

Review Article

Triptolide: Novel Anticancer Agent for Chemoresistant Cancer Cells that are Caspase-3 Deficient

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***Corresponding author:** Halaby R, Department of Biology and Molecular Biology, USA**Received:** November 17, 2014; **Accepted:** December 09, 2014; **Published:** January 12, 2015**Abstract**

Cancer is a major cause of death worldwide and there are over 100 different types of cancers. The current treatment strategies for cancer patients such as surgery, chemotherapy and radiation or the combination of radiotherapy and chemotherapy may have successfully increased five-year survival rates. However, the long-term survival rates remain poor due to cancer relapse, tumor resistance and metastasis. Treatments such as chemotherapy and radiation are known to have untoward side effects on normal cells. Therefore, it is imperative to develop safer and noninvasive treatments. The use of natural products in the fight against cancer is one possible way of slowing tumor growth and may allow us to design more effective therapies. In this review, we examine the central role played by the key apoptotic executioner protease, caspase-3 and propose molecular mechanisms by which cancer cells can evade anticancer treatments by mutating this protein. Triptolide, a Chinese herb, has been used for over 200 years to treat anti-inflammatory and autoimmune disorders. A considerable body of data indicates that triptolide has potent anticancer properties. We provide supporting evidence demonstrating that caspase-3 independent cell, lysosomal-mediated death pathways induced by triptolide, a Chinese herb, may provide effective novel antitumor treatment modalities.

Keywords: Caspase-3; Chemoresistance; Triptolide; Apoptosis; Lysosomes

Abbreviations

LMP: Lysosomal Membrane Permeabilization

Introduction

Cancer is a global disease that results in a considerable health care burden. While great strides have been made in anticancer treatment modalities, there are still a significant number of treatment-resistant cancers that ultimately lead to metastatic disease. Therapies such as radiation and chemotherapy are known to have serious side effects; therefore it is imperative to develop safer treatment alternatives. The key executioner protease, caspase-3, plays a pivotal role in regulating apoptotic pathways and a considerable body of literature indicates that mutations in this protein confer a survival, chemoresistance phenotype in tumor cells. In this paper, we provide supporting data for caspase-3's effect on survival in cancer cells and propose a novel mechanism to treat resistant cancer cells using a natural product.

Triptolide

Triptolide is an extract from the Chinese herb *Tripterygium wilfordii* Hook F. Long used in traditional Chinese medicine, the herb is purported to have immunosuppressive and anti-inflammatory properties and triptolide has been identified as a major chemical component governing these properties [1, 2]. Triptolide is a diterpenoid triepoxide. It is well documented that triptolide has a broad spectrum ability to inhibit proliferation and induce apoptosis of various cancer cell lines *in vitro* and prevent tumor growth and metastases *in vivo* [3- 8]. Triptolide shows anticancer activity in cells derived from both hematological malignancies and solid tumors, such as HL-60, T cell lymphoma [9], U937, OCI-AML3 [10],

Kasumi-1 and SKNO-1 cells [11], human hepatocellular carcinoma SMMC-7721 cells and cell lines of multiple myeloma, breast, gastric, prostate, lung, oral, colon, pancreatic and cervical cancers [3, 6, 12-14], cholangiocarcinoma [15] and neuroblastoma [16, 17]. The *in vivo* experiments have also demonstrated triptolide's therapeutic efficacy in several model systems including cholangiocarcinoma in a hamster model [18] and xenografts of human melanoma, breast cancer, bladder cancer, gastric carcinoma [17], pancreatic cancer [18] and neuroblastoma in nude mice [8].

Since triptolide has epoxide moieties, it is conceivable that this compound could bind to a certain cellular protein via formation of covalent bond. In 1974, Kupchan *et al.* [19] suggested that the 14b hydroxyl along with the [9, 11] epoxide might be responsible for the observed antitumor activity. In 2007, McCallum *et al.* [20] discovered that triptolide could bind specifically and irreversibly through the epoxide moieties to a 90 kDa nuclear protein, which may be a transcriptional regulator or somehow involved in turnover of a critical transcriptional regulator, such that its covalent modification prevented a key step in transcription. Recently, Titov *et al.* [21] reported that triptolide covalently bound to a human 90 kD protein, XPB (also known as ERCC3) which is a subunit of the transcription factor TFIIH, and inhibited its DNA-dependent ATPase activity, which led to the inhibition of RNA polymerase II-mediated transcription and likely nucleotide excision repair. The identification of XPB as the target of triptolide accounts for the majority of the known biological activities of triptolide. In human gastric and prostatic epithelial cells [22, 23] and HL-60 leukemia cells [24], triptolide-caused proliferation inhibition and apoptosis induction may be primarily mediated by its modulation of p53, a nuclear phosphor protein which acts as a tumor

Table 1: Targets of triptolide for its antitumor effects.

Target gene	Tumor/Cell Line	Reference(s)
Caspase 3, 8, and 9	Multiple myeloma cells	[39]
XIAP	Leukemic cell lines, acute myeloid leukemia	[36]
bcr-abl	K562 cells	[31]
Bax, Bcl-2	Glioma cells, HL-60	[12, 40]
p53, p21(waf1/cip1), bax	Gastric cancer cells	[12]
NFκB	Human anaplastic thyroid carcinoma cells	[4]
MKP-1, ERK-1/2, JNK-1/2, p38 MAPK	NSCLC, hippocampal cells	[41, 42]
PI3K	Human fibrosarcoma	[43]
HSP70, HSF1	Pancreatic cancer cells	[18, 44, 45]
5-LOX	Pancreatic cancer cells	[46]
ADAM10	Leukemic cell lines	[47]
RNA polymerase	Human non-small cell lung cancer cell line	[48]
Jak2, Mcl-1	Human myeloproliferative disorder cells	[49]
Histone methyltransferase	Myeloma	[50]

suppressor. Nuclear factor κB (NF-κB) is a transcription factor that can promote cell survival, stimulate growth and reduce susceptibility to apoptosis via up regulation of various targeted proteins [25-30]. However, other reports suggest that triptolide inhibits the DNA binding ability of NF-κB or cytokine-stimulated NF-κB activity [31, 32]. In multiple myeloma cells, triptolide decreases histone H3K9 and H3K27 methylation via the down-regulation of histone methyltransferases SUV39H1 and EZH2, respectively and reduces the expression of HDAC8, leading to increase of the histone H3 and H4 acetylation [33]. Triptolide also inhibits the activity of RNA polymerase, resulting in the general transcription inhibition [34].

In a cDNA array analysis, Zhao *et al.* (33) demonstrated that triptolide inhibited the expression of genes involved in cell cycle progression and cell survival, such as cyclins D1, B1 and A1, Cdc-25; Bcl-X and c-Jun. Triptolide reduced the expression of apoptosis antagonists XIAP, Bcl-2 and Mcl-1 [35]. Triptolide induced caspase-dependent apoptosis of leukemia and cervical cancer cells [6, 15, 36] and triggered caspase-independent autophagic cell death in pancreatic cancer cells [15]. Leuenroth *et al.* [37] identified calcium (Ca²⁺) channel polycystin-2 (PC2) as a putative direct target of triptolide in a mouse model of polycystic kidney disease (PKD). Triptolide may perturb multiple targets and interfere with multiple signaling pathways and potentiate activities of other antitumor agents such as Apo2/TRAIL, tumor necrosis factor α and other chemotherapeutic agents.

The known targets of triptolide for promoting its antitumor effects are shown in Table 1, reviewed in Liu [38]. The exact mechanisms by which triptolide regulates these molecular targets have yet to be fully elucidated. A better understanding of these processes should lay a foundation for the development of triptolide in chemotherapeutic treatments.

Triptolide and Chemoresistance

Ovarian cancer is currently the leading cause of mortality among gynecological malignant tumors, with epithelial ovarian cancer (EOC) being the most common, accounting for >85% of all cases [51].

The majority of ovarian cancers are diagnosed at an advanced stage, mostly due to a lack of effective screening strategies and difficulties in obtaining a diagnosis [52]. Despite the progress that has been made in prolonging remission by the combination of surgical resection and platinum-based chemotherapy, the overall survival of patients with advanced disease is rarely >30%. The poor prognosis in the treatment of ovarian cancer is mainly attributed to chemoresistance [53]. Tumor cells may dampen the cytotoxic effects of anticancer drugs via several mechanisms, including increased drug efflux, drug inactivation, alteration in the drug target and increased DNA repair [54, 55]. As a result, efforts have been directed towards the development of novel agents in an attempt to ameliorate the lethality of this malignancy. Recent studies on the chemoresistance of ovarian cancer have indicated that a decreased susceptibility of the cancer to apoptosis is strongly associated with drug resistance. Thus, novel strategies involving less toxic agents that are able to either enhance the antitumor effects of cisplatin or overcome chemoresistance to the drug are highly desirable.

Constitutively activated nuclear factor (NF)-κB may be critical in the development of drug resistance in ovarian cancer cells [56]. NF-κB is known to suppress apoptosis through the induction of anti-apoptotic proteins, including Bcl-2 and X-linked inhibitor of apoptosis protein (XIAP), leading to a resistance to cancer therapy and a poor prognosis [57-59]. Intriguingly, numerous anticancer drugs, including the DNA-damaging agent cisplatin are able to simultaneously stimulate NF-κB activation, as they trigger the cell death process in neoplasm cells [57, 58, 60]. Therefore, the inhibition of NF-κB may be useful in increasing the sensitivity of cells to chemotherapy-dependent apoptosis and reversing drug resistance in ovarian cancer.

Although first-line platinum-based chemotherapy following an apparent curative resection has improved survival length, severe adverse side-effects and drug resistance have emerged as the major impediments to effective ovarian cancer therapy [61]. The pleiotropic anticancer activities of triptolide have attracted a great deal of research interest. Notably, triptolide has also been identified to be effective in

the induction of apoptosis in drug-resistant multiple myeloma [62] and cervical cancer [63] cells. A recent study investigated whether triptolide treatment was able to exhibit a cytotoxic effect on platinum-resistant ovarian cancer cells [64]. The results demonstrated that triptolide reduced the growth of the platinum-resistant ovarian cancer cells by inducing apoptosis, evidenced by the externalization of membrane-bound phosphatidylserine and the cleavage of caspase 3 [64]. The results also showed that the addition of a low concentration of triptolide greatly increased the cytotoxicity of cisplatin against the SKOV3^{PT} cells, which is consistent with previous studies [62-64]. Triptolide circumvents drug resistance and enhances the antitumor effect of 5-fluorouracil [63, 65]. The understanding of the molecular mechanisms by which triptolide inhibits cancer cell growth will shed new insights for cancer therapy.

The HER2 gene, also known as *neu* (in mouse) or *erbB2*, encodes a 185-kDa transmembrane receptor tyrosine kinase and is a member of the epidermal growth factor receptor family [65]. Over expression of HER2 is found in approximately 30% of human breast cancers and in many other cancer types [66]. HER2 phosphorylates downstream substrates and activates a variety of signaling cascades, including phosphatidylinositol-3 kinase (PI3K), serine/threonine-specific protein kinase (Akt) and Ras/mitogen-activated protein kinase (MAPK) pathways. These regulatory signaling cascades promote cell survival, tumor growth, and metastasis [67, 68]. Triptolide reduces PI3K activity, which is a downstream of HER2 signaling pathway. More importantly, triptolide blocks the activity of NF- κ B, which activates HER2 gene transcription [69, 70]. Thus, it is hypothesized that triptolide-induced tumor regression is attributed to its repression on HER2 activity.

The activation of anti-apoptotic effectors, such as NF- κ B, can cause the resistance of cancer cells to cytotoxic therapy [71-73]. Therefore, compounds that inhibit NF- κ B stimulation could overcome chemotherapy resistance. One phase I and pharmacological study of F60008, a semi-synthetic derivative of triptolide was performed in patients with advanced solid tumors [74]. In 2009, Kitzen et al. [74] enrolled 20 patients in the above study who received a total of 35 cycles. For one cycle, F60008 was given intravenously as a weekly infusion for 2 weeks every 3 weeks. The most frequent hematological side effect was mild grade 1-2 anemia. Non-hematological toxicities included constipation, fatigue, vomiting, diarrhea and nausea, which were all grades 1-2. There are few clinical trials of triptolide in solid tumors, and further clinical studies and trials are warranted to investigate its clinical applications.

Triptolide Enhances the Effects of Chemotherapy

Triptolide sensitizes several cancer cell lines to chemotherapy *in vivo* and *in vitro*. Triptolide highlights the synergistic anti-tumor effect in cells in combination with many cytotoxic drugs. The synergistic anti-tumor effect of triptolide and cisplatin or 5-FU down-regulates cancer cell viability in liver cancer cell lines *in vitro* and in nude mice and induces higher levels of apoptosis compared to single treatments [75]. Furthermore, cells treated with triptolide plus cisplatin or 5-FU exhibit a marked production of intracellular ROS and caspase-3 activity, down-regulate Bcl-2 expression and up-regulate Bax expression [76]. Previous studies showed that

combined-agent-treated groups almost stopped growing and tumor weights *in vivo* were much lighter than with single-drug treatment [77]. Triptolide in combination with sorafenib is superior to single drug treatment in inducing apoptosis and down-regulating viability via decreasing NF- κ B activity [78]. Tumor growth inhibition rates in combined-agent-treated groups in a nude mouse model are increased compared to single drug treatment [78]. Triptolide combined with oxaliplatin (OXA) effectively inhibits proliferation in the colon cancer cell line SW480 and induces cell apoptosis [79]. The mechanism partly involves the inhibition the expression of target genes in the cell cycle and nuclear translocation of β -catenin [79]. Moreover, combined-agent-treated groups in a nude mouse model significantly suppressed tumor growth [79]. Triptolide in combination with temozolomide significantly up-regulates the percentage of apoptotic cells in glioma-initiating cells via up-regulation of NF- κ B transcriptional activity and increased expression of downstream genes [80]. Triptolide was demonstrated to synergize with CPT-11, a topoisomerase inhibitor [81], with doxorubicin (by blocking p-21-mediated growth arrest and accumulating cells in G2-M [32], and with Mylotarg [36] on leukemic cell lines. Triptolide reduced the expression of cell cycle and survival regulators in tumors, such as cyclins D1, B1, and A1, cdc-25, bcl-x, and c-jun [33]. Triptolide enhanced anthracycline toxicity *in vitro* and it cooperated with AraC to induce apoptosis on THP1 leukemic cells and primary AML blast cells [82]. Triptolide synergistically enhanced the antitumor effect of cisplatin in cisplatin-resistant human bladder cancer cells [83]. Minnelide a water-soluble pro-drug of triptolide, decreased cell viability of both platinum sensitive and resistant epithelial ovarian cancer cells *in vitro* [84].

The combination of triptolide with non-cytotoxic drugs also has synergistic effects in numerous types of cancer cells. The main mechanism of the triptolide-enhancing apoptosis effect of dexamethasone is that triptolide affects the PI3k/Akt/NF- κ B pathway, mitogen-activated protein kinase signaling pathway and Bcl-2 expression [85]. The synergistic antitumor effect of triptolide and iron deficiency anemia in acute myelocytic leukemia cells is due to induction of reactive oxygen species and the inhibition of the Nrf2 and hypoxia inducible factor-1 α pathways [86]. The synergistic antitumor effect of triptolide and aspirin in cervical cancer involves a reduction in cyclin E expression, up-regulation of Bax and P21 expression, the inhibition of cell proliferation and induction of cell apoptosis [87].

Caspase-3

When activated, caspase-3 can cleave the vast majority of polypeptides that undergo proteolysis in apoptotic cells [88, 89]. The activated caspase-3 acts as the critical effector in both intrinsic and extra cellular apoptotic pathways by triggering a series of downstream apoptotic cascade [90-92]. Caspase-3 is the ultimate executioner caspase that is essential for the nuclear changes associated with apoptosis, including chromatin condensation [93]. The pro-apoptotic enzyme caspase-3 is activated at a point of convergence for the intrinsic and extrinsic apoptosis induction pathways [94], so its activity should give a reliable measure of ongoing levels of apoptosis in tumor samples. Thus, apoptotic pathways depend upon activation of effector caspases, in particular caspase-3, for the final execution of apoptosis. Therefore, it might be expected that high levels of active caspase3 reflect proper functioning of one or both identified

apoptosis pathways, resulting in relatively chemotherapy-sensitive neoplastic cells and a favorable response to chemotherapy [94].

Loss of caspase-3 expression may represent an important mechanism of cell survival and chemoresistance by various cancer cells. The ability of cells to evade apoptosis is one of the essential hallmarks of cancer cells. This feature allows cancer cells to become non-responsive to anticancer therapies [95]. Normal breast parenchyma and primary breast tumor samples, obtained from patients undergoing breast surgery, lacked caspase-3 expression in the majority of breast cancer patients [96]. Low caspase-3 activity was shown to correlate with poor response to chemotherapy and clinical outcome in colon cancer patients [97]. Likewise, a significant percentage of neuroblastomas lack caspase-3 mRNA and protein [98]. Another study reported that in nasopharyngeal carcinoma patients treated with curative intent, absence of active caspase-3-positive neoplastic cells predicted rapid fatal outcome and was associated with poor response to radiotherapy and high T and N stage at time of presentation [99].

Resistance to apoptosis is a key characteristic of neoplastic cells, and response to chemotherapy is thought to be related to the capacity to restore the apoptotic program in a given tumor cell [100, 101]. Reports demonstrated that the level of caspase-3 expression decreased as gastro carcinogenesis progressed and became undetectable in the majority of malignant samples examined [102, 103]. It is therefore implicated that the down-regulated caspase-3 expression may be one of the important intrinsic factors that confer on gastric cancer and other cancer cells, an apoptosis resistant property. Caspase-3 activity is a final effector of the apoptotic program. Being a late step in the apoptotic cascade, it is likely that its variations reflect the accumulated effect of small variations in a number of genes harboring distinct functions such as DNA damage sensors, cell cycle checkpoints, DNA repair and the apoptotic cascade itself, among others [97].

Lysosomal-Mediated Cell Death

Lysosomes, discovered over fifty years ago, are the major cell digestive organelles [104, 105]. They contain a number of hydrolases that are capable of breaking down nucleic acids, proteins, carbohydrates and lipids. Today, it is clear that lysosomes and lysosomal proteases can be involved in apoptosis. Among lysosomal proteases, the role of cathepsins in cancer progression is especially well documented [105-107]. Following their release into the cytosol, they cleave Bid and degrade anti apoptotic Bcl-2 proteins, thereby triggering the mitochondrial pathway of apoptosis, with lysosomal membrane permeabilization (LMP) being the critical step in this pathway [107, 108]. How LMP is modulated by the complex Bcl-2 protein network, however, is still unclear. Various insults, including oxidative stress and DNA damage, may lead to the limited release of cathepsins that culminate in the induction of apoptosis [109-112]. Hsp70 has been implicated in playing an important role for inhibiting LMP to promote the survival of stressed cells [113]. However, blocking cathepsins by small molecule inhibitors has been shown to significantly delay cancer progression in a number of mouse cancer models as well as to sensitize tumor cells to other chemotherapeutic agents [114]. Lysosomal-mediated apoptosis is still largely under investigation and not fully understood.

LMP and Cancer

Immortalization and transformation have been shown to increase the susceptibility of mouse embryonic fibroblasts to lysosome-dependent cell death induced by anticancer agents [115]. This effect is mediated through cathepsin B over expression and increased cathepsin-dependent cell death. Lysosomal maturation, size and activity are tightly regulated by PI3K [116], an enzyme that is activated in many cancers. Inhibition of PI3K induces the translocation of cathepsin B to the cytosol and may sensitize endothelial cells to TNF- α -induced apoptosis [117, 118]. Hsp70 may promote tumorigenesis by stabilizing lysosomal membranes and by protecting cells against lysosomal membrane permeabilization (LMP) induced by hypoxia, non-inflammatory cytokines, oxidative stress, irradiation or anticancer drugs [119]. The reasons for the increased susceptibility of cancer cell lysosomes to LMP are not understood. As one possibility, relatively large lysosomes, as found in cancer cells [120], may be more fragile than normal-sized lysosomes [121]. Moreover, cancer cells exhibit higher metabolic rates and an increased turnover of iron-containing proteins, leading to the lysosomal accumulation of iron, with consequent iron-mediated sensitization to ROS-induced LMP [122]. Cancer cells often produce elevated ROS levels and the associated higher rate of spontaneous cathepsin release from lysosomes may facilitate cell death induction [123]. On theoretical grounds, all these factors render lysosomes from cancer cells particularly susceptible to the therapeutic induction of LMP. However, this speculation awaits experimental verification.

Triptolide Induces Lysosomal-Mediated Cell Death in Caspase-3 Deficient Breast Cancer Cells

Among the various in vitro cell line models available for breast cancer, the MCF-7 cell line presents distinctive properties that may help shed new light on the mechanism of action of triptolide. In particular, MCF-7 is an estrogen receptor-positive cell line that lacks caspase-3 and beclin-1 [124, 125]. Thus, it represents a cell model with compromised apoptotic machinery and low autophagic activity that might influence cellular response to anticancer drug treatment. We demonstrated that MCF-7 cells treated with triptolide undergo an atypical, apoptotic death that is dependent on LMP because they lack caspase-3 [126]. This cell death was accompanied by chromatin condensation, over expression of cleaved caspase-7 and cleaved caspase-9 proteins and up-regulation of cathepsin B in the cytosolic fractions of experimental cells [126]. To our knowledge, this was the first report in the literature of triptolide-induced lysosomal membrane permeability as an anticancer treatment.

Conclusions

Caspase-3 is a key executioner protein in both the intrinsic and extrinsic apoptotic pathways. Several studies have confirmed that this protein is a desirable target for cancer cells to mutate because the resulting phenotype promotes cell survival, and thus chemoresistance. Further studies are warranted to decipher the mechanisms by which triptolide exerts its antitumor effects via a lysosomal-mediated mechanism and a caspase-3 independent manner. We hope that triptolide will provide more benefit in the treatment of malignant tumors in the future as a prospective anticancer drug candidate.

References

- Li FQ, Lu XZ, Liang XB, Zhou HF, Xue B, Liu XY, et al. Triptolide, a Chinese herbal extract, protects dopaminergic neurons from inflammation-mediated damage through inhibition of microglial activation. *J Neuroimmunol*. 2004; 148: 24-31.
- Qiu D, Kao PN. Immunosuppressive and anti-inflammatory mechanisms of triptolide, the principal active diterpenoid from the Chinese medicinal herb *Tripterygium wilfordii* Hook. f. *Drugs R D*. 2003; 4: 1-18.
- Chen YW, Lin GJ, Chia WT, Lin CK, Chuang YP, Sytwu HK. Triptolide exerts anti-tumor effect on oral cancer and KB cells in vitro and in vivo. *Oral Oncol*. 2009; 45: 562-568.
- Zhu W, Hu H, Qiu P, Yan G. Triptolide induces apoptosis in human anaplastic thyroid carcinoma cells by a p53-independent but NF-kappaB-related mechanism. *Oncol Rep*. 2009; 22: 1397-1401.
- Li H, Takai N, Yuge A, Furukawa Y, Tsuno A, Tsukamoto Y, et al. Novel target genes responsive to the anti-growth activity of triptolide in endometrial and ovarian cancer cells. *Cancer Lett*. 2010; 297: 198-206.
- Kim MJ, Lee TH, Kim SH, Choi YJ, Heo J, Kim YH. Triptolide inactivates Akt and induces caspase-dependent death in cervical cancer cells via the mitochondrial pathway. *Int J Oncol*. 2010; 37: 1177-1185.
- Wu PP, Liu KC, Huang WW, Ma CY, Lin H, Yang JS, et al. Triptolide induces apoptosis in human adrenal cancer NCI-H295 cells through a mitochondrial-dependent pathway. *Oncol Rep*. 2011; 25: 551-557.
- Antonoff MB, Chugh R, Borja-Cacho D, Dudeja V, Clawson KA, Skube SJ, et al. Triptolide therapy for neuroblastoma decreases cell viability in vitro and inhibits tumor growth in vivo. *Surgery*. 2009; 146: 282-290.
- Chan EW, Cheng SC, Sin FW, Xie Y. Triptolide induced cytotoxic effects on human promyelocytic leukemia, T cell lymphoma and human hepatocellular carcinoma cell lines. *Toxicol Lett*. 2001; 122: 81-87.
- Carter BZ, Mak DH, Schober WD, Dietrich MF, Pinilla C, Vassilev LT, et al. Triptolide sensitizes AML cells to TRAIL-induced apoptosis via decrease of XIAP and p53-mediated increase of DR5. *Blood*. 2008; 111: 3742-3750.
- Zhou GS, Hu Z, Fang HT, Zhang FX, Pan XF, Chen XQ, et al. Biologic activity of triptolide in t(8;21) acute myeloid leukemia cells. *Leuk Res*. 2011; 35: 214-218.
- Jiang XH, Wong BC, Lin MC, Zhu GH, Kung HF, Jiang SH, et al. Functional p53 is required for triptolide-induced apoptosis and AP-1 and nuclear factor-kappaB activation in gastric cancer cells. *Oncogene*. 2001; 20: 8009-8018.
- Wang Z, Jin H, Xu R, Mei Q, Fan D. Triptolide downregulates Rac1 and the JAK/STAT3 pathway and inhibits colitis-related colon cancer progression. *Exp Mol Med*. 2009; 41: 717-727.
- Tengchaisri T, Chawengkirttikul R, Rachaphaew N, Reutrakul V, Sangsuwan R, Sirisinha S. Antitumor activity of triptolide against cholangiocarcinoma growth in vitro and in hamsters. *Cancer Lett*. 1998; 133: 169-175.
- Mujumdar N, Mackenzie TN, Dudeja V, Chugh R, Antonoff MB, Borja-Cacho D, et al. Triptolide induces cell death in pancreatic cancer cells by apoptotic and autophagic pathways. *Gastroenterology*. 2010; 139: 598-608.
- Antonoff MB, Chugh R, Skube SJ, Dudeja V, Borja-Cacho D, Clawson KA, et al. Role of Hsp-70 in triptolide-mediated cell death of neuroblastoma. *J Surg Res*. 2010; 163: 72-78.
- Yang S, Chen J, Guo Z, Xu XM, Wang L, Pei XF, et al. Triptolide inhibits the growth and metastasis of solid tumors. *Mol Cancer Ther*. 2003; 2: 65-72.
- Phillips PA, Dudeja V, McCarroll JA, Borja-Cacho D, Dawra RK, Grizzle WE, et al. Triptolide induces pancreatic cancer cell death via inhibition of heat shock protein 70. *Cancer Res*. 2007; 67: 9407-9416.
- Kupchan SM, Schubert RM. Selective alkylation: a biomimetic reaction of the antileukemic triptolides? *Science*. 1974; 185: 791-793.
- McCallum C, Kwon S, Leavitt P, Shen DM, Liu W, Gurnett A. Triptolide binds covalently to a 90 kDa nuclear protein. Role of epoxides in binding and activity. *Immunobiology*. 2007; 212: 549-556.
- Titov DV, Gilman B, He QL, Bhat S, Low WK, Dang Y, et al. XPB, a subunit of TFIIH, is a target of the natural product triptolide. *Nat Chem Biol*. 2011; 7: 182-188.
- Wang BY, Cao J, Chen JW, Liu QY. Triptolide induces apoptosis of gastric cancer cells via inhibiting the overexpression of MDM2. *Med Oncol*. 2014; 31: 270.
- Xiaowen H, Yi S. Triptolide sensitizes TRAIL-induced apoptosis in prostate cancer cells via p53-mediated DR5 up-regulation. *Mol Biol Rep*. 2012; 39: 8763-8770.
- Wei YS, Adachi I. Inhibitory effect of triptolide on colony formation of breast and stomach cancer cell lines. *Zhongguo Yao Li Xue Bao*. 1991; 12: 406-410.
- Lee YS, Song YS, Giffard RG, Chan PH. Biphasic role of nuclear factor-kappa B on cell survival and COX-2 expression in SOD1 Tg astrocytes after oxygen glucose deprivation. *J Cereb Blood Flow Metab*. 2006; 26: 1076-1088.
- Kim BH, Yoon JH, Yang JI, Myung SJ, Lee JH, Jung EU, et al. Guggulsterone attenuates activation and survival of hepatic stellate cell by inhibiting nuclear factor kappa B activation and inducing apoptosis. *J Gastroenterol Hepatol*. 2013; 28: 1859-1868.
- Chen MC, Lee NH, Ho TJ, Hsu HH, Kuo CH, Kuo WW, et al. Resistance to irinotecan (CPT-11) activates epidermal growth factor receptor/nuclear factor kappa B and increases cellular metastasis and autophagy in LoVo colon cancer cells. *Cancer Lett*. 2014; 349: 51-60.
- Zhao B, Ma Y, Xu Z, Wang J, Wang F, Wang D, et al. Hydroxytyrosol, a natural molecule from olive oil, suppresses the growth of human hepatocellular carcinoma cells via inactivating AKT and nuclear factor-kappa B pathways. *Cancer Lett*. 2014; 347: 79-87.
- Guzmán EA1, Maers K, Roberts J, Kemami-Wangun HV, Harmody D, Wright AE. The marine natural product microsclerodermin A is a novel inhibitor of the nuclear factor kappa B and induces apoptosis in pancreatic cancer cells. *Invest New Drugs*. 2014; .
- Loganathan R, Selvaduray KR, Nesaretnam K, Radhakrishnan AK. Tocotrienols promote apoptosis in human breast cancer cells by inducing poly(ADP-ribose) polymerase cleavage and inhibiting nuclear factor kappa-B activity. *Cell Prolif*. 2013; 46: 203-213.
- Lou YJ, Jin J. Triptolide down-regulates bcr-abl expression and induces apoptosis in chronic myelogenous leukemia cells. *Leuk Lymphoma*. 2004; 45: 373-376.
- Chang WT, Kang JJ, Lee KY, Wei K, Anderson E, Gotmare S, et al. Triptolide and chemotherapy cooperate in tumor cell apoptosis. A role for the p53 pathway. *J Biol Chem*. 2001; 276: 2221-2227.
- Zhao G, Vaszar LT, Qiu D, Shi L, Kao PN. Anti-inflammatory effects of triptolide in human bronchial epithelial cells. *Am J Physiol Lung Cell Mol Physiol*. 2000; 279: L958-966.
- Pan J. RNA polymerase - an important molecular target of triptolide in cancer cells. *Cancer Lett*. 2010; 292: 149-152.
- Kiviharju TM, Lecane PS, Sellers RG, Peehl DM. Antiproliferative and proapoptotic activities of triptolide (PG490), a natural product entering clinical trials, on primary cultures of human prostatic epithelial cells. *Clin Cancer Res*. 2002; 8: 2666-2674.
- Carter BZ, Mak DH, Schober WD, McQueen T, Harris D, Estrov Z, et al. Triptolide induces caspase-dependent cell death mediated via the mitochondrial pathway in leukemic cells. *Blood*. 2006; 108: 630-637.
- Leuenroth SJ, Okuhara D, Shotwell JD, Markowitz GS, Yu Z, Somlo S, et al. Triptolide is a traditional Chinese medicine-derived inhibitor of polycystic kidney disease. *Proc Natl Acad Sci U S A*. 2007; 104: 4389-4394.
- Liu Q. Triptolide and its expanding multiple pharmacological functions. *Int Immunopharmacol*. 2011; 11: 377-383.
- Yinjun L, Jie J, Yungui W. Triptolide inhibits transcription factor NF-kappaB and induces apoptosis of multiple myeloma cells. *Leuk Res*. 2005; 29: 99-105.

40. Lin J, Chen LY, Lin ZX, Zhao ML. The effect of triptolide on apoptosis of glioblastoma multiforme (GBM) cells. *J Int Med Res.* 2007; 35: 637-643.
41. Tai CJ, Wu AT, Chiou JF, Jan HJ, Wei HJ, Hsu CH, et al. The investigation of mitogen-activated protein kinase phosphatase-1 as a potential pharmacological target in non-small cell lung carcinomas, assisted by non-invasive molecular imaging. *BMC Cancer.* 2010; 10: 95.
42. Koo HS, Kang SD, Lee JH, Kim NH, Chung HT, Pae HO. Triptolide Inhibits the Proliferation of Immortalized HT22 Hippocampal Cells Via Persistent Activation of Extracellular Signal-Regulated Kinase-1/2 by Down-Regulating Mitogen-Activated Protein Kinase Phosphatase-1 Expression. *J Korean Neurosurg Soc.* 2009; 46: 389-396.
43. Miyata Y, Sato T, Ito A. Triptolide, a diterpenoid triepoxide, induces antitumor proliferation via activation of c-Jun NH2-terminal kinase 1 by decreasing phosphatidylinositol 3-kinase activity in human tumor cells. *Biochem Biophys Res Commun.* 2005; 336: 1081-1086.
44. Westerheide SD, Kawahara TL, Orton K, Morimoto RI. Triptolide, an inhibitor of the human heat shock response that enhances stress-induced cell death. *J Biol Chem.* 2006; 281: 9616-9622.
45. Whitesell L, Lindquist S. Inhibiting the transcription factor HSF1 as an anticancer strategy. *Expert Opin Ther Targets.* 2009; 13: 469-478.
46. Zhou GX, Ding XL, Huang JF, Zhang H, Wu SB. Suppression of 5-lipoxygenase gene is involved in triptolide-induced apoptosis in pancreatic tumor cell lines. *Biochim Biophys Acta.* 2007; 1770: 1021-1027.
47. Soundararajan R, Sayat R, Robertson GS, Marignani PA. Triptolide: An inhibitor of a disintegrin and metalloproteinase 10 (ADAM10) in cancer cells. *Cancer Biol Ther.* 2009; 8: 2054-2062.
48. Vispé S, DeVries L, Créancier L, Besse J, Bréand S, Hobson DJ, et al. Triptolide is an inhibitor of RNA polymerase I and II-dependent transcription leading predominantly to down-regulation of short-lived mRNA. *Mol Cancer Ther.* 2009; 8: 2780-2790.
49. Chen Q, Lu Z, Jin Y, Wu Y, Pan J. Triptolide inhibits Jak2 transcription and induces apoptosis in human myeloproliferative disorder cells bearing Jak2V617F through caspase-3-mediated cleavage of Mcl-1. *Cancer Lett.* 2010; 291: 246-255.
50. Zhao F, Chen Y, Li R, Liu Y, Wen L, Zhang C. Triptolide alters histone H3K9 and H3K27 methylation state and induces G0/G1 arrest and caspase-dependent apoptosis in multiple myeloma in vitro. *Toxicology.* 2010; 267: 70-79.
51. Cannistra SA. Cancer of the ovary. *N Engl J Med.* 2004; 351: 2519-2529.
52. Shepherd JE. Current strategies for prevention, detection, and treatment of ovarian cancer. *J Am Pharm Assoc (Wash).* 2000; 40: 392-401.
53. Agarwal R, Kaye SB. Ovarian cancer: strategies for overcoming resistance to chemotherapy. *Nat Rev Cancer.* 2003; 3: 502-516.
54. Siddik ZH. Cisplatin: mode of cytotoxic action and molecular basis of resistance. *Oncogene.* 2003; 22: 7265-7279.
55. Brabec V, Kasparkova J. Modifications of DNA by platinum complexes. Relation to resistance of tumors to platinum antitumor drugs. *Drug Resist Updat.* 2005; 8: 131-146.
56. White KL, Rider DN, Kalli KR, Knutson KL, Jarvik GP, Goode EL. Genomics of the NF- κ B signaling pathway: hypothesized role in ovarian cancer. *Cancer Causes Control.* 2011; 22: 785-801.
57. Nakanishi C, Toi M. Nuclear factor-kappaB inhibitors as sensitizers to anticancer drugs. *Nat Rev Cancer.* 2005; 5: 297-309.
58. Li F1, Sethi G. Targeting transcription factor NF-kappaB to overcome chemoresistance and radioresistance in cancer therapy. *Biochim Biophys Acta.* 2010; 1805: 167-180.
59. Baud V, Karin M. Is NF-kappaB a good target for cancer therapy? Hopes and pitfalls. *Nat Rev Drug Discov.* 2009; 8: 33-40.
60. Yeh PY, Chuang SE, Yeh KH, Song YC, Ea CK, Cheng AL. Increase of the resistance of human cervical carcinoma cells to cisplatin by inhibition of the MEK to ERK signaling pathway partly via enhancement of anticancer drug-induced NF kappa B activation. *Biochem Pharmacol.* 2002; 63: 1423-1430.
61. Berkenblit A, Cannistra SA. Advances in the management of epithelial ovarian cancer. *J Reprod Med.* 2005; 50: 426-438.
62. Yang M, Huang J, Pan HZ, Jin J. Triptolide overcomes dexamethasone resistance and enhanced PS-341-induced apoptosis via PI3k/Akt/NF-kappaB pathways in human multiple myeloma cells. *Int J Mol Med.* 2008; 22: 489-496.
63. Chen YW, Lin GJ, Chuang YP, Chia WT, Hueng DY, Lin CK, et al. Triptolide circumvents drug-resistant effect and enhances 5-fluorouracil antitumor effect on KB cells. *Anticancer Drugs.* 2010; 21: 502-513.
64. Zhong YY, Chen HP, Tan BZ, Yu HH, Huang XS. Triptolide avoids cisplatin resistance and induces apoptosis via the reactive oxygen species/nuclear factor- κ B pathway in SKOV3(PT) platinum-resistant human ovarian cancer cells. *Oncol Lett.* 2013; 6: 1084-1092.
65. Eccles SA. The epidermal growth factor receptor/ErbB/HER family in normal and malignant breast biology. *Int J Dev Biol.* 2011; 55: 685-696.
66. Tsang RY, Finn RS. Beyond trastuzumab: novel therapeutic strategies in HER2-positive metastatic breast cancer. *Br J Cancer.* 2012; 106: 6-13.
67. Yarden Y, Sliwkowski MX. Untangling the ErbB signalling network. *Nat Rev Mol Cell Biol.* 2001; 2: 127-137.
68. Hynes NE, Lane HA. ERBB receptors and cancer: the complexity of targeted inhibitors. *Nat Rev Cancer.* 2005; 5: 341-354.
69. Zhu W, Ou Y, Li Y, Xiao R, Shu M, Zhou Y, et al. A small-molecule triptolide suppresses angiogenesis and invasion of human anaplastic thyroid carcinoma cells via down-regulation of the nuclear factor-kappa B pathway. *Mol Pharmacol.* 2009; 75: 812-819.
70. Kang DW, Lee JY, Oh DH, Park SY, Woo TM, Kim MK, et al. Triptolide-induced suppression of phospholipase D expression inhibits proliferation of MDA-MB-231 breast cancer cells. *Exp Mol Med.* 2009; 41: 678-685.
71. Zhang K, Chen J, Chen D, Huang J, Feng B, Han S, et al. Aurora-A promotes chemoresistance in hepatocellular carcinoma by targeting NF-kappaB/microRNA-21/PTEN signaling pathway. *Oncotarget.* 2014; .
72. Yang YI, Ahn JH, Lee KT, Shih IeM, Choi JH. RSF1 is a positive regulator of NF- κ B-induced gene expression required for ovarian cancer chemoresistance. *Cancer Res.* 2014; 74: 2258-2269.
73. Zhou W, Fu XQ, Zhang LL, Zhang J, Huang X, Lu XH, et al. The AKT1/NF-kappaB/Notch1/PEN axis has an important role in chemoresistance of gastric cancer cells. *Cell Death Dis.* 2013; 4: e847.
74. Kitzen JJ, de Jonge MJ, Lamers CH, Eskens FA, van der Biessen D, van Doorn L, et al. Phase I dose-escalation study of F60008, a novel apoptosis inducer, in patients with advanced solid tumours. *Eur J Cancer.* 2009; 45: 1764-1772.
75. Meng C, Zhu H, Song H, Wang Z, Huang G, Li D, et al. Targets and molecular mechanisms of triptolide in cancer therapy. *Chin J Cancer Res.* 2014; 26: 622-626.
76. Li Y, Hu S. Triptolide sensitizes liver cancer cell lines to chemotherapy in vitro and in vivo. *Panminerva Med.* 2014; 56: 211-220.
77. Westfall SD, Nilsson EE, Skinner MK. Role of triptolide as an adjunct chemotherapy for ovarian cancer. *Chemotherapy.* 2008; 54: 67-76.
78. Alsaied OA, Sangwan V, Banerjee S, Krosch TC, Chugh R, Saluja A, et al. Sorafenib and triptolide as combination therapy for hepatocellular carcinoma. *Surgery.* 2014; 156: 270-279.
79. Liu Y, Xiao E, Yuan L, Li G. Triptolide synergistically enhances antitumor activity of oxaliplatin in colon carcinoma in vitro and in vivo. *DNA Cell Biol.* 2014; 33: 418-425.
80. Sai K, Li WY, Chen YS, Wang J, Guan S, Yang QY, et al. Triptolide synergistically enhances temozolomide-induced apoptosis and potentiates inhibition of NF- κ B signaling in glioma initiating cells. *Am J Chin Med.* 2014; 42: 485-503.

81. Fidler JM, Li K, Chung C, Wei K, Ross JA, Gao M, et al. PG490-88, a derivative of triptolide, causes tumor regression and sensitizes tumors to chemotherapy. *Mol Cancer Ther.* 2003; 2: 855-862.
82. Pigneux A, Mahon FX, Uhalde M, Jeanneteau M, Lacombe F, Milpied N, et al. Triptolide cooperates with chemotherapy to induce apoptosis in acute myeloid leukemia cells. *Exp Hematol.* 2008; 36: 1648-1659.
83. Ho JN, Byun SS, Lee S, Oh JJ, Hong SK, Lee SE, et al. Synergistic Antitumor Effect of Triptolide and Cisplatin in Cisplatin Resistant Human Bladder Cancer Cells. *J Urol.* 2014; .
84. Rivard C, Geller M, Schnettler E, Saluja M, Vogel RI, Saluja A, et al. Inhibition of epithelial ovarian cancer by Minnelide, a water-soluble pro-drug. *Gynecol Oncol.* 2014; 135: 318-324.
85. Huang X, Yang M, Jin J . Triptolide enhances the sensitivity of multiple myeloma cells to dexamethasone via microRNAs. *Leuk Lymphoma.* 2012; 53: 1188-1195.
86. Liu Y, Chen F, Wang S, Guo X, Shi P, Wang W, et al. Low-dose triptolide in combination with idarubicin induces apoptosis in AML leukemic stem-like KG1a cell line by modulation of the intrinsic and extrinsic factors. *Cell Death Dis.* 2013; 4: e948.
87. Chen RH, Tian YJ . Enhanced anti-tumor efficacy of aspirin combined with triptolide in cervical cancer cells. *Asian Pac J Cancer Prev.* 2013; 14: 3041-3044.
88. Park IC, Park MJ, Rhee CH, Lee JI, Choe TB, Jang JJ, et al. Protein kinase C activation by PMA rapidly induces apoptosis through caspase-3/CPP32 and serine protease(s) in a gastric cancer cell line. *Int J Oncol.* 2001; 18: 1077-1083.
89. Li X, Guo M, Mori E, Mori T. Active roles of caspase-3 in human gastric carcinoma cell death by apoptosis inducing nucleosides from CD57+HLA-DRbright natural suppressor cell line. *Int J Oncol.* 2001; 18: 837-842.
90. Varghese J, Khandre NS, Sarin A . Caspase-3 activation is an early event and initiates apoptotic damage in a human leukemia cell line. *Apoptosis.* 2003; 8: 363-370.
91. Miyake Y, Kakeya H, Kataoka T, Osada H . Epoxycyclohexenone inhibits Fas-mediated apoptosis by blocking activation of pro-caspase-8 in the death-inducing signaling complex. *J Biol Chem.* 2003; 278: 11213-11220.
92. Kaufmann SH, Earnshaw WC . Induction of apoptosis by cancer chemotherapy. *Exp Cell Res.* 2000; 256: 42-49.
93. Woo M, Hakem R, Soengas MS, Duncan GS, Shahinian A, Kagi D, et al. Essential contribution of caspase 3/CPP32 to apoptosis and its associated nuclear changes. *Genes Dev.* 1998; 12: 806-819.
94. Brown JM, Attardi LD . The role of apoptosis in cancer development and treatment response. *Nat Rev Cancer.* 2005; 5: 231-237.
95. Susnow N, Zeng L, Margineantu D, Hockenbery DM . Bcl-2 family proteins as regulators of oxidative stress. *Semin Cancer Biol.* 2009; 19: 42-49.
96. Devarajan E, Sahin AA, Chen JS, Krishnamurthy RR, Aggarwal N, Brun AM, et al. Down-regulation of caspase 3 in breast cancer: a possible mechanism for chemoresistance. *Oncogene.* 2002; 21: 8843-8851.
97. de Oca J, Azuara D, Sanchez-Santos R, Navarro M, Capella G, Moreno V, et al. Caspase-3 activity, response to chemotherapy and clinical outcome in patients with colon cancer. *Int J Colorectal Dis.* 2008; 23: 21-27.
98. Iolascon A, Borriello A, Giordani L, Cucciolla V, Moretti A, Monno F, et al. Caspase 3 and 8 deficiency in human neuroblastoma. *Cancer Genet Cytogenet.* 2003; 146: 41-47.
99. Oudejans JJ, Harijadi A, Cillessen SA, Busson P, Tan IB, Dukers DF, et al. Absence of caspase 3 activation in neoplastic cells of nasopharyngeal carcinoma biopsies predicts rapid fatal outcome. *Mod Pathol.* 2005; 18: 877-885.
100. Sen S, D'Incalci M . Apoptosis. Biochemical events and relevance to cancer chemotherapy. *FEBS Lett.* 1992; 307: 122-127.
101. Hao X, Du M, Bishop AE, Talbot IC . Imbalance between proliferation and apoptosis in the development of colorectal carcinoma. *Virchows Arch.* 1998; 433: 523-527.
102. Kania J, Konturek SJ, Marlicz K, Hahn EG, Konturek PC . Expression of survivin and caspase-3 in gastric cancer. *Dig Dis Sci.* 2003; 48: 266-271.
103. Yoo NJ, Kim HS, Kim SY, Park WS, Kim SH, Lee JY, et al. Stomach cancer highly expresses both initiator and effector caspases; an immunohistochemical study. *APMIS.* 2002; 110: 825-832.
104. de Duve C . The lysosome turns fifty. *Nat Cell Biol.* 2005; 7: 847-849.
105. Luzio JP, Pryor PR, Bright NA . Lysosomes: fusion and function. *Nat Rev Mol Cell Biol.* 2007; 8: 622-632.
106. Linder S, Shoshan MC . Lysosomes and endoplasmic reticulum: targets for improved, selective anticancer therapy. *Drug Resist Updat.* 2005; 8: 199-204.
107. Johansson AC, Appelqvist H, Nilsson C, Kägedal K, Roberg K, Ollinger K . Regulation of apoptosis-associated lysosomal membrane permeabilization. *Apoptosis.* 2010; 15: 527-540.
108. ÅEesen MH, Pegan K, Spes A, Turk B . Lysosomal pathways to cell death and their therapeutic applications. *Exp Cell Res.* 2012; 318: 1245-1251.
109. Eno CO, Zhao G, Venkatanarayan A, Wang B, Flores ER, Li C . Noxa couples lysosomal membrane permeabilization and apoptosis during oxidative stress. *Free Radic Biol Med.* 2013; 65: 26-37.
110. Hornick JR, Vangveravong S, Spitzer D, Abate C, Berardi F, Goedegebuure P, et al. Lysosomal membrane permeabilization is an early event in Sigma-2 receptor ligand mediated cell death in pancreatic cancer. *J Exp Clin Cancer Res.* 2012; 31: 41.
111. Nowak R, Tarasiuk J. Retaining cytotoxic activity of anthracycline CO1 against multidrug resistant cells is related to the ability to induce concomitantly apoptosis and lysosomal death of leukaemia HL60/VINC and HL60/DOX cells. *J Pharm Pharmacol.* 2013; 65: 855-867.
112. Li LJ, Zhong LF, Jiang LP, Geng CY, Zhu TZ, Xu YH, et al. Lysosomal membrane permeabilization contributes to elemene emulsion-induced apoptosis in A549 cells. *Free Radic Res.* 2011; 45: 1232-1240.
113. Kirkegaard T, Roth AG, Petersen NH, Mahalka AK, Olsen OD, Moilanen I, et al. Hsp70 stabilizes lysosomes and reverts Niemann-Pick disease-associated lysosomal pathology. *Nature.* 2010; 463: 549-553.
114. Shree T, Olson OC, Elie BT, Kester JC, Garfall AL, Simpson K, et al. Macrophages and cathepsin proteases blunt chemotherapeutic response in breast cancer. *Genes Dev.* 2011; 25: 2465-2479.
115. Fehrenbacher N, Gyrd-Hansen M, Poulsen B, Felbor U, Kallunki T, Boes M, et al. Sensitization to the lysosomal cell death pathway upon immortalization and transfection. *Cancer Res.* 2004; 64: 5301-5310.
116. Mousavi SA, Brech A, Berg T, Kjekken R . Phosphoinositide 3-kinase regulates maturation of lysosomes in rat hepatocytes. *Biochem J.* 2003; 372: 861-869.
117. Madge LA, Li JH, Choi J, Pober JS . Inhibition of phosphatidylinositol 3-kinase sensitizes vascular endothelial cells to cytokine-initiated cathepsin-dependent apoptosis. *J Biol Chem.* 2003; 278: 21295-21306.
118. Li JH, Pober JS . The cathepsin B death pathway contributes to TNF plus IFN-gamma-mediated human endothelial injury. *J Immunol.* 2005; 175: 1858-1866.
119. Rohde M, Daugaard M, Jensen MH, Helin K, Nylandsted J, Jäättelä M . Members of the heat-shock protein 70 family promote cancer cell growth by distinct mechanisms. *Genes Dev.* 2005; 19: 570-582.
120. Glunde K, Guggino SE, Solaiyappan M, Pathak AP, Ichikawa Y, Bhujwala ZM . Extracellular acidification alters lysosomal trafficking in human breast cancer cells. *Neoplasia.* 2003; 5: 533-545.
121. Ono K, Kim SO, Han J . Susceptibility of lysosomes to rupture is a determinant for plasma membrane disruption in tumor necrosis factor alpha-induced cell death. *Mol Cell Biol.* 2003; 23: 665-676.

122. Eaton JW, Qian M . Molecular bases of cellular iron toxicity. *Free Radic Biol Med.* 2002; 32: 833-840.
123. Gyrd-Hansen M, Nylandsted J, Jäättelä M . Heat shock protein 70 promotes cancer cell viability by safeguarding lysosomal integrity. *Cell Cycle.* 2004; 3: 1484-1485.
124. Semenov DV, Aronov PA, Kuligina EV, Potapenko MO, Richter VA. Oligonucleosome DNA fragmentation of caspase 3 deficient MCF-7 cells in palmitate-induced apoptosis. *Nucleosides Nucleotides Nucleic Acids.* 2004; 23: 831-836.
125. Jänicke RU, Sprengart ML, Wati MR, Porter AG . Caspase-3 is required for DNA fragmentation and morphological changes associated with apoptosis. *J Biol Chem.* 1998; 273: 9357-9360.
126. Owa C, Messina ME Jr, Halaby R. Triptolide induces lysosomal-mediated programmed cell death in MCF-7 breast cancer cells. *Int J Womens Health.* 2013; 5: 557-569.