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p53 Plays a Regulatory Role in Differentiation and Apoptosis of Central Nervous System-Associated Cells

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This study demonstrated the involvement of the tumor suppressor protein p53 in differentiation and programmed cell death of neurons and oligodendrocytes, two cell types that leave the mitotic cycle early in development and undergo massive-scale cell death as the nervous system matures. We found that primary cultures of rat oligodendrocytes and neurons, as well as of the neuronal PC12 pheochromocytoma cell line, constitutively express the p53 protein. At critical points in the maturation of these cells in vitro, the subcellular localization of p53 changes: during differentiation it appears mainly in the nucleus, whereas in mature differentiated cells it is present mainly in the cytoplasm. These subcellular changes were correlated with changes in levels of immunoprecipitated p53. Infection of cells with a recombinant retrovirus encoding a C-terminal p53 miniprotein (p53 DD), previously shown to act as a dominant negative inhibitor of endogenous wild-type p53 activity, inhibited the differentiation of oligodendrocytes and of PC12 cells and protected neurons from spontaneous apoptotic death. These findings suggest that p53, upon receiving appropriate signals, is recruited into the nucleus, where it plays a regulatory role in directing primary neurons, oligodendrocytes, and PC12 cells toward either differentiation or apoptosis in vitro.

Cells of the central nervous system leave the mitotic cycle and differentiate early in development (77). At a certain stage during the development and differentiation of the vertebrate nervous system, massive-scale death of neurons and oligodendrocytes occurs as part of the normal physiological process (6, 63). The affected cells are thought to be neurons that have failed to form connections with a target and thus lack target-derived support, such as specific neurotrophic factors (63), and oligodendrocytes that have failed to obtain the growth factors, cytokines, and neurotrophic factors necessary for their survival (2, 5–7, 10, 43, 48, 58, 66). Death of neurons and oligodendrocytes has also been observed in certain pathological conditions. Death of oligodendrocytes has been observed, for example, in multiple sclerosis (67). Neuronal death occurs in degenerative disorders such as Alzheimer's disease (19) and after traumatic injuries (78), in which damage to axons leads to their degeneration and eventually to the death of the neuronal cell bodies (1, 30, 53). This neuronal cell death was recently shown to occur via apoptosis (26, 62), probably because of deprivation of target-derived trophic support.

We recently showed that the wild-type (wt) p53 tumor suppressor gene is involved in apoptotic death of oligodendrocytes (20). We therefore examined the possibility that wt p53, known to be involved in both apoptosis (76, 81) and differentiation (23, 74) in other systems, might also play a role in differentiation of oligodendrocytes as well as in death and differentiation of neurons.

We show here that the wt p53 protein changes its subcellular localization upon maturation and differentiation of neurons and oligodendrocytes in vitro and that inactivation of endogenous wt p53 blocks both apoptosis and differentiation of these cells. Moreover, changes in the subcellular localization of p53 were found to be correlated with changes in levels of the protein upon cell maturation and differentiation. No changes in p53 mRNA levels were observed.

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MATERIALS AND METHODS

Preparation of mature rat hippocampal neuron cultures. Low-density rat hippocampal neuronal cultures were prepared by the procedure of Goslin and Banker (28). Coverslips were cleaned and sterilized in porcelain racks in such a way that they did not adhere to one another (Cohen cover glass staining racks; Thomas Scientific). Coverslips were placed in staining racks, rinsed in distilled water (four rinses, 1 min each) to remove dust, and transferred to concentrated HNO_3 for 36 h. After being rinsed in distilled water (four changes over 3 h) and sterilized with dry heat (overnight at 225°C), small coverslips (13-mm diameter) were transferred to 24-well dishes, one coverslip per well, and larger ones (22- to 24-mm-diameter) were transferred to 9-cm-diameter petri dishes, nine coverslips per plate, arranged so that they did not overlap. To support the coverslips above the glia during coculturing, paraffin dots were placed on dishes and UV irradiation (30 min) was applied before the coverslips were introduced. One milligram of poly-L-lysine hydrobromide (Sigma) (molecular weight, 30,000 to 70,000) per ml was dissolved in 0.1 M borate buffer (pH 8.5), filtered, sterilized, and used to coat each coverslip overnight. The poly-L-lysine was then removed, coverslips were rinsed in distilled water (two washes, 2 h each), plating medium (Eagle's minimal essential medium with Earle's salts containing extra glucose [600 mg/liter] and 10% horse serum) was added, and the dishes were stored in an incubator. Astroglial cultures were prepared from the brains of neonatal rats according to the method of Goslin and Banker (28). In each well or petri dish 10^5 or 10^6 cells were plated, respectively. Glial cultures were fed with plating medium twice a week and were used after reaching confluence, about 2 weeks after plating. One day before use the plating medium was removed, neuronal maintenance medium (minimal essential medium containing N_2 supplements) (8, 9) was added, and incubation was continued. The desired number of viable rat hippocampal nerves (from E18 embryos) were plated on the poly-L-lysine-treated coverslips. After 3 to 4 h, when most of the neurons were attached, the coverslips were transferred to the dishes containing the glial cells in maintenance medium, in such a way that the neuronal side was facing the glia, which support neuronal survival and development. To reduce glial proliferation, 1-β-D-arabinofuranosylcytosine (cytosine arabinoside) (Calbiochem) (final concentration, 5×10^{-6} M) was added to the cultures 2 days after plating. At various time points cells were fixed, treated with specific antibodies, and photographed.

Growth of the PC12 cell line. PC12 cells were grown either in enriched medium (Dulbecco's modified Eagle's medium [DMEM] supplemented with 8% horse serum, 8% fetal calf serum [FCS], glutamine, and gentamicin), in which the cells proliferated, or in nonenriched medium (DMEM supplemented with 1% horse serum, glutamine, and gentamicin) in the presence of nerve growth factor (NGF) (50 ng/ml), which caused the cells to differentiate. At various time points

cells were fixed, stained with appropriate antibody and with 4′,6-diamidino-2-phenylindole (DAPI), and photographed.

Preparation of mature rat brain oligodendrocyte cultures. Rat brain cultures highly enriched with oligodendrocytes were prepared by a modification of the procedure of McCarthy and deVellis (47). Cells dissociated from the cerebral cortices of 2-day-old Wistar rats were cultured for 8 days in poly-D-lysine-coated Nunc tissue culture flasks (two brains per flask; 85-cm^2 surface area) in DMEM containing 7.5% FCS, 2 mM glutamine, and antibiotics. The medium was changed every 2 days. After 8 days, flasks were shaken for 8 h at 37°C on a rotary platform and the medium, containing mostly macrophages, was discarded. Fresh medium was added to the flasks, which were then shaken for an additional 16 h at 37°C. The cells shaken off were mostly progenitor cells for oligodendrocytes and type 2 astrocytes and were collected and seeded (1.5×10^6 cells per 9-cm-diameter plate containing nine 24-mm-diameter glass coverslips). To encourage oligodendrocyte proliferation, cells in the logarithmic phase of growth were seeded in an appropriate medium (B104 cells maintained in DGA medium [10% FCS, 2 mM L-glutamine, and antibiotics in DMEM^{++}]). For preparation of the proliferative medium, DGA medium was removed, cells were washed twice with DMEM, and Raff's modification of Bottenstein's and Sato's defined medium (SATO medium) (8, 64) was added for 2 to 3 days to cells in the logarithmic phase of growth, after which the medium was collected, filtered, and diluted 1:3 in SATO medium. This enabled the cells to continue proliferating without differentiating. To encourage oligodendrocyte development, B104 proliferating medium was replaced with SATO medium. At various time points cells were fixed, stained with appropriate antibody, and photographed.

Immunostaining of cells. Cultures were prepared as described above. Cells were collected and seeded (25,000 cells per 13-mm-diameter glass coverslip). At various times cells were fixed in methanol for 5 min at −20°C, treated with cold acetone at 4°C for 2 min, and washed in phosphate-buffered saline (PBS). In order to avoid nonspecific immunostaining, cells were incubated with 3% FCS in PBS for 30 min in a moist chamber at 37°C. Following PBS washes, monoclonal antibody PAb421, specific to p53 (31), was applied to coverslips for 30 min in a moist chamber at 37°C. The coverslips were washed with PBS and stained for 30 min at room temperature by a specific second antibody (fluorescein isothiocyanate-labeled goat anti-mouse immunoglobulin G plus immunoglobulin M) (Jackson ImmunoResearch Laboratories), diluted 1:30 in 3% FCS–PBS, and then fluorescently stained with DAPI (5 mg/ml; Sigma) (to allow visualization of the nuclei) diluted 1:10,000 in PBS for 15 min at room temperature. The cells were washed three times with PBS, mounted in a solution of glycerol containing 22 mM 1,4-diazabicyclo(2,2,2)octane (Sigma) to prevent fading (35), and photographed.

Infection with retroviral vectors. Rat brain cultures highly enriched with oligodendrocytes were prepared and seeded in a proliferative medium as described above. This enabled the cells to continue proliferating without differentiating. Two days after being seeded, cells were infected with retroviruses expressing either LXSNp53DD or G418 resistance only (LXSN). Generation of these retroviral vectors has been described elsewhere (29). Infection was allowed to proceed for 2.5 h, after which the virus was washed out and fresh medium was added. Selection with G418 was performed for 3 days (starting on the day after infection) by daily addition of 1 mg of G418 per ml. At the end of the third day of selection, cells were fixed and stained as described above. Rat hippocampal neuronal cultures were prepared and seeded as described above. Since the neurons when prepared continued to divide to a limited extent (one to two divisions only) before differentiation, the cells were infected a few hours after being seeded in order to let the virus infect proliferating cells. G418-resistant cells were allowed to mature for 8 days and were then fixed and stained as described above.

Protein extract preparation and immunoprecipitation. Protein analysis was performed as described previously (45). For determination of protein levels, cell extracts were prepared by the addition of 54 K buffer (50 mM Tris [pH 8.0], 150 mM NaCl, 5 mM EDTA, 0.5% Nonidet P-40). Immunoprecipitation of 200 μg of each extract was performed by the following procedure. For preclearing, 50 μl of control medium (DMEM plus 10% serum) for monoclonal antibodies was added and incubated for 30 min on ice. To this was added 30 μl of protein A–Sepharose CL-4B (Pharmacia Biotech), with gentle mixing and further incubation for 10 min on ice followed by centrifugation for 3 min at 4°C. Monoclonal hybridoma supernatant (50 μl) was added, mixed gently, incubated on ice for 10 min, and centrifuged for 3 min at 4°C. The supernatant was discarded and the pellet was washed three times with 900 μl of buffer B (50 mM Tris [pH 7.4], 500 mM NaCl, 5 mM EDTA, 0.5% Nonidet P-40) and then once with buffer C (50 mM Tris [pH 7.4], 150 mM NaCl, 5 mM EDTA). The pellet was resuspended in 20 μl of protein sample buffer (10% glycerol, 5% β-mercaptoethanol, 3% sodium dodecyl sulfate [SDS], 180 mM Tris [pH 6.8]) and processed by Western blot (immunoblot) analysis.

Western blot analysis. Samples were subjected to SDS-polyacrylamide gel electrophoresis. Gels were blotted onto nitrocellulose for 2 h at 200 mA and incubated for 2 h with PBS containing 10% (vol/vol) milk. The immobilized proteins were detected with the appropriate antibodies as follows. The blots were incubated for 2 h in PBS containing 10% milk and then for 4 h in an identical solution containing the appropriate antibody and then washed three times, once for 15 min and twice for 10 min each time, in PBS containing 0.05% Tween 20 and 10% milk; this procedure was followed by a 1-h incubation with an appropriate horseradish peroxidase-conjugated second antibody in PBS containing 10% milk and the same washes as before without the milk. All steps were performed at room temperature. Bands were visualized by the enhanced-chemiluminescence system (Amersham).

Preparation and analysis of RNA. Total cellular RNA was isolated by the RNAzol B method (13) with the aid of a Biotec Laboratories kit. All steps were performed at 4°C or on ice. Polyadenylated [poly(A)$^+$] RNA was purified by oligo(dT) magnetic beads with the aid of a Dynabeads kit (Dynal). RNA was quantified by determining the A_{260} or A_{280}.

Poly(A)$^+$ RNA (about 4 μg) was denatured at 65°C for 5 min in denaturing buffer consisting of 6% formaldehyde–50% formamide (vol/vol) in 3-[*N*-morpholino]propanesulfonic acid (MOPS) buffer (20 mM MOPS [pH 7.0], 5 mM sodium acetate, 1 mM EDTA). RNA was then size fractionated by electrophoresis through a 1% agarose–formaldehyde gel and transferred to GeneScreen Plus membrane (DuPont, New England Nuclear) according to the method of Lehrach et al. (41). Gels were blotted in 10× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate) and left overnight. Blots were baked for 1 h at 80°C in a vacuum. The relative amounts and quality of the transferred RNA were assessed by staining of the blot with methylene blue (0.04% in 0.5 M sodium acetate [pH 5.2]) or by hybridizing with a glyceraldehyde-3-phosphate dehydrogenase probe (18). Prehybridization was performed at 42°C for 4 to 24 h in buffer containing 50% formamide, 5× SSC (pH 7.2), 5× Denhardt's buffer (0.1% polyvinylpyrrolidone, 0.1% Ficoll, and 0.1% bovine serum albumin), 1% SDS (pH 7.2), 50 mM Na_2HPO_4/NaH_2PO_4 (pH 7.0), and 300 mg of denatured herring sperm DNA per ml. The blots were hybridized at 42°C for 16 to 24 h in buffer containing the same materials but with smaller proportions of Denhardt's buffer (1×) and Na_2HPO_4/NaH_2PO_4 (20 mM), in the presence of an appropriate ^{32}P-labeled gel-purified fragment randomly primed (22) with the aid of a Megaprime DNA labeling system (Amersham). The probe used was a p53 cDNA clone (BS 19A). Blots were washed twice at room temperature in 2× SSC–0.1% SDS for 10 min each time and in 0.1× SSC–0.1% SDS at 50°C for 30 to 60 min. The blots were then exposed to X-ray film.

RESULTS

Involvement of p53 in neuronal differentiation. We recently showed that oligodendrocytes express wt p53 and that this protein is directly involved in apoptotic death of these cells (20). To further investigate the possible functions of p53 in neural cells, we first examined both oligodendrocytes and neurons at the transcript level. Total RNA was prepared from primary rat brain oligodendrocytes and from primary neurons. The RNA preparations were subjected to reverse transcriptase PCR analysis (21), using rat p53-specific primers. A band of the expected size was observed at all stages of differentiation and maturation in both cell types (data not shown).

We next examined the subcellular localization of the p53 protein in neurons. Cultures enriched with rat hippocampal neurons were grown at a low density. At different stages of their differentiation and maturation, cells were fixed and stained with the p53-specific monoclonal antibody PAb421 (31), which recognizes rat wt p53, and with DAPI, which allows visualization of the cell nuclear border. Soon after seeding, p53 was detected mainly in the cell nuclei and was barely seen in the cytoplasm, which at this stage occupied relatively little space in these cells (Fig. 1A3, B3, and F). Further differentiation and maturation of these cells were accompanied by a gradual reduction of p53 immunostaining in the nucleus relative to the cytoplasmic staining (Fig. 1C3 and D3). In fully mature neurons, p53 immunostaining was observed predominantly in the cytoplasm (Fig. 1E3 and G). It should be noted that in these experiments the cultures were treated with cytosine arabinoside. As neurons do not proliferate under the conditions of our experiment, they were expected to be unaffected by this addition. This was confirmed by the similar results obtained when p53 was assayed in cytosine arabinoside-free medium (data not shown).

The primary neurons used in the experiments described above, even at the earliest stage in vitro, are already committed to differentiation; consequently, the use of this culture system did not allow us to examine the localization of p53 in uncommitted neuronal progenitors and/or in proliferating neurons

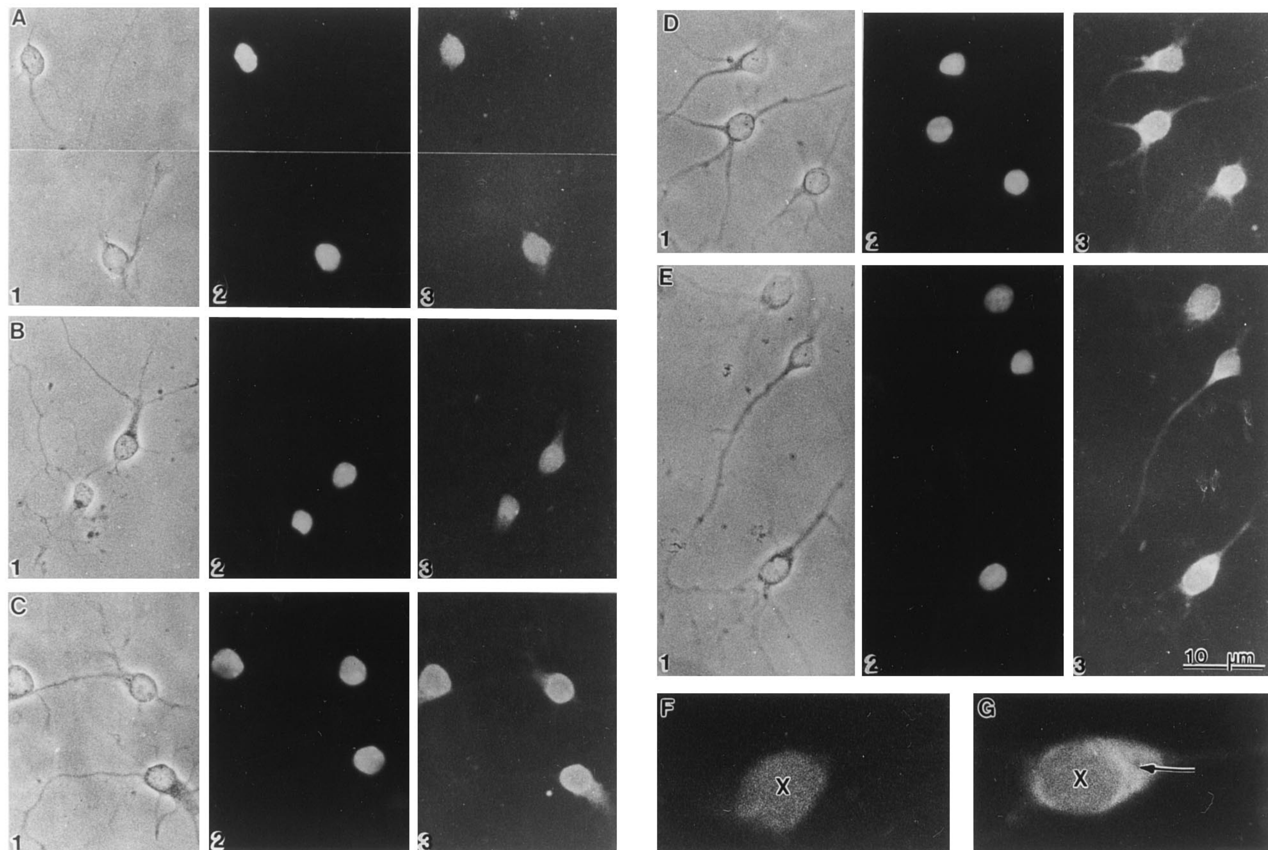

FIG. 1. Changes in p53 subcellular localization associated with maturation and differentiation of neurons. Rat hippocampal neuron cultures were grown at a low density so that individual neurons could be easily observed. At the desired stage of development, the cells were fixed, stained with DAPI (panel 2) and monoclonal antibody PAb421 (panel 3), and photographed at a magnification of ×1,600. Neurons were inspected at 1 (A), 2 (B), 3 (C), 4 (D), and 7 (E) days in culture. The same fields were also visualized by phase contrast (panel 1). The experiment was performed in duplicate and repeated three times. (F and G) High-magnification photographs of a neuron shown in panel A3 at an early stage of postseeding, where the p53 staining is predominantly in the nucleus (cross) (F), and of a neuron shown in panel E3, where the p53 staining is more pronounced in the cytoplasm (arrow) than in the nucleus (G).

prior to their differentiation. We therefore repeated the experiment using PC12 pheochromocytoma cells, which proliferate as long as they are grown in enriched medium but start to differentiate when transferred to a nonenriched medium in the presence of NGF. Immunostaining showed that p53 was barely detectable in the nuclei of PC12 cells as long as they were proliferating (Fig. 2A2 and E). However, 16 h after the addition of NGF, when the cells had become spindle shaped and had extended small processes indicative of an early stage of differentiation, high levels of p53 immunoreactivity were found in the nuclei (Fig. 2B2 and F). This stage apparently corresponded to the stage at which the primary neurons were first observed in culture (Fig. 1A3). At 26 h after the addition of NGF to PC12 cells, p53 was still detected in the nucleus, but some of the cells already showed weaker nuclear staining (Fig. 2C2). In fully differentiated PC12 cells, as in the primary neurons (Fig. 1E3), p53 was again barely detectable in the nucleus (Fig. 2D2). Thus, the findings in PC12 cells complemented those in primary neurons and suggested that at an early stage of neuronal differentiation p53 accumulates in the nucleus, where it may participate in activation of other genes directing the cell towards differentiation. Once the cells have become committed to differentiation, p53 is presumably no longer needed in the nucleus, and its nuclear level declines.

To verify that p53 is directly involved in the commitment of neuronal cells to differentiation, we employed a dominant negative mutant of p53, the p53 DD miniprotein (29, 71), to interfere with the activity of endogenous wt p53 in PC12 cells. The DD miniprotein comprises the last 89 residues of wt mouse p53, including the dimerization domain, and acts as an inhibitor of coexpressed wt p53 (71). The underlying mechanism appears to be based, at least in part, on the formation of functionally impaired mixed oligomers between the DD miniprotein and the full-length wt p53 (65, 66). We used the retroviral vector LXSN expressing the DD miniprotein (LXSNp53DD) (29). A similar retroviral vector, LXSN encoding G418 resistance only, was used as a control. PC12 cells were seeded and infected with LXSNp53DD or LXSN. After 24 h the medium was replaced by nonenriched medium, as described in the legend to Fig. 3, and NGF was added (50 ng/ml). At this stage selection with G418 was started as well. Three days later the cells were again fed with NGF. On day 6 of culture, when cells are usually fully differentiated, the surviving cells were fixed and immunostained with PAb421. Each experiment was carried out in duplicate; LXSNp53DD-infected cells were used in three experiments, while control-infected cells were used in five experiments. In each infected group, the number of PC12 cells with neurite extensions was counted and calculated as a percentage of the total number of cells (about 1,000 in each experiment). The results showed a substantial reduction in the percentage of differentiating neurons in cultures infected with the DD-expressing retrovirus

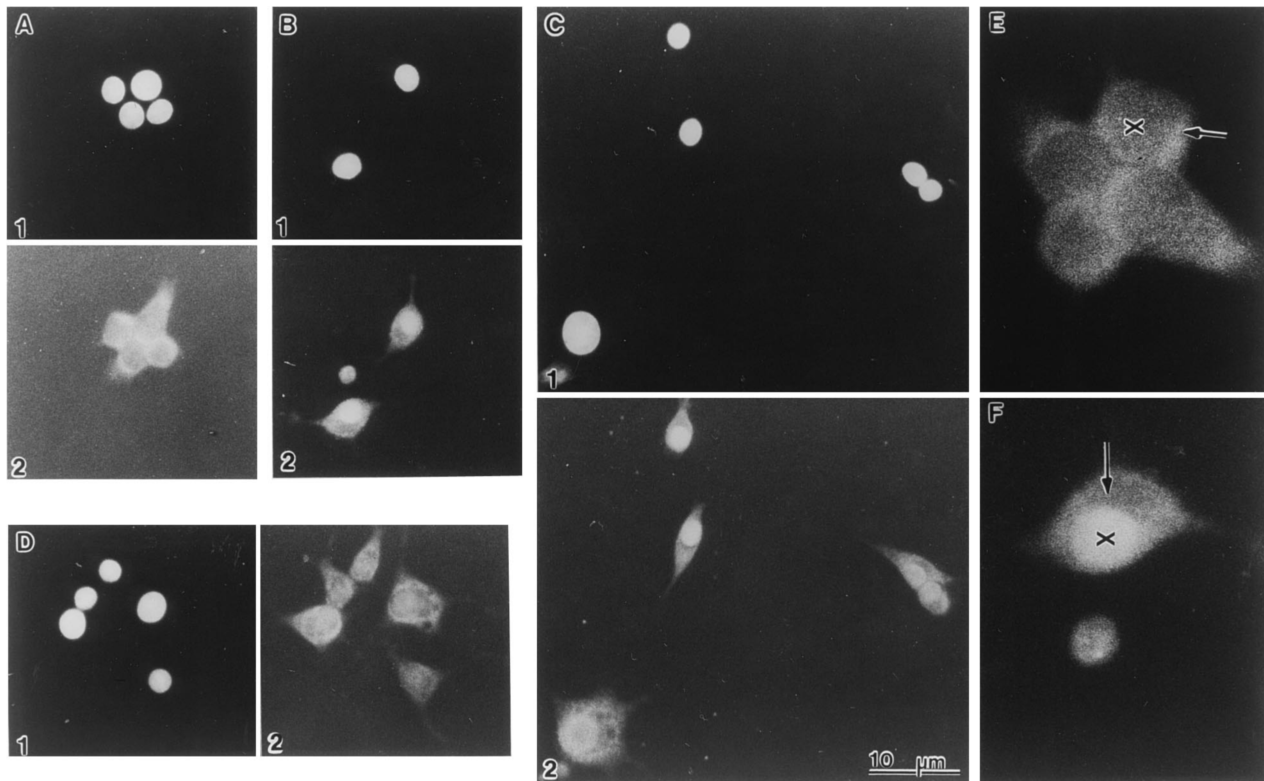

FIG. 2. Changes in p53 subcellular localization in PC12 cells upon NGF starvation and in the presence of NGF. Cells were seeded at 20,000 cells per 13-mm-diameter glass coverslip and grown either in enriched medium in which the cells proliferated or in nonenriched medium in the presence of NGF (50 ng/ml), which causes the cells to differentiate (see Materials and Methods). At various time points cells were fixed, stained with monoclonal antibody PAb421 and DAPI, and photographed at a magnification of ×1,600. (Panel 1) DAPI-stained cells photographed with a UV filter; (panel 2) PAb421-stained cells. The same field was photographed for both panels for each of the following experimental groups: proliferating PC12 cells maintained in enriched medium (A) and PC12 cells maintained in nonenriched medium in the presence of NGF for 16, 26, and 40 h (B to D, respectively). The experiment was performed in duplicate and repeated three times. (E and F) High-magnification photographs of PC12 cells shown in panels A2 and B2, respectively, depicting p53 immunoreactivity predominantly in the cytoplasm (arrow) of proliferating cells and predominantly in the nucleus (cross) of differentiating cells, respectively.

compared with cultures infected with the control retrovirus (to 4.4% ± 0.3% and 63.0% ± 4.1%, respectively [means ± standard errors]; $P < 0.0001$ [considered highly significant] by unpaired *t* test). The small percentage of DD-infected PC12 cells that differentiated may represent cells that were already committed to differentiation at the time of infection.

Involvement of p53 in apoptotic death of neurons. The above-described observations suggest that p53 plays an important role in neuronal differentiation. In view of the temporal association between neuronal differentiation and apoptotic death during nervous system development (14, 15, 60, 61), and the known dual activity of p53 in differentiation and apoptosis in other systems, it was of interest to determine whether p53 is also involved in the spontaneous death of neurons. Previous studies in our laboratory have demonstrated an involvement of p53 in apoptotic death of oligodendrocytes (20). Again we employed the dominant negative p53 DD miniprotein. Because retroviruses can infect only proliferating cells, we used cultures enriched with low-density rat primary hippocampal neurons, which continue to divide (though to a limited extent) prior to differentiation (see Materials and Methods). G418-resistant cells infected with either the control or the DD-expressing retrovirus were allowed to mature for 8 days, and the cells were then fixed and fluorescently stained with DAPI for visualization of apoptotic morphology. The same cells were stained with PAb421 for visualization of p53. Each experiment was carried out in duplicate; three experiments were performed for each group.

In cultures infected with the control retrovirus LXSN, 75.0% ± 2.9% (mean ± standard error) of the cells showed apoptotic morphology. In contrast, the number of apoptotic cells in cultures infected with the DD-expressing retrovirus was only 20.0% ± 2.1%, indicating substantial protection against apoptosis. In this experiment apoptotic cells were counted as a fraction of the total cells (about 400 in each experiment) in culture, rather than as a fraction of successfully infected cells only. Thus, the extent of protection by DD is actually an underestimate. Examination of the cells successfully expressing the DD miniprotein, as visualized by staining with PAb421, revealed almost complete protection from apoptosis.

p53 mRNA and protein expression during maturation and differentiation of PC12 cells. To examine whether maturation-associated changes in p53 are also manifested at the transcript level, poly(A)$^+$ RNA from NGF-treated PC12 cells at different stages of differentiation was prepared and subjected to Northern (RNA) blotting and hybridization with a p53 probe. The results showed constitutive expression of p53 with no change in its transcript levels, in contrast to results for control hybridization with glyceraldehyde-3-phosphate dehydrogenase, during differentiation and maturation of the cells (Fig. 3). Immunoprecipitation of proteins extracted from PC12 cells with PAb421 (p53-specific monoclonal antibody), followed by West-

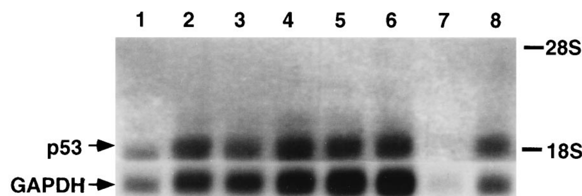

FIG. 3. Expression of p53 transcripts during differentiation of PC12 cells. Shown are results of Northern blot analysis of poly(A)$^+$ RNA prepared at different stages of maturation and differentiation of PC12 cells (see Materials and Methods). The blot was hybridized with a mouse p53 cDNA probe or a glyceraldehyde-3-phosphate dehydrogenase (GAPDH) probe to indicate the amount of mRNA in each lane. Lane 1, proliferating PC12 cells maintained in enriched medium; lanes 2 to 7, PC12 cells in nonenriched medium in the presence of NGF for 8, 16, 24, 40, and 48 h and for 6 days, respectively; lane 8, PC12 cells in nonenriched medium without NGF for 6 days. 18S and 28S rRNAs were used as molecular weight markers, and their positions are indicated.

ern blot analysis with the same antibodies, revealed an increase in the total amounts of p53 16 h after differentiation was induced (Fig. 4). Total p53 levels remained high until 40 h after onset of the differentiation signal and then started to decline (Fig. 4). The elevation and reduction of p53 levels in PC12 cells coincided with the enhancement and reduction of p53 nuclear immunostaining, respectively (Fig. 2).

p53 in differentiation of oligodendrocytes. We recently showed that oligodendrocytes express wt p53 and that this protein is directly involved in apoptotic death of these cells (20). To determine whether p53 is involved not only in apoptotic death but also in differentiation of oligodendrocytes, we used cultures enriched with rat brain oligodendrocyte progenitors, which were maintained in culture medium that allowed them either to proliferate or to differentiate (see Materials and Methods). At different time points the cells were fixed and immunostained with the p53-specific antibody PAb421. The results showed that as long as the cells were proliferating (Fig. 5A1), and soon after initiation of differentiation (6 h after the change to SATO differentiating medium) (Fig. 5B1), the level of p53 immunostaining in the nucleus was low relative to the level of the cytoplasmic staining (Fig. 5A3 and B3, respectively). However, 24 h after the differentiating medium was added, p53 immunoreactivity was found predominantly in the nuclei of cells undergoing differentiation (Fig. 5C3 and E). In fully differentiated cells, i.e., in cells maintained in the differentiating medium for 7 days, p53 immunostaining in the nucleus was again barely detectable relative to the cytoplasmic staining (Fig. 5D3 and F). Thus, differentiation of oligodendrocytes was associated with a transient elevation of the p53 level in the nucleus. It should be noted that p53 appeared in the nucleus well before morphological differentiation became discernible.

The involvement of p53 in the commitment of oligodendrocytes to differentiation was further substantiated by again employing the dominant negative p53 DD miniprotein in a retroviral vector and a control retrovirus expressing G418 resistance only. Rat brain cultures enriched with oligodendrocytes were maintained in culture media that allow their proliferation or differentiation (see Materials and Methods). The day after being seeded, cells were infected with retrovirus expressing either LXSNp53DD or G418 resistance only (LXSN), as described in Materials and Methods. At the end of the seventh day of selection with G418, surviving cells were fixed, immunostained with PAb421, and counted. In both infected cultures the total number of cells (about 500 in each experiment) and the number of cells with neurite extensions were counted. Each experiment was carried out in duplicate; five experiments were performed for each group. Early interference with the activity of endogenous wt p53 in primary oligodendrocyte cultures, by infection with the DD-expressing retrovirus, significantly blocked neurite extension, i.e., differentiation. The results showed a marked reduction in the percentage of differentiating oligodendrocytes in the cultures infected with the DD-expressing retrovirus compared with the control (13.83% $\pm$ 4.18% and 80.50% $\pm$ 6.43%, respectively [means $\pm$ standard errors]; $P < 0.001$ [considered highly significant] by the unpaired t test).

DISCUSSION

The results of this study suggest that p53 acts as a regulatory protein in neurons and oligodendrocytes in culture. The possible involvement of p53 in differentiation was inferred from the changes in its subcellular localization and specifically its appearance in the nucleus at distinct stages of differentiation of these cells. These changes in p53 immunoreactivity in the nucleus were accompanied by changes in p53 protein levels. In addition, direct evidence for the involvement of p53 in differentiation and apoptosis came from the inhibition of these processes following the blocking of endogenous wt p53 activity in the various neural cell types.

Studies with various experimental models have indicated that p53 may be functionally regulated by its differential subcellular localization, through a well-controlled nuclear translocation mechanism (25, 27, 46, 56, 57, 72, 73, 75, 80). p53 was found to play an important role as a cell cycle "checkpoint" protein, arresting cells primarily in G_1 in response to DNA damage (32, 37, 40). Studies have shown that induced expression of wt p53 in tumor cells can have one of the following results: (i) suppression of cell proliferation (4, 12, 16, 49, 50, 52), (ii) induction of apoptosis (76, 81), or (iii) induction of differentiation (23, 74). Interestingly, wt p53-induced apoptosis in myeloid leukemia cells could be greatly reduced by administration of the cytokine interleukin-6 (81), which normally induces differentiation in this particular cell line. Livingstone et al. (42) postulated that the p53 gene may behave like some of the cell cycle checkpoint genes in *Saccharomyces cerevisiae*, which are dispensable during optimal growth conditions but can arrest the growing cell in G_2 or G_1 (32, 33). It seems likely that p53 operates through different pathways, involving activation of different genes and proteins leading either to apoptosis or to differentiation, and that the pathway chosen by the cell depends on the cell type and the levels of wt p53 expressed (17).

The physiological involvement of p53 in differentiation and death of neurons and oligodendrocytes might operate through distinct p53-activated pathways. For example, the role of p53 in

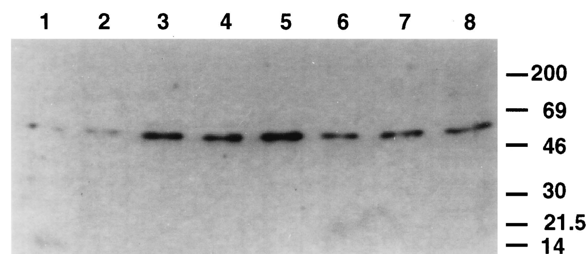

FIG. 4. Expression of p53 during differentiation of PC12 cells. Protein extracts prepared at different stages of maturation and differentiation of PC12 cells were subjected to Western blot analysis (see legend to Fig. 3). The extracts were immunoprecipitated and visualized with PAb421. Lanes 1 to 8, PC12 cells subjected to the same treatments as for Fig. 3. Molecular weight markers were run in parallel, and their positions are indicated (in thousands).

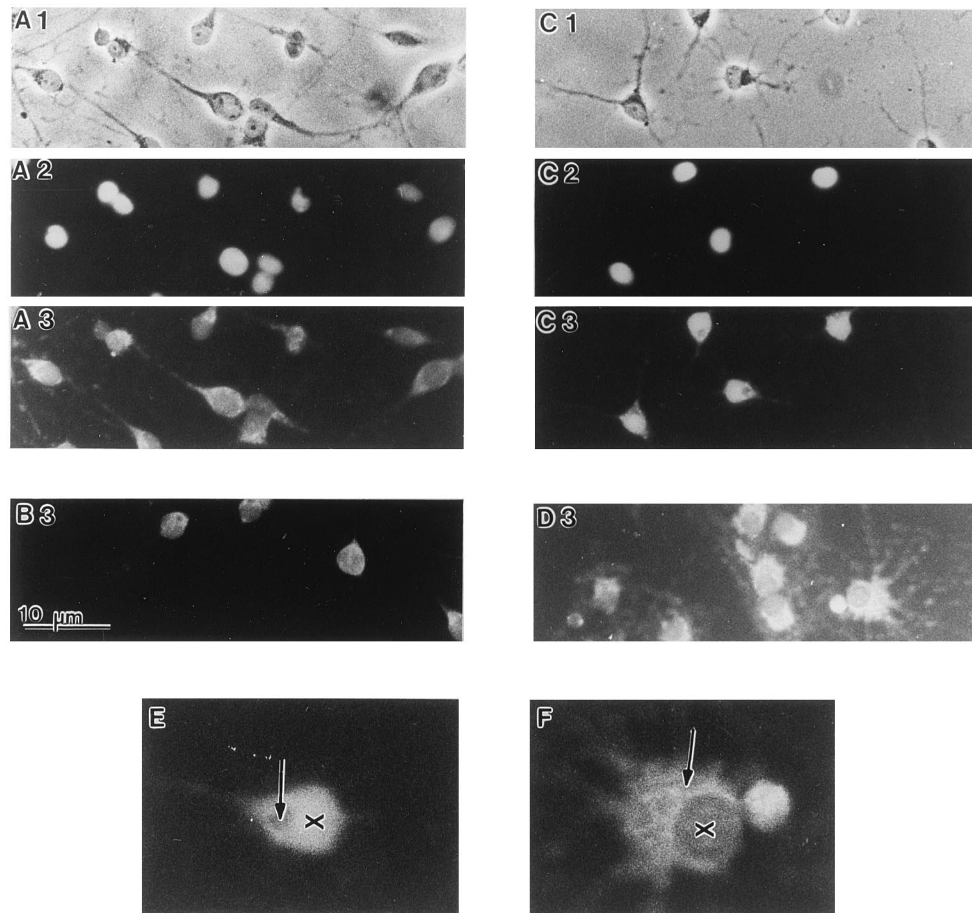

FIG. 5. Changes in subcellular localization of p53 immunoreactivity from the cytoplasm to the nucleus are associated with differentiation and maturation of oligodendrocytes. Enriched progenitor rat brain oligodendrocyte cultures, grown in culture media that maintained the cells at different stages of differentiation and maturation (as described in Materials and Methods), were fixed, stained with monoclonal antibody PAb421 (directed against p53) and DAPI, and photographed at a magnification of ×1,600. (A) Proliferating progenitor oligodendrocytes maintained in proliferative B104 medium. (B) Proliferating oligodendrocytes grown in B104 proliferative medium and then incubated for 6 h in SATO medium. (C) Proliferating oligodendrocytes grown in B104 proliferative medium and then incubated for 24 h in SATO medium. (D) Fully differentiated oligodendrocytes. (Panel 1) phase-contrast photographs; (panel 2) DAPI-stained oligodendrocytes; (panel 3) PAb421-stained oligodendrocytes (same fields for all panels). The experiment was performed in duplicate and repeated three times. The phase-contrast photographs were taken through the UV filter, which enables visualization of DAPI staining as well. (E and F) High-magnification photographs of oligodendrocytes shown in panels C3 and D3, respectively, depicting p53 staining predominantly in the nucleus (cross) of differentiating oligodendrocytes (E) and barely in the nucleus compared with that in the cytoplasm (arrow) of mature oligodendrocytes (F).

promoting differentiation may require the specific transactivation of differentiation-related genes, while its apoptosis-promoting effect may be independent of such activity. Indeed, it was recently reported that p53 can induce apoptosis without the transactivation of transcription (11, 34, 79). Moreover, although p53 is found to be constitutively expressed, it may normally remain nonfunctional until the cell receives appropriate signals. It is possible that the phosphorylation state and/or the conformation of p53 may differ under conditions inducing apoptosis or differentiation.

Among the p53-regulated genes that might contribute to apoptosis a likely candidate is *bax*, which was recently shown to be regulated by p53 (55). This gene belongs to the *bcl-2* family and contains the two conserved motifs termed *bcl-2* homology 1 (BH1) and *bcl-2* homology 2 (BH2) domains, but it antagonizes the survival-promoting effect of *bcl-2* (59). bax forms either homodimers or heterodimers with bcl-2 and, when overproduced, promotes apoptosis (38). Overexpression of wt p53 up-regulates bax expression in cells undergoing p53-induced apoptosis (54, 70). High levels of bax were found in some populations of neurons that are particularly sensitive to cell death induced by transient global ischemia. In the nervous system, it was shown that within 30 min to 3 h after an ischemic episode, immunostaining for bax was markedly increased in neurons undergoing apoptosis (39). From 3 h after the episode and onwards, neurons with morphological evidence of degeneration contained reduced levels of bcl-2. Also of interest is the observation that both p53 and tumor growth factor β1, which cause apoptosis in M1 cells, down-regulate *bcl-2*, whereas only p53 affects *bax* expression (70). In the latter study, ectopic *bcl-2* expression abolished apoptosis mediated by tumor growth factor β1 but not by p53; this difference may be due to the up-regulation of *bax* in the case of p53-mediated apoptosis. p53 was also shown to regulate the expression of *bcl-2* (54), which is expressed in neurons as well (24, 51). Overexpression of *bcl-2* in neural cells was found to reduce the rate of insult-induced death (36, 44, 82). Taken together, these studies suggest that *bax* might be a key p53 effector gene in mediating the p53 apoptotic response, probably in conjunction with limiting levels of bcl-2.

The fact that studies with p53 null mice appeared to show that normal neuronal functioning is not affected by blockage of endogenous wt p53 does not necessarily contradict the observations in this study. This is because even in normal cells p53 is not the only player and there may be "backup" genes that take over in its absence, perhaps even more so in p53 null mice, which are considered to develop normally in the absence of p53. Indeed, the observation that p53 null mice develop exencephalic brains (3, 68) is indicative of abnormal brain development as a result of the absence of p53, further pointing to an important role for p53 in neuronal development. Moreover, it should be borne in mind that physiological backup genes are less likely to be present in vitro than in vivo, and thus dependence on a single gene, in this case p53, is more pronounced in vitro.

Understanding the mechanism underlying death of neurons and oligodendrocytes may yield a new insight into certain pathological conditions. Neuronal death occurs in degenerative disorders such as Alzheimer's disease (19) and after traumatic injuries (78). Death of oligodendrocytes has been observed, for example, in multiple sclerosis (67). Induction of p53 was shown to be associated with neuronal damage in the central nervous system (69). Recent studies in our laboratory have shown involvement of p53 in apoptotic death of oligodendrocytes, which is induced by dimeric interleukin-2 (20).

Future studies should be aimed at elucidating the mechanisms governing apoptosis and differentiation in which p53 is involved and at identifying additional intracellular and nuclear components that dictate the final cellular outcome of p53-mediated signaling. Such information can be expected to provide important insights into pathological mechanisms in the nervous system and may yield a more effective approach to treatment of injured nerves.

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REFERENCES

1. **Allcutt, D., M. Berry, and J. Sievers.** 1984. A qualitative comparison of retinal ganglion cell axons to optic nerve crush in neonate and adult mice. Dev. Brain Res. **16:**231–240.
2. **Arakawa, Y., M. Sendtner, and H. Thoenen.** 1990. Survival effect of ciliary neurotrophic factor (CNTF) on chick embryonic motoneurons in culture: comparison with other neurotrophic factors and cytokines. J. Neurosci. **10:** 3507–3515.
3. **Armstrong, J. F., M. H. Kaufman, D. J. Harrison, and A. R. Clark.** 1995. High-frequency developmental abnormalities in p53-deficient mice. Curr. Biol. **5:**931–936.
4. **Baker, S. J., S. Markowitz, E. R. Fearon, J. K. V. Willson, and B. Vogelstein.** 1990. Suppression of human colorectal carcinoma cell growth by wild-type p53. Science **249:**912–915.
5. **Barres, B. A., I. K. Hart, H. S. Coles, J. F. Burne, J. T. Voyvodic, W. D. Richardson, and M. C. Raff.** 1992. Cell death and control of cell survival in the oligodendrocyte lineage. Cell **70:**31–46.
6. **Barres, B. A., M. C. Raff, F. Gaese, I. Bartke, G. Dechant, and Y. A. Barde.** 1994. A crucial role for neurotrophin-3 in oligodendrocyte development. Nature (London) **367:**371–375.
7. **Barres, B. A., R. Schmid, M. Sendtner, and M. C. Raff.** 1993. Multiple extracellular signals are required for long-term oligodendrocyte survival. Development **118:**283–295.
8. **Bottenstein, J. E., and G. H. Sato.** 1979. Growth of a rat neuroblastoma cell line in serum-free supplemented medium. Proc. Natl. Acad. Sci. USA **76:** 514–517.
9. **Bottenstein, J. E., and G. H. Sato (ed.).** 1985. Cell culture in the neurosciences, p. 3–44. Plenum Press, New York.
10. **Buchman, V. L., and A. M. Davies.** 1993. Different neurotrophins are expressed and act in a developmental sequence to promote the survival of embryonic sensory neurons. Development **118:**989–1001.
11. **Caelles, C., A. Helmberg, and M. Karin.** 1994. p53-dependent apoptosis in the absence of transcriptional activation of p53-target genes. Nature (London) **370:**220–223.
12. **Chen, P.-L., Y. Chen, R. Bookstein, and W.-H. Lee.** 1990. Genetic mechanisms of tumor suppression by the human p53 gene. Science **250:**1576–1580.
13. **Chomczynski, P., and N. Sacchi.** 1987. Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. Anal. Biochem. **162:**156–159.
14. **Cowan, W. M.** 1973. Neuronal death as a regulative mechanism in the control of cell number in the nervous system, p. 19–41. *In* M. Rockstein (ed.), Development and aging of the nervous system. Academic Press, New York.
15. **Cowan, W. M., J. W. Fawcett, D. D. O'Leary, and B. B. Stanfield.** 1984. Regressive events in neurogenesis. Science **225:**1258–1265.
16. **Diller, L., J. Kassel, C. E. Nelson, M. A. Gryka, G. Litwak, M. Gebhardt, B. Bressac, M. Ozturk, S. J. Baker, B. Vogelstein, and S. H. Friend.** 1990. p53 functions as a cell cycle control protein in osteosarcomas. Mol. Cell. Biol. **10:**5772–5781.
17. **Donehower, L. A., and A. Bradley.** 1993. The tumor suppressor p53. Biochim. Biophys. Acta **1155:**181–205.
18. **Dugaiczyk, A., J. A. Haron, E. M. Stone, O. E. Dennison, K. N. Rothblum, and R. J. Schwartz.** 1983. Cloning and sequencing of a deoxyribonucleic acid copy of glyceraldehyde-3-phosphate dehydrogenase messenger ribonucleic acid isolated from chicken muscle. Biochemistry **22:**1605–1613.
19. **Duguid, J. R., C. W. Bohmot, N. Liu, and W. W. Tourtellotte.** 1989. Changes in brain gene expression shared by scrapie and Alzheimer disease. Proc. Natl. Acad. Sci. USA **86:**7260–7264.
20. **Eizenberg, O., A. Faber-Elman, E. Gottlieb, M. Oren, V. Rotter, and M. Schwartz.** 1995. Direct involvement of p53 in programmed cell death of oligodendrocytes. EMBO J. **14:**1136–1144.
21. **Erlich, H. A.** 1989. PCR technology: principles and applications for DNA amplification. Stockton Press, New York.
22. **Feinberg, A., and B. Vogelstein.** 1984. A technique for radiolabeling restriction endonuclease fragments to high specific activity. Anal. Biochem. **137:** 266–267.
23. **Feinstein, E., R. P. Gale, J. Reed, and E. Canaani.** 1992. Expression of the normal p53 gene induces differentiation of K562 cells. Oncogene **7:**1853–1857.
24. **Ferrer, I., A. Tortosa, E. Condom, R. Blanco, A. Macaya, and A. Planas.** 1994. Increased expression of bcl-2 immunoreactivity in the developing cerebral cortex of the rat. Neurosci. Lett. **179:**13–16.
25. **Gannon, J. V., and D. P. Lane.** 1991. Protein synthesis required to anchor a mutant p53 protein which is temperature-sensitive for nuclear transport. Nature (London) **349:**802–806.
26. **García-Valenzuela, E., W. Gorczyca, Z. Darzynkiewicz, and S. C. Sherma.** 1994. Apoptosis in adult retinal ganglion cells after axotomy. J. Neurobiol. **25:**431–438.
27. **Ginsberg, D., D. Michael-Michalovitz, D. Ginsberg, and M. Oren.** 1991. Induction of growth arrest by a temperature-sensitive p53 mutant is correlated with increased nuclear localization and decreased stability of the protein. Mol. Cell. Biol. **11:**582–585.
28. **Goslin, K., and G. Banker.** 1991. Rat hippocampal neurons in low density culture, p. 251–281. *In* G. Banker and K. Goslin (ed.), Culturing nerve cells. MIT Press, Cambridge, Mass.
29. **Gottlieb, E., R. Haffner, T. von Ruden, E. W. Wagner, and M. Oren.** 1994. Down-regulation of wild-type p53 activity interferes with apoptosis of IL-3-dependent hematopoietic cells following IL-3 withdrawal. EMBO J. **13:** 1368–1374.
30. **Grafstein, B., and N. A. Ingoglia.** 1982. Intracranial transection of the optic nerve in adult mice: preliminary observations. Exp. Neurol. **76:**318–330.
31. **Harlow, E., L. V. Crawford, D. C. Pim, and N. M. Williamson.** 1981. Monoclonal antibodies specific for simian virus 40 antigens. J. Virol. **39:**861–869.
32. **Hartwell, L.** 1992. Defects in a cell cycle checkpoint may be responsible for the genomic instability of cancer cells. Cell **71:**543–546.
33. **Hartwell, L. H., and T. A. Weinert.** 1989. Checkpoints: controls that ensure the order of cell cycle events. Science **246:**629–633.
34. **Haupt, Y., S. Rowan, E. Shaulian, K. H. Vousden, and M. Oren.** 1995. Induction of apoptosis in HeLa cells by transactivation-deficient p53. Genes Dev. **9:**2170–2183.
35. **Johnson, G. D., R. S. Davidson, K. C. McNamee, D. G. Russell, and E. J. Holborow.** 1982. Fading of immunofluorescence during microscopy: a study of the phenomenon and its remedy. J. Immunol. Methods **55:**231–242.
36. **Kane, D. J., T. A. Sarafin, R. Anton, H. Hahn, E. Butler-Gralla, V. J. Selverstone, T. Ord, and D. E. Bredesen.** 1993. Bcl-2 inhibition of neural death: decreased generation of reactive oxygen species. Science **262:**1274–1277.
37. **Kastan, M. B., O. Onyekwere, D. Sidransky, B. Vogelstein, and R. W. Craig.** 1991. Participation of p53 in the cellular response to DNA damage. Cancer Res. **51:**6304–6311.
38. **Korsmeyer, S. J., J. R. Shutter, D. J. Veis, D. E. Merry, and Z. N. Oltvai.** 1993. Bcl-2/bax: a rheostat that regulates an antioxidant pathway and cell death. Semin. Cancer Biol. **4:**327–332.

39. **Krajewski, S., J. K. Mai, M. Krajewska, M. Sikorska, M. J. Mossakowski, and J. C. Reed.** 1995. Upregulation of bax protein levels in neurons following cerebral ischemia. J. Neurosci. **15:**6364–6376.
40. **Lane, D. P.** 1992. Worrying about p53. Curr. Biol. **2:**581–583.
41. **Lehrach, H., D. Diomond, J. M. Wosney, and H. Boedtker.** 1977. RNA molecular weight determination by gel electrophoresis under denaturing conditions: a critical reexamination. Biochemistry **16:**4743–4751.
42. **Livingstone, L. R., A. White, J. Sprouse, E. Livanson, T. Jacks, and T. D. Tlsty.** 1992. Altered cell cycle arrest and gene amplification potential accompany loss of wild-type p53. Cell **70:**923–935.
43. **Louis, J.-C., E. Magal, S. Takayama, and S. Varon.** 1993. CNTF protection of oligodendrocytes against natural and tumor necrosis factor-induced death. Science **259:**689–692.
44. **Mah, S. P., L. T. Zhong, Y. Liu, A. Roghani, R. H. Edwards, and D. E. Bredesen.** 1993. The protooncogene bcl-2 inhibits apoptosis in PC12 cells. J. Neurochem. **60:**1183–1186.
45. **Maltzman, W., M. Oren, and A. J. Levine.** 1981. The structural relationships between 54000-molecular weight cellular tumor antigens detected in viral- and nonviral-transformed cells. Virology **112:**145–156.
46. **Martínez, J., I. Georgoff, J. Martínez, and A. J. Levine.** 1991. Cellular localization and cell cycle regulation by a temperature sensitive-mutant p53 protein. Genes Dev. **5:**151–159.
47. **McCarthy, K. D., and J. J. deVellis.** 1980. Preparation of separate astroglial and oligodendroglial cell cultures from rat cerebral tissue. J. Cell Biol. **85:**890–902.
48. **McKinnon, R. D., T. Matsui, M. Dubois-Dalcq, and S. A. Aaronson.** 1990. FGF modulates the PDGF-driven pathway of oligodendrocyte development. Neuron **5:**603–614.
49. **Mercer, W. E., M. Amin, G. J. Sauve, E. Appella, S. J. Ullrich, and J. Romano.** 1990. Wild-type p53 is antiproliferative in SV40-transformed hamster cells. Oncogene **5:**973–980.
50. **Mercer, W. E., M. T. Shields, M. Amin, G. J. Sauve, E. Appella, and S. J. Ullrich.** 1990. Negative growth regulation in a glioblastoma tumor cell line that conditionally expresses human wild-type p53. Proc. Natl. Acad. Sci. USA **87:**6166–6170.
51. **Merry, D. E., D. J. Veis, W. F. Hickey, and S. J. Korsmeyer.** 1994. Bcl-2 protein expression is widespread in the developing nervous system and retained in the adult PNS. Development **120:**301–311.
52. **Michalovitz, D., O. Halevy, and M. Oren.** 1990. Conditional inhibition of transformation and of cell proliferation by a temperature-sensitive mutant of p53. Cell **62:**671–680.
53. **Misantone, L. J., M. Gershenbaum, and M. Murray.** 1984. Viability of retinal ganglion cells after optic nerve crush in rats. J. Neurocytol. **13:**449–465.
54. **Miyashita, T., S. Krajewski, M. Krajewska, H. G. Wang, H. K. Lin, D. A. Liebermann, B. Hoffman, and J. C. Reed.** 1994. Tumor suppressor p53 is a regulator of bcl-2 and bax in gene expression in vitro and in vivo. Oncogene **9:**1799–1805.
55. **Miyashita, T., and J. C. Reed.** 1995. Tumor suppressor p53 is a direct transcriptional activator of the human bax gene. Cell **80:**293–299.
56. **Moll, U. M., M. LaQuaglia, J. Benard, and G. Riou.** 1995. Wild-type p53 protein undergoes cytoplasmic sequestration in undifferentiated neuroblastomas but not in differentiated tumors. Proc. Natl. Acad. Sci. USA **92:**4407–4411.
57. **Moll, U. M., G. Riou, and A. J. Levine.** 1992. Two distinct mechanisms alter p53 in breast cancer: mutation and nuclear exclusion. Proc. Natl. Acad. Sci. USA **89:**7262–7266.
58. **Noble, M., K. Murray, P. Strobant, M. D. Waterfield, and P. Riddle.** 1988. Platelet-derived growth factor promotes division and motility and inhibits premature differentiation of the oligodendrocyte/type-2 astrocyte progenitor cell. Nature (London) **333:**560–562.
59. **Oltvai, Z. N., C. L. Milliman, and S. J. Korsmeyer.** 1993. Bcl-2 heterodimerizes in vivo with a conserved homolog, bax, that accelerates programmed cell death. Cell **74:**609–619.
60. **Oppenhaim, R. W.** 1981. Neuronal cell death and some related regressive phenomena during neurogenesis: a selective historical review and progress report, p. 74–133. *In* W. M. Cowan (ed.), Studies in developmental neurobiology: essays in honor of Victor Hamburger. Oxford University Press, New York.
61. **Oppenhaim, R. W.** 1991. Cell death during development of the nervous system. Annu. Rev. Neurosci. **14:**453–501.
62. **Quigley, H. A., R. W. Nickells, L. A. Kerrigan, M. E. Pease, D. J. Thibault, and D. J. Zack.** 1995. Retinal ganglion cell death in experimental glaucoma and after axotomy occurs by apoptosis. Invest. Ophthalmol. Visual Sci. **36:** 774–786.
63. **Raff, M. C., B. A. Barres, J. F. Burne, H. S. Coles, Y. Ishizaki, and M. D. Jacobson.** 1993. Programmed cell death and the control of cell survival: lessons from the nervous system. Science **262:**695–700.
64. **Raff, M. C., R. H. Miller, and M. Noble.** 1983. A glial progenitor cell that develops in vitro into an astrocyte or an oligodendrocyte depending on culture medium. Nature (London) **303:**390–396.
65. **Reed, M., Y. Wang, G. Mayr, M. E. Anderson, J. F. Schwedes, and P. Tegtmeyer.** 1993. p53 domains: suppression, transformation, and transactivation. Gene Expr. **3:**95–107.
66. **Richardson, W. D., N. Pringle, M. J. Mosley, B. Watermark, and M. Dubois-Dalcq.** 1988. A role for platelet-derived growth factor in normal gliogenesis in the central nervous system. Cell **53:**309–319.
67. **Rodríguez, M., B. W. Scheithauer, G. Forbes, and P. J. Kelly.** 1993. Oligodendrocyte injury is an early event in lesions of multiple sclerosis. Mayo Clin. Proc. **68:**627–636.
68. **Sah, V. P., L. D. Attardi, G. J. Mulligan, B. O. Williams, R. T. Bronson, and T. Jacks.** 1995. A subset of p53-deficient embryos exhibit exencephaly. Nat. Genet. **19:**175–180.
69. **Sakhi, S., A. Bruce, N. Sun, G. Tocco, M. Baudry, and S. S. Schreiber.** 1994. p53 induction is associated with neuronal damage in the central nervous system. Proc. Natl. Acad. Sci. USA **91:**7525–7529.
70. **Selvakumaran, M., H.-K. Lin, T. Miyashita, H. G. Wang, S. Krajewski, J. C. Reed, B. Hoffman, and D. Liebermann.** 1994. Immediate early up-regulation of bax expression by p53 but not TGF-β1: a paradigm for distinct apoptotic pathways. Oncogene **9:**1791–1798.
71. **Shaulian, E., A. Zauberman, D. Ginsberg, and M. Oren.** 1992. Identification of a minimal transforming domain of p53: negative dominance through abrogation of sequence-specific DNA binding. Mol. Cell. Biol. **12:**5581–5592.
72. **Shaulsky, G., A. Ben-Ze'ev, and V. Rotter.** 1990. Subcellular distribution of the p53 protein during the cell cycle of Balb/c 3T3 cells. Oncogene **5:**1707–1711.
73. **Shaulsky, G., N. Goldfinger, A. Ben-Ze'ev, and V. Rotter.** 1990. Nuclear accumulation of p53 protein is mediated by several nuclear localization signals and plays a role in tumorigenesis. Mol. Cell. Biol. **10:**6565–6577.
74. **Shaulsky, G., N. Goldfinger, A. Peled, and V. Rotter.** 1991. Involvement of wild-type p53 in pre-B-cell differentiation in vitro. Proc. Natl. Acad. Sci. USA **88:**8982–8986.
75. **Shaulsky, G., N. Goldfinger, M. S. Tosky, A. J. Levine, and V. Rotter.** 1991. Nuclear localization is essential for the activity of p53 protein. Oncogene **6:**2055–2065.
76. **Shaw, P., R. Bovey, S. Tardy, R. Sahli, B. Sordat, and J. Costa.** 1992. Induction of apoptosis by wild-type p53 in a human colon tumor-derived cell line. Proc. Natl. Acad. Sci. USA **89:**4495–4499.
77. **Temple, S., and M. C. Raff.** 1986. Clonal analysis of oligodendrocyte development in culture: evidence for a developmental clock that counts cell divisions. Cell **44:**773–779.
78. **Vantini, G., and S. D. Skaper.** 1992. Neurotrophic factors: from physiology to pharmacology? Pharmacol. Res. **26:**1–15.
79. **Wagner, A. J., J. M. Kokontis, and N. Hay.** 1994. Myc-mediated apoptosis requires wild-type p53 in a manner independent of cell cycle arrest and the ability of p53 to induce $p21^{waf1/cip1}$. Genes Dev. **8:**2817–2830.
80. **Yonish-Rouach, E., D. Ginsberg, and M. Oren.** 1993. The role of tumor suppressor genes in apoptosis, p. 187–202. *In* M. Lavin and D. Watters (ed.), Programmed cell death. Harwood, Chur, Switzerland.
81. **Yonish-Rouach, E., D. Resnitzky, J. Lotem, L. Sachs, A. Kimchi, and M. Oren.** 1991. Wild-type p53 induces apoptosis of myeloid leukemic cells that is inhibited by interleukin-6. Nature (London) **352:**345–347.
82. **Zhong, L.-T., T. Sarafian, D. J. Kane, A. C. Charles, S. P. Mah, R. H. Edwards, and D. E. Bredesen.** 1993. Bcl-2 inhibits death of central neural cells induced by multiple agents. Proc. Natl. Acad. Sci. USA **90:**4533–4537.