the ARF/MDM2/MDM4/p53 pathway is disrupted in 78% of GBMs.[121]

Additional genomic alterations may also contribute to the neutralization of p53 activity in GBM. A novel member of the Bcl2 family, Bcl2-like 12 (Bcl2L12), is overexpressed in almost all primary GBMs and has been found to inhibit p53-mediated cellular senescence and cell death by binding to and impairing p53 transactivation potential.[132,133] Genomic loss of ATM and CHEK2, two components of the DNA damage response required for p53-mediated apoptosis in response to radiation, is found in 13.3% and 22% of GBMs, respectively.[134] The tumor suppressor CHD5, located at 1p36 that is deleted in GBM with 1p loss, has been shown to modulate p53 function by increasing expression of ARF.[135] Considering the large number of factors that can regulate p53 activity,[42] it is likely that upon further examination, additional genomic alterations in GBM will be shown to neutralize p53 function.

Similar to MB, intense effort is under way to generate a comprehensive molecular classification of GBM with the hope that this may ultimately lead to improved clinical outcome. A pilot study by the Cancer Genome Atlas (TCGA) Research Network has catalogued genomic alterations and gene expression profiles from a large cohort of morphologically similar GBMs. This work has led to the identification of 4 subgroups of GBM: proneural, neural, classical, and mesenchymal.[136] Although present in 3 of the 4 subgroups, p53 mutations were predominantly found in the proneural subgroup of GBM. Significantly, 3 of the 4 known secondary GBMs included in the study were of the proneural subgroup, consistent with the idea that p53 loss is an early event in this group of GBMs. It has yet to be established whether GBM subgroups originate from NSCs that pursue different differentiation pathways or whether they reflect tumor initiation in different stem-like precursor cells. In light of the recently identified roles for p53 in NSC self-renewal and cell fate decisions, loss of p53 in NSCs may serve to increase NSC proliferation and self-renewal, leading to an expanded pool of precursor cells in which differentiation is compromised, thereby fostering their transformation into BTSCs for GBM. Supporting this idea, prenatal treatment with ENU results in the formation of glioblastoma-like tumors in 60% of $p53^{-/-}$ mice.[54]

GBM has proven to be highly resistant to most conventional therapeutic approaches, and for the majority of cases, surgery followed by chemo- and radiotherapy is largely palliative. Our improved understanding of GBM pathogenesis has, to date, failed to translate into more effective targeted therapies, and there has been little improvement in mean survival time for the past 10 years.[137] Treatment difficulties include intratumoral heterogeneity as well as putative BTSC subpopulations. New strategies to specifically target BTSCs are particularly needed as these cells are highly efficient at DNA repair and typically express elevated levels of multiple-drug-resistant transporter genes, thereby causing them to be highly resistant to radiation and chemotherapeutic agents. In human GBM xenografts, $CD133^{+}$ tumor cells were found enriched after ionizing radiation treatment.[138] BTSCs are therefore likely to play an important role in GBM multidrug resistance and tumor regrowth. Efforts to develop therapeutics specifically targeted to the known genetic lesions of GBM have, to date, been largely unsuccessful. Thus, there is an immediate need for the identification of new drug targets and for the development of multimodality approaches to treat this devastating disease.

Strategies to increase the function of p53 in tumor cells are currently being explored and may improve the treatment of GBM. As a critical component of the cellular response to stress, p53 function is required for the cytotoxic effects of many commonly used cancer drugs and radiation. Loss of p53 function correlates with a decrease in sensitivity to these same genotoxic agents and an increase in tumor cell survival.[139] The *p53* status of individual tumor types is frequently an important prognostic indicator for the likelihood of successful treatment with radiation and some chemotherapeutics. Adjuvant therapeutics that augment p53 function are predicted to sensitize tumor cells to cancer therapies that rely on p53 for their efficacy.[140] Different therapeutic strategies will be needed depending on whether p53 is mutated, deleted, or wild-type but functionally inactivated by viral or cellular p53 regulatory proteins.[141] In this regard, pharmacological compounds (e.g., CP-31398, APR-246) have been identified that promote the correct folding and reactivation of p53 mutant proteins.[142,143] An adenoviral-mediated gene transfer strategy is

being explored to reintroduce wild-type p53 function in *p53*-null tumor cells. Yet another strategy is to use MDM2 antagonists (e.g., nutlin-3, RITA) that reduce the MDM2-p53 interaction and impair MDM2-mediated proteolytic degradation of p53. These agents have been demonstrated to potentiate p53 function in cultured tumor cells and *in vivo* xenograft models and enhance the chemosensitivity of tumor cells to DNA damaging agents.[144-146] Importantly, in mice, even a moderate ~20% reduction in the level of MDM2 is sufficient to sensitize mice to the lethal effects of ionizing radiation due to the enhancement of p53 function,[40] suggesting that even modest gains in p53 function may sensitize tumor cells to radiation and chemotherapeutic agents. More work is required to evaluate whether enhanced p53 activity in GBM tumor cells, particularly BTSCs, can potentiate cell death in response to current treatment modalities.

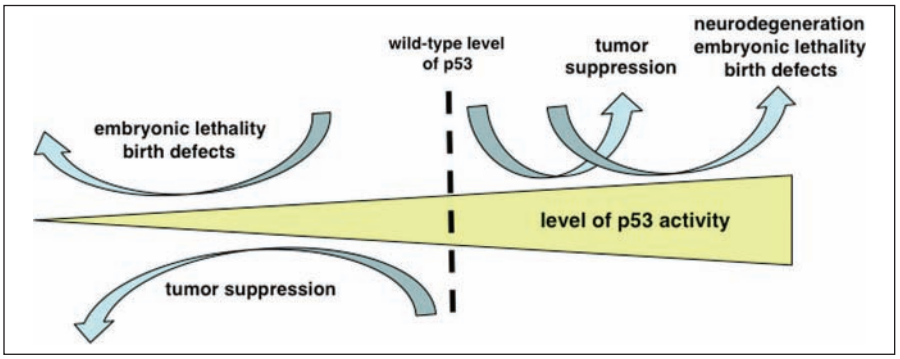

Figure 1. The level of p53 as a determinant of life and death. Elevating basal p53 function beyond its normal wild-type level can enhance tumor suppression. However, as p53 levels continue to rise, the benefits of enhanced tumor suppression become increasingly negated by the deleterious effects of the antigrowth properties of p53 in developing and homeostatic tissues. Chronic or aberrantly elevated p53 function is implicated in the neurodegeneration associated with several central nervous system pathologies, including Alzheimer and Parkinson diseases in adults and congenital birth defects and lethality in developing embryos. Conversely, loss of p53 function increases lifelong susceptibility to cancer and may abrogate a critical developmental checkpoint in the embryo.

Contribution of p53 to CNS Pathology

Although important for tumor suppression, p53-mediated neuronal apoptosis is associated with neurological damage that occurs following acute insults such as ischemia, traumatic brain injury, or exposure to neurotoxins. Sustained p53-mediated apoptosis has also been implicated in chronic neurodegenerative pathologies, including Parkinson, Alzheimer, and Huntington diseases.[147,148] Neuronal cells of the developing CNS, in particular, are highly sensitive to genotoxic stress, and the transactivation and apoptotic functions of p53 are rapidly increased throughout the embryonic brain following exposure to genotoxic agents such as gamma irradiation[9,149] or the xenobiotic, TCDD.[150] Mouse embryos lacking *p53* treated with these same genotoxic agents exhibit an increased incidence of teratological malformations.[149,150] In the embryo, p53 may suppress teratogenesis by limiting the propagation of severely damaged cells in the developing CNS and promote death of the organism in cases where the genotoxic damage is too great (Figure 1).

As a suppressor of teratogenesis, p53 may reduce the incidence of congenital defects in surviving embryos. However, in some cases, perhaps more than are currently appreciated, the potent growth inhibitory properties of p53 may also underlie the CNS pathology of human congenital disorders. Evidence for a key role of p53 in congenital birth defects is provided by data from *in vivo* models. In *Tcof1*$^{+/-}$ mice, a model of Treacher Collins syndrome, elevated apoptosis in the neuroepithelium is rescued by the loss of p53,[151] thus ameliorating the craniofacial abnormalities typically associated with this disorder. A point mutant in the human *Pax3* gene is associated with Waardenburg syndrome.[152] The Pax3 transcription factor is highly expressed in the neuroepithelium and neural crest, and Pax3-deficient *Splotch* (*Sp/Sp*) mice exhibit neural tube defects, including exencephaly, spina bifida aperta, or both, which coincides with increased p53-dependent apoptosis in the neuroepithelium.[153-155] Intriguingly, it has been recently demonstrated that within a panel of zebrafish carrying mutations in essential housekeeping genes, all had up-regulation of multiple p53 target genes. These mutants all exhibited increased neuronal apoptosis, which could be inhibited by the injection of anti-p53 morpholinos.[156] Together, these studies in mice and zebrafish illustrate the sensitivity of the neuroepithelium and neural crest cells to p53-mediated apoptosis induced in response to mutations in genes with roles in a diverse range of essential processes.

How do so many gene mutations lead to p53 activation? As discussed previously, in some cases, an impaired ability to repair spontaneous DNA breaks due to loss of DNA repair pathway proteins (e.g., *Lig4*, *XRCC4*, *Pol*β) may result in an elevated level of endogenous genotoxic stress that is sufficient to trigger a p53-dependent DNA damage response. However, in many cases, the gene mutation is not predicted to directly lead to an increase in genotoxic stress. We propose that many of these gene mutations may perturb the network of negative regulators that normally limit p53 function, thereby lowering the threshold for p53 activation in response to spontaneous genotoxic stress or suboptimal trophic support.

For example, constitutive expression of the Notch1 intracellular domain (NICD) in the developing mammalian brain selectively promotes p53-dependent apoptosis

in a subset of neural precursor cells but not in immature or mature postmitotic cells.[157] Although the mechanism by which constitutive Notch1 signaling triggers p53 activation in neural progenitor cells is not yet well understood, the suppression of PI3K/AKT-mediated enhancement of MDM2 function may contribute. Upon growth factor stimulation, activated AKT phosphorylates MDM2 at Ser166 and Ser186 through both direct and indirect mechanisms, thereby enhancing MDM2-mediated inhibition of p53.[105-108] In cultured cells, NICD signaling through activation of the CBF1 transcription factor regulates expression of PTEN,[158] which encodes a lipid phosphatase that dephosphorylates and inactivates PI3K. The direct regulation of PTEN expression by Notch signaling is consistent with the finding that NICD overexpression reduces the level of total and phosphorylated AKT hepatocellular carcinoma cells (HCC), thereby enhancing p53 stability through the attenuation of MDM2-mediated proteasomal degradation of p53. Active Notch1 signaling sensitizes HCC cells to p53-mediated apoptosis in response to tumor necrosis factor–related apoptosis-inducing ligand (TRAIL).[159] Taken together, these observations suggest that by inhibiting the AKT-mediated modulation of MDM2 function, Notch signaling may lower the threshold for p53 activation, thus sensitizing neural precursor cells to apoptotic stimuli.

In the aforementioned *Splotch* (*Sp/Sp*) mice, loss of Pax3 results in a post-transcriptional increase in p53 protein levels.[153,160] Recent studies demonstrate that Pax3 enhances the rate of p53 degradation. Pax3 can down-regulate a p53 mutant incapable of binding MDM2,[161] thereby implicating Pax3 in the regulation of other negative regulators of p53 stability (e.g., PirH2 or Cop1). The chromatin silencing factor, Sirt1, antagonizes p53 function by promoting its deacetylation,[162] and mice deficient in Sirt1 also exhibit exencephaly that correlates with p53 hyperacetylation and enhanced p53-dependent apoptosis.[163] Mutations in more than 150 genes, encoding proteins involved in a wide variety of biochemical pathways and cellular processes, result in NTDs in mice.[164] To date, only a few NTD mutants have been tested for an interaction with p53. It is tempting to speculate that, as with zebrafish,[156] many of these NTD mutations canalize on the MDM2/MDM4/p53 axis.

Together, these findings suggest that p53 functions in a developmental checkpoint in the CNS by integrating environmental and genetic information. Further understanding of the contribution of active p53 to human disease may yield new points of therapeutic intervention.

Concluding Remarks

Our knowledge of p53 function is immense, but the continued discovery of new roles for p53 in diverse cellular processes suggests that the majority of what we need to learn is still hidden from us. In particular, the recent demonstration that p53-mediated neuronal apoptosis is a major contributing factor to the lethality of mice and zebrafish that carry mutations in a wide range of housekeeping genes invites the further investigation of p53 pathway modifications in human congenital birth defects. Therapeutic strategies to inactivate p53 have not been very actively pursued, but such strategies do exist, and it remains to be determined whether there are windows of opportunity in which their application may mitigate some developmental malformations. In contrast, numerous therapeutic strategies to activate p53 are being explored. Although initial studies in animal models show that enhancing p53 function can be effective at de-bulking xenograft tumors, it remains to be determined whether this approach is effective at eliminating rare brain tumor stem cells. Moreover, issues regarding the delivery of p53 enhancing agents to tumors in the brain will need to be addressed. The decision as to whether to use a p53-targeted therapeutic approach in a tumor is predicted to be guided, in part, by advances in the classification of tumors, such as those described for medulloblastoma and glioblastoma. Among the recently identified functions for p53, perhaps the most exciting is the role of p53 in the regulation of neural precursor cell self-renewal, differentiation, and cell fate. New mouse models and tools to identify and track neural precursor cells *in vivo* will be required to further elucidate the role of p53 in this cell type. New information about the molecular and environmental factors that regulate p53 function in neural precursor cells may provide novel insight into how the loss of p53 promotes CNS tumorigenesis.

Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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