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The redox-sensitive protein HMGB1: intracellular and extracellular roles

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HMGB1 is a non-histone nuclear protein that is primarily located in the nucleus. It can be translocated to the cytoplasm and secreted into the extracellular space upon stimulation. In the nucleus, HMGB1 functions as a DNA chaperone; in the cytoplasm, it participates in autophagy and mitochondrial homeostasis; and in the extracellular environment, it acts as a damage-associated molecular pattern. HMGB1 consists of three cysteine residues (C²³, C⁴⁵ and C¹⁰⁶) and is a redox-sensitive protein that exists in distinct redox isoforms: reduced (Re-HMGB1), disulfide (Ds-HMGB1), oxidized (Ox-HMGB1) and dimerized (Di-HMGB1). The localization-specific functions of HMGB1 are regulated by its redox state, which is involved in preventing DNA damage, inflammation, cell death and survival, and various inflammatory disorders. This Review describes the oxidation mechanisms of HMGB1 and summarizes its functional roles in the nucleus, cytoplasm and extracellular space depending on its redox status. We further describe the oxidation states of HMGB1 released during different forms of cell death, the distinct redox isoform of HMGB1 and its possible association with various diseases. This Review provides insights into therapeutic strategies targeting redox-sensitive damage-associated molecular patterns in inflammatory and autoimmune pathologies.

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INTRODUCTION

Damage-associated molecular patterns (DAMPs) are endogenous danger signals generated in response to pathogen invasion, cellular damage or oxidative stress. Under normal physiological conditions, DAMPs do not trigger immune responses. However, excessive release of DAMPs, or changes in their biochemical or structural properties, can activate the immune system by engaging innate immune receptors and initiating inflammation. DAMPs are classified on the basis of their molecular characteristics as follows: (1) nucleic acids, including both intracellular and extracellular DNA and RNA, as well as mitochondrial DNA; (2) cellular proteins, including HMGB1, heat shock proteins (HSPs), S100 proteins, thioredoxin (Trx), peroxiredoxins (Prxs) and cytokines such as interleukin (IL)-1 α and IL-1 β ; (3) ions, including potassium (K⁺) and calcium (Ca²⁺), which are recognized as ionic DAMPs; (4) glycans, including hyaluronan and decorin, which are key representatives of carbohydrate DAMPs; and (5) metabolites, including adenosine triphosphate (ATP), uric acid and succinate^{1–4}. Pathogen-associated molecular pattern (PAMPs) are conserved molecular structures uniquely expressed by bacteria, viruses and other infectious microorganisms. These pathogen-specific motifs are recognized by a host's innate immune system, facilitating rapid and efficient detection of invading pathogens. Representative PAMPs include lipopolysaccharide (LPS), lipoteichoic acid (LTA), peptidoglycan, viral double-stranded RNA and single-stranded RNA, β -glucan, mannans, flagellin and unmethylated cytosine-phosphate-guanine (CpG) DNA motifs⁵. Beyond

these categories, certain DAMPs can form complexes with PAMPs that potentiate immune responses. For example, HMGB1–LPS or HMGB1–LTA complexes can be formed, significantly increasing inflammatory signaling⁶. DAMPs and PAMPs are sensed by a variety of pattern recognition receptors that are expressed in both immune and non-immune cells. Intracellular sensing of DAMPs activates cell-intrinsic sterile inflammatory responses, which may facilitate cellular repair but can become pathogenic if persistently activated⁷. Major DAMP- and PAMP-sensing receptors include Toll-like receptors (TLRs), receptor for advanced glycation endproducts (RAGE), nucleotide-binding oligomerization domain-like receptors (NLRs), retinoic acid-inducible gene I-like receptors (RLRs) and cyclic GMP–AMP synthase-stimulator of interferon genes (cGAS–STING). Activation of these receptors leads to the production of inflammatory cytokines and chemokines, the recruitment of immune cells and the amplification of the inflammatory response^{1,4}. DAMPs and their receptors play central roles in the pathogenesis of various diseases⁷. By excessively activating immune responses, DAMPs contribute to autoimmune diseases such as rheumatoid arthritis and systemic lupus erythematosus^{8,9}. DAMPs are also involved in metabolic disorders, including type 2 diabetes and obesity^{2,10}, as well as neurodegenerative conditions, such as Alzheimer's disease¹¹. In the tumor micro-environment (TME), DAMPs can modulate immune activity and influence cancer progression and metastasis¹² (Fig. 1). Understanding the multifaceted roles of DAMPs provides valuable insights into immune regulation and highlights potential

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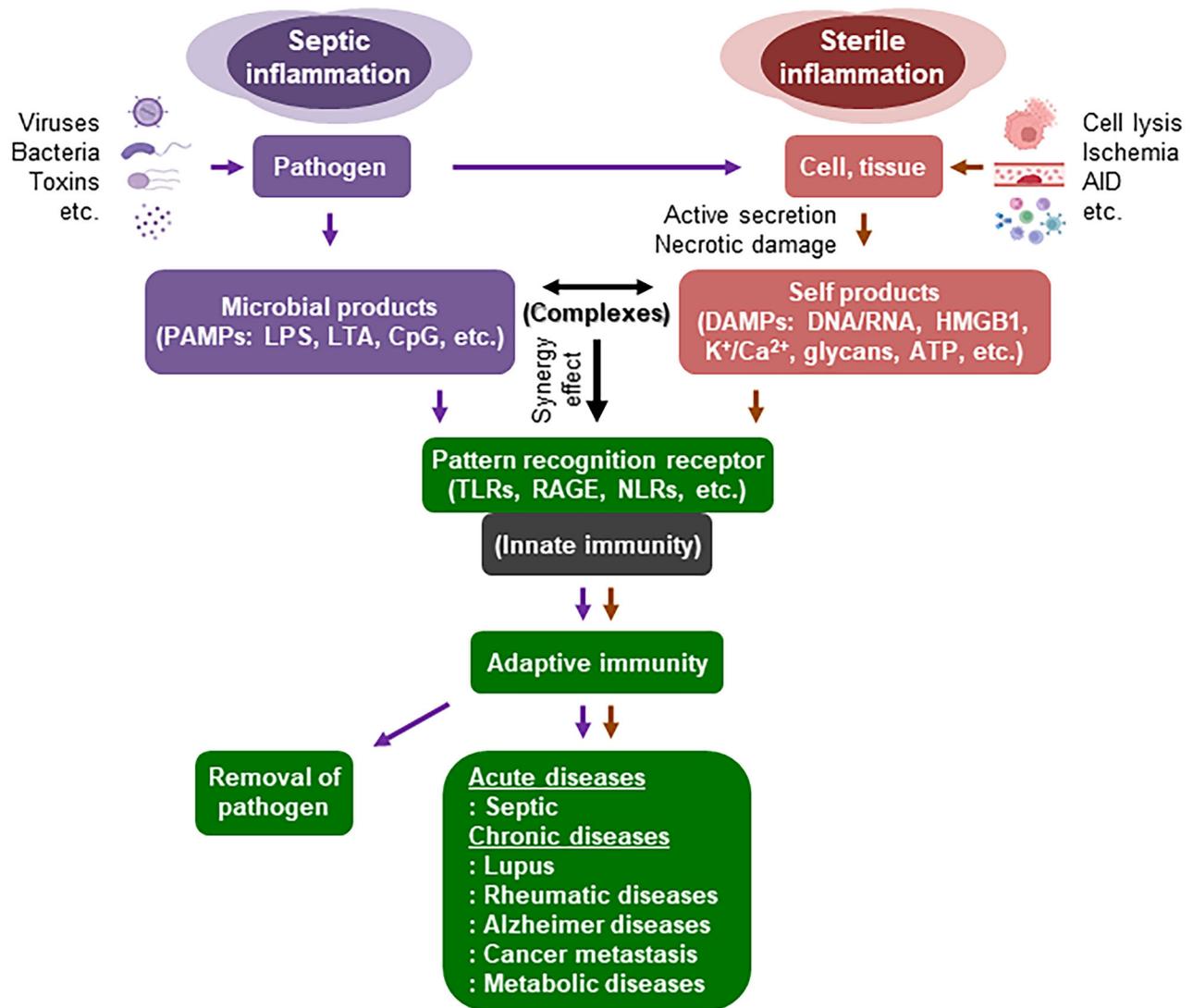


Fig. 1 Distinct and overlapping pathways in septic and sterile inflammation. Septic inflammation is initiated by pathogens such as viruses, bacteria or microbial toxins, leading to the release of PAMPs (LPS, LTA, and CpG). By contrast, sterile inflammation results from tissue or cellular damage caused by ischemia or AID, leading to the active secretion or necrotic release of self-derived DAMPs (HMGB1, HSPs, S100, Prx, and Trx). Both PAMPs and DAMPs activate pattern recognition receptors (PRRs) such as TLRs and RAGE, initiating innate immune responses. These signals act independently, synergistically or as molecular complexes to enhance immune activation. Subsequent adaptive immune responses contribute to pathogen clearance; however, if unresolved, they may lead to disease progression. While septic inflammation is primarily linked to infection-related pathologies, sterile inflammation also regulates intracellular signaling and contributes to tissue homeostasis. AID, autoimmune diseases.

therapeutic targets for a range of inflammatory and immune-mediated diseases.

HMG proteins are non-histone chromatin-binding proteins that are characterized by their rapid mobility during electrophoresis and their ability to bind to DNA. There are three major subfamilies of these proteins: HMGA, HMGB and HMGN. Each subfamily has distinct structural motifs, nuclear localization patterns and biological functions^{13,14}. HMGA proteins contain three AT-hook DNA-binding domains that bind preferentially to AT-rich regions of DNA. They influence gene regulation during development and differentiation by modulating chromatin architecture. HMGB proteins consist of two HMG-box domains (box A and box B) and an acidic C-terminal containing a string of glutamic and aspartic acid residues. HMGN proteins contain a nucleosome-binding domain that allows them to bind specifically to nucleosomes. This interaction modulates histone structure and enhances the accessibility of transcription factors, thereby promoting transcriptional activity^{13,14}.

HMGB1 is a ubiquitously expressed, non-histone nuclear protein that is abundantly expressed in almost all cell types that contain a nucleus. First discovered in 1973 in calf thymus tissue, HMGB1 was identified as a nuclear protein involved in chromatin architecture¹⁵. In 1999, Wang et al. demonstrated its immunological significance when they showed that HMGB1 acts as a proinflammatory mediator in LPS-mediated endotoxemia¹⁶. HMGB1 is released during necrotic cell death and promotes innate immune responses¹⁷. Over the past 26 years, extensive research has been conducted to identify the role of HMGB1 in inflammation, its secretion mechanism, the interaction with endogenous host molecules and PAMPs, and intracellular function. HMGB1 is a redox-sensitive protein, and its immune functions vary depending on its oxidation state. This Review aims to investigate HMGB1's oxidation mechanisms and elucidate its roles and functions under different oxidation states from immunological and pathological perspectives.

REACTIVE OXYGEN SPECIES (ROS) GENERATION IN INFLAMMATION AND ROS-SENSITIVE DAMPS

Inflammatory conditions are closely associated with increased intracellular ROS production, which plays a pivotal role in modulating immune responses and contributing to tissue damage. During inflammation, immune cells such as neutrophils and macrophages undergo a 'respiratory burst', which leads to a significant increase in ROS production. This process is primarily mediated by the activation of nicotinamide adenine dinucleotide phosphate hydrogen (NADPH) oxidase complexes, particularly NADPH oxidase 2 (NOX2), which generate superoxide anions and other ROS¹⁸. Mitochondria are another major source of ROS during inflammatory responses. Inflammatory stimuli can disrupt mitochondrial electron transport chains, leading to electron leakage and subsequent ROS generation. These ROS further amplify inflammatory signaling pathways, including nuclear factor kappa-light-chain-enhancer of activated B cells (NF- κ B), nod-like receptor family pyrin domain-containing 3 (NLRP3) inflammasome, mitogen-activated protein kinase (MAPK), and Janus kinase 2 (JAK2)-signal transducer and activator of transcription 3 (STAT3) pathways^{19,20}. Excessive ROS production can overwhelm cellular antioxidant defenses, resulting in oxidative stress. This condition can damage proteins, lipids and DNA, contributing to cell dysfunction and death and exacerbating inflammatory conditions²⁰.

During inflammation, increased intracellular ROS can oxidize proteins involved in immune signaling. These redox modifications can modulate immune responses and contribute to disease progression. For example, ROS can increase IL-8 expression, thereby enhancing its chemotactic activity toward neutrophils. Antioxidants such as *N*-acetylcysteine can suppress IL-8 production, suggesting redox-sensitive regulation²¹. In rhabdomyosarcoma cells, inhibition of nuclear receptor 4A1 leads to the accumulation of ROS, which subsequently induces IL-24 expression in a ROS-dependent manner, thereby suppressing cellular proliferation, survival and migration²². Oxidation also leads to the formation of malondialdehyde (MDA) adducts on proteins, generating neo-epitopes that can trigger autoimmune responses, as seen in rheumatoid arthritis. Elevated levels of antibodies against MDA-modified proteins correlate with disease activity in autoimmune conditions²³. Furthermore, ROS levels influence macrophage polarization: high ROS promote the proinflammatory M1 phenotype, while antioxidants promote the anti-inflammatory M2 phenotype²⁴. HMGB1 is also a redox-sensitive protein that influences various inflammatory functions depending on its oxidation state¹⁴.

HMGB1 STRUCTURE

HMGB1 is composed of 215 amino acids organized into two domains: the A box (amino acids (a.a.) 1–79) and the B box (a.a. 89–162). These domains exhibit anti- and proinflammatory properties, respectively. The B box domain activates immune cells via TLR4 and RAGE receptors²⁵. The acidic tail (a.a. 186–215) is rich in acidic residues that regulate the DNA-binding activity of the A/B boxes and facilitate transcriptional stimulation by interacting with transcriptional cofactors. Notably, a short peptide (a.a. 201–205) in this region exhibits antimicrobial activity^{14,26}. The A and B boxes bind to the minor groove of DNA and induce bending and unwinding of the DNA in the nucleus, acting as DNA chaperones²⁷. This function facilitates transcription regulation, replication, recombination and DNA repair^{13,14,28}. HMGB1 plays critical roles in both intracellular chromatin architecture and extracellular inflammatory signaling when secreted. It has two nuclear localization signals (NLS1, a.a. 28–44; NLS2, a.a. 179–185) that are essential for the nuclear import of newly translated HMGB1. The segment a.a. 89–108 is crucial for binding to TLR4, which facilitates innate immune activation and cytokine

production in response to tissue damage or infection²⁵. The C-terminal region of B box (a.a. 150–183) constitutes the binding site for RAGE. The HMGB1–RAGE interaction promotes cell migration, survival and sustained inflammatory responses, contributing to chronic inflammation, tumor progression and metastasis²⁹. The A box domain (a.a. 1–15) and overlapping regions within the B box (a.a. 80–96) interact with bacterial LPS and transfer it to CD14³⁰. These HMGB1-derived peptides can bind to distinct domains of LPS and have been shown to inhibit LPS-induced inflammatory responses in murine endotoxemia models. This suggests a potential anti-inflammatory therapeutic application³¹ (Fig. 2a).

The redox-sensitive protein HMGB1 contains three cysteine residues (C²³, C⁴⁵ and C¹⁰⁶) and four distinct redox isoforms: reduced (Re)-HMGB1, disulfide (Ds)-HMGB1, oxidized (Ox)-HMGB1 and dimeric (Di)-HMGB1. The following sections focus on the localization-specific functions of the different HMGB1 redox forms in DNA damage, inflammation, cell death and survival, and various inflammatory disorders. The structure of the HMGB1 A box was modeled using the Modeller 10.7 program based on the structural templates from the Protein Data Bank (PDB IDs: 4QR9, 2MSY, 2RTU and 8I9M). The HMGB1 A box comprises three α -helices (helices I, II and III) and two loops (loops I and II). Two cysteine residues (Cys²³ and Cys⁴⁵) are structurally proximal and capable of forming an intramolecular disulfide bond in response to ROS (Fig. 2b).

HMGB1 secretion and activity are tightly regulated by various post-translational modifications (PTMs), which modulate its DNA-binding ability, intracellular localization and secretion mechanisms. In response to ROS, PrxI and PrxII are oxidized, which activates their sensor function and transmits oxidative signals to HMGB1. HMGB1 oxidation leads to the formation of a disulfide bond between its Cys²³ and Cys⁴⁵ residues. This results in a conformational change that facilitates nucleocytoplasmic translocation³². Under inflammatory conditions or in high-glucose environments, lactate levels within cells increase, activating the histone acetyltransferases p300/CBP. These enzymes catalyze the lactylation of HMGB1, enhancing its cytoplasmic accumulation and secretion³³. Inducible nitric oxide (NO) synthase is activated during inflammation and generates NO, which induces S-nitrosylation of HMGB1 at Cys¹⁰⁶ in the brain microglia and macrophages. This HMGB1 modification facilitates its secretion into the extracellular space, promoting proinflammatory and neurodegenerative effects³⁴. Classical protein kinase C (cPKC) plays a critical role in inflammatory signaling by phosphorylating multiple serine residues (Ser^{35,39,42,46,53,181}) in HMGB1^{35–37}. Calcium/calmodulin-dependent protein kinase (CaMK) IV also induces HMGB1 phosphorylation. This phosphorylation weakens the nuclear retention signal of HMGB1, promoting its translocation to the cytoplasm³⁸. Acetylation of HMGB1 at Lys^{28,30,182–185} by poly(ADP-ribose) polymerase-1 (PARP1) in response to inflammatory stimuli reduces its nuclear localization and promotes its translocation to the cytoplasm³⁹. HMGB1 is glycosylated at Asn^{37,134,135}, a modification that promotes its nucleocytoplasmic translocation and secretion⁴⁰. Additionally, methylation at Lys⁴³ has been identified as a regulatory modification that contributes to HMGB1 translocation and secretion in response to cellular stress⁴¹. The poly(ADP-ribosylation) of HMGB1 by PARP at its C-terminal acidic tail reduces its affinity for binding to DNA and its secretion. These modifications are induced during DNA damage and stress responses, which emphasizes HMGB1's role as a DAMP in cellular stress and necrosis⁴². Ubiquitination of HMGB1 at its B-box by RING finger protein 186 (RNF186) and synoviolin 1 (SYVN1) leads to its degradation and reduces secretion. Conversely, deubiquitination by ubiquitin-specific peptidase (USP)12 and USP13, which directly bind to HMGB1, inhibits its ubiquitination. This prevents degradation and enhances stability, leading to increased cytoplasmic accumulation and extracellular release of HMGB1^{43–46}. After undergoing

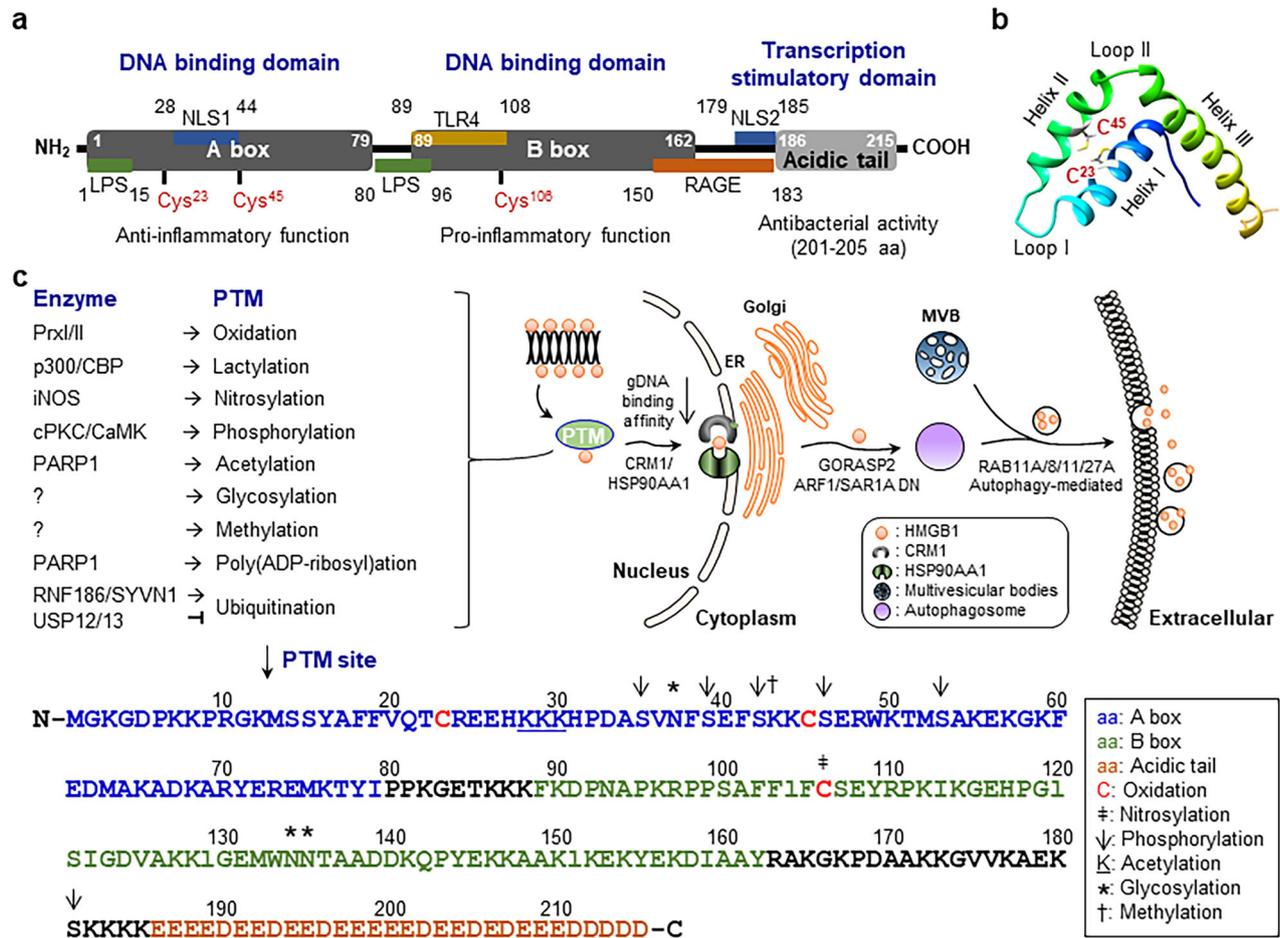


Fig. 2 Structure of HMGB1 and PTMs of HMGB1. **a**, Schematic structure of HMGB1. HMGB1 consists of two DNA-binding domains (A box and B box) and C-terminal acidic tail with transcriptional activity. The A box includes Cys²³ and Cys⁴⁵ forming intramolecular disulfide bonds, linked to anti-inflammatory function. The B box contains redox-sensitive Cys¹⁰⁶, critical for proinflammatory signaling. The acidic tail is associated with antibacterial activity. Two NLS regions (NLS1: a.a. 28–44, NLS2: a.a. 179–185) facilitate nuclear import. Key binding sites for LPS, TLR4 and RAGE are indicated. **b**, Model structure of HMGB1 A box. The structural model (PDB ID: 4QR9, 2MSY, 2RTU and 8I9M) was generated using the Modeller 10.7 program. The A box comprises three α -helices (I–III) and two loops (I and II). Cys²³ and Cys⁴⁵ are spatially close, capable of intramolecular disulfide bond formation in response to ROS. **c**, Enzymes and specific PTM sites of HMGB1. Multiple enzymes induce distinct PTMs of HMGB1: oxidation, lactylation, nitrosylation, phosphorylation, acetylation, glycosylation, methylation, poly(ADP-ribosyl)ation, ubiquitination and deubiquitination. PTMs modulate HMGB1 subcellular localization, genomic DNA (gDNA)-binding affinity and secretion via ER–Golgi, MVBs or autophagy. Key proteins involved in HMGB1 trafficking include CRM1/HSP90AA1, GORASP2, ARF1/SAR1A, and Rab11A/8/11/27A. The full-length HMGB1 sequence is shown with annotated domains: A box (blue), B box (green) and acidic C tail (orange). PTM sites are indicated: oxidation (red C), nitrosylation (‡), phosphorylation (↓), acetylation (K), glycosylation (*) and methylation (†). Domain-specific PTMs regulate HMGB1 structure, localization and function. ER, endoplasmic reticulum; MVBs, multivesicular bodies; iNOS, inducible NO synthase.

various PTMs, HMGB1 translocates to the cytoplasm through chromosome region maintenance 1 (CRM1) and HSP90 α family class A member 1 (HSP90AA1), where it is secreted via unconventional pathways and packaged into secretory autophagosomes and lysosomes^{39,47}. Autophagy-mediated HMGB1 secretion involves diverse components of the autophagy machinery, vesicular trafficking systems and membrane fusion proteins, including Golgi reassembly stacking protein 2 (GORASP2), ADP ribosylation factor 1 (ARF1), secretion-associated Ras-related GTPase 1A (SAR1A) and Ras-related protein Rab11A/8/11/27A⁴⁷ (Fig. 2c). These pathways are regulated so that HMGB1 functions as a DAMP to activate immune responses through diverse receptors.

INTRACELLULAR ROLE OF HMGB1 OXIDATION

Accumulation of ROS inside cells under stressful conditions such as inflammation, infection or hyperglycemia induces redox-sensitive modifications in a variety of proteins, including HMGB1.

HMGB1 oxidation mechanism

One of the most prominent redox modifications of HMGB1 is the formation of an intramolecular disulfide bond between Cys²³ and Cys⁴⁵ (Ds-HMGB1). This process is tightly regulated by the antioxidant enzymes PrxI and PrxII. These enzymes contain catalytic thiol groups that react with ROS, such as hydrogen peroxide (H₂O₂). The peroxidatic cysteine in PrxI and PrxII oxidizes to sulfenic acid (Cys–SOH) when exposed to H₂O₂, which then reacts with a resolving cysteine to form an intra- or intermolecular disulfide bond. The oxidized PrxI and PrxII then transfer this oxidative signal to HMGB1 via thiol–disulfide exchange. This leads to the formation of a disulfide bond between HMGB1's Cys²³ and Cys⁴⁵ (reaction: Prx–S–S + HMGB1–(SH)₂ → Prx–(SH)₂ + HMGB1–S–S)³². The Ds-HMGB1 isoform exhibits proinflammatory properties and preferentially activates TLR4 on immune cells. Concurrently, the Trx system can counterbalance this oxidation. Trx contains a conserved Cys–X–X–Cys motif that reduces Ds-HMGB1 through thiol–disulfide exchange (Trx–(SH)₂ + HMGB1–S–S → Trx–S–S + HMGB1–(SH)₂), forming Re-HMGB1. Oxidized Trx is

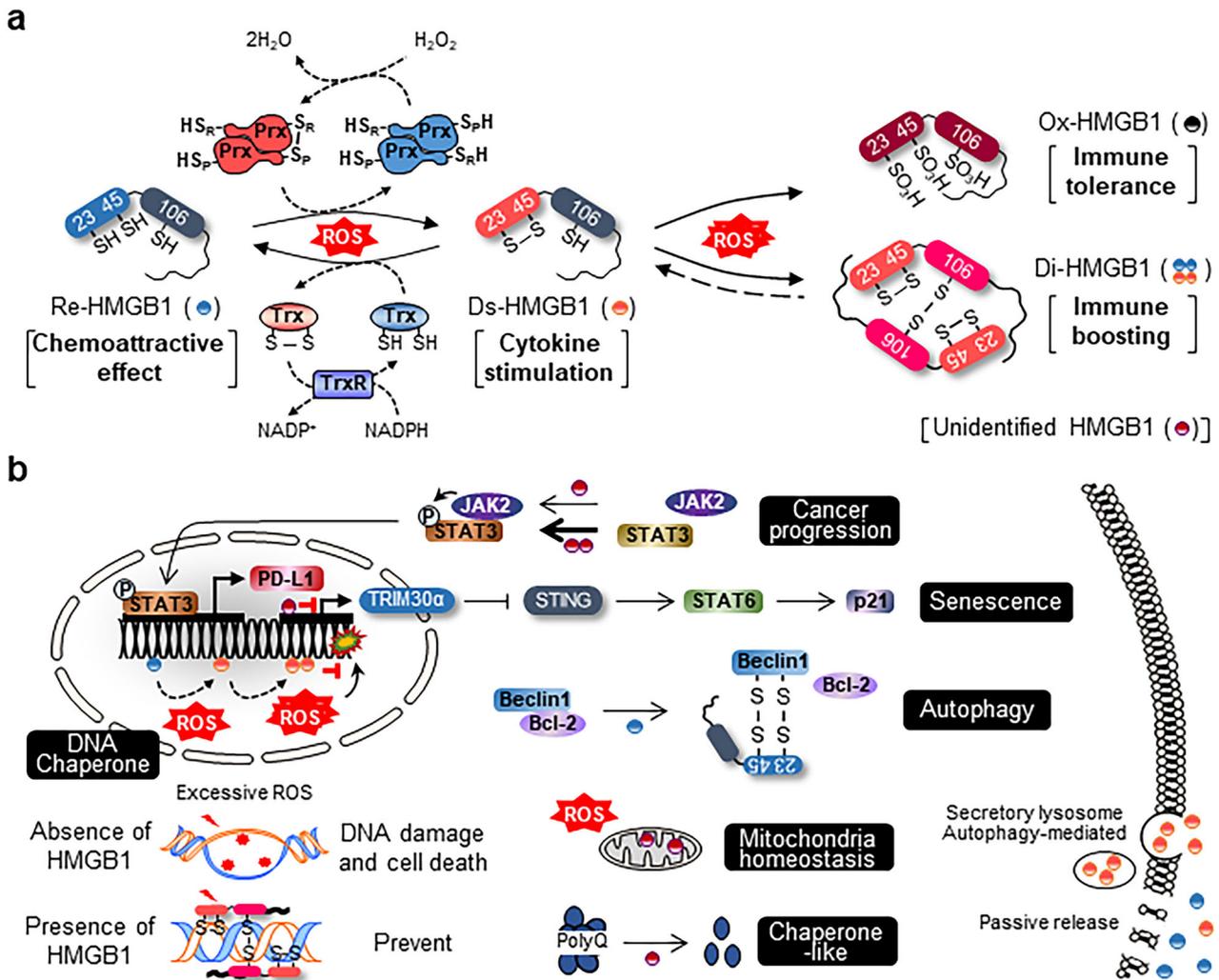


Fig. 3 Redox regulation and intracellular function with resulting immune signaling of HMGB1. **a**, Schematic of redox-dependent transitions of HMGB1 in the nucleus. Under oxidative conditions, Prx/II oxidize HMGB1 via thiol–disulfide exchange, generating Ds-HMGB1 (Cys²³–Cys⁴⁵). The Trx system (Trx/TrxR) restores Re-HMGB1. In the presence of excessive ROS, Cys²³, Cys⁴⁵ and Cys¹⁰⁶ of HMGB1 are oxidized to sulfonic acid (–SO₃H), leading to immune tolerance. Alternatively, the Cys¹⁰⁶–Cys¹⁰⁶ intermolecular disulfide bond forms Di-HMGB1 with enhanced proinflammatory properties. **b**, Cellular functions of HMGB1 redox isoforms. In the nucleus, HMGB1 acts as a DNA chaperone and regulates oxidative stress responses. Under excessive ROS, HMGB1 absence leads to DNA damage and cell death, while its presence promotes genome stability. HMGB1 modulates PD-L1 expression via the JAK2–STAT3 pathway and influences STING-mediated signaling through TRIM30α, promoting senescence (via p21) autophagy (Re-HMGB1 via Beclin1–Bcl-2 complex disruption), and mitochondrial homeostasis. HMGB1 also exhibits chaperone-like activity in preventing PolyQ aggregation. Extracellular release occurs via secretory lysosomes, autophagy-mediated pathways or passive release.

subsequently regenerated by Trx reductase (TrxR) using electrons from NADPH (Trx–S–S + NADPH + H⁺ → Trx–(SH)₂ + NADP⁺). Re-HMGB1 exhibits chemoattractive activity, particularly in recruiting immune cells under stress resolution conditions⁴⁸. By contrast, under conditions of prolonged or excessive oxidative stress, HMGB1 undergoes irreversible hyperoxidation. Its cysteine residues progressively oxidize through sulfinic acid (–SO₂H) and sulfonic acid (–SO₃H) forms to create Ox-HMGB1. Ox-HMGB1 undergoes significant conformational changes resulting in the loss of DNA binding and receptor interactions. This renders the protein immunologically inert. This sulfonyl form of HMGB1 contributes to immune tolerance, particularly in late-stage inflammation or apoptotic contexts⁴⁹. Interestingly, Cys¹⁰⁶ of HMGB1 can also form an intermolecular disulfide bond, leading to dimerization of HMGB1 molecules (HMGB1–SH + HMGB1–SH → HMGB1–S–S–HMGB1), forming Di-HMGB1. Di-HMGB1 exhibits an enhanced proinflammatory capacity compared with the monomer. It shows stronger binding to receptors, such as TLR2 and TLR4, and

potentiates cytokine release from immune cells⁶ (Fig. 3a). However, the precise mechanism remains undefined. These redox-driven modifications not only determine HMGB1's localization (nuclear versus cytoplasmic) and secretion, but also critically modulate its immunological function. Depending on the oxidative environment, HMGB1 can transition from an immune-activating DAMP to an immunologically silent form.

Function of intracellular HMGB1

In the nucleus, HMGB1 acts as a DNA chaperone that facilitates nucleosome remodeling and stabilizes chromatin structure. Under mild oxidative stress, ROS induce the formation of a disulfide bond between Cys²³ and Cys⁴⁵ via Prx/II, generating the Ds-HMGB1 form³². Under conditions of excessive ROS accumulation, HMGB1 undergoes dimerization via an intermolecular disulfide bond between Cys¹⁰⁶ and Cys¹⁰⁶, resulting in an antiparallel dimer configuration. Di-HMGB1 strongly binds to DNA, preventing hydroxyl radical-induced DNA damage and enhancing cell

survival⁵⁰. In addition, nuclear HMGB1 contributes to cancer cell senescence by modulating the STING pathway through the suppression of TRIM30 α , a negative regulator of STING. By downregulating TRIM30 α expression, HMGB1 enhances STING signaling, leading to increased STAT6 phosphorylation and upregulation of p21⁵¹ (Fig. 3b).

HMGB1 also functions in the cytoplasm, where it plays a critical role in maintaining mitochondrial homeostasis. Multiple studies have demonstrated that cytoplasmic HMGB1 regulates mitochondrial quality control by modulating autophagy, mitophagy, mitochondrial dynamics and DNA repair mechanisms. Re-HMGB1 binds directly to Beclin-1, promoting the initiation of autophagy. This redox-sensitive interaction displaces Bcl-2 from Beclin-1, thereby facilitating the formation of autophagosomes and the clearance of dysfunctional mitochondria (mitophagy), a process essential for mitochondrial quality control and energy balance⁵². In addition, HMGB1 has been shown to promote mitochondrial fusion by downregulating dynamin-related protein 1 (Drp1) through the C-X-C chemokine receptor type 4 (CXCR4)-proteasome subunit β type-5 (PSMB5) axis, independently of the nuclear factor erythroid 2-related factor 2 (NRF2) pathway. This prevents excessive mitochondrial fragmentation and supports cellular homeostasis⁵³. Cytoplasmic HMGB1 directly interacts with JAK2 and STAT3, enhancing JAK2-STAT3 complex formation and promoting STAT3 phosphorylation in breast cancer cells. This signaling cascade induces the expression of STAT3 target genes, including PD-L1, thereby facilitating tumor cell migration and invasion. Notably, Di-HMGB1 was found to more effectively potentiate JAK2-STAT3 activation than its monomeric HMGB1⁵⁴. HMGB1 can also inhibit the aggregation of proteins such as polyglutamine (PolyQ), which induces neurodegenerative disorders. This indicates its chaperone-like activity⁵⁵ (Fig. 3b). Therefore, investigating Di-HMGB1 in the nucleus and cytoplasm could provide critical insights into regulating cell survival and death and its pathophysiological relevance in cancer therapies. Cytoplasmic HMGB1 can be released either actively through unconventional secretion pathways involving secretory autophagosomes and lysosomes or passively as a result of cell death^{14,39,47}.

EXTRACELLULAR ROLE OF HMGB1 OXIDATION

HMGB1 oxidation mechanism

Both the reduced and oxidized forms of HMGB1 are present in the extracellular space. LPS and LTA are well-characterized PAMPs that initiate immune responses. These molecules act as binding platforms that promote extracellular HMGB1 dimerization, a process primarily driven by ROS-dependent oxidation of Cys¹⁰⁶. ROS selectively oxidize the thiol group of Cys¹⁰⁶ in HMGB1. First, it is converted to sulfenic acid (-SOH), which then facilitates disulfide bond formation between two HMGB1 molecules at this site: $\text{HMGB1-SH} + \text{HMGB1-SH} + \text{ROS} \rightarrow \text{HMGB1-S-S-HMGB1}$. LPS and LTA promote the spatial proximity of HMGB1 molecules in the extracellular environment. The resulting HMGB1 dimer demonstrates an enhanced binding affinity for TLR2 and TLR4, which sustains NF- κ B activation and drives robust proinflammatory cytokine release⁶ (Fig. 4a).

Function of extracellular HMGB1

Depending on its redox state, HMGB1 interacts with distinct receptors to exert its function as a DAMP, contributing to immune responses by promoting chemotaxis or stimulating the release of proinflammatory cytokines (Fig. 4b). HMGB1 facilitates proinflammatory signaling by interacting with TLR2. Di-HMGB1 binds more strongly to TLR2 than monomeric HMGB1, further enhancing NF- κ B signaling⁶. HMGB1 has been shown to interact with LTA and facilitate its transfer to CD14 and TLR2. This amplifies the

production of proinflammatory cytokines, such as tumor necrosis factor (TNF- α) and IL-6⁵⁶. Ds-HMGB1 has also been shown to negatively affect the maturation of oligodendrocyte progenitor cells via TLR2 signaling⁵⁷. Yang et al. demonstrated that Ds-HMGB1, but not Re- or Ox-HMGB1, directly binds to MD2/TLR4 and induces the release of the proinflammatory cytokine TNF- α ⁵⁸. In addition, HMGB1 binds to LPS, promoting its transfer to CD14 and TLR4 and augmenting LPS-induced TNF- α production in human monocytes³⁰. Synthetic peptides HPep1 and HPep6, derived from the A box and B box domains of HMGB1, block LPS transfer to CD14, reduce cellular uptake in RAW264.7 cells and suppress LPS-induced TNF- α production in human peripheral blood mononuclear cells and a mouse endotoxemia model³¹. Di-HMGB1 has a higher binding affinity to TLR4 than monomeric HMGB1 and markedly enhances NF- κ B signaling⁵. A pivotal study by Das et al. elucidated that extracellular HMGB1 binds to TLR5, initiating a proinflammatory signaling cascade via the MyD88-dependent pathway. The C-terminal acidic tail of HMGB1 plays a crucial role in facilitating this binding to TLR5⁵⁹. Both Re- and Ds-HMGB1 have been shown to interact directly with RAGE. Hepatocyte-derived Ds-HMGB1 interacts with RAGE to activate proinflammatory signaling in alcohol-associated liver disease⁶⁰. Extracellular Re-HMGB1 enhances the senescence-associated secretory phenotype (SASP), which includes IL-6, IL-8 and TNF- α , via RAGE-mediated NF- κ B signaling⁶¹. In addition, the HMGB1-IL-1 β complex has been shown to strongly enhance NF- κ B signaling through the activation of extracellular signal-regulated kinase (ERK)1/2 and p38 mitogen-activated protein kinase pathways⁶². Re-HMGB1 forms a hetero-complex with the C-X-C motif chemokine ligand 12 (CXCL12), which subsequently binds to the CXCR4, enhancing chemotactic signaling and promoting inflammatory cell recruitment⁶³. Yanai et al.'s study elucidates the role of HMGB proteins, including HMGB1, as universal sentinels in nucleic acid-mediated innate immune responses. HMGB1 binds to various nucleic acids, such as DNA and RNA, and facilitates their recognition by TLRs, including TLR3, TLR7 and TLR9. This binding enhances the activation of downstream signaling pathways, leading to the production of type I interferons and proinflammatory cytokines⁶⁴. Re-HMGB1 has also been shown to modulate TLR3 signaling pathways. In keratinocytes, pretreatment with Re-HMGB1 attenuates polyinosinic:polycytidylic acid [poly(I:C)]-induced activation of TLR3 signaling⁶⁵. Sosa et al. investigated the immunological factors contributing to varied liver transplant outcomes among female Hispanic patients with non-alcoholic steatohepatitis. The study identified elevated HMGB1 levels and increased TLR4, TLR8 and TLR9 activation in this patient population⁶⁶. Ds-HMGB1 has been identified as the variant that promotes TLR9-mediated inflammatory responses. Ds-HMGB1 engages both the TLR4 and TLR9 pathways, leading to the polarization of macrophages toward a proinflammatory phenotype⁶⁷. HMGB1 in complex with immunogenic DNA or RNA is recognized by cytosolic sensors such as RIG-I, melanoma differentiation-associated gene 5 (MDA5), DNA-dependent activator of interferon regulatory factors (DAI) and absent in melanoma 2 (AIM2), leading to the activation of innate immune responses⁶⁴ (Fig. 4b). Conversely, the interaction between HMGB1 and its receptors has also been implicated in anti-inflammatory signaling. Specifically, its interaction with TIM-3 on dendritic cells (DCs) inhibits the endosomal accumulation of nucleic acids, thereby reducing the immunogenicity of nucleic acids released from dying tumor cells. This mechanism has been associated with diminished efficacy of DNA vaccination and chemotherapy⁶⁸. Furthermore, both LPS and LTA have been shown to induce intermolecular dimerization of HMGB1. The resulting LPS-Di-HMGB1 and LTA-Di-HMGB1 complexes exhibited markedly enhanced proinflammatory activity compared with their respective monomeric counterparts, LPS-HMGB1 and LTA-HMGB1. Notably, these dimeric complexes more effectively amplified TLR4- and TLR2-mediated signaling, respectively,

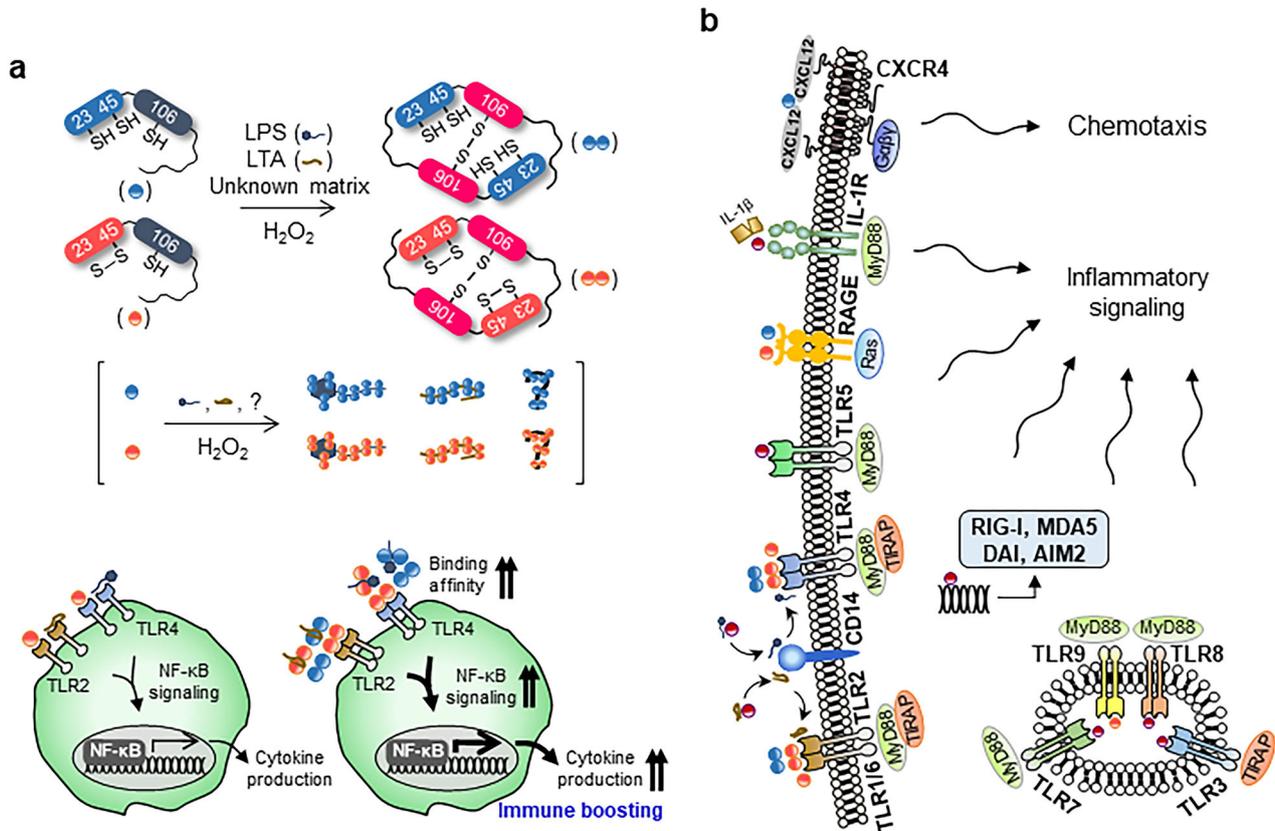


Fig. 4 Extracellular redox regulation of HMGB1 and receptor-mediated immune activation. **a**, Redox state of HMGB1 under different conditions. In the extracellular space, H₂O₂ and bacterial components (LPS and LTA) promote oxidation and spatial proximity of HMGB1, leading to Cys¹⁰⁶-Cys¹⁰⁶ dimerization. Di-HMGB1 enhances binding affinity for TLR2 and TLR4, promoting NF-κB-dependent cytokine production and proinflammatory signaling. Redox-dependent dimerization functions as an immune-boosting mechanism during infection and inflammation. **b**, Extracellular HMGB1 interacts with multiple immune-regulating receptors: TLRs, RAGE, IL-1R and CXCR4. These interactions activate downstream signaling pathways that trigger chemotaxis and inflammatory responses. Cytosolic DNA sensors RIG-I, MDA5, DAI and AIM2 also respond to HMGB1-associated stimuli.

resulting in elevated downstream inflammatory cytokine production⁶. These findings suggest that dimerization of HMGB1 may induce conformational rearrangements that enhance its affinity for bacterial and potentially viral components such as RNA and DNA molecules, thereby facilitating a more stable and efficient transfer of these ligands to their corresponding receptors. This implies that HMGB1 dimerization serves a structural mechanism that potentiates PAMP recognition and amplifies innate immune signaling cascades under oxidative or inflammatory conditions. The redox states of HMGB1 play distinct and critical roles in modulating inflammatory signaling pathways.

HMGB1 AND CELL DEATH

Both Re- and Ds-HMGB1 can be passively released during cell death. Once extracellular, these redox isoforms exert distinct functions, promoting either survival or further cell death. This section examines the mechanisms underlying HMGB1 release and how its redox-dependent functions affect cell fate.

Release of various oxidized forms of HMGB1 by cell death

Necrosis is triggered by stimuli such as hypoxia, DNA damage or alkylating agents. DNA damage activates PARP1, which facilitates the translocation of HMGB1 from the nucleus to the cytoplasm prior to cell death (Fig. 5a). PARP1-deficient cells do not exhibit HMGB1 translocation following DNA damage⁴². Early in necrosis, HMGB1 is primarily released in its reduced form. As necrosis progresses, rising ROS levels oxidize HMGB1⁶⁹. Necroptosis is

initiated by signals such as TNF-α in the presence of caspase inhibitors, including z-VAD-fmk. This triggers the phosphorylation of receptor-interacting serine/threonine kinase (RIPK)1/RIPK3 and the activation of mixed-lineage kinase domain-like pseudokinase (MLKL), resulting in HMGB1 translocation and release⁷⁰. Mitochondria generate ROS when disrupted during necroptosis and further oxidize HMGB1⁷¹. Autophagic cell death, which is often induced by toxins such as diphtheria toxin-EGF in glioblastoma cells, can lead to HMGB1 release without compromising membrane integrity. Knockdown of autophagy genes (*ATG5*, *ATG7* and *ATG12*) inhibits HMGB1 release despite cell death; however, the oxidation state of HMGB1 was not specified⁷². Apoptosis is generally considered immunologically silent. HMGB1 remains sequestered in apoptotic vesicles, preventing its release into the extracellular space⁷³. In addition, apoptosis-associated ROS generation results in HMGB1 hyperoxidation, rendering it immunologically inactive⁴⁹. Ferroptosis, a form of cell death dependent on iron and lipid peroxidation, is induced by type I and type II activators, such as erastin and RSL3, respectively. These inducers promote HMGB1 acetylation and autophagy-mediated release. Deletion of autophagy genes or the use of inhibitors prevents the release of acetylated HMGB1⁷⁴. Pyroptosis is driven by inflammasome-activated caspase-1/11 and gasdermin-mediated pore formation. This process leads to the release of HMGB1 alongside IL-1β and IL-18. Protein kinase R (PKR) is essential for inflammasome activation⁷⁵. Ds-HMGB1 is the predominant form released during pyroptosis^{76,77} (Fig. 5a). PANoptosis is a combined form of pyroptosis, apoptosis and

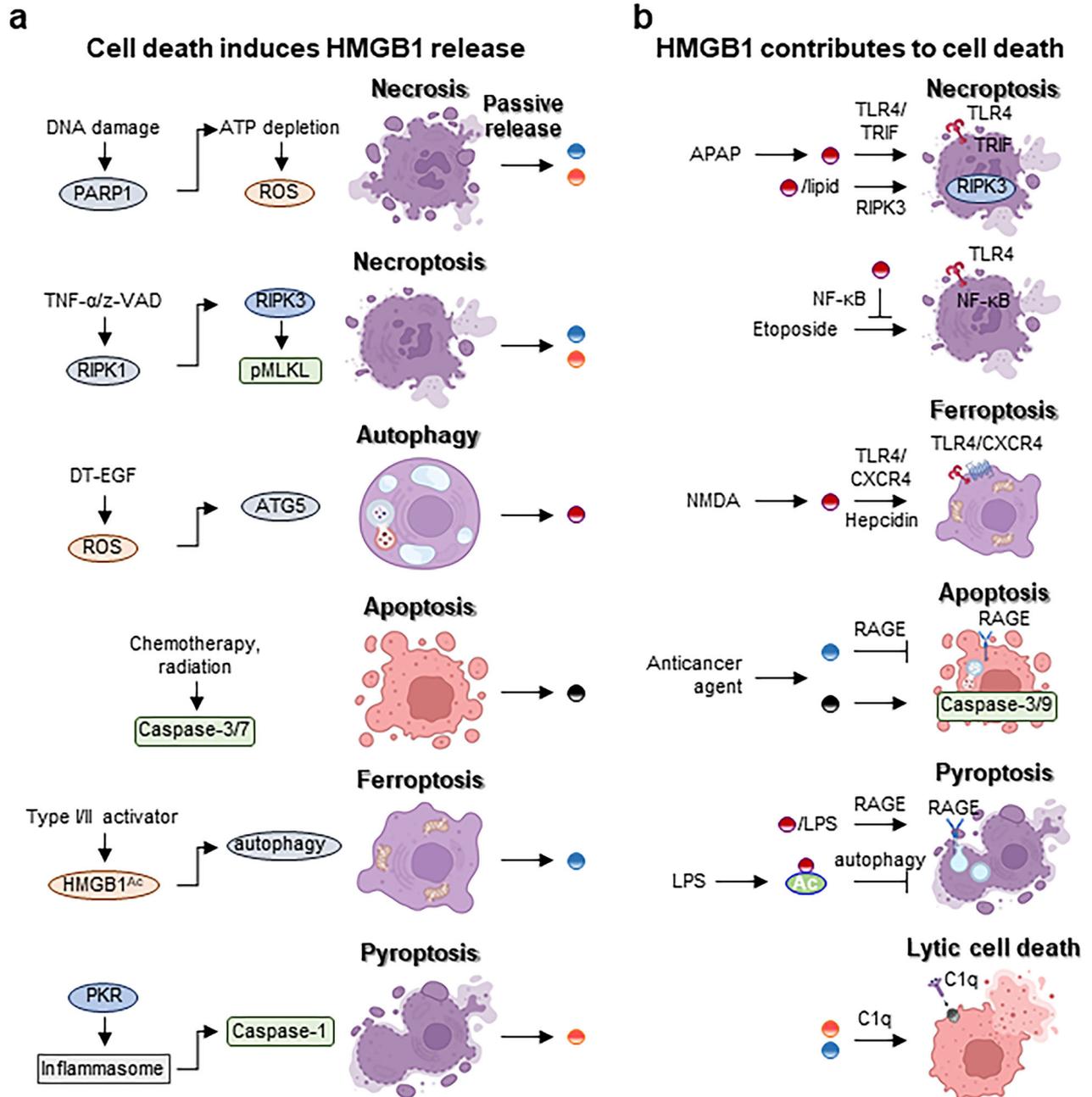


Fig. 5 Bidirectional relationship between HMGB1 and regulated cell death. **a**, Multiple forms of regulated cell death trigger passive release of HMGB1 into the extracellular space. Each cell death involves distinct upstream signals such as PARP1 activation, caspase cleavage, autophagy induction or inflammasome activation. The redox state of released HMGB1 varies according to the type of cell death and oxidative context. **b**, Released HMGB1 modulates various cell death pathways in an autocrine or paracrine manner. HMGB1 amplifies necroptosis via TLR4/TRIF-mediated signaling pathway, and HMGB1–lipid complex engages RIPK3 phosphorylation to promote necroptosis. HMGB1 activates ferroptosis through hepcidin upregulation via TLR4–CXCR4 signaling. Ox-HMGB1 facilitates apoptosis through caspase-3/9 activation, while Re-HMGB1 promotes Beclin-1-dependent autophagy via RAGE. HMGB1 drives pyroptosis through RAGE-mediated endocytosis and caspase-1 activation. In lytic death, both Re- and Ds-HMGB1 activate C1q and complement-mediated MAC deposition. HMGB1^{Ac}, HMGB1 acetylation.

necroptosis triggered during severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection. It results in the release of multiple HMGB1 PTM forms, including Ds-HMGB1⁷⁸. Extracellular HMGB1 binds directly to the SARS-CoV-2 spike protein, causing RAGE-mediated infection by endocytosis⁷⁹.

HMGB1 induces diverse cell death

Under certain extracellular conditions or in specific cell types, HMGB1 also actively contributes to the induction of various forms of cell death

(Fig. 5b). Acetaminophen (*N*-acetyl-*para*-aminophenol, APAP) treatment leads to the release of HMGB1 from necrotic hepatocytes. Extracellular HMGB1 then acts in a feed-forward manner to exacerbate hepatocyte necrosis. In HepaRG cells, HMGB1 induces cell death via TLR4 and its adaptor protein, TIR-domain-containing adapter-inducing interferon- β (TRIF), leading to the activation of RIPK3⁸⁰. In addition, HMGB1 released from damaged or necrotic cells indirectly amplifies injury by recruiting neutrophils. These neutrophils exacerbate tissue damage and necrosis, establishing a vicious cycle of

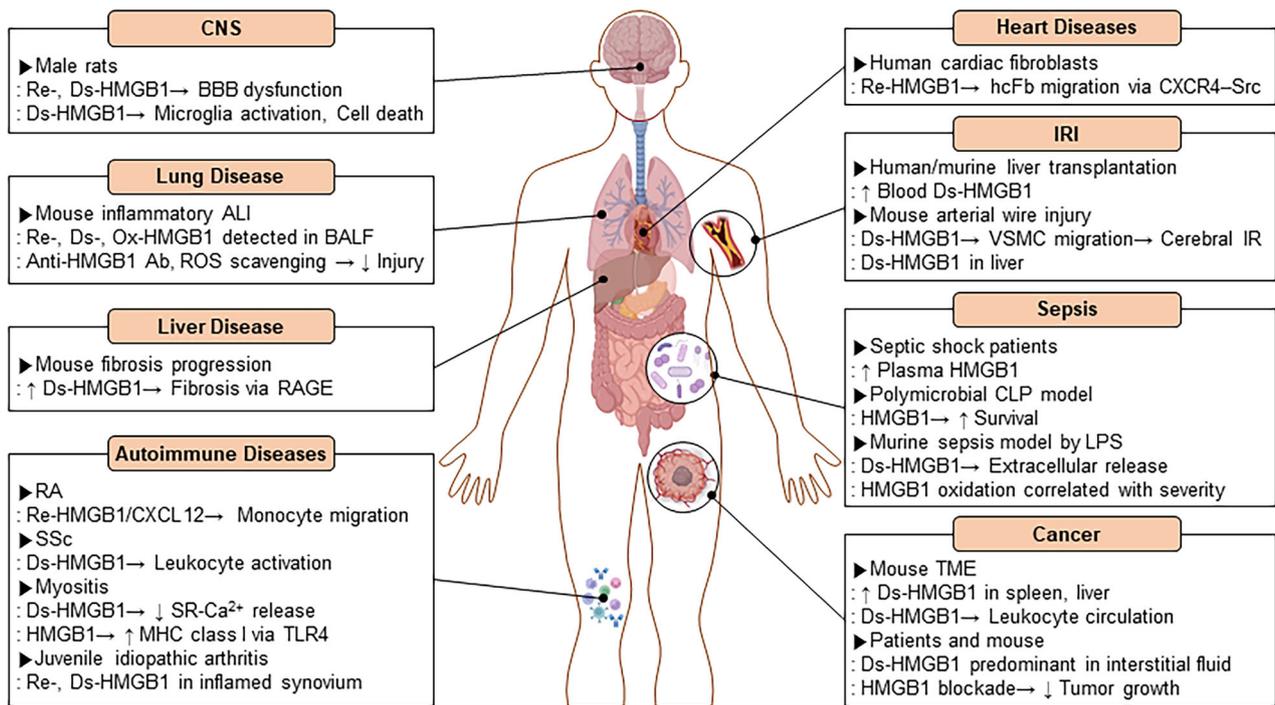


Fig. 6 Disease-specific distribution of HMGB1 redox isoforms in human and experimental models. A schematic of distinct HMGB1 redox forms in various pathological contexts. The redox status of HMGB1 varies spatially and temporally across diseases, modulating immune responses and tissue pathology. CNS, central nervous system; BBB, blood–brain barrier; ALI, acute lung injury; BALF, bronchoalveolar lavage fluid; RA, rheumatoid arthritis; SSc, systemic sclerosis; SR-Ca²⁺, sarcoplasmic reticulum Ca²⁺; hcFb, human cardiac fibroblast; VSMC, vascular smooth muscle cell.

injury amplification⁸¹. Extracellular HMGB1 forms complexes with bacterial lipid components, such as lipid IVa and lipid A, which are recognized and activated through TLR4–TRIF–RIPK3 signaling pathways during MLKL-mediated necroptosis⁸². Conversely, HMGB1 prevents necroptosis via NF- κ B activation in acute myeloid leukemia cells treated with etoposide and z-VAD-fmk, suggesting context-dependent outcomes⁸³. In cerebral ischemia, *N*-methyl-D-aspartate treatment induces HMGB1 secretion, and extracellular HMGB1 activates ferroptosis in astrocytes through TLR4–CXCR4 signaling and hepcidin upregulation. This induces intracellular iron overload, lipid peroxidation and ferroptosis, contributing to neuronal injury⁸⁴. The redox form of HMGB1 determines whether it induces apoptosis or autophagy in cancer cells. Anticancer agents such as melphalan and paclitaxel induce the secretion of Re-HMGB1 and Ox-HMGB1, and Re-HMGB1 binds to RAGE and stimulates Beclin-1-dependent autophagy to promote cell survival. However, Ox-HMGB1 induces intrinsic apoptosis via caspase-3/9 pathways, thereby enhancing treatment-induced cytotoxicity⁸⁵. HMGB1 can either promote or suppress pyroptosis. When HMGB1 binds to LPS, the complex is internalized via a RAGE- and dynamin-dependent pathway. This disrupts lysosomal membranes, allowing LPS to escape into the cytoplasm and activate the noncanonical inflammasome via caspase-11, inducing pyroptosis⁸⁶. In LPS-stimulated RAW264.7 macrophages, cytoplasmic acetylated HMGB1 promotes early autophagy, whereas extracellular HMGB1 induces late-stage pyroptosis⁸⁷. Both Re- and Ds-HMGB1 forms bind to complement component C1q, activating the antibody-independent classical complement cascade and ultimately forming C3a and C5b-9 membrane attack complexes (MACs). These complexes contribute to sterile inflammation and further cell death⁸⁸ (Fig. 5b). Re-HMGB1 induces senescence in fibroblasts through the RAGE-dependent NF- κ B and JAK–STAT signaling pathways⁶¹. Although the redox states of HMGB1 released during different cell death processes are increasingly well defined, the specific redox isoform that induces subsequent cell death is not fully characterized.

HMGB1 balances between cell death and survival

HMGB1 serves as a critical regulator of cell fate, modulating the balance between survival and regulated cell death. Its function is tightly controlled by its redox state, subcellular localization and receptor interactions such as RAGE and TLRs. Under cellular stress, nuclear Re-HMGB1 translocates to the cytoplasm, where it binds to Beclin-1, disrupting the Beclin-1–Bcl-2 complex. This process promotes autophagy, a protective mechanism that preserves mitochondrial integrity, prevents apoptosis and contributes to therapy resistance in cancer cells⁸⁹. In addition, extracellular Re-HMGB1 induces Beclin1-dependent autophagy through RAGE binding, whereas Ox-HMGB1 can promote apoptosis via caspase3/9 intrinsic pathway. Under certain chemotherapeutic conditions, cytosolic HMGB1 shifts from promoting autophagy to directly activating apoptosis when autophagic pathways are overwhelmed⁸⁵. In macrophages, HMGB1 initially promotes autophagy in response to LPS stimulation. However, sustained or excessive stimulation results in the extracellular release of HMGB1, promoting caspase-1-mediated pyroptosis⁸⁷. HMGB1 also plays a pivotal role in modulating the balance between cellular senescence and apoptosis. In B16 mouse melanoma cells treated with doxorubicin, knocking out HMGB1 led to enhanced apoptosis accompanied by decreased p21 expression. Conversely, HMGB1 overexpression shifted the cellular response from apoptosis to senescence, which correlated with increased p21 levels⁹⁰. Thus, depending on cellular context, timing and its redox states, HMGB1 functions as either a survival factor or an inflammatory cell-death trigger.

HMGB1 OXIDATION AND IMMUNE DISORDERS

The redox state of HMGB1 is associated with time and tissues in certain pathological conditions⁹¹. Multiple studies in animal models and clinical settings have demonstrated that specific HMGB1 redox states contribute to disease pathogenesis (Fig. 6). Intracortical injection of Re- and Ds-HMGB1 induces blood–brain

barrier disruption in rats, and Ds-HMGB1 increases microglia activation and apoptosis in the central nervous system⁹². Elevated HMGB1 levels have been observed in traumatic brain injury⁹³, epilepsy⁹⁴, Alzheimer's disease⁹⁵ and Parkinson's disease⁹⁶. Maria Entezari et al. reported that, in hyperoxic lung injury, all redox forms of HMGB1 are present in bronchoalveolar lavage fluid, with Re-HMGB1 predominating extracellularly. Inhibiting HMGB1 secretion or neutralizing anti-HMGB1 antibody mitigates hyperoxia-induced acute lung injury⁹⁷. In carbon tetrachloride-induced liver fibrosis, Ds-HMGB1 levels increase during injury, whereas Ox-HMGB1 levels increase during resolution. Only Ds-HMGB1 drives fibrogenesis via RAGE. In severe acute pancreatitis, Ds-HMGB1 correlates with severe inflammation, gut barrier dysfunction, bacterial translocation and organ damage⁹⁸. Redox regulation of HMGB1 plays an important role in autoimmune diseases. In rheumatoid arthritis, for example, elevated Trx/Trx reductase (TrxR) maintains HMGB1 in its reduced form, thereby enhancing monocyte migration via CXCL12. Blocking Trx or JAK2/COX2 increases HMGB1 oxidation and reduces inflammation⁹⁹. In addition, oxidative stress induces Ds-HMGB1 from platelets, activating neutrophils via TLR4-driven ROS production and autophagy in systemic sclerosis¹⁰⁰. In inflammatory myositis, only Ds-HMGB1, not Re-HMGB1, impairs calcium signaling via TLR4 and induces major histocompatibility complex (MHC) I upregulation in muscle fibers¹⁰¹. Lundbäck et al. demonstrate that Re- and Ds-HMGB1 are prevalent in the inflamed synovial fluid of patients with juvenile idiopathic arthritis¹⁰². HMGB1 has also been associated with cardiac dysfunction. In cardiac fibroblasts, the Re- or '3S' non-oxidizable HMGB1 mutant induces migration via CXCR4 interaction and proto-oncogene tyrosine-protein kinase Src activation, unlike Ds-HMGB1¹⁰³. In human liver transplantation, elevated levels of Ds-HMGB1 in portal blood correlate with the severity of ischemia-reperfusion injury (IRI) and drive TNF- α release via TLR4¹⁰⁴. In murine models, HMGB1 neutralization reduces TLR4-dependent injury in renal and cerebral IRI^{105,106} and Ds-HMGB1 induces smooth muscle migration via TLR4-MyD88/NF- κ B, promoting intimal hyperplasia¹⁰⁷. Following cerebral IRI, Ds-HMGB1 stimulates hepcidin via TLR4/RAGE in the liver, causing iron dysregulation and damage¹⁰⁸. HMGB1 is one of the major inflammatory mediators of sepsis, along with TNF- α , IL-1, IL-6 and IL-8. In septic shock patients, high circulating HMGB1 levels correlate with disease severity, proinflammatory cytokine balance and mortality. Anti-HMGB1 treatment improves survival in models of cecal ligation and puncture¹⁰⁹. In LPS-induced murine sepsis, HMGB1 oxidation mirrors disease severity and correlates with impaired mitochondrial function and increased cytokine release¹¹⁰. HMGB1 redox isoforms play context-dependent roles in cancer. Ds-HMGB1 is closely associated with proinflammatory signaling and promotes tumor growth by encouraging cancer cell proliferation, migration and metastasis. In the TME, Ds-HMGB1 is predominantly enriched in the spleen and liver, whereas its levels are markedly reduced in cachectic skeletal muscle tissues. In addition, Ds-HMGB1 induces leukocyte circulation and infiltration and is the predominant isoform in the interstitial fluids of the TME⁹¹. Blocking HMGB1 drastically reduces tumor growth by decreasing monocytic/granulocytic myeloid-derived suppressor cells and regulatory T lymphocytes. This increases the M1/M2 macrophage ratio and activates DCs and plasmacytoid DCs in the TME¹¹¹ (Fig. 6). Moreover, Di-HMGB1 can be detected not only in the serum but also in the liver tissue of mice following intraperitoneal injection of LPS, as well as in subcutaneous tumor tissues exposed to ultraviolet irradiation⁵⁰. These findings suggest that Di-HMGB1 formation is not confined to a single tissue type but rather occurs in various organs under conditions of elevated ROS. Given its potent proinflammatory activity, Di-HMGB1 may also contribute to the pathogenesis of immune disorders associated with excessive or chronic inflammation. These findings support the possibility that oxidative stress associated with

infection or cellular injury can promote Di-HMGB1 generation across multiple tissues.

CONCLUDING REMARKS AND PERSPECTIVES

HMGB1 is a prototypical DAMP that plays a critical role in inflammation and immune regulation. Its biological functions are governed by subcellular localization and redox status. HMGB1 contains three redox-sensitive cysteine residues that undergo specific oxidative modifications, which enable distinct functional roles, such as maintaining genomic integrity in the nucleus, regulating autophagy and mitochondrial homeostasis in the cytoplasm, and modulating immune responses in the extracellular environment. Re-HMGB1 predominantly facilitates tissue repair via CXCR4 signaling. By contrast, Ds-HMGB1 activates TLRs and RAGE, driving proinflammatory cytokine release. Furthermore, HMGB1 release mode and its redox state are tightly coupled to distinct forms of cell death, including necrosis, necroptosis, autophagy, apoptosis, ferroptosis and pyroptosis. These redox transitions determine the immunological fate of the dying cell and shape the surrounding inflammatory milieu. Pathophysiologically, redox-specific HMGB1 isoforms play different roles in various immune-related disorders. Ds-HMGB1 is associated with chronic inflammation, cytokine storms and disease exacerbation in conditions such as sepsis, autoimmune diseases, neuroinflammation and cancer. This emerging HMGB1 dimerization mechanism represents a poorly explored yet potentially significant mode of redox-dependent immunoregulation, particularly in oxidative microenvironments associated with infection and tissue injury. In vitro experiments demonstrated that an increase in ROS levels induces conformational changes of Re-HMGB1 to Ds-HMGB1 and Di-HMGB1. Furthermore, in vivo studies confirmed the presence of both Ds-HMGB1 and Di-HMGB1 in ROS-inducing conditions, such as LPS injection or ultraviolet irradiation. However, the oxidation threshold of Ds-HMGB1 and Di-HMGB1 in the parenchyma or interstitial fluid across time and space in pathological conditions remains uncharacterized. The variation in HMGB1 oxidation states in specific tissues and conditions is likely to provide crucial insights into the underlying mechanisms of HMGB1-mediated inflammation.

REFERENCES

- Vénéreau, E., Ceriotti, C. & Bianchi, M. E. DAMPs from cell death to new life. *Front. Immunol.* **6**, 422 (2015).
- Chen, G. Y. & Nuñez, G. Sterile inflammation: sensing and reacting to damage. *Nat. Rev. Immunol.* **10**, 826–837 (2010).
- Chen, F. et al. DAMPs in immunosenescence and cancer. *Semin. Cancer Biol.* **106–107**, 123–142 (2024).
- Kono, H. & Rock, K. L. How dying cells alert the immune system to danger. *Nat. Rev. Immunol.* **8**, 279–289 (2008).
- Mogensen, T. H. Pathogen recognition and inflammatory signaling in innate immune defenses. *Clin. Microbiol. Rev.* **22**, 240–273 (2009).
- Kwak, M. S. et al. ROS anchor PAMPs-mediated extracellular HMGB1 self-association and its dimerization enhances pro-inflammatory signaling. *Redox Biol.* **80**, 103521 (2025).
- Huang, Y., Jiang, W. & Zhou, R. DAMP sensing and sterile inflammation: intracellular, intercellular and inter-organ pathways. *Nat. Rev. Immunol.* **24**, 703–719 (2024).
- Rock, K. L., Latz, E., Ontiveros, F. & Kono, H. The sterile inflammatory response. *Annu. Rev. Immunol.* **28**, 321–342 (2010).
- Kiriakidou, M. & Ching, C. L. Systemic lupus erythematosus. *Ann. Intern. Med.* **172**, Itc81–itc96 (2020).
- Hotamisligil, G. S. Inflammation and metabolic disorders. *Nature* **444**, 860–867 (2006).
- Castro-Gomez, S. & Heneka, M. T. Innate immune activation in neurodegenerative diseases. *Immunity* **57**, 790–814 (2024).
- Zitvogel, L., Kepp, O. & Kroemer, G. Immune parameters affecting the efficacy of chemotherapeutic regimens. *Nat. Rev. Clin. Oncol.* **8**, 151–160 (2011).

13. Lee, S. A., Kwak, M. S., Kim, S. & Shin, J. S. The role of high mobility group box 1 in innate immunity. *Yonsei Med. J.* **55**, 1165–1176 (2014).
14. Kwak, M. S. et al. Immunological significance of HMGB1 post-translational modification and redox biology. *Front. Immunol.* **11**, 1189 (2020).
15. Goodwin, G. H. & Johns, E. W. Isolation and characterisation of two calf-thymus chromatin non-histone proteins with high contents of acidic and basic amino acids. *Eur. J. Biochem.* **40**, 215–219 (1973).
16. Wang, H. et al. HMG-1 as a late mediator of endotoxin lethality in mice. *Science* **285**, 248–251 (1999).
17. Scaffidi, P., Misteli, T. & Bianchi, M. E. Release of chromatin protein HMGB1 by necrotic cells triggers inflammation. *Nature* **418**, 191–195 (2002).
18. Herb, M. & Schramm, M. Functions of ROS in macrophages and antimicrobial immunity. *Antioxidants* **10**, 313 (2021).
19. Canton, M. et al. Reactive oxygen species in macrophages: sources and targets. *Front. Immunol.* **12**, 734229 (2021).
20. Liu, J. et al. Reactive oxygen species (ROS) scavenging biomaterials for anti-inflammatory diseases: from mechanism to therapy. *J. Hematol. Oncol.* **16**, 116 (2023).
21. Vlahopoulos, S., Boldogh, I., Casola, A. & Brasier, A. R. Nuclear factor-kappaB-dependent induction of interleukin-8 gene expression by tumor necrosis factor alpha: evidence for an antioxidant sensitive activating pathway distinct from nuclear translocation. *Blood* **94**, 1878–1889 (1999).
22. Hedrick, E., Mohankumar, K., Lacey, A. & Safe, S. Inhibition of NR4A1 promotes ROS accumulation and IL24-Dependent growth arrest in Rhabdomyosarcoma. *Mol. Cancer Res.* **17**, 2221–2232 (2019).
23. Thiele, G. M. et al. Malondialdehyde-acetaldehyde adducts and anti-malondialdehyde-acetaldehyde antibodies in rheumatoid arthritis. *Arthritis Rheumatol.* **67**, 645–655 (2015).
24. Griess, B., Mir, S., Datta, K. & Teoh-Fitzgerald, M. Scavenging reactive oxygen species selectively inhibits M2 macrophage polarization and their pro-tumorigenic function in part, via Stat3 suppression. *Free Radic. Biol. Med.* **147**, 48–60 (2020).
25. Li, J. et al. Structural basis for the proinflammatory cytokine activity of high mobility group box 1. *Mol. Med.* **9**, 37–45 (2003).
26. Gong, W., Li, Y., Chao, F., Huang, G. & He, F. Amino acid residues 201–205 in C-terminal acidic tail region plays a crucial role in antibacterial activity of HMGB1. *J. Biomed. Sci.* **16**, 83 (2009).
27. Stros, M. DNA bending by the chromosomal protein HMG1 and its high mobility group box domains. Effect of flanking sequences. *J. Biol. Chem.* **273**, 10355–10361 (1998).
28. Stros, M. HMGB proteins: interactions with DNA and chromatin. *Biochim. Biophys. Acta* **1799**, 101–113 (2010).
29. Sims, G. P., Rowe, D. C., Rietdijk, S. T., Herbst, R. & Coyle, A. J. HMGB1 and RAGE in inflammation and cancer. *Annu. Rev. Immunol.* **28**, 367–388 (2010).
30. Youn, J. H., Oh, Y. J., Kim, E. S., Choi, J. E. & Shin, J. S. High mobility group box 1 protein binding to lipopolysaccharide facilitates transfer of lipopolysaccharide to CD14 and enhances lipopolysaccharide-mediated TNF-alpha production in human monocytes. *J. Immunol.* **180**, 5067–5074 (2008).
31. Youn, J. H. et al. Identification of lipopolysaccharide-binding peptide regions within HMGB1 and their effects on subclinical endotoxemia in a mouse model. *Eur. J. Immunol.* **41**, 2753–2762 (2011).
32. Kwak, M. S. et al. Peroxiredoxin-mediated disulfide bond formation is required for nucleocytoplasmic translocation and secretion of HMGB1 in response to inflammatory stimuli. *Redox Biol.* **24**, 101203 (2019).
33. Yang, K. et al. Lactate promotes macrophage HMGB1 lactylation, acetylation, and exosomal release in polymicrobial sepsis. *Cell Death Differ.* **29**, 133–146 (2022).
34. Yang, R. et al. Posttranslational S-nitrosylation modification regulates HMGB1 secretion and promotes its proinflammatory and neurodegenerative effects. *Cell Rep.* **40**, 111330 (2022).
35. Youn, J. H. & Shin, J. S. Nucleocytoplasmic shuttling of HMGB1 is regulated by phosphorylation that redirects it toward secretion. *J. Immunol.* **177**, 7889–7897 (2006).
36. Oh, Y. J. et al. HMGB1 is phosphorylated by classical protein kinase C and is secreted by a calcium-dependent mechanism. *J. Immunol.* **182**, 5800–5809 (2009).
37. Oh, Y. J. et al. CKD712, (S)-1-(alpha-naphthylmethyl)-6,7-dihydroxy-1,2,3,4-tetrahydroisoquinoline, inhibits the lipopolysaccharide-stimulated secretion of HMGB1 by inhibiting PI3K and classical protein kinase C. *Int. Immunopharmacol.* **11**, 1160–1165 (2011).
38. Zhang, X. et al. Calcium/calmodulin-dependent protein kinase (CaMK) IV mediates nucleocytoplasmic shuttling and release of HMGB1 during lipopolysaccharide stimulation of macrophages. *J. Immunol.* **181**, 5015–5023 (2008).
39. Bonaldi, T. et al. Monocytic cells hyperacetylate chromatin protein HMGB1 to redirect it towards secretion. *EMBO J.* **22**, 5551–5560 (2003).
40. Kim, Y. H. et al. N-linked glycosylation plays a crucial role in the secretion of HMGB1. *J. Cell Sci.* **129**, 29–38 (2016).
41. Ito, I., Fukazawa, J. & Yoshida, M. Post-translational methylation of high mobility group box 1 (HMGB1) causes its cytoplasmic localization in neutrophils. *J. Biol. Chem.* **282**, 16336–16344 (2007).
42. Ditsworth, D., Zong, W. X. & Thompson, C. B. Activation of poly(ADP)-ribose polymerase (PARP-1) induces release of the pro-inflammatory mediator HMGB1 from the nucleus. *J. Biol. Chem.* **282**, 17845–17854 (2007).
43. Shin, J. et al. USP13 regulates HMGB1 stability and secretion through its deubiquitinase activity. *Mol. Med.* **28**, 164 (2022).
44. Li, H. et al. Deubiquitylase USP12 induces pro-survival autophagy and bortezomib resistance in multiple myeloma by stabilizing HMGB1. *Oncogene* **41**, 1298–1308 (2022).
45. Yao, W. et al. Epigenetic regulator KDM4D restricts tumorigenesis via modulating SYVN1/HMGB1 ubiquitination axis in esophageal squamous cell carcinoma. *Front. Oncol.* **11**, 761346 (2021).
46. Du, J. et al. Ubiquitination of cytoplasmic HMGB1 by RNF186 regulates hepatic lipophagy in non-alcoholic fatty liver disease. *Metabolism* **152**, 155769 (2024).
47. Kim, Y. H. et al. Secretory autophagy machinery and vesicular trafficking are involved in HMGB1 secretion. *Autophagy* **17**, 2345–2362 (2021).
48. Pirani, E. et al. Tumor cells express and maintain HMGB1 in the reduced isoform to enhance CXCR4-mediated migration. *Front. Immunol.* **15**, 1358800 (2024).
49. Kazama, H. et al. Induction of immunological tolerance by apoptotic cells requires caspase-dependent oxidation of high-mobility group box-1 protein. *Immunity* **29**, 21–32 (2008).
50. Kwak, M. S. et al. Reactive oxygen species induce Cys106-mediated anti-parallel HMGB1 dimerization that protects against DNA damage. *Redox Biol.* **40**, 101858 (2021).
51. Lee, J. J. et al. HMGB1 orchestrates STING-mediated senescence via TRIM30A modulation in cancer cells. *Cell Death Discov.* **7**, 28 (2021).
52. Kang, R. et al. Metabolic regulation by HMGB1-mediated autophagy and mitophagy. *Autophagy* **7**, 1256–1258 (2011).
53. Zhang, S. et al. Recombinant high-mobility group box 1 (rHMGB1) promotes NRF2-independent mitochondrial fusion through CXCR4/PSMB5-mediated Drp1 degradation in endothelial cells. *Oxid. Med. Cell. Longev.* **2021**, 9993240 (2021).
54. Han, J. Y., Rhee, W. J. & Shin, J. S. Cytoplasmic HMGB1 promotes the activation of JAK2–STAT3 signaling and PD-L1 expression in breast cancer. *Mol. Med.* **31**, 197 (2025).
55. Min, H. J. et al. Chaperone-like activity of high-mobility group box 1 protein and its role in reducing the formation of polyglutamine aggregates. *J. Immunol.* **190**, 1797–1806 (2013).
56. Kwak, M. S. et al. HMGB1 binds to lipoteichoic acid and enhances TNF- α and IL-6 production through HMGB1-mediated transfer of lipoteichoic acid to CD14 and TLR2. *J. Innate Immun.* **7**, 405–416 (2015).
57. Ved, R. et al. Disulfide HMGB1 acts via TLR2/4 receptors to reduce the numbers of oligodendrocyte progenitor cells after traumatic injury in vitro. *Sci. Rep.* **11**, 6181 (2021).
58. Yang, H. et al. MD-2 is required for disulfide HMGB1-dependent TLR4 signaling. *J. Exp. Med.* **212**, 5–14 (2015).
59. Das, N. et al. HMGB1 activates proinflammatory signaling via TLR5 leading to allodynia. *Cell Rep.* **17**, 1128–1140 (2016).
60. Ge, X. et al. A protein complex of liver origin activates a pro-inflammatory program that drives hepatic and intestinal injury in alcohol-associated liver disease. *Cell. Mol. Gastroenterol. Hepatol.* **18**, 101362 (2024).
61. Shin, J. W. et al. Propagation of senescent phenotypes by extracellular HMGB1 is dependent on its redox state. *Metabolism* **168**, 156259 (2025).
62. García-Armandis, I. et al. High mobility group box 1 potentiates the pro-inflammatory effects of interleukin-1 β in osteoarthritic synoviocytes. *Arthritis Res. Ther.* **12**, R165 (2010).
63. Schiraldi, M. et al. HMGB1 promotes recruitment of inflammatory cells to damaged tissues by forming a complex with CXCL12 and signaling via CXCR4. *J. Exp. Med.* **209**, 551–563 (2012).
64. Yanai, H. et al. HMGB proteins function as universal sentinels for nucleic-acid-mediated innate immune responses. *Nature* **462**, 99–103 (2009).
65. Mori, H. et al. Reduced-HMGB1 suppresses poly(I:C)-induced inflammation in keratinocytes. *J. Dermatol. Sci.* **90**, 154–165 (2018).
66. Sosa, R. A. et al. Immune features of Disparate Liver transplant outcomes in female hispanics with nonalcoholic steatohepatitis. *Transplant. Direct* **9**, e1550 (2023).
67. Terry, A. Q. et al. Disulfide-HMGB1 signals through TLR4 and TLR9 to induce inflammatory macrophages capable of innate-adaptive crosstalk in human liver transplantation. *Am. J. Transplant.* **23**, 1858–1871 (2023).
68. Chiba, S. et al. Tumor-infiltrating DCs suppress nucleic acid-mediated innate immune responses through interactions between the receptor TIM-3 and the alarmin HMGB1. *Nat. Immunol.* **13**, 832–842 (2012).

69. Urbonaviciute, V. et al. Oxidation of the alarmin high-mobility group box 1 protein (HMGB1) during apoptosis. *Autoimmunity* **42**, 305–307 (2009).
70. Yu, C. et al. Role of HMGB1 in TNF- α combined with Z-VAD-fmk-induced L929 cells necroptosis. *Biochem. Genet.* **60**, 598–610 (2022).
71. Kaczmarek, A., Vandenabeele, P. & Krysko, D. V. Necroptosis: the release of damage-associated molecular patterns and its physiological relevance. *Immunity* **38**, 209–223 (2013).
72. Thorburn, J. et al. Autophagy regulates selective HMGB1 release in tumor cells that are destined to die. *Cell Death Differ.* **16**, 175–183 (2009).
73. Schiller, M. et al. During apoptosis HMGB1 is translocated into apoptotic cell-derived membranous vesicles. *Autoimmunity* **46**, 342–346 (2013).
74. Wen, Q., Liu, J., Kang, R., Zhou, B. & Tang, D. The release and activity of HMGB1 in ferroptosis. *Biochem. Biophys. Res. Commun.* **510**, 278–283 (2019).
75. Lu, B. et al. Novel role of PKR in inflammasome activation and HMGB1 release. *Nature* **488**, 670–674 (2012).
76. Yang, H., Wang, H., Chavan, S. S. & Andersson, U. High mobility group box protein 1 (HMGB1): the prototypical endogenous danger molecule. *Mol. Med.* **21**, S6–S12 (2015).
77. Murao, A., Aziz, M., Wang, H., Brenner, M. & Wang, P. Release mechanisms of major DAMPs. *Apoptosis* **26**, 152–162 (2021).
78. Kwak, M. S. et al. SARS-CoV-2 infection induces HMGB1 secretion through post-translational modification and PANoptosis. *Immune Netw.* **23**, e26 (2023).
79. Jung, S. F. et al. Direct interaction of HMGB1 with SARS-CoV-2 facilitates its infection via RAGE-dependent endocytosis. *iScience* **28**, 113063 (2025).
80. Minsart, C. et al. New insights in acetaminophen toxicity: HMGB1 contributes by itself to amplify hepatocyte necrosis in vitro through the TLR4-TRIF-RIPK3 axis. *Sci. Rep.* **10**, 5557 (2020).
81. Huebener, P. et al. The HMGB1/RAGE axis triggers neutrophil-mediated injury amplification following necrosis. *J. Clin. Invest.* **125**, 539–550 (2015).
82. Meng, R. et al. High mobility group box 1 enables bacterial lipids to trigger receptor-interacting protein kinase 3 (RIPK3)-mediated necroptosis and apoptosis in mice. *J. Biol. Chem.* **294**, 8872–8884 (2019).
83. Liu, Y. et al. Extracellular HMGB1 prevents necroptosis in acute myeloid leukemia cells. *Biomed. Pharmacother.* **112**, 108714 (2019).
84. Davaanyam, D. et al. HMGB1 induces hepcidin upregulation in astrocytes and causes an acute iron surge and subsequent ferroptosis in the postischemic brain. *Exp. Mol. Med.* **55**, 2402–2416 (2023).
85. Tang, D. et al. HMGB1 release and redox regulates autophagy and apoptosis in cancer cells. *Oncogene* **29**, 5299–5310 (2010).
86. Deng, M. et al. The endotoxin delivery protein HMGB1 mediates caspase-11-dependent lethality in sepsis. *Immunity* **49**, 740–753 (2018).
87. Shang, J. et al. HMGB1 mediates lipopolysaccharide-induced macrophage autophagy and pyroptosis. *BMC Mol. Cell Biol.* **24**, 2 (2023).
88. Kim, S. Y. et al. High-mobility group box 1-induced complement activation causes sterile inflammation. *Front. Immunol.* **9**, 705 (2018).
89. Tang, D. et al. Endogenous HMGB1 regulates autophagy. *J. Cell Biol.* **190**, 881–892 (2010).
90. Lee, J. J., Park, I. H., Rhee, W. J., Kim, H. S. & Shin, J. S. HMGB1 modulates the balance between senescence and apoptosis in response to genotoxic stress. *FASEB J.* **33**, 10942–10953 (2019).
91. Ferrara, M. et al. Oxidation of HMGB1 is a dynamically regulated process in physiological and pathological conditions. *Front. Immunol.* **11**, 1122 (2020).
92. Aucott, H. et al. Neuroinflammation in response to intracerebral injections of different HMGB1 Redox Isoforms. *J. Innate Immun.* **10**, 215–227 (2018).
93. Okuma, Y. et al. Anti-high mobility group box-1 antibody therapy for traumatic brain injury. *Ann. Neurol.* **72**, 373–384 (2012).
94. Fu, L. et al. Therapeutic effects of anti-HMGB1 monoclonal antibody on pilocarpine-induced status epilepticus in mice. *Sci. Rep.* **7**, 1179 (2017).
95. Fujita, K. et al. HMGB1, a pathogenic molecule that induces neurite degeneration via TLR4-MARCKS, is a potential therapeutic target for Alzheimer's disease. *Sci. Rep.* **6**, 31895 (2016).
96. Sasaki, T. et al. Anti-high mobility group box 1 antibody exerts neuroprotection in a rat model of Parkinson's disease. *Exp. Neurol.* **275**, 220–231 (2016).
97. Entezari, M. et al. Inhibition of extracellular HMGB1 attenuates hyperoxia-induced inflammatory acute lung injury. *Redox Biol.* **2**, 314–322 (2014).
98. Yang, R., Tenhunen, J. & Tonnessen, T. I. HMGB1 and Histones play a significant role in inducing systemic inflammation and multiple organ dysfunctions in severe acute pancreatitis. *Int. J. Inflamm.* **2017**, 1817564 (2017).
99. Cecchinato, V. et al. Redox-mediated mechanisms Fuel Monocyte responses to CXCL12/HMGB1 in active rheumatoid arthritis. *Front. Immunol.* **9**, 2118 (2018).
100. Maugeri, N. et al. Oxidative stress elicits platelet/leukocyte inflammatory interactions via HMGB1: a candidate for microvessel injury in systemic sclerosis. *Antioxid. Redox Signal.* **20**, 1060–1074 (2014).
101. Zong, M. et al. TLR4 as receptor for HMGB1 induced muscle dysfunction in myositis. *Ann. Rheum. Dis.* **72**, 1390–1399 (2013).
102. Lundbäck, P. et al. Characterization of the inflammatory properties of actively released HMGB1 in juvenile idiopathic arthritis. *Antioxid. Redox Signal.* **24**, 605–619 (2016).
103. Di Maggio, S. et al. Non-oxidizable HMGB1 induces cardiac fibroblasts migration via CXCR4 in a CXCL12-independent manner and worsens tissue remodeling after myocardial infarction. *Biochim. Biophys. Acta Mol. Basis Dis.* **1863**, 2693–2704 (2017).
104. Sosa, R. A. et al. Disulfide high-mobility group box 1 drives Ischemia-Reperfusion injury in human liver transplantation. *Hepatology* **73**, 1158–1175 (2021).
105. Wu, H. et al. HMGB1 contributes to kidney ischemia reperfusion injury. *J. Am. Soc. Nephrol.* **21**, 1878–1890 (2010).
106. Wang, C. et al. Inhibiting HMGB1 reduces cerebral ischemia reperfusion injury in diabetic mice. *Inflammation* **39**, 1862–1870 (2016).
107. Cai, J. et al. HMGB1-driven inflammation and intimal Hyperplasia after arterial injury involves cell-specific actions mediated by TLR4. *Arterioscler. Thromb. Vasc. Biol.* **35**, 2579–2593 (2015).
108. Davaanyam, D., Seol, S. I., Oh, S. A., Lee, H. & Lee, J. K. Hepatocyte activation and liver injury following cerebral ischemia promote HMGB1-mediated hepcidin upregulation in hepatocytes and regulation of systemic iron levels. *Exp. Mol. Med.* **56**, 2171–2183 (2024).
109. Stevens, N. E. et al. Therapeutic targeting of HMGB1 during experimental sepsis modulates the inflammatory cytokine profile to one associated with improved clinical outcomes. *Sci. Rep.* **7**, 5850 (2017).
110. Abdulmahdi, W. et al. HMGB1 redox during sepsis. *Redox Biol.* **13**, 600–607 (2017).
111. Hubert, P. et al. Extracellular HMGB1 blockade inhibits tumor growth through profoundly remodeling immune microenvironment and enhances checkpoint inhibitor-based immunotherapy. *J. Immunother. Cancer* **9**, e001966 (2021).

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COMPETING INTERESTS

The authors declare no competing interests.

ADDITIONAL INFORMATION

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