Available online at www.sciencedirect.com
ScienceDirect
Biochemical and Biophysical Research Communications 353 (2007) 92–97
BBRC
www.elsevier.com/locate/ybbrc

c-Jun induces apoptosis of starved BM2 monoblasts by activating cyclin A-CDK2

Petr Vaňhara [a], Vítězslav Bryja [b], Viktor Horváth [c], Alois Kozubík [c], Aleš Hampl [d,e,f], Jan Šmarda [a,*]

[a] Institute of Experimental Biology, Faculty of Science, Masaryk University, Brno, Czech Republic
[b] Department of Medical Biochemistry and Biophysics, Karolinska Institute, Stockholm, Sweden
[c] Institute of Biophysics, Academy of Sciences of the Czech Republic, Brno, Czech Republic
[d] Institute of Biology, Faculty of Medicine, Masaryk University, Brno, Czech Republic
[e] Center for Cell Therapy and Tissue Repair, Charles University, Prague, Czech Republic
[f] Institute of Experimental Medicine, Academy of Sciences of the Czech Republic, Prague, Czech Republic

Received 19 November 2006
Available online 4 December 2006

Abstract

c-Jun is one of the major components of the activating protein-1 (AP-1), the transcription factor that participates in regulation of proliferation, differentiation, and apoptosis. In this study, we explored functional interactions of the c-Jun protein with several regulators of the G1/S transition in serum-deprived v-*myb*-transformed chicken monoblasts BM2. We show that the c-Jun protein induces expression of cyclin A, thus up-regulating activity of cyclin A-associated cyclin-dependent kinase 2 (CDK2), and causing massive programmed cell death of starved BM2cJUN cells. Specific inhibition of CDK2 suppresses frequency of apoptosis of BM2cJUN cells. We conclude that up-regulation of cyclin A expression and CDK2 activity can represent important link between the c-Jun protein, cell cycle machinery, and programmed cell death pathway in leukemic cells.

Keywords: Jun; Cyclin A; CDK2; Apoptosis; v-*myb*-transformed monoblasts

c-Jun is a major subunit of transcription factor AP-1, which controls the key cellular processes, including proliferation, differentiation, apoptosis, and oncogenic transformation. The c-*jun* gene belongs to a group of immediate early genes that are activated in response to a variety of cytokines through MAP or JNK/SAPK kinase pathways. It codes for the c-Jun protein that participates in control of cell cycle and apoptosis, thus having major impact on carcinogenesis [1–3]. It has been shown that c-Jun is associated with endometrial cancer [4], breast cancer [5,6], and many types of leukemia [7,8]. In addition, the Bcr-Abl protein chimera can activate the Jun N-terminal kinase (JNK), thus activating transcription of the *jun* gene in chronic myeloid leukemia (CML) [9]. Dominant negative c-Jun mutants can impair the Bcr-Abl-induced transformation [9]. The c-Jun can also interact with Pml transcription coactivator [10] and p73 protein, thus enhancing cell response to various DNA-damaging agents, such as *cis*-platin [11,12].

To address the role of the c-Jun protein in leukemic cells, we previously established and partially characterized the v-*myb*-transformed chicken monoblasts [13] inducibly expressing c-*jun* from human metallothionein II_A promoter (BM2cJUN) [14]. Exogenous c-Jun significantly suppressed growth and induced differentiation of BM2cJUN cells [14]. In this study, we show that c-Jun can also induce apoptosis of these cells once they are cultured in serum-deprived conditions. This effect is mediated by cyclin A that is

* Corresponding author. Fax: +420 54949 5533.
E-mail address: smarda@sci.muni.cz (J. Šmarda).

0006-291X/$ - see front matter © 2006 Elsevier Inc. All rights reserved.
doi:10.1016/j.bbrc.2006.11.124

significantly up-regulated in c-Jun-expressing BM2cJUN cells and causes activation of the CDK2 kinase.

Materials and methods

Cell cultivation. The line of v-*myb*-transformed chicken monoblasts (BM2), its c-*jun*-expressing derivative (BM2cJUN) and the empty-vector transfected BM2CD4 cells were described earlier [13–15]. The cells were cultured in DME medium as described elsewhere [14] supplemented with either 5% fetal calf and 5% chicken sera (normal serum, NS) or 0.2% fetal calf and 0.2% chicken sera (low serum, LS). The cells were seeded at a starting density of 2×10^5 cells/ml (NS) or 4×10^5 cells/ml (LS), respectively, and cultured for 48 h. Then, they were treated with 1×10^{-4} M $ZnCl_2$ for the indicated time periods to induce expression of exogenous c-*jun*. To specifically block the CDK2 activity, cells were treated with 75 μM roscovitine for the same time as $ZnCl_2$. All chemicals and reagents were purchased from Sigma–Aldrich, Prague, Czech Republic.

SDS–PAGE and immunoblotting. Harvested cells were collected by centrifugation, washed in 1× PBS and resuspended in RIPA lysis buffer containing 50 mM Tris–Cl (pH 7.4), 150 mM NaCl, 1 mM EDTA, 0.5% NP-40, 50 mM NaF, 8 mM glycerolphosphate, and protease inhibitors (200 mM PMSF, 100 mM TPCK, 100 mMDTT, 1 μg/μl leupeptin, 1 μg/μl aprotinin, and 10 μg/μl trypsin inhibitor) [16]. Fifteen micrograms of cellular protein extracts quantified by Bio-Rad protein assay (Bio-Rad Laboratories, Hercules, CA, USA) was mixed with 2× Laemmli sample buffer (100 mM Tris, pH 6.8, 4% SDS, 200 mM DTT, 20% glycerol, and 0.1% bromophenol blue) boiled for 3 min, resolved by 10% SDS–polycrylamide gel electrophoresis (SDS–PAGE), electroblotted to 0.45 μm PVDF membrane (BiotraceTM, Pall Life Sciences, NY, USA), and incubated with one of the polyclonal antibodies diluted 1:500–1:1000 (anti-c-Jun sc-44, anti-CDK2 sc-163, anti-CDK4 sc-601G, anti-CDK6 sc-177, anti-cyclin A sc-751, anti-cyclin D1 sc-450, anti-cyclin D2 sc-593, and anti-cyclin E sc-481, all purchased from Santa Cruz Biotechnology, Santa Cruz, CA, USA and anti-actin A5060, Sigma–Aldrich, Prague, Czech Republic) at 4 °C overnight. The blots were developed using horseradish peroxidase-conjugated secondary antibodies (A6667 for sc-751, sc-593, sc-481, sc-44, A5060; A4914 for sc-163; A4174 for sc-601G; and A9044 for sc-450) (dilution 1:5000) (Sigma–Aldrich, Prague, Czech Republic), and ECL Plus Western Blotting Detection System (Amersham Biosciences, Vienna, Austria) according to the manufacturer's protocol. At least three independent experiments were performed.

CDK2 kinase assay. Hundred micrograms of cell extracts was precleared using RIPA-washed G-protein beads (Sigma–Aldrich, Prague, Czech Republic). Supernatants were incubated with 5 μl of anti-CDK2 (sc-163), anti-cyclin A (sc-751) or anti-cyclin E (sc-481) antibody at 4 °C for 1 h. Then, 25 μl of RIPA-washed G-proteins was added and incubated overnight at 4 °C. Pellets were washed four times in RIPA buffer and twice in kinase buffer (50 mM Hepes, pH 7.5, 10 mM $MgCl_2$, 10 mM $MnCl_2$, and 10 mM glycerolphosphate). Reaction was initiated by mixing 25 μl of the sample with 25 μl of the kinase buffer solution containing histone H1 (Sigma–Aldrich, Prague, Czech Republic) (2.5 μg) and 0.1 μl [^{32}P]ATP (100 μCi/ml). The mixture was incubated at 37 °C for 30 min. Then, the reaction was stopped by addition of 50 μl of 2× Laemmli buffer. Each sample was loaded on 10% SDS–polyacrylamide gel and analyzed by SDS–PAGE. The gel was fixed in a mixture of 50% methanol, 40% H_2O and 10% acetic acid, dried and exposed to the film for autoradiography [16]. Four independent experiments were performed.

Analysis of nuclear morphology. Suspension of $2–3 \times 10^6$ cells was centrifuged, washed in PBS and fixed in a mixture of 75% methanol and 25% acetic acid at −20 °C for at least 1 h. Then, the cells were collected by centrifugation, resuspended in 100 μl of supernatant and dropped on a slide. The cells were stained with propidium iodide (20 mg/ml) and nuclear morphology was assessed using fluorescence microscopy. Frequency of cells with fragmented nuclei in a population of at least 250 cells was determined and assessed by analysis of variance (ANOVA). Three independent experiments were performed.

Analysis of phosphatidylserine externalization. Cells were cultured either in NS or LS medium for 48 h. Then, the cells were treated with 1×10^{-4} M $ZnCl_2$ for 6 h and harvested. Vital staining of apoptotic cells was performed using FITC-conjugated annexin V (Roche Diagnostics, Mannheim, Germany) and propidium iodide. The cells were washed twice with staining buffer (10 mM Hepes, 140 mM NaCl, 10 mM $CaCl_2$, pH 7.4), incubated with annexin V-FITC (0.5 μl/sample) and propidium iodide (5 μg/ml) for 15 min and analyzed by flow cytometry (FACS Calibur, Becton–Dickinson, 488 nm argon laser for excitation). At least 10,000 viable cells were collected for each sample. For analysis, the cell population was gated using forward versus side scatter parameters. Flow cytometric data were analyzed using CellQuest 3.0 software (Becton–Dickinson). Results were evaluated as a percentage of double-negative cells (intact), annexin V-positive cells (early apoptotic), and double-positive (necrotic or late apoptotic) cells.

Results and discussion

We described earlier that ectopically expressed c-Jun down-regulates proliferation of v-*myb*-transformed BM2cJUN monoblasts by inducing arrest in G1 phase of

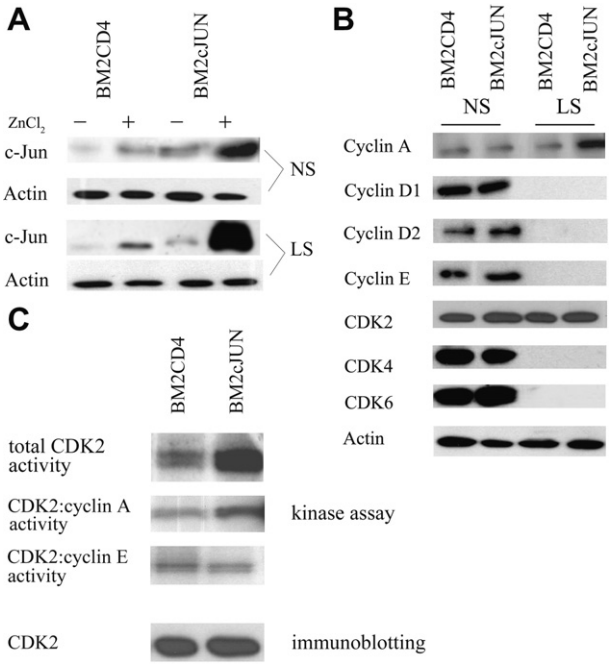

Fig. 1. (A) $ZnCl_2$ induces expression of c-Jun in BM2cJUN cells. BM2cJUN and the empty vector-transfected BM2CD4 cells grown in normal serum (NS) and low serum (LS) conditions were treated with $ZnCl_2$ (1×10^{-4} M) for 24 h. Extracts of harvested cells were resolved in 10% SDS–PAGE and analysed by immunoblotting with the Jun-specific antibody. Anti-actin antibody was used to control for sample loading. (B) Expression of cyclin A is increased in BM2cJUN cells grown in LS conditions. Extracts of zinc-treated BM2cJUN cells were analysed by SDS–PAGE and immunoblotting using antibodies specific for indicated regulators of G1/S transition. (C) Activity of cyclin A-associated CDK2 increases in BM2cJUN cells. The cells grown in LS conditions were treated with $ZnCl_2$ for 24 h. To control for CDK2 expression, SDS–PAGE and immunoblotting were performed as described. To measure the CDK2 activity, the protein was immunoprecipitated from harvested cells using anti-CDK2 antibody (upper box) or co-immunoprecipitated using anti-cyclin A (middle box) or anti-cyclin E (lower box) antibodies. Activity of CDK2 was determined by kinase assay as described in Materials and methods.

the cell cycle [14]. BM2cJUN cells contain exogenous c-*jun* placed under control of human metallothionein II_A promoter making the c-*jun* expression inducible with heavy metals [14]. In order to investigate the molecular processes controled by c-Jun in BM2cJUN cells, we determined expression and/or activity of several regulators of G1/S transition—cyclins A, D1, D2, E and kinases CDK2, CDK4, CDK6. Since c-Jun as well as the other regulators of G1/S transition can be activated by serum growth factors, the study was performed in serum-starved BM2cJUN cells cultured in low serum (LS) conditions. To test the level of c-*jun* expression in serum-starved BM2cJUN cells,

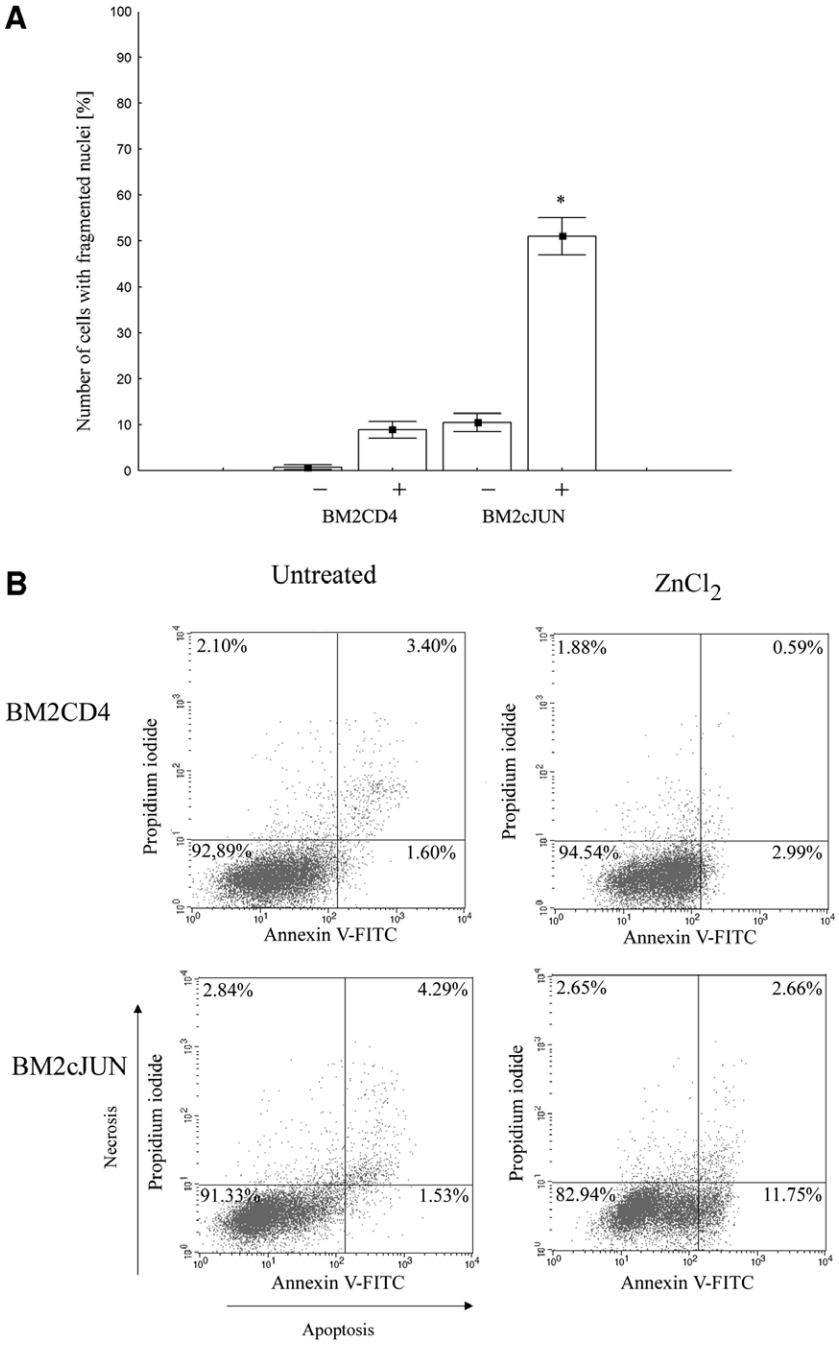

Fig. 2. c-Jun induces apoptosis of BM2cJUN cells. (A) BM2CD4 and BM2cJUN cells were cultured in LS conditions for 48 h, and treated with 1×10^{-4}M $ZnCl_2$ (+) or left untreated (−) for next 24 h. The cells were fixed, stained with propidium iodide and examined by fluorescence microscopy to enumerate cells with fragmented nuclei. Data were processed by ANOVA. At least 250 cells were analysed in each sample. The data represent mean values of six independent experiments. Error bars indicate standard deviations. Asterisk denotes a significant difference ($p < 0.05$) from BM2CD4 control. (B) c-Jun increases externalization of phosphatidylserine (PS) in BM2cJUN cells. The cells were cultured as described (A), and treated with $ZnCl_2$ for 6 h. The exposure of PS on the cell surface was assessed by FACS using FITC-conjugated annexin V probe. Cell viability was measured by propidium iodide staining. Typical results of three independent experiments are shown.

the cells were treated with zinc chloride for 24 h and extracts of harvested cells were analyzed by SDS–PAGE followed by immunoblotting. The level of c-Jun was compared with BM2cJUN cells cultured in normal serum (NS), and with control BM2CD4 cells. We detected low amount of c-Jun in zinc-treated BM2CD4 cells suggesting that expression of endogenous c-*jun* can be partially activated in BM2 cells treated with zinc ions (Fig. 1A). In contrast, the level of the c-Jun protein dramatically increased in zinc-treated BM2cJUN cells irrespective of the presence or absence of the sera. Then, we tested expression of cyclin A, cyclin D1, cyclin D2, cyclin E, CDK4, and CDK6 in zinc-treated BM2cJUN and BM2CD4 cells cultured in NS and LS conditions. Cyclin D1, cyclin D2, cyclin E, CDK4, and CDK6 were clearly down-regulated in serum-starved BM2CD4 as well as BM2cJUN cells (Fig. 1B). CDK2 was produced in similar levels by BM2CD4 and BM2cJUN cells independently of the serum growth factors. In contrast, the level of cyclin A was clearly increased in zinc-treated BM2cJUN cells but not in control BM2CD4 cells (Fig. 1B). These data demonstrate that the c-Jun protein can specifically control the level of cyclin A but not any other cyclins/CDKs regulating entry of starved BM2 monoblasts to the S phase of the cell cycle. This observation prompted us to investigate whether cyclin A can affect activity of the cyclin A-associated kinase CDK2. We cultured BM2cJUN and BM2CD4 cells in LS conditions, and measured activity of CDK2 in cell extracts by kinase assay. We found that the CDK2 activity was significantly higher in zinc-treated BM2cJUN cells cultured in LS conditions than in similarly treated control BM2CD4 cells (Fig. 1C) and untreated BM2cJUN cells (not shown). CDK2 can be activated by either cyclin E or cyclin A. To specify the type of cyclin that activates CDK2 in BM2cJUN cells, we co-immunoprecipitated CDK2 using cyclin A- or cyclin E-specific antibody and performed the kinase assays again. While the kinase activity of the cyclin E-CDK2 in BM2cJUN cells was the same as in BM2CD4 cells, activity of the cyclin A-CDK2 was higher in BM2cJUN cells than in control BM2CD4 cells (Fig. 1C). These results document that the c-Jun protein induces expression of cyclin A, thus activating CDK2 kinase in serum-deprived BM2cJUN monoblasts.

To test frequency of apoptosis occurring in serum-starved BM2cJUN cells, we examined nuclear morphology of serum-deprived BM2cJUN and control BM2CD4 cells treated with $ZnCl_2$ for 24 h. We found that about 10% of zinc-treated BM2CD4 cells contained fragmented nuclei (Fig. 2A). In contrast, the apoptotic nuclear fragmentation occurred in about 55% of zinc-treated BM2cJUN cells. This difference was statistically significant ($p < 0.05$) (Fig. 2A). In addition, analysis of phosphatidylserine externalization confirmed high frequency of apoptosis occurring in BM2cJUN cells treated with zinc chloride for only 6 h (12%), while untreated BM2cJUN cells (1.5%) and zinc-treated BM2CD4 cells (3%) retained significantly lower frequency of apoptosis (Fig. 2B). Induction of apoptosis in starving- and c-*jun*-expressing BM2cJUN cells was also confirmed by analysis of DNA fragmentation by agarose gel electrophoresis (not shown).

High rate of apoptosis can be associated with cyclin A-mediated up-regulation of CDK2 in BM2cJUN cells. To test this hypothesis, we treated BM2cJUN cells with the ATP-competitive isopropylpurine analogue, the roscovitine—2-(*R*)-(1-ethyl-2-hydroxyethylamino)-6-benzylamino-9-purine. Roscovitine acts as specific inhibitor of CDK2 [17,18] possessing the pro-apoptotic effects [19]. Frequency of apoptosis increased in roscovitine- and zinc-treated BM2CD4 and roscovitine-treated BM2cJUN cells, but it significantly decreased ($p < 0.0001$) in roscovitine- and zinc-treated BM2cJUN cells (Fig. 3). This suggests that it is the CDK2 activity that is responsible for high rate of apoptosis in these cells.

The c-Jun protein is considered to drive the cell fate either towards apoptosis or survival [20–25]. Pro-apoptotic effects of the c-Jun protein were documented in several cell types including neurons [26–28], fibroblasts and erythroleukemic cells [29]. At the same time, c-Jun can activate expression of cyclin A that regulates G1/S transition of the cell cycle [30]. Cyclins, CDKs, and their inhibitors are often involved in regulation of programmed cell death [31]. Up-regulation of the CDK2 activity seems to be a common mechanism of initiation of apoptosis. For example, increased rate of cyclin A expression and CDK2 activity were found in HeLa and FaO rat hepatoma cells undergoing apoptosis induced by various chemical reagents [32]. Activation of CDK2, but not CDK1 occurs also in

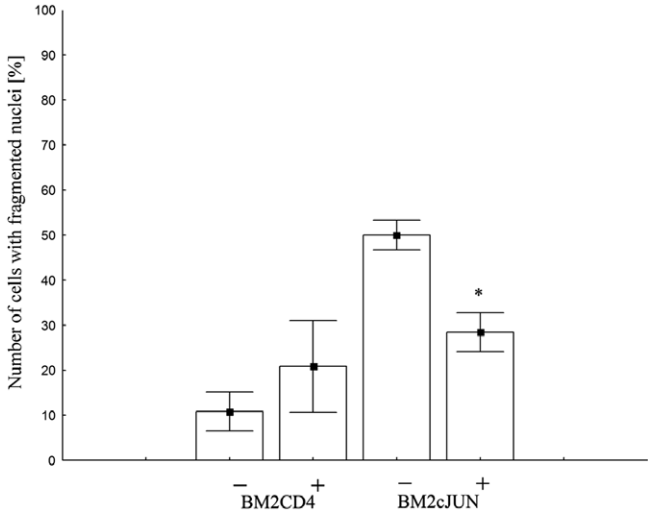

Fig. 3. Roscovitine suppresses c-Jun-induced apoptosis of BM2cJUN cells. The cells were cultured in LS for 48 h and treated with $ZnCl_2$ (−) or $ZnCl_2$ and roscovitine (+) for 24 h. The cells containing fragmented nuclei were enumerated as described in the legend to Fig. 2A. The data represent mean values from three independent experiments. Error bars indicate standard deviations. At least 250 cells were analysed in each sample. Asterisk denotes a significant difference ($p < 0.0001$) from BM2cJUN cells grown in the absence of roscovitine as determined by ANOVA.

mature non-cycling $CD4^+CD8^+$ thymocytes undergoing apoptosis [33]. This effect can be suppressed by specific inhibition of the CDK2 activity by ATP-competitive chemicals, such as olomoucine [34]. In addition, the CDK2 activity is essential for cell death-associated chromatin condensation, and other features of apoptosis [35]. Our study suggests that c-Jun can activate expression of cyclin A, thus up-regulating activity of CDK2 and inducing apoptosis of starved v-*myb*-transformed monoblasts. This provides a new link between the c-Jun protein and apoptotic pathway in leukemic monoblasts.

Acknowledgments

We thank Petr Dvořák for support, Iveta Nevřivá for technical assistance, Eva Ondroušková, Sabina Ševčíková, and Jarmila Navrátilová for helpful discussions and Clare Parish for stylistic revision. This work was supported by Grants 301/06/0036 of the Grant Agency of the Czech Republic (J.Š.), MSM0021622415 (J.Š.), 1M0538 (A.H.), and FRVŠ 111/2006 (P.V.) of the Ministry of Education, Youth and Sports of the Czech Republic and AVOZ50390512 of the Academy of Sciences of the Czech Republic (A.H.).

References

[1] P.K. Vogt, Jun, the oncoprotein, Oncogene 20 (2001) 2365–2377.
[2] H.J. Rahmsdorf, Jun: transcription factor and oncoprotein, J. Mol. Med. 74 (1996) 725–747.
[3] E. Shaulian, M. Karin, AP-1 in cell proliferation and survival, Oncogene 20 (2001) 2390–2400.
[4] A.M. Bamberger, K. Milde-Langosch, E. Rossing, C. Goemann, T. Loning, Expression pattern of the AP-1 family in endometrial cancer: correlations with cell cycle regulators, J. Cancer Res. Clin. Oncol. 127 (2001) 545–550.
[5] J.M. Gee, A.F. Barroso, I.O. Ellis, J.F. Robertson, R.I. Nicholson, Biological and clinical associations of c-jun activation in human breast cancer, Int. J. Cancer 89 (2000) 177–186.
[6] K. Milde-Langosch, A.M. Bamberger, C. Methner, G. Rieck, T. Loning, Expression of cell cycle-regulatory proteins rb, p16/MTS1, p27/KIP1, p21/WAF1, cyclin D1 and cyclin E in breast cancer: correlations with expression of activating protein-1 family members, Int. J. Cancer 87 (2000) 468–472.
[7] J. Rangatia, R.K. Vangala, S.M. Singh, A.A. Peer Zada, A. Elsasser, A. Kohlmann, T. Haferlach, D.G. Tenen, W. Hiddemann, G. Behre, Elevated c-Jun expression in acute myeloid leukemia inhibits C/EBPalpha DNA binding via leucine zipper domain interaction, Oncogene 22 (2003) 4760–4764.
[8] S. Mathas, M. Hinz, I. Anagnostopoulos, D. Krappmann, A. Lietz, F. Jundt, K. Bommert, F. Mechta-Grigoriou, H. Stein, B. Dorken, C. Scheidereit, Aberrantly expressed c-Jun and JunB are a hallmark of Hodgkin lymphoma cells, stimulate proliferation and synergize with NFκB, EMBO J. 21 (2002) 4104–4113.
[9] A.B. Raitano, J.R. Halpern, T.M. Hambuch, C.L. Sawyers, The Bcr-Abl leukemia oncogene activates Jun kinase and requires Jun for transformation, Proc. Natl. Acad. Sci. USA 92 (1995) 11746–11750.
[10] P. Salomoni, R. Bernardi, S. Bergmann, A. Changou, S. Tuttle, P.P. Pandolfi, The promyelocytic leukemia protein PML regulates c-Jun function in response to DNA damage, Blood 105 (2005) 3686–3690.
[11] W.H. Toh, M.M. Siddique, L. Boominathan, K.W. Lin, K. Sabapathy, c-Jun regulates the stability and activity of the p53 homologue, p73, J. Biol. Chem. 279 (2004) 44713–44722.
[12] I. Sanchez-Perez, P. Perona, Lack of c-Jun activity increases survival to cisplatin, FEBS Lett. 453 (1999) 151–158.
[13] C. Moscovici, N. Zeller, M.G. Moscovici, Continuous lines of AMV-transformed nonproducer cells: growth and oncogenic potential in the chicken embryo, in: R.F. Revoltella, C. Basilico, G.M. Potori, G. Rovera, J.H. Subak-Sharpe (Eds.), Expression of differentiated function in cancer cells, Raven Press, New York, 1982, pp. 325–449.
[14] S. Sevcikova, K. Soucek, L. Kubala, V. Bryja, J. Smarda, Differential effects of v-Jun and c-Jun proteins on v-myb-transformed monoblasts, Cell. Mol. Life Sci. 59 (2002) 1690–1705.
[15] J. Smarda, J.S. Lipsick, c-Myb prevents TPA-induced differentiation and cell death in v-Myb transformed monoblasts, Oncogene 9 (1994) 237–245.
[16] R.J. Sheaff, Regulation of mammalian cyclin-dependent kinase 2, in: W.G. Dunphy (Ed.), Methods in Enzymology: Cell Cycle Control, vol. 283, Academic Press, San Diego, pp. 173–193.
[17] W.F. De Azevedo, S. Leclerc, L. Meijer, L. Havlicek, M. Strnad, S.H. Kim, Inhibition of cyclin-dependent kinases by purine analogues: crystal structure of human cdk2 complexed with Roscovitine, Eur. J. Biochem. 243 (1997) 518–526.
[18] F. Alessi, S. Quarta, M. Savio, F. Riva, L. Rossi, L.A. Stivala, A.I. Scovassi, L. Meijer, E. Prosperi, The cyclin-dependent kinase inhibitors olomoucine and roscovitine arrest human fibroblasts in G1 phase by specific inhibition of CDK2 kinase activity, Exp. Cell Res. 245 (1998) 8–18.
[19] H. Edamatsu, C.L. Gau, T. Nemoto, L. Guo, F. Tamanoi, Cdk inhibitors, roscovitine and olomoucine, synergize with farnesyltransferase inhibitor (FTI) to induce efficient apoptosis of human cancer cell lines, Oncogene 19 (2000) 3059–3068.
[20] D.A. Liebermann, B. Gregory, B. Hoffman, AP-1 (Fos/Jun) transcription factors in hematopoietic differentiation and apoptosis, Int. J. Oncol. 12 (1998) 685–700.
[21] J. Hess, P. Angel, M. Schorpp-Kistner, AP-1 subunits: quarrel and harmony among siblings, J. Cell Sci. 117 (2004) 5965–5973.
[22] M. Ameyar, M. Wisnievska, J.B. Weitzman, A role for AP-1 in apoptosis: the case for and against, Biochimie 85 (2003) 747–752.
[23] R. Eferl, M. Sibilia, F. Hilberg, A. Fuchsbichler, I. Kufferath, B. Guertl, R. Zenz, E.F. Wagner, K. Zatloukal, Functions of c-Jun in liver and heart development, J. Cell Biol. 145 (1999) 1049–1061.
[24] H. Le-Niculescu, E. Bonfoco, Y. Kasuya, F.X. Claret, D.R. Green, M. Karin, Withdrawal of survival factors results in activation of the JNK pathway in neuronal cells leading to Fas ligand induction and cell death, Mol. Cell. Biol. 19 (1999) 751–763.
[25] C.G. Besirli, E.M. Johnson, JNK-independent activation of c-Jun during neuronal apoptosis induced by multiple DNA-damaging agents, J. Biol. Chem. 278 (2003) 22357–22366.
[26] J. Ham, C. Babij, J. Whitfield, C.M. Pfarr, D. Lallemand, M. Yaniv, M.L.L. Rubin, A c-Jun dominant negative mutant protects sympathetic neurons against programmed cell death, Neuron 14 (1995) 927–939.
[27] S. Estus, W.J. Zaks, R.S. Freeman, M. Gruda, R. Bravo, E.M. Johnson Jr., Altered gene expression in neurons during programmed cell death: identification of c-jun as necessary for neuronal apoptosis, J. Cell Biol. 127 (1994) 1717–1727.
[28] M. Palmada, S. Kanwal, N.J. Rutkoski, C. Gustafson-Brown, R.S. Johnson, R. Wisdom, B.D. Carter, c-jun is essential for sympathetic neuronal death induced by NGF withdrawal but not by p75 activation, J. Cell Biol. 158 (2002) 453–461.
[29] Y.H. Kim, M. Takahashi, E. Suzuki, E. Niki, Apoptosis induced by hydrogen peroxide under serum deprivation and its inhibition by antisense c-jun in F-MEL cells, Biochem. Biophys. Res. Commun. 271 (2000) 747–752.
[30] M. Katabami, H. Donninger, F. Hommura, V.D. Leaner, I. Kinoshita, J.F. Chick, M.J. Birrer, Cyclin A is a c-Jun target gene and is necessary for c-Jun-induced anchorage-independent growth in RAT1a cells, J. Biol. Chem. 280 (2005) 16728–16738.

[31] A. Borgne, R.M. Golsteyn, The role of cyclin-dependent kinases in apoptosis, Prog. Cell Cycle Res. 5 (2003) 453–459.
[32] W. Meikrantz, S. Gisselbrecht, S.M. Tam, R. Schlegel, Activation of cyclin A-dependent protein kinases during apoptosis, Proc. Natl. Acad. Sci. USA 91 (1994) 3754–3758.
[33] A. Hakem, T. Sasaki, I. Kozieradzki, J.M. Penninger, The Cyclin-dependent Kinase Cdk2 regulates thymocyte apoptosis, J. Exp. Med. 189 (1999) 957–967.
[34] A. Appert-Collin, B. Hugel, R. Levy, N. Niederhoffer, G. Coupin, Y. Lombard, P. Andre, P. Poindron, J.P. Gies, Cyclin dependent kinase inhibitors prevent apoptosis of postmitotic mouse motoneurons, Life Sci. 79 (2006) 484–490.
[35] K.J. Harvey, D. Lukovic, D.S. Ucker, Caspase-dependent Cdk activity is a requisite effector of apoptotic death events, J. Cell Biol. 148 (2000) 59–72.