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Upregulation of Daxx Mediates Apoptosis in Response to Oxidative Stress

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Abstract Oxidative stress induces apoptosis in a variety of cell types by as yet unclear signaling mechanisms. The Daxx protein is reportedly involved in apoptosis through its interactions with Fas, transforming growth factor-β receptor, and promyelocytic leukemia protein (PML). Here, we explored the possible roles of Daxx in oxidative stress-induced apoptosis. We found that both the mRNA and protein levels of Daxx markedly increased when cells underwent apoptosis after H_2O_2 treatment. Pretreatment with the cell-permeable antioxidant, N-acetyl cysteine, prevented cells from H_2O_2-induced Daxx upregulation and subsequent apoptosis, indicating that the endogenous oxidant regulated Daxx expression. Furthermore, suppression of endogenous Daxx expression by antisense oligonucleotide technology inhibited oxidative stress-induced apoptosis in HeLa cells. Taken together, these results suggest that Daxx acts as an intermediary messenger of pro-apoptotic signals triggered by oxidative stress. J. Cell. Biochem. 96: 330–338, 2005.

Key words: Daxx; apoptosis; oxidative stress; antisense oligonucleotide; redox

Reactive oxygen species (ROS), such as H_2O_2, $OH^{\bullet}$, and $O_2^{\bullet-}$, are potentially dangerous by-products generated during normal intracellular metabolism in mitochondria. In addition, ROS may be produced by a variety of cytosolic enzyme systems, including those involving members of the NADPH oxidase family, and by a number of external agents such as ionizing radiation and chemotherapeutics [Finkel and Holbrook, 2000]. Intracellular ROS levels are maintained at acceptable levels by antioxidant enzymes including superoxide dismutase, catalase, glutathione peroxidase, and peroxiredoxin. Oxidative stress caused by disruption of this balance triggers apoptosis by damaging proteins, lipids, and DNA [Chandra et al., 2000]. However, the detailed signaling process by which cells progress from the initial damage to cell death is still unclear.

Daxx was first identified as a death-associated protein involved in Fas-induced apoptotic signaling. Upon binding with the Fas death domain, Daxx was shown to activate apoptosis signal-regulating kinase (ASK1), which in turn activates the Jun-N-terminal kinase (JNK) pathway [Yang et al., 1997; Chang et al., 1998]. Daxx is reportedly involved in transforming growth factor (TGF)-β-induced apoptotic signaling through direct binding with TGF-β receptor type II and subsequent activation of JNK [Perlman et al., 2001]. In addition, Daxx is also involved in UV-induced apoptosis via activation of JNK [Wu et al., 2002]. In addition to this cytosolic signaling, Daxx functions within the nucleus, where it interacts with PML, translocates to the PML nuclear body, and facilitates the induction of apoptosis [Ishov et al., 1999; Torii et al., 1999; Li et al., 2000a; Zhong et al., 2000].

Abbreviations used: ROS, reactive oxygen species; BSO, buthionine-(S,R)-sulfoximine; JNK, jun-N-terminal kinase; NAC, N-acetyl cysteine; PML, promyleoma leukemia protein; TGF, transforming growth factor; Gy, gray.

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In contrast to these pro-apoptotic roles, Daxx also appears to function in cell survival. The targeted disruption of Daxx in mice led to extensive apoptosis and embryonic lethality [Michaelson et al., 1999]. Similarly, increased apoptosis was observed in cells depleted of Daxx with small interfering RNA (siRNA) technology [Chen and Chen, 2003; Michaelson and Leder, 2003]. In addition, Daxx also appears to negatively regulate transcription factors such as ETS1, Pax3, and Pax5 via direct interactions [Hollenbach et al., 1999; Li et al., 2000b; Emelyanov et al., 2002]. The repressor activities of Daxx were abrogated when the protein was separated from the chromatin by sequestration in the nuclear body [Li et al., 2000a], suggesting a possible switch from anti-apoptotic to pro-apoptotic function of Daxx. Although some of these reported functions remain controversial, the previous reports indicate that Daxx may participate in multiple cellular functions through binding with several target molecules and/or translocation to different subcellular compartments.

Here, we have investigated the involvement of Daxx in oxidative stress-induced apoptosis by altering extra- and intra-cellular redox state, examining Daxx expression, and characterizing morphology in HeLa and HEK 293 cells. In addition, we have used antisense oligonucleotides to reduce endogenous Daxx level and thereby assess Daxx function. We found that endogenous Daxx is upregulated as an early response to oxidative stress, and that suppression of this increased expression inhibits oxidative stress-induced apoptosis. These findings demonstrate that Daxx expression is necessary for apoptotic signaling triggered by oxidative stress.

MATERIALS AND METHODS

Cell Culture Conditions and Treatments

HeLa and HEK 293 cells were grown in Dulbecco's modified Eagle's medium (Invitrogen) with 10% fetal bovine serum (Invitrogen) in a 5% CO_2 atmosphere at 37°C. For initiation of apoptotic stimuli, cells of ~50% confluency were treated with 1 mM H_2O_2. For the perturbation of intracellular redox states, cells were preincubated in media containing 100 μM buthionine-(S,R)-sulfoximine (BSO) or 40 mM N-acetyl cysteine (NAC) for 12 h, then washed and incubated with fresh media prior to treatment with H_2O_2.

Caspase-3 Assay

The caspase-3 activity of cell extracts (~10^6 cells) was determined with the synthetic substrate Ac-Asp-Glu-Val-Asp-7-amino-4-methyl coumarin (DEVD-AMC; Peptron), according to the manufacturer's instructions. The AMC fluorescence released by active caspase-3 was measured at an excitation wavelength of 360 nm and an emission wavelength of 460 nm.

Western Blot Analysis

At the indicated times after the various treatments, cells were harvested and lysed in 20 mM HEPES, pH 7.5, 50 mM NaCl, 10% glycerol, 0.5% Triton X-100, 0.1 mM Na_3VO_4, 1 mM NaF, 0.1 mM 4-(2-aminoethyl)-benzenesulfonyl fluoride hydrochloride, 2 μg/ml leupeptin, and 5 μg/ml aprotinin. Cell extracts were separated by SDS–PAGE and transferred to a nitrocellulose membrane (Schleicher and Schuell). Membranes were blocked with 5% skim milk in TTBS buffer (20 mM Tris, pH 7.4, and 150 mM NaCl containing 0.05% Tween 20), and then sequentially probed with rabbit anti-Daxx antibody (Santa Cruz Biotechnology) and goat anti-actin antibody (Santa Cruz Biotechnology). Blots were washed three times with TTBS and incubated with peroxidase-conjugated anti-rabbit IgG antibody (Upstate Biotechnologies), or anti-goat IgG antibody (Sigma), then developed with a chemiluminescence detection system (Pierce).

Northern Blot Analysis

Total RNA was isolated from cells with the Easy–BLUE™ RNA extraction kit (Intron, Inc.). Fifteen micrograms of RNA was resolved on a formaldehyde-containing agarose gel, transferred onto Hybond N nylon membranes (Amersham Biosciences) in 10 × SSC (1 × SSC is 150 mM NaCl plus 15 mM sodium citrate) for 12 h, and cross-linked to the membranes by exposure to UV light. The probes, consisting of the 3′-end 640-bp region of the Daxx cDNA and the 5′-end 540-bp region of the β-actin respectively, were labeled with the Rediprime DNA labeling kit (Amersham Biosciences), according to the manufacturer's recommendation. The nylon membranes were incubated for 4 h at 65°C in prehybridization solution contain-

ing 100 μg/ml denatured salmon sperm DNA, and hybridized with the ^{32}P-labeled cDNA probe in hybridization buffer for 2 h at 65°C [Sambrook and Russel, 2001]. The membranes were washed for 5 min at room temperature in 2× SSC-0.5% SDS, for 15 min at room temperature in 2× SSC-0.1% SDS, for 30 min at 37°C in 0.1× SSC-0.5% SDS, and finally for 30 min at 65°C in 0.1× SSC.

Real-Time Quantitative PCR

To generate cDNA, 5 μg of isolated total RNA was reverse transcribed by the avian myeloblastosis virus reverse transcriptase system and an oligo dT(15) primer (Promega), according to the manufacturer's protocol. The cDNA was used in a real-time quantification assay with a LightCycler PCR machine (Roche). Each PCR reaction (20 μl final volume) contained 10 ng of cDNA template, 500 nM of each primer (forward, 5′-GAG TGG GAA GGA AGG CGG AG-3′ and reverse, 5′-GTT GCA GAA CTC CGC CGA GG-3′), and the 1× LightCycler FastStart DNA Master SYBR Green I buffer (Roche). Samples were quantified by fluorescence measurement at the end of the elongation phase of each cycle. Reactions were performed in duplicate, and the mean threshold cycle was calculated and normalized to that of β-actin.

Immunofluorescence Microscopy

HeLa cells grown on cover slips were treated with H_2O_2 and incubated for 5 h, at which point they began to show drastic morphological changes. Cells were then washed three times with ice-cold PBS for 5 min each, fixed with 3.7% paraformaldehyde in PBS for 10 min, and permeabilized with 0.2% Trition X-100 in PBS for 15 min at room temperature. The samples were incubated with blocking solution (TTBS containing 2% BSA and 5% horse serum) and anti-Daxx polyclonal antibody diluted 1:2,000 in blocking solution for 1 h each. The samples were then washed with TTBS and incubated for 30 min with TRITC-conjugated anti-rabbit IgG (Sigma) diluted 1:400 in blocking solution. After being washed with TTBS, the samples were incubated with 4′,6-diamidino-2-phenylindole (DAPI) for DNA staining, and finally mounted on glass plates with 10% glycerol for fluorescence microscopy. All fluorescence images were obtained using a Zeiss AxioCam HRc connected to a Zeiss Axioskop fluorescence microscope.

Cell Death Assays

Annexin V, a specific reagent for phosphatidylserine, was used to stain the apoptotic cells, because early apoptotic event is characterized by the export of phosphatidylserine to the outer leaflet of the plasma membrane. Cells were cultured on coverslips for 36 h, treated with H_2O_2, incubated for another 5 h, washed with PBS, and subsequently washed with buffer (10 mM HEPES, pH 7.4, 140 mM NaCl, and 2.5 mM $CaCl_2$). The samples were then stained with Annexin V-fluorescein isothiocyanate (FITC) diluted 1:40 in the same buffer for 15 min at room temperature, and washed sequentially with buffer and PBS. After samples were fixed with 3.7% paraformaldehyde, cells were observed under a fluorescence microscope. For the Trypan Blue dye exclusion assay, pooled floating and adherent cells were resuspended in PBS containing 0.4% Trypan Blue dye, and the percentage of cells that failed to exclude the dye was determined using light microscope. To assess the chromatin condensation, cells were fixed, permeabilized, stained with Hoechst dye, and then observed under fluorescence microscope. For DNA fragmentation analysis, cells grown on 100 mm dish for 2 days were treated with or without 1 mM H_2O_2 for various times and genomic DNA was extracted with G-DEX^{TM} solution (Intron, Inc.) according to manufacturer's instructions. Equal amount of extracted DNA was separated on 1.5% agarose gels and visualized by ethidium bromide staining under UV light.

Antisense Oligonucleotide Treatment

Antisense and sense Daxx phosphothioate oligonucleotides were designed following the previous report [Gongora et al., 2001] and produced by Bioneer, Inc. The sequences of the antisense and sense oligonucleotides were 5′-CGGTGGCCATGGGGGTTC-3′ and 5′-AATTT-GAACCCCCATGGC-3′, respectively. Cells were preloaded with 3 μM of the phosphothioate oligonucleotides for 48 h, transferred to fresh media, stimulated with 1 mM H_2O_2, and cultured for another 5–7 h before assessing cell death.

RESULTS

Oxidative Stress Induces Daxx Expression and Apoptosis

Considering that oxidative stress triggers apoptosis in various cell types and Daxx is

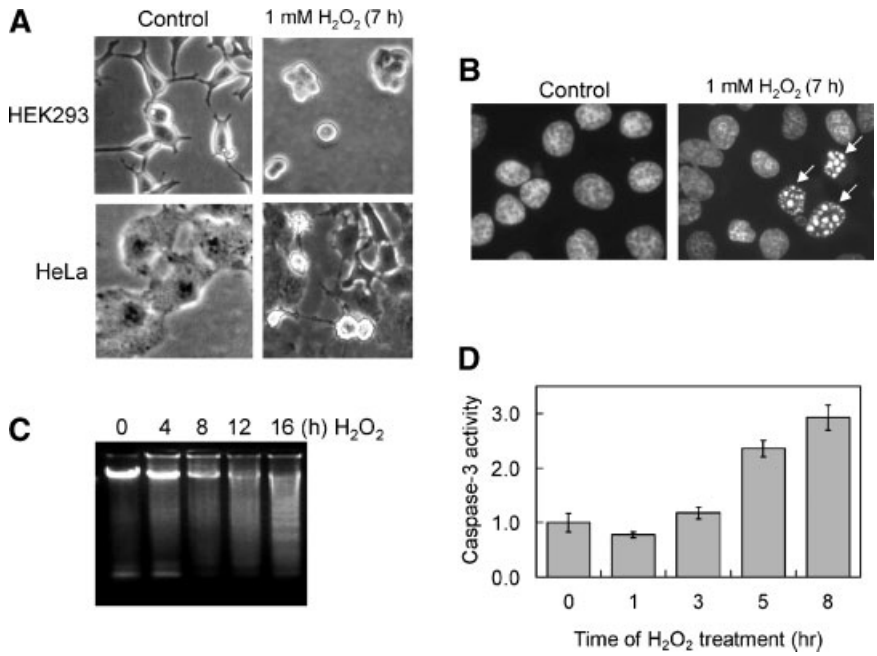

Fig. 1. Oxidative stress induces cell death. **A**: HeLa and HEK 293 cells at ~50% confluency were treated with 1 mM H_2O_2 and further incubated for 7 h. Cell morphologies were observed by phase-contrast microscopy. **B**: Nuclear morphology was examined by Hoechst staining of HeLa cells. Arrows indicate chromatin condensation of apoptotic cells. **C**, **D**: HeLa cells were collected at the indicated times after H_2O_2 treatment. Genomic DNA was prepared and separated on 1.5% agarose gels and DNA fragmentation was visualized by ethidium bromide staining (C). The caspase-3 activities of the cell lysates were analyzed by detecting DEVD-AMC cleavage. Data are expressed as the mean ± SD of three independent experiments (D).

involved in various extracellular stimuli-induced apoptotic events, we investigated the role of Daxx in oxidative stress-induced apoptosis. To induce apoptosis, HeLa and HEK 293 cells were treated with 1 mM H_2O_2. Within 7 h after treatment, both cell types showed morphological changes reflecting apoptosis, such as membrane blebbing, cell rounding, and subsequent detachment from the dish (Fig. 1A). Apoptosis was further confirmed by assessing nuclear morphology, genomic DNA stability, Annexin V staining, and caspase-3 activity. H_2O_2 treatment induced typical apoptotic chromatin condensation (Fig. 1B), time-dependent genomic DNA fragmentation (Fig. 1C), marked increase of Annexin V-positive cells (see below), and elevated caspase-3 activity up to about threefold that of control levels (Fig. 1D). These results were similar in HeLa (Fig. 1B) and HEK 293 cells (data not shown), indicating typical apoptotic events.

To investigate the role of Daxx in oxidative stress-induced apoptosis, we examined the levels of Daxx mRNA and protein in cells undergoing apoptosis after H_2O_2 treatment. The level of Daxx protein in the cell lysates was determined by Western blot analysis with anti-Daxx antibody (Fig. 2A). Following the initiation of oxidative stress, we observed an obvious, time-dependent increase of Daxx protein in both cell types (Fig. 2A). To examine the levels and subcellular localization of Daxx protein in intact cells, we performed immunofluorescent staining of endogenous Daxx before and after H_2O_2 treatment. Increased Daxx protein levels were observed throughout the cells following treatment, with the increase being most pronounced in the cell nuclei (Fig. 2B). Next, Northern blotting was performed to determine whether this increase of Daxx protein was due to elevated transcription of the Daxx gene. The level of Daxx mRNA was markedly increased in RNA pools isolated from cells treated with H_2O_2, as compared with those from untreated cells (Fig. 2C). This treatment-associated increase in Daxx mRNA levels was confirmed by real-time PCR analysis (Fig. 2D). Therefore, we conclude that Daxx is transcriptionally upregulated during H_2O_2-induced apoptosis.

To determine whether endogenous Daxx levels increase in response to other apoptotic stimuli, we next analyzed cells that had been treated with UV, cisplatin, etoposide, or staurosporine. These stimuli, however, did not

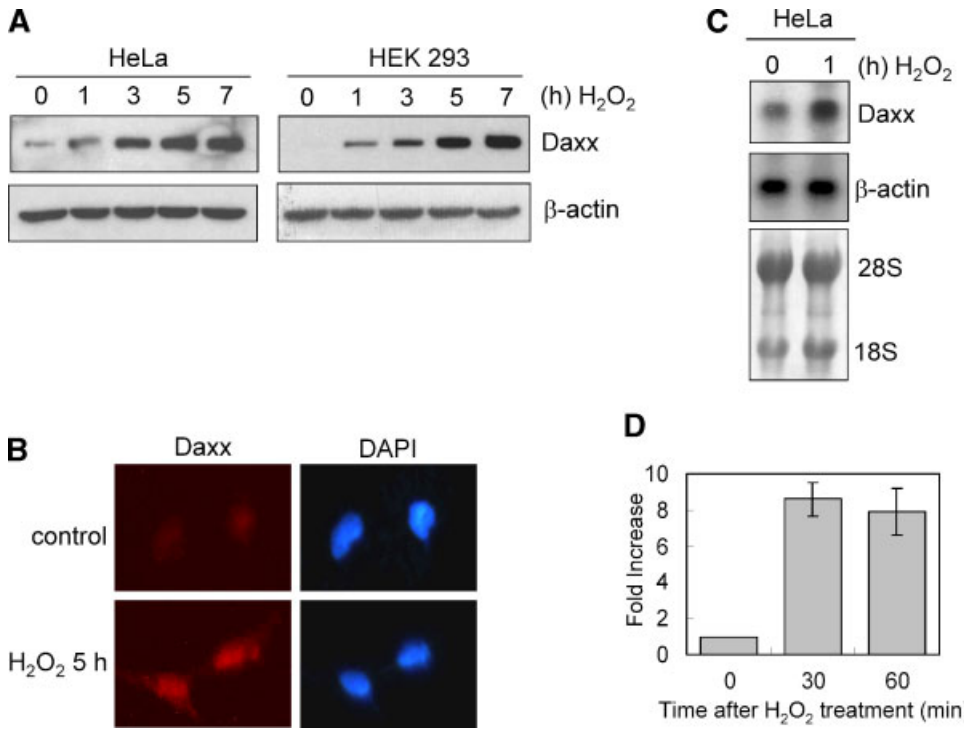

Fig. 2. Increases of Daxx protein and mRNA levels in response to ROS-generating stimuli. **A**: HeLa and HEK 293 cells were treated with 1 mM H_2O_2. At the indicated times after stimulation, cells lysates were analyzed by Western blotting with anti-Daxx and anti-actin antibodies. **B**: HeLa cells grown on cover slips were treated with 1 mM H_2O_2, incubated for additional 5 h, fixed with paraformaldehyde, permeabilized with Triton X-100, and immunostained with anti-Daxx antibody and TRITC-conjugated anti-rabbit IgG, followed by DAPI staining for nuclei. **C**: Northern blot analysis was performed on total RNA (15 μg/lane) prepared from HeLa cells at 1 h after stimulation using probes specific to the Daxx cDNA and the β-actin cDNA, respectively. The lower panel shows methylene blue-stained 18s and 28s rRNAs, which were used for normalization. **D**: RNA was isolated at time 0, 0.5, and 1 h after H_2O_2 treatment, and Daxx mRNA levels were determined by quantitative real-time PCR with β-actin used as the normalizing control. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

significantly affect Daxx levels, even though the treated cells showed similar apoptotic morphologies (data not shown). Therefore, Daxx induction seems to be an oxidative stress-specific response rather than a common mechanism for various apoptosis pathways.

Intracellular Redox State Controls Daxx Expression

It is known that extracellular oxidative stress results in perturbation of the intracellular redox balance. The ratio of reduced to oxidized glutathione is a well-known indicator of cellular redox state, and is strictly conserved from prokaryotes to higher eukaryotes. To determine whether the Daxx upregulation was caused by an alteration of intracellular redox state triggered by oxidative stress, we examined Daxx expression in cells in which this balance was shifted. Preincubation with BSO, a specific inhibitor of glutathione biosynthesis, lowers the intracellular concentration of reduced glutathione, whereas preincubation with N-acetyl cysteine, a precursor for glutathione biosynthesis, raises the intracellular concentration of reduced glutathione [Halliwell and Gutteridge, 1999]. We found that depletion of intracellular glutathione with BSO pretreatment increased Daxx expression when cells were stimulated with H_2O_2 (Fig. 3A, lane 4 compared with lane 2). In contrast, the increase of glutathione by N-acetyl cysteine blocked the induction of Daxx expression following H_2O_2 stimulation (Fig. 3B, lane 4 compared with lane 2). And finally, our Annexin V assay revealed that N-acetyl cysteine blocked H_2O_2-induced apoptosis (Fig. 3C). Together, these results suggest that the intracellular redox state tightly controls the expression of endogenous Daxx, and that the level of Daxx expression might modulate apoptosis.

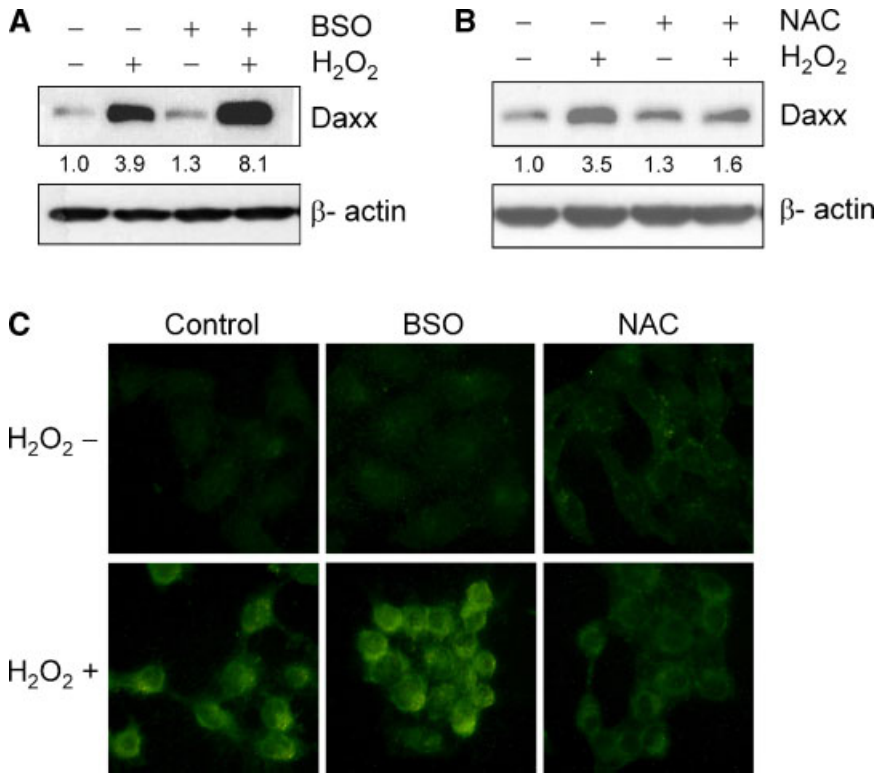

Fig. 3. The effect of intracellular redox state on Daxx expression levels. **A**, **B**: HeLa cells pretreated with 100 μM buthionine-(S,R)-sulfoximine (BSO) (A) or 40 mM N-acetyl cysteine (NAC) (B) for 12 h were stimulated with 1 mM H_2O_2 and incubated for additional 7 h. Western blot analysis of the cell lysates was performed as described in Figure 2, and band intensities were digitized by densitometric analysis. **C**: HeLa cells grown on cover slips were pretreated with BSO or NAC as described above, stimulated with 1 mM H_2O_2 and incubated for additional 5 h. Cells were stained with Annexin V-FITC for 15 min, fixed with 3.7% paraformaldehyde for 10 min, and then observed under a fluorescence microscope. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

Daxx Upregulation Is Required for Oxidative Stress-Induced Apoptosis

Because oxidative stress triggers apoptosis in parallel with the induction of Daxx expression, we questioned whether Daxx expression is necessary for oxidative stress-induced apoptosis. To address this, we suppressed Daxx expression in HeLa cells by treatment with antisense oligonucleotides, and then examined cell morphology and viability following H_2O_2 treatment. Cells preloaded with the controls (mock pretreatment or sense oligonucleotides) showed typical apoptotic features (Fig. 4B,C) and increased Daxx protein expression in response to H_2O_2 treatment (Fig. 4A). In contrast, cells preloaded with the Daxx antisense oligonucleotides showed a remarkable reduction in the extent of apoptosis in response to treatment with H_2O_2 (Fig. 4B,C). Because Annexin V staining detects the early stages of apoptosis and the later stages of Trypan Blue, these two assays showed different cell populations. However, both assays revealed a consistent pattern of reduced cell death in the presence of the Daxx antisense oligonucleotides. This reduction corresponded with the loss of Daxx induction (Fig. 4A, lane 6), indicating that Daxx upregulation is required for oxidative stress-induced apoptosis.

DISCUSSION

Reactive oxygen species (ROS) can elicit cell death. However, the signaling pathways that mediate this process remain elusive. ROS are generated via different pathways, including oxidative phosphorylation in mitochondria and intracellular signalings initiated by many external stimuli such as cytokines, ionizing radiation, and chemotherapeutic agents [Chandra et al., 2000; Finkel and Holbrook, 2000]. Daxx is involved in the receptor-mediated [Yang et al., 1997; Perlman et al., 2001] and extracellular stress-induced apoptotic pathways [Wu et al., 2002]. Here, we demonstrate the involvement of

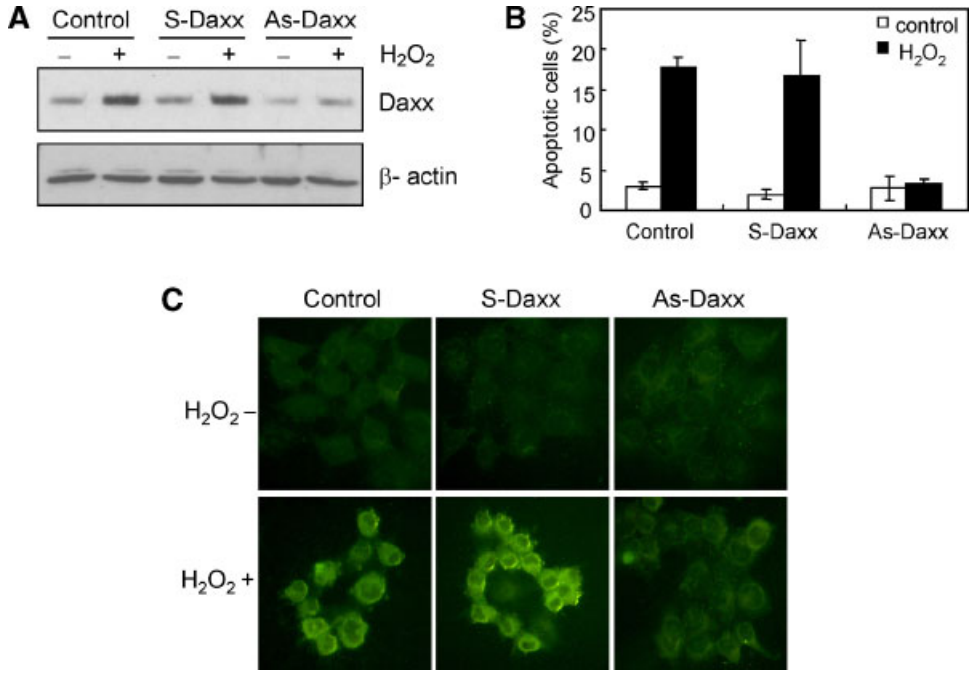

Fig. 4. Daxx upregulation is required for ROS-induced apoptosis. **A**: HeLa cells preloaded for 48 h with 3 μM sense (S-Daxx) or antisense (As-Daxx) oligonucleotides, or mock preloaded were treated with or without 1 mM H_2O_2 and cultured for an additional 7 h. Harvested cells were lysed and equal amounts of proteins were subjected to Western blot analysis. **B**: Each aliquot of harvested cells was washed once with PBS and stained with same volume of Trypan Blue solution (0.4%) for 10 min at room temperature. Blue-colored apoptotic cells were counted. Data are expressed as the mean ± SD of three independent experiments. **C**: HeLa cells cultured on cover slips were preloaded with S-Daxx or AS-Daxx oligonucleotides for 48 h, treated with 1 mm H_2O_2, incubated for additional 5 h, and then stained with Annexin V as described in Figure 3C. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

Daxx in ROS-induced apoptosis in HeLa and HEK 293 cells. We found that H_2O_2 treatment rapidly induced the expression of endogenous Daxx at the transcription level. Inhibition of Daxx upregulation with antisense oligonucleotides prevented H_2O_2-triggered apoptosis, indicating that Daxx upregulation is necessary for H_2O_2-induced apoptosis.

Investigation of additional apoptotic stimuli revealed that anti-Fas antibody, UV, cisplatin, etoposide, and staurosporine did not affect Daxx expression (data not shown). It is widely accepted that UV-radiation [Kulms et al., 2002] and chemotherapeutic drugs such as adriamycin, etoposide, and cisplatin [Miyajima et al., 1997; Siitonen et al., 1999; Chandra et al., 2000] induce apoptosis through generation of ROS. However, these stimuli did not induce Daxx expression in our experiments, suggesting that chemotherapeutic drugs and UV may induce apoptosis in quite a different manner from that induced by H_2O_2. Interestingly, it was recently reported that ROS generation is not required for chemotherapeutic drug-induced apoptosis [Senturker et al., 2002], thus supporting our hypothesis that oxidative stress, but not all other apoptotic stimuli, induces endogenous Daxx expression, leading to apoptosis.

The involvement of receptor-interacting protein (RIP) and tumor necrosis factor receptor (TNFR)-associated factor 2 (TRAF2) in oxidative stress-induced cell death has been well defined [Shen et al., 2004]; disruption of either gene abolished oxidative stress-induced cell death. Daxx is a Fas-associated protein [Yang et al., 1997], and RIP and TRAF2 are both key effector molecules of death signaling associated with Fas [Stanger et al., 1995] or TNF death receptor [Ting et al., 1996; Baud and Karin, 2001]. Thus, even though the expression of RIP and TRAF2 proteins seems constitutive [Shen et al., 2004], whereas Daxx is inducible upon oxidative stress, we speculate that these three molecules may function cooperatively in regulating oxidative stress-induced cell death.

The function of Daxx in apoptosis is controversial because contradictory results have been obtained from studies using transient overexpression, antisense, siRNA, and knockout technologies. Ectopic Daxx overexpression revealed

that the protein may function in a pro-apoptotic role [Chang et al., 1998; Torii et al., 1999; Zhong et al., 2000; Perlman et al., 2001]. However, complete or near-complete depletion of endogenous Daxx by knockout or siRNA technology revealed an anti-apoptotic role for Daxx [Michaelson et al., 1999; Chen and Chen, 2003; Michaelson and Leder, 2003], whereas incomplete depletion of endogenous Daxx by antisense technology revealed pro-apoptotic roles for Daxx in interferon- [Gongora et al., 2001], TGF-β- [Perlman et al., 2001], and ROS-induced [this study] apoptosis. It is unclear why Daxx shows both pro- and anti-apoptotic functions. One possibility is that Daxx might have dual functions depending on its cellular localization and concentration. At higher levels, Daxx might play a pro-apoptotic role in the PML nuclear body or cytoplasm through quantitative interactions with the apoptotic machinery. In contrast, at lower levels, Daxx might play an anti-apoptotic role as a transcription repressor through tight binding to its cognate chromatin structure. If this is the case, partial depletion of Daxx might then result in cell survival, whereas full depletion might result in cell death. We, therefore, hypothesize that cells fine-tune Daxx levels depending on the cell state, in order to modulate downstream signaling.

Many evolutionarily conserved stress-response genes are transcriptionally regulated. For example, the protective heat shock and antioxidant proteins, which are involved in repair and survival, are induced by stress. Their opponents, the pro-apoptotic genes, may also be transcriptionally regulated to amplify the stress response and lead to programmed cell death. For example, the genes for Fas ligand, Fas, Bak, CHOP, tumor necrosis factor receptor, and procaspases are induced by various apoptotic stimuli [Ossina et al., 1997; Le-Niculescu et al., 1999; Brachat et al., 2000]. Here, we propose that Daxx should be included as a new member of this second group of stress-response genes.

Oxidative stress activates the transcription factors OxyR in *Escherichia coli* [Zheng et al., 1998] and SigR in *Streptomyces coelicolor* [Kang et al., 1999] by covalent modification, resulting in elevated expression of antioxidant genes such as thioredoxin, thioredoxin reductase, glutaredoxin, glutathione reductase, and hydroperoxidase, which help to protect cells from stress. The Atf1 transcription factor in *Schizosaccharomyces pombe* upregulates the mRNA expression of antioxidant enzymes such as catalase, glutathione peroxidase, and Cu,Zn-superoxide dismutase, in response to oxidative stress [Buck et al., 2001; Lee et al., 2002]. The eukaryotic transcription factors Yap1 in *Saccharomyces cerevisiae* and AP-1 in mammalian cells also seem to play modulatory roles in protecting cells from oxidative stress [Abate et al., 1990; Kuge et al., 1997; Delaunay et al., 2000]. Similarly, we propose a Daxx-controlling transcription factor. Daxx upregulation by oxidative stress implies the existence of an oxidative stress-response element upstream of the Daxx coding region, and the possible involvement of a novel transcription factor responsible for binding this element and functioning as a transcriptional regulator of this stress-response gene. Based on this model, our future work will focus on uncovering the molecular mechanism for transcriptional regulation of the Daxx gene.

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