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Daxx overexpression in T-lymphoblastic Jurkat cells enhances caspase-dependent death receptor- and drug-induced apoptosis in distinct ways

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Abstract

The role of Daxx, in particular, its ability to promote or hinder apoptosis, still remains controversial. 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. We thus demonstrate that ectopic expression of Daxx substantially increases 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). Analysis of the molecular changes induced in the extrinsic and intrinsic apoptosis pathways reveals that augmentation of apoptosis by Daxx overexpression is conveyed by distinctly different mechanisms. Although enforced apoptosis caused by ectopic Daxx expression is caspase-dependent in both cases, major differences between Fas/TRAIL-induced apoptosis and doxorubicin-induced apoptosis are observed in expression patterns of X-linked inhibitor of apoptosis (XIAP), p53, Bid, ZIP kinase, and prostate apoptosis response gene 4 (Par-4). Moreover, we could show that addition of a CD95 blocking antibody to the clones treated with doxorubicin was able to increase apoptosis as compared to doxorubicin treatment alone and was accompanied by an enhancement of the mitochondrial branch of apoptosis. In conclusion, we here outline the major molecular mechanisms underlying the apoptosis-promoting effect of Daxx in neoplastic lymphocytes and demonstrate fundamental molecular differences elicited by the overexpression of Daxx in the extrinsic and intrinsic signaling pathways.

Keywords: Daxx; Fas; TRAIL; Doxorubicin; Apoptosis

1. Introduction

The death associated protein (Daxx) has emerged as a multifunctional protein implicated in a growing number of important apoptotic pathways. Originally, a yeast two hybrid screening identified Daxx as a Fas-binding protein depicting Daxx as a proapoptotic protein able to enhance the extrinsic pathway of apoptosis through Jun N-terminal kinase activation [1]. This observation has since been supplemented by a large number of subsequent experiments demonstrating a variety of different functions of Daxx in the cytoplasmatic as well as the nuclear subcellular localization. In the cytoplasmatic compartment

Abbreviations: Daxx, death-associated protein; Par-4, prostate apoptosis response gene 4; XIAP, X-linked inhibitor of apoptosis; cIAP, cellular inhibitor of apoptosis; Caspases, cysteine proteases that cleave after aspartatic acids; PARP, Poly-(ADP-ribose)-polymerase; Bcl-2, B-cell lymphoma gene 2; Bax, Bcl-associated X-protein; Bax, Bak, Bid, proapoptotic members of the Bcl-2 family; Bcl-2, Bcl-xL, anti-apoptotic members of the Bcl-2 family; IC 50, inhibiting concentration 50%; JC-1, 5,5',6,6'-tetrachloro-1,1',3,3'-tetraethyl-benzimidazol-carbocyanine-iodide.

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Daxx has been described as an interaction partner of apoptosis signal-regulating kinase 1 (ASK1), an apical kinase in the JNK and p38 pathway system, thereby acting as a promoter of apoptosis [2]. Furthermore, the functional interaction of Daxx with the Fas receptor is supported by the observation that the proapoptotic activity of Daxx can be inhibited by c-FLIP$_L$ [3], the long form of the FLICE inhibitor protein.

In the nuclear compartment, Daxx has been depicted as a transcriptional repressor, interacting among others with nuclear proteins such as the centromeric protein CENP-C as well as Pax-3, Pax-5, histone deacetylases and PML in nuclear bodies [4–8]. In addition, there is evidence that HIPK1 kinase could translocate Daxx from the PML nuclear bodies to the cytoplasm, thereby regulating its repressive activity [9]. Another mechanism of Daxx-controlled apoptosis was lately put forward by Kawai et al. [10] involving the phosphorylation of Daxx and the proapoptotic protein prostate apoptosis response gene 4 (Par-4) by ZIP kinase in nuclear bodies. Nevertheless, the exact role of Daxx in apoptosis is still controversial and even the proapoptotic properties of Daxx have been put into question by data from Michaelson et al. [11,12] showing anti-apoptotic attributes of Daxx in experiments with Daxx −/− mice and RNAi.

After demonstrating the importance of Daxx as an important factor in association with good response to drug-induced apoptosis in lymphoma cells [13], we were interested in the influence of Daxx overexpression on the extrinsic and intrinsic apoptosis pathways and drug-induced apoptosis in this cell entity. We therefore stably transfected Jurkat T-cells with a Daxx-overexpressing construct or a respective mock vector. Taking into account the relevance of extrinsic apoptosis signaling in Jurkat T-cells, we carried out apoptosis induction experiments with the death receptor agonists Fas and TRAIL and in parallel experiments induced apoptosis with doxorubicin (DOX), an established drug in lymphoma treatment.

Thereby, we could demonstrate that overexpression of Daxx strongly promotes apoptosis as compared to mock transfected cells upon treatment with Fas/TRAIL agonists as well as with doxorubicin. Interestingly, the enhancement of apoptosis by Daxx overexpression in these cells proved to be caspase-dependent and furthermore involved distinctly different mechanisms, depending on the apoptotic stimulus: death receptor agonist or cytotoxic drug.

2. Materials and methods

2.1. Cell culture

Jurkat T-cells were cultured at 37 °C under 5% CO_2 in RPMI 1640 (Gibco, Paisley, Scotland) supplemented with 10% fetal calf serum (Gibco), 2 mM L-glutamine (Gibco), and a 1% penicillin–streptomycin mixture (Gibco). For all experiments cells were seeded in a concentration of 25×10^4 cells/ml.

2.2. Antibodies

The anti-pro-caspase-6 antibody was purchased from Becton Dickinson (Heidelberg, Germany), the anti-pro-caspase-7 antibody and anti-Bid antibody from PharMingen (Heidelberg, Germany), the anti-α/β-tubulin antibody from Dunn (Asbach, Germany). The anti-PARP antibody was from Roche (Mannheim, Germany). The anti-pro-caspase-3, -8, -9 antibodies, anti-ZIP kinase, anti-p53, anti-Par-4, anti-Bcl-xL, anti-Bax, anti-Bak and the secondary alkaline-phosphatase or horseradish-peroxidase conjugated antibodies were obtained from Santa Cruz Biotechnology (California, USA), the anti-pro-caspase-10 antibody (4C1) from Medical and Biological Laboratories (Naka-ku, Nagoy, Japan). The anti-Daxx antibody was from Serotec (Oxford, UK), the anti-cIAP-1 and XIAP antibodies from R&D Systems (Wiesbaden, Germany). The anti-CD95 antibody was from Immunotec (Marseille, France). The anti-Bcl-2 antibody was from Dako (Denmark).

2.3. Construction of plasmids and stable transfection

The pCMV-Tag2b expression vector containing the entire open reading frame of Daxx was a gift of Dr. Thomas G. Hofmann (Heinrich-Pette-Institute for Experimental Virology and Immunology, University of Hamburg, Germany). Daxx cDNA was excised by digestion at *Eco*RI and *Rho*I and subsequently cloned into the pcDNA3.1+expression vector (Invitrogen, Carlsbad). Jurkat cells were transfected using TransFast™ Transfection Reagent, selected by treatment with G418 (Serva, Heidelberg, Germany) and cloned under limiting dilution conditions. Clones were assessed for successful pcDNA3.1+/Daxx or pcDNA3.1+control vector transfection by PCR using 5′-ctc act ata ggg aga ccc aag c-3′(sense) and 5′-ggc tgg caa cta gaa ggc aca a-3′(antisense) or or 5′-ttt cga cca gcc ttc tca ttc t-3′(antisense), respectively. Two mock transfected clones (PC-1, PC-2) and three Daxx-overexpressing clones (Daxx-1, Daxx-2, Daxx-3) were chosen for all experiments. In addition, Daxx overexpression was confirmed by Western blotting.

2.4. Induction and detection of apoptosis

Apoptosis of Daxx-transfected Jurkat cells and mock transfected control cells was induced by incubation with 250 ng/ml of the Fas/CD95 agonistic antibody (CH11) (Immunotec) or with 100 ng/ml of soluble recombinant human Trail/APO2L ligand comprising the residues 114–281 (Calbiochem, Darmstadt, Germany) as recommended by the manufacturer and tested elsewhere [14,15]. Blocking of the Fas receptor was carried out with CD95 Fas blocking antibody ZB4 (Immunotec) at a concentration of 1 μg/ml