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Regulation of c-Jun N-terminus kinase activity by Daxx after hydrogen peroxide treatment

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Daxx has been implicated in the regulation of c-Jun N-terminus Kinase (JNK) activity and apoptosis, however, data on Daxx effect on apoptosis are controversial. According to many reports, Daxx participates in activation of JNK and acts as a proapoptotic protein, while others state that Daxx may block activation of JNK and apoptosis. In this study, we investigated hydrogen peroxide-mediated JNK activation and its dependence on the expression level of Daxx protein. Our data show that Daxx overexpression does not effect JNK activation. We have also found that cell lines generated from Daxx gene knockout mice are still capable to respond to H_2O_2 treatment by JNK activation. Reconstitution of wild type Daxx protein expression in Daxx knocked out cells prolongs H_2O_2-induced JNK activation. Moreover, expression of Daxx mutant consisting of its C-terminus dramatically reduces JNK stimulation. Our findings provide an evidence that upon exposure to hydrogen peroxide Daxx prolongs JNK activation dynamics, and the C-terminus of Daxx alone is able to decrease JNK activation and probably participates in the regulation of JNK activation.

Abbrevations. MAPK, mitogen activated protein kinase; ASK1, apoptosis signal regulating kinase 1; JNK, c-Jun N-terminal kinase; ROS, reactive oxygen species.

Key words: Daxx, JNK, hydrogen peroxide

INTRODUCTION

The cell responds to changes of the environment in different ways depending of the type and strength of stimuli. The mitogen-activated protein kinase (MAPK) cascades convey, amplify, and integrate signals from a variety of extracellular stimuli received at the cell surface [1]. The MAPK family consists of three major groups possessing different features and functions. Among MAP kinases, p42/44 extra-cellular regulating kinases (ERKs) are activated by various cytokines and growth factors and play the central role in cell growth and differentiation. On the other hand, c-Jun N-terminal kinases (JNKs) and p38 MAP kinases are usually activated by physical or chemical stress, such as reactive oxygen, UV radiation, heat shock and by proinflammatory cytokines, and these cascades control stress adaptation, cell death and survival. Activated MAPKs translocate to the nucleus where they phosphorylate a variety of target transcription factors [1, 2].

One of the stresses, oxidative stress, is implicated in aging and in a wide range of diseases of the nervous, respiratory, cardiovascular, and gastrointestinal systems in humans [3, 4]. Exposure of the cell to a slight dose of reactive oxygen at the or for a short time induces cell proliferation, migration, or survival, while a high concentration of reactive oxygen or a long time of exposure invoke in the cell necrosis or apoptosis. Numerous studies have shown that in a variety of cell types JNK activates and modulates cell response to H_2O_2 or intracellular reactive oxygen species (ROS). Activation of JNK is regulated by multiple upstream signaling pathways [2]. Beside JNK cascade, ROS also alter the activities and localizations of a wide range of other signal transduction proteins. Recent studies have shown that in the regulation of JNK activation directly involved is the apoptosis-implicated death-associated protein Daxx [6]. Daxx overexpression and RNA interference studies have reported that Daxx is essential for stress-induced cell death and is able to regulate the JNK pathway. However, the function of Daxx in the regulation of apoptosis is not clear [5, 7–9]t. In recent reports, Daxx has been found to interact with and activate the upstream JNK kinase ASK1 after a number of apoptotic inductors [5, 6, 10–12].

In this study, we investigated the role of Daxx in hydrogen peroxide (H_2O_2) induced JNK activation in several cell types. Our results have demonstrated that overexpression of Daxx in the A431 cell line has no effect in JNK activation. However, JNK activation by hydrogen peroxide dramatically decreases in long-time activation in

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Daxx knocked out mouse cells (Daxx-/-). In addition, deletion of the N-terminal part of Daxx (deletion of mutant Daxx-C) significantly impaired JNK activation by H_2O_2.

MATERIALS AND METHODS

Cell culture and DNA constructs. The expression vector pLXSN-Daxx was obtained by cloning the amplified cDNA human Daxx gene into pLXSN using *EcoRI-XhoI* restriction sites. A431 cells overexpressing Daxx were established by infection with virus expression vectors encoding cDNA of Daxx (A-Daxx) or the empty vector pLXSN (A-N) using the retrovirus-mediated transfection–infection protocol following selected by using neomycin (SIGMA, USA). Daxx-/-, DaxxWT, and DaxxC mouse embryonic stem cell lines were described elsewhere [13]. All cell lines were cultured in a DMEM medium containing 10% (vv) fetal calf serum, penicillin (100 Uml) and streptomycin (100 μ/ml) in a 5% CO_2 atmosphere.

Antibodies. Anti-Daxx, anti-JNK and AP-conjugated secondary antibodies were from Santa Cruz. Anti-active JNK antibody was purchased from Promega. Anti-Uba2 antiserum was produced as described previously [14].

Preparation of cell lysate Western blotting. Cell culture containing about 3.0×10^6 cells per dish were synchronized in DMEM medium for 24 h and treated with 300 μM or 1mM H_2O_2 (SIGMA, USA). Cells were lysed in EB lysis buffer (10 mM TrisHCl, pH 7.4, 50 mM NaCl, 5 mM EDTA, 50 mM NaF, 1% Triton X-100, 20 mg/ml Aprotonin, 1 mM PMSF, 2 mM Na_3VO_4, phosphatase inhibitor cocktail II (SIGMA)). The lysate was clarified by centrifugation at 20000 *g* for 15 min. Samples were analyzed by SDS-PAGE, transferred onto PVDF membrane, and probed with the relevant antibodies.

RESULTS

We analyzed JNK activation by hydrogen peroxide (H_2O_2) in different cell lines. We explored A431 cell line with (A-Daxx) or without (A-N) overexpression of Daxx to determinate the influence of the amount of Daxx required for JNK activation (Fig. 1). Activation of JNK with 300 μM of H_2O_2 did not show significant differences between A-N and A-Daxx at different time points of treatment, because endogenous Daxx was sufficient for a complete activation of JNK. Further we tested JNK activation in murine Daxx knockout (Daxx-/-) cells and cells reconstituted with wild-type Daxx (DaxxWT) (Fig. 2). In Daxx-/- cells, JNK activity was peaked at 30 min and at 120 min decreased until the basal level, while in DaxxWT cells JNK remained active for two hours. Apparently depletion of Daxx did not abolish JNK activation, but Daxx was critical for a long-time activation of JNK.

Equally expressed Uba2 was used to compare the expression of Daxx with the total amount of protein.

To investigate the role of Daxx molecule in JNK activation, we used Daxx-/- cells reconstituted with truncated mutants of Daxx that maintain C-terminus of a molecule consisting of 628-740 amino acids (DaxxC) (Fig. 2). Surprisingly, JNK activity after treatment with H_2O_2 in DaxxC cells was very weak. These data suggest that the C-terminus of Daxx is directly or indirectly involved in the downregulation of JNK activity.

DISCUSSION

Accumulating evidence indicates the importance of Daxx in activation of JNK in the cell. The molecular mechanism of JNK cascade activation by Daxx, has been established, in which Daxx acts as an activator of MAPKK kinase ASK1. However, the effect of Daxx in response to various stresses remains controversial. According to the latest publications, Daxx may act either as an activator or suppressor [7]. Noteworthy, to deplete Daxx in the cell, in the experiments RNA interference was used. However, according to our data, even low amounts of Daxx are sufficient to activate JNK. In our experiments we avoided such a risk by using Daxx-/- cells. For the first time we have demonstrated JNK activation by hydrogen peroxide in Daxx-/- cells. Our study clearly shows that Daxx is essential in H_2O_2-induced JNK activation.

In the light of a recent report demonstrating that upon UV stress the dominant negative mutant of Daxx

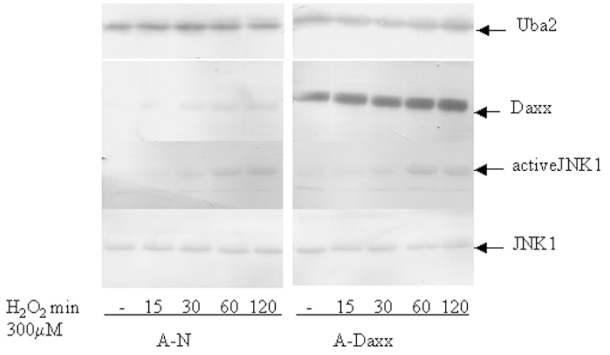

Fig. 1. Hydrogen peroxide activates JNK in A-N and A-Daxx cell lines. Time-depending JNK activation was tested after treatment with 300 μM of H_2O_2. Cell lysates were subjected to Western blot analysis with a specific anti-active JNK antibody. Western blot with anti-Daxx shows the level of endogenous and overexpressed Daxx in the cells.

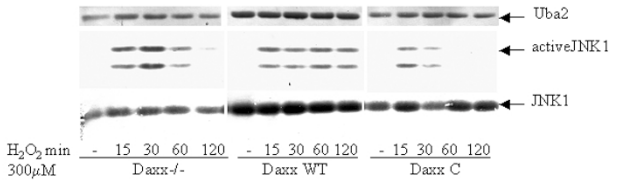

Fig. 2. Daxx-regulated activation of JNK by H_2O_2 in murine Daxx-/- cells. JNK activation with 300 μM H_2O_2 was tested in Daxx-/-, DaxxWT and DaxxC cells at different times. Western blot analysis with anti-active JNK antibody shows JNK activation level. Western blot with Uba2 and JNK specific antibodies refers to equal amounts of proteins in cell lysate

(C-terminus of Daxx) inhibits JNK activation [9], we have studied the activation of JNK by H_2O_2 DaxxC cells. Our findings suggest that the C-terminus of Daxx is critical for JNK activation and participates in the regulation of JNK activity after treatment with H_2O_2.

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References

1. Hommes D W, Pappelenbosch M.P, van Deventer S J H. Gut 2003; 52: 144–151.
2. Davis R J. Cell 2000; 103: 239–52.
3. Das D K, Maulik N, Engelman R M J Cell Mol Med 2004; 8(1): 144–52.
4. Rahman I. Curr Drug Targets Inflamm Allergy 2002; 1(3): 291–315.
5. Khelifi A F, Stagno M, Alcontres D, Salomoni P. Cell Death and Differentiation 2005; 12: 724–33.
6. Song J J, Lee Y J. Cell Biochem 2005; 170(1): 61–72.
7. Michaelson J S. Apoptosis 2000; 5: 217–20.
8. Chen L-Y, Chen J.D. MCB 2003; 23(20): 7108–21.
9. Wu S, Loke H.N, Rehemtulla A. Neoplasia 2002; 4(6): 486–92.
10. Ohiro Y, Usheva A, Kobayashi S, Dyffy S, Nantz R, Gius D, Horikoshi N. MCB 2003; 23: 322–34.
11. Song J J, Lee Y J. Cell Biochem 2004; 92(6): 1257–70.
12. Michaelson J S, Leder P. Journal of Cell Science 2002; 116(2): 3807–20.
13. Ishov A M, Vladimirova O V, Maul G G. Journal of Cell Science 2004; 117(17): 3807–20.
14. Tunaitis V, Ger M, Stoškus M, Valius M. Biologija 2005; 1: 24–6.

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C-JUN N-GALO KINAZĖS AKTYVUMO REGULIAVIMAS DAXX'U PAVEIKUS VANDENILIO PEROKSIDU

Santrauka

Daxx poveikis JNK aktyvavimui ir apoptozės indukcijai jau yra žinomas, bet duomenys gana prieštaringi. Daugelyje straipsnių Daxx aktyvuoja JNK ir veikia kaip proapoptotinis baltymas, tačiau kai kurie autoriai Daxx įvardija kaip JNK aktyvavimą slopinantį ir antiapoptotinį baltymą. Šiame darbe mes tyrėme JNK aktyvavimo vandenilio peroksidu priklausomybę nuo Daxx ekspresijos. Mūsų gautais duomenimis, padidinta Daxx baltymo ekspresija A431 ląstelėse nekeičia JNK aktyvavimo. Tyrime naudotas pelės ląsteles su nokautuotu Daxx genu (Daxx-/-) paveikus H_2O_2 JNK aktyvacija išlieka, tačiau ilgalaikis JNK aktyvavimas vyksta tik DaxxWT ląstelėse. Be to, Daxx-/- ląstelėse, ekspresuojančiose N galo delecinį Daxx mutantą iš molekulės 628-740 amino rūgščių (DaxxC), JNK aktyvavimas paveikus H_2O2 stipriai sumažėjo. Visi šie duomenys rodo, kad Daxx prailgina JNK aktyvavimą, o Daxx baltymo C dalis tiesiogiai ar netiesiogiai blokuoja JNK aktyvavimą vandenilio peroksidu.