Daxx inhibits stress-induced apoptosis in cardiac myocytes

Renata Zabalova^{1,2}, Emma Swettenham¹, Jaromira Chladova², Lan-Feng Dong¹, Jiri Neuzil^{1,2}

¹*Apoptosis Research Group, School of Medical Science, Griffith University and Griffith Institute of Health and Medical Research, Southport, Queensland, Australia*

²*Molecular Therapy Group, Institute of Molecular Genetics, Czech Academy of Sciences, Czech Republic*

The role of the death-associated protein Daxx in modulation of apoptosis induced in cardiac myocytes by oxidative stress was studied. Exposure of cultured cardiomyocyte-like cells to oxidative stress or simulated hypoxia increased the level of accumulated ROS and apoptosis. Under conditions of sub-apoptotic stimulation of cardiac myocytes, there was no increase in the level of the Daxx protein, but it translocated from the nucleus to the cytoplasm. Daxx overexpression protected the cells from apoptosis, while they were sensitised to cell death following its down-regulation by siRNA. Moreover, lowering the level of the Daxx protein sensitised cardiac myocytes to spontaneous apoptosis, suggesting that the protein may also have a pro-survival role under physiological conditions. Finally, it was shown that DJ-1, a protein suggested previously to sequester Daxx in the nucleus under conditions of oxidative stress (thereby preventing its cytosolic translocation), was localised solely in the cytoplasm of cardiac myocytes. This indicates that the protein does not modulate the apoptosis regulatory activity of Daxx in cardiac myocytes by its nuclear sequestration. Taken together, Daxx plays a protective role in cultured cardiomyocyte-like cells, at least under the conditions used.

Keywords: Daxx, apoptosis, cardiomyocytes, oxidative stress

Introduction

Heart failure is the leading cause of death in industrialised countries, with myocardial infarction being the main reason.¹ The physiology of myocardial infarction is well known and has been extensively studied. At the molecular level, however, the process of ischaemia-reperfusion is rather intriguing and still not completely understood.² It triggers many signalling pathways and produces biologically active mediators.²⁻⁴

Reactive oxygen species (ROS) are important mediators of the deleterious effects of ischaemia-reperfusion.⁵ Increase in the level of ROS creates oxidative stress, which causes cell damage with ensuing cell death by way of activating various signalling pathways that, in many cases, converge at apoptosis induction.^{6,7} While the signalling mechanisms have not been completely unravelled, it has been suggested that the protein Daxx plays an important role in the oxidative stress-induced apoptotic pathways.^{4,8}

Daxx (death-associated protein) is a multi-functional protein, ubiquitously expressed and highly conserved in mammals.⁹⁻¹¹ Under normal conditions, Daxx is found in the nucleus, where it localises to sub-nuclear structures referred to as promyelocytic leukaemia protein (PML) bodies or ND-10.¹¹⁻¹⁴ In

Correspondence to: Jiri Neuzil, Apoptosis Research Group, School of Medical Science and Griffith Institute of Health and Medical Research, Griffith University, Southport, 9716 Queensland, Australia.
Tel: +61 2 555 29109; Fax: +61 2 555 28444;
E-mail: j.neuzil@griffith.edu.au

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response to oxidative stress, UV radiation or other stimuli, Daxx translocates into the cytoplasm, where it can partake in various signalling pathways.^{15,16} Studies have demonstrated that Daxx exerts both pro- and anti-apoptotic functions.^{9,11,17–20} It is involved in different apoptotic routes, such as the Fas-mediated apoptotic pathway^{9,10,21} or the TGF β -induced apoptotic signalling,^{15,16} and also appears to function in cell survival as well as in transcriptional co-repression.^{13,18–25}

One of the most studied and important pathways involving Daxx is the aforementioned Fas-mediated pro-apoptotic signalling pathway,^{26,27} which is known to respond to oxidative stress and to trigger apoptosis. Daxx has been found to translocate from the nucleus into the cytoplasm in response to oxidative stress, bind to the Fas receptor, activate down-stream kinases (*e.g.* ASK1), and induce apoptosis via the JNK pathway.^{9,10,28,29} Various studies have shown an increased level of Daxx due to hypoxia and other forms of oxidative stress, leading to more pronounced apoptosis in cell lines.^{4,8} However, the majority of these studies have been performed with malignant cell lines.

Oxidative stress has been implicated to play an important role in myocardial infarction, and Daxx responds to, and is activated by, oxidative stress.^{4,8,16} Therefore, apoptosis induced in the heart after myocardial infarction by oxidative stress would seem to implicate the protein. Hence, we decided to study the role of Daxx in cardiac myocytes to gain better understanding of the role of this protein in cardiac cells in response to oxidative stress and to determine if it modulates cell survival.

Materials and Methods

Cell culture conditions and treatment

H9c2 rat cardiomyoblasts were obtained from the American Type Culture Collection (ATCC; Rockville, MD, USA) and cultured in complete Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS), 100 U/ml penicillin and 100 μ g/ml streptomycin. The HL-1 mouse atrial cardiac muscle cells were provided by Dr W.C. Claycomb and cultured in the complete Claycomb medium supplemented with 10% FBS, 0.1 mM noradrenaline, 2 mM glutamine, and antibiotics.³⁰ The human endothelial cell line EAhy 926 was maintained in DMEM supplemented with 10% serum, the HAT supplement and antibiotics,³¹ while the human mesothelioma cell lines MM-BI and Meso-2 were maintained in DMEM plus 10% serum and

antibiotics. The cells were treated with various concentrations of H₂O₂ and CoCl₂ in order to establish the sub-apoptotic to apoptotic levels of the stressors.

Apoptosis detection

Apoptosis was quantified using the annexin V-FITC method, which detects phosphatidyl serine in the early phases of apoptosis.³³ At the indicated times, cells were harvested, incubated with the annexin V mixture (200 μ l PBS, 20 μ l annexin-binding buffer [10 $\times$], 2 μ l annexin V-FITC; PharMingen), and analysed by flow cytometry (FACScalibur, Becton Dickinson) for green fluorescence.

Assessment of ROS formation

To monitor cellular production of ROS, a cell-permeable probe, dihydroethidium (DHE; Molecular Probes) was used. At the indicated times, cells were harvested, resuspended in the DHE mixture (5 μ M solution of DHE in PBS), incubated in the dark at 37°C for 15 min, and analysed by flow cytometry.

Cell transfections

H9c2 cells were seeded at a density of 2×10^5 cells/well in a 6-well tissue culture plate and transfected with 2 μ g of pcDNA3 (negative control) or pcDNA3-Daxx (provided by Dr L. Andera, Institute of Molecular Genetics, Prague, Czech Republic) using Lipofectamine-2000 (Invitrogen) according to the manufacturer's instructions. Cells were incubated for 4 h in OptiMEM, which was then replaced by complete growth media. After 48 h, DMEM supplemented with G418 was added to the cells and the selection process was allowed to continue for 3 weeks.

Small interfering RNA (siRNA) transfections

The cells were seeded in the growth medium without antibiotics and allowed to reach 50% confluency. The cells were then transfected with 60 pmol of non-silencing siRNA (negative control) and Daxx siRNA (Prologo) using Oligofectamine as the transfection reagent according to the manufacturer's instructions (Invitrogen), essentially as described elsewhere.³² RT-PCR was used to assess the efficacy of Daxx down-regulation, which was 70–80% (data not shown).

Western blot analysis

Cells were treated as indicated and lysed in whole cell lysis buffer³² or in buffers for the cytoplasmic (10 mM

HEPES, pH 7.8, 10 mM KCl, 2 mM $MgCl_2$, 0.1 mM EDTA, 1 mM DTT and 0.1 mM PMSF) and nuclear fractions (50 mM HEPES, pH 7.8, 50 mM KCl, 300 mM NaCl, 0.1 mM EDTA, 1 mM DTT, 0.1 mM PMSF and 25% glycerol, v/v). Cell lysates were separated by SDS-PAGE and transferred to a nitrocellulose membrane, which was incubated with anti-Daxx IgG (Cell Signaling), anti-PARP IgG, or anti-DJ-1 IgG (both Santa Cruz). After incubation

with HRP-conjugated secondary IgG (Santa Cruz), the blots were developed using the ECL kit (Pierce).

Flow cytometry

The level of expression of the Daxx protein in cells transfected with pcDNA3-Daxx or Daxx siRNA was routinely assessed by flow cytometry using the FACScalibur instrument (Becton Dickinson). In brief,

Figure 1 Cardiomyocyte-like cells are susceptible to oxidative stress and simulated hypoxia. H9c2 (A,B,D,E) and HL-1 cells (C,F,G) were plated to reach about 50% confluency, and exposed to H_2O_2 (A,D,F) or $CoCl_2$ (B,C,E,G) at the concentration (μ M) and for the times (h) shown, or for the period of 12 h (D–F). The cells were then assessed by flow cytometry for ROS generation using the fluorescent label DHE (expressed as increase in mean fluorescence intensity, MFI) (A–C) or for apoptosis levels using annexin V-FITC (D–G). The data are mean values $\pm$ SD derived from three independent experiments. *Statistically different data from the control in (A–C) and from the 'zero' concentration in (D–G), with $P < 0.05$

Figure 2 Daxx is expressed in CEM-like cells and responds to stress. (A) HL-1, H9c2, EAhy 926, MM-BI and Meso-2 cells were probed for the level of the Daxx protein by Western blotting. (B) Daxx expression was assessed by Western blotting in HL-1 cells after treatment with CoCl_2 and H_2O_2 at concentrations (μM) as shown. (C) H9c2 cells were fractionated into the cytosolic (C) and nuclear compartment (N), and probed for Daxx protein expression by Western blotting. The nuclear protein PARP was used as a control for the purity of the fractions. (D) H9c2 cells were plated on cover slips, treated for 12 h with 250 μM H_2O_2 and assessed for Daxx compartmentalisation using confocal microscopy. Arrows indicate nuclei positive or negative for the protein. (E) HL-1 cells were exposed to CoCl_2 at 500 μM and for times shown, and assessed by Western blotting for the level of HIF-1 α . The amount of loaded protein was equal for all samples in all Western blots performed. Images shown are representative of at least three independent experiments

cells were fixed with NBF, permeabilised using a 0.2% solution of saponin, and incubated with anti-Daxx IgG followed by incubation with FITC-conjugated secondary IgG (Santa Cruz). The relative level of expression of Daxx was based on the percentage of cells with high and low green fluorescence.

Confocal microscopy

Cells were plated on cover-slips, allowed to recuperate for 24 h, and incubated for 30 min at room temperature with neutral-buffered formalin (NBF; 3.7% formaldehyde in PBS). The cells were then permeabilised with a mixture of PBS, 2% FCS and 0.2% saponin for 30 min at room temperature, incubated with the primary IgGs (diluted in PBS with 2% FCS and 0.2% saponin) for 60 min at room temperature, and incubated further with a secondary antibody (FITC- or Cy3-conjugated IgG; Santa Cruz) in the same manner. Cells were then mounted using

Vectashield with DAPI and observed under the TCS-SP microscope (Leica).

Statistical analysis

Images shown are representative of at least three independent experiments. The data shown are expressed as mean $\pm$ SD ($n = 3$). Statistically significant differences were considered at $P < 0.05$, as calculated using the Student's t -test.

Results and Discussion

Daxx is an intriguing protein with contradictory functions, possibly depending on its compartmentalisation, level and cell type. Reports to date mostly show Daxx to modulate apoptosis in cancer cells.^{4,9-11} Data on the possible role of Daxx in non-malignant cells, such as cardiac myocytes, are scant.³⁴⁻³⁶ In this

Figure 3 Increase of Daxx levels suppresses susceptibility of cardiomyocyte-like cells to stress. Daxx-overexpressing cells (Daxx) or cells transfected with the empty vector (EV) were exposed for 12 h or as shown to H₂O₂ (A,C) or CoCl₂ (B,D,E) at the concentrations shown (μ M). The cells were then assessed for ROS accumulation (A,B), apoptosis induction (C,D), and for Daxx compartmentalisation in the cytoplasmic (C) and nuclear fractions (N). (E) The insert in (A) shows the level of expression of the Daxx protein in parental cells (1), cells transfected with the empty vector (2) and with pcDNA3-Daxx (3). The data are mean values $\pm$ SD derived from three independent experiments. *Indicates statistically different data from the control; **statistically different data for the treated cells transfected with pcDNA3 and pcDNA3-Daxx, with $P < 0.05$.

study we explored the role of the protein in apoptosis induced in two types of cardiomyocyte-like cells undergoing simulated ischaemia-reperfusion.

First, we established conditions of stress which would result in toxicity to cardiac myocytes. As a form of oxidative stress we used H₂O₂, that can undergo conversion to ROS catalysed, for example, by the cardiomyocyte myoglobin,³⁷ and CoCl₂, which has been reported to mimic hypoxia by augmenting ROS generation during normoxia.^{38,39} There was a significant increase in ROS formation in both cardiomyocyte-like H9c2 and HL-1 cells in response to the treatments (Fig. 1A–C). We next sought to establish to what extent stress conditions leading to ROS accumulation affect the level of apoptosis in cardiac myocytes. Apoptosis increased to some extent in both cell lines in response to treatment with both H₂O₂ and CoCl₂ (Fig. 1D–G), indicating that oxidative

stress activates pathways resulting in apoptosis induction.

Various studies have shown that Daxx is a nuclear protein localised in sub-nuclear structures called PML bodies, expressed in different, mainly malignant, cell lines.^{8–13} Western blot analysis revealed that the Daxx protein is ubiquitously expressed in various cell lines, including the endothelial cell line EAhy926 and the malignant mesothelioma cell lines MM-BI and Meso-2, as well as in the cardiomyocyte-like HL-1 and H9c2 cells (Fig. 2A). Western blotting and confocal microscopy revealed nuclear localisation of the Daxx protein (Fig. 2C,D); the micrographs suggest that Daxx is not localised in the PML bodies in cardiac myocytes, but dispersed throughout the nucleus. Different studies have reported that Daxx, in response to oxidative stress, increases its expression and/or translocates from the nucleus into the cytoplasm,

Figure 4 Daxx down-regulation promotes apoptosis in cardiomyocyte-like cells. HL-1 cells were treated with NS siRNA or Daxx siRNA, and assessed for apoptosis without treatment (A) or after exposure to H_2O_2 at the concentration (μM) and times (B) shown, or to $CoCl_2$ for 24 h and at the concentration shown (μM) (C). Controls in (B,C) show spontaneous apoptosis of cells pre-treated with Daxx siRNA and exposed to the vehicle only. All data in (B,C) are presented after subtraction of spontaneous apoptosis (HL-1 cells with no siRNA pre-treatment and no treatment) to indicate levels of expression of the Daxx protein in control cells (1), and in cells exposed to NS siRNA (2) or Daxx siRNA (3). The data are mean values $\pm$ SD derived from three independent experiments. *Indicates statistically different data from the control, with $P < 0.05$

Figure 5 Expression of Daxx and DJ-1 in cardiomyocyte-like cells. (A) H9c2 cells were plated on cover slips and probed by confocal microscopy for the expression of Daxx using Cy3 (red) and DJ-1 using FITC (green) before and after 12 h exposure to $250 \mu M H_2O_2$. H9c2 cells were exposed to $CoCl_2$ (B) or H_2O_2 (C) at the concentration (μM) and for times shown, and probed for DJ-1 expression using Western blotting. The amount of loaded protein was equal for all samples in all Western Blots performed. The images are representative of three independent experiments

which appears to be dependent on the cell type.^{4,7,14} We found no increase in the level of the Daxx protein in cardiac myocytes in response to CoCl_2 treatment, although it appeared to decrease at high concentration of the stressor (Fig. 2B), possibly due to a more advanced apoptosis stage with non-specific proteolytic events. Expression of Daxx did not appreciably change after H_2O_2 exposure of the cells either (Fig. 2B). Exposure of cardiac myocytes to H_2O_2 caused translocation of the protein into the cytosol (Fig. 2D), where it can partake in processes such as the oxidative stress-induced Fas-mediated apoptotic pathway.^{25,26} That CoCl_2 does cause simulated hypoxia is shown in Figure 2E, documenting accumulation of the hypoxia-responsive transcription factor HIF-1 α upon exposure of cardiac myocytes to the stressor.

The above data reveal changes in the localisation of the Daxx protein in cardiac myocytes upon their exposure to stress, although it is not clear whether Daxx is pro- or anti-apoptotic in this cell type. We, therefore, manipulated the levels of the protein in cardiac myocytes. Overexpression of the Daxx protein lowered the levels of ROS accumulated in the cells exposed to both H_2O_2 and CoCl_2 (Fig. 3A,B), and it resulted in less apoptosis in the stress-induced cells (Fig. 3C,D), when compared to their non-transfected counterparts (*cf.* Fig. 1). Transfection of the cells with pcDNA3-Daxx resulted in the appearance of the protein in the cytosol even in unstressed cells, with more Daxx mobilised to the cytosol upon exposure of the Daxx-overexpressing cells to CoCl_2 (Fig. 3E).

Conversely, we studied apoptosis in cardiac myocytes in which the expression of the Daxx protein was down-regulated by treatment with Daxx siRNA. Lowering the level of the Daxx protein itself in cardiac myocytes sensitised the cells to spontaneous apoptosis (Fig. 4A), indicating that the protein may act as a survival factor in cardiac myocytes under conditions of homeostasis. Pre-treatment of cardiac myocytes with Daxx siRNA further sensitised the cells to both H_2O_2 - and CoCl_2 -induced apoptosis (Fig. 4B,C). Although this result contradicts most published data on the role of Daxx, it needs to be emphasized that those were obtained from experiments with malignant cells, while we used the non-malignant cardiac myocyte-like cell lines in our study.

An interesting aspect of the mechanism by which Daxx regulates apoptosis is the role of the redox-sensitive protein DJ-1,⁴⁰ which has been suggested to sequester Daxx in the nucleus under conditions of oxidative stress, thereby preventing its mobilisation to the cytoplasm.⁴¹ We assessed DJ-1 compartmentalisation in cardiac myocytes and found it to be solely cytosolic, as documented by confocal microscopy of H9c2 cells; there was no change in

DJ-1 protein localisation following H_2O_2 treatment of the cells (Fig. 5A). On the other hand, the same treatment showed mobilisation of the originally nuclear Daxx to the cytosol, where it may co-localise with DJ-1 (Fig. 5A; *cf.* Fig. 2D). Western blotting of DJ-1 in HL-1 cells exposed to either CoCl_2 or H_2O_2 revealed appearance of the protein at a larger size (Fig. 5B,C), indicative of its SUMOylation as reported earlier.¹²

We show, in this report, that Daxx translocates from the nucleus to the cytosol upon exposure of cardiomyocyte-like cells to stress. Further, we document that the protein appears to be anti-apoptotic in these cells, since its increase makes the cells less susceptible and its down-regulation sensitises them to stress-induced apoptosis. Interestingly, too, down-regulation of the Daxx protein itself resulted in spontaneous apoptosis in cardiac myocytes, suggesting that the protein may have a pro-survival role under physiological conditions. Moreover, we document that, in cardiac myocytes, DJ-1 does not sequester Daxx in the nucleus following an oxidative stress insult, as suggested in a recent report.⁴¹ In fact, it appears that the notion that DJ-1 may prevent Daxx from cytosolic translocation is based on experiments in which the protein was overexpressed in cells by their transfection and found in the nucleus.⁴¹ In parental cells with natural endogenous DJ-1, the protein was, therefore, found only in the cytosol (V. Dawson, personal communication), consistent with its location documented in this report (*cf.* Fig. 5A).

Conclusions

Daxx plays an anti-apoptotic role in model cells of cardiac myocytes, potentially protecting the cells under conditions of oxidative stress.

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References

1. Yellon DM, Hausenloy DJ. Myocardial reperfusion injury. *N Engl J Med* 2000; **357**: 1121–1135.
2. Neuzil J, Rayner BS, Lowe HC, Witting PK. Oxidative stress in myocardial ischemia reperfusion injury: a renewed focus on a long-

- standing area of research. *Redox Report* 2005; **10**: 185–195.
3. Nishida M, Maruyama Y, Tanaka R, Kontani K, Nagao T, Kurose H. G_{al} and G_{ox} are target proteins of reactive oxygen species. *Nature* 2000; **408**: 492–495.
4. Kim KS, Hwang HA, Chae SK, Ha H, Kwon KS. Upregulation of Daxx mediates apoptosis in response to oxidative stress. *J Cell Biochem* 2005; **96**: 330–338.
5. Neuzil J, Widen C, Gellert N et al. Mitochondria transmit apoptotic signals in cardiomyocyte-like cells and isolated hearts exposed to experimental ischemia-reperfusion injury. *Redox Report* 2007; **12**: 148–162.
6. Chandra J, Samali A, Orrenius S. Triggering and modulation of apoptosis by oxidative stress. *Free Radic Biol Med* 2000; **29**: 323–333.
7. Mascareno E, El-Shafei M, Maulik N, Sato M, Guo Y, Das DK, Siddiqui MA. JAK/STAT signaling is associated with cardiac dysfunction during ischemia and reperfusion. *Circulation* 2001; **104**: 325–329.
8. Song JJ, Lee YJ. Catalase, but not MnSOD, inhibits glucose deprivation-activated ASK1-MEK-MAPK signal transduction pathway and prevents relocalization of Daxx: hydrogen peroxide as a major second messenger of metabolic oxidative stress. *J Cell Biochem* 2003; **90**: 304–314.
9. Yang X, Khosravi-Far R, Chang HY, Baltimore D. Daxx, a novel Fas-binding protein that activates JNK and apoptosis. *Cell* 1997; **89**: 1067–1076.
10. Chang HY, Nishitoh H, Yang X, Ichijo H, Baltimore D. Activation of apoptosis signal-regulating kinase 1 (ASK1) by the adaptor protein Daxx. *Science* 1998; **281**: 1860–1863.
11. Zhong S, Salomoni P, Ronchetti S, Guo A, Ruggero D, Pandolfi PP. Promyelocytic leukemia protein (PML) and Daxx participate in a novel nuclear pathway for apoptosis. *J Exp Med* 2000; **191**: 631–640.
12. Ishov AM, Sotnikov AG, Negorev D et al. PML is critical for ND10 formation and recruits the PML-interacting protein Daxx to this nuclear structure when modified by SUMO-1. *J Cell Biol* 1999; **147**: 221–234.
13. Li H, Leo C, Zhu J, Wu X, O'Neil J, Park EJ, Chen JD. Sequestration and inhibition of Daxx-mediated transcriptional repression by PML. *Mol Cell Biol* 2000; **20**: 1784–1796.
14. Salomoni P, Pandolfi PP. The role of PML in tumor suppression. *Cell* 2000; **108**: 165–170.
15. Perlman R, Schiemann WP, Brooks MW, Lodish HF, Weinberg RA. TGF- β -induced apoptosis is mediated by the adaptor protein Daxx that facilitates JNK activation. *Nat Cell Biol* 2001; **3**: 708–714.
16. Khelifi AF, D'Alcontres MS, Salomoni P. Daxx is required for stress-induced cell death and JNK activation. *Cell Death Differ* 2005; **12**: 724–733.
17. Chang HY, Yang X, Baltimore D. Dissecting Fas signaling with an altered-specificity death-domain mutant: requirement of FADD binding for apoptosis but not Jun N-terminal kinase activation. *Proc Natl Acad Sci USA* 1999; **281**: 1860–1863.
18. Michaelson JS, Bader D, Kuo F, Kozak C, Leder P. Loss of Daxx, a promiscuously interacting protein, results in extensive apoptosis in early mouse development. *Genes Dev* 1999; **13**: 1918–1923.
19. Chen LY, Chen JD. Daxx silencing sensitizes cells to multiple apoptotic pathways. *Mol Cell Biol* 2003; **23**: 7108–7121.
20. Michaelson JS, Leder P. RNAi reveals anti-apoptotic and transcriptionally repressive activities of DAXX. *J Cell Sci* 2003; **116**: 345–352.
21. Ko YG, Kang YS, Park H et al. Apoptosis signal-regulating kinase 1 controls the proapoptotic function of death-associated protein (Daxx) in the cytoplasm. *J Biol Chem* 2001; **276**: 39103–39106.
22. Lehembre F, Muller S, Pandolfi PP, Dejean A. Regulation of Pax3 transcriptional activity by SUMO-1-modified PML. *Oncogene* 2001; **19**: 1–9.
23. Kiriakidou M, Driscoll DA, Lopez-Guisa JM, Strauss JF. Cloning and expression of primate Daxx cDNAs and mapping of the human gene to chromosome 6p21.3 in the MHC region. *DNA Cell Biol* 1997; **16**: 1289–1298.
24. Michaelson JS. The Daxx enigma. *Apoptosis* 2000; **5**: 217–220.
25. Kim EJ, Park JS, Um SJ. Identification of Daxx interacting with p73, one of the p53 family, and its regulation of p53 activity by competitive interaction with PML. *Nucleic Acids Res* 2003; **31**: 5356–5367.
26. Krammer PH. CD95(APO-1/Fas)-mediated apoptosis: live and let die. *Adv Immunol* 1999; **71**: 163–210.
27. Hsu H, Xiong J, Goeddel DV. The TNF receptor 1-associated protein TRADD signals cell death and NF- κ B activation. *Cell* 1995; **81**: 495–504.
28. Zhang J, Gao JX, Salojin K et al. Regulation of fas ligand expression during activation-induced cell death in T cells by p38 mitogen-activated protein kinase and c-Jun NH2-terminal kinase. *J Exp Med* 2000; **191**: 1017–1030.
29. Curtin JF, Cotter TG. Live and let die: regulatory mechanisms in Fas-mediated apoptosis. *Cell Signal* 2003; **15**: 983–992.
30. Claycomb WC, Lanson NA, Stallworth et al. HL-1 cells: a cardiac muscle cell line that contracts and retains phenotypic characteristics of the adult cardiomyocyte. *Proc Natl Acad Sci USA* 1998; **95**: 2979–2984.
31. Dong LF, Swettenham E, Eliasson J et al. Vitamin E analogs inhibit angiogenesis by selective apoptosis induction in proliferating endothelial cells: the role of oxidative stress. *Cancer Res* 2007; **67**: 11906–11913.
32. Stapelberg M, Gellert N, Swettenham E et al. α -Tocopheryl succinate inhibits malignant mesothelioma by disrupting the FGF autocrine loop: the role of oxidative stress. *J Biol Chem* 2005; **280**: 25369–25376.
33. Weber T, Dalen H, Andera L et al. Mitochondria play a central role in apoptosis induced by α -tocopheryl succinate, an agent with anticancer activity. *Biochemistry* 2003; **42**: 4277–4291.
34. Yaniv G, Shilkrot M, Larisch S, Binah O. Hydrogen peroxide predisposes neonatal rat ventricular myocytes to Fas-mediated apoptosis. *Biochem Biophys Res Commun* 2005; **336**: 740–746.
35. Jung YS, Kim HY, Lee YJ, Kim E. Subcellular localization of Daxx determines its opposing functions in ischemic cell death. *FEBS Lett* 2007; **581**: 843–852.
36. Roubille F, Combes S, Leal-Sanchez J et al. Myocardial expression of a dominant-negative form of Daxx decreases infarct size and attenuates apoptosis in an *in vivo* mouse model of ischemia/reperfusion injury. *Circulation* 2007; **116**: 2709–2717.
37. Witting PK, Liao WQ, Harris MJ, Neuzil J. Expression of wild-type and the Y103F variant of human myoglobin in H9c2 myocyte-like cells enhances toxicity toward added hydrogen peroxide. *Biochem Biophys Res Commun* 2006; **348**: 485–493.
38. Chandel NS, Maltepe E, Goldwasser E, Mathieu CE, Simon MC, Schumacker PT. Mitochondrial reactive oxygen species trigger hypoxia-induced transcription. *Proc Natl Acad Sci USA* 1998; **95**: 11715–11720.
39. Ivan M, Kondo K, Yang H et al. HIF α targeted for VHL-mediated destruction by proline hydroxylation: implications for O₂ sensing. *Science* 2001; **292**: 464–468.
40. Dawson TM, Dawson VL. Molecular pathways of neurodegeneration in Parkinson's disease. *Science* 2003; **302**: 819–822.
41. Junn E, Taniguchi H, Jeong BS, Zhao X, Ichijo H, Mouradian MM. Interaction of DJ-1 with Daxx inhibits apoptosis signal-regulating kinase 1 activity and cell death. *Proc Natl Acad Sci USA* 2005; **102**: 9691–9696.