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Sodium arsenite down-regulates the expression of X-linked inhibitor of apoptosis protein via translational and post-translational mechanisms in hepatocellular carcinoma

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ABSTRACT

X-linked inhibitor of apoptosis protein (XIAP) is a member of the inhibitors of apoptosis protein (IAP) family, and has been reported to exhibit elevated expression levels in hepatocellular carcinoma (HCC) and promote cell survival, metastasis and tumor recurrence. Targeting XIAP has proven effective for the inhibition of cancer cell proliferation and restoration of cancer cell chemosensitivity. Arsenic (or sodium arsenite) is a potent anti-tumor agent used to treat patients with acute promyelocytic leukemia (APL). Additionally, arsenic induces cell growth inhibition, cell cycle arrest and apoptosis in human HCC cells. In this study, we identified XIAP as a target for sodium arsenite-induced cytotoxicity in HCC. The exposure of HCC cell lines to sodium arsenite resulted in inhibition of XIAP expression in both a dose- and time-dependent manner. Sodium arsenite blocked the *de novo* XIAP synthesis and the activity of its internal ribosome entry site (IRES) element. Moreover, treatment with sodium arsenite decreased the protein stability of XIAP and induced its ubiquitin–proteasomal degradation. Overexpression of XIAP attenuated the pro-apoptotic effect of sodium arsenite in HCC. Taken together, our data demonstrate that sodium arsenite suppresses XIAP expression via translational and post-translational mechanisms in HCC.

1. Introduction

Hepatocellular carcinoma (HCC) is the fifth most common cancer and the third most common cause of death from cancer worldwide. Although HCC that is detected at an early stage can be managed with surgical resection or transplantation in select cases, advanced tumors respond poorly to currently available medical therapies [1]. Deregulation of the balance between cell proliferation and cell death is a common pro-tumorigenic theme in human hepatocarcinogenesis, which predominantly results from the over-activation of anti-apoptotic pathways [2]. Therefore, therapeutic strategies that selectively inhibit anti-apoptotic signals in liver tumor cells have the potential to become powerful tools for the treatment of HCC [2].

The inhibitors of apoptosis protein (IAP) represent a large family of endogenous caspase inhibitors [3]. XIAP is the most well-studied of the eight human IAPs and has been characterized as the most potent caspase inhibitor, functioning by directly binding to caspases through the baculovirus IAP repeat (BIR) domains [4]. The expression of XIAP is regulated at the level of translation. The 5′-untranslated region (UTR) of XIAP mRNA is reported to contain a 162-nucleotide (nt) internal ribosome entry site (IRES) sequence that is critical for the cap-independent translation of XIAP [5]. In addition, the C-terminal fragment of XIAP, which contains the BIR3 and RING finger domains, has been reported to be involved in the autoubiquitination and degradation of XIAP [6–8]. The XIAP BIR3 domain contains two autoubiquitination sites, Lys^{322} and Lys^{328} [9], whereas the RING domain possesses E3 ubiquitin ligase activity and is required for the autoubiquitination of XIAP [8,9]. Previous studies have demonstrated that XIAP expression is elevated in a variety of human tumors, including esophageal carcinoma [10], clear cell renal carcinoma [11], ovarian carcinoma [12] and lymphoma [13]. Furthermore, XIAP expression has been established as a risk factor in apoptosis resistance, invasiveness, metastasis, and tumor recurrence in HCC patients, suggesting that molecular targeting of XIAP has the potential to suppress HCC metastasis and recurrence [14].

Arsenic (or sodium arsenite) has been shown to be an effective inducer of cellular apoptosis in the clinical trials of acute promyelocytic leukemia (APL) patients; it is also approved by the US Food and Drug Administration for the treatment of relapsed APL that is refractory to therapy with all-trans retinoic acid (Food and Drug Administration (2000) FDA approves arsenic trioxide for leukemia treatment (http://www.fda.gov/bbs/topics/ANSWERS/ANS01040.html)). Moreover, arsenic trioxide (or sodium arsenite) also exhibits therapeutic

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potential and antitumor activity in a variety of solid tumors including HCC [15–17]. Accumulating evidence indicates that arsenic treatment potently induces cell growth inhibition, cell cycle arrest and apoptosis in human HCC cells [18,19].

In this study, we examined whether XIAP was a target of sodium arsenite in HCC. Our data demonstrated that sodium arsenite down-regulated the expression of XIAP in HCC via translational and post-translational mechanisms. The observed decrease in XIAP translation may result from the inhibition of IRES element activity, whereas the post-translational regulation of XIAP results from ubiquitin–proteasomal degradation. Moreover, over-expression of XIAP impaired the pro-apoptotic effect of sodium arsenite in HCC.

2. Materials and methods

2.1. Antibodies and reagents

The rabbit anti-XIAP antibody was purchased from Cell Signaling Technology. The mouse anti-GAPDH antibody was purchased from Santa Cruz Biotechnology. Cycloheximide (CHX) was purchased from Sigma. The dual-luciferase reporter assay system was purchased from Promega.

2.2. Cell culture

Huh7 and BEL-7402 cell lines were cultured in DMEM (Sigma) supplemented with 10% fetal bovine serum, 100 U/ml penicillin and 0.1 mg/ml streptomycin at 37 °C with 5% CO_2.

2.3. Construction of plasmids and transient cell transfection

The full-length amplified XIAP gene was inserted into the pEGFP-N3 vector to generate the pEGFP-XIAP plasmid. Transient transfection was performed using Lipofectamine™ 2000 (Invitrogen) according to the manufacturer's instructions.

2.4. Dual-luciferase reporter gene assay

Dual-luciferase reporter gene assay (Promega) was performed according to the manufacturer's instructions, using a Lumat LB 9507 luminometer (EG&G Berthold, Bad Wildbad, Germany).

2.5. [^{35}S]-methionine incorporation assay

The cells were incubated with DMEM depleted of methionine for 30 min and then labeled with [^{35}S]-methionine (Perkin Elmer). The cell extracts were subjected to immunoprecipitation with the XIAP antibody. The immunoprecipitates were eluted and applied to SDS–PAGE. Then, the gels were dried and visualized by phosphoimaging (Fujifilm).

2.6. Morphological analysis of apoptosis by fluorescent staining

Morphological analysis of apoptosis by fluorescent staining was performed according to our previous report [20]. Briefly, the cells grown on glass coverslips were fixed with 4% paraformaldehyde/PBS, permeabilized in 0.1% Triton X-100/PBS, and stained with Hoechst 33258. After the coverslips were washed in PBS, positive nuclei were counted. Normal nuclei and apoptotic nuclei (identified by their condensed new moon appearance or fragmented chromatin) were easily distinguished.

2.7. Statistical analysis

All data are presented as the mean ± standard deviation (SD). Statistical significance was determined using Student's *t*-test. *P*-values < 0.05 were considered statistically significant.

3. Results

3.1. Sodium arsenite down-regulates the protein expression level of XIAP in HCC

XIAP functions as a potent suppressor of apoptosis by inhibiting the activities of caspase enzymes. Furthermore, XIAP has been reported to be elevated in HCC and promote cell survival, metastasis and tumor recurrence [14]. To determine whether XIAP is a target of sodium arsenite-induced cytotoxicity, we first examined the effect of sodium arsenite on XIAP expression in HCC cells (Huh7 and BEL-7402). As shown in Fig. 1A, the protein expression level of XIAP decreased in a dose-dependent manner when HCC cells were exposed to increasing concentrations of sodium arsenite. Moreover, sodium arsenite induced a rapid loss of XIAP protein as early as 1 h after treatment and promoted the decrease of XIAP expression in a time-dependent manner (Fig. 1B).

3.2. Sodium arsenite inhibits the de novo protein synthesis of XIAP and its IRES activity

To explore the mechanism by which sodium arsenite modulated the expression of XIAP, we first employed quantitative real-time PCR to determine whether the mRNA expression levels of XIAP were affected. As shown in Supplementary Fig. 1, treatment with sodium arsenite showed little effect on the mRNA transcript levels of XIAP, suggesting that sodium arsenite modulated the expression of XIAP at the post-transcriptional level.

To understand whether sodium arsenite modulated the expression of XIAP at the translational level, a [^{35}S]-methionine incorporation assay was performed to detect the rate of *de novo* XIAP synthesis. As shown in Fig. 2A, treatment with sodium arsenite significantly suppressed the *de novo* protein level of XIAP. The effect of sodium arsenite on the XIAP IRES was also assessed using the bicistronic pcDNA/Fluc/IRES/Rluc reporter construct, which we described previously [21]. As shown in Fig. 2B, treatment with sodium arsenite significantly suppressed the XIAP IRES-mediated activity of the Renilla luciferase reporter in a time-dependent manner. Similar results were also observed in CHX-treated cells (Supplementary Fig. 2). However, treatment with PP242, a potent inhibitor of cap-dependent translation, showed little effect on the translation and expression of XIAP, suggesting that the protein synthesis of XIAP in HCC cells is IRES-mediated rather than cap-dependent (Supplementary Fig. 3). Taken together, these results indicate that sodium arsenite inhibits the translation of XIAP and that this inhibition may occur via suppression of XIAP IRES activity.

3.3. Sodium arsenite decreases the stability of XIAP and promotes its ubiquitin–proteasomal degradation

Evidence suggests that, in response to apoptotic stimuli, XIAP is capable of autoubiquitination, which leads to proteasomal degradation [7]. To determine whether proteasomal degradation was involved in the rapid loss of XIAP protein upon sodium arsenite treatment, the stability of XIAP was evaluated by cycloheximide chase. As shown in Fig. 3A, the turnover of XIAP protein was accelerated in cells treated with sodium arsenite (half-life of approximately 1 h) compared with control cells (half-life of greater than 1.5 h).

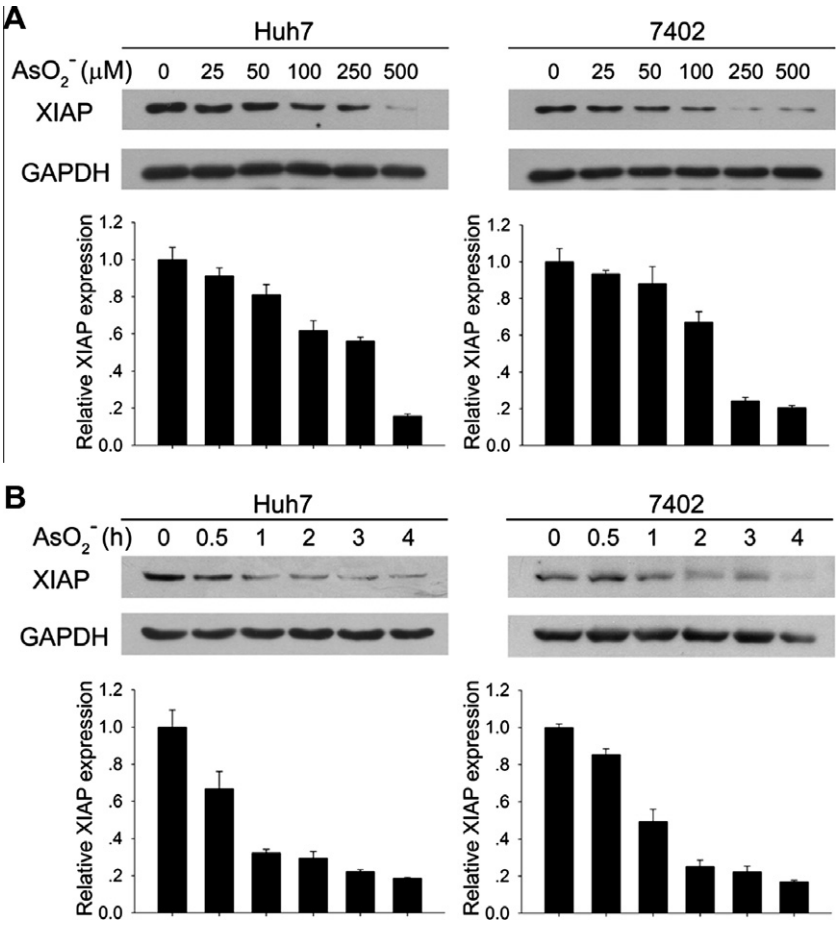

Fig. 1. Sodium arsenite down-regulates the expression of XIAP in HCC. (A) Sodium arsenite suppresses the expression of XIAP in a dose-dependent manner. Huh7 and BEL-7402 cells were treated with sodium arsenite at various concentrations for 4 h, and cell lysates were analyzed by Western blot to determine XIAP expression. (B) Sodium arsenite suppresses the expression of XIAP in a time-dependent manner. Huh7 and BEL-7402 cells were treated with sodium arsenite at 500 μM for the indicated lengths of time, and cell lysates were analyzed by Western blot to determine XIAP expression. Bands were quantified by densitometry scanning, and the value of XIAP/GAPDH in the absence of sodium arsenite was set to 1.0.

We next determined whether sodium arsenite promoted the degradation of XIAP through the ubiquitin–proteasomal pathway. As shown in Fig. 3B, the administration of sodium arsenite promoted the ubiquitination of XIAP, both in the presence and absence of the proteasome inhibitor MG132. Moreover, MG132 suppressed sodium arsenite-induced down-regulation of XIAP expression. These results suggest that sodium arsenite decreases the stability of XIAP and promotes its ubiquitin–proteasomal degradation.

3.4. Overexpression of XIAP attenuates the pro-apoptotic effect of sodium arsenite

To determine whether the down-regulation of XIAP protein expression contributes to the cytotoxicity of sodium arsenite, we examined the effect of XIAP over-expression on sodium arsenite-induced HCC cell apoptosis. As shown in Fig. 4, over-expression of XIAP attenuated sodium arsenite-induced PARP cleavage as well as cellular apoptosis in HCC cells. These results suggest that down-regulation of XIAP contributes to sodium arsenite-induced cytotoxicity in HCC.

4. Discussion

In this study, we reported that XIAP was a target of sodium arsenite-induced cytotoxicity in HCC. Our results demonstrated that sodium arsenite suppressed the expression of XIAP through inhibiting its *de novo* protein synthesis and promoting its ubiquitin–proteasomal degradation. Furthermore, overexpression of XIAP partially rescued cellular apoptosis induced by sodium arsenite.

Previous reports suggest that the IRES motif potentiates XIAP-mediated cytoprotection [22]. This IRES element is located in the 5′-untranslated region (UTR) of the XIAP mRNA and mediates the translation of XIAP during cell proliferation [23]. Under certain conditions of cellular stress, such as serum deprivation and low-dose radiation, the activity of the XIAP IRES is enhanced, leading to increased XIAP expression and resistance to cellular apoptosis [5]. However, in our study, we found that sodium arsenite treatment suppressed the IRES activity of XIAP in HCC cells, suggesting that the regulation of XIAP IRES activity is dependent upon the type of cellular stress. Our data also demonstrated that treatment with the cap inhibitor PP242 produced little effect on XIAP translation and expression in HCC cells, suggesting that XIAP translation may be entirely IRES-dependent (Supplementary Fig. 3). However, in human cholangiocarcinoma, translation of XIAP during hypoxia is dependent on the cap-binding protein eIF4E [24]. Thus, the mechanisms of XIAP translation initiation may vary between different types of tumors.

Our results demonstrated that sodium arsenite also impaired the stability of XIAP protein and promoted its ubiquitin–proteasomal degradation. The C-terminal fragment of XIAP, which contains the BIR3 and RING finger domains, is involved in the autoubiquitination

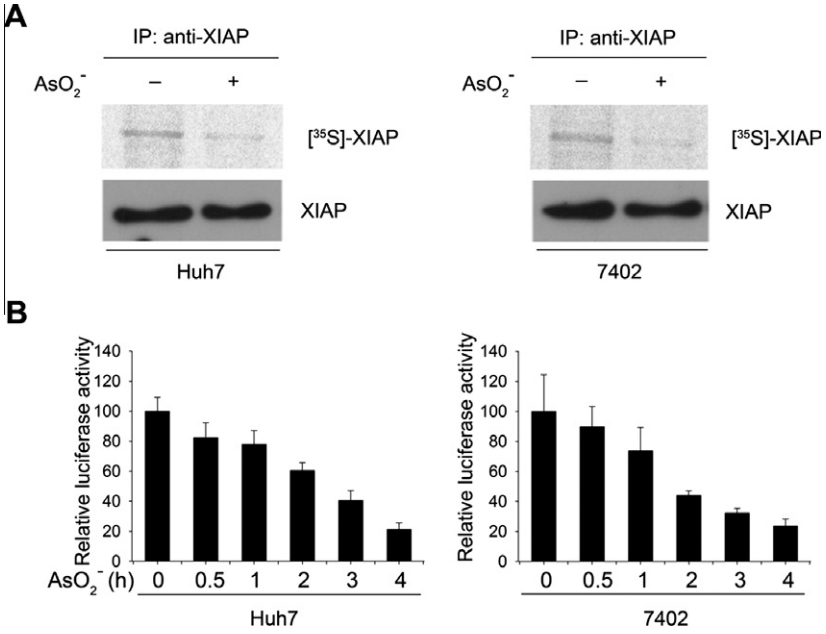

Fig. 2. Sodium arsenite inhibits the *de novo* protein synthesis of XIAP and XIAP IRES activity. (A) Sodium arsenite inhibits the *de novo* protein synthesis of XIAP. Cells were treated with sodium arsenite (500 μM) for 1 h, followed by a [^{35}S]-methionine incorporation assay. (B) Sodium arsenite inhibits the IRES activity of XIAP. The bicistronic pcDNA-Fluc-IRES-Rluc reporter was transfected into cells, and the cells were then treated with sodium arsenite for the indicated lengths of time. Renilla luciferase activities were measured as described in Section 2.

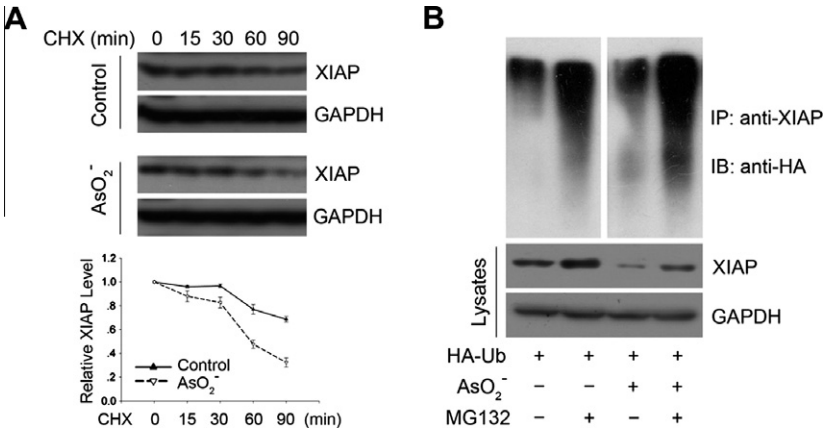

Fig. 3. Sodium arsenite decreases the stability of XIAP and promotes its ubiquitin–proteasomal degradation. (A) Sodium arsenite decreases the stability of XIAP in HCC cells. BEL-7402 cells were treated with 50 μg/ml CHX in the presence or absence of sodium arsenite (500 μM) for the indicated lengths of time. XIAP expression levels were quantified by densitometric analysis and statistically analyzed from three independent experiments. GAPDH was used as an internal control. (B) Sodium arsenite promotes the ubiquitin–proteasomal degradation of XIAP in HCC cells. BEL-7402 cells were transfected with a pcDNA3-HA-ubiquitin construct. At 24 h after transfection, the cells were treated with sodium arsenite and/or MG132 for an additional 4 h. The cell lysates were subjected to immunoprecipitation, and the precipitated complexes were separated and detected with an anti-HA antibody.

and degradation of XIAP [8]. Interestingly, we found that the addition of an EGFP tag to the C-terminus protected exogenously expressed XIAP protein from undergoing sodium arsenite-induced down-regulation (Fig. 4A). Our results are supported by previous reports indicating that the addition of a large tag, such as 6× Myc or enhanced green fluorescent protein (EGFP), around ubiquitination sites can stabilize proteins and suppress their ubiquitin–proteasomal degradation [25,26]. However, the ability of sodium arsenite to promote the degradation of XIAP by inducing autoubiquitination remains to be investigated. A previous study demonstrated that the phosphorylation of XIAP by AKT could protect XIAP from autoubiquitination and degradation [27]. Additionally, arsenic treatment down-regulates both AKT activity and total AKT protein [28]. Therefore, it is likely that decreased AKT activity contributes to the reduced XIAP stability induced by sodium arsenite.

HCC generally exhibits an inherent high resistance to chemotherapeutic drugs. As a result of producing only marginal anti-tumor activity and showing no survival benefit in HCC patients, the systemic and selective intra-arterial administration of chemotherapeutic agents is not recommended in clinical practice. Studies have repeatedly described XIAP as a survival factor in mammalian cancer [29]. As a result, XIAP antagonists, such as SMAC mimetics and AIBM-peptides, have been designed to block its anti-apoptotic function and induce tumor cell death [30,31]. Another approach is the knockdown of XIAP expression with siRNAs or antisense oligonucleotides, which inhibits cancer cell proliferation and restores chemosensitivity in a variety of malignant cell lines [32–35]. Our results demonstrate that sodium arsenite decreases the expression of XIAP in HCC, rendering it a potential therapy that may be used in synergy with other agents for the treatment of HCC.

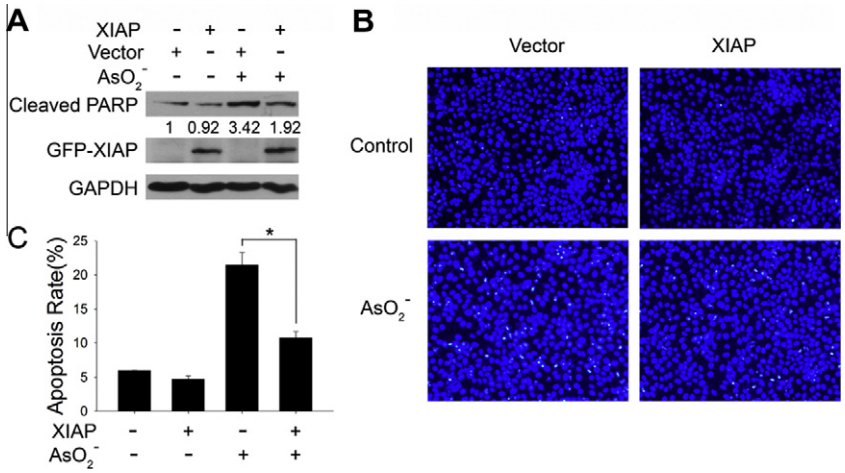

Fig. 4. Overexpression of XIAP attenuates the pro-apoptotic effect of sodium arsenite. (A) Overexpression of XIAP decreases sodium arsenite-induced cleavage of PARP. BEL-7402 cells were transfected with XIAP or control plasmids and treated with sodium arsenite (500 μM) for 1 h. PARP cleavage was analyzed with an antibody that recognizes the cleaved form of PARP. GAPDH was used as a loading control, and the ratio of cleaved PARP/GAPDH was measured for each sample, as indicated. (B and C) Overexpression of XIAP partially protects HCC cells form sodium arsenite-induced apoptosis. The cells were treated as in (A), stained with Hoechst 33258 and examined under a fluorescence microscope to identify apoptotic cells. At least 300 cells were examined randomly.

Acknowledgments

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bbrc.2012.05.066.

References

[1] T.F. Greten, M.P. Manns, F. Korangy, Immunotherapy of HCC, Rev. Recent Clin. Trials 3 (2008) 31–39.
[2] I. Fabregat, Dysregulation of apoptosis in hepatocellular carcinoma cells, World J. Gastroenterol. 15 (2009) 513.
[3] Q.L. Deveraux, J.C. Reed, IAP family proteins – suppressors of apoptosis, Genes Dev. 13 (1999) 239–252.
[4] Y. Shi, Mechanisms of caspase activation and inhibition during apoptosis, Mol. Cell 9 (2002) 459–470.
[5] M. Holcik, R.G. Korneluk, Functional characterization of the X-linked inhibitor of apoptosis (XIAP) internal ribosome entry site element: role of La autoantigen in XIAP translation, Mol. Cell. Biol. 20 (2000) 4648–4657.
[6] A.J. Schile, M. Garcia-Fernandez, H. Steller, Regulation of apoptosis by XIAP ubiquitin–ligase activity, Genes Dev. 22 (2008) 2256–2266.
[7] Y. Yang, S. Fang, J.P. Jensen, A.M. Weissman, J.D. Ashwell, Ubiquitin protein ligase activity of IAPs and their degradation in proteasomes in response to apoptotic stimuli, Science 288 (2000) 874–877.
[8] Q.L. Deveraux, E. Leo, H.R. Stennicke, K. Welsh, G.S. Salvesen, J.C. Reed, Cleavage of human inhibitor of apoptosis protein XIAP results in fragments with distinct specificities for caspases, EMBO J. 18 (1999) 5242–5251.
[9] H. Shin, K. Okada, J.C. Wilkinson, K.M. Solomon, C.S. Duckett, J.C. Reed, G.S. Salvesen, Identification of ubiquitination sites on the X-linked inhibitor of apoptosis protein, Biochem. J. 373 (2003) 965–971.
[10] S. Zhang, F. Ding, A. Luo, A. Chen, Z. Yu, S. Ren, Z. Liu, L. Zhang, XIAP is highly expressed in esophageal cancer and its downregulation by RNAi sensitizes esophageal carcinoma cell lines to chemotherapeutics, Cancer Biol. Ther. 6 (2007) 973–980.
[11] U. Ramp, T. Krieg, E. Caliskan, C. Mahotka, T. Ebert, R. Willers, H.E. Gabbert, C.D. Gerharz, XIAP expression is an independent prognostic marker in clear-cell renal carcinomas, Hum. Pathol. 35 (2004) 1022–1028.
[12] A. Mansouri, Q. Zhang, L.D. Ridgway, L. Tian, F.X. Claret, Cisplatin resistance in an ovarian carcinoma is associated with a defect in programmed cell death control through XIAP regulation, Oncol. Res. 13 (2003) 399–404.
[13] N. Akyurek, Y. Ren, G.Z. Rassidakis, E.J. Schlette, L.J. Medeiros, Expression of inhibitor of apoptosis proteins in B-cell non-Hodgkin and Hodgkin lymphomas, Cancer 107 (2006) 1844–1851.
[14] Y.H. Shi, W.X. Ding, J. Zhou, J.Y. He, Y. Xu, A.A. Gambotto, H. Rabinowich, J. Fan, X.M. Yin, Expression of X-linked inhibitor-of-apoptosis protein in hepatocellular carcinoma promotes metastasis and tumor recurrence, Hepatology 48 (2008) 497–507.
[15] B. Liu, S. Pan, X. Dong, H. Qiao, H. Jiang, G.W. Krissansen, X. Sun, Opposing effects of arsenic trioxide on hepatocellular carcinomas in mice, Cancer Sci. 97 (2006) 675–681.
[16] H.W. Chiu, Y.S. Ho, Y.J. Wang, Arsenic trioxide induces autophagy and apoptosis in human glioma cells in vitro and in vivo through downregulation of survivin, J. Mol. Med. (Berl.) 89 (2011) 927–941.
[17] R.J. Griffin, B.W. Williams, N.A. Koonce, J.C. Bischof, C.W. Song, R. Asur, M. Upreti, Vascular disrupting agent arsenic trioxide enhances thermoradiotherapy of solid tumors, J. Oncol. 2012 (2012) 934918.
[18] M. Fei, M. Lu, Y. Wang, Y. Zhao, S. He, S. Gao, Q. Ke, Y. Liu, P. Li, X. Cui, A. Shen, C. Cheng, Arsenic trioxide-induced growth arrest of human hepatocellular carcinoma cells involving FOXO3a expression and localization, Med. Oncol. 26 (2009) 178–185.
[19] S. Othumpangat, M. Kashon, P. Joseph, Sodium arsenite-induced inhibition of eukaryotic translation initiation factor 4E (eIF4E) results in cytotoxicity and cell death, Mol. Cell. Biochem. 279 (2005) 123–131.
[20] Z. Li, H. Wang, H. Zong, Q. Sun, X. Kong, J. Jiang, J. Gu, Downregulation of beta1,4-galactosyltransferase 1 inhibits CDK11(p58)-mediated apoptosis induced by cycloheximide, Biochem. Biophys. Res. Commun. 327 (2005) 628–636.
[21] Y. Hao, X. Kong, Y. Ruan, H. Gan, H. Chen, C. Zhang, S. Ren, J. Gu, CDK11p46 and RPS8 associate with each other and suppress translation in a synergistic manner, Biochem. Biophys. Res. Commun. 407 (2011) 169–174.
[22] M. Holcik, C. Lefebvre, C. Yeh, T. Chow, R.G. Korneluk, A new internal-ribosome-entry-site motif potentiates XIAP-mediated cytoprotection, Nat. Cell Biol. 1 (1999) 190–192.
[23] M. Holcik, N. Sonenberg, Translational control in stress and apoptosis, Nat. Rev. Mol. Cell Biol. 6 (2005) 318–327.
[24] C. Marienfeld, Y. Yamagiwa, Y. Ueno, V. Chiasson, L. Brooks, F. Meng, T. Patel, Translational regulation of XIAP expression and cell survival during hypoxia in human cholangiocarcinoma, Gastroenterology 127 (2004) 1787–1797.
[25] J. Yang, Y. Hong, W. Wang, W. Wu, Y. Chi, H. Zong, X. Kong, Y. Wei, X. Yun, C. Cheng, K. Chen, J. Gu, HSP70 protects BCL2L12 and BCL2L12A from N-terminal ubiquitination-mediated proteasomal degradation, FEBS Lett. 583 (2009) 1409–1414.
[26] A. Ciechanover, N-terminal ubiquitination, Methods Mol. Biol. 301 (2005) 255–270.
[27] H.C. Dan, M. Sun, S. Kaneko, R.I. Feldman, S.V. Nicosia, H.G. Wang, B.K. Tsang, J.Q. Cheng, Akt phosphorylation and stabilization of X-linked inhibitor of apoptosis protein (XIAP), J. Biol. Chem. 279 (2004) 5405–5412.
[28] K.K. Mann, M. Colombo, W.H. Miller Jr., Arsenic trioxide decreases AKT protein in a caspase-dependent manner, Mol. Cancer Ther. 7 (2008) 1680–1687.
[29] H. Kashkar, X-linked inhibitor of apoptosis: a chemoresistance factor or a hollow promise, Clin. Cancer Res. 16 (2010) 4496–4502.
[30] S. Ramachandiran, J. Cain, A. Liao, Y. He, X. Guo, L.H. Boise, H. Fu, L. Ratner, H.J. Khoury, L. Bernal-Mizrachi, The Smac mimetic RMT5265.2HCL induces apoptosis in EBV and HTLV-I associated lymphoma cells by inhibiting XIAP

and promoting the mitochondrial release of cytochrome C and Smac, Leuk. Res. 36 (2012) 784–790.
[31] N. Edison, T.H. Reingewertz, Y. Gottfried, T. Lev, D. Zuri, I. Maniv, M.J. Carp, G. Shalev, A. Friedler, S. Larisch, Peptides mimicking the unique ARTS-XIAP binding site promote apoptotic cell death in cultured cancer cells, Clin. Cancer Res. 18 (2012) 2569–2578.
[32] M. Chawla-Sarkar, S.I. Bae, F.J. Reu, B.S. Jacobs, D.J. Lindner, E.C. Borden, Downregulation of Bcl-2, FLIP or IAPs (XIAP and survivin) by siRNAs sensitizes resistant melanoma cells to Apo2L/TRAIL-induced apoptosis, Cell Death Differ. 11 (2004) 915–923.
[33] D.C. McManus, C.A. Lefebvre, G. Cherton-Horvat, M. St-Jean, E.R. Kandimalla, S. Agrawal, S.J. Morris, J.P. Durkin, E.C. Lacasse, Loss of XIAP protein expression by RNAi and antisense approaches sensitizes cancer cells to functionally diverse chemotherapeutics, Oncogene 23 (2004) 8105–8117.
[34] C. Jiang, T. Tan, X.P. Yi, H. Shen, Y.X. Li, Lentivirus-mediated shRNA targeting XIAP and survivin inhibit SW1990 pancreatic cancer cell proliferation in vitro and in vivo, Mol. Med. Rep 4 (2011) 667–674.
[35] J.J. Ma, B.L. Chen, X.Y. Xin, XIAP gene downregulation by small interfering RNA inhibits proliferation, induces apoptosis, and reverses the cisplatin resistance of ovarian carcinoma, Eur. J. Obstet. Gynecol. Reprod. Biol. 146 (2009) 222–226.