RNAi reveals anti-apoptotic and transcriptionally repressive activities of DAXX

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Summary

The function of DAXX, a highly conserved mammalian gene, has remained controversial; this is due, in part, to its identification in a variety of yeast two-hybrid screens. Targeted deletion in the mouse revealed that DAXX is essential for embryonic development. Furthermore, the increased levels of apoptosis observed in Daxx-knockout embryos and embryonic stem cell lines suggested that DAXX functions in an anti-apoptotic capacity. In contrast, overexpression studies showed that DAXX may promote apoptosis. Additional studies showed that, when overexpressed, DAXX could function as a transcriptional repressor. To clarify these matters, we have used RNAi to deplete endogenous DAXX and thereby assess DAXX function in cell lines previously tested in overexpression studies. Increased apoptosis was observed in DAXX-depleted cells, showing DAXX to be anti-apoptotic. The apoptosis induced by the absence of DAXX was rescued by Bel-2 overexpression. In addition, transcriptional de-repression was observed in RNAi-treated cells, indicating the ability of endogenous DAXX to repress gene expression and allowing for the identification of novel targets of DAXX repression, including nuclear factor κB (NF-κB) and E2F1-regulated targets. Thus, depletion of DAXX by RNAi has verified the crucial role of endogenous DAXX as an anti-apoptotic regulator, and has allowed the identification of probable physiological targets of DAXX transcriptional repression.

Key words: DAXX, RNAi, Apoptosis, Transcriptional repression, NF-κB, E2F1

Introduction

Daxx is a highly conserved mammalian gene that is essential in the mouse (Michaelson et al., 1999). DAXX protein is predominantly located in the nucleus, where its speckled distribution coincides with promyelocytic leukemia oncopgenic oomains (PODs or ND10s) (Ishov et al., 1999; Li et al., 2000a; Torii et al., 1999). However, its identification in a variety of yeast two-hybrid screens has resulted in the assignment of DAXX to a multiplicity of putative cellular functions (reviewed by Michaelson, 2000).

Many reports have implicated DAXX in apoptosis, but whether it functions as a pro- or anti-apoptotic molecule has been a matter of dispute. Targeted deletion of Daxx in the mouse results in severe developmental abnormalities in knockout embryos, highlighted by extensive apoptosis at embryonic day (e) 7.5 and e8.5 (Michaelson et al., 1999). Daxx-knockout embryonic stem cell (ES) lines are similarly characterized by elevated levels of apoptosis (Michaelson et al., 1999). Together, these studies imply that DAXX plays a protective role in preventing apoptosis. Additional evidence implicating DAXX as anti-apoptotic include a report showing the ability of DAXX to inhibit CD43-mediated apoptosis in hematopoietic cells (Cermak et al., 2001), and a recent suggestion that histone deacetylase inhibitor-induced apoptosis may be associated with downregulation of DAXX in acute promyelocytic leukemia cells (Amin et al., 2001).

Overexpression studies, however, suggested that DAXX is capable of inducing apoptosis. DAXX, initially identified as interacting with the Fas receptor death domain, was shown to enhance Fas-mediated apoptosis when overexpressed (Yang et al., 1997). DAXX was proposed to function in a pathway independent of Fas-associated death domain and to mediate apoptosis through activation of the Jun N-terminal kinase (JNK) pathway via apoptosis signal-regulating kinase 1 (Chang et al., 1998; Chang et al., 1999; Ko et al., 2001; Yang et al., 1997). Similarly, a role for DAXX in transforming growth factor β (TGF-β)-induced apoptosis and associated JNK activation was shown in B-cell lymphomas and hepatocytes (Perlman et al., 2001). Interestingly, Fas-mediated apoptosis and JNK signaling was shown to be independent of DAXX in lymphoid cells (Villunger et al., 2000).

Other studies have proposed that the ability of DAXX to induce apoptosis relies not on the ability of DAXX to interact with Fas, but rather on a nuclear apoptotic pathway, consistent with DAXX localization studies. Furthermore, it was suggested that DAXX facilitates the induction of apoptosis in primary splenocytes and keratinocytes from within PODs and only in the presence of PML (Zhong et al., 2000). Similarly, Torii et al. report that the ability of DAXX to facilitate Fas-induced apoptosis requires DAXX localization to PODs (Torii et al., 1999).

The ability of DAXX to function as a transcriptional repressor has also been shown. DAXX was shown to bind to Pax3, a member of the paired box homeodomain family of transcription factors, and on overexpression DAXX repressed Pax3-mediated transcriptional activity (Hollenbach et al.,
For FACs analysis, HeLa cells were plated at 3.5 x 10^5 per well of a six-well dish (or 8 x 10^5 l of 20 ml sample was analyzed on an Automat LB953 luminometer) following DNA transfection. Cells were collected 48, 72 or 96 hours following RNAi treatment, washed in PBS and fixed in 70% ethanol. Samples were incubated with anti-DAXX polyclonal antibody was used as described (Michaelson et al., 1999). Monoclonal anti-β-actin diluted 1:5000 (Sigma) and rabbit anti-poly-ADP ribose polymerase diluted 1:500 (Santa-Cruz) were also used as secondary antibodies, followed by detection by electrochemiluminescence (ECL).

**FACS analysis**

Cells were collected 48, 72 or 96 hours following RNAi treatment, washed in PBS and fixed in 70% ethanol. Samples were incubated with RNase A (0.5 mg/ml) and propidium iodide (5 µg/ml), followed by analysis on a FACs Calibur flow cytometer (Becton-Dickinson). A minimum of 1.5 x 10^5 cells were analyzed for each FACS experiment sample. For GFP experiments, a minimum of 5 x 10^4 cells were analyzed.

**Reporter assays**

Lysates were prepared from six-well dishes 24 hours following DNA transfection in 250 µl Passive Lysis Buffer (Promega; Madison, WI), and a 50 µl sample was analyzed on an Automat LB953 luminometer (Berthold) with automatic injection of luciferase reagent (Fischer). All samples were co-transfected with 50-100 ng β-galactosidase reporter construct, pCMVβ (MacGregor and Caskey, 1989). Luciferase values were normalized for transfection efficiency by measuring β-galactosidase activity using the Galacto-star system (Tropix). All transfections were performed in triplicate.

**Materials and Methods**

**Cell culture and transfections**

Cells were grown in DMEM media (Gibco) containing 10% bovine serum (Sigma) and 4 mM L-glutamine in the presence of antibiotics at 37°C with 5% CO2. HeLa (5 x 10^4), U2OS (6 x 10^5) and 293 (7.5 x 10^5) cells were grown in six-well dishes for 24 hours in medium without antibiotics before RNA transfection. Cells were transfected with 10 µl of 20 µM double-stranded RNA in Opti-Mem media (Gibco) using 3 µl Oligofectamine. Specifically, transfection of 21-23-nucleotide double-stranded RNAs into human and mouse cell lines was shown to efficiently suppress the expression of target genes, either endogenous or overexpressed.

Our analysis of cell lines depleted of DAXX by RNAi has revealed increased levels of apoptosis, confirming the role of DAXX as an anti-apoptotic protein. Furthermore, transcriptional studies in DAXX-depleted cells have shown that endogenous DAXX represses transcriptional activity, and has allowed for the identification of probable physiological targets of DAXX repression.

**RNA oligonucleotides**

21-nucleotide RNA oligonucleotides were obtained from Dharmacon Research.

hDx1: CCCUCCCACACACCUCUCdTdT, GGAGAGG-UGUGGGGAGGdIdTdT
GenBank AF015956 bp 531-551.

hDx2: GGAGUUUGGACUCUGAGAAdTdT, UCUGAGAGAU-CCAACUccdTdT
GenBank AF015956 bp 678-798.

mDx2: GGAGUUUGGACUGAGAGCdTdT, GCUCUGACA-GGUCCAACUccdTdT

RNA oligonucleotides were annealed as described previously (Elbashir et al., 2001).

**DNA constructs**

The Daxx expression vector contains full-length mouse Daxx with a C-terminal myc epitope tag expressed under the control of the chicken β-actin promoter in the pCAGGS vector (Niwa et al., 1991). pEGFP-C1 (Clontech) was used for expression of green fluorescent protein (GFP). The Bcl-2 expression vector contained full-length human Bcl-2 cloned into pCDNA3.1. cMet-luciferase (Met-luc) contains luciferase under control of the cMet promoter (Epstein et al., 1996). E2F1-luc and SP1-luc were kind gifts from P. Farnham (University of Wisconsin Medical School, Madison, WI) (Slansky et al., 1993). Other luciferase reporter constructs were obtained from Stratagene.

**Western blot analysis**

Protein extracts were prepared in non-denaturing buffer [1% NP40, 0.15 M NaCl, 0.01 M NaPO4, 2 mM EDTA and 50 mM NaF with Complete protease inhibitors cocktail (Boehringer Mannheim)]. Samples were electrophoresed on 8% polyacrylamide gels and then transferred to a polyvinylidine fluoride (PVDF) Immobilon-P membrane (Millipore). Affinity purified anti-DAXX polyclonal antibody was used as described (Michaelson et al., 1999). Monoclonal anti-β-actin diluted 1:5000 (Sigma) and rabbit anti-poly-ADP ribose polymerase diluted 1:500 (Santa-Cruz) were also used as primary antibodies. Horseradish peroxidase linked sheep anti-rabbit and antimouse immunoglobulin G (Amersham) were used as secondary antibodies, followed by detection by electrochemiluminescence (ECL).

Depletion of DAXX by RNAi

RNAs were used to deplete cell lines of endogenous DAXX protein. Two sets of 21-basepair double-stranded RNA oligonucleotides (hDx1 and hDx2) corresponding to human DAXX were tested by transfection into HeLa cells. Western blot analysis of cell lysates collected 72 hours after transfection revealed that hDx2 effected near complete depletion of endogenous DAXX protein, whereas hDx1 resulted in a modest decrease in DAXX (Fig. 1A).

**Results**

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bands and β-actin control were unaffected by RNAi treatment, indicating the specificity of the RNAi effect and the equivalent amounts of protein loaded on the gel. Subsequent studies used exclusively hDx2 as an efficient means to deplete cells of endogenous DAXX. A time-course study revealed that the RNAi effect of hDx2 lasted 5 days post-transfection, with the effect largely dissipated by 9 days (Fig. 1B). The RNAi effect was evident as early as 48 hours post-transfection but was not apparent at 24 hours (data not shown).

The specificity of the hDx2 effect was confirmed using a control RNA oligonucleotide (mDx2), the mouse homolog of hDx2 which differs in sequence by three nucleotides plus an additional nucleotide. Indeed, mDx2 had no effect on DAXX protein levels (Fig. 1C). Transient transfection of mouse Daxx cDNA following RNAi treatment revealed that hDx2 had no effect on accumulation of overexpressed mouse DAXX, whereas mDx2 treatment prevented overexpression (Fig. 1D). These studies confirmed the species specificity of hDx2 and mDx2. As shown in Fig. 1E,F, hDx2 was also effective in U2OS and 293 cells, with the resulting depletion of DAXX protein being slightly more pronounced following a second round of transfection. Taken together, these studies show that treatment of human cell lines with hDx2 results in a specific and significant depletion of endogenous DAXX protein.

Increased levels of apoptosis upon depletion of DAXX by RNAi

Whether DAXX functions as a pro- and/or anti-apoptotic molecule has been a matter of dispute in the literature (reviewed by Michaelson, 2000). Although results from knockout studies show that DAXX probably plays a role in preventing apoptosis in the early embryo and in ES cells, overexpression studies suggested that DAXX may function as a pro-apoptotic molecule in other cell types. Our ability to deplete DAXX using RNAi provided the opportunity to directly assess the function of DAXX in cell lines previously analyzed in overexpression studies (Chang et al., 1998; Chang et al., 1999; Ko et al., 2001; Torii et al., 1999; Yang et al., 1997).

The cell-cycle profile of HeLa cells transfected with hDx2 was analyzed by FACS. The sub-G1 peak, indicative of the apoptotic fraction due to fragmented DNA content, was modestly yet significantly increased in hDx2-compared with mock-transfected cells (Fig. 2A). The increased levels of apoptosis were evident as early as 48 hours post-transfection and were more pronounced 72 and 96 hours following RNAi transfection. The levels observed in the DAXX-depleted cells were similar to those observed in Daxx knockout ES cell lines (Michaelson et al., 1999). Elevated levels of apoptosis were similarly observed following transfection of hDx2 into 293 cells (data not shown). To verify that the increased apoptosis observed in HeLa cells was not a nonspecific RNAi effect, mDx2 was used in a similar experiment and showed no apoptotic effect (Fig. 2B).

As an additional method of assessing apoptosis, cleavage of the caspase target PARP was analyzed by western blot following RNAi treatment. In contrast to extracts prepared from mDx2-transfected HeLa cells, in which only full-length PARP (112 kDa) was evident, depletion of DAXX by hDx2 treatment resulted in the reproducible appearance of the cleaved version of PARP (85 kDa) (Fig. 2C), indicating activation of the caspase cascade.

It is well documented that Bcl-2, the founding member of a conserved family of proteins that regulate cell death,
functions to inhibit apoptosis (reviewed by Adams and Cory, 2001). We tested whether overexpression of Bcl-2 could rescue the apoptosis induced by DAXX depletion. HeLa cells were transfected with Bcl-2 or vector control 4 hours post-RNAi treatment. Measurement by FACS analysis revealed a significant rescue of the apoptotic fraction following transfection with Bcl-2 (Fig. 3A). The rescue was not complete, as expected, given the limitation of transfection efficiency. To monitor transfected cells only, HeLa cells were co-transfected with GFP in addition to Bcl-2 or vector control following RNAi treatment. FACS analysis on GFP-positive staining cells revealed a complete rescue of apoptotic cells (Fig. 3B). Note that in addition to rescue of hDx2-induced apoptosis, Bcl-2 also rescued the apoptotic fraction generated presumably as a result of GFP toxicity (Liu et al., 1999), which was also evident in mock-transfected cells.

**RNAi depletion of DAXX results in transcriptional de-repression**

Previous studies have shown that DAXX, when overexpressed, can mediate transcriptional repression (Emelyanov et al., 2002; Hollenbach et al., 2002; Hollenbach et al., 1999; Li et al., 2000a; Li et al., 2000b). We have assessed the ability of endogenous DAXX to mediate repression by measuring transcriptional activity of putative targets in cells depleted of DAXX by RNAi. Following RNAi treatment, HeLa cells were transfected with Met-luc (Epstein et al., 1996), a luciferase reporter gene driven by the Pax-3-regulated c-Met (hepatocyte growth factor) promoter. Cells treated with hDx2 RNAi revealed significantly increased levels of luciferase activity relative to mock- or mDx2-treated cells, indicating that depletion of endogenous DAXX causes de-repression of the c-Met promoter (Fig. 4A).

To show that the de-repression was solely a function of the loss of DAXX, an attempt was made to revert the de-repression by reconstituting cells with increasing levels of DAXX. Reconstitution was accomplished by transfection with mouse
RNAi reveals DAXX-dependent activities

Daxx, which is unaffected by hDx2 treatment (see Fig. 1D). In mock-treated cells, transfection of mouse Daxx cDNA resulted in decreased activity of c-Met (Fig. 4B), indicative of repression of the c-Met promoter and consistent with previous studies showing the repressive effects of DAXX overexpression (Hollenbach et al., 1999; Li et al., 2000b). In hDx2-treated cells, where increased levels of c-Met activity were observed (Fig. 4B), transfection of increasing levels of Daxx resulted in an incremental decreases in luciferase activity (Fig. 4B). In the presence of a high concentration of transfected Daxx (2.0 μg), significant repression of Met-luc was observed. These results confirm that loss of DAXX is responsible for the de-repression observed in the RNAi experiments.

The ability of endogenous DAXX to mediate repression of several other luciferase reporter constructs was tested. Following depletion of DAXX by hDx2, significantly increased luciferase activity from reporters driven by NF-κB and E2F1 elements (NFκB-luc and E2F1-luc) was observed relative to mDx2-treated cells (Fig. 5A), indicating that NF-κB and E2F1 targets are probably repressed by endogenous DAXX. In contrast, a basal promoter element (TATA-luc) showed no difference in luciferase activity when treated with hDx2 as compared with mDx2 (Fig. 5A). Similarly, RNAi treatment had no significant effect on the transcriptional activity of several reporter constructs, including AP1-luc and others (Fig. 5A). Thus, it is likely that endogenous DAXX does not regulate this subset of promoters in the given cellular context.

The ability of DAXX to repress the panel of reporter constructs was also tested using DAXX overexpression studies. When DAXX was overexpressed, the Met-, NF-κB- and E2F1-driven luciferase activities were accordingly repressed, but there was no significant effect on TATA-luc activity (Fig. 5B). These results are consistent with the effect of DAXX observed in the RNAi studies (Fig. 5A). However, in overexpression studies, DAXX was capable of moderately repressing AP1-luc, SP1-luc and cAMP-responsive element (CRE)-luc, reporters of DAXX-dependent activities.
that were not accordingly de-repressed following depletion of endogenous DAXX (Fig. 5A). Taken together, these studies probably identify a set of physiological targets of DAXX, which include the Pax-3 regulated c-Met, as well as NF-κB and E2F1 targets.

Discussion

In this report, we have used RNAi technology to assess the function of endogenous DAXX. Our results show that DAXX protects cells from apoptosis, thus confirming an anti-apoptotic role for DAXX. Furthermore, these studies illustrate the ability of endogenous DAXX to repress transcriptional targets.

We have taken advantage of the power of RNAi to evaluate the function of DAXX. Only recently has RNAi been shown to be effective in inhibiting gene expression in mammalian systems (Caplen et al., 2001; Elbashir et al., 2001). As a technical application, our studies exploit RNAi as a tool to evaluate mammalian gene function, namely apoptosis and transcriptional regulation. Recently, a few examples of successful use of RNAi in assessing cell growth and division were reported (Du et al., 2001; Harborth et al., 2001; Ohta et al., 2002). Our studies have further taken advantage of the specificity of RNAi to enable human cells depleted of DAXX to be reconstituted with mouse DAXX. By reintroducing DAXX into RNAi-depleted cells, we could show the specificity of the RNAi effect in mediating transcriptional de-repression. This type of reconstitution experiment is a powerful and effective method that will undoubtedly prove useful in validating phenotypes observed following RNAi treatment.

The ability to effect nearly complete depletion of endogenous protein was crucial for these studies. The observations that Daxx heterozygous mice develop normally and that Daxx heterozygous embryos as well as ES cells show no increase in apoptosis, despite the reduced levels of DAXX protein (Michaelson et al., 1999), suggest that modest levels of DAXX within the cell are probably sufficient for normal gene function. Therefore, it is likely that a relatively complete reduction in protein levels is required to achieve functional depletion of DAXX. Previous studies have attempted to use antisense technology to assess DAXX function. For example, in a study by Gongora and colleagues (Gongora et al., 2001), the significant levels of residual DAXX evident following antisense treatment make it difficult to interpret the effect observed on interferon-induced apoptosis in pro-B cells. Antisense studies were also employed to assess TGF-β-induced apoptosis in murine hepatocytes; although protection from apoptosis was observed, the extent to which depletion of DAXX was achieved was not reported (Perlman et al., 2001). The RNAi studies reported here show nearly complete depletion of DAXX and thus enable a more meaningful interpretation of resulting phenotypes.

RNAi studies have allowed us to evaluate the function of endogenous DAXX, a matter that until now has been somewhat controversial. Previously, results from the Daxx-knockout studies revealed that embryos and ES cell lines lacking DAXX have increased levels of apoptosis (Michaelson et al., 1999). In contrast to the knockout findings, several studies suggested that when overexpressed, DAXX could enhance Fas-mediated apoptosis (Chang et al., 1998; Chang et al., 1999; Ko et al., 2001; Yang et al., 1997), although not in lymphoid cells (Villunger et al., 2000). Other studies argued for DAXX mediating apoptosis from within the nucleus (Torii et al., 1999; Zhong et al., 2000). Taken together, the accumulating data were consistent with the notion that DAXX might have a dual function with respect to apoptosis, depending on the cellular context. Although DAXX may function in an anti-apoptotic capacity in development and possibly in the lymphoid system, it might be pro-apoptotic in fibroblasts and other cell types. The RNAi data presented here, however, suggest that DAXX also has an anti-apoptotic effect in cell lines such as HeLa and 293, in which overexpression studies previously showed the opposite effect. We deduce that DAXX may be anti-apoptotic in a variety of contexts, given that many of the studies arguing for a pro-apoptotic role used overexpression methodology. Nevertheless, the possibility that DAXX may be pro-apoptotic under certain circumstances cannot be ruled out.

Indeed, the levels of apoptosis that we observed here are consistent with the percentage of apoptotic cells measured in the Daxx-knockout ES cell lines (Michaelson et al., 1999). Although a truncated DAXX transcript and polypeptide observed in the knockout was of potential concern, we have recently generated a true DAXX null and the phenotype mimics that of the original knockout (J.S.M. and P.L., unpublished observations). Nevertheless, although our results suggest that the absence of DAXX results in a consistent level of apoptosis in several cellular contexts, the levels of apoptosis observed both in the RNAi studies and in the knockout ES lines are relatively modest. It is possible that cells lacking DAXX are only susceptible to apoptosis at a particular phase of the cell cycle. Alternatively, it is conceivable that cells with an exceedingly low level of DAXX can escape apoptosis. Finally, as discussed below, apoptosis may be secondary to the transcriptional effects of DAXX, in which case the apoptotic outcome may be dictated by fine differences in the transcriptional status of a given cell.

Significantly, we have found that Bcl-2 can rescue cells from the apoptosis induced by the absence of DAXX. This observation confirms that the death induced by DAXX depletion is indeed apoptotic. Furthermore, this finding indicates that cells lacking DAXX are subject to an apoptotic pathway that is Bcl-2 dependent. This would imply that the absence of DAXX cannot directly induce effector caspase activation. The caspase cascade is, nevertheless, probably involved in the apoptotic effect, given our observation of PARP cleavage in RNAi-treated cells. The ability of Bcl-2 to rescue the apoptotic effect of DAXX depletion is not surprising, given the ability of Bcl-2 to rescue many apoptotic responses, including that presumably induced by GFP overexpression (Liu et al., 1999) as observed in this study (Fig. 3B).

The RNAi studies also show the role of endogenous DAXX in mediating transcriptional repression. It was previously observed that overexpression of DAXX resulted in repression of targets of various transcription factors, such as ETS and Pax family members (Hollenbach et al., 1999; Li et al., 2000a; Li et al., 2000b). Our RNAi data show that endogenous DAXX can indeed mediate transcriptional repression of a variety of targets, including the c-Met promoter regulated by Pax 3. It is likely, however, that in our experimental system, regulation of the c-Met promoter is through other factors, given that Pax-3 is not expressed in HeLa cells. In cases where we observed transcriptional de-repression following RNAi treatment, such
as Met-luc, NF-κB-luc and E2F1-luc, we detected corresponding levels of repression when DAXX was overexpressed. These probably represent true targets of DAXX. In contrast, several targets showed no change with RNAi treatment, indicating the specificity of DAXX repression and arguing against DAXX being a general repressor. Interestingly, modest repression of AP1-luc, SP1-luc and CRE-luc was observed with DAXX overexpression. We consider it likely that these are not physiological targets of DAXX, but rather that the repression observed is an artifact of overexpression. Alternatively, they may be potential targets of DAXX repression under certain circumstances. We believe that RNAi thus provides an advantageous method to identify physiological targets of transcriptional regulators.

Our results suggest that AP1 transcriptional regulation is probably not a physiological target of DAXX. Previous studies had suggested that DAXX mediated JNK activation (Chang et al., 1998; Perlman et al., 2001; Yang et al., 1997), a process which results in increased transcriptional activity of AP-1 (reviewed by Davis, 2000). However, in our RNAi studies we observed no effect on AP-1 and, upon overexpression of DAXX, we actually detected modest repression of AP-1. Our findings thus do not support the claim that DAXX induces JNK activation. JNK activation by DAXX has similarly been challenged by several other studies in both lymphoid (Villunger et al., 2000) and fibroblast cell lines (Charette et al., 2000; Hofmann et al., 2001; Torri et al., 1999).

The question remains regarding the link between the role of DAXX in protecting from apoptosis and its function in transcriptional repression. It is possible that these represent independent activities of DAXX. However, our data provide a potential explanation for how the transcriptional effects of DAXX may dictate an apoptotic outcome. For example, the strong repression of E2F1 targets by DAXX and its de-repression in the absence of DAXX correlate with the positive role for E2F1 in inducing apoptosis (reviewed by Black and Azizkhan-Clifford, 1999). Furthermore, the repression of NF-κB target genes by DAXX may reduce the expression of pro-apoptotic genes, which, in the absence of DAXX, are then upregulated to induce apoptosis. Alternatively, it is conceivable that the activation of NF-κB in the absence of DAXX, as observed in our transcriptional studies, may represent a response to apoptotic signals. Such a mechanism would suggest that the transcriptional activities of DAXX are secondary to its apoptotic function. Future experimentation will be required to dissect the interplay between the role of DAXX in apoptosis and its function in transcriptional repression.

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