Influence of Environmental Conditions on c-Jun N-terminal Kinase Mediated Apoptosis of HL60 Cells by Anti-Cancer Drugs

Eun-Hye HUR, Mun Jung KANG, Sung-Doo KIM, Sung-Nam LIM, Dae-Young KIM,
Jung-Hee LEE, Kyoo-Hyung LEE, and Je-Hwan LEE*

Department of Hematology, Asan Medical Center, University of Ulsan College of Medicine, Seoul 138-736, Republic of Korea

(Received December 30, 2009; Revised January 11, 2010; Accepted January 11, 2010)

Abstract — Activation of JNK has long been associated with the apoptotic response induced by various anti-cancer drugs including doxorubicin, vinblastine, and etoposide. In this study, we examined and compared patterns of apoptosis and JNK activation according to three different anti-cancer drugs (daunorubicin, vinblastine, and etoposide) and two different sources of HL60 cells (Jackson Laboratory and ATCC). HL60 cells from Jackson Laboratory (HL60/RPMI) were maintained in RPMI 1640 containing 5% fetal bovine serum and those from ATCC (HL60/IMDM) in IMDM containing 20% fetal bovine serum as to each manufacture's guideline. In general, HL60/RPMI cells were more sensitive to anti-cancer drugs compared to HL60/IMDM cells, demonstrated by the XTT and flow cytometric analyses. Apoptotic pathways after treatment with anti-cancer drugs seemed to be different between HL60/RPMI (daunorubicin and etoposide, caspase 3 dependent, but caspase 8 or 9 independent; vinblastine, caspase 3 independent) and HL60/IMDM (caspase 3 and caspase 9 dependent). The expression of apoptotic protein, BID, was consistent with caspase 3 activation. Immunoblotting of phospho-JNK and JNK kinase assay showed JNK activation by all three anti-cancer drugs in HL60/RPMI, while JNK activation was observed only in vinblastine-treated cells in HL60/IMDM. Our study results suggest that in vitro environmental conditions have a significant influence on JNK mediated apoptosis of HL60 cells by anti-cancer drugs and in vitro culture conditions are important factors in JNK or possibly other MAPK related studies.

Keywords: JNK, Anticancer drugs, HL60

INTRODUCTION

c-Jun N-terminal kinase (JNK), also known as stress-activated MAP kinases (SAPK), represents one of three groups of mitogen-activated protein kinases (MAPK) that are involved in the regulation of cell proliferation, differentiation, and apoptosis (Davis, 2000; Nishina *et al.*, 2004; Weston and Davis, 2007; Dhanasekaran and Reddy, 2008). JNK is a key regulator of many cellular events and has pro- or anti-apoptotic functions. Whether the activation of JNK leads to cell proliferation or apoptosis depends on cell type, nature of death stimulus, duration of its activation and the activity of other signaling pathway (Lin and Dibling, 2002; Liu and Lin, 2005; Dhanasekaran and Reddy, 2008). JNK can be activated by several different stimuli including

growth factors, cytokines, and stress factors such as ultra-violet irradiation, heat shock, osmotic shock, and many cytotoxic or genotoxic agents (Davis, 2000; Weston and Davis, 2007; Dhanasekaran and Reddy, 2008). Multiple splice variants of JNKs encoded by three distinct genes, namely JNK1, JNK2, and JNK3, have been identified (Davis, 2000; Johnson and Nakamura, 2007). JNK1 and JNK2 are ubiquitously expressed, but the expression of JNK3 is mainly restricted to brain, heart, and testis. There are different substrate specificities among isoforms; therefore, different JNK isoforms may play specific roles, but there is also complementation between the JNK genes (Davis, 2000; Cui *et al.*, 2007).

Anti-cancer drugs are considered to mediate cell death by activating key elements of the apoptosis program and the cellular stress response (Herr and Debatin, 2001). MAPK signaling pathways have been implicated in the response of tumor cell to anti-cancer drugs (Fan and

*Corresponding author

Tel: +82-2-3010-3218 Fax: +82-2-3010-6961
E-mail: jhlee3@amc.seoul.kr

Chambers, 2001; Boldt *et al.*, 2002). Among the pathways, activation of JNK has long been associated with the apoptotic response induced by various anti-cancer drugs including doxorubicin (Osborn and Chambers, 1996; Yu *et al.*, 1996; Brantley-Finley *et al.*, 2003; Panaretakis *et al.*, 2005; Lagadinou *et al.*, 2008), vinblastine (Osborn and Chambers, 1996; Fan *et al.*, 2001; Brantley-Finley *et al.*, 2003; Kolomeichuk *et al.*, 2008), etoposide (Osborn and Chambers, 1996; Brantley-Finley *et al.*, 2003), cytarabine (Sampath and Plunkett, 2000; Sampath *et al.*, 2006), and other agents (Choi and Lee, 2009). However, opposing functions of JNK have also been evidenced for anti-cancer drugs. JNK may play a protective role in some situations (Potapova *et al.*, 1997; Wisdom *et al.*, 1999), and inhibition of JNK2 expression was shown to induce apoptosis of human tumor cell (Potapova *et al.*, 2000). In acute myeloid leukemia cells, multidrug resistance has been related to JNK activity (Potapova *et al.*, 2000; Cripe *et al.*, 2002; Do *et al.*, 2007). Cytarabine induced apoptosis in HL60 cells was preceded by the activation of JNK, but the inhibition of JNK activation did not block apoptosis of HL60 cells by cytarabine (Stadheim *et al.*, 2000). Thus, JNK signaling may induce apoptosis or promote cell survival and proliferation after treatment with anti-cancer drugs and may be dispensable for apoptosis. The effects of JNK on cellular responses to anti-cancer drugs appear to depend on the cell type, the class of anti-cancer drugs, the context of other signals received by the cell, and environmental conditions. We have studied the role of JNK activation on the effects of anti-cancer drugs and found different patterns of apoptosis according to different anti-cancer drugs and different sources of the same cells (HL60). Our results indicate that in vitro environmental conditions are important in the studies of JNK mediated apoptosis by anti-cancer drugs.

MATERIALS AND METHODS

Reagents

All of three anti-cancer drugs were used for the study: daunorubicin, vinblastine, and etoposide (Sigma-Aldrich, Saint Louis, MO). The drugs are common anti-leukemic agents and the main mechanism of action is inhibition of topoisomerase II in daunorubicin and etoposide, and inhibition of the assembly of microtubules in vinblastine. For the concentrations of the agents in this study, we made reference to a prior study, which investigated JNK activation by anti-cancer drugs (Brantley-Finley *et al.*, 2003). Primary antibodies for the immunoblot assays, which were against human antigens, and Chaps Cell Extract Buffer were purchased from Cell Signaling Technology (Danvers, MA).

The sodium 3'-[1-(phenylaminocarbonyl)-3,4-tetrazolium]-bis (4-methoxy-6-nitro) benzene sulfonic acid hydrate (XTT) assay was performed with Cell Proliferation Kit II (Roche Applied Science, Switzerland).

Cell lines and culture media

Two difference sources of HL60 cells were used for the study: HL60 cells from ATCC (Manassas, VA), which were maintained in Iscove's Modified Dulbecco's Medium (IMDM; ATCC) containing 20% fetal bovine serum and 1% L-glutamine, and those from the Jackson Laboratory (Sacramento, CA), which were maintained in RPMI Medium 1640 (Invitrogen, Carlsbad, CA) containing 5% fetal bovine serum and 1% L-glutamine. The cells were passaged twice a week.

Assays for cellular viability

Cellular viability was evaluated using the XTT assay and flow cytometric analysis. The XTT assay is based on the cleavage of the yellow tetrazolium salt XTT to form an orange formazan dye by metabolically active viable cells (Gerlier and Thomasset, 1986; Jost *et al.*, 1992). For the assay, 1.5×10^3 HL60 cells were split onto each well of 96-well microplates. The XTT labeling mixture was added 72 hours after treatment of each anti-cancer drug and the microplates were incubated for 4 hours at 37°C. The absorbance was measured using an ELISA reader at 480 nm with a reference wave-length at 650 nm.

For flow cytometric analysis of cellular viability (Krishan, 1975), 1×10^6 cells re-suspended in 1.5 ml complete medium were put in a 15 ml polypropylene tube and 1.5 ml serum free medium including each anti-cancer drug was added. Twenty-four hours later, the cells were fixed with cold ethanol (70%) overnight at 4°C, and were stained with 60 mcg/ml propidium iodide (PI; Sigma-Aldrich) containing 10 units/ml RNase ONE (Promega, Madison, WI) for 30 minutes. The cells were incubated at 4°C until analysis. The percentages of PI stained cells were measured using a fluorescence-activated cell sorting flow cytometer (Becton Dickinson, San Jose, CA) and inbuilt software.

JNK assay and immunoblot analysis

Total JNK protein and activated phosphor-JNK protein were assayed with immunoblot analysis. The cells were lysed with Chaps Cell Extract Buffer and protein concentration was determined using a Protein Assay Reagent A (Bio-Rad Laboratories, Hercules, CA). The cell lysates (50 mcg) were fractionated by SDS-PAGE, and were transferred to nitrocellulose membrane by iBlot™ Dry Blotting System (Invitrogen). Membranes were blocked in 5% skim

milk in TBST buffer and were incubated overnight with anti-JNK or anti-phospho-JNK antibodies (Thr183/Tyr185) at 4°C. The blots were visualized with SuperSignal West Pico Chemiluminescent Substrate (Thermo Scientific, Waltham,

MA). Immunoblot analyses were also performed using other primary antibodies for caspases, PARP, Bid, Bax, acetylated histone H3, survivin, and actin with the same protocol.

Fig. 1. Cellular viability and caspase activation after treatment of anti-cancer drugs on two different sources of HL60 cells (HL60/RPMI and HL60/IMDM). DNR: daunorubicin, VBL: vinblastine, ETO: etoposide. (A) XTT assay. The IC₅₀ values for daunorubicin, vinblastine, and etoposide were 0.04 μM, 0.02 μM, and 0.2 μM in HL60/RPMI, while those were 0.2 μM, 0.1 μM, and 3 μM in HL60/IMDM. (B) Flow cytometric analysis with PI staining. The percent proportions of apoptotic cells 24 hours after treatment of anti-cancer drugs are presented. (C) Caspase pathway activation.

JNK kinase assay was performed 24 hours after treatment of anti-cancer drugs. After the cells were rinsed with ice-cold PBS, 0.5 ml ice-cold 1X Cell Lysis Buffer (Cell Signaling Technology) and 1 mM phenylmethylsulfonyl fluoride were added, and procedure of freezing and thawing was repeated three times. The lysates were centrifuged at 4°C, 14,000 rpm for 10 minutes and the supernatants were adjusted to 500 mcg of protein in 0.5 ml, then stored at -80°C. For immunoprecipitation with immobilized c-Jun substrate, 0.5 ml cell lysates were incubated with 500 mcg GST-C-Jun bead slurry (kindly provided by prof. E.J. Choi,

Korea University) overnight at 4°C. The immunoprecipitates were centrifuged at 4°C, 5,000 rpm for 3 min to removed supernatant and washed two times with 500 mcl 1X Cell Lysis Buffer, then washed two times with 500 mcl 1X Kinase Buffer (Cell Signaling Technology). The washed immunoprecipitates were suspended in 50 mcl of 1X Kinase Buffer supplemented with 200 mcM ATP and incubated at 30°C for 30 min. The reaction was stopped by addition with 20 mcl 2X SDS Sample Buffer (Bio-Rad Laboratories). The lysates were heated in a boiling water bath for 5 min and subsequently subjected to SDS-PAGE

Fig. 2. Activation of JNK by anti-cancer drugs. DNR: daunorubicin, VBL: vinblastine, ETO: etoposide. (A) Changes of intracellular JNK and phospho-JNK amounts 1, 3.5, and 7 hours after treatment of anti-cancer drugs on HL60/RPMI and HL60/IMDM. Of double bands, upper band is for JNK2 (55 KD) and lower one is for JNK1/3 (46 KD). (B) Results of JNK kinase assay after treatment of anti-cancer drugs on HL60/RPMI. IP: immunoprecipitation, IB: immunoblotting.

on a 12% polyacrylamide gel, then followed by immunoblot analysis.

RESULTS

Cellular viability after the treatment of anti-cancer drugs on two different sources of HL60 cells

Sensitivity to anti-cancer drugs was different between HL60 cells from ATCC (HL60/IMDM) and those from Jackson Laboratory (HL60/RPMI) (Fig. 1). The cellular proliferation rates 72 hours after treatment with anti-cancer drugs were evaluated with the XTT assay, which indicated that the IC₅₀ values for daunorubicin, vinblastine, and etoposide were lower in HL60/RPMI than in HL60/IMDM (0.04 mcM vs 0.2 mcM for daunorubicin, 0.02 mcM vs. 0.1 mcM for vinblastine, and 0.2 mcM vs. 3 mcM for etoposide) (Fig. 1A). The results mean that HL60/RPMI has higher sensitivity to anti-cancer drugs than HL60/IMDM. Flow cytometric analysis with PI staining 24 hours after treatment with anti-cancer drugs showed similar findings to the XTT assay (Fig. 1B). The proportion of apoptotic cells after etoposide treatment was much higher in HL60/RPMI than in HL60/IMDM (52.4% vs. 20.4%). Caspases play a crucial role in the execution of the apoptotic process (Philchenkov, 2004; Lavrik *et al.*, 2005). We observed different patterns of caspase activation after treatment of anti-cancer drugs between HL60/RPMI and HL60/IMDM (Fig. 1C). In HL60/RPMI, daunorubicin and etoposide caused the activation of caspase 3 and poly(ADP-ribose) polymerase (PARP), but vinblastine did not. In HL60/IMDM, however, all three drugs caused the activation of caspase 3 and PARP. Cleavage of caspase 9 by anti-cancer drugs was observed only in HL60/IMDM (not in HL60/RPMI), while caspase 8 was not activated both in HL60/RPMI and HL60/IMDM.

Activation of JNK by anti-cancer drugs

We observed the changes of intracellular JNK and phospho-JNK amounts 1, 3.5, and 7 hours after treatment of anti-cancer drugs (Fig. 2A). Intracellular amounts of total JNK protein did not change after treatment of anti-cancer drugs in HL60/IMDM, while amounts of JNK proteins in HL60/IMDM increased after treatment of anti-cancer drugs. Regarding JNK isoforms, amounts of JNK1/3 and JNK2 were similar in HL60/RPMI, while amount of JNK2 was less than JNK1/3 amount in HL60/IMDM. The patterns of JNK activation, measured by phospho-JNK, were different according to anti-cancer drugs and sources of cells. In HL60/RPMI, JNK was well activated after treatment of all three anti-cancer drugs although vinblastine induced acti-

Fig. 3. Expression of apoptotic proteins by anti-cancer drugs. D: daunorubicin, V: vinblastine, E: etoposide.

vation of JNK decreased more rapidly than daunorubicin or etoposide induced activation. In HL60/IMDM, only vinblastine caused activation of JNK. JNK kinase assay also showed increase of JNK kinase activity after treatment of each anti-cancer drug in HL60/RPMI (Fig. 2A).

Expression of apoptotic proteins by anti-cancer drugs

BH3 interacting domain death agonist (BID) and Bcl-2-associated X protein (Bax) are pro-apoptotic members of the Bcl-2 protein family. BID expression patterns were correlated with activation patterns of caspase 3. In HL60/RPMI, BID expression was upregulated by daunorubicin or etoposide, but not by vinblastine, while Bax expression was not changed after treatment of anti-cancer drugs (Fig. 3). In HL60/IMDM, BID or Bax was constitutively expressed and was not changed by anti-cancer drugs. Survivin is a member of the inhibitor of apoptosis family and the survivin protein functions to inhibit caspase activation. Survivin expression decreased in HL60/RPMI by etoposide, but it was not changed in other situations.

DISCUSSION

In this study, we examined and compared patterns of apoptosis and JNK activation according to three different anti-cancer drugs (daunorubicin, vinblastine, and etoposide) and two different sources of the same cells (HL60/RPMI and HL60/IMDM). HL60 cells of each source were maintained with different culture media that were recommended by each manufacture's guideline. Surprisingly, patterns of apoptosis and JNK activation by anti-cancer drugs were significantly different between two sources of cells. In general, HL60/RPMI cells were more sensitive to anti-cancer drugs compared to HL60/IMDM cells, demonstrated by the XTT and flow cytometric analyses. Apoptotic pathways after treatment with anti-cancer drugs seemed to be different between HL60/RPMI (daunorubicin and etoposide, caspase 3 dependent, but caspase 8 or 9 in-

dependent; vinblastine, caspase 3 independent) and HL60/IMDM (caspase 3 and caspase 9 dependent). The anti-cancer drugs that were used in this study have been known to induce apoptosis, at least in part, through caspase 3 activation (Gamen *et al.*, 1997; Benjamin *et al.*, 1998; Tashiro *et al.*, 1998; Turnbull *et al.*, 1999). In this study, however, we could not find caspase 3 activation after vinblastine treatment in HL60/RPMI cells and expression of apoptotic protein, BID, was consistent with caspase 3 activation. The findings indicate that diverse apoptotic pathways are present in response to anti-cancer drug (Herr and Debatin, 2001) and there may be different cellular responses according to status of the cells even though the same anti-cancer drug (vinblastine in this study) is treated to the same cell type (HL60 cells in this study). Previous studies have shown that anti-cancer drugs induce the activation of JNK pathway, which may contribute to apoptosis or proliferation of cells (Fan and Chambers, 2001; Herr and Debatin, 2001; Boldt *et al.*, 2002). Immunoblotting of phospho-JNK and JNK kinase assay showed JNK activation by all three anti-cancer drugs in HL60/RPMI, while JNK activation was observed only in vinblastine-treated cells in HL60/IMDM. The findings suggest that the role of JNK pathway in the apoptosis after treatment with anti-cancer drugs may be various according to the cell type, nature of anti-cancer drug, and cellular status (Fan and Chambers, 2001; Herr and Debatin, 2001; Boldt *et al.*, 2002; Brantley-Finley *et al.*, 2003). The various patterns of apoptosis and JNK activation by anti-cancer drugs in this study may be originated from different in vitro environmental conditions between two sources of cells, not from source itself. There was difference in the concentration of fetal bovine serum in the culture media; 5% in HL60/RPMI and 20% in HL60/IMDM. Fetal bovine serum contains a variety of growth factors and cellular response to anti-cancer drugs may be affected by the concentration of fetal bovine serum in the media. In addition, RPMI and IMDM may provide different environmental conditions in cell culture system.

In conclusion, our study results suggest that in vitro environmental conditions have a significant influence on JNK mediated apoptosis of HL60 cells by anti-cancer drugs and in vitro culture conditions are important factors in JNK or possibly other MAPK related studies.

ACKNOWLEDGMENTS

This research was supported by the Converging Research Center Program through the National Research Foundation of Korea (NRF) funded by the Ministry of

Education, Science and Technology (KRF-2005-041-E00190).

REFERENCES

- Benjamin, C. W., Hiebsch, R. R. and Jones, D. A. (1998). Caspase activation in MCF7 cells responding to etoposide treatment. *Mol. Pharmacol.* **53**, 446-450.
- Boldt, S., Weidle, U. H. and Kolch, W. (2002). The role of MAPK pathways in the action of chemotherapeutic drugs. *Carcinogenesis*. **23**, 1831-1838.
- Brantley-Finley, C., Lyle, C. S., Du, L., Goodwin, M. E., Hall, T., Szewo, D., Kaushal, G. P. and Chambers, T. C. (2003). The JNK, ERK and p53 pathways play distinct roles in apoptosis mediated by the antitumor agents vinblastine, doxorubicin, and etoposide. *Biochem. Pharmacol.* **66**, 459-469.
- Choi, J. H. and Lee, K. T. (2009). Costunolide-induced apoptosis in human leukemia cells: involvement of c-jun N-terminal kinase activation. *Biol. Pharm. Bull.* **32**, 1803-1808.
- Cripe, L. D., Gelfanov, V. M., Smith, E. A., Spigel, D. R., Phillips, C. A., Gabig, T. G., Jung, S. H., Fyffe, J., Hartman, A. D., Kneebone, P., Mercola, D., Burgess, G. S. and Boswell, H. S. (2002). Role for c-jun N-terminal kinase in treatment-refractory acute myeloid leukemia (AML): signaling to multidrug-efflux and hyperproliferation. *Leukemia* **16**, 799-812.
- Cui, J., Zhang, M., Zhang, Y.Q. & Xu, Z. H. (2007). JNK pathway: diseases and therapeutic potential. *Acta Pharmacol. Sin.* **28**, 601-608.
- Davis, R. J. (2000). Signal transduction by the JNK group of MAP kinases. *Cell* **103**, 239-252.
- Dhanasekaran, D. N. and Reddy, E. P. (2008). JNK signaling in apoptosis. *Oncogene* **27**, 6245-6251.
- Do, J. H., Oh, S. H., Song, E. J., Chung, J. S., Kang, C. D. and Lee, E. Y. (2007). Treatment outcome of multidrug resistance related mRNA expression and c-jun-N-terminal kinase activity in patients with acute myeloid leukemia. *Korean J. Lab. Med.* **27**, 229-236.
- Fan, M. and Chambers, T. C. (2001). Role of mitogen-activated protein kinases in the response of tumor cells to chemotherapy. *Drug Resist. Updat.* **4**, 253-267.
- Fan, M., Goodwin, M. E., Birrer, M. J. & Chambers, T. C. (2001). The c-Jun NH(2)-terminal protein kinase/AP-1 pathway is required for efficient apoptosis induced by vinblastine. *Cancer Res.* **61**, 4450-4458.
- Gamen, S., Anel, A., Lasierra, P., Alava, M. A., Martinez-Lorenzo, M. J., Pineiro, A. and Naval, J. (1997). Doxorubicin-induced apoptosis in human T-cell leukemia is mediated by caspase-3 activation in a Fas-independent way. *FEBS Lett.* **417**, 360-364.
- Gerlier, D. and Thomasset, N. (1986). Use of MTT colorimetric assay to measure cell activation. *J. Immunol. Methods* **94**, 57-63.
- Herr, I. and Debatin, K.M. (2001). Cellular stress response and apoptosis in cancer therapy. *Blood* **98**, 2603-2614.
- Johnson, G. L. and Nakamura, K. (2007). The c-jun kinase/stress-activated pathway: regulation, function and role in human disease. *Biochim. Biophys. Acta* **1773**, 1341-1348.

- Jost, L. M., Kirkwood, J. M. and Whiteside, T. L. (1992). Improved short- and long-term XTT-based colorimetric cellular cytotoxicity assay for melanoma and other tumor cells. *J. Immunol. Methods* **147**, 153-165.
- Kolomeichuk, S. N., Bene, A., Upreti, M., Dennis, R. A., Lyle, C. S., Rajasekaran, M. & Chambers, T. C. (2008). Induction of apoptosis by vinblastine via c-Jun autoamplification and p53-independent down-regulation of p21WAF1/CIP1. *Mol. Pharmacol.* **73**, 128-136.
- Krishan, A. (1975). Rapid flow cytofluorometric analysis of mammalian cell cycle by propidium iodide staining. *J. Cell. Biol.* **66**, 188-193.
- Lagadinou, E. D., Ziros, P. G., Tsopra, O. A., Dimas, K., Kokkinou, D., Thanopoulou, E., Karakantza, M., Pantazis, P., Spyridonidis, A. and Zoumbos, N. C. (2008). c-Jun N-terminal kinase activation failure is a new mechanism of anthracycline resistance in acute myeloid leukemia. *Leukemia* **22**, 1899-1908.
- Lavrik, I. N., Golks, A. and Krammer, P. H. (2005). Caspases: pharmacological manipulation of cell death. *J. Clin. Invest.* **115**, 2665-2672.
- Lin, A. and Dibling, B. (2002). The true face of JNK activation in apoptosis. *Aging Cell* **1**, 112-116.
- Liu, J. and Lin, A. (2005). Role of JNK activation in apoptosis: a double-edged sword. *Cell Res.* **15**, 36-42.
- Nishina, H., Wada, T. and Katada, T. (2004). Physiological roles of SAPK/JNK signaling pathway. *J. Biochem.* **136**, 123-126.
- Osborn, M.T. and Chambers, T.C. (1996). Role of the stress-activated/c-Jun NH2-terminal protein kinase pathway in the cellular response to adriamycin and other chemotherapeutic drugs. *J. Biol. Chem.* **271**, 30950-30955.
- Panaretakis, T., Laane, E., Pokrovskaja, K., Bjorklund, A. C., Moustakas, A., Zhivotovsky, B., Heyman, M., Shoshan, M. C. and Grand, D. (2005). Doxorubicin requires the sequential activation of caspase-2, protein kinase Cdelta, and c-Jun NH2-terminal kinase to induce apoptosis. *Mol. Biol. Cell.* **16**, 3821-3831.
- Philchenkov, A. (2004). Caspases: potential targets for regulating cell death. *J. Cell. Mol. Med.* **8**, 432-444.
- Potapova, O., Gorospe, M., Dougherty, R. H., Dean, N. M., Gaarde, W. A. and Holbrook, N. J. (2000). Inhibition of c-Jun N-terminal kinase 2 expression suppresses growth and induces apoptosis of human tumor cells in a p53-dependent manner. *Mol. Cell. Biol.* **20**, 1713-1722.
- Potapova, O., Haghighi, A., Bost, F., Liu, C., Birrer, M. J., Gjerset, R. and Mercola, D. (1997). The Jun kinase/stress-activated protein kinase pathway functions to regulate DNA repair and inhibition of the pathway sensitizes tumor cells to cisplatin. *J. Biol. Chem.* **272**, 14041-14044.
- Sampath, D., Cortes, J., Estrov, Z., Du, M., Shi, Z., Andreeff, M., Gandhi, V. and Plunkett, W. (2006). Pharmacodynamics of cytarabine alone and in combination with 7-hydroxystaurosporine (UCN-01) in AML blasts in vitro and during a clinical trial. *Blood* **107**, 2517-2524.
- Sampath, D. and Plunkett, W. (2000). The role of c-Jun kinase in the apoptotic response to nucleoside analogue-induced DNA damage. *Cancer Res.* **60**, 6408-6415.
- Stadheim, T.A., Saluta, G.R. and Kucera, G.L. (2000). Role of c-Jun N-terminal kinase/p38 stress signaling in 1-beta-D-arabinofuranosylcytosine-induced apoptosis. *Biochem. Pharmacol.* **59**, 407-418.
- Tashiro, E., Simizu, S., Takada, M., Umezawa, K. and Imoto, M. (1998). Caspase-3 activation is not responsible for vinblastine-induced Bcl-2 phosphorylation and G2/M arrest in human small cell lung carcinoma Ms-1 cells. *Jpn. J. Cancer Res.* **89**, 940-946.
- Turnbull, K. J., Brown, B. L. and Dobson, P. R. (1999). Caspase-3-like activity is necessary but not sufficient for daunorubicin-induced apoptosis in Jurkat human lymphoblastic leukemia cells. *Leukemia* **13**, 1056-1061.
- Weston, C. R. and Davis, R. J. (2007). The JNK signal transduction pathway. *Curr. Opin. Cell. Biol.* **19**, 142-149.
- Wisdom, R., Johnson, R. S. and Moore, C. (1999). c-Jun regulates cell cycle progression and apoptosis by distinct mechanisms. *EMBO J.* **18**, 188-197.
- Yu, R., Shtil, A. A., Tan, T. H., Roninson, I. B. and Kong, A. N. (1996). Adriamycin activates c-jun N-terminal kinase in human leukemia cells: a relevance to apoptosis. *Cancer Lett.* **107**, 73-81.