·Review·

Progress on understanding the subcellular localization of Daxx

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[Abstract] As a highly conserved nuclear protein, Daxx plays an important role in transcriptional control, tumorigenesis, viral infection, and other processes. Daxx can be localized in promyelocytic leukemia nuclear bodies (PML–NBs), nucleoplasm, nucleoli, cytoplasm, and heterochromatin. The subcellular localization of Daxx can be changed by modification or by interacting with other proteins. Under cellular stress, Daxx can interact with many kinds of molecules, and thus affect its downstream signaling pathway. The purpose of this review is to discuss the subcellular localization of Daxx under different conditions and its translocation between subcellular compartments.

Key words: Daxx, subcellular localization, translocation, stress

Daxx is a fatty acid synthase (Fas) death domain-associated protein, identified by Yang *et al*.[1] in 1997, that activates the c-Jun N-terminal kinase (JNK) pathway and induces cell apoptosis. As a nuclear protein, it mainly interacts with promyelocytic leukemia (PML) protein and is then co-localized in PML nuclear bodies (PML-NBs), which are also known as PML oncogenic domains or nuclear domain 10s (ND10s). As an adapter in protein interactions, Daxx regulates the transcription of numerous cytokines and is involved in various cell reactions. Daxx is an important regulator protein that interacts with a number of proteins, such as histone deacetylase 1 (HDAC1), HDAC2, androgen receptor (AR), Smad4, tumor suppressor gene p53, paired box gene 3 (Pax3), and Axin. When cells are stressed, Daxx can interact with different molecules and thereby regulate the expression of multiple stress-related genes. This may be a self-protective mechanism in cells under stress. Herein we reviewed and summarized the research progress on the subcellular localization of Daxx.

Structural features of the Daxx molecule

Daxx is a highly conservative protein present in both mammals and humans and extensively distributed in normal and tumor cells, including hepatic, renal, and cardiac cells, and the 293 and HeLa cell lines. Mouse Daxx (mDaxx) consists of 739 amino acids, with a molecular weight of 81.4 kDa, while human Daxx (hDaxx) consists of 740 amino acids, with a molecular weight of 81.3 kDa. The homology of the amino acid sequence between hDaxx and mDaxx is 69%. The full length of the hDaxx gene is 315 kb, containing seven exons and six introns. Due to post-transcriptional modifications, hDaxx can be seen in three variations, with molecular weights of 70 kDa, 97 kDa, and 120 kDa, respectively. hDaxx has four domains, namely, two double-spiral domains, one domain rich in acidic amino acid, and one rich in serine/proline/threonine. These domains are closely related to the regulation of hDaxx transcription. Among them, the serine/proline/threonine-rich domain can mediate the interaction of Daxx with numerous molecules, and therefore its dominant negative mutant is often used to study the functions of wild-type Daxx.[2]

Subcellular localization of Daxx

Daxx can be seen in subcellular compartments including PML-NBs, nucleoplasm, nucleoli, and cytoplasm. By modifying or interacting with other proteins, Daxx can be translocated from one subcellular compartment to another, and thus either promotes or prevents apoptosis and regulates transcription.

Daxx localization in PML-NBs

Daxx and PML modified by small ubiquitin-like modifier 1 (SUMO1) are co-localized in PML-NBs. As the main protein components of PML-NBs, Daxx and PML are closely associated with the formation and maintenance of the spherical structure of PML-NBs and the localization of other proteins in PML-NBs. Using co-immunoprecipitation, Lin *et al*.[3] revealed for the first

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time that Daxx could bind to PML that had been modified by SUMO1 and was thus localized in PML-NBs. As_2O_3 can increase the SUMO modification of PML, and thereby help localize Daxx in PML-NBs. The SUMO-interacting motif (SIM) ^{733}I-I-V-L- S-D-S-D^{740} of Daxx binds to the groove on the surface of the SUMO1 molecule formed by the β sheet (aa 33–39) and part of the α helix (aa 45–54). However, Jung *et al*.[4] showed that Daxx was an individual and integral nuclear protein that was independent on PML or p53. Daxx may also be a scaffold protein of PML-NBs like PML. Intranuclear Daxx is essential in holding the various components of PML-NBs, and blocked Daxx expression can result in the dissociation of PML-NBs.

Daxx localization in the nucleus

Daxx is mainly a nuclear protein. When HeLa cells are separated into different components, such as cytoplasm and mitotic chromosomal segments, Daxx is mainly seen in the nuclear components. In nuclear, cytoplasmic, low-density microsome, high-density microsome, and cytoplasmic membrane components extracted from NIH-3T3 mice fibroblasts, Daxx is also aggregated in the nucleus, with a small portion of Daxx seen in low-density microsomes. Immunofluorescent detection for endogenous Daxx in the 3T3 fibroblasts revealed that the fluorescence localized mainly in the nucleus, while merely dim spotty fluorescence was seen in the cytoplasm.[5]

Daxx localization in the nucleolus

In human tumor cell lines, the p14^{ARF} protein is generally localized in the nucleolus. As a factor of tumor suppression, it has a critical role in tumorigenesis, cell apoptosis, and proto-oncogene activation. Ivanchuk *et al*.[6] found that, on an endogenous expression level, the N-terminal of alternative reading frame (ARF) could bind to the N-terminal of Daxx and was thereby co-localized in the nucleolus. The N-terminal of Daxx (aa 391–398) may be a signal for its nucleolus localization. In addition, immunofluorescent analysis on transfected U2OS cells suggested that Daxx, ARF, and human double minute 2 (HDM2) created a complex, which was partially co-localized both in the nucleolus and in the spotty structures around nucleolus. ARF can promote SUMO modification of HDM2. However, when ARF is co-expressed with Daxx, Daxx inhibits the SUMO modification of HDM2 mediated by ARF. We assume that the localization of Daxx in the nucleolus induced by ARF may have an important effect in regulating ARF activity and thus influence downstream signaling pathways. ARF induces ubiquitination and SUMO modification of Daxx, indicating that, under stress, the post-translational modification of Daxx is very important in regulating its activity. The co-expression of HDM2 and Daxx also induces Daxx ubiquitination, suggesting that Daxx may be one of the substrates for the HDM2 E3 ligase. When the three molecules are co-expressed, Daxx ubiquitination is increased. Furthermore, Daxx can be localized in the nucleolus by interacting with the nucleolus microspherule protein of 58 kDa (MSP58).[7]

Daxx localization in the cytoplasm

The death domain of Fas can activate apoptosis signal-regulating kinase 1 (ASK1), while ASK1 promotes Daxx translocation from the nucleolus to the cytoplasm. Translocated Daxx thereby reacts with the death domain of Fas and mediates cell apoptosis via the Fas-Daxx-ASK1-JNK1 signaling pathway.[1] Sharma *et al*.[8] obtained contradictory results, which revealed that in CRL2571 cells, 4-hydroxynonenal (HNE) treatment induced fast and stable up-regulation of Daxx. When Daxx expression was interrupted by small interfering RNA (siRNA), CRL2571 cells became more susceptible to apoptosis induced by toxic Fas antibodies. This demonstrates that the binding of Daxx to Fas is not necessary in the apoptotic signaling pathway mediated by Fas. Awasthi *et al*.[9] further confirmed that HNE bound to Daxx and induced Daxx translocation from the nucleolus to the cytoplasm, where Daxx bound to Fas in the cytoplasm and inhibited apoptosis.

MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine) is a neurotoxin that induces the loss of dopaminergic neurons in mice. In the brain, MPTP is oxidized by dopaminergic neurons into 1-methyl-4-phenyl pyridinium (MPP+), which has a high affinity toward the dopamine transporter and can lead to selective uptake by dopaminergic neurons. Karunakaran et al used MPP+ to treat human neuroblastoma for 12 h and found that Daxx translocated from the nucleus to the cytoplasm.[10] Treatment with N-acetylcysteine or α -lipoic acid 1 h before MPP^+ treatment could inhibit Daxx translocation. In mice, MPTP dosing also induced similar Daxx translocation. Daxx translocated from the nucleolus to the cytoplasm in the neurons of substantia nigra pars compacta, establishing Daxx translocation and its functions in vivo for the very first time.

MPTP exposure activates mouse ASK1 and thus leads to the phosphorylation of downstream mitogen-activated protein kinase kinase 4 (MKK4, also known as SEK1) and JNK. Daxx may be phosphorylated via the ASK1-SEK1-JNK1 pathway and translocated into the cytoplasm. DJ-1 is a protein sensitive to oxidation and reduction seen in familial Parkinson's disease. Under normal circumstances, the interaction between DJ-1 and Daxx can prevent Daxx translocation from the nucleolus to the cytoplasm and thereby maintain normal levels of ASK1 activity and protect the cells. When DJ-1 is mutated (such as L166P), Daxx can be translocated from the nucleolus to the cytoplasm and thus induce apoptosis, which may be one of the pathogenic mechanisms in Parkinson's disease.[11] MPTP exposure can downregulate DJ-1 levels in intranuclear and extranuclear compartments. Hence, it is highly possible that low intranuclear levels of DJ-1 result in the dissociation of DJ-1 from Daxx and subsequently the translocation of Daxx, which then reacts with ASK1 in the cytoplasm and triggers a cell death cascade.[10]

Daxx localization in heterochromatin

In PML-/- cells, Daxx binds to dense chromatin. When cells are transfected with plasmid and overexpress PML, Daxx can be recruited onto PML-NBs again. The α -thalassemia/mental retardation syndrome X-linked (ATRX) protein is a member of the SNF-2 family and an ATP-dependent chromatin remodeling protein. Daxx and ATRX create a complex when mediated by ATRX phosphorylation. Thereby, ATRX accumulates in PML-NBs at the G_1 and G_2 phases, and also concentrates in dense chromatin at the end of the S phase.[12] Daxx can also bind to

histone deacetylase 2 (HDAC2), core histone, and the chromatin-associated protein Dek. The binding of Daxx to chromatin is critical in its inhibition of transcription.[5]

Regulation of Daxx localization

Daxx is translocated from one subcellular compartment to another by modifying or interacting with other proteins. The N-terminal of Daxx binds to the N-terminal of ARF and is therefore co-localized in the nucleolus. ARF induces SUMO modification of Daxx. Presumably the localization of the N-terminal of Daxx may be affected by the SUMO modification, but this has yet to be demonstrated.[6] When PML is either absent or not modified by SUMO1, Daxx can be translocated from the PML-NBs to dense chromatin. When PML is restored to normal levels, Daxx can be translocated back to the PML-NBs. When cells are infected with certain viruses, viral proteins (such as adenovirus E1B-55K oncoprotein[13]) can result in the dissociation of PML-NBs and changes in their spherical structures, and consequently prevent Daxx from binding to PML and being localized in the PML-NBs; instead, Daxx is localized in the chromatin. These changes help viruses accumulate in the PML-NBs, where viral genes are replicated and transcripted.

The nuclear-transporting protein Importin α 3 can bind to various cargo proteins in the cytoplasm and then import these proteins via the pore complex into the nucleus. Yeung *et al*.[2] found that Importin α 3 mediated the nuclear import of Daxx. Daxx interacted with two nuclear localization signal (NLS) sites on Importin α 3 via NLS-1 and NLS-2, with NLS-2 as the main binding site. Daxx is mainly localized in the nucleus, but it can be translocated into the cytoplasm by interacting with numerous proteins. For example, ASK1,[1] HNE,[9] and JNK[10] can promote Daxx translocation from the nucleus to the cytoplasm, whereas Carboxyl-terminus of HSC70-interacting protein (CHIP) inhibits Daxx translocation into the cytoplasm.[14] CHIP is a co-chaperonin and ubiquitin ligase. Expression of wild-type CHIP inhibits Daxx translocation into the cytoplasm. CHIP triggers ASK1 degradation by ubiquitination and subsequently results in less Daxx in the cytoplasm and more Daxx in the nucleus. Cytoplasmic Daxx activates ASK1. When ASK1 is decreased, Daxx can be relocalized into the nucleus and counteracts apoptosis. The interaction between DJ-1 and Daxx also inhibits Daxx translocation into the cytoplasm.[11]

Daxx localizations and its regulation under stress

Unders stress, Daxx expression and localization are modified by the interaction with various molecules, and therefore influences downstream signaling pathways. These changes in Daxx may be a self-protective mechanism in cells under stress.

Glucose deprivation-induced stress

Chromosome region maintenance protein (CRM1) is a member of the β -karyopherin family that mediates the export of nuclear protein. Song *et al*.[15] proved that CRM1 bound to Daxx and mediated Daxx translocation into the cytoplasm when glucose was deprived. Daxx bound to CRM1 via a nuclear export signal (NES) (aa 565-575). They also found that the tryptophan 621 of Daxx might react with the hydrophobic region formed by the α helix in the C-terminal via Van der Waal's force and thus conceal the NES of Daxx, and thereby inhibit its binding to CRM1. The phosphorylation of the serine 667 could interfere with such an effect and reveal NES, promoting the binding of Daxx and CRM1. Moreover, this change was essential in the translocation of Daxx into the cytoplasm and its subsequent binding to ASK1.

Oxidative stress

HNE is a small signaling molecule that is produced in lipid peroxidation under oxidative stress. It is involved in the regulation of the cell cycle as well as in the expression of a number of genes. At higher levels of concentration, HNE induces apoptosis in numerous kinds of cells. HNE also induces Daxx translocation from the nucleus to the cytoplasm, where Daxx binds to Fas and then inhibits the apoptosis mediated by Fas. This may be a self-protective mechanism in cells under stress.[9] Jung *et al*.[4] revealed that chemical hypoxia treatment could induce Daxx expression and translocation into the cytoplasm in H9c2 cells, as well as in L6 cells, HeLa cells, Chinese hamster fibroblast cell line PS120, and myocardial cells in neonatal rats. They studied subcellular localizations of Daxx using the nuclear export inhibitor leptomycin B (LMB) and Daxx mutants localized in different compartments of the cells. In H92c cells, Daxx mutants W621A and S667A were localized in the cytoplasm and the nucleus, respectively, and the localization was not susceptible to the influence of chemical hypoxia. However, with chemical hypoxia, LMB treatment could prevent the nuclear export of Daxx mutants and endogenous Daxx. Both treatment with LMB and transfection with wild-type Daxx or Daxx S667A in H9c2 cells could inhibit cell death induced by chemical hypoxia, but transfection with Daxx W621A made cells more susceptible to cell death. Similar findings were seen for HeLa cells. They also found that transfection with wild-type Daxx or Daxx S667A could inhibit the activation of JNK under chemical hypoxia, whereas transfection with Daxx W621A activated JNK (with or without chemical hypoxia). These findings suggested that intranuclear Daxx inhibited JNK, while cytoplasmic Daxx activated JNK. Inhibition on JNK activation could suppress the nuclear export of Daxx, while the blocked nuclear export of Daxx inhibited the activation of JNK, creating a positive feedback loop.

In another study, they found that hydrogen exchanger isoform-1 (NHE-1) interacted with Daxx and mediated cell death induced by chemical hypoxia.[16] Ezrin/radixin/moesin (ERM) can bind to NHE-1 and localize NHE-1 on actin cytoskeleton and activate the cell survival signaling pathway PI3K/Akt-1. Daxx can also bind to NHE-1. Moreover, the binding activity is significantly enhanced by stressors that include ischemia, chemical hypoxia, and glucose deprivation. Under chemical hypoxia, Daxx is exported from the nucleus and co-localized in the cytoplasm with NHE-1, and thus prevents the binding of ERM to NHE-1 and inhibits the activation of the ERM →PI3K →Akt-1 pathway. The Daxx-NHE-1 interaction improves the H+ transport rate as mediated by NHE-1 and increases the intracellular Ca^{2+} level, leading to cell death. When PS120/NHE-1 cells are transfected with Daxx mutants localized in the nucleus and the cytoplasm,

respectively, it is found that only cytoplasmic Daxx can be co-localized with NHE-1, suggesting that subcellular localization of Daxx affects the Daxx-NHE-1 interaction.

In untreated THP1 macrophages, the level of Daxx expression is low and mainly seen in the cytoplasm. When THP1-derived macrophages are treated with oxidized low-density lipoprotein (oxLDL), Daxx concentration in the nucleus increases from 8% to 58%, demonstrating that oxLDL induces Daxx import from the cytoplasm into the nucleus. On the other hand, probucol can effectively suppress the induction on Daxx by oxLDL, as well as the nuclear import of Daxx.[17]

Peptidylprolyl isomerase (Pin1) is a pivotal effector in a number of oncogenic signaling pathways, and is generally overexpressed in numerous human tumors, for instance, the amplification of the Pin1 gene and the overexpression of the protein are seen in cervical cancer cell lines and cervical cancer tissue.[18] Pin1 catalyzes the cis/trans isomerization of the phosphorylated (Ser/Thr)-Pro motif and consequently changes its functions. Ryo *et al.*[19] found that when Pin1 was overexpressed in malignant glioma cells A172, the mRNA level of Daxx was not changed, but Daxx protein decreased, suggesting that Pin1 influenced the stability of Daxx. Further study confirmed that Pin1 bound to the phosphorylated Ser178-Pro motif of Daxx and promoted Daxx degradation via a ubiquitin-proteasome pathway, and subsequently inhibited the apoptosis induced by oxidative stress. They also found that Pin1 and Daxx were co-localized in PML-NBs without H_2O_2 stimulus. When stimulated by H_2O_2, both of them were partially translocated into the cytoplasm.

Conclusions

Daxx is a multifunctional protein that can be localized in subcellular compartments including PML-NBs, nucleoplasm, nucleoli, and cytoplasm. Daxx can be translocated from one subcellular compartment to another, and thus influences its downstream signaling pathways. Daxx regulates the expression of multiple stress-related genes; meanwhile the localization and expression of Daxx are also changed. The subcellular localization of Daxx affects its biologic activity. However, the particular way Daxx localization influences its functions, as well as the relationship between its localization and functions, is not yet conclusive and needs further exploration.

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