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## HMGB1 in Cancer: Good, Bad, or Both?

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### Abstract

Forty years ago, high mobility group box 1 (HMGB1) was discovered in calf thymus and named according to its electrophoretic mobility in polyacrylamide gels. Now, we know that HMGB1 performs dual functions. Inside the cell, HMGB1 is a highly conserved chromosomal protein acting as a DNA chaperone. Outside of the cell, HMGB1 is a prototypical damage-associated molecular pattern, acting with cytokine, chemokine, and growth factor. During tumor development and in cancer therapy, HMGB1 has been reported to play paradoxical roles in promoting both cell survival and death by regulating multiple signaling pathways, including inflammation, immunity, genome stability, proliferation, metastasis, metabolism, apoptosis, and autophagy. Here, we review the current knowledge of both HMGB1's oncogenic and tumor suppressive roles and the potential strategies that target HMGB1 for the prevention and treatment of cancer.

### Introduction

High mobility group box 1 (HMGB1) was first isolated and characterized in calf thymus in 1973 and is named for its electrophoretic mobility on polyacrylamide gels. HMGB1 contains two DNA-binding HMG-box domains (N-terminal A and central B) and an acidic C-terminal tail (Fig. 1A). In most cells, HMGB1 is located in the nucleus, where it acts as a DNA chaperone to help maintain nuclear homeostasis. HMGB1 was later discovered to express on cell surface membranes, cytosol, and mitochondria, and release into the extracellular space. HMGB1 has many biological functions inside as well as outside the cell (Fig. 1B), and plays a significant role in many diseases, especially inflammatory diseases and cancer (1–3).

Cancer development is a multi-step process. As cells become more abnormal, they gain new capabilities. In 2011, Douglas Hanahan and Robert Weinberg described ten functional capabilities of cancers that they called “the hallmarks of cancer” (Fig. 2A) (4). Evidence that HMGB1 dysfunction is associated with each hallmark of cancer and contributes to cancer development and therapy is increasing (1). However, HMGB1 has paradoxically been reported to both promote cell survival and cell death by regulating multiple cancer signaling pathways (Fig. 2B). This review describes recent advances in our understanding of HMGB1

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regulation and function; that they affect cancer biology and influence the strategies that target HMGB1 for the prevention and treatment of cancer.

## Nuclear Function of HMGB1

HMGB1 is stored in the nucleus as a result of the presence of two lysine-rich nuclear localization sequences (NLSs) located in the A box and in the B box (Fig. 1A). Hyperacetylation of NLSs promotes HMGB1 translocation from the nucleus to the cytosol, and the subsequent release of HMGB1. The HMG boxes enable HMGB1 to bind different DNA structures without sequence-specificity and act as a DNA chaperone. HMGB1 is the structural protein of chromatin and regulates nuclear homeostasis and genome stability in several ways (Fig. 1B). **1) Nucleosome structure and dynamics.** Nucleosome is the basic unit of chromatin, consisting of a short length of DNA wrapped around a core of histone proteins. HMGB1 binds to nucleosomes at the dyad axis, promotes nucleosome sliding, relaxes nucleosome structure, and makes chromatin more accessible by its ability to bend DNA (5). **2) Gene transcription.** HMGB1 knockout mice show a defect in the transcriptional enhancement of the glucocorticoid receptor and die shortly after birth. HMGB1 has been found to increase the binding affinity of many sequence-specific transcription factors to their cognate DNA, such as p53, p73, the retinoblastoma protein (RB), nuclear factor- $\kappa$ B (NF- $\kappa$ B), and the estrogen receptor. **3) DNA repair.** Loss of HMGB1 increases DNA damage and decreases DNA repair efficiency in response to chemotherapy, irradiation, and oxidative stress. HMGB1 directly binds to a variety of bulky DNA lesions and allows it to participate in DNA repair pathways including nucleotide excision repair, base excision repair, mismatch repair, and double strand break repair via nonhomologous end-joining (6). **4) Gene recombination.** V(D)J recombination is a mechanism of genetic recombination in the early stages of immunoglobulin and T cell receptor production of the immune system. HMGB1 is required for efficient V(D)J recombination by enhancing recombination activating gene (RAG) binding to the recombination signal sequences (RSSs). **5) Telomere homeostasis.** A telomere is a region of repetitive nucleotide sequences at the end of a chromosome. Loss of HMGB1 reduces telomerase activity, decreases telomere length, and increases chromosomal instability.

## Mechanism of HMGB1 Release

In addition to its role in the nucleus, HMGB1 also functions as a damage-associated molecular pattern (DAMP) when passively released from dead, dying, or injured cells or when actively secreted from immune cells or cancer cells in response to exogenous and endogenous stimuli such as endotoxin, CpG DNA, double-stranded RNA (dsRNA), tumor necrosis factor (TNF)- $\alpha$ , interleukin (IL)-1, interferons (IFN)- $\gamma$ , hydrogen peroxide, adenosine triphosphate (ATP), and hypoxia (Fig. 3). In addition, macrophage engulfment of apoptotic cells may induce significant active HMGB1 release, suggesting a direct interplay between dying cells and immune cells, which also induces HMGB1 release (7).

Depending on the inducing stimulus, the mechanism of HMGB1 secretion and release can vary. HMGB1 lacks a leader signal sequence and its secretion needs a non-classical, secretory lysosome-mediated exocytosis (8). Several mechanisms have been recently proposed to explain the mechanism of HMGB1 secretion and release. **1) Post transcriptional modifications (PTMs)** (Fig. 4A). Following stimuli, the HMGB1 protein is modified by different PTMs, such as acetylation, ADP-ribosylation, methylation, phosphorylation, and oxidation, which regulate HMGB1 secretion. However, we still do not know whether these PTMs are competitively, cooperatively, or independently regulated. **2) Secondary messengers** (Fig. 4A). Several of the secondary messengers, such as cytosolic free calcium, reactive oxygen species (ROS), and nitric oxide, regulate HMGB1 secretion.

**3) Nuclear export receptor** (Fig. 4A). Chromosome-region maintenance 1 (CRM1) directly mediates HMGB1 export from the nucleus (9). **4) Pyroptosis** (Fig. 4B). Pyroptosis is an inflammatory cell death and is typically triggered by caspase-1 after its activation by various inflammasomes. dsRNA-dependent protein kinase (PKR) is implicated in inflammation and immune dysfunction by interfering with many signaling pathways (10). PKR-mediated inflammasome activation is required for DAMP and pathogen-associated molecular pattern (PAMP)-induced HMGB1, IL-1  $\beta$ , and IL-18 release in macrophages. **5) Apoptosis** (Fig. 4C). An early study suggests that the chromatin of apoptotic cells sequesters HMGB1 and prevents inflammation (11). However, HMGB1 also can be released by apoptotic cells at a late stage (12). It has been shown that nuclear DNA and histones are released during apoptosis, and they are well-known binding partners of HMGB1 in the nucleus. The mechanism for HMGB1 release undergoing apoptosis partly involves caspase-3/7-mediated mitochondrial ROS production (13). **6) Necrosis** (Fig. 4D). HMGB1 release occurs passively as cell permeability breaks down upon necrosis (11). In addition, poly(ADP-ribose) polymerase 1 (PARP1)-mediated ATP depletion regulates HMGB1 release in necrosis (14), suggesting that PARP1 is a potential therapy target in inflammatory disease. **7) Autophagy** (Fig. 4E). Autophagy is a lysosome-mediated, self-degrading process and plays an important role in sustaining cellular homeostasis under stress. The ATG5-mediated classical autophagy pathway has been reported to promote HMGB1 release in starvation and lipopolysaccharide (LPS) treatment (15, 16). This process requires ROS signaling (15).

## HMGB1 Signaling Pathways

Once released, extracellular HMGB1 binds to several cell surface receptors to activate the downstream signaling pathway (e.g., NF- $\kappa$ B, IFN regulatory factor-3 (IRF3), and phosphatidylinositol 3-kinase [PI3K]) to produce a functional response, such as activation of innate immune cells, induction of proinflammatory cytokines and type I IFNs, stimulation of cell adhesion and migration, inhibition of phagocytosis, promotion of cell proliferation and angiogenesis, and induction of autophagy (17, 18). These receptors include the receptor for advanced glycation end products (RAGE), Toll-like receptors (TLRs, such as TLR2, TLR4, and TLR9), Mac-1, syndecan-1 (CD138), phosphacan protein-tyrosine phosphatase (PPTP)- $\zeta/\beta$ , CD24, chemokine (C-X-C motif) ligand 4 (CXCL4), T cell immunoglobulin mucin-3 (TIM-3), and possibly others. Of these receptors, CD24 and TIM-3 act as negative receptors and inhibit immune activity of HMGB1 in macrophages and tumor-associated dendritic cells (TADCs), respectively (19, 20). Apart from a direct receptor interaction, HMGB1 may form heterocomplexes with other immune co-activators such as IL-1, CXCL12, DNA, nucleosome, or LPS that generate synergistic responses in inflammation and immunity.

In 1995, RAGE was the first receptor demonstrated to bind HMGB1 (21). At the time, the functions of RAGE in HMGB1 signaling were unknown, but it was later discovered that HMGB1 signaling through RAGE mediates chemotaxis and migration, proliferation and differentiation of immune and cancer cells, and upregulation of cell surface receptors and autophagy. The cytoplasmic tail is believed to be essential for intracellular signaling of RAGE, including RAGE-mediated energy metabolism (22). In addition, RAGE provides a functional platform for crosstalk with other HMGB1 receptors. For example, interplay between RAGE and TLR9 is critical for HMGB1-DNA complex-activated immune responses in dendritic cells (DCs) (23). Interplay between Mac-1 and RAGE is required for HMGB1-mediated adhesive and migratory neutrophil functions (24). Interplay between syndecan-1, PPTP- $\zeta/\beta$ , and RAGE is required for neurite outgrowth.

Apart from RAGE, HMGB1 binding of TLR2 and TLR4 also results in NF- $\kappa$ B activation. TLR4 may be more important for HMGB1-induced macrophage activation and proinflammatory cytokine release (25). TLR4-deficient animals are significantly protected

from ischemia-reperfusion injury to the liver, kidney, and heart, suggesting that TLR4 plays a critical role in sterile inflammation (26). Determining the functional relationship between RAGE and TLR4 in inflammation and immunity is still of great interest.

## HMGB1 Redox States

Recent studies underscore the importance of redox modification in the regulation of HMGB1 translocation, release, and activity in disease (27). Three cysteines are encoded within the HMGB1, two vicinal cysteines in box A (C23 and C45) and a single one in box B (C106) (Fig. 1A). Replacement of Cys23 and/or 45 with serines did not affect the nuclear distribution of the mutant proteins, while C106S and triple cysteine mutations impaired nuclear localization of HMGB1, allowing entry of some of the protein into the cytosol. Moreover, increased endogenous and exogenous ROS promotes HMGB1 translocation and release (28) (Fig. 4).

Of note, the redox status of HMGB1 distinguishes its cytokine-inducing and chemokine activity (29) (Fig. 3). Initial studies suggest that reduced C106 is necessary for the binding of HMGB1 to TLR4 to stimulate cytokine release and inflammation (25). A recent study suggests that a disulfide bond between C23 and C45 is also required for HMGB1 cytokine activity (29). Mutations of C45 or C23 abolish the cytokine activity of HMGB1. In contrast, all-cysteine-reduced HMGB1 does not have TLR4-dependent cytokine activity, but binds to CXCL4 to induce inflammatory cell recruitment and chemotaxis by the CXCL12 receptor (30). ROS oxidizes the HMGB1 at C106 released from apoptotic cells, thereby neutralizing its cytokine-inducing activity and promoting tolerance in DCs (13). Finally, all-cysteine-oxidized HMGB1 prevents HMGB1's cytokine or chemotactic activity (29). Thus, redox modifications are crucial for HMGB1 functionality as a mediator during infection and sterile inflammation. As described below, redox also regulates HMGB1 function and activity in autophagy.

## HMGB1 as an Autophagy Regulator

It is clear that autophagy, literally meaning “self-eating”, is not only a crucial biological process from physiology to pathology, but is also a promising and daunting target for several diseases, including inflammatory disease, neurodegeneration, and cancer. The role of autophagy in cancer is complex and is likely dependent on tumor type, stage, genetic context, and tumor microenvironment (31). On one hand, autophagy acts as a tumor suppressor by preventing genome instability, limiting oxidative stress, reducing intratumoral necrosis-dependent inflammation, and inhibiting angiogenesis (Fig. 5A). On the other hand, autophagy acts as a survival mechanism that can promote the growth of established tumors and resistance to anticancer treatment by providing substrates for metabolism, removing damaged organelles and proteins, and diminishing apoptosis (Fig. 5A). Thus, defining the context-specific role for autophagy in cancer and the mechanisms involved will be important to guide autophagy-based therapeutic intervention.

During autophagy, double-membrane structures called autophagosomes engulf cytosol or organelles and deliver them to lysosomes (called autolysosomes) to be degraded and recycled (Fig. 5A). It is now known that the complex and fine-tuned process of autophagy is regulated by many proteins, including HMGB1. HMGB1 has transcription-dependent and transcription-independent proautophagic activities (Fig. 5B). Heat shock protein beta-1 (HSPB1), a regulator of actin cytoskeleton dynamics, is a direct transcriptional target of HMGB1 in autophagy (32). Suppression expression of HSPB1 and HMGB1 inhibits the dynamics of autophagy as well as mitophagy, a form of selective autophagy to remove damaged mitochondria (32). During stress, including starvation, oxidative stress, and chemotherapy, HMGB1 translocates from the nucleus to the cytoplasm and binds to Beclin

1, which initiates autophagosome formation (15). Furthermore, the intra-molecular disulfide bridge (C23–C45) of HMGB1 is required to bind to Beclin 1 and sustain autophagy (15). Once released, reduced HMGB1 triggers autophagy in a RAGE-dependent manner in cancer cells, which promotes tumor cell proliferation (33). In contrast, oxidized HMGB1 induces apoptosis (33). Thus, both intracellular and extracellular HMGB1 are critical in regulating autophagy (Fig. 5B). Given the dual role of autophagy in cancer, HMGB1-mediated autophagy may have a similar role in cancer biology that needs further investigation.

## HMGB1's Pro-tumor Roles in Tumorigenesis

### Sustenance of the Inflammatory Microenvironment

Tumor-infiltrating leucocytes, as well as cytokine-related signaling pathways, are critical components in the development of the inflammatory tumor microenvironment (TME). The inflammatory TME can promote neoplastic transformation and support tumor growth, invasion, and metastases. Infiltrating leucocytes, as well as the cancer cells themselves, have the ability to secrete HMGB1 under hypoxia, injury, inflammatory stimuli, or environmental factors (34). In turn, extracellular HMGB1 can activate proinflammatory signaling pathways, such as the NF- $\kappa$ B and inflammasome pathways, to induce proinflammatory cytokine release. This loop will accelerate inflammatory responses, tumor formation, and metastasis. Both RAGE and TLR4 are involved in the HMGB1-mediated inflammatory response in tumorigenesis (35, 36), but the crosstalk between these receptors is not well-known.

### Fulfillment of Metabolic Requirements

Cancer requires more energy that allows for rapid, invasive, and metastatic growth. While normal cells generate ATP through a combination of oxidative and glycolytic metabolism, cancer cells dramatically accelerate and reprogram their metabolism to better fit the energy requirement. This is one of cancer's most common phenotypes, called the "Warburg effect". HMGB1 has been implicated in tumor energy metabolism (22, 32). Recombinant exogenous HMGB1 or endogenous HMGB1 in necrotic tumor cell lysates increased ATP production and pancreatic tumor cell proliferation, providing a direct link between inflammation and energy metabolism with the TME (22). Extracellular HMGB1 increases mitochondrial RAGE expression and translocation, which in turn increases mitochondrial complex I activity and ATP production (22). Loss of RAGE in mice prevents tumorigenesis in the skin, pancreas, and intestine (35, 37, 38) and inhibits ATP production for tumor growth (37). Loss of HMGB1 increases mitochondrial injury and decreases ATP production (32). The crosstalk between the HMGB1-RAGE signaling pathway and other key cancer metabolic signaling pathways is still unknown.

### Promotion of Invasion and Metastasis

Most cancer deaths are caused by tumor invasion and metastasis rather than the primary tumor itself. In the clinic, expression of RAGE is closely associated with the invasive and metastatic activity of cancers such as gastric cancer (39) and colorectal cancer (40). In animals, inhibition of RAGE-HMGB1 interaction suppresses tumor growth and metastasis by activation of mitogen-activated protein kinases and the NF- $\kappa$ B pathway, which results in the expression of matrix metalloproteinases (MMPs), such as MMP2 and MMP9 (41). MMPs degrade extracellular matrix proteins and play a major role in tumor invasion and metastasis. *In vitro*, suppression of RAGE and HMGB1 by antisense S-oligodeoxynucleotide or HMGB1 150–183 peptide (RAGE-binding motif) inhibits growth, migration, and invasion in cancer cells (42). These studies suggest that HMGB1-RAGE signaling plays a major role in tumor invasion and metastasis.

## Inhibition of Antitumor Immunity

Cancer immunity surveillance is considered to be an important host defense process to inhibit carcinogenesis and to maintain cellular homeostasis. As a multifunctional cytokine, HMGB1 has been characterized with both immunosuppressive and immune-activation properties, which depends on receptors, targeted cells, and redox state (27, 43). DCs are professional antigen-presenting cells and constitute several subsets with distinct phenotypes and crucial roles in promoting both immune tolerance and immunity. HMGB1 has the ability to induce apoptosis in macrophage-derived DCs, which diminish host anti-cancer immunity (44). In addition, tumor cell-derived HMGB1 suppresses naturally-acquired CD8<sup>+</sup> T cell-dependent antitumor immunity, partly by enhancing tumor-associated Treg to produce IL-10 (45). More recently, HMGB1 promotes tumor-infiltrating T cell-expressed lymphotoxin  $\alpha$ 1 $\beta$ 2, which leads to the recruitment of CD11b<sup>+</sup>F4/80<sup>+</sup> macrophages into the tumor for tumor promotion by providing growth factors and supporting angiogenesis (46). The downstream signaling pathway of HMGB1-mediated tumor immune suppression needs to be elucidated in future studies.

## Promotion of Angiogenesis

A complex balance between pro- and anti-angiogenic factors exists in tumor microenvironments and regulates vessel formation. In a fast-growing tumor, the diffusion distance from the existing vascular supply increases, resulting in hypoxia. The regulation of angiogenesis by hypoxia is well-studied in cancer. Hypoxia causes translocation and release of HMGB1 and increases expression of RAGE in TME (47, 48). Once released, HMGB1 binds to RAGE and activates the NF- $\kappa$ B pathway to induce expression of proangiogenic growth factors (e.g., vascular endothelial growth factor) and their receptors (49). Thus, using HMGB1 antibody or knockdown of RAGE inhibits tumor angiogenesis and metastasis *in vitro* and *in vivo* (49, 50).

## HMGB1's Anti-tumor Roles in Tumorigenesis

### Interactions with Tumor Suppressor

Intracellular HMGB1 functions as a tumor suppressor in breast cancer by directly binding to RB (51) (Fig. 2B). RB is a well-known tumor suppressor protein that is dysfunctional in many cancers. RB has three functionally distinct binding domains and interacts with its regulatory proteins with a conserved LXCXE motif. HMGB1 binds to RB by the LXCXE motif, which in turn enhances RB-mediated transcription repression such as E2F and cyclin A1 (51). HMGB1 causes RB-dependent G1 arrest and apoptosis induction, and overexpression of HMGB1 inhibits RB positive breast cancer growth *in vitro* and prevents tumorigenicity in subcutaneous tumor models *in vivo* (51). These findings suggest that endogenous HMGB1, as a RB-associated protein, suppresses breast tumorigenesis. Whether HMGB1 interplays with other tumor suppressors (e.g., p53) or oncogenes (e.g., KRAS) to regulate tumorigenesis is of great interest.

### Increaser of Genome Instability

As a major driving force for tumorigenesis, genomic instability, which includes both numerical (e.g., aneuploidy) and structural chromosomal abnormalities, occurs early in the development of most cancers. HMGB1 has an important role in modulating genome stability. HMGB1 deficiency in cells results in genome instability (52, 53). Telomeres are essential for chromosome stability by protecting them from recombination and degradation. Loss of HMGB1 displays telomere shortening (52, 54). The mechanisms by which HMGB1 maintains telomere lengthening are involved in regulation of telomerase activity by binding to telomerase (54). However, the cooperative action of HMGB1 and telomerase in regulating telomere length and function still remains unknown. Topoisomerase II $\alpha$

(Topo II $\alpha$ ), an enzyme that controls and alters the topologic states of DNA during transcription, is implicated in chromosome replication, segregation, and recombination. HMGB1 not only binds Topo II $\alpha$  to stimulate its enzymatic activity, but also promotes its expression (55). Moreover, the upregulation of Topo II $\alpha$  by HMGB1 is inhibited by RB (55), suggesting that interplay between HMGB1 and RB regulates Topo II $\alpha$  expression and subsequently, genome stability. In addition, HMGB1-mediated DNA damage repair contributes genome stability (6). These findings suggest that loss of HMGB1 in tissue may cause genome instability and tumorigenesis (Fig. 2B).

### Increaser of Autophagy

Several studies have indicated that defective autophagy-associated genes (e.g., Beclin1, ATG5, UVRAG, Bif-1) in mice increase genome instability, inflammation, oxidative stress, and mitochondrial injury, which contribute to tumorigenesis (56–59). It is well demonstrated that autophagy is a negative regulator of inflammasome activation by promoting its degradation or diminishing ROS production. Autophagy-deficient mice lose their ability to remove damaged mitochondria, which in turn induces ROS production and inflammasome activation (60). However, the mechanism by which autophagy deficiency compromises genomic stability is still unclear. One possibility is that autophagy has the ability to remove damaged DNA, which in turn prevents chromosomal instability. Given that HMGB1 is a critical regulator of autophagy and mitophagy (15, 32), loss of HMGB1 leading to autophagy deficiency may cause genome instability and inflammation, which lead to tumorigenesis (Fig. 2B).

### HMGB1's Protective Roles in Anticancer Therapy

HMGB1-mediated immunogenic cell death (ICD) contributes to immune-mediated eradication of tumors during chemotherapy (e.g., anthracyclines) or radiotherapy (61–63). ICD is characterized by the release of dying cancer cells or cell surface exposure of DAMPs (e.g., calreticulin, heat shock proteins, ATP, and HMGB1), which are helpful for the maturation, antigen uptake, and presentation of DCs and serve as powerful immunological adjuvants to active cytotoxic T lymphocyte response. Autophagy-mediated ATP release also contributes to ICD development (64). Blocking the HMGB1- TLR4 pathway inhibits ICD-associated anticancer immune responses upon chemotherapy both *in vitro* and *in vivo* (63) (Fig. 2B). However, HMGB1 released from necrotic cancer cells treated with chemotherapy enhances regrowth and metastasis of remnant cancer cells in a RAGE-dependent way (65). Thus, blocking HMGB1-RAGE signaling increases the effectiveness of chemotherapy (66). These studies suggest that TLR4 in DC is critical for HMGB1-mediated ICD and tumor clearance, whereas RAGE in cancer cells is important for HMGB1-mediated survival after chemotherapy. Although both apoptotic and necrotic cells have the ability to release HMGB1, HMGB1 released from apoptotic cells is immunity-tolerant (13). Moreover, HMGB1 binding to TIM-3 in TADCs is important for evasion of the immune system by tumor cells in response to chemotherapy and vaccines (20), suggesting that a balance mechanism between TLR4 and TIM-3 exists in DCs (Fig. 2B). Of note, a recent study suggests that ICD-mediated tumor clearance works only in tumor cell line transplantation models or the immunogenic 3- methylcholanthrene fibrosarcoma model, but not spontaneous mammary tumor models (67). In addition, TLR2, but not TLR4 in TADCs, mediates T cell-dependent brain tumor regression in antibrain cancer immunotherapy (68). Thus, defining the context-specific role for extracellular HMGB1 in chemotherapy and immunotherapy, including ICD, and the mechanisms involved will be important to optimal therapeutic outcome.

## HMGB1's Negative Roles in Anticancer Therapy

Induction of cell death and inhibition of cell growth are the main targets of cancer therapy. A number of studies have demonstrated that suppression of HMGB1 expression by RNAi increased the anticancer activity of cytotoxic agents, whereas overexpression of HMGB1 expression by gene transfection increased drug resistance (69–71). HMGB1 expression regulates chemotherapeutic response and resistance by interfering with autophagy and the apoptotic pathway (Fig. 2B). HMGB1 increases pro-survival autophagy in a Beclin 1-dependent way in chemotherapy, whereas HMGB1 inhibits both intrinsic and extrinsic programmed cell death/apoptosis in a caspase-dependent way in cancer cells. In some cases, knockout HMGB1 in fibroblasts inhibits antimetabolite drug-induced apoptosis (72), suggesting that HMGB1 plays distinct roles in apoptosis depending on cell types. The cross-talk between apoptosis and autophagy regulates cell death and determines cell fate in anticancer therapy. Upregulation of apoptosis inhibits autophagy, whereas upregulation of autophagy inhibits apoptosis during chemotherapy. HMGB1 and p53 are capable of physical interaction, and a region of inducible structure in the p53 transactivation domain (residues 38–61) is the essential element for binding to the A box (residues 7–74) (Fig.1A) (73). Interplay between HMGB1 and p53 regulates apoptosis and autophagy in clone cancer cells after treatment with DNA-damaging anticancer drugs (70). DNA damage promotes interactions between p53 and HMGB1 in the nucleus and cytoplasm. Loss of p53 increases cytosolic HMGB1, leading to increased binding to Beclin 1, thereby promoting autophagy and decreasing apoptosis; In contrast, loss of HMGB1 increases cytosolic p53 and apoptosis and decreases autophagy (70). These findings provide new insights into the HMGB1-p53 signaling and cancer cell response to DNA damage.

## HMGB1-Targeting Therapeutic Agents

Apart from genetic inhibition or overexpression of HMGB1 expression in cancer cells, several HMGB1-targeting agents have been used in experimental cancer research. These agents including sRAGE, HMGB1 neutralizing antibody, A box protein, platinating agent, ethyl pyruvate, quercetin, and glycyrrhizin. sRAGE acts as a decoy to prevent RAGE signaling and has been used successfully in blocking the HMGB1-RAGE signaling pathway in animal tumor models (41). HMGB1 neutralizing antibody and A box protein can block activity of extracellular HMGB1 in tumor therapy (71). Platinating agents such as cisplatin and oxaliplatin have the ability to retain HMGB1 within the nucleus by conformational changes in the double helix to which HMGB1 binds quite stably (74). Ethyl pyruvate, the first HMGB1 inhibitor used in animal models of sepsis by inhibition of NF- $\kappa$ B pathway, inhibits liver tumor growth (75). In addition, glycyrrhizin and quercetin, potential HMGB1 inhibitors by directly binding to HMGB1 or inhibition of PI3K, improve the effectiveness of anticancer agents in several different tumor models (33, 71). Further investigation is needed to evaluate these therapies and their possible role in clinical practice.

## Conclusions and Perspectives

We have described how HMGB1 seemingly plays both oncogenic and tumor suppressing roles during tumor development and therapy (Fig. 2B). In many cases, extracellular HMGB1 acts as a pro-tumor protein due to its cytokine, chemokine, and growth factor activity, whereas intracellular HMGB1 acts as an anti-tumor protein due to its ability to sustain genome stability and autophagy activity during tumor growth. In contrast, extracellular HMGB1 may contribute to ICD-associated antitumor immunity in the early stages of chemotherapy, but promote residual tumor cell survival in the late stages of chemotherapy. Moreover, suppression of intracellular HMGB1 expression inhibits autophagy and increases apoptosis, which boosts the effectiveness of anticancer treatment. Thus, the strategies that



target HMGB1 for the prevention and treatment of cancer may vary at different stages. Perhaps we should be thinking about the design of tumor-selective modulators of HMGB1. Given the major role of HMGB1 in nucleus, HMGB1 could be a sensible target for hereditary cancers such as breast cancer that are characterized by the presence of genomic instability (51). Knowledge of the signal transduction and regulation of HMGB1 release is one of the hot topics in immunity and cancer research. HMGB1 can induce immune responses by itself, as well as in association with other endogenous and exogenous molecules. In addition, dynamic PTMs of HMGB1, as well as receptor selection, might shift their activity from immunity activation to immunity tolerance. However, the roles of PTMs of HMGB1 in tumorigenicity and anticancer therapies still remain largely unknown. In addition, it remains unclear whether HMGB1 mutations exist. Further work is needed to understand the molecular basis and context-dependent role of HMGB1 as an anti- or pro-tumor protein in cancer. Also, it is important to establish HMGB1-associated spontaneous tumor models, although many xenograph or transplantable tumor models have been used.

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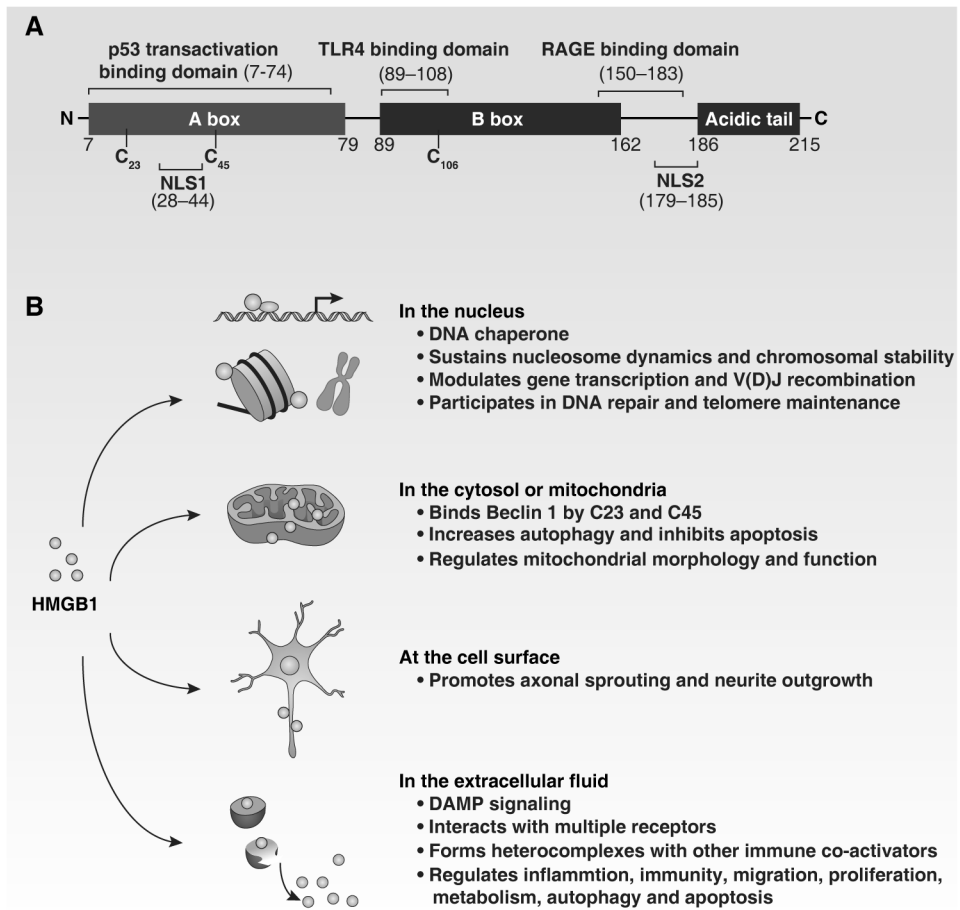
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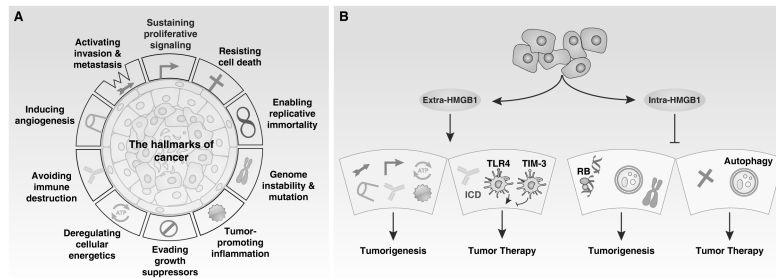
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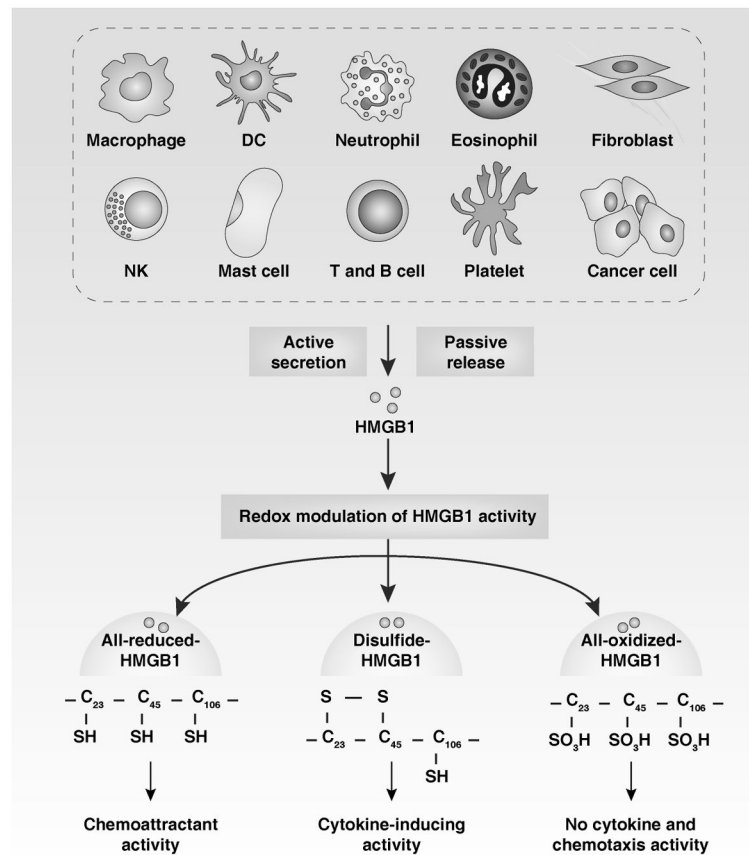
### Figure 1. Structure and function of HMGB1

(A) HMGB1 is structurally composed of three different domains: two homologous DNA-binding domains (box A and box B) and a negatively charged C-terminal domain. Residues 150–183 are responsible for binding to RAGE, whereas residues 89–108 and residues 7–74 are responsible for binding to TLR4 and p53 transactivation domain, respectively. Two nuclear localization signals (NLS1 and NLS2) control nuclear transport of HMGB1. In addition, HMGB1 contains three redox-sensitive cysteine residues (C23, C45 and C106), which are important for HMGB1 activity. (B) HMGB1 has multiple roles based on its location, as indicated.



**Figure 2. The dual roles of HMGB1 in cancer**

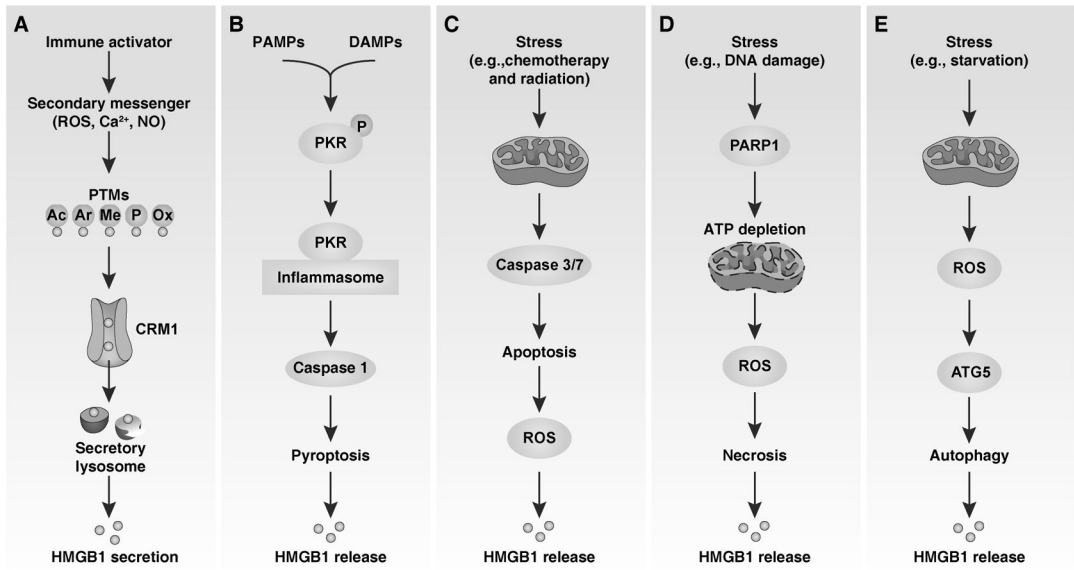
(A) The hallmarks of cancer comprise ten biological capabilities acquired during the multistep development of human tumors. Deregulation of HMGB1 is associated with the hallmarks of cancer. Figure modified from Hanahan and Weinberg (4). (B) HMGB1 acts as an anti- or pro-tumor protein in tumor development and therapy. Extracellular HMGB1 promotes proliferation, inflammation, energy metabolism, and angiogenesis, and inhibits host anti-cancer immunity, which contributes to tumorigenesis. In contrast, extracellular-HMGB1 is important for the immunogenic cell death (ICD) of cancer cells and stimulates antitumor immunity response during chemotherapy or radiotherapy. HMGB1's binding to TLR4 increases antitumor immunity, whereas binding to TIM-3 inhibits antitumor immunity in DCs. Intracellular HMGB1 prevents genome instability and enhances RB function in tumorigenesis. In addition, HMGB1 is a critical regulator of autophagy. Loss of HMGB1 inhibits autophagy and increases apoptosis. Suppression of autophagy promotes tumorigenesis and increases the effectiveness of anticancer therapy.



**Figure 3. HMGB1 release and redox activity**

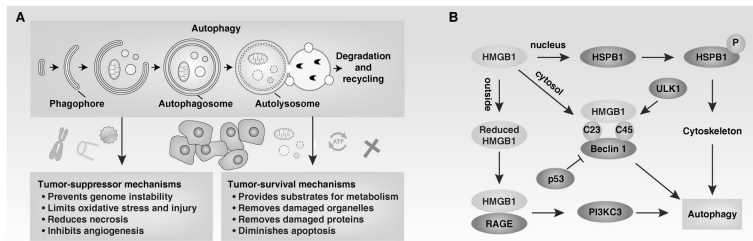
HMGB1 can be passively released or actively secreted from the indicated immune and cancer cells under stress. The activity of HMGB1 varies with the redox states of the cysteine residues (C23, C45 and C106). All- reduced-HMGB1 has sole chemokine activity, whereas disulfide-HMGB1 has only cytokine activity, and all-oxidized-HMGB1 has neither.





**Figure 4. Mechanism of HMGB1 release**

(A) Endogenous and exogenous immune activators can produce secondary messenger molecules that will induce post-translational modifications (PTMs) of HMGB1 and subsequently CRM1-mediated HMGB1 nuclear export. Finally, HMGB1 is secreted by non-classical, lysosome-mediated exocytosis. “Ac” = acetylation, “Ar” = ADP-ribosylation, “Me” = methylation, “P” = phosphorylation, “Ox” = oxidation. (B) In pyroptosis, PAMPs and DAMPs promote the autophosphorylation and activation of PKR. Active PKR physically interacts with inflammasome and promotes the release of HMGB1 in a caspase 1-dependent way. (C) In apoptosis, chemotherapy and radiation promote activation of caspase-3 and caspase-7, which impair the mitochondrial respiratory chain, induce production of ROS, and result in HMGB1 release. (D) In necrosis, DNA damage activates PARP-1, which leads to ATP depletion, mitochondrial ROS production, and subsequent HMGB1 release. (E) In autophagy, starvation promotes ROS production, which leads to HMGB1 release in an ATG5-dependent way.



### Figure 5. The HMGB1-mediated autophagy pathway

(A) The dual roles of autophagy in cancer. The main process of autophagy includes formation and maturation of the phagophore, autophagosome, and autolysosome, which lead to the degradation and recycling of the sequestered contents. During tumor development and in cancer therapy, autophagy promotes both tumor suppression and tumor survival. (B) Multiple roles of HMGB1 in autophagy. Nuclear HMGB1 modulates the expression of heat shock protein  $\beta$ -1 (HSPB1). Loss of either HMGB1 or HSPB1 results in a phenotypically similar deficiency in mitophagy and autophagy. This pathway requires phosphorylation of HSPB1 (both Ser15 and Ser86) to modulate actin polymerization and reorganization. Cytoplasmic HMGB1 is a novel Beclin 1-binding protein active in autophagy. HMGB1 C23 and C45 is required for HMGB1 binding to Beclin 1. p53 and unc-51-like kinase 1 (ULK1) have opposing roles in regulation of the HMGB1- Beclin 1 complex formation in cancer cells. The ULK1 kinase is an essential component of the core autophagy machinery. Extracellular HMGB1 induces autophagy by receptor for advanced glycation end products (RAGE), and this role is dependent on HMGB1's redox state.