The tumor suppressor protein p53 is required for neurite outgrowth and axon regeneration

Simone Di Giovanni^{1,2,*}, Chad D Knights³,
Mahadev Rao³, Alexander Yakovlev²,
Jeannette Beers², Jason Catania³, Maria
Laura Avantsaggiati^{3,4,*} and Alan I Faden^{2,4}

¹Laboratory for NeuroRegeneration and Repair, Hertie Institute for Clinical Brain Research, University of Tuebingen, Germany,

²Department of Neuroscience, Georgetown University Medical Center, Washington DC, USA and ³Lombardi Cancer Center, Georgetown University Medical Center, Washington DC, USA

Axon regeneration is substantially regulated by gene expression and cytoskeleton remodeling. Here we show that the tumor suppressor protein p53 is required for neurite outgrowth in cultured cells including primary neurons as well as for axonal regeneration in mice. These effects are mediated by two newly identified p53 transcriptional targets, the actin-binding protein Coronin 1b and the GTPase Rab13, both of which associate with the cytoskeleton and regulate neurite outgrowth. We also demonstrate that acetylation of lysine 320 (K320) of p53 is specifically involved in the promotion of neurite outgrowth and in the regulation of the expression of Coronin 1b and Rab13. Thus, in addition to its recognized role in neuronal apoptosis, surprisingly, p53 is required for neurite outgrowth and axonal regeneration, likely through a different post-translational pathway. These observations may suggest a novel therapeutic target for promoting regenerative responses following peripheral or central nervous system injuries.

The EMBO Journal (2006) 25, 4084–4096. doi:10.1038/sj.emboj.7601292; Published online 31 August 2006

Subject Categories: neuroscience; molecular biology of disease

Keywords: axon regeneration; coronin 1b; neurite outgrowth; p53; Rab13

Introduction

Axonal injuries induce delayed biochemical alterations that may result in either cell death or survival/restoration, including successful target re-innervation (Yakovlev and Faden, 1995; Kalb and Strittmatter, 2000; Dumont *et al*, 2001;

Makwana and Raivich, 2005). Functional recovery reflects, in part, the number of surviving cells and fiber tracts, the extent of neural plasticity, and the presence of a permissive environment for regeneration. Such processes are orchestrated by time-regulated gene expression changes, with an earlier phase associated with inflammation, extension of axonal damage and cell death, and a later one linked to axon outgrowth and regeneration (Kubo *et al*, 2002; Bareyre and Schwab, 2003; Di Giovanni *et al*, 2003, 2005a; Glanzner *et al*, 2004).

Some of the genes related to neurite and axon outgrowth and regeneration play a dual role in both cell death and in cell survival following central nervous system (CNS) or peripheral nerve injury (Benowitz and Routtenberg, 1997; Hughes *et al*, 1999; Emery *et al*, 2003). One example of this duality of effects is provided by the transcription factor c-jun, which has been implicated in cell death and nerve degeneration in various models of CNS and peripheral nerve injury, but more recently has also been shown to be required for axonal regeneration following nerve transection (Raivich *et al*, 2004).

Another transcription factor with pleiotropic functions is the tumor suppressor protein p53, which serves as a key determinant of cell fate following exposure to a variety of insults (Schuler and Green, 2005; Vousden and Prives, 2005). p53 functions as a DNA-binding, sequence-specific transcription factor that activates the expression of multiple genes, causing either reversible cell-cycle arrest or apoptosis. The proapoptotic activity of p53 often occurs when cells are exposed to severe genotoxic stress, whereas its effects on cell-cycle arrest may occur after moderate DNA damage, or after nutrient depletion (Bates *et al*, 1999; Jones *et al*, 2005).

The transcriptional activity of p53 is increased in neuronal precursors of the developing mouse brain, whereas it is reduced in cells undergoing terminal differentiation (Rogel *et al*, 1985; Schmid *et al*, 1991). However, high levels of p53 mRNA have also been detected in the developing brain in areas showing little or no apoptosis (Gottlieb *et al*, 1997; Komarova *et al*, 1997), and a percentage of p53-deficient mice exhibit neuronal abnormalities, particularly defects in neural tube closure (Armstrong *et al*, 1995). Studies in neuronal-like cells have also suggested that p53 plays a role in cell survival following NGF administration in PC-12 cells, and that the interaction of p53 with neuronal-specific transcription factors, such as Brn-3a, may determine a shift from cell death to neuronal survival (Hudson *et al*, 2004, 2005). Thus, because of its dual actions on cell cycle and cell death, p53 plays a versatile role in the regulation of cellular growth and differentiation.

We have recently employed temporal gene expression profiling after experimental spinal cord injury (SCI) in rats (Di Giovanni *et al*, 2005b), through which we identified a cluster of temporally correlated genes, including factors known to promote neurite outgrowth and axonal regeneration. This cluster includes the actin-binding protein Coronin

*Corresponding authors. Simone Di Giovanni, Laboratory for NeuroRegeneration and Repair, Hertie Institute for Clinical Brain Research, University of Tuebingen, Otfried-Mueller Strasse 27, D-72076 Tuebingen, Germany. Tel.: +49 0 7071 29 80449; Fax: +49 0 7071 29 4521; E-mail: simone.digiovanni@medizin.uni-tuebingen.de or Maria Laura Avantsaggiati, Lombardi Cancer Center, Georgetown University, 3970 Reservoir Road, Washington DC, 20057, USA. Tel.: +1 202 687 9199; Fax: +1 202 687 6402; E-mail: ma364@georgetown.edu

⁴These authors contributed equally to this work

Received: 23 February 2006; accepted: 27 July 2006; published online: 31 August 2006

1b, and the GTPase Rab13, which we showed to be required for physiological neurite outgrowth in PC-12 cells and dorsal root ganglion neurons (Di Giovanni *et al*, 2005b). The temporally coordinated activation of these genes suggested that their expression might be regulated by a common transcription factor. In this study, we have explored this possibility, and we demonstrate that p53 regulates the expression of both Coronin 1b and Rab13. Moreover, p53 is required for neurite outgrowth and maturation in cultured neurons, as well as for axonal regeneration following facial nerve transection. These activities are mediated by acetylation of p53 at position K320, which we have recently shown to be involved in p53-mediated resumption of proliferation and cell survival.

Results

In silico promoter analysis and experimental evidence identify p53 as a transcription factor for Coronin 1b and Rab13

We recently demonstrated that the actin-binding Coronin 1b and the small GTPase Rab13 are temporally coregulated following experimental SCI during the phase of nerve regeneration and recovery (Di Giovanni *et al*, 2005b). Importantly, these genes are also coexpressed with the proneurite outgrowth and proaxonal regeneration protein Gap-43 (growth-

associated protein-43) in axons after injury, and are required for the promotion of neurite outgrowth in PC-12 cells as well as in primary neurons (Di Giovanni *et al*, 2005b). These findings led us to hypothesize that their expression may be regulated by common transcription factors. In order to verify this hypothesis, we performed an '*in silico*' computer-based analysis of the predicted promoters of Rab13 and Coronin 1b genes. We found that rat Coronin 1b and Rab13 have multiple common p53 transcription binding sites (TBS). Significantly, such p53-binding motifs were conserved in both the mouse and human genes, thus implying that they are physiologically relevant (Figure 1A).

To assess whether p53 can bind to these promoter regions *in vivo* and within a chromatin environment, chromatin immunoprecipitation (ChIP) assays were performed. For these experiments, we took advantage of a p53 null cell line (H1299), where the expression of p53 is controlled by a tetracycline-inducible system. As shown (Figure 1B), p53 is bound to the '*in silico*' predicted TBS of endogenous Coronin 1b and Rab13 *in vivo*. Moreover, in human embryonic kidney cells (293), in rat pheochromocytoma PC-12 cells, and in the p53-inducible H1299 cells, both messenger RNAs and protein levels of Coronin 1b and Rab13 were coordinately upregulated following p53 expression (Figures 1C and D). Indeed, neither Coronin 1b nor Rab13 were expressed in H1299 cells

Figure 1 p53 binds to the TBS of Coronin 1b and Rab13, and regulates their expression. (A) Shown is a schematic illustrating the TBS for p53 on Coronin 1b and Rab13. DNA sequences and location from the transcription starting site are also reported. MS stands for matrix similarity, and a score between 0.7 and 1 is considered highly significant. p53 matching P-value for the described TBS is highly significant. For details about interpretation of these data see: <http://www.genomatix.de>. (B) ChIP assays demonstrate *in vivo* interaction of WTp53 with DNA-binding elements derived from the human Coronin 1b and Rab13 promoters. H1299-WTp53 cells were left untreated (–) or treated for 48 h (48 h) with tetracycline to induce the expression of native p53. Following induction of p53, cells were crosslinked with formaldehyde and immunoprecipitated with a polyclonal antibody recognizing p53. The 'input' samples represent equal fractions of extract collected prior to precipitation from which DNA was extracted and used as a control. WAF1 (p21) promoter region was used as a positive control for p53 DNA binding. (C) Data from real-time RT-PCR experiments show increased mRNA expression levels for Coronin 1b and Rab13 in H1299 cells with tetracycline p53-inducible system 8 h after p53 activation. Similar results were obtained in PC-12 cells (data not shown). (**t*-test *P*-values <0.01; ***P*-values <0.001). (D) Protein expression data from immunoblotting experiments at the 24 h time point in H1299 cells that have a p53 tetracycline-inducible expression system (p53 tet-ind) and in PC-12 cells (p53 WT, siRNA, DN) (similar data were obtained in 293 cells, not shown). Induction (p53 tet-ind, and p53 WT) or repression (p53DN and p53 siRNA) of p53 activity, respectively, triggers or suppresses expression of Coronin 1b and Rab13.

in the absence of tetracycline, and overexpression of p53 in 293 or PC-12 cell lines resulted in a marked induction of their levels (Figure 1D). Conversely, either a p53-specific RNAi or a p53 dominant-negative construct (p53DN), previously shown to inhibit p53-dependent transcription (Yakovlev *et al*, 2004), nearly completely abrogated the expression of these two proteins in response to NGF treatment. Thus, the expression levels of Coronin 1b and Rab13 are regulated by p53.

p53 and its target genes Coronin 1b and Rab13 are expressed sequentially during neurite outgrowth

The above results lead to the prediction that a correlation should exist between p53 expression and the levels of Coronin 1b or Rab13 during neurite outgrowth and neuronal maturation. To test this possibility, we used PC-12 cells treated with NGF or rat embryonic primary cortical neurons (RCN) exposed to neuronal medium (B27). NGF mediates neurite outgrowth and cytoskeleton remodeling in PC-12 cells, whereas cultured RCN undergo maturation, neurite elongation, sprouting and expression of postmitotic neuronal-specific proteins in the presence of B27. In PC-12 cells exposed to NGF, we observed an early induction of nuclear p53, which was paralleled by elevations in the expression levels of Coronin 1b and Rab13, as well as of Gap-43 (Figure 2A and B). The latter is a cytoskeleton-bound protein found in growth cones and commonly used as a marker of neurite outgrowth, maturation and regeneration (Figure 2A).

The levels of p53 are regulated predominantly at a post-translational level, via a regulatory feedback with the ubiquitin ligase MDM2. Thus, we examined whether the expression of MDM2 changes during neurite outgrowth. We found that a marked reduction of MDM2 protein levels preceded p53 activation (Figure 2C). Such downregulation might explain, at least in part, the observed p53 induction in response to growth factors. In RCN, the expression of p53 peaked in the nuclei 2 days after the addition of growth factors, and this coincided with the time of greatest increase of Coronin 1b, Rab13, and Gap-43 protein levels (Figure 2D–F) at the cortical cytoskeleton. Therefore, p53 seems to act as a switch to turn on Coronin 1b and Rab13 expression levels in RCN, and its baseline expression levels appear to be sufficient to maintain Coronin 1b and Rab13 protein levels over longer periods of time. Alternatively, p53 post-translational modifications such as acetylation, which might increase the transcriptional activity of p53, as well as other transcription factors, might be involved in the sustained, long-term expression of Coronin 1b and Rab13. Thus, in different experimental systems there is a tight temporal correlation between the pattern of expression of p53 and that of Coronin 1b and Rab13.

p53 is required for neurite outgrowth

To further evaluate the potential role of p53 for neurite outgrowth, we first studied the effects of inhibiting p53 activity with the p53DN construct. The read-outs for these experiments were the NGF-mediated induction of neurite outgrowth in PC12 cells, and neurite outgrowth and maturation in cultured embryonic RCN. Stably transfected p53DN PC12 cells or RCN infected with a p53DN-lentiviral vector were treated with growth factors, and their phenotypes were analyzed. As it can be seen, inhibition of p53 signaling blocked the NGF-mediated increase of Coronin 1b and Rab13 expression (Figure 3A). Strikingly, in contrast to

naive PC-12 cells, the p53DN-transfected cells did not produce neurites following NGF treatment but, rather, demonstrated neurite length and number similar to that of untreated cells (Figure 4B and C). Similar results were also obtained by inhibiting p53 expression with a specific siRNA (data not shown). Likewise, expression of the p53DN in RCN markedly reduced the expression of both Coronin 1b and Rab13 (Figure 4A, Supplementary Figure 1), and significantly inhibited neurite outgrowth and branching (Figure 4B and C). Gap-43 levels were also decreased in cells expressing the p53DN construct. Thus, in the absence of p53, RCN cells fail to complete the maturation program in response to growth factors.

To more definitely assess the impact of p53-mediated control of Coronin 1b and Rab13 on neurite outgrowth, a rescue experiment was subsequently performed. In PC-12 cells stably transfected with p53DN, transfection of Coronin 1b and Rab13, which localized at high levels at the cortical cytoskeleton and at the growth cone (Figure 5A, Supplementary Figure 2), significantly restored the differentiated phenotype to a similar extent with and without previous administration of NGF, suggesting that NGF signaling is completely blocked in p53DN stably expressing cells and that Coronin 1b and Rab13 act downstream of p53 in such pathway (Figure 5A and B). These results support the conclusion that p53 is required for neurite outgrowth, and that such effects largely reflect p53-mediated modulation of Coronin 1b and Rab13 expression.

Specific acetylation of p53 at lysine 320 preferentially induces Coronin 1b and Rab13 expression, binds to their promoter elements and promotes neurite outgrowth

The highly undifferentiated human (NT2/D1) teratocarcinoma cells differentiate into several cell types, including neurons, following retinoic acid (RA) administration, and develop neuritic and synaptic networks. Thus, they are a very good model to study the molecular mechanisms of neurite outgrowth (Pleasure *et al*, 1992). Importantly, it was shown previously that although p53 transcriptional activity is required for NT2/D1 cellular differentiation, RA treatment induces a paradoxical reduction of p53 total protein levels (Curtin *et al*, 2001; Curtin and Spinella, 2005). This observation suggests that specific post-translational modifications may be required to regulate p53 activity, especially in conditions where the total levels of the protein are diminished. We have recently shown that in epithelial cells, specific acetylation of p53 at lysine 320 (K320) favors cell survival, unlike acetylation of lysine 372 (K372), which promotes cell death (Knights *et al*, 2006). Therefore, we examined whether specific acetylation events of p53 occur during neuronal maturation and neurite outgrowth. To this end, we first measured total p53 protein levels following RA treatment in NT2-D1 cells. Consistent with previous studies, we found that RA induced indeed a reduction of total p53 levels, which corresponded with the time of expression of the neuronal and neurite outgrowth markers β -3 tubulin and Gap-43. This also coincided with a net increase in the levels of expression of the p53 targets Coronin 1b and Rab13 (Figure 6A). Despite the lower p53 levels induced by RA, acetylation of K320 but not of K372 of p53 was markedly increased, and RA treatment also clearly induced the expression levels of the acetyltrans-

Figure 2 p53, Coronin 1b and Rab13 are coregulated during neurite outgrowth. (A) Protein expression temporal profiling in PC-12 cells after NGF. Western blot shows early induction (4–24 h) of the expression of p53, Rab13, Coronin 1b and Gap-43 after NGF. Bar graphs show densitometry data (the expression level for each protein and time point represents the intensity of each band of the immunoblot as a ratio value to β -actin levels). (B) Immunocytochemistry shows increased nuclear expression of p53 in PC-12 cells 24 h after NGF. (C) Protein temporal profiling for MDM2 by immunoblotting shows marked suppression by 4 h following NGF in PC-12 cells. (D) Immunoblotting shows protein expression temporal profiling in E16 RCN during maturation in Neurobasal medium. p53 expression is highest at day 2 corresponding to the highest increase in expression for Coronin 1b, Rab13, and Gap-43. Bar graphs show densitometry data (the expression level for each protein and time point represents the intensity of each band of the immunoblot as a ratio value to β -actin levels). (E, F) Immunocytochemistry shows nuclear expression of p53 (E) and at the cortical cytoskeleton (colocalize with tubulin) for Coronin 1b and Rab13 (F) in RCN.

ferase PCAF, which is known to acetylate p53 at K320 (Knights *et al*, 2006).

To test the possibility that acetylation of K320 is specifically responsible for the regulation of the expression levels of Coronin 1b and Rab13, we used a p53 null cell line (H1299) where the expression of native p53 or of p53 acetylation

mutants at positions 320 (p53K320Q) and 372 (p53K372Q) was reconstituted with a tetracycline-inducible system. Substitution of lysine (K) for glutamine (Q) mimics the effects of a constitutive acetylation (Knights *et al*, 2006), likely because acetylation neutralizes the positive charge of lysine, whereas glutamine recapitulates this effect. The results of

Figure 3 p53 is required for neurite outgrowth in PC-12 cells and modulates the expression of Coronin 1b and Rab13. **(A)** Immunoblotting shows protein temporal expression profiling in WT PC-12 cells versus p53DN stably transfected cells after NGF. Increase in protein expression for Coronin 1b and Rab13 in WT is repressed in p53DN cells. **(B)** Immunofluorescence documents that p53DN stable-transfected PC-12 do not develop neurites following NGF (72 h), whereas WT PC-12 develop long neurites. **(C)** Bar graphs show that p53DN cells exposed to NGF have neurite length comparable to untreated PC-12 (number of experiments: 3, total number of cells: about 300, average values: **t*-test *P*-values <0.001)

these experiments were striking. Indeed, the p53K320Q acetylation mimic strongly induced the expression of Coronin 1b and Rab13, whereas p53K372Q had only a minimal effect (Figure 6B). To gain more confidence that truly acetylated p53 at lysine 320 (acetyl-K320 p53) is capable of a direct interaction with the p53-binding sequences contained in the promoter regions of Coronin 1b and Rab13, we performed a ChIP assay in cells expressing native p53. As it can be seen, the comparison of ChIP reactions performed with the antibody recognizing total p53 (FL-393), and the antibody recognizing exclusively p53 populations acetylated at position 320 (anti-acetyl-320), showed that acetylated p53 binds to these promoters with very high affinity. In addition, overexpression of the acetyltransferase PCAF resulted in a net increase in binding of acetylated p53 to these promoters. Thus, collectively, these results demonstrate that p53 populations acetylated at lysine 320 bind to the promoter regions of Coronin 1b and Rab13 (Figure 6C), and regulate the expression of these two genes.

Then, to explore whether p53K320 is really important for neuronal maturation and neurite outgrowth, NT2-D1 cells were transfected with the plasmid expressing the p53 acetylation mimics p53K320Q or p53K372Q. The expression of Gap-43 and of the p53 target genes Coronin 1b and Rab13 was then examined. Strikingly, we found that only cells transfected with p53K320Q, but not with p53K372Q expressed Gap-43, and displayed increased expression of Coronin 1b and Rab13 (Figure 7A). In order to address the specificity of the effect of the p53K320Q mimic, a p53K320R mutant, where lysine was replaced with arginine, was employed in similar experiments and results were compared to cells treated with RA-GFP or transfected with GFP only. Arginine has the same charge as lysine, but it prevents acetylation, and therefore is a useful control for the glutamine

substitution (see for example, Knights *et al*, 2006). In this case, the expression levels of Coronin 1b and Rab13, while induced by RA, were comparable to control cells transfected with GFP (Figure 7B). Thus, these results strongly support the notion that p53 molecules acetylated at position 320 are specifically responsible for the regulation of Coronin 1b and Rab13 during neurite outgrowth.

Finally, to validate the concept that p53 acetylation at lysine 320 plays a role in neurite outgrowth in a model system more relevant to the mammalian CNS, we tested the effects of the mutants p53K320Q and p53K320R in rat primary cortical neurons. Cultured primary neurons were transfected with the vectors expressing these p53 acetylation mutants, and their effect compared to those exerted by GFP. As it can be seen, the p53K320Q mutant was able to increase total neurite length by $40 \pm 12\%$ (s.d.), and the length of the longest neurite by $31 \pm 14\%$, while total number of neurites was not significantly affected (Figure 8A and B). In fact, we observed an opposite effect following expression of the p53K320R mutant. This led to a reduction of $31 \pm 11\%$ of the total average of neurite length, with the length of the longest neurite impaired by $41 \pm 12\%$, and only modestly affected the number of neurites by $22 \pm 8\%$ (Figure 8A and B).

Overall these data show that acetylated p53 at lysine 320 binds to the Coronin 1b and Rab13 promoter elements and plays an important role in neurite outgrowth in different neuronal cells and signaling pathways.

p53 is required for physiological nerve regeneration

It has been proposed that the biological pathways subserving neurite outgrowth are partially recapitulated by axonal regeneration following nerve injury (Benowitz and Routtenberg, 1997; Emery *et al*, 2003). Based upon this

Figure 4 p53 is required for neurite outgrowth and maturation in embryonic rat cortical neurons and influences the expression of Coronin 1b and Rab13. (A) Immunoblotting shows inhibition of protein expression for Coronin 1b and Rab13, following infection with p53DN lentivirus at day 1 in RCN. Reduction in protein levels for Gap-43 suggests impaired maturation. (B) Immunofluorescence at day 5 in RCN after infection with p53DN or eGFP lentivirus. Shown is the inhibition of neurite outgrowth and branching when p53DN (V5 + β-3-tubulin) is expressed in the nucleus versus eGFP-infected cell (eGFP + β-3-tubulin). (C) Bar graphs show the inhibitory effects of p53DN infection (days 1–5) on RCN neuritic network (number of experiments: 3, total number of cells: about 240, average values: **t*-test *P*-values <0.01; ***P*-values <0.001).

conceptualization and our *in vitro* studies, we next asked whether p53 plays a role in axonal regeneration *in vivo*. To this end, we compared the extent of nerve regeneration in mice lacking both copies of the p53 alleles (p53^{−/−}) and in wild-type (WT) animals after facial nerve axotomy, an established model for regeneration (Raivich *et al*, 2004). The

Figure 5 Coronin 1b and Rab13 rescue neurite outgrowth in p53DN cells. (A) Overexpression of Coronin 1b-GFP or Rab13-GFP in p53DN PC-12 cells exposed or not to NGF compared to control GFP-transfected cells. Both proteins are localized at the cortical cytoskeleton and at the growth cone and partially rescue neurite outgrowth. p53DN is localized in the nucleus (V5), as shown by colocalization with the nuclear marker Hoechst. (B) Bar graphs show neurite measurements, which demonstrate the effects of Coronin 1b and Rab13 on neurite outgrowth in p53DN cells compared to control (number of experiments: 3, total number of cells: about 240, average values: **t*-test *P*-values <0.01; ***P*-values <0.001).

extent of regeneration was assessed by counting fluorescent motor neurons in the facial motor nuclei with the retrograde nerve tracer FluoroGold 28 days after injury. Strikingly, there was a significant decrease in the number of regenerating fibers in p53 null mice as compared to WT animals. The percentage of FluoroGold-positive neurons, corresponding to

Figure 6 Acetylated p53 at lysine 320 is induced following RA during neurite outgrowth, binds to promoter regions of Coronin 1b and Rab13, and preferentially triggers their expression levels. **(A)** Immunoblotting shows induction of p53K320 and its acetyltransferase PCAF, but not of p53K372, following RA administration (5 days) in NT2-D1 cells (left). Coronin 1b and Rab13 are also induced by RA along with the neuronal and neurite outgrowth markers β -3-tubulin and Gap-43. **(B)** Protein expression data from immunoblotting experiments at the 24 and 48 h time point in p53 null cell line (H1299) where the expression of native p53 or of p53 acetylation mutants at position 320 (p53K320Q) and 372 (p53K372Q) was reconstituted with a tetracycline-inducible system. Lysine (K) in place of glutamine (Q) mimics the effects of a constitutive acetylation. Expression of the p53K320Q mutant versus K372Q preferentially triggers the expression of Coronin 1b and Rab13. **(C)** ChIP assays demonstrate *in vivo* interaction of WTP53 and of lys320-p53 with DNA-binding elements derived from the human Coronin 1b and Rab13 promoters. H1299-WTP53 cells were left untreated (–) or treated for 48 h (48 h) with tetracycline to induce the expression of native p53 with or without overexpression of full-length PCAF. Following induction of p53, cells were crosslinked with formaldehyde and immunoprecipitated with a polyclonal antibody recognizing p53 or lys320-p53. The ‘input’ samples represent equal fractions of extract collected prior to precipitation from which DNA was extracted and used as a control. WAF1 (p21) promoter region was used as a positive control for p53 DNA binding. Preferential binding to Coronin 1b and Rab13 promoter elements of lys320-p53 is visible following the overexpression of PCAF.

regenerating fibers, was $32.8\% \pm 1.5$ (s.e.) in the axotomized side compared to the control side in WT mice, versus $14 \pm 1.6\%$ (s.e.) in the p53 null mice (Figure 9A and B); this difference was highly significant (*t*-test *P*-value < 0.0001).

As p53 can play a role in apoptosis and elimination of neurons following axotomy (Kiryu-Seo *et al*, 2005), we counted the number of surviving facial motor neurons (FMN) at 28 days postaxotomy. This analysis showed about 10% fewer surviving FMN in WT ($70.8 \pm 7\%$ (s.e.)) versus p53 $^{-/-}$ mice ($80.2 \pm 9\%$) compared to control unoperated side (Figure 9C). This difference is not statistically significant,

but suggests that there are fewer cells available for target reinnervation in the WT mouse. As the WT shows also more regenerating neurons than the null counterpart, together these data suggest that p53 plays a dual role in the elimination of highly damaged cells and in axon regeneration of surviving neurons. Also, the presence of fewer cells available for target reinnervation in WT magnifies the difference between the number of regenerating neurons in the WT versus p53 null mice following axotomy.

In agreement with our *in vitro* studies, immunofluorescence experiments in WT mice also showed that the expres-

Figure 7 Mimicking p53 acetylation mutant at lysine 320 promotes expression of Coronin 1b and Rab13 along with the proneurite outgrowth protein Gap-43 in NT2-D1 cells. (A) Overexpression of the p53K320Q mutant versus K372Q is performed by transfecting NT2-D1 cells. Immunoblotting shows induction of the proneurite outgrowth proteins Gap-43, Coronin 1b and Rab13 only in the K320Q after 5 days in culture. GFP-transfected cells are used as negative control. Bar graphs show densitometry data (the expression level for each protein and time point represents the intensity of each band of the immunoblot as a ratio value to β -actin levels). (B) Overexpression of the p53K320R mutant versus the effects of RA in NT2-D1 cells. Immunoblotting shows induction of the proneurite outgrowth proteins and p53 targets Coronin 1b and Rab13 only in the RA-treated cells after 5 days in culture. p53K320R does not induce protein expression changes compared to GFP-transfected cells, which are used as negative control. Bar graphs show densitometry data (the expression level for each protein and time point represents the intensity of each band of the immunoblot as a ratio value to β -actin levels).

sion of Coronin 1b and Rab13 was clearly induced preferentially in axonal sprouts in regenerating facial nuclei in WT, but not in p53 null mice (Figure 9D). Thus, p53 is required for the expression of Coronin1b and Rab13 in axonal sprouts during nerve regeneration.

Discussion

In this study, we have demonstrated that p53 promotes neurite outgrowth *in vitro* and nerve regeneration *in vivo* through molecular mechanisms that involve the activation of the actin-binding protein Coronin 1b and the GTPase Rab13. We recently showed that Coronin 1b and Rab13 were co-expressed at neurite tips, in association with the known proplasticity factor Gap-43, after experimental spinal cord trauma as well as in cell culture models of neurite outgrowth. These factors were part of a larger cluster of genes, identified using microarrays methodology, which were highly coordinately regulated after SCI (Di Giovanni *et al*, 2005b). A search for potential common regulatory elements within this gene cluster identified the tumor suppressor protein p53 as a potential transcriptional regulator. We demonstrated that p53, a protein implicated in neuronal apoptosis, promotes neurite outgrowth *in vitro* and axonal regeneration *in vivo*. Indeed, p53 is required for neurite outgrowth and regeneration: primary neurons and PC-12 cells expressing either dominant-negative forms or siRNA specific for p53 are unable to develop neurites and p53 null mice show impaired nerve regeneration following axotomy. In tissue culture cells, these

effects are mediated, at least in part, by p53-dependent transcriptional activation of Coronin 1b and Rab13. The latter proteins both play an important role in cytoskeleton remodeling and have diverse cellular functions. The ability of Coronin 1b to interact with actin and promote its polymerization might underlie its biological actions, as it could favor cell-to-cell communications, affect localization of receptors on the cell membrane and facilitate remodeling of the cytoskeleton at the growth cone (De Hostos, 1999; Humphries *et al*, 2002; Oku *et al*, 2003; Rybak and Clemen, 2005). Rab13, which belongs to the trans-Golgi network, may play a role in synaptic vesicle transport, similar to several other members of Rab family (Tang, 2001). These activities of Coronin 1b and Rab13 are likely to be important for neurite outgrowth, maturation, and regeneration.

The effects of p53 activation in the nervous system, as in other cells, appear to be pleiotropic. Previous work has shown that p53 is capable of modulating complex molecular pathways that potentially lead to neuronal cell death, DNA repair, and differentiation (for a review, see Miller *et al*, 2000; Oren, 2003; Harms *et al*, 2004). Also, a role for p53 in PC-12 cells in survival and cell-cycle arrest following growth factors has been proposed, including Trk-A receptor- and MAPK cascade-dependent p53 activation (Montano, 1997; Poluha *et al*, 1997; Hughes *et al*, 2000). In one report (Hughes *et al*, 2000), p53 activity was not associated with neurite outgrowth in PC-12 cells. Nevertheless, these findings are in contradiction with previous reports and our data (Montano, 1997; Poluha *et al*, 1997), which showed that p53 activity was

Figure 8 Overexpression of the acetylation mimicking p53K320Q mutant promotes neurite outgrowth in primary cortical neurons, while the p53K320R mutant inhibits it. **(A)** Cultured primary rat cortical neurons (E18) were transfected at day 3 for 72 h with either GFP, p53K320Q, or p53K320R mutant plasmid DNAs. Both GFP and immunostaining with anti-human p53 antibodies (Cell Signaling AB1, AB6) were used to detect p53 expression, and to identify nuclear and neurites localization. Both control GFP and the p53 mutant proteins were expressed in the nucleus and along the processes. Experiments were performed using co-transfections with p53 mutants and GFP, which also allow the complete identification of processes along with the use of the neuronal-specific staining for β -3 tubulin. Representative images of transfected neurons show that overexpression of p53K320Q enhances neurite outgrowth, while overexpression of p53K320R impairs it as compared to control GFP-transfected cells. **(B)** Bar graphs show quantitation of neurite outgrowth measurements following transfections expressed as average percentage values of control GFP-transfected cells (data obtained from three different experiments for about 210 total neurons counted for each condition) (**t*-test *P*-values < 0.01).

actually required for neurite outgrowth. Differences may be due to the use of different mutant p53 proteins and PC-12 cell lines. Also, we were the first to use RNA interference for p53 in neurite outgrowth assays through which we showed that silencing p53 expression blocks NGF-mediated neurite outgrowth in PC-12 cells.

Most importantly, in addition to the identification of the novel proneurite outgrowth p53 targets Coronin 1b and Rab13, here we show that specific post-translational modifications of p53 promote neurite outgrowth and that p53 is required for physiological axonal regeneration *in vivo*.

How does p53 switch among its multiple biological functions? p53 activity is well known to be regulated by multiple post-translational modifications, including phosphorylation and acetylation. The C-terminal segment of p53 is acetylated at different residues by at least two different acetyltransferases, p300/CBP, and PCAF. p300/CBP acetylate lysines at position 370, 372, 373 and 382 of p53, while PCAF acetylates

a single residue at position 320 (Avantaggiati *et al*, 1997; Lill *et al* 1997; Xu, 2003; Bode and Dong, 2004).

We recently have found that acetylation at lysine 320 specifically promotes survival and proliferation in epithelial cells, unlike acetylation of lysine 372 that triggers cell death. These different biological outcomes are due to the ability of each acetylation event to influence protein-protein interactions and to re-direct the activity of p53 onto selected sets of promoters that act either in favor of cell survival or by promoting cell death (Knights *et al*, 2006). Consistent with the idea that acetylation of K320 serves as a prosurvival signal, we have shown here that acetylation of this residue, but not of lysine 372 of p53, was specifically induced by signals that stimulate neurite outgrowth and preceded neuronal maturation in NT2-D1 cells. Importantly, this acetylation event coincided with an enhancement of the expression levels of PCAF. Similar changes were also observed in PC-12 cells during neurite outgrowth following NGF (data not

Figure 9 p53 is required for axonal regeneration and for Coronin 1b and Rab13 expression in axonal sprouts. (A) FluoroGold fluorescence in the facial nucleus in p53 WT versus p53^{-/-} on the control (ctr) and transected side (trans) 28 days after facial nerve cut. Note the strong reduction in the number of FluoroGold-positive neurons in p53^{-/-} compared to p53 WT after nerve cut (trans). (B) Bar graphs show quantitation of retrograde labeling of facial motor neurons 28 days after facial nerve cut. Shown is the overall ratio of labeled neurons in the operated/unoperated side after sectioning of the entire facial nucleus (five mice per group, **t*-test *P*-values <0.001). (C) Bar graphs show quantitation of surviving neurons 28 days following facial transection in WT versus p53 null (^{-/-}) mice. Shown is the overall ratio of surviving facial motor neurons in the operated/unoperated side (five mice per group). Counting revealed about 10% fewer FMN in p53^{-/-} versus WT (80.2 ± 9% (s.e.) in null versus 70.8 ± 7% in WT of surviving FMN in transected compared to control side). (D) Immunoperoxidase for Coronin 1b and Rab13 in the facial nucleus in p53 WT versus p53^{-/-} in the control (ctr) and transected side (trans) 28 days after facial nerve cut. Expression of both Coronin 1b and Rab13 is increased in axonal sprouts (arrows) following axotomy in WT (a, b and e, f), but not in p53 null mice (c, d and g, h).

shown). Significantly, p53 acetylated at lysine 320 was also able to bind to the promoter elements of the target genes Coronin 1b and Rab13 as shown by ChIP assay, and binding was enhanced by the expression of PCAF, which acetylates p53 at K320. This event has a functional implication as the expression of a mutant form of p53 that mimics constitutive acetylation at position 320 (p53K320Q), but not of a form that does not allow acetylation at the same site (p53K320R), was able to increase the levels of both Coronin 1b and Rab13 p53 target proteins and to induce Gap-43 as a marker of neurite outgrowth in NT2-D1 cells. Strikingly, delivery of p53K320Q in cultured primary cortical neurons was able to promote neurite outgrowth, while neurons expressing the p53K320R mutant had impaired neurite development, suggesting a possible dominant-negative effect of this specific mutant. Thus, acetylation of p53 at position 320 is involved in p53-mediated induction of Coronin 1b and Rab13 as well as in promotion of neurite outgrowth.

Furthermore, facial nerve transection experiments performed in WT versus p53 null mice clearly showed that p53 is also required for physiological axon regeneration *in vivo*. This correlated with the fact that the p53 transcriptional targets Coronin 1b and Rab13 were expressed in axonal sprouts in the facial nuclei following nerve cut only in WT mice. Together, our data strongly argue that the proneurite outgrowth activity of p53, which occurs in tissue culture cells through the activation of Coronin 1b and Rab13, is recapitulated *in vivo* following nerve transection and are required for axonal regeneration.

In conclusion, our work establishes strong evidence for a novel role of p53 in neurite outgrowth and nerve regeneration and provides the molecular framework for such a function. Modulation of this p53-dependent pathway may provide a novel therapeutic target for facilitating neuroplasticity and neuroregeneration after injuries to central or peripheral nervous system.

Materials and methods

In silico transcription factor binding site (TFBS) analysis

Sequences of selected genes Coronin 1b and Rab13 were analyzed using the FrameWorker, a part of the complex Genomatix software tool (<http://www.genomatix.de/matinspector.html>, Munich, Germany), that allows extracting a common *framework* of elements from a set of DNA sequences. In our case, these elements were TFBS within predicted promoter regions of the genes, since this tool is designed for the comparative analysis of promoter sequences. This software returns the most complex models that are common to the input sequences (and satisfying the user parameters). All elements that occur in the same order and in a certain distance range in the input sequences are identified, thus providing a model of transcriptional regulation of gene activity. The output is a set of candidate transcription factors ranked by *P*-values according to their probability of binding to the selected TFBS.

ChIP assays

ChIPs were performed as described elsewhere (Ogawa *et al*, 2002). Briefly, 4.5×10^7 H1299-WT^{p53} cells were grown in the absence or presence of tetracycline for 48 h and subsequently exposed to a 1% formaldehyde–PBS solution for 13 min at room temperature. Following cell lysis, extracts were sonicated to shear DNA between the lengths of 300–800 bp. Chromatin solutions were precipitated overnight with rotation using 2 µg of rabbit polyclonal anti-p53 antibody (FL393, Santa Cruz) or rabbit polyclonal anti-lys320-p53 antibody (Upstate). Also, in a set of experiments transfection of full-length PCAF plasmid DNA expressed from pcDNA vector was performed at the moment of tetracycline induction as described previously (Knights *et al*, 2006). On the following day, protein A agarose beads that had been previously blocked with salmon sperm DNA and BSA were added to each reaction to precipitate antibody complexes. The precipitated complexes were washed and then incubated at 65°C overnight in parallel with ‘input’ samples to reverse the crosslinking. DNA was isolated by P:C:I extraction, which was followed by ethanol precipitation in the presence of glycogen. The resulting DNA was then used in ChIP PCR reactions. The primers used for amplification of p53-responsive elements were as follows: WAF1-P21—5′-TCA CCA TTC CCC TAC CCC ATG CTG CTC-3′, 3′-AAG TTT GCA ACC ATG CAC TTG AAT GTG-5′; Coronin 1b—5′-GTC ATT CCC ACT CCA TGG AG-3′, 3′-CTC AGT CCT CTG CTG GTC C-5′; and Rab13—5′-CTT CAA GTT GCT GAT CG-3′, 3′-GAG AAG GAG GTC ACA GGA G-5′. Standard PCR parameters were used with annealing temperatures of 55°C for Rab13 and 51°C for Coronin 1b and P21. All PCR reactions were run using 35 cycles.

Real-time reverse transcriptase (RT)-PCR

We studied Coronin 1b and Rab13 by real-time PCR using cDNAs extracted from H1299 cell lines controlled by a tetracycline p53-inducible expression system, 8 and 24 h after p53 induction by administration of tetracycline. Fluorophore-labeled LUX primers (forward) and their unlabeled counterparts (reverse) were provided by Invitrogen. LUX primers were designed matching the probe set sequences for human Coronin 1b, and Rab13 and all primers were designed using the software called LUX Designer (Invitrogen, www.invitrogen.com/lux). For more experimental details see online Supplementary data.

Cell cultures

Cell lines. Human epithelial 293, H1299, and pheochromocytoma PC-12 cell lines were grown in Dulbecco's modified medium supplemented with 10% fetal bovine serum, 100 U/ml penicillin, and 100 mg/ml streptomycin (Invitrogen) in a humidified atmosphere of 5% CO₂ in air at 37°C. The cultures were maintained in a logarithmic phase by passage every 2–3 days. For more experimental details see online Supplementary data.

Rat primary cortical neurons. Cortical neuronal cultures were derived from rat embryonic cortices as reported previously (Di Giovanni *et al*, 2005c). For more experimental details see online Supplementary data.

Clones and transfection/infection experiments

PC-12 cells were stable transfected with pcDNA3 vector expressing dominant-negative forms of p53 as described previously (Yakovlev

et al, 2004). For more experimental details see online Supplementary data.

Construction of replication-incompetent lentiviral vectors was performed using ViraPower expression system (Invitrogen), according to the recommendation of the manufacturer. For more experimental details see online Supplementary data.

Rat Coronin 1b and Rab13 were cloned in a GFP expression vector under the control of a CMV promoter and used for transfection experiments.

Tetracycline-inducible vectors were employed in H1299 cells, and were purchased from Invitrogen. Generation of these cell lines was carried out accordingly to the manufacturer's instructions (Invitrogen). The p53 mutants used for the tetracycline-inducible system and for transfection experiments in NT2-D1 cells and primary cortical neurons were constructed by employing a *Pfu*-polymerase-based, site-directed mutagenesis (Stratagene), followed by cloning of the amplified cDNAs into the pcDNA/TO4 vector (Invitrogen). The primers used for mutagenesis were as follows: p53Q320—5′-CCC CAG CCA AAG CAG AAA CCA CTG GAT GGA GAA-3′; p53Q372—5′-AGC CAC CTG CAG TCC CAA CAG GGT CAG TCT ACC-3′; p53R320—5′-CCC CAG CCA AAG AGG AAA CCA CTC GAG GGA GAA-3′, 5′-TTC TCC CTC GAG TGG TTT CCT CTT TGG CTG GGG-3′.

A Flag or GFP-encoding sequence was fused in frame to the N-terminus of p53 for immunodetection of the protein.

Transfection experiments were performed using Lipofectamine 2000 (Invitrogen) for PC-12, 293, and H1299.

Lentiviral constructs were used to infect RCN. Expression of transfected or infected p53DN DNAs was detected by antibodies against the V5-tag, expressed in these expression vectors or by GFP visualization, which represented the tag protein for the corresponding control empty vectors (Figures 3B, C and 4B, C).

Neurite outgrowth was evaluated in neurons and PC-12 cells considering the number of neurites per cell, the average neurite length, and the length of the longest neurite per cell. Measurements were conducted in at least three different experiments by two different operators. In between 200 and 300 cells for each experimental condition were counted both in the case of primary neurons and PC-12 cells in the several experiments where neurite measurements were performed (see legends for details). Statistical data analysis reflects the results of the combined experiments. Cells were viewed using a CCD camera and analyzed with the image analysis software AxioVision 3.1 (Zeiss).

RNA interference

For RNA interference experiments for p53, we employed a plasmid-based siRNA system (GeneSuppressor System, Imgenex, IMG-803). For additional experimental details see online Supplementary data.

Antibodies

See online Supplementary data.

Immunoblotting and immunocytochemistry

See online Supplementary data.

p53 null mice and facial nerve transection

Six-week-old homozygous p53^{−/−} mice FVB.129S2 (B6)-Trp53^{tm1Tyj}, and FVB.129S2 (B6) control mice were obtained from Jacksons laboratories. These animals do not present any known developmental abnormalities, and are completely undistinguishable from WT mice. They feed normally, reproduce properly, and show normal motor activity. Importantly, mice homozygous for the Trp53^{tm1Tyj} mutation often develop tumors (principally lymphomas and osteosarcoma) by the fifth–sixth month of age and die within a month from the disease onset. Our experiments were performed starting at 6 weeks of age in homozygous p53 null and WT mice. Mice were males, all tumors free throughout the experiments, and were five per group. It is important to note that no adverse reaction to surgery has been reported in the literature, and we did not encounter any abnormalities following facial axotomy. The genotype of p53 null mice was confirmed by Southern blot analysis. Facial nerve transection was performed as previously described (Raivich *et al*, 2004). Briefly, mice were anesthetized by intramuscular injection of a combination of xylazine (13 mg/kg) and ketamine HCl (85 mg/kg). The surgical site was shaved and disinfected. A skin incision was made behind the left ear and the facial nerve exposed, and transected about 2 mm posterior to the

foramen stylomastoideum as detailed previously. The same incisions were also made on the contralateral side for sham-operated intra-animal controls. The wounds were sutured, and the animals were allowed to recover in a darkened room on isothermal pads warmed at 37°C for an estimated 30 min.

Regeneration in facial nuclei in the brainstem following facial nerve transection was evaluated by retrograde axonal tracing with FluoroGold. Three days before being killed, mice from each group were anesthetized and 15 µl of 3% FluoroGold was injected through a micropipette with a tip diameter of 25 µm into the whisker pads bilaterally. After 3 days, the mice were deeply anesthetized and perfused with 0.9% saline solution followed by 4% paraformaldehyde in 0.1 M phosphate buffer. The brain stem was removed and placed in 30% sucrose for 24 h. The entire facial nuclei were sectioned and reconstructed and all sections of both facial nuclei per each animal were evaluated.

Transverse serial frozen sections, 12 µm thick, were cut and mounted on slides, protected by coverslips, and photographed under a fluorescence microscope. Only labeled neurons with visible nuclei were counted and taken to represent the number of regenerated motor neurons. The number of total motor neurons and of regenerating motor neurons in facial nuclei after facial nerve axotomy was studied by counting the number of total cells (cresyl

violet stained) and of retrograde labeled motor neurons comparing the uninjured side in each animal for intra-animal control, to the transected side, in both p53 null and WT mice, which resulted in ratio values. Thus, the overall ratio of FluoroGold-positive neurons was compared between mutant and WT animals. The Abercrombie formula for cell counting was included in our analysis to correct for tissue volume.

Supplementary data

Supplementary data are available at *The EMBO Journal* Online (<http://www.embojournal.org>).

Acknowledgements

This work was supported by the National Institute of Health Contract NIH-NINDS-01 (NS-1-2339) (to AIF) and by grants from NIH ROI CA83979, CA030716 and CA102746 (to MLA). We are grateful to Dr Bert Vogelstein for kindly providing a construct expressing a dominant-negative form of p53. We thank Dr Vilen Movsesyan and Bogdan Stoica for providing primary rat cortical neurons, Andrea Sabino for technical assistance, and Dr Jorge Garay for performing the facial nerve transection.

References

- Armstrong JF, Kaufman MH, Harrison DJ, Clarke AR (1995) High-frequency developmental abnormalities in p53-deficient mice. *Curr Biol* **5**: 931–936
- Avantaggiati ML, Ogryzko V, Gardner K, Giordano A, Levine AS, Kelly K (1997) Recruitment of p300/CBP in p53-dependent signal pathways. *Cell* **89**: 1175–1184
- Bareyre FM, Schwab ME (2003) Inflammation, degeneration and regeneration in the injured spinal cord: insights from DNA microarrays. *Trends Neurosci* **26**: 555–563
- Bates S, Hickman ES, Vousden KH (1999) Reversal of p53-induced cell-cycle arrest. *Mol Carcinogen* **24**: 7–14
- Benowitz LI, Routtenberg A (1997) Gap-43: an intrinsic determinant of neuronal development and plasticity. *Trends Neurosci* **20**: 84–91
- Bode AG, Dong Z (2004) Post-translational modification of p53 in tumorigenesis. *Nat Rev Cancer* **4**: 793–805
- Curtin JC, Dragnev KH, Sekula D, Christie AJ, Dmitrovsky E, Spinella MJ (2001) Retinoic acid activates p53 in human embryonal carcinoma through retinoid receptor-dependent stimulation of p53 transactivation function. *Oncogene* **20**: 2559–2569
- Curtin JC, Spinella MJ (2005) P53 in human embryonal carcinoma: identification of a transferable, transcriptional repression domain in the N-terminal region of p53. *Oncogene* **24**: 1481–1490
- de Hostos EL (1999) The coronin family of actin-associated proteins. *Trends Cell Biol* **9**: 345–350
- Di Giovanni S, De Biase A, Yakovlev A, Finn T, Beers J, Hoffman EP, Faden AI (2005a) *In vivo* and *in vitro* characterization of novel neuronal plasticity factors identified following spinal cord injury. *J Biol Chem* **280**: 2084–2091
- Di Giovanni S, Faden AI, Yakovlev A, Duke-Cohan JS, Finn T, Thoun M, Knobloch S, De Biase A, Bregman BS, Hoffman EP (2005b) Neuronal plasticity after spinal cord injury: identification of a gene cluster driving neurite outgrowth. *FASEB J* **19**: 153–154
- Di Giovanni S, Knobloch SM, Brandoli C, Aden SA, Hoffman EP, Faden AI (2003) Gene profiling in spinal cord injury shows role of cell cycle in neuronal death. *Ann Neurol* **53**: 454–468
- Di Giovanni S, Movsesyan V, Ahmed F, Cernak I, Schinelli S, Stoica B, Faden AI (2005c) Cell cycle inhibition provides neuroprotection and reduces glial proliferation and scar formation after traumatic brain injury. *Proc Natl Acad Sci USA* **102**: 8333–8338
- Dumont RJ, Okonkwo DO, Verma S, Hurlbert RJ, Boulos PT, Ellegala DB, Dumont AS (2001) Acute spinal cord injury, part I: pathophysiologic mechanisms. *Clin Neuropharmacol* **24**: 254–264
- Emery DL, Royo NC, Fischer I, Saatman KE, McIntosh TK (2003) Plasticity following injury to the adult central nervous system: is recapitulation of a developmental state worth promoting? *J Neurotrauma* **20**: 1271–1292
- Glanzer JG, Haydon PG, Eberwine JH (2004) Expression profile analysis of neurodegenerative disease: advances in specificity and resolution. *Neurochem Res* **29**: 1161–1168
- Gottlieb E, Haffner R, King A, Asher G, Gruss P, Lonai P, Oren M (1997) Transgenic mouse model for studying the transcriptional activity of the p53 protein: age- and tissue-dependent changes in radiation-induced activation during embryogenesis. *EMBO J* **16**: 1381–1390
- Harms K, Nozell S, Chen X (2004) The common and distinct target genes of the p53 family transcription factors. *Cell Mol Life Sci* **61**: 822–842
- Kiryu-Seo S, Hirayama T, Kato R, Kiyama H (2005) Noxa is a critical mediator of p53-dependent motor neuron death after nerve injury in adult mouse. *J Neurosci* **25**: 1442–1447
- Hudson CD, Morris PJ, Latchman DS, Budhram-Mahadeo VS (2005) Brn-3a transcription factor blocks p53-mediated activation of proapoptotic target genes Noxa and Bax *in vitro* and *in vivo* to determine cell fate. *J Biol Chem* **280**: 11851–11858
- Hudson CD, Podesta J, Henderson D, Latchman DS, Budhram-Mahadeo V (2004) Coexpression of Brn-3a POU protein with p53 in a population of neuronal progenitor cells is associated with differentiation and protection against apoptosis. *J Neurosci Res* **78**: 803–814
- Hughes AL, Gollapudi L, Sladek TL, Neet KE (2000) Mediation of nerve growth factor-driven cell cycle arrest in PC-12 cells by p53. Simultaneous differentiation and proliferation subsequent to p53 functional inactivation. *J Biol Chem* **275**: 37829–37837
- Hughes PE, Alexi T, Walton M, Williams CE, Dragunow M, Clark RG, Gluckman PD (1999) Activity and injury-dependent expression of inducible transcription factors, growth factors and apoptosis-related genes within the central nervous system. *Prog Neurobiol* **57**: 421–450
- Humphries CL, Balcer HI, D'Agostino JL, Winsor B, Drubin DG, Barnes G, Andrews BJ, Goode BL (2002) Direct regulation of Arp2/3 complex activity and function by the actin binding protein coronin. *J Cell Biol* **159**: 993–1004
- Jones RG, Plas DR, Kubek S, Buzzai M, Mu J, Xu Y, Birnbaum MJ, Thompson CB (2005) AMP-activated protein kinase induces a p53-dependent metabolic checkpoint. *Mol Cell* **18**: 283–293
- Kalb RK, Strittmatter SM (2000) *Neurobiology of Spinal Cord Injury*. New Jersey: Humana Press, pp 113–158
- Komarova EA, Chernov MV, Franks R, Wang K, Armin G, Zelnick CR, Chin DM, Bacus SS, Stark GR, Gudkov AV (1997) Transgenic mice with p53-responsive lacZ: p53 activity varies dramatically during normal development and determines radiation and drug sensitivity *in vivo*. *EMBO J* **16**: 1391–1400
- Knights C, Catania J, Di Giovanni S, Muratoglu S, Perez R, Schwartzbeck A, Quong A, Zhang X, Beerman T, Pestell RG, Avantaggiati ML (2006) Distinct p53 acetylation cassettes differ-

- entially influence gene expression patterns and cell fate. *J Cell Biol* **173**: 533–544
- Kubo T, Yamashita T, Yamaguchi A, Hosokawa K, Tohyama M (2002) Analysis of genes induced in peripheral nerve after axotomy using cDNA microarrays. *J Neurochem* **82**: 1129–1136
- Lill NL, Grossman SR, Ginsberg D, DeCaprio J, Livingston DM (1997) Binding and modulation of p53 by p300/CBP coactivators. *Nature* **387**: 823–827
- Makwana M, Raivich G (2005) Molecular mechanisms in successful peripheral regeneration. *FEBS J* **272**: 2628–2638
- Miller FD, Pozniak CD, Walsh GS (2000) Neuronal life and death: an essential role for the p53 family. *Cell Death Differ* **7**: 880–888
- Montano X (1997) P53 associates with trk tyrosine kinase. *Oncogene* **15**: 245–256
- Ogawa H, Ishiguro HK, Gaubatz S, Livingston DM, Nakatani Y (2002) A complex with chromatin modifiers that occupies E2F- and Myc-responsive genes in G0 cells. *Science* **296**: 1132–1136
- Oku T, Itoh S, Okano M, Suzuki A, Suzuki K, Nakajin S, Tsuji T, Nauseef WM, Toyoshima S (2003) Two regions responsible for the actin binding of p57, a mammalian coronin family actin-binding protein. *Biol Pharm Bull* **26**: 409–416
- Oren M (2003) Decision making by p53: life, death and cancer. *Cell Death Differ* **10**: 431–442
- Poluha W, Schonhoff CM, Harrington KS, Lachyankar MB, Crosbie NE, Bulseco DA, Ross AH (1997) A novel, nerve growth factor-activated pathway involving nitric oxide, p53, and p21WAF1 regulates neuronal differentiation of PC-12 cells. *J Biol Chem* **272**: 24002–24007
- Pleasure SJ, Page C, Lee VH (1992) Pure, postmitotic, polarized human neurons derived from NTera2 cells provide a system for expressing exogenous proteins in terminally differentiated neurons. *J Neurosci* **12**: 1802–1815
- Raivich G, Bohatschek M, Da Costa C, Iwata O, Galiano M, Hristova M, Nateri AS, Makwana M, Riera-Sans L, Wolfer DP, Lipp HP, Aquizzi A, Wagner EF, Behrens A (2004) The AP-1 transcription factor c-Jun is required for efficient axonal regeneration. *Neuron* **43**: 57–67
- Rogel A, Popliker M, Webb CG, Oren M (1985) P53 cellular tumor antigen: analysis of mRNA levels in normal adult tissues, embryos, and tumors. *Mol Cell Biol* **5**: 2851–2855
- Rybakin V, Clemen CS (2005) Coronin proteins as multifunctional regulators of the cytoskeleton and membrane trafficking. *Bioessays* **27**: 625–632
- Schmid P, Lorenz A, Hameister H, Montenarh M (1991) Expression of p53 during mouse embryogenesis. *Development* **113**: 857–865
- Schuler M, Green DR (2005) Transcription, apoptosis, p53: catch-22. *Trends Genet* **21**: 182–187
- Tang BL (2001) Protein trafficking mechanisms associated with neurite outgrowth and polarized sorting in neurons. *J Neurochem* **79**: 923–930
- Vousden KH, Prives C (2005) P53 and prognosis; new insights and further complexity. *Cell* **120**: 7–11
- Xu Y (2003) Regulation of p53 responses by post-translational modifications. *Cell Death Differ* **10**: 400–403
- Yakovlev AG, Di Giovanni S, Wang G, Liu W, Stoica B, Faden AI (2004) BOK and NOXA are essential mediators of p53-dependent apoptosis. *J Biol Chem* **279**: 28367–28374
- Yakovlev AG, Faden AI (1995) Molecular biology of CNS injury. *J Neurotrauma* **12**: 767–777