

Review

Advances in the study of the biological function of Daxx

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Death domain-associated protein (Daxx) was originally identified as a protein that specifically binds to the death domain of the transmembrane death receptor Fas (also called CD95) in the cytoplasm and potentiates Fas-induced apoptosis. Over expression of Daxx enhances Fas-mediated apoptosis and activates the Jun N-terminal kinase (JNK) pathway. Daxx was found in the nucleus where it localizes to promyelocytic leukaemia (PML) oncogenic domains (PODs). As a highly conserved nuclear protein, Daxx plays an important role in proapoptosis, antiapoptosis and transcriptional regulation. This article reviews the latest advances in the study of the biological function of Daxx.

Key words: Death domain-associated protein (Daxx), cell apoptosis, transcriptional regulation, biological function.

INTRODUCTION

As a highly conserved nuclear protein, Death domain-associated protein (Daxx) was originally identified as a protein that specifically binds to the death domain of the transmembrane, death receptor FAS (also called CD95) in the cytoplasm and potentiates FAS-induced apoptosis (Yang et al., 1997). A large proportion of Daxx molecules are nuclear, however, associate with the promyelocytic leukaemia (PML) nuclear body (PML-NB) and other subnuclear domains. Daxx has been suggested to function as a pro-apoptotic protein downstream of FAS through activation of the c-Jun-N-terminal kinase (JNK) pathway in a FADD-independent manner (Yang et al., 1997). As a transcriptional regulator, Daxx regulates the transcription of a variety of factors and expresses different biological effects.

In recent years, both domestic and foreign researchers took a lot of research and explorations of Daxx, which

were about apoptosis, anti-apoptosis and transcriptional regulation. This paper reviews the latest advances in the study of the biological function of Daxx.

THE BIOLOGICAL CHARACTERISTICS OF DAXX

Daxx is a highly conserved protein. Human Daxx protein is composed by 740 amino acids and its molecular weight is 81.4 KD. Mouse Daxx protein is composed of 739 amino acids, and its molecular weight is 81.3 kD. Daxx between the human and murine proteins has 69% identity. The total length of Daxx gene is about 315 kb and it has 7 exons and 6 introns. Because of the different post-transcriptional modification, Daxx molecules have three forms of existence, their molecular weight were 70, 97 and 120 ku, respectively. Daxx contains four regions,

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which are two N-terminal paired amphipathic helices (PAHs); a coiled-coil domain; an acidic region and a C-terminal serine/proline/threonine rich region. These structure domains make close association with transcription of Daxx.

Daxx is ubiquitously expressed throughout the body, such as liver, kidney, heart, 293 cells, HeLa cell, etc. In addition, Daxx is also expressed in chronic lymphocytic leukemia (CLL) (Alkan et al., 2005).

DAXX AND PROAPOPTOSIS

Daxx was initially identified as a protein associated with the death domain of the Fas receptor and involved in Fas-mediated apoptosis as well as the activation of the JNK pathway. However, Daxx-mediated apoptosis signaling pathway, at present, is still not entirely clear. Confirming the initial yeast two-hybrid screen, glutathione S-transferase (GST) pull-down experiments showed the C-terminal portion of Daxx as interacting with the Fas receptor (YANG et al., 1997).

In order to further investigate the mechanism of Daxx, yeast two-hybrid technique was used to screen the intracellular proteins interacting with Daxx (Liu et al., 2012), and 13 positive colonies and three proteins interacting with Daxx were obtained. One of the candidate proteins was identified as ferritin, heavy polypeptide 1 (FTH1). The interaction between Daxx and FTH1 was further supported by GST pull-down and co-immunoprecipitation, respectively. Then, Daxx was determined to induce apoptosis through the Fas-Daxx-ASK1-JNK1 signaling pathway. Schepers et al. (2005) found out that an inhibitory role of HSP27 in VP-16-mediated activation of the ASK1/p38/JNK pathway after HSP27 combine with Daxx, which is in the monocytic acute myeloblastic leukemia (AML). As a highly conserved nuclear protein, Daxx deletion mutant (aa 501 to 625) has been known to be an inducer of apoptosis, and Song et al. (2004) observed that the Bax-dependent mitochondrial death signaling pathway plays an important role in Daxx 501 to 625-induced apoptosis. What is more, Daxx 501 to 625-induced apoptosis is mediated through the ASK1-MEK-JNK/p38-Bim-Bax-dependent caspase pathway.

Muromoto et al. (2010) found out that knock down of Daxx attenuated lactate dehydrogenase leakage from cells, indicating that Daxx positively regulates cell death during gp130/STAT3-mediated cell proliferation.

In exploring whether Daxx mediates oxidized low-density lipoprotein (Ox-LDL)-induced apoptosis in macrophage, He et al. (2010) found out that Ox-LDL up-regulated the expression of Daxx mRNA and caveolin-1, increased the accumulation of intercellular cholesterol and induced the apoptosis of RAW264.7 macrophages. However, Ox-LDL-induced intercellular cholesterol accumulation and apoptosis in RAW264.7 cells were prevented by Daxx siRNA. Ox-LDL also induced caveolin-1 expression and this effect was significantly

suppressed by Daxx siRNA. It can be concluded that Daxx mediates Ox-LDL-induced cholesterol accumulation and apoptosis in macrophages by up-regulating caveolin-1 expression. We found out that over-expression of Daxx facilitated HepG2 cells apoptosis induced by hydrogen peroxide (Tuo et al., 2008). Furthermore, there may be a synergetic relation with apoptosis and increase of JNK activity. In order to elucidate the functional relevance of Daxx in apoptosis signaling of malignant lymphocytes, Jurkat T-cells were stably transfected with a Daxx-expressing vector or with the respective Daxx-negative control vector. Boehrer et al. (2005) demonstrated that ectopic expression of Daxx substantially increased the rate of apoptosis upon incubation with death receptor agonists such as Fas and TRAIL, as well as upon incubation with the cytotoxic drug doxorubicin (DOX). Although, enforced apoptosis caused by ectopic Daxx expression is caspase-dependent in both cases, major differences between Fas/Tumor necrosis factor-related apoptosis-inducing ligand (TRAIL)-induced apoptosis and doxorubicin-induced apoptosis are observed in the expression patterns of X-linked inhibitor of apoptosis (XIA-P), p53, Bid, ZIP kinase and prostate apoptosis response gene 4 (Par-4). They outlined the major molecular mechanisms underlying the apoptosis-promoting effect of Daxx in neoplastic lymphocytes and demonstrated fundamental molecular differences elicited by the overexpression of Daxx, in the extrinsic and intrinsic signaling pathways. Sodium hydrogen exchange isoform 1 (NHE1) (Jung et al., 2008) was identified as a Daxx-interacting protein. During ischemic stress, Daxx translocates from the nucleus to the cytoplasm, where it colocalizes with NHE1. Daxx binds to the ezrin/radixin/moesin-interacting domain of NHE1, in competition with ezrin.

As most studies on the role of Daxx in cell death have been conducted using tumour cell lines, Khelifi et al. (2005) analyzed the function of Daxx in physiological settings. They employed RNA interference to down regulate Daxx in primary fibroblasts. Remarkably, Daxx-depleted cells are resistant to cell death induced by both ultraviolet (UV) irradiation and oxidative stress. Furthermore, the down regulation of Daxx results in impaired MKK/JNK activation. This is the evidence that Daxx promotes cell death and JNK activation in physiological conditions.

DAXX AND ANTIAPOPTOSIS

Despite the reports advocating a pro-cell-death function for Daxx, several studies have suggested a potential anti-apoptotic function for Daxx.

Michaelson and Leder. (2003) found out that Daxx was essential to the embryonic development and to be anti-apoptotic, based on the result those large quantities of embryonic stem cells apoptosis when Daxx gene was knocked out from the mouse embryo. The HeLa cell

sensitize to apoptosis in Daxx-knockout HeLa cell. Daxx can exert the anti-apoptotic function through regulating the two targets in Daxx, NF κ B and E2F1.

Chen and Chen (2003) investigated the role of Daxx in Fas- and stress-induced apoptosis by small interfering RNA-mediated Daxx silencing in mammalian cells, and found out that Daxx silences strongly sensitized cells to Fas- and stress-induced apoptosis. These data strongly suggest that Daxx may inhibit Fas and stress-mediated apoptosis by suppressing pro-apoptotic gene expression outside of PML domains. Chaudhary et al. (2010) demonstrated that 4-hydroxynonenal (HNE) modification induced phosphorylation of Daxx at Ser668 and Ser671 to facilitate its cytoplasmic export, which consistently, induced apoptosis in HepG2 cells. However, the use of small interfering RNA-mediated Daxx silencing could induce apoptosis in HepG2 cells, these aspects indicated a potential antiapoptotic function for Daxx. Cermák et al. (2002) showed that over expression of Daxx inhibited anti-CD43 monoclonal antibody (mAb) MEM-59-induce apoptosis of the myeloid progenitor-derived cell line TF-1. Zhang et al. (2010) discovered that down-regulation of MDM2 in acute lymphocytic leukemia cells by berberine occurred at a posttranslational level through modulation of Daxx, which disrupted the MDM2–DAXX–HAUSP interactions and thereby promoted MDM2 self-ubiquitination and degradation. These results suggest a potential anti-apoptotic function for Daxx.

In human cells, Daxx interacted with heat shock factor 1 (HSF1) essentially only during stress, that is, when factor trimerization occurred. Daxx is an important mediator of HSF1 activation. Boellmann et al. (2004) suggest a model in which Daxx released from its nuclear stores during stress, opposes repression of HSF1 transactivation competence by multichaperone complex through its interaction with trimerized HSF1. Over expressed HSF1, known to be largely trimeric, only marginally increased HSF1 activity on its own, but potentiated the activating effect of Daxx overexpression.

Zobalova et al. (2008) found out that exposure of cultured cardiomyocyte-like cells to oxidative stress or simulated hypoxia increased the level of accumulated reactive oxygen species (ROS) and apoptosis. Under the conditions of sub-apoptotic stimulation of cardiac myocytes, there was no increase in the level of the Daxx protein, but its translocation from the nucleus to the cytoplasm was increased. Daxx over expression protect the cells from apoptosis, while they are sensitized to cell death following its down-regulation by siRNA. Moreover, lowering the level of the Daxx protein sensitized cardiac myocytes to spontaneous apoptosis, suggesting that the protein may also have a pro-survival role under physiological conditions. Daxx plays a protective role in cultured cardiomyocyte-like cells, at least under the conditions used.

The small ubiquitin-related modifier-1 (SUMO)-1 is an important posttranslational regulator of different signaling

pathways and is involved in the formation of promyelocytic leukemia (PML) protein nuclear bodies (NBs). Meinecke et al. (2007) showed that the increased expression of SUMO-1 in rheumatoid arthritis (RA) synovial fibroblasts (SFs) contributes to the resistance of these cells against Fas-induced apoptosis through increased sumoylation of nuclear PML protein and increased recruitment of the transcriptional repressor Daxx to PML NBs.

DAXX AS A TRANSCRIPTIONAL REGULATOR

Daxx has been shown to function as a transcriptional regulator via phosphorylation or its interaction with various cytoplasmic and nuclear proteins. Daxx is unlocated in PODs in the absence of PML or under normal circumstances, a condition in which PODs are destroyed. Daxx can localize to regions of condensed chromatin, when Daxx interacts with several transcription factors, including Pax3 (Lehembre et al., 2001), ETS1 (La et al., 2004). Daxx can inhibit their transcription activity, thus gene transcription is repressed and the cell apoptosis does not occur. In B cell lines, Daxx binding to transcription factor Pax5 and activating transcription of B cells shows the role of transcriptional co-activator. Park et al. (2007) found out that physical interaction between Daxx and p65 provides a functional framework for the inhibition of p65 acetylation by p300/CBP and subsequent repression of NF- κ B transcriptional activity.

Daxx functions as a negative AR co-regulator through direct protein-protein interactions. Studies by Lin et al. (2004) revealed that the binding between Daxx and the DNA-binding domain of the ubiquitination AR shows marked suppression on the activity of the AR DNA-binding both *in vitro* and *in vivo*. Moreover, ubiquitination of AR at its amino-terminal domain is involved in Daxx interaction and trans-repression.

Chang et al. (2005) observed that Daxx suppresses Smad4-mediated transcriptional activity by direct interaction with the ubiquitination Smad4 and identified a novel role of Daxx in regulating TGF- β signaling pathway. Daxx also suppressed STAT3-mediated transcriptional activation, while over expression of Daxx inhibited IL-6/STAT3-mediated gene expression (Kenji et al., 2007). Boellmann et al. (2004) found that over expression of Daxx enhanced basal transcription activity of HSF1 in the condition of a stress. Zhang et al (2010) found out that the transcriptional inhibition of HSF4b is associated with Daxx. HSF4b can interact with Daxx in the nucleus and their association is regulated by the phosphorylation of HSF4b/S299. Thus, we can know the binding between Daxx and different transcription factor or related protein can influence the transcription.

Several recent studies have indicated that Daxx is a mediator of lymphocyte death and/or growth suppression, although, the detailed mechanism is unclear. Muromoto

et al. (2010) found out that Daxx suppresses the gp130/STAT3-dependent cell growth and that Daxx endogenously interacts with STAT3 and inhibits the DNA-binding activity of STAT3. Moreover, small-interfering RNA-mediated knockdown of Daxx enhanced the expression of STAT3-target genes and accelerated the STAT3-mediated cell cycle progression. In addition, knockdown of Daxx-attenuated lactate dehydrogenase leakage from cells, indicating that Daxx positively regulates cell death during gp130/STAT3-mediated cell proliferation.

CONCLUSION AND PROSPECTS

Currently, the precise function of Daxx in cell death and transcriptional regulation is not fully understood. While apoptosis appears to be an underlying theme in many of the Daxx studies, the role of Daxx in preventing and/or inducing apoptosis is as yet unclear. However, several studies had indicated that Daxx played significant roles in proapoptosis, antiapoptosis and transcriptional regulation. Certainly, the biochemical function and mechanism of Daxx are in progress and will be critical in further elucidating the functional role of Daxx. In addition, it will bring some novel ideas in prevent and tie at viral infection, cancer, leukemia and cardiovascular disease.

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