

Review Article

HMGB1 is a therapeutic target for leukemia

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Abstract: High mobility group box 1 (HMGB1) is a nuclear DNA-binding protein, which functions as Damage Associated Molecular Pattern molecule (DAMP) when released from cells under conditions of stress, such as injury and infection. Recent studies indicate that HMGB1 plays an important role in leukemia pathogenesis and chemotherapy resistance. Serum HMGB1 is increased in childhood acute lymphocytic leukemia as compared to healthy control and complete remission groups. Moreover, HMGB1 is a negative regulator of apoptosis in leukemia cells through regulation of Bcl-2 expression and caspase-3 activity. As a positive regulator of autophagy, intracellular HMGB1 interacts with Beclin 1 in leukemia cells leading to autophagosome formation. Additionally, exogenous HMGB1 directly induces autophagy and cell survival in leukemia cells. Experimental strategies that selectively target HMGB1 effectively reverse and prevent chemotherapy resistance in leukemia cells, suggesting that HMGB1 is a novel therapeutic target in leukemia.

Keywords: HMGB1, leukemia, apoptosis, autophagy, chemotherapy

Introduction

The tumor microenvironment refers to the unique properties of the stromal tissue environment conferred by the interactions between tumor and host cells [1, 2]. Radiotherapy and some chemotherapeutic agents lead to injury and necrosis resulting in changes on the cell surface of cancer cells with concomitant release of soluble mediators (e.g. DAMPs) that trigger an immune response [3]. These DAMPs can have either a pro-tumor or antitumor effect depending on the tumor type, microenvironment and signaling receptors [4]. DAMPs are localized to the nucleus and cytoplasm [e.g. high mobility group box 1 (HMGB1)], cytoplasm (e.g. S100 proteins), exosomes (e.g. heat shock proteins), and extracellular matrix (e.g. hyaluronic acid). Examples of non-protein DAMPs include adenosine triphosphate, uric acid, heparin sulfate and DNA. When DAMPs are released from the reducing environment within the cell following tissue injury, they encounter an oxidizing milieu resulting in their functional denaturation [5]. As a prototypic DAMP, HMGB1 is released after various

types of cell stress such as those induced by cancer treatments including chemotherapy, radiation therapy and immunotherapy. Our recent studies suggest that HMGB1 is important in the pathogenesis of leukemia [6-12], a type of cancer of the blood or bone marrow characterized by an abnormal increase in white blood cells. Understanding the mechanism of HMGB1 regulation during chemotherapy will provide insights into developing more effective treatments for all types of leukemia.

Structure and functions of redox sensitive HMGB1 protein

HMGB1 is present in almost all metazoans and plants and is a relatively small protein of 215 amino acid residues. Structurally, the protein is organized into three distinct domains: two tandem HMG box domains (A box and B box) and a 30 amino acid-long acidic C-terminal tail (**Figure 1A**). Functionally, the A and B boxes are DNA-binding domains. Similar to other HMG-box proteins, HMGB1 is targeted to particular DNA sites in chromatin by either protein-protein interac-

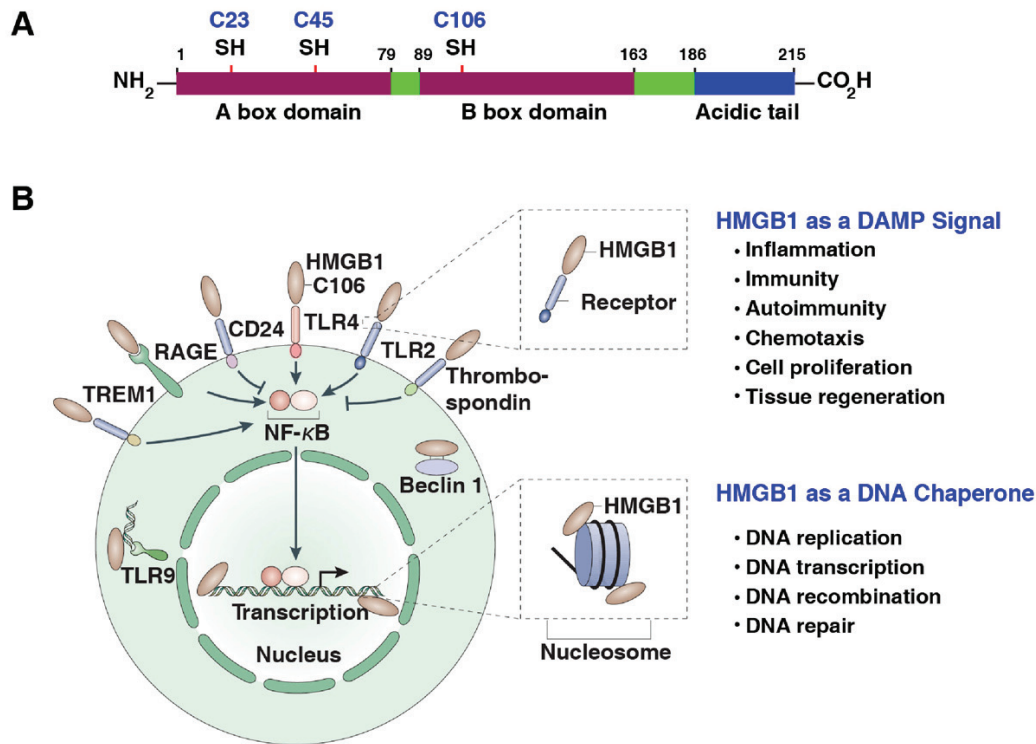


Figure 1. Structure and functions of HMGB1 protein. (A) HMGB1 is composed of two homologous DNA-binding domains (termed A box and B box) and a highly negatively charged C-terminal domain. There are oxidation-sensitive unpaired cysteines at positions 23, 45, and 106. (B) As a DNA chaperone, HMGB1 participates in DNA replication, recombination, transcription and repair. As a DAMP signal, HMGB1 mediates the response to inflammation, immunity, chemotaxis, and tissue regeneration.

tions or recognition of specific DNA structures [13, 14]. The B box contains “cytokine” activity by inducing macrophage secretion of proinflammatory cytokines [15]. This cytokine activity is antagonized by recombinant A box [16]. There are oxidation-sensitive unpaired cysteines at positions 23, 45, and 106. The two Cys23-Cys45 residues can rapidly form an intramolecular disulfide bond with standard redox potential, whereas Cys106 appears to be critical for the nucleocytoplasmic shuttling of HMGB1 [5, 17, 18].

As a DNA chaperone (**Figure 1B**), HMGB1 participates in DNA replication, recombination, transcription, and repair [13, 14]. HMGB1 gene deficient mice are born with several defects and die shortly after birth of hypoglycemia initially believed to be caused by deficient glucocorticoid receptor function [19]. As a prototypic DAMP (**Figure 1B**), HMGB1 is passively released

from injured and necrotic cells [20, 21] and is actively secreted by inflammatory cells [22]. HMGB1 binds with high affinity to several receptors such as the receptor for advanced glycation end products (RAGE), Toll-like receptors (TLR)-2, 4, 9, the triggering receptor expressed on myeloid cells 1 (TREM1) and the negative signaling receptors, thrombospondin and CD24, mediating the response to infection, immunity, autoimmunity, chemotaxis, cell proliferation and tissue regeneration [23, 24]. Additionally, HMGB1 forms complexes with DNA, lipopolysaccharide, interleukin-1, and nucleosomes to augment inflammation [25].

HMGB1 is associated with the hallmarks of cancer

Unlike other DAMPs, HMGB1 has unique roles in cancer [26-28]. HMGB1 expression is greater in tumor cells than in normal surrounding epi-

thelium in a large variety of human neoplasms including lymphoma, melanoma and cancers of the breast, cervix, colon, liver, lung, and pancreas [26-28]. Moreover, serum levels of HMGB1 are significantly increased in patients with several cancers [27]. Overexpression of HMGB1 is associated with each of the hallmarks of cancer [29] including unlimited replicative potential, ability to develop blood vessels (angiogenesis), evasion of programmed cell death (apoptosis), self-sufficiency in growth signals, insensitivity to inhibitors of growth, inflammation, tissue invasion and metastasis, and enhancement of inflammation [27]. HMGB1 receptors have various roles in the regulation of tumor therapy. For example, RAGE but not TLR4 promotes pancreatic tumor cell survival following genotoxic or metabolic stress [30]. Knock-down of HMGB1 or RAGE by specific shRNA increases gemcitabine sensitivity in pancreatic cancer cells [31]. Moreover, blockade of HMGB1-RAGE signaling suppresses tumor growth and metastases in C6 gliomas [32] and DMBA (7,12-dimethyl-benz[a]anthracene) / TPA (12-O-tetradecanoylphorbol-13 acetate)-induced skin carcinogenesis [33]. Thus, HMGB1-RAGE signaling represents an important potential target for cancer therapeutics [34]. In contrast, a recent study indicates that the interaction between HMGB1 and TLR4 in dendritic cells increases tumor antigen presentation and antitumor immune responses in EL4 thymoma, CT26 colon cancer, and MCA205 sarcoma tumor cells treated with chemotherapy and radiotherapy [35]. Our recent study demonstrates that the redox state of HMGB1 is critical to its ability to regulate cell death and survival in cancer cells [36]. Reduced HMGB1 induces autophagy and cell survival, whereas oxidized HMGB1 induces apoptosis and cell death [36]. Moreover, oxidized HMGB1 at C106 has tolerogenic activities through TLR4 signaling in dendritic cells [37] and macrophages [38]. Thus, it is not surprising that various groups report release of HMGB1 as “good” or “bad” for cancer therapy depending on the tumor context.

Clinical studies of HMGB1 in acute lymphoblastic leukemia

Acute lymphoblastic leukemia (ALL) is the most frequent cancer found in children. Karyotyping of leukemic cells identifies recurrent chromosome rearrangements. These are usually translocations that activate genes encoding tran-

scription factors regulating B- or T-cell differentiation. This type of cancer usually progresses quickly if it is not treated. The goal of remission-induction therapy is to eradicate more than 99 percent of the initial burden of leukemia cells and to restore normal hematopoiesis and normal performance status. Three-drug induction therapy using vincristine, corticosteroids (prednisone or dexamethasone), and L-asparaginase in conjunction with intrathecal therapy results in complete remission rates of greater than 95% [39]. Our recent study indicates that serum levels of HMGB1 were significantly higher in children with ALL in the initial treatment group ($n = 15$, $43.78 \pm 4.62 \mu\text{g/ml}$) compared with those in the healthy control group ($n = 15$, $0.60 \pm 0.48 \mu\text{g/ml}$, $p < 0.01$) and ALL complete remission group ($n = 15$, $0.89 \pm 0.62 \mu\text{g/ml}$, $p < 0.01$) [40]. There was no significant difference in HMGB1 levels between the healthy control group and ALL complete remission group ($p > 0.05$) [40], suggesting that HMGB1 may reflect the stages of hemocyte differentiation and maturation.

HMGB1 as an antiapoptotic protein in leukemia cells

Apoptosis has now been widely accepted as a prominent tumor-suppression mechanism [41]. There are at least two broad pathways that lead to apoptosis: an extrinsic pathway triggered by cell death receptors (such as Fas), and an intrinsic pathway triggered by the mitochondrial release of cytochrome c and its subsequent complex formation with Apaf-1, ATP, and caspase-9 [41]. Bcl-2 family proteins are critical regulators of apoptosis [42]. These proteins govern mitochondrial outer membrane permeabilization (MOMP) and can be either pro-apoptotic (e.g. Bax, BAD, Bak and Bok) or anti-apoptotic (e.g. Bcl-2, Bcl-xL, and Bcl-w).

An early study demonstrated that HMGB1 is able to suppress cell death induced by Bak in yeast and protects against apoptosis from Bak and Bax killing and overexpression of Caspase-8, UV radiation, and activation of the death receptors, CD95 and TRAIL, in mammalian cells [43], suggesting a wide role for HMGB1 in the regulation of various apoptosis pathway. HMGB1 overexpression inhibits ADM-induced apoptosis in leukemia K562 cells by regulating the protein level of Bcl-2 and the activities of Caspase-3 and Caspase-9 [11]. Adriamycin

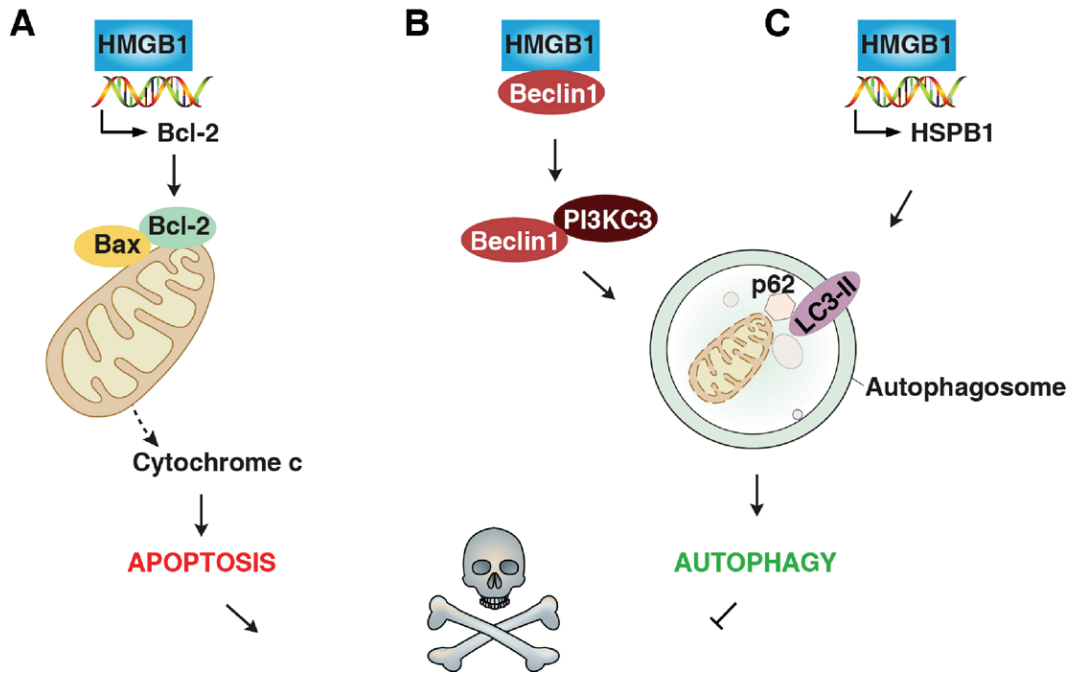


Figure 2. HMGB1 regulates apoptosis and autophagy. (A) HMGB1 inhibits apoptosis. HMGB1 promotes Bcl-2 expression, which inhibits Bax translocation from cytosol to mitochondria and cytochrome c release from mitochondria to cytosol. (B, C) HMGB1 promotes autophagy. Cytosolic HMGB1 binds Beclin 1 (B) and nuclear HMGB1 promotes HSPB1 expression (C), which promotes autophagosome formation and dynamic intracellular trafficking during autophagy and mitophagy.

(doxorubicin) is a chemotherapeutic agent used to treat leukemia and bladder, breast and head and neck cancer. Adriamycin induces a concentration- and time-dependent upregulation of Bcl-2 expression in myeloma and leukemia cells [44]. Bcl-2 overexpression inhibits adriamycin-induced apoptosis [45]. Notably, overexpression of HMGB1 by gene transfection increases adriamycin-induced Bcl-2 protein expression and prevents the activities of apoptotic effectors Caspase-3 and Caspase-9 in K562 myeloid leukemia cells [11]. Consistent with these findings, suppression of HMGB1 by shRNA in adriamycin resistant leukemia cells, K562/A02, decreases Bcl-2 expression and restores the sensitivity of K562/A02 to adriamycin [10]. These findings suggest that HMGB1 functions as an antiapoptotic protein in leukemia cells through regulation of Bcl-2 protein expression (**Figure 2A**).

HMGB1 as a proautophagic protein in leukemia cells

Autophagy is a dynamic homeostatic process of protein and organelle degradation which

is typically observed during nutrient deprivation [46, 47]. Recently, interest in autophagy has been renewed among oncologists, as many types of cancer cells enhance autophagy following chemotherapy and radiation therapy [48–51]. During autophagy, cytosolic constituents are sequestered into autophagosomes, which subsequently fuse with lysosomes (namely, autolysosomes) where their contents are degraded. Constitutive autophagy is a homeostatic mechanism necessary for intracellular recycling, energy homeostasis [52], development and differentiation [53]. The upregulation of autophagy in response to stress is an important mechanism by which damaged proteins and organelles are removed in preparation for anabolism and cell division [54]. Increased levels of autophagy have been implicated in numerous human diseases including cancer [55]. For example, autophagy promotes the growth of BCR-ABL-associated leukemogenesis [56], and inhibition of autophagy by potential autophagy inhibitors or shRNA enhances anticancer agent-induced cytotoxicity in several leukemia cell lines [57–60]. These findings suggest a specific role of

autophagy in leukemia development and therapy [61].

HMGB1 is a novel autophagy regulator and promotes autophagy through multiple mechanisms. In the nucleus, HMGB1 regulates the expression of heat shock protein β -1 (HSPB1, also denoted HSP27 in human) [62]. As a cytoskeleton regulator, HSPB1 is important for dynamic intracellular trafficking during autophagy and mitophagy. Thus, inhibition of the HMGB1-HSPB1 pathway impairs elimination of damaged mitochondria via mitophagy (**Figure 2C**) [62]. In the cytosol, HMGB1 activates autophagy by binding to Beclin 1 and displacing its inhibitory partner, Bcl-2 (**Figure 2B**) [63, 64]. In addition, exogenous HMGB1 in its reduced form induces autophagy [36, 65].

In HL-60, Jurkat and U937 leukemia cells, treatment with anticancer agents such as vincristine, adriamycin, cytosine arabinoside, arsenic trioxide, etoposide, camptothecin, staurosporine and cycloheximide results in HMGB1 release into the extracellular environment [9, 66]. Importantly, we have demonstrated that HMGB1-neutralizing antibodies, potential HMGB1 release inhibitors (e.g. quercetin) and knockdown of HMGB1 by RNAi increase the sensitivity of leukemia cells to these chemotherapeutic agents [6, 7, 9]. Moreover, pretreatment with exogenous HMGB1 protein increases drug resistance in leukemia cells, supporting a potential prosurvival role for HMGB1 in cells exposed to chemotherapy [9]. Knockdown of Beclin 1 or inhibition of autophagy with bafilomycin A1 reverses exogenous HMGB1-induced drug resistance, suggesting that autophagy is required for HMGB1-mediated chemotherapy resistance in leukemia cells [7, 9]. Indeed, intracellular and extracellular HMGB1 protein is a directly inducer of autophagy in leukemia cells. HMGB1 increases the interaction of Beclin 1/ class III phosphoinositide 3-kinase (PI3KC3) and Atg5-Atg12-Atg16 complex formation which are required for vesicle nucleation, phagophore membrane elongation and autophagosome formation (**Figure 2B**) [7, 9]. Moreover, overexpression HMGB1 in leukemia cells increases the transcriptional activity of JNK and ERK [6], which are involved in the regulation of Beclin 1-PI3KC3 complexes. These findings suggest that HMGB1 functions a proautophagic protein in leukemia cells through regulation of Beclin 1-PI3KC3 complex formation and associated signal transduction.

Conclusion

HMGB1 is a DNA-binding nuclear protein, actively released following cytokine stimulation as well as passively during cell injury and death. HMGB1 is overexpressed and released following chemotherapy and radiation therapy in many cancer cells including leukemia cells. As a critical antiapoptotic and proautophagic protein, HMGB1 enhances programmed cell survival and limits programmed cell death during leukemia therapy. In contrast, depletion or inhibition of HMGB1 in leukemia cells markedly increases drug sensitivity to anticancer agents. Thus, inhibiting HMGB1 could be used to augment current leukemia treatments, as many tumors cells upregulate autophagy and downregulate apoptosis to withstand chemotherapeutic treatment.

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References

- [1] Meads MB, Hazlehurst LA and Dalton WS. The bone marrow microenvironment as a tumor sanctuary and contributor to drug resistance. *Clinical cancer research: an official journal of the American Association for Cancer Research* 2008; 14: 2519-2526.
- [2] Whiteside TL. The tumor microenvironment and its role in promoting tumor growth. *Oncogene* 2008; 27: 5904-5912.
- [3] Tesniere A, Apetoh L, Ghiringhelli F, Joza N, Panaretakis T, Kepp O, Schlemmer F, Zitvogel L and Kroemer G. Immunogenic cancer cell death: a key-lock paradigm. *Curr Opin Immunol* 2008; 20: 504-511.
- [4] Srikrishna G, Freeze HH. Endogenous damage-associated molecular pattern molecules at the crossroads of inflammation and cancer. *Neoplasia* 2009; 11: 615-628.
- [5] Rubartelli A, Lotze MT. Inside, outside, upside

- down: damage-associated molecular-pattern molecules (DAMPs) and redox. *Trends Immunol* 2007; 28: 429-436.
- [6] Zhao M, Yang M, Yang L, Yu Y, Xie M, Zhu S, Kang R, Tang D, Jiang Z, Yuan W, Wu X and Cao L. HMGB1 regulates autophagy through increasing transcriptional activities of JNK and ERK in human myeloid leukemia cells. *BMB reports* 2011; 44: 601-606.
- [7] Yang L, Yu Y, Kang R, Yang M, Xie M, Wang Z, Tang D, Zhao M, Liu L, Zhang H and Cao L. Up-regulated autophagy by endogenous HMGB1 promotes chemoresistance in leukemia cells. *Leukemia & lymphoma* 2011.
- [8] Liu L, Yang M, Kang R, Wang Z, Zhao Y, Yu Y, Xie M, Yin X, Livesey KM, Loze MT, Tang D and Cao L. DAMP-mediated autophagy contributes to drug resistance. *Autophagy* 2011; 7: 112-114.
- [9] Liu L, Yang M, Kang R, Wang Z, Zhao Y, Yu Y, Xie M, Yin X, Livesey KM, Lotze MT, Tang D and Cao L. HMGB1-induced autophagy promotes chemotherapy resistance in leukemia cells. *Leukemia* 2011; 25: 23-31.
- [10] Xie M, Kang R, Yu Y, Zhu S, He YL, Xu WQ, Tang DL and Cao LZ. [Enhancive effect of HMGB1 gene silence on adriamycin-induced apoptosis in K562/A02 drug resistance leukemia cells]. *Zhonghua xue ye xue za zhi* 2008; 29: 549-552.
- [11] Yu Y, Xie M, He YL, Xu WQ, Zhu S and Cao LZ. [Role of high mobility group box 1 in adriamycin-induced apoptosis in leukemia K562 cells]. *Ai zheng* 2008; 27: 929-933.
- [12] Kang R, Tang DL, Cao LZ, Yu Y, Zhang GY and Xiao XZ. [High mobility group box 1 is increased in children with acute lymphocytic leukemia and stimulates the release of tumor necrosis factor-alpha in leukemic cell]. *Zhonghua er ke za zhi* 2007; 45: 329-333.
- [13] Lange SS, Vasquez KM. HMGB1: the jack-of-all-trades protein is a master DNA repair mechanic. *Mol Carcinog* 2009; 48: 571-580.
- [14] Thomas JO, Travers AA. HMGB1 and 2, and related "architectural" DNA-binding proteins. *Trends Biochem Sci* 2001; 26: 167-174.
- [15] Li J, Kokkola R, Tabibzadeh S, Yang R, Ochani M, Qiang X, Harris HE, Czura CJ, Wang H, Ulloa L, Warren HS, Moldawer LL, Fink MP, Andersson U, Tracey KJ and Yang H. Structural basis for the proinflammatory cytokine activity of high mobility group box 1. *Mol Med* 2003; 9: 37-45.
- [16] Yang H, Ochani M, Li J, Qiang X, Tanovic M, Harris HE, Susarla SM, Ulloa L, Wang H, DiRaimo R, Czura CJ, Roth J, Warren HS, Fink MP, Fenton MJ, Andersson U and Tracey KJ. Reversing established sepsis with antagonists of endogenous high-mobility group box 1. *Proceedings of the National Academy of Sciences of the United States of America* 2004; 101: 296-301.
- [17] Tang D, Kang R, Zeh HJ, 3rd and Lotze MT. High-mobility group box 1, oxidative stress, and disease. *Antioxidants & Redox Signaling* 2011; 14: 1315-1335.
- [18] Hoppe G, Talcott KE, Bhattacharya SK, Crabb JW and Sears JE. Molecular basis for the redox control of nuclear transport of the structural chromatin protein Hmg1. *Experimental cell research* 2006; 312: 3526-3538.
- [19] Calogero S, Grassi F, Aguzzi A, Voigtlander T, Ferrier P, Ferrari S and Bianchi ME. The lack of chromosomal protein Hmg1 does not disrupt cell growth but causes lethal hypoglycaemia in newborn mice. *Nat Genet* 1999; 22: 276-280.
- [20] Scaffidi P, Misteli T and Bianchi ME. Release of chromatin protein HMGB1 by necrotic cells triggers inflammation. *Nature* 2002; 418: 191-195.
- [21] Tsung A, Sahai R, Tanaka H, Nakao A, Fink MP, Lotze MT, Yang H, Li J, Tracey KJ, Geller DA and Billiar TR. The nuclear factor HMGB1 mediates hepatic injury after murine liver ischemia-reperfusion. *J Exp Med* 2005; 201: 1135-1143.
- [22] Wang H, Bloom O, Zhang M, Vishnubhakat JM, Ombrellino M, Che J, Frazier A, Yang H, Ivanova S, Borovikova L, Manogue KR, Faist E, Abraham E, Andersson J, Andersson U, Molina PE, Abumrad NN, Sama A and Tracey KJ. HMG-1 as a late mediator of endotoxin lethality in mice. *Science* 1999; 285: 248-251.
- [23] Lotze MT, Tracey KJ. High-mobility group box 1 protein (HMGB1): nuclear weapon in the immune arsenal. *Nature reviews. Immunology* 2005; 5: 331-342.
- [24] Andersson U, Tracey KJ. HMGB1 is a therapeutic target for sterile inflammation and infection. *Annual review of immunology* 2011; 29: 139-162.
- [25] Bianchi ME. HMGB1 loves company. *J Leukoc Biol* 2009; 86: 573-576.
- [26] Sims GP, Rowe DC, Rietdijk ST, Herbst R and Coyle AJ. HMGB1 and RAGE in inflammation and cancer. *Annu Rev Immunol* 2010; 28: 367-388.
- [27] Tang D, Kang R, Zeh HJ, 3rd and Lotze MT. High-mobility group box 1 and cancer. *Biochim Biophys Acta* 2010; 1799: 131-140.
- [28] Ellerman JE, Brown CK, de Vera M, Zeh HJ, Billiar T, Rubartelli A and Lotze MT. Masquerader: high mobility group box-1 and cancer. *Clin Cancer Res* 2007; 13: 2836-2848.
- [29] Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. *Cell* 2011; 144: 646-674.
- [30] Kang R, Tang D, Schapiro NE, Livesey KM, Farkas A, Loughran P, Bierhaus A, Lotze MT and Zeh HJ. The receptor for advanced glycation end products (RAGE) sustains autophagy and limits apoptosis, promoting pancreatic tumor cell survival. *Cell Death Differ* 2010; 17: 666-676.

- [31] Tang D, Kang R, Livesey KM, Zeh HJ, 3rd and Lotze MT. High mobility group box 1 (HMGB1) activates an autophagic response to oxidative stress. *Antioxidants & Redox Signaling* 2011; 15: 2185-2195.
- [32] Taguchi A, Blood DC, del Toro G, Canet A, Lee DC, Qu W, Tanji N, Lu Y, Lalla E, Fu C, Hofmann MA, Kislinger T, Ingram M, Lu A, Tanaka H, Hori O, Ogawa S, Stern DM and Schmidt AM. Blockade of RAGE-amphoterin signalling suppresses tumour growth and metastases. *Nature* 2000; 405: 354-360.
- [33] Gebhardt C, Riehl A, Durchdewald M, Nemeth J, Furstenberger G, Muller-Decker K, Enk A, Arnold B, Bierhaus A, Nawroth PP, Hess J and Angel P. RAGE signaling sustains inflammation and promotes tumor development. *J Exp Med* 2008; 205: 275-285.
- [34] Sims GP, Rowe DC, Rietdijk ST, Herbst R and Coyle AJ. HMGB1 and RAGE in inflammation and cancer. *Annual review of immunology* 2010; 28: 367-388.
- [35] Apetoh L, Ghiringhelli F, Tesniere A, Obeid M, Ortiz C, Criollo A, Mignot G, Maiuri MC, Ullrich E, Saulnier P, Yang H, Amigorena S, Ryffel B, Barrat FJ, Saftig P, Levi F, Lidereau R, Nogues C, Mira JP, Chompret A, Joulin V, Clavel-Chapelon F, Bourhis J, Andre F, Delaloge S, Tursz T, Kroemer G and Zitvogel L. Toll-like receptor 4-dependent contribution of the immune system to anticancer chemotherapy and radiotherapy. *Nat Med* 2007; 13: 1050-1059.
- [36] Tang D, Kang R, Cheh CW, Livesey KM, Liang X, Schapiro NE, Benschop R, Sparvero LJ, Amoscato AA, Tracey KJ, Zeh HJ and Lotze MT. HMGB1 release and redox regulates autophagy and apoptosis in cancer cells. *Oncogene* 2010; 29: 5299-5310.
- [37] Kazama H, Ricci JE, Herndon JM, Hoppe G, Green DR and Ferguson TA. Induction of immunological tolerance by apoptotic cells requires caspase-dependent oxidation of high-mobility group box-1 protein. *Immunity* 2008; 29: 21-32.
- [38] Yang H, Hreggvidsdottir HS, Palmblad K, Wang H, Ochani M, Li J, Lu B, Chavan S, Rosas-Ballina M, Al-Abed Y, Akira S, Bierhaus A, Erlandsson-Harris H, Andersson U and Tracey KJ. A critical cysteine is required for HMGB1 binding to Toll-like receptor 4 and activation of macrophage cytokine release. *Proc Natl Acad Sci USA* 2010; 107: 11942-11947.
- [39] Pui CH, Evans WE. Treatment of acute lymphoblastic leukemia. *The New England journal of medicine* 2006; 354: 166-178.
- [40] Kang R, Tang DL, Cao LZ, Yu Y, Zhang GY and Xiao XZ. [High mobility group box 1 is increased in children with acute lymphocytic leukemia and stimulates the release of tumor necrosis factor-alpha in leukemic cell]. *Zhonghua Er Ke Za Zhi* 2007; 45: 329-333.
- [41] Igney FH, Krammer PH. Death and anti-death: tumour resistance to apoptosis. *Nat Rev Cancer* 2002; 2: 277-288.
- [42] Youle RJ, Strasser A. The BCL-2 protein family: opposing activities that mediate cell death. *Nature reviews. Molecular cell biology* 2008; 9: 47-59.
- [43] Brezniceanu ML, Volp K, Bosser S, Solbach C, Lichter P, Joos S and Zornig M. HMGB1 inhibits cell death in yeast and mammalian cells and is abundantly expressed in human breast carcinoma. *Faseb J* 2003; 17: 1295-1297.
- [44] Tu Y, Xu FH, Liu J, Vescio R, Berenson J, Fady C and Lichtenstein A. Upregulated expression of BCL-2 in multiple myeloma cells induced by exposure to doxorubicin, etoposide, and hydrogen peroxide. *Blood* 1996; 88: 1805-1812.
- [45] Huigsloot M, Tjeldens IB, Mulder GJ and van de Water B. Differential regulation of doxorubicin-induced mitochondrial dysfunction and apoptosis by Bcl-2 in mammary adenocarcinoma (MTLn3) cells. *The Journal of biological chemistry* 2002; 277: 35869-35879.
- [46] Klionsky DJ, Emr SD. Autophagy as a regulated pathway of cellular degradation. *Science* 2000; 290: 1717-1721.
- [47] Yang Z, Klionsky DJ. Eaten alive: a history of macroautophagy. *Nat Cell Biol* 2010; 12: 814-822.
- [48] Amaravadi RK, Lippincott-Schwartz J, Yin XM, Weiss WA, Takebe N, Timmer W, DiPaola RS, Lotze MT and White E. Principles and current strategies for targeting autophagy for cancer treatment. *Clinical cancer research* 2011; 17: 654-666.
- [49] Dikic I, Johansen T and Kirkin V. Selective autophagy in cancer development and therapy. *Cancer research* 2010; 70: 3431-3434.
- [50] Kimmelman AC. The dynamic nature of autophagy in cancer. *Genes & development* 2011; 25: 1999-2010.
- [51] Livesey KM, Tang D, Zeh HJ and Lotze MT. Autophagy inhibition in combination cancer treatment. *Current opinion in investigational drugs* 2009; 10: 1269-1279.
- [52] Rabinowitz JD, White E. Autophagy and metabolism. *Science* 2010; 330: 1344-1348.
- [53] Mizushima N, Levine B. Autophagy in mammalian development and differentiation. *Nature cell biology* 2010; 12: 823-830.
- [54] Kroemer G, Marino G and Levine B. Autophagy and the integrated stress response. *Mol Cell* 2010; 40: 280-293.
- [55] Levine B, Kroemer G. Autophagy in the pathogenesis of disease. *Cell* 2008; 132: 27-42.
- [56] Altman BJ, Jacobs SR, Mason EF, Michalek RD, Macintyre AN, Colloff JL, Ilkayeva O, Jia W, He YW and Rathmell JC. Autophagy is essential to suppress cell stress and to allow BCR-Abl-mediated leukemogenesis. *Oncogene* 2011.
- [57] Carew JS, Nawrocki ST, Kahue CN, Zhang H,

- Yang C, Chung L, Houghton JA, Huang P, Giles FJ and Cleveland JL. Targeting autophagy augments the anticancer activity of the histone deacetylase inhibitor SAHA to overcome Bcr-Abl-mediated drug resistance. *Blood* 2007; 110: 313-322.
- [58] Kamitsuji Y, Kuroda J, Kimura S, Toyokuni S, Watanabe K, Ashihara E, Tanaka H, Yui Y, Watanabe M, Matsubara H, Mizushima Y, Hiraumi Y, Kawata E, Yoshikawa T, Maekawa T, Nakahata T and Adachi S. The Bcr-Abl kinase inhibitor INNO-406 induces autophagy and different modes of cell death execution in Bcr-Abl-positive leukemias. *Cell death and differentiation* 2008; 15: 1712-1722.
- [59] Yogalingam G, Pendergast AM. Abl kinases regulate autophagy by promoting the trafficking and function of lysosomal components. *The Journal of biological chemistry* 2008; 283: 35941-35953.
- [60] Bellodi C, Lidonnici MR, Hamilton A, Helgason GV, Soliera AR, Ronchetti M, Galavotti S, Young KW, Selmi T, Yacobi R, Van Etten RA, Donato N, Hunter A, Dinsdale D, Tirro E, Vigneri P, Nicotera P, Dyer MJ, Holyoake T, Salomoni P and Calabretta B. Targeting autophagy potentiates tyrosine kinase inhibitor-induced cell death in Philadelphia chromosome-positive cells, including primary CML stem cells. *The Journal of clinical investigation* 2009; 119: 1109-1123.
- [61] Helgason GV, Karvela M and Holyoake TL. Kill one bird with two stones: potential efficacy of BCR-ABL and autophagy inhibition in CML. *Blood* 2011; 118: 2035-2043.
- [62] Tang D, Kang R, Livesey KM, Kroemer G, Biliyar TR, Van Houten B, Zeh HJ, 3rd and Lotze MT. High-mobility group box 1 is essential for mitochondrial quality control. *Cell Metabolism* 2011; 13: 701-711.
- [63] Tang D, Kang R, Livesey KM, Cheh CW, Farkas A, Loughran P, Hoppe G, Bianchi ME, Tracey KJ, Zeh HJ, 3rd and Lotze MT. Endogenous HMGB1 regulates autophagy. *The Journal of cell biology* 2010; 190: 881-892.
- [64] Kang R, Livesey KM, Zeh HJ, Loze MT and Tang D. HMGB1: a novel Beclin 1-binding protein active in autophagy. *Autophagy* 2010; 6: 1209-1211.
- [65] Tang D, Loze MT, Zeh HJ and Kang R. The redox protein HMGB1 regulates cell death and survival in cancer treatment. *Autophagy* 2010; 6: 1181-1183.
- [66] Bell CW, Jiang W, Reich CF and Pisetsky DS. The Extracellular Release of HMGB1 during Apoptotic Cell Death. *Am J Physiol Cell Physiol* 200; 291: C1318-1325.