

Review

BAG3: a new therapeutic target of human cancers?

Huayuan Zhu, Peng Liu and Jianyong Li

Department of Hematology, The First Affiliated Hospital of Nanjing Medical University, Nanjing, People's Republic of China

Summary. Bcl-2-associated athanogene (BAG) family proteins share the BAG domain, which is characterized by their interaction with a variety of partners (heat shock proteins, steroid hormone receptors, Raf-1 and others) and is involved in regulating a number of cellular processes. BAG3, also known as CAIR-1 or Bis, mediates protein delivery to proteasome and modulates apoptosis by interfering with cytochrome c release, apoptosome assembly and other events in the cellular death program. Moreover, it takes part in the processes of cell adhesion and migration. It has been shown that, in human cancer cells, including lymphocytic and myeloblastic leukemic cells, BAG3 sustains cell survival and underlies resistance to chemotherapy, through down-modulation of apoptosis. BAG3 knocking down could enhance the effectiveness of chemotherapy. This review summarizes the physiological and pathological roles of BAG3 in cancer cells and its potential as a therapeutic target of human malignancies.

Key words: Cancer, Apoptosis, Adhesion, Migration, Leukemia

Introduction

Bcl-2-associated athanogene (BAG)-family proteins were originally identified by their ability to associate with the anti-apoptotic protein, Bcl-2 (Takayama et al., 1995). In humans, there are six BAG family members, including BAG1 (and its various isoforms), BAG2, BAG3 (CAIR-1; Bis), BAG4 (SODD), BAG5 and BAG6 (Scythe, BAT3) (Takayama and Reed, 2001). All of these proteins contain a BAG domain near their C terminus, except BAG5, which has five putative BAG

domains (Briknarova et al., 2001; Rosati et al., 2007). Among them, BAG-3 is a stress- and survival-related protein which was shown to be up-regulated upon cellular stress such as exposure to high temperature, heavy metals and CAI, an inhibitor of non-voltage regulated calcium influx and calcium-regulated adhesion / motility (Kohn et al., 1994; Doong et al., 2002). Moreover, BAG3 is involved in a number of cellular processes, including cell proliferation, apoptosis, adhesion and migration (Takayama et al., 1999; Takayama and Reed, 2001; Kassis et al., 2006). Evidence has indicated that BAG3 was highly expressed in different human cancer tissues, such as thyroid carcinoma, pancreatic cancer, prostate cancer, and leukemic cells (Liao et al., 2001; Romano, et al., 2003a,b; Bonelli et al., 2004; Chiappetta et al., 2007; Staibano et al., 2010). However, there is no or low expression of BAG3 in normal tissues (Liao et al., 2001; Chiappetta et al., 2007). BAG3 acts as a pro-survival and anti-apoptotic protein in different cancer cells, including leukemic cells. It can also enhance cell adhesion and migration to promote tumoral invasion. Furthermore, several reports, including our investigation, showed that inhibition of BAG3 expression could potentiate the efficiency of chemotherapy through different mechanisms (Rosati et al., 2007; Liu et al., 2009). The above observations indicate that BAG3 may be a potential therapeutic target of human cancers. This review illustrates experimental evidence that assigns to BAG3 a role in modulating cancer cell survival, apoptosis, adhesion and migration. We will also discuss the possibility of BAG3 as a candidate target of tumor therapy

Structures and physiologic functions

BAG3 gene, also known as CAIR-1 or Bis, is located in 10q25, encoding a 74 kDa cytoplasmatic protein, which is particularly concentrated in the rough endoplasmic reticulum. A slightly different molecular

weight or a doublet form can be observed in some cell types and following cell exposure to stressors (Doong et al., 2002; Pagliuca et al., 2003). It has three distinct protein interaction motifs: a WW domain, a proline-rich (PXXP) domain, and a BAG domain (Takayama and Reed, 2001; Doong et al., 2002). Although there has been no function identified yet for the WW domain, the PXXP region has diverse functions. It binds SH3 domains and interacts with focal adhesion kinase (FAK) and its downstream partners to mediate cell invasion and tumor progression (Vidal et al., 2001; Brabek et al., 2005; Kassis et al., 2006). The BAG domain, which is shared as an evolutionarily conserved motif within BAG family proteins and which binds and regulates Hsp70/Hsc70 molecular chaperones, is comprised of three anti-parallel α helices (Briknarova et al., 2001). The first and second helices ($\alpha 1$ and $\alpha 2$) bind the serine/threonine kinase Raf-1. The second and third helices ($\alpha 2$ and $\alpha 3$) interact with the ATPase domain of Hsc70/Hsp70 with TNF-R1 (Antoku et al., 2001; Sondermann et al., 2001; Song et al., 2001; Takayama and Reed, 2001; Briknarova et al., 2002). Recently, Fuchs et al and Hishiya et al identified two conserved IPV (Ile-Pro-Val) motifs in Bag3 which mediate its binding to the molecular chaperone HspB8 (heat-shock protein B8) (Fuchs et al., 2010; Hishiya et al., 2011).

Protein degradation is mediated by the proteasome and autophagy pathways. These processes are regulated by the interaction of BAG3 and its partner Hsps. Through the control of protein quality, BAG3 affects many cellular processes, including cell survival, apoptosis, migration and adhesion (Gamerding et al., 2009). BAG3 knockout mice show muscle defects resulting in fulminant myopathy, which is characterized by non-inflammatory myofibrillar degeneration, and may be due to the lack of BAG3-stimulated autophagy (Homma et al., 2006; Youn et al., 2008). Selcen et al. showed that a heterozygous p.Pro209Leu mutation in BAG3 could cause severe autosomal dominant childhood muscular dystrophy (Selcen et al., 2009). Furthermore, a novel role has been ascribed to BAG3 in controlling the turnover of misfolded protein by interacting with HspB8. In BAG3-HspB8 complex, HspB8 is responsible for recognizing the misfolded proteins, whereas BAG3 might recruit and activate the macroautophagy machinery in close proximity to the chaperone-loaded substrates, at least in part through its PXXP domain (Carra et al., 2008). Recently, Fuchs et al. have identified the existence of two conserved IPV (Ile-Pro-Val) motifs in the BAG3 central region between the WW (Trp-Trp) domain and PXXP region, which is necessary for BAG3 binding to its partners (HspB8 or HspB6) and mediates clearance of aggregated polyglutamine-containing protein Htt43Q (huntingtin exon 1 fragment with 43 CAG repeats) (Fuchs et al., 2010).

BAG3 in cancer cell survival and apoptosis

BAG3 has also been reported to play a remarkable

anti-apoptotic role by enhancing Bcl-2 activity and promoting survival of cancer cells (Takayama et al., 1995; Lee et al., 1999; Bonelli et al., 2004). Inhibiting the expression of BAG3 can promote the apoptosis of thyroid cancer cells, colon cancer cells and kidney cancer cells (Chiappetta et al., 2007; Du et al., 2008; Jacobs and Marnett, 2009; Wang et al., 2009). Romano et al. indicated that BAG3 depresses the apoptosis of B cell chronic lymphocytic leukemia (B-CLL) cells by inhibiting cytochrome c release and caspase-3 activity (Romano, et al., 2003a). Moreover, the anti-apoptotic effect of BAG3 is regulated by proteasome. The application of MG-132 to inhibit the 20S subunit of the proteasome can reduce BAG3 degradation and potentiate the anti-apoptotic effect of BAG3 (Virador et al., 2009), whereas BAG3 knockdown by siRNA sensitized cancer cells to MG-132 (Wang et al., 2008). However, the mechanism by which proteasome inhibitors affect the expression of BAG3 is diverse. HSF1 directly binds to the MG132-responsive motif on the BAG3 promoter, and the activation of HSF1 leads to the up-regulation of BAG3 upon MG-132 treatment (Du et al., 2009). Du et al. indicated that the regulation of BAG3 by proteasome inhibitor is also caspase-dependent (Du et al., 2008). The study from the same group also found that the JNK pathway was associated with the protective response against proteasome inhibition, by mediating induction of BAG3 (Wang et al., 2009). Interestingly, the level of BAG3 mRNA was decreased by about 3.1 fold when treated with all-trans retinoic acid and zoledronic acid in ovarian cancer cell lines (Karabulut et al., 2010). Our previous data showed that another proteasome inhibitor, Bortezomib, upregulated BAG3 expression in human leukemic cells, and that silencing the BAG3 gene by shRNA can sensitize leukemic cells to Bortezomib-induced apoptosis (Liu et al., 2009). These data confirm the anti-apoptotic effect of BAG3 and support the therapeutic role of BAG3 inhibition in cancer treatment.

Recently, it was found that BAG3 can be regulated by other proteins such as WT1 is involved in the processes of cell survival. Cesaro et al. showed that WT1 protein regulates BAG3 expression and that a WT1-mediated increase in BAG3 protein levels contributes to the prosurvival role of WT1 in leukemic cells (Cesaro et al., 2010). It was also demonstrated that BAG3 altered the interaction between Hsp70 and IKK γ , increasing availability of IKK γ and protecting it from proteasome-dependent degradation, which in turn results in increased NF- κ B activity and cell survival (Ammirante et al., 2010).

BAG3 in cancer cell adhesion and migration

Over-expression of BAG3 in MDA435 human breast cancer cells results in a significant decrease in migration and adhesion to matrix molecules, and this effect was reversed by deleting the BAG3 PXXP domain, indicating that the PXXP domain of BAG3 plays roles in the regulation of breast cancer cell migration and adhesion (Kassis et al., 2006). Furthermore, BAG3

down-regulates the expression of CCN1, a known AP-1 target, to modulate tumor cell adhesion and migration. Therefore, CCN1 may be necessary for adhesion and matrix-related signaling in cancer cells, abrogating a negative signal of the PXXP domain when BAG3 is intact (Kassis et al., 2009). Kassis et al showed that BAG-3 may regulate cell adhesion and migration through inhibiting focal adhesion proteins (Kassis et al., 2006). It was indicated that homozygous BAG3-deficient mouse embryonic fibroblasts (MEF) exhibit delayed formation of filopodia and focal adhesion complexes when freshly plated, and that BAG3-deficient MEFs show reduced cell motility in culture. Over-expression of BAG3 can increase motility of Cos7 cells and several types of human cancer cells, including breast cancer MCF7 and prostate cancer DU145 and ALVA31 cells (Iwasaki et al., 2007). Recently, Iwasaki and his colleagues indicated that BAG3 regulates cell adhesion and motility activity by interacting with PDZ domain containing guanine nucleotide exchange factor 2 (PDZGEF2). Briefly, the PPDY motif of C-terminus of PDZGEF2 binds with the WW domain of BAG3 to induce activation of Rap1 and thereby increases integrin-mediated cell adhesion (Iwasaki et al., 2010).

Possible roles of BAG3 in human leukemia

The expression of BAG3 in human leukemic cells has been reported by several groups. In 2003, Romano et al. firstly reported BAG3 protein was significantly expressed in 29 CLL patients (Romano et al., 2003a) and 11 childhood acute lymphoblastic leukemia (ALL) patients (Romano et al., 2003b). The up-regulated BAG3 gene expression was also observed in Busulfan-resistant human chronic myeloid leukemia B5/Bu2506 cells compared with their parental B5 cells (Valdez et al., 2008). Our unpublished results showed that BAG3 expression was significantly increased in 46 CLL patients compared with 20 healthy controls ($p=0.01$). Moreover, there was a higher level of BAG3 expression in drug-resistant patients compared with drug-responsive patients ($p=0.002$). Though the expression level of BAG3 has no statistically significant differences among CLL patient subgroups who were divided according to established prognostic factors such as disease stage and cytogenetics analysis, BAG3 expression was increased in the IgVH unmutated, CD38 positive, ZAP-70 positive and Binet C groups of CLL patients (Chen et al., 2010). Our latest data showed that the expression of BAG3 is markedly increased in 29 acute monocytic leukemia (AML) patients ($p=0.002$, unpublished data).

BAG3 can influence chemotherapy-induced apoptosis in neoplastic cells. The first evidence came from the study of primary leukemic cells from 29 CLL patients. Romano et al. found that down-regulation of BAG3 by antisense oligodeoxynucleotides (ODN) resulted in a more than 40% increase in both cytochrome c release and caspase 3 activity. The fludarabine-induced apoptosis percentages rose by more than 100%

when the primary leukemia cells were incubated with antisense ODN (Romano et al., 2003a). The pro-survival effect of BAG3 has also been concluded in 11 children acute lymphoblastic leukemic cell samples. The addition of any of three antisense, but not that of nonsense, ODNs can result in a >40% increase in caspase 3 activity and an enhancement by more than 93% of apoptotic elements in primary cultures, either untreated or incubated with cytosine arabinoside (Romano et al., 2003b).

Our previous investigations showed that bortezomib up-regulated BAG3 level in HL-60, U937, Jurkat, and MO7-e human leukemia cell lines. Moreover, BAG3 gene knockdown greatly sensitized generation of apoptosis by bortezomib in HL-60 and U937 leukemia cells (Liu et al., 2009). These results indicate that BAG3 gene up-regulation can partly account for bortezomib-resistance in some patients. In a nude mouse mode which was generated using HL-60 stably expressing shBAG3, though the BAG3 silencing alone had no effect on leukemic cell growth *in vivo*, it greatly enhanced the antitumor activity of bortezomib, which was demonstrated by the decreased tumor growth and weight at autopsy (Liu et al., 2009). These data indicated that BAG3 is a potential therapeutic target against leukemia, by which chemotherapeutic drugs can enhance clinical effects in the treatment of leukemia.

Conclusion

BAG3 expression is up-regulated in human tumoral cells. It regulates a number of cellular processes, including apoptosis and adhesion, as well as migration in cancer cells. Inhibition of BAG3 expression enhances the effectiveness of chemotherapy against cancer cells. Therefore, BAG3 may be a new marker for cancer prognosis and may be a novel therapeutic target of human malignancies.

Acknowledgements. This work was supported by National Natural Science Foundation of China (30500603, 30871104, 30971295, 81170485, 81170486), International Cooperation Program of Jiangsu Province (BZ2010041), the Program for Development of Innovative Research Team in the First Affiliated Hospital of NJMU and A Project Funded by the Priority Academic Program Development of Jiangsu Higher Education Institutions.

References

- Ammirante M., Rosati A., Arra C., Basile A., Falco A., Festa M., Pascale M., d'Avenia M., Marzullo L., Belisario M.A., De Marco M., Barbieri A., Giudice A., Chiappetta G., Vuttariello E., Monaco M., Bonelli P., Salvatore G., Di Benedetto M., Deshmane S.L., Khalili K., Turco M.C. and Leone A. (2010). IKK γ protein is a target of BAG3 regulatory activity in human tumor growth. *Proc. Natl. Acad. Sci. USA* 107, 7497-7502.
- Antoku K., Maser R.S., Scully W.J. Jr, Delach S.M. and Johnson D.E. (2001). Isolation of Bcl-2 binding proteins that exhibit homology with

- BAG-1 and suppressor of death domains protein. *Biochem. Biophys. Res. Commun.* 286, 1003-1010.
- Bonelli P., Petrella A., Rosati A., Romano M.F., Lerose R., Pagliuca M.G., Amelio T., Festa M., Martire G., Venuta S., Turco M.C. and Leone A. (2004). BAG3 protein regulates stress-induced apoptosis in normal and neoplastic leukocytes. *Leukemia* 18, 358-360.
- Brabek J., Constancio S.S., Siesser P.F., Shin N.Y., Pozzi A. and Hanks S.K. (2005). Crk-associated substrate tyrosine phosphorylation sites are critical for invasion and metastasis of SRC-transformed cells. *Mol. Cancer. Res.* 3, 307-315.
- Briknarova K., Takayama S., Brive L., Havert M.L., Knee D.A., Velasco J., Homma S., Cabezas E., Stuart J., Hoyt D.W., Satterthwait A.C., Llinas M., Reed J.C. and Ely K.R. (2001). Structural analysis of BAG1 cochaperone and its interactions with Hsc70 heat shock protein. *Nat. Struct. Biol.* 8, 349-352.
- Briknarova K., Takayama S., Homma S., Baker K., Cabezas E., Hoyt D.W., Li Z., Satterthwait A.C. and Ely K.R. (2002). BAG4/SODD protein contains a short BAG domain. *J. Biol. Chem.* 277, 31172-31178.
- Carra S., Seguin S.J. and Landry J. (2008). HspB8 and Bag3: a new chaperone complex targeting misfolded proteins to macroautophagy. *Autophagy* 4, 237-239.
- Cesaro E., Montano G., Rosati A., Crescitelli R., Izzo P., Turco M.C. and Costanzo P. (2010). WT1 protein is a transcriptional activator of the antiapoptotic bag3 gene. *Leukemia* 24, 1204-1206.
- Chen H.Y., Liu P., Sun M., Wu L.Y., Zhu H.Y., Qiao C., Dong H.J., Zhu D.X., Xu W. and Li J.Y. (2010). Bag3 gene expression in chronic lymphocytic leukemia and its association with patients' prognosis. *Zhongguo. Shi.Yan. Xue. Ye. Xue. Za. Zhi.* 18, 838-842.
- Chiappetta G., Ammirante M., Basile A., Rosati A., Festa M., Monaco M., Vuttariello E., Pasquinelli R., Arra C., Zerilli M., Todaro M., Stassi G., Pezzullo L., Gentilella A., Tosco A., Pascale M., Marzullo L., Belisario M.A., Turco M.C. and Leone A. (2007). The antiapoptotic protein BAG3 is expressed in thyroid carcinomas and modulates apoptosis mediated by tumor necrosis factor-related apoptosis-inducing ligand. *J. Clin. Endocrinol. Metab.* 92, 1159-1163.
- Doong H., Vrailas A. and Kohn E.C. (2002). What's in the 'BAG'?--A functional domain analysis of the BAG-family proteins. *Cancer Lett.* 188, 25-32.
- Du Z.X., Meng X., Zhang H.Y., Guan Y. and Wang H.Q. (2008). Caspase-dependent cleavage of BAG3 in proteasome inhibitors-induced apoptosis in thyroid cancer cells. *Biochem. Biophys. Res. Commun.* 369, 894-898.
- Du Z.X., Zhang H.Y., Meng X., Gao Y.Y., Zou R.L., Liu B.Q., Guan Y. and Wang H.Q. (2009). Proteasome inhibitor MG132 induces BAG3 expression through activation of heat shock factor 1. *J. Cell. Physiol.* 218, 631-637.
- Fuchs M., Poirier D.J., Seguin S.J., Lambert H., Carra S., Charette S.J. and Landry J. (2010). Identification of the key structural motifs involved in HspB8/HspB6-Bag3 interaction. *Biochem. J.* 425, 245-255.
- Gamerding M., Hajieva P., Kaya A.M., Wolfrum U., Hartl F.U. and Behl C. (2009). Protein quality control during aging involves recruitment of the macroautophagy pathway by BAG3. *EMBO J.* 28, 889-901.
- Hishiya A., Salman M.N., Carra S., Kampinga H.H. and Takayama S. (2011). BAG3 directly interacts with mutated alphaB-crystallin to suppress its aggregation and toxicity. *PLoS. One* 6, e16828.
- Homma S., Iwasaki M., Shelton G.D., Engvall E., Reed J.C. and Takayama S. (2006). BAG3 deficiency results in fulminant myopathy and early lethality. *Am. J. Pathol.* 169, 761-773.
- Iwasaki M., Homma S., Hishiya A., Dolezal S.J., Reed J.C. and Takayama S. (2007). BAG3 regulates motility and adhesion of epithelial cancer cells. *Cancer. Res.* 67, 10252-10259.
- Iwasaki M., Tanaka R., Hishiya A., Homma S., Reed J.C. and Takayama S. (2010). BAG3 directly associates with guanine nucleotide exchange factor of Rap1, PDZGEF2, and regulates cell adhesion. *Biochem. Biophys. Res. Commun.* 400, 413-418.
- Jacobs A.T. and Marnett L.J. (2009). HSF1-mediated BAG3 expression attenuates apoptosis in 4-hydroxynonenal-treated colon cancer cells via stabilization of anti-apoptotic Bcl-2 proteins. *J. Biol. Chem.* 284, 9176-9183.
- Karabulut B., Karaca B., Varol U., Muslu U., Cakar B., Atmaca H., Kisim A., Uzunoglu S. and Uslu R. (2010). Enhancing cytotoxic and apoptotic effect in OVCAR-3 and MDAH-2774 cells with all-trans retinoic acid and zoledronic acid: a paradigm of synergistic molecular targeting treatment for ovarian cancer. *J. Exp. Clin. Cancer. Res.* 29, 102.
- Kassis J.N., Guancial E.A., Doong H., Virador V. and Kohn E.C. (2006). CAIR-1/BAG-3 modulates cell adhesion and migration by downregulating activity of focal adhesion proteins. *Exp. Cell Res.* 312, 2962-2971.
- Kassis J.N., Virador V.M., Guancial E.A., Kimm D., Ho A.S., Mishra M., Chuang E.Y., Cook J., Gius D. and Kohn E.C. (2009). Genomic and phenotypic analysis reveals a key role for CCN1 (CYR61) in BAG3-modulated adhesion and invasion. *J. Pathol.* 218, 495-504.
- Kohn E.C., Felder C.C., Jacobs W., Holmes K.A., Day A., Freer R. and Liotta L.A. (1994). Structure-function analysis of signal and growth inhibition by carboxyamido-triazole, CAI. *Cancer. Res.* 54, 935-942.
- Lee J.H., Takahashi T., Yasuhara N., Inazawa J., Kamada S. and Tsujimoto Y. (1999). Bis, a Bcl-2-binding protein that synergizes with Bcl-2 in preventing cell death. *Oncogene* 18, 6183-6190.
- Liao Q., Ozawa F., Friess H., Zimmermann A., Takayama S., Reed J.C., Kleeff J. and Buchler M.W. (2001). The anti-apoptotic protein BAG-3 is overexpressed in pancreatic cancer and induced by heat stress in pancreatic cancer cell lines. *FEBS Lett.* 503, 151-157.
- Liu P., Xu B., Li J. and Lu H. (2009). BAG3 gene silencing sensitizes leukemic cells to Bortezomib-induced apoptosis. *FEBS Lett.* 583, 401-406.
- Pagliuca M.G., Lerose R., Cigliano S. and Leone A. (2003). Regulation by heavy metals and temperature of the human BAG-3 gene, a modulator of Hsp70 activity. *FEBS Lett.* 541, 11-15.
- Romano M.F., Festa M., Pagliuca G., Lerose R., Bisogni R., Chiurazzi F., Storti G., Volpe S., Venuta S., Turco M.C. and Leone A. (2003a). BAG3 protein controls B-chronic lymphocytic leukaemia cell apoptosis. *Cell. Death Differ.* 10, 383-385.
- Romano M.F., Festa M., Petrella A., Rosati A., Pascale M., Bisogni R., Poggi V., Kohn E.C., Venuta S., Turco M.C. and Leone A. (2003b). BAG3 protein regulates cell survival in childhood acute lymphoblastic leukemia cells. *Cancer Biol. Ther.* 2, 508-510.
- Rosati A., Ammirante M., Gentilella A., Basile A., Festa M., Pascale M., Marzullo L., Belisario M.A., Tosco A., Franceschelli S., Moltedo O., Pagliuca G., Lerose R. and Turco M.C. (2007). Apoptosis inhibition in cancer cells: a novel molecular pathway that involves BAG3 protein. *Int. J. Biochem. Cell Biol.* 39, 1337-1342.
- Selcen D., Muntoni F., Burton B.K., Pegoraro E., Sewry C., Bite A.V. and Engel A.G. (2009). Mutation in BAG3 causes severe dominant

BAG3 in cancer

- childhood muscular dystrophy. *Ann. Neurol.* 65, 83-89.
- Sondermann H., Scheufler C., Schneider C., Hohfeld J., Hartl F.U. and Moarefi I. (2001). Structure of a Bag/Hsc70 complex: convergent functional evolution of Hsp70 nucleotide exchange factors. *Science* 291, 1553-1557.
- Song J., Takeda M. and Morimoto R.I. (2001). Bag1-Hsp70 mediates a physiological stress signalling pathway that regulates Raf-1/ERK and cell growth. *Nat. Cell. Biol.* 3, 276-282.
- Staibano S., Mascolo M., Di Benedetto M., Vecchione M.L., Ilardi G., Di Lorenzo G., Autorino R., Salerno V., Morena A., Rocco A., Turco M.C. and Morelli E. (2010). BAG3 protein delocalisation in prostate carcinoma. *Tumour. Biol.* 31, 461-469.
- Takayama S. and Reed J.C. (2001). Molecular chaperone targeting and regulation by BAG family proteins. *Nat. Cell. Biol.* 3, E237-241.
- Takayama S., Sato T., Krajewski S., Kochel K., Irie S., Millan J.A. and Reed J.C. (1995). Cloning and functional analysis of BAG-1: a novel Bcl-2-binding protein with anti-cell death activity. *Cell* 80, 279-284.
- Takayama S., Xie Z. and Reed J.C. (1999). An evolutionarily conserved family of Hsp70/Hsc70 molecular chaperone regulators. *J. Biol. Chem.* 274, 781-786.
- Valdez B.C., Murray D., Ramdas L., de Lima M., Jones R., Kornblau S., Betancourt D., Li Y., Champlin R.E. and Andersson B.S. (2008). Altered gene expression in busulfan-resistant human myeloid leukemia. *Leuk. Res.* 32, 1684-1697.
- Vidal M., Gigoux V. and Garbay C. (2001). SH2 and SH3 domains as targets for anti-proliferative agents. *Crit. Rev. Oncol. Hematol.* 40, 175-186.
- Virador V.M., Davidson B., Czechowicz J., Mai A., Kassis J. and Kohn E.C. (2009). The anti-apoptotic activity of BAG3 is restricted by caspases and the proteasome. *PLoS One* 4, e5136.
- Wang H.Q., Liu H.M., Zhang H.Y., Guan Y. and Du Z.X. (2008). Transcriptional upregulation of BAG3 upon proteasome inhibition. *Biochem. Biophys. Res. Commun.* 365, 381-385.
- Wang H.Q., Liu B.Q., Gao Y.Y., Meng X., Guan Y., Zhang H.Y. and Du Z.X. (2009). Inhibition of the JNK signalling pathway enhances proteasome inhibitor-induced apoptosis of kidney cancer cells by suppression of BAG3 expression. *Br. J. Pharmacol.* 158, 1405-1412.
- Youn D.Y., Lee D.H., Lim M.H., Yoon J.S., Lim J.H., Jung S.E., Yeum C.E., Park C.W., Youn H.J., Lee J.S., Lee S.B., Ikawa M., Okabe M., Tsujimoto Y. and Lee J.H. (2008). Bis deficiency results in early lethality with metabolic deterioration and involution of spleen and thymus. *Am. J. Physiol. Endocrinol. Metab.* 295, E1349-1357.

Accepted September 21, 2011