Peg3/Pw1 Is a Mediator between p53 and Bax in DNA Damage-induced Neuronal Death*

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Neuronal cell death after DNA damage requires p53 and Bax, but the mechanism by which p53 activation leads to Bax translocation and cell death in neurons is not known. We report here that Peg3/Pw1 is up-regulated after DNA damage in cortical neurons in a p53-dependent manner. Overexpression of Peg3/Pw1 leads to decreased neuronal viability. The deleterious effect of Peg3/Pw1 on neuronal survival is abrogated by deletion of either p53 or Bax, indicating an essential role for both in Peg3/Pw1-mediated neuronal death. Moreover, overexpression of a Peg3/Pw1 dominant negative protein inhibits Bax translocation and neuronal cell death after DNA damage. These findings implicate Peg3/Pw1 as a mediator between p53 and Bax in a neuronal cell death pathway activated by DNA damage. Previous observations in our laboratory have demonstrated that p53 and Bax are essential components of the cell death pathway activated in neurons after DNA damage due to exposure to ionizing radiation or the topoisomerase I inhibitor, camptothecin (1–3). We and others have also obtained evidence for a downstream role for caspase activation in this cell death pathway under some, but not all circumstances (4–6). Numerous other agents that are released from mitochondria as a result of Bax translocation have been identified as potential mediators of caspase-independent cell death pathways (7), although their role in neuronal cell death is poorly understood. Moreover, the mechanism by which p53 activation leads to Bax translocation is not known. Although Bax expression can be up-regulated at the transcriptional level by p53, we and others have shown that DNA damaging agents result in Bax translocation without causing a significant increase in the levels of Bax protein in cultured postnatal cortical neurons (2, 3, 8). Thus, additional mechanisms must exist that link p53 activation to Bax translocation. Identification of molecules that serve as intermediaries between p53 and Bax would increase our understanding of neuronal death mechanisms and possibly provide new therapeutic targets.

One such candidate molecule is Peg3/Pw1, a protein that was originally identified as being involved in the development of the myogenic and neuronal lineages (9). Peg3/Pw1 is thought to be a multifunctional protein with primarily nuclear localization (9, 10). The protein contains 12 putative Kruppel-type zinc finger DNA-binding domains, an RER protein interaction domain, and two proline-rich repeat domains, suggesting that Peg3/Pw1 may act as a transcription factor (9, 11). The Peg3/Pw1 gene is an imprinted gene that is expressed ubiquitously from the paternal allele in embryos, and mRNA hybridization studies in mouse and human tissues suggest that it is expressed at highest levels in the ovary, placenta, testis, and brain (9, 11–13). Multiple isoforms with differential localization have been identified in humans, although only a single isoform has been reported in mice (14). Peg3/Pw1 is highly conserved between mouse and human, suggesting an important and conserved function throughout evolution (12). Disruption of the paternal allele of mouse Peg3/Pw1 results in behavioral abnormalities, growth retardation, and alterations in the number of oxytocin neurons in the hypothalamus (15).

More recently, Peg3/Pw1 was identified as a gene product that is specifically expressed in fibroblasts undergoing p53-mediated apoptosis when compared with cells undergoing p53-mediated growth arrest (16, 17). Evidence suggests that it may cooperate with another p53-regulated gene product, Siah1, to mediate Bax translocation and cell death in a fibroblast cell line (16). In neurons, Peg3/Pw1 expression is up-regulated in vivo and in vitro by hypoxia (18). This observation is of interest because of evidence suggesting that neuronal death occurring after hypoxic insults is p53-dependent (19, 20).

Given the putative role of Peg3/Pw1 in p53-mediated apoptosis in fibroblasts and neurons, we have chosen to examine its role in p53-mediated neuronal cell death occurring after DNA damage. We report here that Peg3/Pw1 is up-regulated after DNA damage in neurons in a p53-dependent manner. Enforced overexpression of Peg3/Pw1 protein leads to decreased neuronal viability, an effect that is abrogated by deletion of either the p53 or Bax genes. Moreover, overexpression of a Peg3/Pw1 dominant negative protein (Peg3-DN) containing a truncated C terminus inhibits both Bax translocation and neuronal death occurring after DNA damage. These findings suggest that Peg3/Pw1 acts as a mediator between p53 and Bax in a neuronal death pathway that is activated by DNA damage.

EXPERIMENTAL PROCEDURES

Animals and Cell Culture—Primary neuronal cell cultures derived from wild-type, p53-deficient, or Bax-deficient animals were used for this study. p53 wild-type and knockout mice on a C57Bl/6 X 129 Sv background were generated as previously described (21). Bax knockout mice were generated from a 129/Sv x C3H background as previously described (22). The genotypes of the mating pairs and all offspring were determined by PCR using DNA extracted from the tail as previously described (23, 24). Primary neuronal cultures were established from newborn mouse cortex as described previously (1, 2, 4). Briefly, cortical tissue was excised, trypsinized, and dissociated by trituration to obtain single cells. Cells were then plated onto poly-d-lysine-coated culture-
FIG. 1. Peg3/Pw1 protein is increased by DNA damage. A, phase-contrast (A1, B1, and C1) and fluorescence (A2, B2, and C2) micrographs of primary cortical neurons under control conditions or after exposure to a topoisomerase 1 inhibitor (camptothecin, 2.5 μM) or glutamate (50 μM). Primary cortical neurons were obtained from newborn wild-type mice and maintained in dissociated cell culture for 4 days in a neurobasal medium with B27 supplements. The cells were then exposed to camptothecin (2.5 μM), glutamate (50 μM), or vehicle control (Me2SO). After 18 h, the cells were washed in phosphate-buffered saline, fixed in 4% paraformaldehyde for 30 min, and stained for Peg3/Pw1 immunoreactivity using a mouse monoclonal anti-Peg3/Pw1 antibody as described under “Experimental Procedures.” A significant increase in Peg3/Pw1 immunoreactivity was noted after exposure to camptothecin, but not glutamate. B, wild-type primary mouse cortical neurons were exposed and maintained in a serum-free medium as described. After 4 days, the cultures were treated for 6 h with camptothecin (CPT, 2.5 μM) or with vehicle control (Me2SO). The cells were then harvested by scraping and the protein analyzed by Western blot as described under “Experimental Procedures.” Western blot analysis confirmed a 2-fold increase in Peg3/Pw1 protein in wild-type neurons after a 6-h exposure to camptothecin. Western blot analyses at later time points revealed a persistent and progressive accumulation of Peg3/Pw1 protein with continued exposure to camptothecin (see Fig. 4A).

2 The abbreviations used are: PBS, phosphate-buffered saline; GFP, green fluorescent protein; EGFP, enhanced green fluorescent protein.

3 X. Wu, unpublished data.
camptothecin paralleled a similar differential induction of Bax protein and caspase activity caused by these agents in cultured postnatal cortical neurons (2, 4).

The induction of Peg3/Pw1 protein was confirmed by Western blot analysis (Fig. 1B). Primary cortical neurons were treated with camptothecin (2.5 μM) for 6 h, and protein extracts were then collected and analyzed by Western blot as described. A 2-fold increase in Peg3/Pw1 protein was observed 6 h after camptothecin exposure. This increase was persistent, and was also observed after 18 h of camptothecin exposure (see Fig. 3A). Thus, both immunocytochemical and Western blot analysis indicated that Peg3/Pw1 protein levels were increased in primary cortical neurons after DNA damage.

**Increased Expression of Peg3/Pw1 Decreases Neuronal Viability**—To determine the effect of increased Peg3/Pw1 expression on neuronal viability, primary mouse cortical neurons were transfected with a vector containing Peg3/Pw1 fused to green fluorescent protein (pEGFP-Peg3/Pw1) in a transient transfection assay. Control cells were transfected with a vector containing only pEGFP. After 72 h, the cultures were examined for the presence of fluorescent cells expressing either the Peg3/Pw1 fusion protein or the GFP control protein. Approximately 5% of the neurons were noted to express GFP (as indicated by the presence of green fluorescence) in control cultures. The EGFP-transfected cells showed the extensive dendritic and axonal outgrowth typical of mouse cortical neurons cultured under these conditions (Fig. 2A). In contrast, most neurons overexpressing the Peg3/Pw1-GFP fusion protein displayed a small, rounded morphology with short, abortive neuritic processes. Co-staining of fixed neurons with Hoechst dye to identify the nucleus confirmed that the overexpressed Peg3/Pw1-GFP protein was primarily nuclear in its location (data not shown), although some expression in the cytoplasm was observed. When measured at either 24 or 72 h, the mean number of neurons per high power field expressing the Peg3/Pw1-GFP fusion protein was significantly less than the mean number of neurons expressing either GFP or a COOH terminus truncated Peg3/Pw1 dominant negative protein (0.6 versus 7.0 versus 6.9, p < 0.003, after 72 h, paired t test). More importantly, the percent decline in the mean number of transfected neurons observed between 24 and 72 h was greater in the EGFP-Peg3/Pw1-transfected cultures (75%) than in the control EGFP-transfected cultures (11%) or the Peg3/Pw1-DN-transfected cultures (13%). Thus, these findings indicated that overexpression of Peg3/Pw1 in primary mouse cortical neurons resulted in a progressive decline in neuronal viability.

**Overexpression of a Peg3/Pw1 Dominant Negative Protein Inhibits Neuronal Death**—To determine whether Peg3/Pw1 is required for execution of the neuronal death pathway activated by DNA damage, wild-type neurons were transfected with a Peg3/Pw1-DN vector (amino acids 1–592) which encodes a COOH terminus truncated Peg3/Pw1 protein (28). This protein lacks 8 of the 12 zinc finger domains and both of the His-Pro repeat domains, but retains the RER protein-interaction domain. This Peg3/Pw1-DN protein has been previously shown to inhibit Peg3/Pw1-mediated NF-κB activation, Peg3/Pw1-mediated apoptosis, and Peg3/Pw1-mediated Bax translocation in fibroblasts (16, 17, 28). The EGFP vector was co-transfected with the Peg3/Pw1-DN vector to facilitate the identification of transfected cells. Control cultures were transfected with the EGFP vector alone. Approximately 24 h after transfection, the cultures were exposed to camptothecin (2.5 μM) for an additional 18 h. Transfected cells were then examined under fluorescence and counted to assess overall survival. In some cases, transfected neurons were fixed and counterstained with Hoechst dye to identify apoptotic nuclei with condensed chromatin. As shown in Fig. 3A, transfected neurons expressing the GFP control vector showed marked somatic disruption and neurite degeneration after camptothecin exposure, characteristic of neurons undergoing DNA damage-induced cell death. In contrast, most neurons expressing the Peg3/Pw1-DN protein and exposed to camptothecin retained an intact soma and ex-
Fig. 3. Inhibition of Peg3/Pw1 function increases neuronal survival after DNA damage. A, primary wild-type cortical neurons were transduced with either a Peg3/Pw1 dominant negative mutant vector (Peg3-DN) or with an EGFP control vector. After 24 h, the cells were exposed to either camptothecin (2.5 μM) or vehicle control for 18 h. As shown in A, viable neurons with intact somata and healthy neurites were seen after camptothecin exposure (2.5 μM) in cultures transfected with the Peg3/Pw1-DN vector when compared with cultures transfected with the EGFP vector. B, primary wild-type cortical neurons were transduced with either a Peg3/Pw1 dominant negative mutant vector (Peg3-DN) or with an EGFP control vector. After 24 h, the cells were exposed to either camptothecin (2.5 μM) or vehicle control for 18 h. The cultures were counterstained with Hoechst 3342 dye and viewed under epifluorescence to identify apoptotic cells exhibiting condensed and fragmented nuclei. The total number of apoptotic and non-apoptotic transfected neurons per high-power field was determined and expressed as a percentage. The experiment was performed in quadruplicate. As discussed in the text, neurons expressing GFP and exposed to camptothecin showed a significant decrease in survival when compared with untreated EGFP-expressing neurons (p < 0.01, paired t test). In contrast, neurons expressing the dominant negative form of Peg3/Pw1 failed to show a significant decline in neuronal viability after 18 h of camptothecin exposure when compared with untreated, Peg3/Pw1-DN-expressing neurons (p > 0.84, paired t test). The percentage of viable neurons expressing Peg3/Pw1-DN protein was significantly greater than the percentage of viable GFP-expressing neurons after camptothecin exposure (p = 0.05, paired t test).

Fig. 4. p53 is necessary but not sufficient for Peg3/Pw1-mediated neuronal death. A, primary cortical neurons derived from wild-type (+/+) or p53-deficient (−/−) mice were dissociated and maintained in a serum-free medium as described. After 4 days, the cultures were treated for 18 h with camptothecin (Campto, 2.5 μM) or with vehicle control (CONTROL). The cells were then harvested by scraping and the protein analyzed by Western blot as described under “Experimental Procedures.” Western blot analysis revealed a significant increase in Peg3/Pw1 protein in wild-type neurons, but not in p53-deficient neurons exposed to camptothecin. B, primary cortical neurons were transduced with an adenovirus containing either the human p53 gene (Ad-p53) or the LacZ β-galactosidase gene (Ad-βgal) at a multiplicity of infection of 125. After −60 h, the cultures were fixed in 4% paraformaldehyde and stained for Peg3/Pw1 immunocytochemistry. Phase-contrast (top row) and fluorescence (bottom row) images are shown. No significant increase in Peg3/Pw1 immunoreactivity was observed. C, dissociated primary cortical neurons derived from wild-type (WT) or p53-deficient (p53−/−) postnatal mice were transduced with a pEGFP-Peg3/Pw1 vector, and subsequently viewed under epifluorescence after 24 or 48 h. The mean number of fluorescent neurons per high power field (×20 objective) was assessed for each condition. Data shown are derived from two separate experiments, and are represented as the mean ± S.E. Each condition was performed in triplicate. A significant decrease in the number of wild-type neurons expressing Peg3/Pw1-GFP was noted between 24 and 48 h (p < 0.05, paired t test), in contrast to p53-deficient neurons expressing Peg3/Pw1-LacZ, which failed to show any decrease in neuronal survival over the same period (p > 0.12, paired t test).

Thus, inhibition of Peg3/Pw1 activity by overexpression of the Peg3/Pw1-DN protein significantly inhibited neuronal death after DNA damage.

Peg3/Pw1-Mediated Neuronal Death Requires a Functional p53 Protein—We have previously shown that neuronal death after DNA damage requires the presence of a functional p53 protein (1–3). To determine whether the observed increase in Peg3/Pw1 expression after DNA damage was p53-dependent, primary cortical neurons derived from wild-type or p53-deficient mice were exposed to camptothecin for 18 h. The protein was then collected and analyzed by Western blot for Peg3/Pw1 expression. Protein extracts from control (vehicle treated) neurons were also analyzed. As shown in Fig. 4A, Peg3/Pw1 expression was up-regulated in wild-type, but not in p53-deficient neurons exposed to camptothecin. Thus, a functional p53 protein is required for Peg3/Pw1 induction after DNA damage in neurons.

Studies in fibroblasts containing a temperature-sensitive mutant form of p53 indicated that p53 expression alone was insufficient to induce Peg3/Pw1 expression (16, 17). To determine whether this was also true in neurons, we transduced...
The experiment was performed in quadruplicate. Under control conditions, neurons displayed a diffuse, cytoplasmic distribution of the Bax-GFP fusion protein. Camptothecin exposure induced a redistribution of Bax-GFP into a punctate pattern, indicating translocation of Bax from the cytoplasm to mitochondria, as previously described (8, 41). In contrast, most neurons expressing both Bax-GFP and Peg3/Pw1-DN failed to show any evidence of Bax translocation. As shown in Fig. 5A, the Bax-GFP fusion protein was diffusely distributed in wild-type neurons at both time points. A statistically significant decline in the number of transfected neurons per high powered field was observed when Peg3/Pw1 expression was increased by other means. Although p53 was required for Peg3/Pw1 induction in neurons after DNA damage, we wondered whether enforced overexpression of Peg3/Pw1 could nevertheless mediate neuronal death in the absence of p53. To investigate this possibility, primary cortical neurons derived from wild-type or p53-deficient mice were transfected with the EGFP-Peg3/Pw1 vector, and the cultures were then examined after either 24 or 48 h to determine the number of transfected neurons that survived. As shown in Fig. 4C, Peg3/Pw1 overexpression significantly decreased neuronal survival in wild-type, but not in Bax-deficient neurons (p < 0.05, paired t test). Thus, a functional Bax protein is required for Peg3/Pw1-mediated neuronal death.

To determine whether Peg3/Pw1 activity mediates Bax activation and translocation after DNA damage in neurons, we co-transfected wild-type neurons with a vector containing a truncated (amino acids 1–592) Peg3/Pw1-DN protein and with a Bax-GFP vector. The neurons were then exposed to camptothecin for 18 h, and the cells were examined to determine the cytoplasmic distribution of the Bax-GFP protein. As shown in Fig. 5B, the Bax-GFP fusion protein was diffusely distributed throughout the neuronal cytoplasm under control conditions. After camptothecin exposure, a redistribution of Bax-GFP into a punctate distribution was observed, indicating translocation of Bax from the cytoplasm to mitochondria, in agreement with observations in fibroblasts (42). These data are consistent with observations in fibroblasts indicating that up-regulation of p53 alone is insufficient to induce Peg3/Pw1 expression (16). Taken together, these findings indicate that p53 is necessary, but not sufficient for Peg3/Pw1 induction after DNA damage in neurons.

DISCUSSION

A Role For Peg3/Pw1 in p53-dependent Neuronal Death after Injury—In previous studies (1–3, 8), we and others have obtained experimental evidence that p53 and Bax are essential components of a neuronal cell death pathway that is activated by DNA damage. Among the downstream effectors of this pathway are caspases, although the importance of these cysteine aspartases to neuronal death after DNA damage may vary depending on the stage of neuronal maturation (4, 5). Other reports indicate that Bax translocation is an important step in the genesis of mitochondrial dysfunction and programmed cell death under numerous circumstances (29, 30). Indeed, camptothecin-induced DNA damage leads to Bax translocation in primary cultured cortical neurons (8). This finding is of interest, given the reported role of Peg3/Pw1 in mediating Bax translocation in cultured fibroblasts undergoing p53-dependent apoptosis. To examine the role of Bax in Peg3/Pw1-dependent neuronal cell death in neurons, primary cortical neurons derived from wild-type or Bax-deficient mice were transfected with the Peg3/Pw1 vector and subsequently examined to determine the number of transfected neurons that survived. As shown in Fig. 5A, Peg3/Pw1 overexpression significantly decreased neuronal survival in wild-type, but not in Bax-deficient neurons (p < 0.05, paired t test). Thus, a functional Bax protein is required for Peg3/Pw1-mediated neuronal death.
The Relationship between p53 and Peg3/Pw1—Although DNA damage-induced up-regulation of Peg3/Pw1 protein was critically dependent upon the presence of p53, overexpression of p53 alone using adenovirus-mediated transduction was insufficient to up-regulate Peg3/Pw1 immunoreactivity in cultured primary cortical neurons. The finding that increased p53 expression is necessary, but not sufficient to induce Peg3/Pw1 protein levels is similar to that observed in fibroblasts containing a temperature-sensitive mutant form of p53 (16), and suggests that cofactors, in addition to p53, are necessary for Peg3/Pw1 up-regulation. This result differs from that obtained by Yamaguchi et al. (18), however, who found a significant up-regulation of Peg3/Pw1 mRNA in SK-N-SH neuroblastoma cells transduced with a p5 adenovirus. The reasons for this discrepancy are unclear, although one possible explanation is that Yamaguchi et al. (18) assayed Peg3/Pw1 mRNA levels, in contrast to the immunocytochemical analysis of Peg3/Pw1 protein expression performed in the current study. Several reports indicate that changes in mRNA levels do not always correspond to changes in protein expression (33). Another explanation for this discrepancy may lie in the fact that Yamaguchi et al. (18) used a neuroblastoma cell line for the adenovirus experiments, in contrast to the primary cortical neurons used in the present study. Using a different neuroblastoma cell line (SH-SY5Y), we have observed differences between neuroblastoma cells and primary neurons in the sensitivity to camptothecin and in the ability of caspase inhibitors to promote survival after DNA damage (4), indicating subtle differences in the p53-dependent cell death pathways activated by DNA damage in neuroblastoma cell lines when compared with primary neurons. In support of this hypothesis, it is notable that Yamaguchi et al. (18) also reported a concomitant increase in Bax mRNA levels in SK-N-SH cells after p53 overexpression. This contrasts with findings from our laboratory and others indicating no change in Bax protein levels in primary neurons after adenovirus-mediated overexpression of p53 or after DNA damage (2, 3, 8). Further studies are clearly needed to determine the factors governing the p53-dependent up-regulation of Peg3/Pw1 in neurons after injury.

The finding that p53-deficient neurons are resistant to cell death caused by enforced overexpression of Peg3/Pw1 suggests that Peg3/Pw1 cooperates with other p53-regulated factors to effect cell death. The pro-apoptotic protein, Bax, is clearly one such factor. The Bax gene contains a p53 consensus binding site in its promoter, and p53 can directly increase Bax transcription (31). Bax protein levels are unchanged in primary neurons after DNA damage, although the p53-dependent cell death pathway has an obligate requirement for Bax in order for it to be executed (2, 3, 8). Because Bax protein is present at significant levels in p53-deficient cortical neurons (2), there must be yet additional p53-regulated factors that are required for Peg3/Pw1 to mediate cell death.

One possible such cofactor is Siah1a (seven in absentia homolog 1), another p53-induced gene product. Siah1 is differentially induced by p53 in a leukemic cell line undergoing growth arrest or apoptosis (34), or by genotoxic stress (35). Interestingly, several members of the Siah1 family have been shown to have ubiquitin targeting activity (36). Other studies suggest that p53 and Siah1 share a common mechanism of tumor suppression and induction of apoptosis that involves protein folding, unfolding, and trafficking (37). Siah1a has previously been shown to bind directly to Peg3/Pw1 by yeast two-hybrid assays and by direct co-immunoprecipitation experiments in a fibroblast cell line (16). Whereas overexpression of Peg3/Pw1 in the absence of p53 does not induce apoptosis in fibroblasts (a finding that parallels our observations in neurons), co-trans-

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3 M. D. Johnson, X. Wu, N. Aithmitti, and R. S. Morrison, unpublished observations.
fection of both Peg3/Pw1 and Siah1 indicates apoptosis in fibroblasts in the absence of a functional p53 protein (16). It will be of interest to determine whether Siah1 also plays a role in neuronal death occurring after DNA damage.

Peg3/Pw1 has also been shown to bind to TRAF2, another RER domain protein that is believed to regulate NF-κB activation through interactions with the tumor necrosis factor superfamily of death receptors. However, the significance of the Peg3/Pw1-NF-κB interaction to cell survival is unclear, since cells derived from mice lacking Peg3/Pw1 show normal NF-κB activation and apoptosis in response to tumor necrosis factor (38).

Fortin et al. (6) have reported that DNA damage in embryonic cortical neurons leads to the p53-dependent up-regulation of APAF-1 expression. APAF-1 is a component of the apoptosis, and cooperates with cytochrome c and ATP to activate caspases (39). It is unlikely that APAF-1 is the p53-regulated factor required for Peg3/Pw1-mediated Bax activation in the paradigm used in the current study, however, since APAF-1-dependent caspase activation generally occurs downstream of Bax translocation (39). Moreover, we and others have shown that postnatal primary cortical neurons such as those used in this study die by a p53-dependent, Bax-dependent, but caspase-independent mechanism (4, 5).

The Relationship between Peg3/Pw1 and Bax—Previous studies in fibroblasts indicated that Peg3/Pw1 overexpression induced Bax translocation, and that the use of antisense mRNA to block Peg3/Pw1 protein expression inhibited such translocation during p53-mediated apoptosis (17). However, it was unclear from those studies whether there was an obligate requirement for Bax in Peg3/Pw1-induced cell death. In the present study, we report that Bax is required for Peg3/Pw1-dependent cell death. Neurons derived from Bax-deficient mice failed to show a decline in viability after enforced overexpression of Peg3/Pw1. We have also found that inhibition of Peg3/Pw1 activity using a truncated form of the Peg3/Pw1 protein inhibits both Bax translocation and neuronal death after DNA damage. This latter finding parallels the results using antisense mRNA obtained previously in fibroblasts (17), and using a dominant negative Peg3/Pw1 protein in SK-N-SH cells exposed to hypoxia (18). Taken together, these data indicate that Peg3/Pw1 and Bax cooperate to bring about neuronal death after DNA damage.

The mechanism by which Peg3/Pw1 causes Bax translocation is unclear. The Peg3/Pw1 protein contains 12 zinc finger domains and an RER protein interaction domain (9). This domain structure, combined with the fact that localization of Peg3/Pw1 is primarily nuclear, suggests that this protein may affect gene transcription. However, it seems unlikely that a direct effect on transcription of the Bax gene is involved in Peg3/Pw1-dependent cell death, given that there is no change in Bax protein levels in cultured postnatal cortical neurons after camptothecin exposure. However, the possibility remains that Peg3/Pw1 may regulate the transcription of other genes that are important for neuronal viability. In this context, it is of interest that another set of nuclear proteins, the cyclin dependent kinases (cdk’s), have been shown to play an important role in neuronal death occurring after DNA damage. Inhibition of cdk’s using cdk inhibitors delays DNA damage-induced neuronal cell death (8, 40). During development, the maximal expression of Peg3/Pw1 in developing myo blastas is cell cycle specific, occurring primarily in late M phase (9). The cdk’s, which are nuclear proteins critical for cell cycle control, would thus be potential candidates for cooperating with Peg3/Pw1 in mediating neuronal cell death.

It is also possible that direct protein/protein interactions in nuclear or extranuclear compartments could be involved in the mechanism of Peg3/Pw1-induced cell death. As mentioned above, potential interactions of Peg3/Pw1 with Siah1, TRAF2, or other as yet unidentified proteins may be involved. Additional studies are needed to elucidate the mechanism by which increased Peg3/Pw1 expression leads to Bax translocation.

A Model for Neuronal Death after DNA Damage—Taken together, these findings detail a striking interdependence of Peg3/Pw1, Bax, and p53 in neuronal death after DNA damage. We propose a model (Fig. 6) in which DNA damage leads to p53 activation, followed by increased expression of Peg3/Pw1 and a yet to be identified cofactor (e.g. Siah1, TRAF2, cdk’s, etc). Peg3/Pw1 would then cooperate with this factor(s) to cause Bax translocation to mitochondria, resulting in mitochondrial dysfunction, a decline in intracellular ATP levels, cytochrome c release, and caspase activation. Based on previous studies, we believe that Bax translocation also activates a caspase-independent cell death pathway that involves other factors (e.g. p53AIP, endonuclease G, etc.) that remain to be specifically identified as having a role in p53-dependent neuronal cell death. Studies are currently underway to elucidate additional details of p53-dependent neuronal cell death pathways after injury.

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