

Biological functions and theranostic potential of HMGB family members in human cancers

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Abstract: The high mobility group box (HMGB) protein family consists of four members: HMGB1, 2, 3, and 4. They share similar amino acid sequences and identical functional regions, especially HMGB1, 2, and 3. The homology in structure may lead to similarity in function. In fact, though their targets may be different, they all possess the fundamental function of binding and distorting target DNAs. However, further research confirmed they are distributed differently in tissues and involved in various distinct physiological and pathological cellular processes, including cell proliferation, division, migration, and differentiation. Recently, the roles of HMGB family members in carcinogenesis has been widely investigated; however, systematic discussion on their functions and clinical values in malignant tumors is limited. In this review, we mainly review and summarize recent advances in knowledge of HMGB family members in terms of structure, distribution, biochemical cascades, and specific mechanisms regarding tumor progression. Importantly, the diagnostic, prognostic, and therapeutic value of these proteins in cancers is discussed. Finally, we envisage the orientation and challenges of this field in further studies.

Keywords: biochemical cascades, biological function, carcinogenesis, HMGB, prognosis

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Introduction

HMGB protein family members, which are less than 30 kDa in length, include HMGB1, 2, 3, and 4. They can bind to DNA in a structure-dependent manner, regardless of specific sequences.¹ HMGB1, 2, and 3, which have similar biochemical structures and share semblable biochemical properties, comprise two HMG-box domains and an acidic tail.² However, HMGB4 is less conserved at the nucleotide sequence level and is specific to mammals. In addition, HMGB4 contains two HMG-boxes but lacks the acid tail.^{3,4} Since research into HMGB4 is limited, we thus mainly focus on recent advances in knowledge of HMGB1, 2, and 3 in human cancers.

Despite the high level of similarity in the amino acid sequences of HMGB1, 2, and 3, they share diverse expression patterns in human tissues.

HMGB1 and 2 are expressed ubiquitously at high levels during embryonic development,^{5,6} while HMGB3 is expressed mainly during embryogenesis.⁷ HMGB4 can be detected only in the developing pancreas and brain.^{8,9} In adult tissues, HMGB1 is universally regarded as a housekeeping gene for its ubiquitous high expression in whole tissue extract assays.^{9,10} However, it is reported that HMGB1 is expressed more highly in testis and lymphoid tissues, and lower in the liver and brain, suggesting a role in the differentiation of various tissues.¹⁰ HMGB2 is expressed mainly in the thymus and testis.⁶ HMGB3 is expressed mainly in hematopoietic stem cells,¹¹ and HMGB4 localizes in neural cells and testis.¹²

These HMGB family members also share similar functions, especially in the nucleus.¹³ Specifically,

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box A identifies and binds to non-B-type DNA structures, and then box B distorts target DNAs by lending, looping, or winding them.¹ These members all play an important role in the construction of nucleoprotein complexes by changing chromatin structure, which leads to combinations of other factors and the modification of multiple DNA.^{14,15} Still, the difference in expression patterns of HMGB family members indicates their possible different roles in various biological processes. For example, HMGB1 participates widely in both embryonic development and adult tissue formation, while other members play a relatively redundant role.⁴

HMGB family members can participate in different pathological progressions, including inflammation,¹⁶ disorders of immunological system,¹⁷ and carcinogenesis.¹⁸ Accumulating studies have reported their significant roles in various cancers, including hepatocellular cancer (HCC),¹⁹ colorectal cancer (CRC),¹⁸ and lung cancer.²⁰ Evidence has also revealed that HMGB family members are widely involved in cellular processes of tumor cells, including proliferation,²¹ metastasis,²² autophagy,²³ apoptosis,¹⁸ and drug resistance.²⁰ In this review, we provide a comprehensive overview of the recent advances in our knowledge of HMGB1, 2, and 3 in human cancers. Particularly, we focus on the potential value of HMGB family members as markers and therapeutic targets in human cancers. We also envisage the challenges and future orientation of this field.

Characteristics of the HMGB family members

Structure

As described above, HMGB1, 2, and 3 are all composed of three structurally and functionally conserved regions.³ The N-terminal A box is a DNA-binding region, which identifies and binds targeted genes in a structure-dependent but sequence-independent manner.¹ In detail, the A box identifies and binds to the AT-rich DNA fragment.¹¹ The Central B box can modulate targeted DNAs by bending them and altering their structures.²⁴ The C-terminal tail contains abundant glutamic and aspartic acid residues and it is relatively less investigated.²⁵ However, the diversity in sequence and length of the tail may influence or even determine the distinctive functions of these three members, which remains to be investigated.²⁶ Another research study highlighted

the role of the C-terminal acidic tail in the antibacterial activity.²⁷ In a word, these three members are more than 80% identical in amino acid sequence, but their diversity in key sites may lead to different chemical and biological properties.

In terms of primary structure, human HMGB1 has 215 amino acid residues.²⁸ The DNA binding regions (A and B boxes) contain nuclear-emigration signals (NES), while the steady domain is located in the nucleus with the assistance of two nuclear-localization signals (NLS1 and NLS2). Residues 7–74 and 89–108 are reported to be associated with the combination of TLR4 and p53 transactivating domains, while residues 150–183 are responsible for RAGE binding.²⁹ In secondary structure, the two HMGB boxes are composed of three alpha-helices and two loops, which subsequently form an “L” shape whose arms form an angle of 80°.³⁰ The short arm is made up of helix I and helix II, whereas the long arm is constructed by helix III as well as an N-terminal unstructured segment that is in parallel with helix III. The A box has higher alpha helix content and is more positively charged than the B box.³¹ In tertiary structure, HMGB1 has three cysteine residues at positions C23, C45, and C106, respectively, that are sensitive to the oxidative situation. Their oxidation–reduction status partly determines their characteristics in biological development. Depending on their form, they are categorized as fully reduced HMGB1 (all-thiol), semi-oxidized HMGB1 (disulfide), or terminally oxidized HMGB1.³² In addition to the redox state, different posttranslational modifications (PTMs) can also play important roles in the alteration of chemical structure and biochemical properties. These PTMs include methylation, acetylation, and phosphorylation.³² In the quaternary structure, native HMGB1 exists in homodimer and oligomer form, while acid-extracted HMGB1 does not.³³ Indeed, the structure of HMGB1 can be altered when the extracting methods are different.³³ In addition, exogenous HMGB1 can bind to other chemicals in the forms of dimer, trimer, tetramer, or oligomer.³⁴

Regarding the structures of HMGB2 and 3, it is universally accepted that they share 80% identity with HMGB1 at the amino acid level and they are each composed of the three special functional regions. HMGB2 is also revealed to be modified by PTMs including phosphorylation by case kinase 2 and acetylation by CREB-binding protein.^{35,36} Whether HMGB3 could be modified by

PTMs is still little known. Unlike HMGB1, few research studies have reported the effects of oxidation–reduction conditions on the structure alteration of HMGB2 and 3. Little information can be obtained from current research on the secondary, tertiary, and quaternary structure of HMGB2 and 3. Thus, identifying the specific structure of HMGB2 and HMGB3 is of primary importance for further investigations.

Distribution

The distribution of these three members from the macro perspective was described above. We also confirmed their expression levels in different tumors and corresponding normal tissues *via* the GEPIA database (<http://gepia.cancer-pku.cn/>).³⁷ Compared with normal tissues, HMGB1 is highly expressed in CHCL, ESCA, LIHC, and LUSC (Figure S1), while the expression of HMGB2 is higher in BLCA, BRCA, CESC, COAD, ESCA, HNSC, KIRP, LUAD, LUSC, PCPG, READ, STAD, and UCES than in normal tissues (Figure S2). Higher expression of HMGB3 is observed in BLCA, BRCA, CESC, COAD, ESCA, HNSC, KIRP, LUAD, LUSC, READ, STAD, and UCEC compared with normal tissues (Figure S3). The distribution of these three members at the cellular level will be also discussed below.

HMGB1 is active both inside and outside cells.³⁸ In the nucleus, it binds to DNA and serves as an architectural chromatin-binding factor.³⁹ HMGB1 can also translocate to the cytoplasm and act as a cytokine.⁴⁰ The lysine residues located in A box (aa28–44) and B box (aa179–185) interact together and form NLSs. The control of NLSs is the main mechanism for the translocation of HMGB1 from the nucleus to the cytoplasm. The monomethylation of lysine 42 can limit the HMGB1 from binding DNAs and set it free, resulting in its tendency to translocate into the cytoplasm.⁴¹ The acetylation of NLS is also pivotal in the nuclear-to-cytoplasmic translocation of HMGB1.⁴² As is shown by immunofluorescence, HMGB1 is concentrated in the cytoplasm after acetylation, while a mutant form of HMGB1 is still localized in the nucleus.⁴² In addition, phosphorylation of serine also plays a significant role in its shuttling from the nucleus to cytoplasm.⁴³ Secretive HMGB1 can either be secreted actively by specific immune cells or released passively when a cell is dead or injured.^{39,44,45} HMGB1 lacks the leader sequence that plays an essential role in the classical secretion pathway in

which endoplasmic reticulum (ER)–Golgi participates.⁴⁶ In hematopoietic cells, secretory lysosomes are generated to assist the active secretion of HMGB1 previously localized and concentrated in the cytoplasm.²⁸ In non-hematopoietic cells, HMGB1 follows the same rule when it translocates from the nucleus to the cytoplasm. Nevertheless, secretory lysosomes are not observed to be employed, and a concrete mechanism has not been elucidated. It is speculated that HMGB1 may cross the plasma membrane with the assistance of membrane transporters.¹⁷ The passive release of HMGB1 occurs in necrotic cells but not apoptotic cells. In addition, the engulfment of dying cells by immune cells, including macrophages, can induce the active secretion of HMGB1.⁴⁷ This indicates the possible correlation and diversity between dying cells and immune cells in the aspect of HMGB1 release or secretion.²³ On the one hand, dying cells can activate immune cells to secrete HMGB1. On the other hand, HMGB1 passively released from dying cells or actively secreted from immune cells comes in different modified forms and exerts different functions, which will be depicted in detail below.^{23,42}

Similar to HMGB1, HMGB2 also distributes both intracellularly and extracellularly.⁴⁸ However, the concrete mechanisms of its localization still need in-depth investigation. As for HMGB3, its extracellular localization and function have not been reported.

Role of HMGB family members in carcinogenesis

HMGB family members have been investigated broadly in the three decades since they were discovered. They are confirmed to take part in different diseases, including inflammatory conditions,^{4,16} autoimmune disease,¹⁷ and cancers.^{18,20} The roles and mechanisms of HMGB family members in different localizations may be various. HMGB1 can play pivotal roles in the nucleus, in the cytoplasm, on the membrane, and outside the cells.⁴⁹ In the nucleus, HMGB1 takes part in nucleosome stability,⁵⁰ nuclear catastrophe,⁵¹ nucleosome release,⁵¹ DNA bending,⁵² and gene transcription.⁵³ Cytosolic HMGB1 is highly expressed and co-localized with lysosome protein in tissue types including colon, liver, gastric, and brain,^{49,54} indicating its significant roles in regulating autophagy in the cytoplasm. Membrane HMGB1 can facilitate cell maturation and repair, including neurite outgrowth,⁵⁵ activation of platelets,⁵⁶ and cell

differentiation.⁵⁷ Extracellular HMGB1 can bind to receptors such as RAGE and TLRs, which can activate downstream cascades and then induce inflammation,⁵⁸ immune disorder,⁵⁹ and malignant transformation.^{60–62}

Similar to HMGB1, HMGB2 plays different roles according to its localization. For example, nuclear HMGB2 participates mainly in DNA bending and gene transcription.^{4,63} Extracellular HMGB2 can also bind to those receptors, but its affinity to target receptors and inflammation-inducing capability are relatively lower compared with HMGB1.⁶⁴ In addition, HMGB2 is reported to facilitate efficient non-viral gene delivery, which is not widely investigated and not reported to occur in the other family members.⁶³ The roles of HMGB2 in the cytoplasm and on the membrane are poorly elucidated.⁴

HMGB3 is merely confirmed to participate in bending targeted DNAs and assisting gene transcription in the nucleus. Its roles in the cytoplasm, on the membrane, and outside the cell are little known.⁴⁹ Table 1 presents the roles of HMGB family members in carcinogenesis. An in-depth comprehension of the HMGB family may provide important clues for developing novel biomarkers and targets for cancer management.

HMGB1

HMGB1 is the most investigated member of the HMGB family.¹⁸ HMGB1 is involved in the pathogenesis of different tumors, including CRC,¹⁸ HCC,⁹⁰ and lung cancer.²⁰ Several biological and pathological processes toward malignant transformation are proved to be associated with HMGB1, including inflammation,¹⁷ dysregulation of immunological system,¹⁷ autophagy,²³ and migration.⁶⁷

Role of HMGB1 in inflammatory and immunological response. Multicellular animals can discriminate between self and non-self and distinguish between dead and live cells, keeping the body in homeostasis. Maintenance of the integrity status depends partly on the smooth functioning of the immune system.¹⁷ Damage-associated molecular patterns (DAMPs) and pathogen-associated molecular patterns (PAMPs) are the two concepts universally accepted to stimulate the innate and adaptive immune system.^{91,92} Dysregulation of the immune system will undermine homeostasis and result in the formation of illegal tissues, including tumors.

HMGB1 is one of the best characterized DAMPs.¹⁷ DAMPs are molecules that are released passively by dead or dying cells undergoing unscheduled death during cellular stress. They can be recognized by pattern recognition receptors (PPRs), such as RAGE and TLRs.⁹³ The interaction between DAMPs and PPRs leads to the activation of downstream signaling pathways and the dysregulation of inflammatory and immunological responses.⁹³ The negatively charged V-domain of RAGE and the positively charged C-terminal of HMGB1 form this ligand–receptor complex.^{94,95} Besides, RAGE was confirmed to form a complex with heparan sulfate, a cell-surface proteoglycan, before binding to HMGB1.⁹⁶ The HMGB1–RAGE, as well as the downstream cascade, is associated closely with cell migratory responses.⁹⁷ The physicochemical mechanism of the interaction between HMGB1 and TLRs (including TLR2, TLR4, and TLR9) is little known,^{98,99} although it is accepted that HMGB1 binding to TLR4 relies on its disulfide form.¹⁰⁰ The disulfide form of HMGB1 can induce release of the cytokine after interacting with TLR4.¹⁰⁰ Fully reduced HMGB1 can also function as a chemoattractant without binding to TLR4, but the concrete receptor remains for identification.¹⁰¹ In addition, HMGB1 can form complexes with abundant TLR ligands, including extracellular DNA material class A cytosine-guanine-rich DNA and the lipopolysaccharide, and these complexes can strengthen the inflammatory responses rather than HMGB1 alone.¹⁰² One of the possible reasons is the co-activation and co-operation of both TLR and RAGE.

HMGB1 is also a kind of soluble proteins that acts as an alarmin and is actively secreted by immune cells such as macrophages.¹⁶ HMGB1's active secretion or negative release is revealed in Figure 1. Active secretion occurs when cells suffer from severe cell stress, not limited to cell damage, such as ischemia or pathogen infection.^{42,103} Under these circumstances, macrophages detect the extracellular PAMPs or DAMPs *via* TLR4, after which NF- κ B is activated and interferon regulatory factors (IRFs) are phosphorylated. They function as transcription factors to promote the transcription of interferon-beta (IFN- β) and assist its eventual secretion. Secreted IFN β then binds to interferon receptors (IFNARs) located on the membranes of adjacent cells, resulting in the stimulation of associated Janus kinases (JAK) and phosphorylation of signal transducer and activator of transcription 1 (STAT1). Phospho-STAT1

Table 1. Role of HMGB family members in carcinogenesis.

Hallmarks	HMGB family	Key findings	References
Angiogenesis	HMGB 1	HMGB1 can promote the expression of neuropilin-1, VEGFA, VEGF receptors -1 and -2 to induce angiogenesis.	van Beijnum <i>et al.</i> ⁶⁵
		HMGB1 can promote the expression of PDGF to induce angiogenesis.	van Beijnum <i>et al.</i> ⁶⁵
		HMGB1 binds to RAGE to activate NF- κ B and then induces angiogenesis.	van Beijnum <i>et al.</i> ⁶⁶
Metastasis	HMGB 1	HMGB1 binds to RAGE and mediates EMT <i>via</i> MMP-7, phosphor-NF- κ B and Snail.	Zhu <i>et al.</i> ⁶⁷
		HMGB1 binds to RAGE and mediates EMT <i>via</i> the production of NF- κ B, p65, iNOS, MMP-9 and phosphorylation of Rac-1, ERK1/2 and AKT.	Kuniyasu <i>et al.</i> ⁶⁸
		HMGB1 binds to TLR4 and upregulates downstream MAPK and PI3K signaling pathways.	Sharma <i>et al.</i> ⁶⁹
		Secreted HMGB1 targets other stromal cells, whose released factors can cause ECM proteolytic degradation.	Gialeli <i>et al.</i> ⁷⁰
		miR-218 can inhibit the expression of HMGB1 and metastasis.	Zhang <i>et al.</i> ⁷¹
		miR-325-3p can suppress the expression of HMGB1 and metastasis.	Yao <i>et al.</i> ⁷²
	miR-142-3p can inhibit the expression of HMGB1 and metastasis.	Xiao and Liu ⁷³	
	HMGB 2	HMGB2 correlates with OCT4 and retains the pluripotent gene expression signature. LncCRCMSL plays as a guide and directs the cytoplasmic maintenance of HMGB2.	Han <i>et al.</i> ⁷⁴
	vIRF1 promotes cell migration by p53- and lnc-OIP5-AS1-mediated downregulation of miR-218-5p, leading to increased expression levels of HMGB2.	Li <i>et al.</i> ⁷⁵	
miR-329 downregulates HMGB2 <i>via</i> the β -catenin pathway, leading to invasion and metastasis in melanoma.	Mo <i>et al.</i> ⁷⁶		
HMGB 3	miR-758 regulates HMGB3, leading to the inhibition of migration, and promotes apoptosis of NSCLC cells.	Zhou <i>et al.</i> ²²	
	miR-513b inhibits the invasion and promotes the apoptosis by negatively targeting HMGB3 and mTOR signaling in NSCLC cells.	Wang <i>et al.</i> ⁷⁷	
	Overexpression of miR-200b targets HMGB3 protein and inhibits HCC cell migration.	Wang <i>et al.</i> ¹⁹	
	Overexpression of miR-205-5p leads to downregulation of HMGB3 and lower abilities in proliferation and metastasis.	Yamada <i>et al.</i> ⁷⁸	
	miR-532-5p directly targets HMGB3 and downregulates Wnt/ β -catenin signaling, inhibiting the proliferation and invasion of bladder cancer cells.	Xie <i>et al.</i> ⁷⁹	
In CRC, HMGB3 promotes growth and migration by regulating Wnt/ β -catenin pathway <i>via</i> c-Myc and MMP-7.	Zhang <i>et al.</i> ⁸⁰		
Proliferation	HMGB 2	HMGB2 upregulates the AR-YY1 mediated transcription and interacts with HOX10, contributing to the proliferation in prostate cancers.	Barreiro-Alonso <i>et al.</i> ⁸¹

(Continued)

Table 1. (Continued)

Hallmarks	HMGB family	Key findings	References
		HMGB2 transcriptionally regulates LDHB and FBP1 and then regulates the Warburg effect, promoting the proliferation and glycolysis of breast cancer.	Fu <i>et al.</i> ²¹
		HMGB2 upregulates p53 or potentiates Wnt/ β -catenin signaling. This can be suppressed by anti-human EGFR2 antibody <i>via</i> the AKT pathway.	Kwon <i>et al.</i> ⁸²
		HMGB2 activates AKT signaling pathway, resulting in the proliferation of cervical carcinoma.	Zhang <i>et al.</i> ⁸³
Senescence	HMGB 2	HMGB2-TOP1cc-cGAS axis regulates SASP and assists cytoplasmic chromatin recognition, enabling response to immune checkpoint blockade.	Zhao <i>et al.</i> ⁸⁴
		Biogenesis can stimulate HMGB1's dsDNA sensing pathway, which can be repressed by p53.	Bianco and Mohr ⁸⁵
		HMGB2 binds to the SASP gene promoter area, preventing HP1 α protein recruitment.	Völp <i>et al.</i> ⁸⁶
Drug resistance	HMGB 2	HMGB2 protein migrates from the cytosol to the nucleus, where it can bind to cis-Pt-DNA adducts in genomic DNA and activates repair system under cisplatin treatment.	van Beijnum <i>et al.</i> ⁸⁷
		A complex including HMGB2 and the coactivator SRC-1 binds to the promoter of DDX18, leading to the chemical resistance.	van Beijnum <i>et al.</i> ⁶⁵
		miR-23b-3p inhibits autophagy <i>via</i> regulating ATG12 and HMGB2 and sensitizes GC cells to chemotherapeutic treatment.	van Beijnum <i>et al.</i> ⁶⁶
		HMGB2 might be involved in the regulation of p53 and MMP-2/TIMP2 and results in the resistance to TMZ chemotherapy.	Lin <i>et al.</i> ⁸⁸
	HMGB 3	HMGB3 deletion attenuates ATR/CHK1/p-CHK1 DNA damage signaling pathway and increases apoptosis and sensitivity to cisplatin.	Xiao and Liu ⁷³
		EGb 761 can sensitize 5FUR CRC cells by inhibiting the EMT phenotype and suppressing HMGB3 expression <i>via</i> the Wnt/ β -catenin pathway.	Gialeli <i>et al.</i> ⁷⁰
Hypoxia	HMGB 3	HOTTIP deficiency represses glycolysis under hypoxic conditions partly by targeting miR-615-3p/HMGB3 axis in NSCLC cells.	Abraham <i>et al.</i> ⁸⁹
		Silence of HMGB3 attenuates HIF1 α and inhibits cell proliferation. HMGB3 silence also suppresses the expression of Nanog, SOX2 and OCT-4 genes/proteins.	Barreiro-Alonso <i>et al.</i> ⁸¹

EMT, epithelial-mesenchymal transition; GC, gastric cancer; HMGB, high mobility group box; NSCLC, non small cell lung carcinoma; PDGF, platelet-derived growth factor; PI3K, phosphatidylinositol 3-kinase; TMZ, temozolomide.

dimers translocate into the nucleus and recruit histone acetylases, leading to acetylation of HMGB1.^{17,42} In this cascade, acetylation prevents HMGB1 from re-entering the nucleus and assists its secretion. This happens in a lysosome-dependent manner in hematopoietic cells or in a lysosome-independent way in non-hematopoietic cells,⁴⁶ whereas the passively released HMGB1

functioning as DAMP leaks out from dead cells without acetylation or other modification.¹⁷

Figure 2 presents the role of HMGB1 in inflammation. Intracellular HMGB1 tends to exist in the reduced state due to the strongly negative reducing redox potential in the cytosol and the nucleus.^{104,105} The released fully reduced HMGB1 interacts with

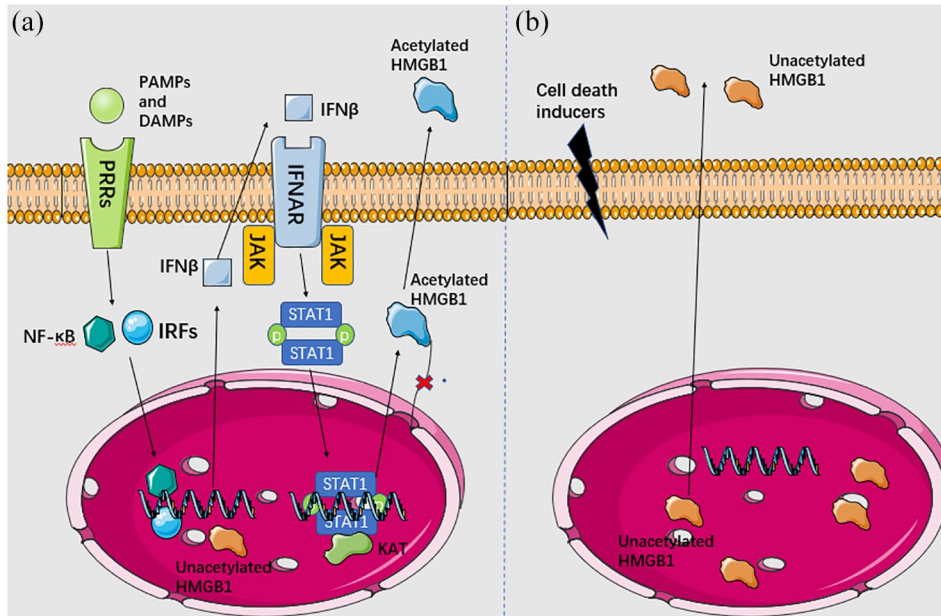


Figure 1. HMGB1 is secreted actively (a) and passively (b) via different cascades. In active secretion, HMGB1 is acetylated or phosphorylated in the nucleus and then translocated into the cytoplasm, where it can then be secreted *via* lysosomes in hematopoietic cells or *via* not confirmed mechanisms in non-hematopoietic cells. In passive release, HMGB1 is released by dead cells confronting with tissue injury. Intracellular HMGB1 exists in the reduced form, while extracellular HMGB1 gradually transforms into fully reduced form. Thin black arrows indicate secretion or activation of the downstream targets. DAMPs, damage-associated molecular patterns; HMGB1, high mobility group box; IFNAR, interferon receptor; IFN β , interferon β ; IRFs, interferon regulatory factors; JAK, Janus kinase; PAMPs, pathogen-associated molecular patterns; PRRs, pattern recognition receptors.

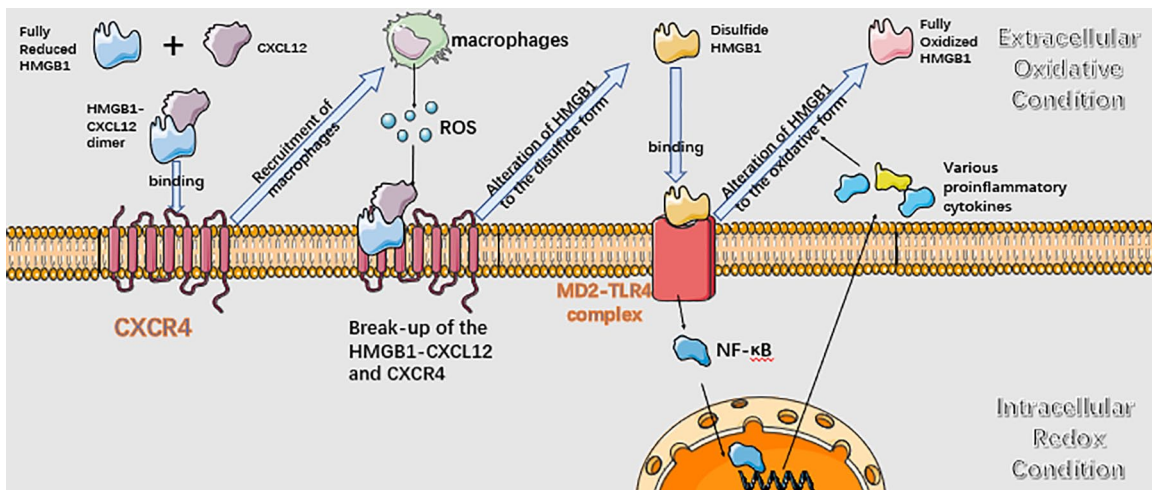


Figure 2. HMGB1 participates in inflammatory processes. HMGB1 exists in redox form after initially being passively released or actively secreted from cells. The redox form of HMGB1 can form a heterocomplex with CXCL12 and this complex binds to CXCR4, and macrophages are recruited after that. Macrophages secrete ROS and transform HMGB1 to disulfide form. The disulfide form of HMGB1 cannot interact with CXCL12 and CXCR. The disulfide HMGB1 binds to MD2-TLR4 complex and activates the NF- κ B signaling pathway, proinflammatory cytokines are transcriptionally activated and secreted extracellularly subsequently. The disulfide HMGB1 is eventually oxidized to fully oxidized form and loses inflammation inducing capability. Black arrows indicate secretion or activation of the downstream targets, while blue arrows represent the alterations of the next inflammatory stage. HMGB1, high mobility group box; ROS, reactive oxygen species.

C-X-C motif chemokine ligand 12 (CXCL12) and forms a heterocomplex, which then binds C-X-C motif chemokine receptor 4 (CXCR4), one 7-transmembrane G-protein-coupled receptor (GPCR).¹⁰⁶ CXCR4 can form dimers, multimers, or heterodimers with other GPCRs. The transformation is induced by different ligands, including the CXCL12, CXCL4, HMGB1-CXCL12 heterocomplex.^{107,108} HMGB1-CXCL12 can promote the recruitment of leukocytes after interacting with CXCR4.¹⁷ The reactive oxygen species (ROS) originating from infiltrating leukocytes and the oxidizing condition in the extracellular milieu assists the transformation of reduced HMGB1 to the disulfide form. The disulfide HMGB1 loses the ability to interact with CXCL12 and cannot stimulate CXCR4.¹⁰⁵ Instead, it interacts with the lymphocyte antigen 96 (MD2)-TLR4 complex and stimulates activation of NF- κ B, inducing the secretion of multiple proinflammatory cytokines and chemokines.¹⁰⁰ The oxidizing environment eventually induces the terminal oxidation of HMGB1 to the sulfonyl form, which no longer promotes chemoattractant and proinflammatory activities. More HMGB1 are secreted by tissue-healing phenotype macrophages after inflammation resolution, which contributes to the repair of damaged tissues by stimulating stem cells and promoting angiogenesis.¹⁰⁰

Inflammation is a hallmark of carcinogenesis. It can induce alteration of the microenvironment, which influences the formation of carcinoma. HMGB1 – a protein central to inflammation and injury – is also involved in tumor biology.¹⁰⁹ Mesothelioma is the most representative exemplary in which HMGB1-induced inflammation participates.¹¹⁰ Asbestos and other mineral fibers cause mesothelioma. The constant secretion of HMGB1 leads to recruitment of inflammatory cells, resulting in the carcinogenesis of mesothelial cells.¹¹¹ The prolonged bio-persistence of the mineral fibers leads to the maintenance of HMGB1-induced inflammation condition.¹¹² Although the concrete mechanism is still obscure, a possibility is that HMGB1-induced recruitment of tissue-healing phenotype macrophages assists the survival of cells with malignant transformation, which simply die out under normal conditions.¹¹³ The growth of colon cancer secondary lesions in the peritoneum, melanoma, and papilloma also result partly from the constant secreted HMGB1-induced inflammation, similar to the process in mesothelioma.^{114,115}

In this process, necrotic cell death in a tumor will induce the release of HMGB1 and the recruitment of macrophages and neutrophils.¹¹⁶ HMGB1 binds to TLR4 and leads to malignant transformation by generating chronic inflammation. Macrophages and neutrophils assist the survival of these cells, which will be recognized and eliminated in normal tissues.¹¹⁷ From this aspect, HMGB1 is a typical oncogene; however, it can also exert anti-tumor function *via* the immune system.¹¹⁸ Immunogenic cell death (ICD) is a form of apoptotic death and it occurs when cancer cells are exposed to radiotherapy or chemotherapeutics.¹¹⁹ What triggers ICD is still obscure, but for sure ICD is characterized by the secretion of HMGB1, calreticulin, and ATP.¹²⁰ Secreted HMGB1 from cells undergoing ICD stimulates the maturation of dendritic cells and the cross-presentation of neoantigens to lymphocytes. B- and T-cell responses are stimulated. The anti-tumor immunological memory is established after that.¹²⁰ ROS triggered by ICD and TLR4 imply the significant role of disulfide HMGB1 in this anti-tumor process.¹²¹

HMGB1 plays a dual role in the inflammatory and immunological response, which can eventually result in tumor formation. The type of release may be one possible reason for its completely contradicting function.¹²² Chronic release can induce the recruitment of macrophages and alter the microenvironment to be suitable for the malignant transformation, while acute massive release can stimulate dendritic cell (DC) activation and induce ICD *via* HMGB1-TLR4 interaction, leading to the suppression of carcinogenesis.¹²² Based on this finding, the method to control its release type and direct its affinity to DC cells can be applied as a therapeutic method.

Role of HMGB1 in autophagy. Autophagy plays a pivotal role in promoting cell survival *via* aggregating proteins and eliminating damaged organelles by degeneration of the lysosome.⁹ Recent evidence has shed light on the role of autophagy in assisting the survival of cancer cells by mitigating genome damage and limiting inflammation and necrosis.^{18,123} HMGB1, widely distributed inside and outside the cell, can induce autophagy through various pathways.¹²⁴ For example, HMGB1 can function as the transcription factor in the nucleus and enhance the expression of heat-shock protein 27 (HSP 27).¹²⁵ HSP 27 is phosphorylated by MAPK activated protein

kinase 2 (MAPKAPK2) and then is enabled to modulate mitophagy, a cargo-specific kind autophagy of mitochondria,¹²⁶ through the Pink/Parkin pathway.¹²⁷ In the cytoplasm, Beclin-1 is validated to participate in the initiation of autophagy.¹²⁸ The formation of the Beclin-1/phosphatidylinositol 3-kinase-III (PI3K-III) complex by Beclin-1 and class III phosphatidylinositol-3 kinase can recruit Atg proteins to the phagophore.¹²⁹ The Beclin-1/PI3K-III complex is reduced after binding between Beclin-1 and Bcl-2.¹³⁰ HMGB1 can promote the phosphorylation of Bcl-2 *via* the extracellular signal-regulated kinase (ERK)/MAPK pathway, resulting in the dissociation of the Beclin-1/Bcl-2 complex.⁴⁰ Conclusively, HMGB1 promotes autophagy by maintaining the formation of Beclin-1/PI3K-III complex in the cytoplasm. Extracellular HMGB1 binds to RAGE on adjacent cells and induces autophagy activity.¹³¹ It is reported Beclin-1-dependent autophagy is stimulated by the binding of HMGB1 and RAGE. The phosphorylation of ERK1/2 and activation of AMPK/mTOR signaling pathway are confirmed to be involved in the HMGB1/RAGE axis induced autophagy.^{132,133}

Role of HMGB1 in apoptosis. Apoptosis – a programmed form of cell death – is always abrogated during carcinogenesis. Two pathways are clarified underlying apoptosis: the extrinsic pathway and the intrinsic pathway.¹⁸ Through the extrinsic pathway, death receptors are activated by external signals such as TNF- α . The adaptor proteins including Fas-associated death domain proteins are recruited by death receptors after that. Downstream activated caspase-8 promotes the expression of caspase-3/-7, therefore provoking the apoptotic cascade.¹³⁴ While in the intrinsic pathway, the release of different proapoptotic proteins, including Bcl-2 associated agonist of cell death (BAD), Bcl-2 interacting killer (BIK), NOXA, and Bcl-2 modifying factor is triggered by cellular stress. These proteins can stimulate the assembly of the Bcl-2 antagonist/killer 1 (BAK)-Bcl-2 associated X (BAX) oligomers localized on the outer mitochondrial membrane.¹³⁵ BAK-BAX oligomers will promote the efflux cytochrome *c* to the cytosol, which activates the recruitment of caspase-9 and then the expression of executioner caspase-3/-7 cascades.¹³⁵

It is reported that HMGB1 may play a significant role in apoptosis by suppressing pro-apoptotic proteins such as caspase-3 and BAX or promoting anti-proapoptotic proteins including Bcl-2.¹³⁶

Bcl-2 is responsible for the suppression of the efflux of cytochrome and the pro-apoptotic proteins. Its activity can also be inhibited by ABT-737, which is a negative target of HMGB1.¹³⁷ Other studies show that HMGB1 can increase the activity of NF- κ B and subsequently promote the expression of c-IAP2, another anti-apoptotic protein. In addition, HMGB1 may also suppress the expression of apoptosome caspase-9 and interfere with the apoptotic machinery.⁸⁶

In fact, the roles of HMGB1 in the autophagy and anti-apoptosis are closely related. Figure 3 presents the roles of HMGB1 in autophagy and apoptosis. Disrupting the association between HMGB1 and Bcl-2 may be an effective manner to regulate autophagy and apoptosis, thus repressing the survival of cancer cells. In addition, the redox status of HMGB1 functions as a checkpoint between apoptosis and autophagy. Oxidized HMGB1 results in the apoptosis of cancer cells while reduced HMGB1 is responsible for the activation of autophagy.¹³⁸ This also indicates another applicable therapeutic method to modify HMGB1 *via* the oxidation-reduction environment.

Role of HMGB1 in angiogenesis. Angiogenesis is another hallmark of cancer. Additional vasculature formation is required for the growth and development of tumors.¹³⁹ Downregulation of HMGB1 by antibody results in the repression of endothelial cell migration and sprouting.¹⁴⁰ Rapid tumor growth leads to low microvessel density and chronic hypoxia, which results in necrotic regions. These necrotic areas tend to overexpress angiogenic growth factors and attract macrophages recruitment and HMGB1 release.⁸⁷ HMGB1 was found to promote the expression of neuropilin-1, endothelial growth factor A (VEGFA), and VEGF receptors 1 and 2 to induce angiogenesis.⁶⁵ In addition, platelet-derived growth factor is another angiogenesis inducing factor regulated by HMGB1.⁶⁵ Interaction between HMGB1 and RAGE leads to activation of NF- κ B, thereby upregulating leukocyte adhesion molecules and the production of angiogenic factors.⁶⁶ TLR4 is also reported to be activated in HMGB1-mediated neovascularization; however, elucidating the exact mechanism requires further investigation.⁸⁸

Role of HMGB1 in cancer metastasis. Tumor invasion and metastasis are the most significant hallmarks of malignant tumors. HMGB1, which is involved in various cascades, can also regulate

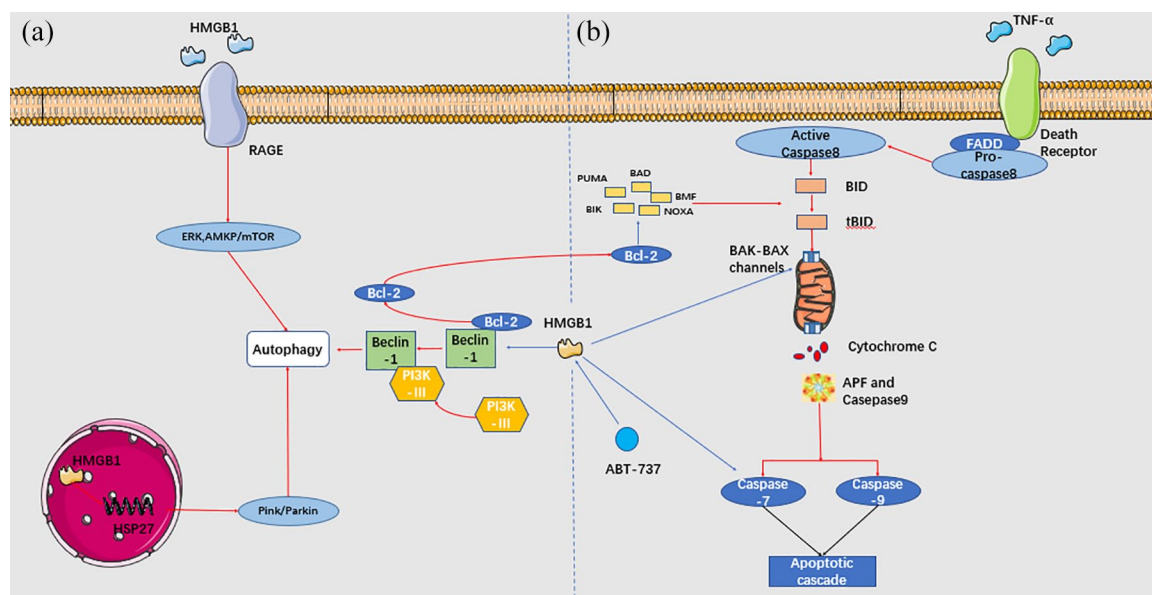


Figure 3. HMGB1 and BCL-2 are involved in autophagy and apoptosis. a. In the nucleus, HMGB1 transcriptionally activates HSP27 and results in autophagy by the Pink/Parkin pathway. In the cytoplasm, HMGB1 leads to the dissociation of Beclin-1-BCL-2 complex. Beclin-1/PI3K-III is formed, which then contributes to the initiation of autophagy. Extracellularly, HMGB1 binds to RAGE and activates downstream ERK and AMPK/mTOR pathway, which induces autophagy. b. HMGB1 exert anti-apoptosis role via the intrinsic and extrinsic pathways. HMGB1 can directly targets BCL-2, BAX-BAK channels, caspase-9, and caspase-3. Besides, HMGB1 indirectly targets cytochrome c and pro-apoptotic proteins via BCL-2. Red arrows represent activation of the downstream targets, while blue arrows represent suppression of the downstream targets. HMGB, high mobility group box.

metastasis.¹⁸ Epithelial-mesenchymal transition (EMT) is a cellular process in which epithelial cells lose cell-cell contacts and apical-basal polarity and acquire the mesenchymal phenotype. These alterations result in reduced interactions among cells and increased invasive capacities.¹⁴¹ As is reported, HMGB1 can induce EMT in a RAGE-dependent manner. HMGB1 interacting with RAGE can upregulate abundant proteins that exert functions as EMT-inducing molecules, including matrix metalloproteinase 7 (MMP-7), phosphor-NF- κ B, and Snail.⁶⁷ The production of NF- κ B/p65, inducible nitric oxide synthase, matrix metalloproteinase 9 (MMP-9), and the phosphorylation of Rac family small GTPase 1 (Rac1), ERK 1/2, and AKT were also reported to be associated with HMGB1/RAGE axis and EMT.⁶⁸ HMGB1 can also interact with the other main receptor TLR4 and enhance the expression of downstream signaling pathways, such as MAPK and PI3K pathways.⁶⁹ In addition, certain microRNAs, including, but not limited to, miR-218,⁷¹ miR-325-3p,⁷² and miR-142-3p,⁷³ can suppress the expression of HMGB1 and its metastasis-inducing ability by directly targeting the 3'

untranslated region of HMGB1 mRNA. Secreted HMGB1 can also target other stromal cells such as myofibroblasts within the microenvironment. Activated myofibroblasts subsequently release various matrix metalloproteinases and growth factors. These factors may cause extracellular matrix (ECM) proteolytic degradation and alter the interaction of cell-cell and cell-ECM, which can, in turn, promote cancer cell migration.⁷⁰

As discussed, HMGB1 is involved in the initiation, development, and progression of tumors. This is consistent with our expectation that it exerts extensive function in the formation of cancer. Paying attention to the expression of HMGB1 and regulating it is advisable in any period during tumorigenesis. Focusing on the alteration of HMGB1 before the cancer is irreversible or untreatable is especially meaningful.

HMGB2

The roles of HMGB2 in carcinogenesis have been gradually elucidated in recent years. HMGB2 plays a role in the chromosomal distorting process

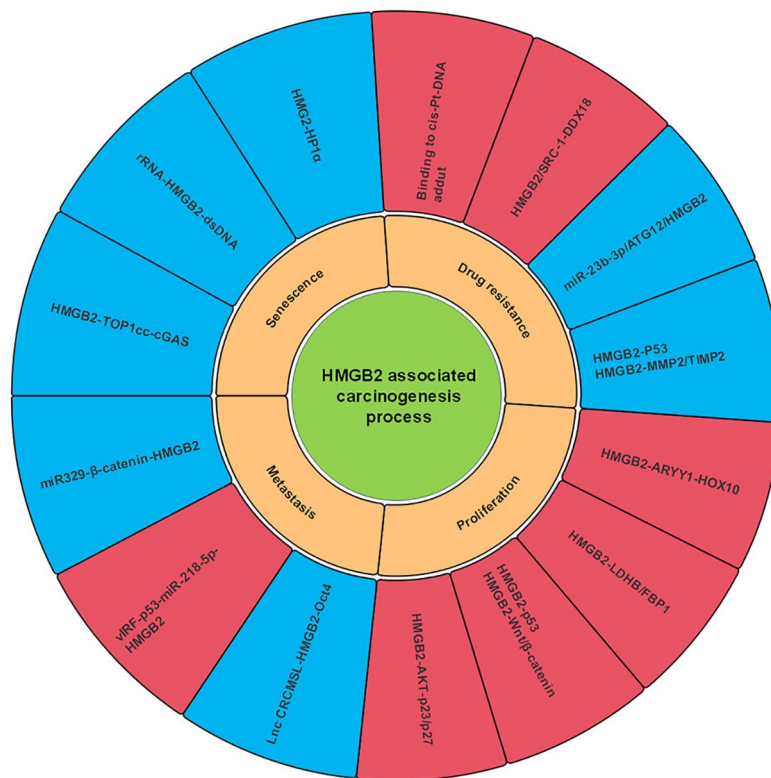


Figure 4. Role of HMGB2 in carcinogenesis. This figure reveals that HMGB2 participates in the processes of carcinogenesis via several signaling pathways. The yellow frameworks represent several cellular processes in carcinogenesis. The blue frameworks represent the negative effects of HMGB2 on the corresponding processes, while the red frameworks represent its positive effects. HMGB, high mobility group box.

in a structure-dependent manner intracellularly like HMGB1.⁸⁹ Its extracellular effects, either triggering the immunological system or recruiting inflammatory factors are more limited than HMGB1, indicating its lower ability to promote the initiation of carcinogenesis.⁴⁸ Its roles in carcinogenesis are presented in Figure 4. In addition to proliferation and metastasis, HMGB2 also participates in the senescence and chemotherapy resistance. This suggests that HMGB2 may be mainly involved in the development and progression of tumors.

Role of HMGB2 in tumor proliferation and metastasis. HMGB2 is reported as an oncogene to induce malignant transformation in various tumors. It can promote the proliferation of different cancer cells, including prostate cancer,⁸¹ breast cancer,²¹ HCC,⁸² and cervical cancer.⁸³ HMGB2 upregulates the androgen receptor (AR)-YY1-mediated transcription and interacts with HOX10 in prostate cancer and subsequently

induces proliferation.⁸¹ In breast cancer, HMGB2 transcriptionally regulates glycolytic enzymes lactate dehydrogenase and fructose-bisphosphatase 1 (FBP1) and then regulates the Warburg effect, which provides essential energy for cancer cell's proliferation.²¹ Furthermore, HMGB2 can promote p53 or potentiate Wnt/ β -catenin pathway, which can be attenuated by anti-human epidermal growth factor receptor 2 antibody *via* the AKT pathway in HCC.⁸² HMGB2 can also activate the AKT pathway by phosphorylating AKT and suppress the expression of p21 and p27 in cervical cancer.⁸³ This suggests that AKT and Wnt pathways are closely associated with HMGB2-mediated proliferation of cancer.

HMGB2 also plays a significant role in tumor invasion and migration. Noncoding RNAs including long noncoding RNA (LncRNA) and micro RNA (miRNA) are closely associated with HMGB2-mediated metastasis. For example, in CRC cells, LncCRCMSL mediates the

cytoplasmic maintenance of HMGB2 and inhibits downstream EMT signaling.⁷⁴ To put it another way, HMGB2-mediated EMT could lead to metastasis.⁷⁴ In human immunodeficiency virus (HIV)-induced Kaposi's sarcoma (KS), HMGB2 is upregulated by vIRF1/p53 and vIRF1/Lnc-OI5P-AS1 mediated miR-218-5p suppression.⁷⁵ miR-329 suppresses the expression of HMGB2 *via* the β -catenin pathway in melanoma.⁷⁶ miRNAs-mediated HMGB2 suppression is involved in these processes, which can eventually suppress metastasis. In addition to noncoding RNAs, CENPU is another co-expression gene of HMGB2, and together they contribute to the invasion and migration of ovarian cancer cells.¹⁴²

Role of HMGB2 in senescence. Senescence is a pivotal carcinoma repression process, in which the propagation of cells suffering from insults is limited.¹⁴³ The termed senescence-associated secretory phenotype (SASP), including different secreted chemokines, cytokines, proteases, and growth factors, is a hallmark of senescence.¹⁴³ Cyclic GMP-AMP synthase (cGAS) senses cytosolic double-strand DNA (dsDNA) in a sequence-independent manner.¹⁴⁴ The cGAS-dsDNA sensing pathway is critical in the regulation of SASP and senescence.¹⁴⁵ Cytoplasmic chromatin fragments (CCF) resulted from nuclear membrane blebbing is the main factor to induce cGAS activation.¹⁴⁵ DNA topoisomerase 1 (TOP1) forms a stable cleavage complex (TOP1cc) *via* its enzymatic effect.⁸⁴ TOP1cc plays a significant role in regulating the recognition of CCF by cGAS. HMGB2 mechanistically stabilizes TOP1cc and subsequently strengthens the binding between cGAS and dsDNA.⁸⁴ A HMGB2-dsDNA sensing pathway is also reported to be stimulated by rRNA biogenesis.⁸⁵ The inhibition of rRNA biogenesis can stabilize p53 and suppress HMGB2 expression in a p53-dependent manner.⁸⁵ HMGB2 is also reported to bind to the SASP gene promoter region directly and prevent heterochromatin protein 1 α (HP1 α) protein recruitment, subsequently regulating senescence. This effect can be inhibited by ATM-p53-p21 axis.¹⁴⁶

Role of HMGB2 in drug resistance. Chemotherapy is always the first-line therapy for various advanced tumors.¹⁴⁷ However, several anti-chemotherapy mechanisms, such as autophagy, anti-apoptosis, and immune defense, can compromise the effects of chemotherapy treatment.¹⁴⁸ HMGB2 is

critically involved in chemotherapy resistance. Under cisplatin treatment, HMGB2 can translocate from the cytoplasm into the nucleus, where it binds to cis-Pt-DNA adducts and initiates the repair system, leading to chemoresistance to cisplatin.¹⁴⁹ Low HMGB2 is reported as a significant predictor for long-term disease-free survival in breast cancer.¹⁵⁰ In the presence of tamoxifen, HMGB2 combines with its coactivator steroid receptor coactivator-1 (SRC-1), and they then bind to the promoter of DEAD-box helicase 18 (DDX18), leading to resistance to tamoxifen.¹⁵⁰ Previously, our laboratory found that miR-23b-3p can inhibit autophagy regulated by HMGB2 and sensitize gastric cancer (GC) cells to chemotherapy.¹⁵¹ Furthermore, HMGB2 can also sensitize glioblastoma cells to temozolomide (TMZ) therapy, partly by downregulation of p53 and matrix metalloproteinase 2 (MMP2). However, HMGB2 cannot induce the expression of O-6-methylguanine-DNA methyltransferase (MGMT) protein. Since MGMT is the major factor in the activation in TMZ therapy, this process suggests that HMGB2 may be another independent factor predicting the therapeutic effect of TMZ.⁶⁵

HMGB3

Structurally, HMGB3 shares more than 80% identical amino acids with HMGB1 and HMGB2.¹⁵² However, its distribution is limited in embryonic development and adult tissues.¹⁵² Like HMGB1 and HMGB2, HMGB3 is also involved in the development and progression of several tumors. Figure 5 presents the roles of HMGB3 in carcinogenesis.

Role of HMGB3 in cancer metastasis. HMGB3 can also regulate cancer metastasis, and this process is often modulated by miRNAs. For example, miR-758 and miR-513b can inhibit non-small lung cancer metastasis by targeting HMGB3.^{22,77} HMGB3 induced metastasis is suppressed by miR-200b and miR-205-5p in HCC and prostate cancer, respectively^{19,78}; while in bladder cancer, miR-532-5p and Wnt/ β -catenin signaling pathway can modulate the expression of HMGB3.⁷⁹ Besides, the Wnt/ β -catenin signaling pathway can also suppress HMGB3 *via* c-Myc and MMP7 in a miRNA-independent manner in CRC metastasis.⁸⁰

Role of HMGB3 in drug resistance. HMGB3 is also involved in chemotherapy resistance.

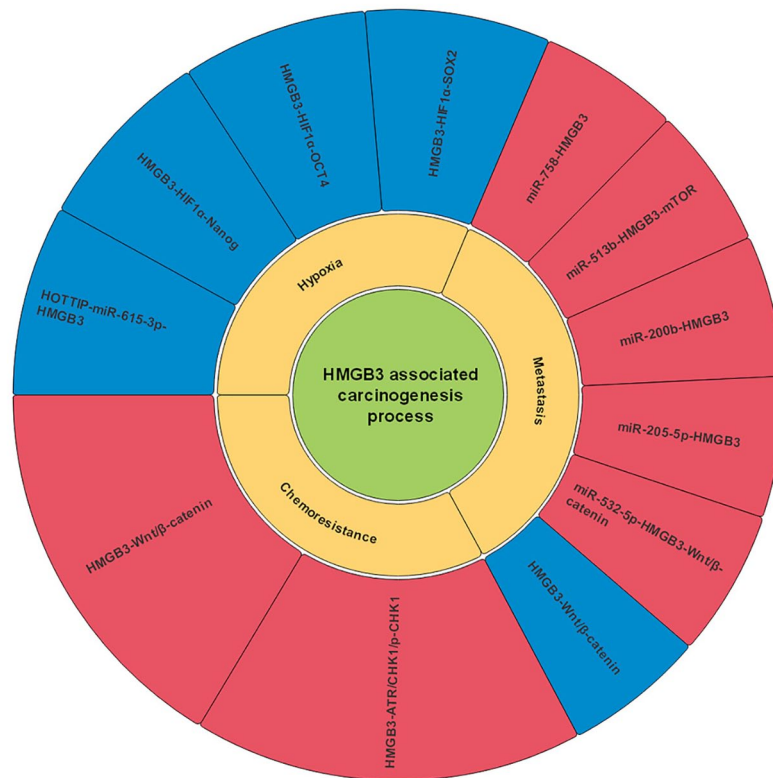


Figure 5. Role of HMGB3 in carcinogenesis. This figure shows that HMGB3 participates in the processes of carcinogenesis through several signaling pathways. The blue frameworks represent the negative effects of HMGB3 on the corresponding processes, while the red frameworks represent its positive effects. HMGB, high mobility group box.

HMGB3 can transcriptionally upregulate serine/threonine kinases ATR and CHK1 and decrease cell apoptosis, leading to cisplatin resistance *via* ATR/CHK1/p-CHK1 DNA damage signaling pathway.¹⁵³ In addition, EGb 761 can suppress HMGB3 expression *via* the Wnt/ β -catenin pathway and improve sensitivity of CRC cells to 5-fluorouracil.¹⁵⁴

Role of HMGB3 in hypoxia. Hypoxia, a pivotal character of solid tumors, drives several malignant processes by mediating the metabolic reprogramming out of oxidative stress.¹⁵⁵ Under hypoxic conditions, HMGB3 is upregulated by HOXA transcript at the distal tip (HOTTIP), and glycolysis is subsequently promoted.¹⁵⁵ It was reported that hypoxia-inducible factor 1 α (HIF1 α) could mediate the upregulation of Nanog, SRY-box transcription factor 2 (Sox2), and organic cation/carnitine transporter 4 (OCT4).¹⁵⁶ Later research revealed that this mediation could be strengthened by HMGB3.¹⁵⁷

Clinical significance of HMGB family members in human cancers

HMGB family members are widely involved in carcinogenesis, increasing evidence also indicate that HMGB family members are promising targets for cancer treatment. Moreover, the diagnostic and prognostic roles of HMGB family members are gradually revealed.

Diagnostic and prognostic role of HMGB family members in human cancers

HMGB family members are investigated in numerous studies. The results indicate that its dysregulation is related to the development of several tumors, including CRC,⁷⁴ HCC,^{158,159} gastric cancer,¹⁶⁰ breast cancer,¹⁶¹ and nasopharyngeal carcinoma.¹⁶² In addition, the expression levels of HMGB family members are associated with the prognosis of human cancers. We analyzed the survival rate of patients with high and low expression of HMGB family members in several tumors

using the Kaplan–Meier-plotter website (<http://kmpplot.com/analysis/>).¹⁶³ The results show that overexpression of HMGB1 is associated with poor prognosis in patients with ECA, HNSC, LUAD, PAAD, and SARC, while its overexpression is related to good prognosis in BLCA, SARC, THCA, and THYM (Figure S4). HMGB2 overexpression is significantly correlated with shorter overall survival (OS) in ECA, KIRC, KIRP, LIHC, LUAD, OV, PADD, and THYM, but its overexpression is an indicator for longer OS in BRCA, CESC, HNSC, LUSC, OV, READ, STAD, TGCT, and THCA (Figure S5). Moreover, patients with higher HMGB3 have shorter OS in BRCA, ECA, KIRC, LIHC, PCPG, SARC, THCA, and UCEC, while patients with higher HMGB3 show a longer OS in CESC, KIRP, LUAD, LUSC, OV, TGCT, and THCA (Figure S6).

HMGB1 plays a significant role in carcinogenesis. Evidence in case–control studies showed that the expression of HMGB1 is significantly higher in the patients with HCC,^{21,82,159} breast cancer,^{21,161} nasopharyngeal carcinoma,¹⁶² and squamous-cell carcinoma of the head and neck,¹⁶⁴ indicating its potential in early cancer diagnosis and prognosis judgment. Specifically, higher expression of HMGB1 is observed in HCC patients with a higher rate of vascular invasion.¹⁵⁹ In addition, the expression of HMGB1 is higher in breast cancer patients with prolonged metastasis-free survival.¹⁶¹ Moreover, HMGB1 is higher expressed in patients with a superior clinical stage in nasopharyngeal carcinoma and squamous-cell carcinoma of the head and neck.^{162,164} The expression level of HMGB2 is also reported to be higher expressed in patients with HCC,⁸² gastric cancer,¹⁶⁵ and breast cancer.²¹ Researchers found that overexpression of HMGB2 is closely associated with shorter OS in HCC,⁸² advanced gastric cancer,¹⁶⁵ and advanced breast cancer.²¹ HMGB3 is found to be significantly different between carcinoma and peritumoral tissues.¹⁶⁰ The concentration of serum HMGB3 is higher in HCC, lower in liver cirrhosis, chronic hepatitis, and the healthy control.¹⁵⁸ The positive expression rate of HMGB3 is higher in the gastric cancer than in the peritumoral tissue.¹⁶⁰

Clearly, HMGB family members are promising diagnostic and prognostic markers in human cancers. Nevertheless, questions remain to be solved. Firstly, the diagnostic and prognostic role of HMGB family members needs to be investigated

in larger-scale case-control or cohort studies. Secondly, the development of fast, effective, and convenient detection techniques and tools are necessary before it can be used in clinical settings.

Therapeutic potential of HMGB family members in human cancers

Many studies highlight the potential role of HMGB family members as novel therapeutic targets for cancer treatment. HMGB1 have been investigated extensively in the pre-clinical stage,¹⁶⁶ although the therapeutic value of HMGB2 and HMGB3 is still obscure because their carcinogenic mechanisms have not been systematically elucidated. Here, we will mainly review and summarize the therapeutic potential of HMGB1 in cancers. In addition, we envisage the future investigation of therapeutic strategies regarding HMGB family members.

Given that HMGB1 can play a dual role in carcinogenesis, current fundamental therapeutic methods targeting HMGB1 are different. Repressing its oncogenic function and stimulating its tumor-repressing effect are both theoretically applicable. Basic research on this hypothesis has been performed extensively, while clinical studies are still limited.¹⁶⁶ We can achieve the former strategy by reducing the release of HMGB1 or disrupting its connection with molecules or receptors that strengthen the process of carcinogenesis.¹⁶⁶ Based on this consideration, sodium salicylate and ethyl pyruvate (EP) were confirmed to switch the glucose deprivation-induced necrosis to autophagy,^{166,167} resulting in the repression of HMGB1 release and prevention of tumor development. EP was also reported to suppress the release of HMGB1 via the NF- κ B signaling pathway,¹⁶⁸ and nicotine could also inhibit NF- κ B signaling and HMGB1 release by binding to the α 7-nicotine acetylcholine receptor (nAChR).⁴⁶ Besides, antibodies to HMGB1 have been shown to block the interaction between HMGB1 and RAGE in animal models. Inflammation and carcinogenesis are repressed afterward.¹⁶⁹ In addition, targeting RAGE is another strategy to prevent the HMGB1–RAGE interaction. This strategy includes the administration of extracellular, soluble domain of RAGE (sRAGE), which is ligand-binding, and preformation of transfected C6 glioma expressing sRAGE or RAGE mutant lacking the cytosolic tail.¹⁷⁰

To activate the tumor-suppressing effect of HMGB1 is to strengthen its ICD-inducing

capability. In fact, HMGB1 stimulating the immunological system is one of the main causes of radiotherapy and chemotherapy to inhibit the development of carcinoma.^{171,172} Chemotherapy drugs including oxaliplatin, mitoxantrone, shikonin, and bortezomib are all reported to induce ICD in an HMGB1-dependent way.^{173–176} However, their ICD-inducing capabilities vary, with the most likely reason being that they share different affinities in assisting HMGB1 binding to TLR4 on DC cells.

In conclusion, for HMGB1, the primary task is to translate the achievements of basic research to clinical application. To achieve this, clinical research must be carried out, and before that, the safety and effectiveness of the corresponding proposals must be confirmed in the preclinical stage. As for HMGB2 and 3, more extensive basic research is still needed, so that sufficient evidence can be employed to choose the most promising strategies.

Potential challenges in future researches regarding to the HMGB family members

There are still many challenges in translating the above findings into clinical uses. Initially, since HMGB family members can be secreted or released in various types that lead to different progression and outcomes,²³ we should detect the main secretive methods in the target carcinoma before performing HMGB-targeted therapy. Different secretion mechanisms may guide us to use up-regulation or down-regulation of HMGB1 expression to achieve the purpose of tumor inhibition. Secondly, multiple receptors can bind to HMGB family members and induce the activation of distinctive downstream cascades.⁴⁹ We can decide on whether to select HMGB as a target after identifying the affinity between HMGB and these receptors in different carcinomas. We can also alter the process toward what we expect partly by changing the affinity. Besides, we should exclude the involvement of other receptors or confirm cooperation of multiple receptors when validating each receptor's downstream cascades and function. To take HMGB1 as an example, recent studies provide us with approaches to address this, one of which is to employ double knockout mice such as RAGE-TLR4 deficient mice, while another would be to conduct parallel studies that employ recombinant HMGB1 lacking other receptors' binding domains so that HMGB1 can bind to specific target receptors.⁹⁷

Finally, redox states and PTMs can influence, or even determine, the roles of HMGB family members in the process or stage in carcinogenesis.¹⁷ Determining the expression level of the main forms would be beneficial; however, current analysis and assay are not sensitive enough to meet our expectations. We should update existing detective means and tools as well as further investigate the roles of different modified forms of HMGB family members in carcinogenesis. If we could accurately assess the redox status and PTMs of HMGB1 in patients and confirm their roles, we may be more confident in predicting patient tumor stage and prognosis. In addition, alteration of such modified forms may be applicable and efficient as a therapeutic target.

Discussion

Our review has summarized the commonalities and disparities of the three HMGB protein family members in structure, distribution, carcinogenesis, and clinical potentials. We have concluded from current research and reviews that the similarity in their amino acids sequence and regions may indicate functional consistency. However, discrimination, either in structure or distribution, partly determines their distinctive roles in biochemical cascades and regulation of carcinogenesis. The broader distribution and higher expression level of HMGB1 than HMGB2 and 3, both in embryonic development and adult tissues, exposes its more extensive roles in biochemical and pathological procedures. This implication was validated by existing research, as reviewed herein.

From the above analysis, extensive and intensive research is necessary for the complete understanding of the HMGB protein family. Based on the confirmed functions of HMGB1, we can analogously investigate the undiscovered effects of the other two members. Initially and primarily, the specific structure and modifying mechanisms of HMGB2 and 3 should be elucidated, since structure plays a critical role in determining function. The comparison between their construction will provide deep insights into the comprehension of their features. Then, their distribution at the cellular level, and the translocation mechanisms of HMGB2 and 3 should be investigated, which will allow their localization to be understood. Furthermore, their involvement in biochemical cascades and carcinogenesis should be investigated at a larger scale and compared more rationally. In addition to focusing on the identity of

their targets, we should also consider their varied affinity. Apart from looking for their connection, we ought to pay attention to how they antagonize each other as well. Their distinctive roles in tumorigenesis indicate that their subtle but critical distinction in conformation matters. We can place emphasis on this difference since directly modifying or even altering the main regions may be effective in their clinical application. Besides, regulating their expression by indirectly targeting other factors in the cascades, or alteration of the oxidation-reduction environment can be another feasible strategy, while existing therapeutic strategies still need to be verified in large-scale preclinical and clinical experiments.

Collectively, the last several decades have witnessed great progression in investigating this abundantly expressed protein family, while further researches are still urgently need. We firmly hold the opinion that this protein family is critically involved in tumorigenesis. In-depth studies can help us to get a better understanding of its roles in carcinogenesis and its clinical potential for cancer management.


Conflict of interest statement

The authors declare that there is no conflict of interest.

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Supplemental material

Supplemental material for this article is available online.

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