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Review article

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The multifunctional protein HMGB1: 50 years of discovery

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Abstract

Fifty years since the initial discovery of HMGB1 in 1973 as a structural protein of chromatin, HMGB1 is now known to regulate diverse biological processes depending on its subcellular or extracellular localization. These functions include promoting DNA damage repair in the nucleus, sensing nucleic acids and inducing innate immune responses and autophagy in the cytosol and binding protein partners in the extracellular environment and stimulating immunoreceptors. In addition, HMGB1 is a broad sensor of cellular stress that balances cell death and survival responses essential for cellular homeostasis and tissue maintenance. HMGB1 is also an important mediator secreted by immune cells that is involved in a range of pathological conditions, including infectious diseases, ischaemia–reperfusion injury, autoimmunity, cardiovascular and neurodegenerative diseases, metabolic disorders and cancer. In this Review, we discuss the signalling mechanisms, cellular functions and clinical relevance of HMGB1 and describe strategies to modify its release and biological activities in the setting of various diseases.

Sections

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Introduction

An identifying characteristic of eukaryotic cells is the presence of a nucleus that stores and segregates the genetic material. In 1973, high-mobility group (HMG) proteins (HMG-1 and HMG-2) were first identified in calf thymus as a class of nonhistone proteins that contribute to packaging DNA into chromosomes[1] (Fig. 1). The phrase 'high-mobility group' was a description of the high electrophoretic mobility of the proteins in polyacrylamide gels. Currently, the HMG Chromosomal Protein Nomenclature Committee classifies HMG proteins into three subfamilies — HMGA (formerly HMG-14 and HMG-17), HMGB (formerly HMG-1 and HMG-2) and HMGN (formerly HMG-I and HMG-Y) — on the basis of their characteristic functional sequence motifs. Many of the HMG proteins are now known to have disparate functions in different locations, such as in the cell membrane, cytosol or nucleus. The most extensively studied HMG family member, HMGB1 (formerly known as HMG-1, amphoterin or SBP-1), is involved in many important physiological and pathological processes.

The *HMGB1* gene has evolved over 525 million years during the development of multicellular animals[2]. The HMGB1 protein is highly conserved, having 99% homology between rodents and humans. Embryonic global deletion of *Hmgb1* or conditional deletion of uterine *Hmgb1* in mice is lethal embryonically or shortly after birth or causes implantation defects, respectively, which indicates a crucial role of HMGB1 in development and reproduction[3,4]. In addition to its architectural function in the nucleus, HMGB1 is also present in the cytosol and can be secreted or released from cells (Box 1). Cytosolic HMGB1 has a major role as a sensor for nucleic acid-induced immune responses[5] and can also function as an endogenous promoter of autophagy[6]. In the extracellular space, HMGB1 is a key immune mediator and functions as a ubiquitous damage-associated molecular pattern (DAMP) during cell death and tissue injury[7]. The release and activity of HMGB1 in sterile inflammation and infection are further regulated by its post-translational modification and by the diversity of receptors to which HMGB1 can bind (Fig. 2). Toll-like receptor 4 (TLR4) and the advanced glycosylation end product-specific receptor (AGER; also known as RAGE) are the most well-studied receptors for HMGB1, binding of which leads to the activation of downstream signalling pathways through nuclear factor-κB (NF-κB) and interferon regulatory factor 3 (IRF3) and the production of immune mediators including cytokines and chemokines. HMGB1 protein has a higher affinity for TLR4 than for AGER[8–11]. However, it should be noted that recombinant HMGB1 protein is commonly purified from *Escherichia coli* and thus may be contaminated with the TLR4 ligand bacterial lipopolysaccharide (LPS); it is therefore controversial whether HMGB1 alone directly binds to TLR4.

Fifty years since the discovery of HMG proteins, an extensive literature on the roles of HMGB1 in immune responses has accumulated. Here, we discuss three related topics. First, we introduce the subcellular localization-dependent functions of HMGB1. Second, we discuss the roles of HMGB1 in immune responses associated with microbial infection, sterile inflammation, ageing and cancer. Third, we summarize potential uses of HMGB1 as a biomarker and drug target for human disease. Our accumulated knowledge of the immune functions of HMGB1 contributes to a broader understanding of the DAMP hypothesis and to developing effective strategies to prevent and treat inflammation-related diseases[12].

Subcellular functions of HMGB1

The subcellular localization of HMGB1 has a key role in determining its function and its interaction with other cellular components. Here, we summarize the functions of HMGB1 in the nucleus, cytosol, cell membrane and extracellular space.

Nuclear HMGB1

HMGB1 is mainly present in the nucleus under physiological conditions and this was the focus of early research[13]. By binding to both DNA and histones, HMGB1 helps to maintain the structure of nucleosomes and limits access of mutagens and photons to DNA, thereby preventing DNA damage. In addition, HMGB1 modulates the strength of the histone–DNA interaction and thereby influences the packaging of DNA into chromatin. By facilitating nucleosome sliding — a mechanism by which DNA can be exposed for access by the transcriptional machinery — HMGB1 can regulate gene expression.

Human HMGB1 is a 215-amino-acid protein consisting of three domains: two positively charged DNA-binding domains (A-box and B-box) and a negatively charged C terminus (acidic tail) (Fig. 3). The A-box (residues 9–79) and B-box (residues 95–163) recognize specific DNA structures (for example, four-way junctions[14] and cisplatin-modified DNA duplexes[15]), rather than specific DNA sequences, unlike transcription factors. As a DNA chaperone, nuclear HMGB1 binds to the minor groove of linear DNA and bends it into a helical structure, which can affect the accessibility of DNA to cellular factors involved in DNA replication, transcription, DNA repair, chromatin remodelling and V(D)J recombination within T cells and B cells[16,17]. The C-terminal acidic tail (residues 186–215), containing aspartic acid or glutamic acid residues, may compete with DNA for binding to A-box and B-box domains of HMGB1, thus mediating autoinhibition under conditions of cellular or nuclear stress[18]. A frameshift variant in the acidic tail of HMGB1, which enhances its accumulation in the nucleolus and leads to nucleolar dysfunction, is found in humans with the rare congenital disorder of brachyphalangy, polydactyly and tibial aplasia syndrome[19]. In addition, heterozygous *HMGB1* loss-of-function variants are associated with a 13q12.3 microdeletion syndrome characterized by intellectual disability, microcephaly and atopic dermatitis[20]. These studies provide direct evidence that mutations affecting the nuclear functions of HMGB1 can cause human disease.

HMGB1 is a redox protein containing three cysteines (C23 and C45 in A-box and C106 in B-box)[21]. The switch between reduced and oxidized forms of A-box affects the DNA-binding activity of HMGB1, with oxidized forms having reduced binding to platinized DNA (DNA treated with platinum-based drugs such as cisplatin)[22]. HMGB1 can also cooperate with histone H1 to promote chromatin remodelling and transcriptional silencing during endotoxin tolerance in vitro[23]. Functionally, the conditional depletion of *Hmgb1* in mouse cells or tissues leads to genomic instability, telomere shortening and the release of nucleosomes, which induce inflammation and activate innate immunity[24,25]. An increase in circulating nucleosomes is associated with immunopathological processes that can lead to lethal infections, autoimmune diseases and tissue damage[26,27]. These findings establish a crucial function of nuclear HMGB1 in maintaining nuclear homeostasis and controlling the release of nuclear DAMPs. It will also be important to further understand the complementary and unique functions of the other HMG family members.

Extracellular HMGB1

In 1999, HMGB1 was identified as a mediator of endotoxin lethality in mice[28], opening a new chapter in the study of the extracellular functions of HMGB1. This study showed that LPS triggers mouse macrophages or human primary peripheral blood mononuclear cells to secrete HMGB1

in a delayed manner that differs from the earlier kinetics of cytokine release[28], which offered promise for expanding the time window for the treatment of patients with sepsis by using HMGB1 antagonists.

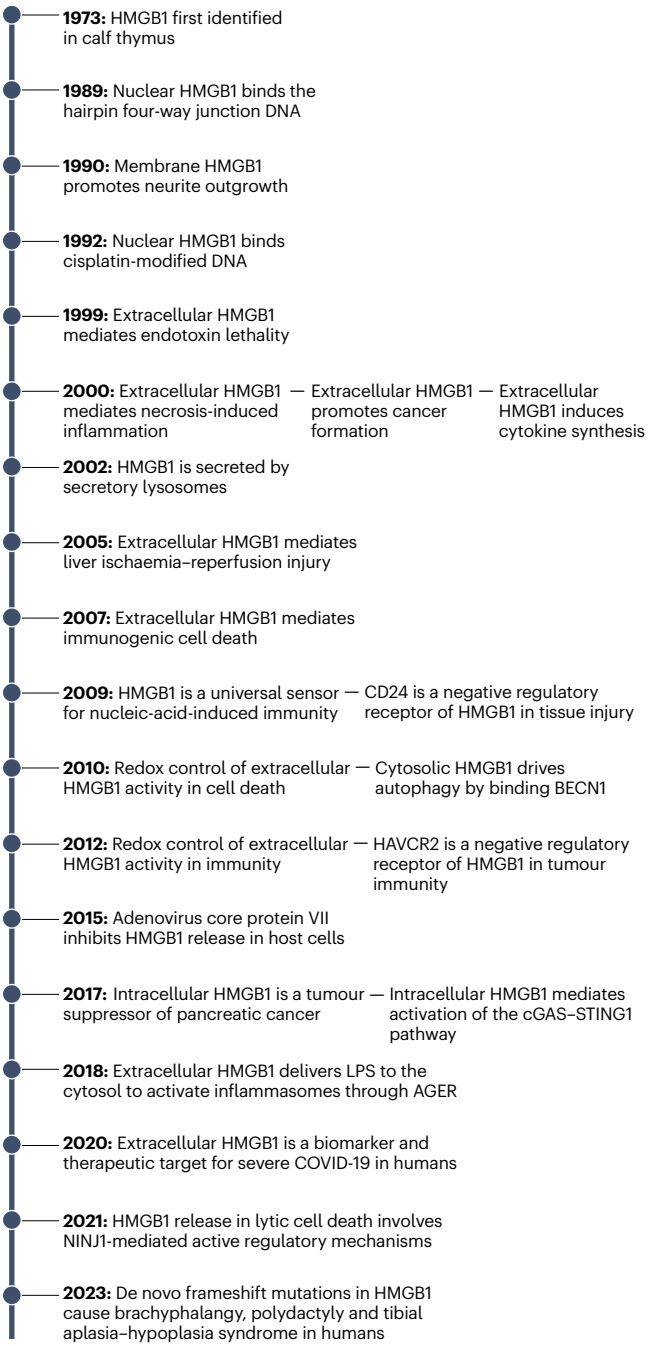


Fig. 1 | Timeline of breakthrough discoveries since HMGB1 was first identified. Key milestones are indicated that have contributed to defining the functions of high-mobility group box 1 (HMGB1) both inside and outside cells. AGER, advanced glycosylation end product-specific receptor; BECN1, beclin 1; cGAS, cyclic GMP–AMP synthase; HAVCR2, hepatitis A virus cellular receptor 2 (best known as TIM3); LPS, lipopolysaccharide; NINJ1, ninjurin 1; STING1, stimulator of interferon response cGAMP interactor 1.

Subsequent studies showed that the secretion of HMGB1 by macrophages and monocytes involves secretory lysosomes[29] (Fig. 4 and Box 1). Secretion of HMGB1 via this pathway is also observed in response to various other inflammatory stimuli (such as interferon-γ (IFNγ)), which can exacerbate the cytokine storm that occurs during sepsis by activating NF-κB and IRF signalling pathways[30].

In addition to immune cells, HMGB1 can be actively secreted by other cell types, including hepatocytes[31] and neurons[32], to mediate inflammatory responses. HMGB1 can also be released passively by all cell types upon cell death or tissue damage. The notion of HMGB1 being a so-called DAMP signal for the immune response was first reported using in vitro models of necrosis induced by heat or freeze–thawing in a human cancer cell line and mouse fibroblasts[33]. The role of HMGB1 as a mediator of sterile inflammation was later identified in a mouse model of liver ischaemia–reperfusion (I/R) injury, by activating the TLR4 pathway[7]. We now know that HMGB1 is released not only by necrotic cells but also by cells undergoing other forms of cell death and stress. Owing to its high levels of expression and rapid translocation between the nucleus and cytoplasm, HMGB1 is likely to be one of the most highly released molecules in response to various cellular stressors[34].

In addition to having cytokine-like or chemokine-like activity, extracellular HMGB1 can also promote inflammation in combination with other molecules (Fig. 2). For example, HMGB1 that is released during apoptosis attaches to nucleosomes, creating a complex that interacts with TLR2 in macrophages and dendritic cells (DCs)[35]. Indeed, HMGB1–nucleosome complexes participate in the pathogenesis of systemic lupus erythematosus by increasing the immunogenicity of nucleosomes released from apoptotic cells[35]. Complexes formed between HMGB1 and DNA stimulate increased cytokine production in macrophages, DCs and B cells by activating AGER and TLR9 in endosomes[36,37]. In addition, HMGB1–LPS not only enhances TLR4-dependent cytokine production but also promotes LPS uptake through AGER and subsequent inflammasome activation in macrophages[31]. Of note, highly purified HMGB1 protein does not induce inflammatory cytokine production in a mouse macrophage cell line or mixed rat glial cultures[38], which suggests that extracellular HMGB1 regulates immunity primarily through the formation of complexes with other factors. Regardless, understanding the cellular or tissue origin of circulating HMGB1 during disease conditions is crucial to comprehending the pathological mechanisms involved in local injury and systemic inflammation.

Cytosolic HMGB1

The release of HMGB1 into the extracellular space is associated with an increased residence of HMGB1 in the cytosol following its translocation from the nucleus. A longstanding open question in HMGB1 biology regards the function of cytosolic HMGB1. In 2010, we found that cytosolic HMGB1 promotes autophagy in mouse fibroblasts or human cancer cells in response to various environmental stresses, including starvation and oxidative damage[6]. Mechanistically, cytosolic HMGB1 was shown to compete with the anti-apoptotic protein B cell lymphoma 2 (BCL-2) to interact with the autophagy core driver beclin 1 (BECN1; the mammalian homologue of yeast Atg6) through C23 and C45 residues in the HMGB1 A-box[6] (Fig. 5). The binding of HMGB1 to nuclear proteins, such as telomeric repeat binding factor 2 (TERF2) and tumour protein p53 (TP53; best known as p53), impairs binding between HMGB1 and BECN1 (refs. 39,40). Furthermore, the association of HMGB1 and nucleotide binding oligomerization domain containing 2 (NOD2) within the cytosol disrupts the association of

NOD2 with receptor interacting serine/threonine kinase 2 (RIPK2), leading to the formation of autophagy-related 16-like 1 (ATG16L1)-dependent autophagosomes in LPS-induced brain microglia[41]. These findings suggest that HMGB1 may regulate membrane vesicle formation during autophagy and stress responses. However, it should be noted that levels of autophagy in the liver are not affected in mice with hepatocyte-specific conditional knockout of *Hmgb1* (ref. 42), which calls into question the role of HMGB1 in autophagy induction. Thus, elucidating the cell type-dependent or tissue-dependent functions of HMGB1 in autophagy and cell stress remains an important challenge.

Autophagy serves as a crucial defence mechanism that promotes cell survival and controls infection[43]. However, excessive autophagy can in some cases lead to cell death, which triggers the release of DAMPs (including HMGB1) and results in inflammation[44]. Furthermore, autolysosomes can facilitate the secretion of leaderless proteins such as HMGB1, SQSTM1 and IL-1β, which in turn promotes further inflammation[45–48] (Fig. 5). Thus, HMGB1-dependent autophagy has been implicated in several inflammatory diseases. For example, in mice with inflammatory bowel disease, autophagy reduces inflammation and associated tissue injury; in this case, cytosolic HMGB1 forms complexes with BECN1 and ATG5 to protect these proteins from pro-apoptotic cleavage mediated by calpain[49]. Deletion of *Hmgb1* in intestinal epithelial cells impairs autophagy and accelerates the development of experimental inflammatory bowel disease[49]. Disruption of the cytosolic HMGB1–BECN1 complex in epithelial cells results in restricted autophagy, leading to increased lung inflammation, in a 1,3-β-glucan-induced mouse model[50]. Furthermore, HMGB1 physically interacts with the transcription factor signal transducer and activator of transcription 3 (STAT3)[51]; absence of *Hmgb1* in the intestine leads to STAT3 overactivation and decreased expression of BECN1 (a repressor target gene of STAT3), thereby suppressing autophagy and causing *Salmonella* colitis in mice[51]. Mice with myeloid cell-specific loss of *Hmgb1* had increased susceptibility to endotoxemia and *Listeria monocytogenes* infection owing to autophagy deficiency[52]. These studies suggest that cytosolic HMGB1-dependent autophagy has an active role in defence against infection in intestinal epithelial cells and myeloid cells.

HMGB1-dependent autophagy also participates in tumour biology by maintaining inflammation and immunosuppression within the tumour microenvironment. For example, HMGB1 promotes the survival of myeloid-derived suppressor cells (MDSCs) by inducing autophagy, which promotes tumour growth in BALB/c mice[53]. HMGB1-induced autophagy also promotes mesothelial cell survival and malignant transformation of associated epithelial cells[54]. The antineoplastic drug doxorubicin, which induces translocation of HMGB1 into the cytosol in human hepatoma cells, results in increased autophagy and therapeutic resistance[55]. Once released from a cell, the reduced form of HMGB1 can further promote autophagy through interaction with AGER, whereas the oxidized form of HMGB1, resulting from increased oxidative stress and apoptotic cell death, loses its autophagy-inducing and immunostimulatory activity[56,57]. These findings suggest that a redox modification feedback mechanism may control HMGB1 activity in the tumour microenvironment.

Another important function of cytosolic HMGB1 is the formation of complexes with free nucleic acids. The absence of *Hmgb1* in mouse embryonic fibroblasts markedly impairs the activation of TLR3, TLR7 and TLR9 by their nucleic acid ligands[5]. Interestingly, immunogenic nucleic acids bind to HMGB1 with higher affinity than less immunogenic nucleic acids. In addition, HMGB2 binds to B-DNA but not RNA, and HMGB3 binds to both DNA and RNA to activate TLR pathways in endosomes[5]. Moreover, HMGB1 can bind to long U-turn DNA to activate the cyclic GMP–AMP synthase (cGAS)–stimulator of interferon response cGAMP interactor 1 (STING1) pathway within the cytosol[58]. This suggests that HMGB proteins function to promote nucleic acid-mediated innate immune responses, including inflammation and cell death. As autophagy necessitates the fusion of autophagosomes with endosomes[59], exploring the relationship between roles of cytosolic HMGB1 in autophagy and the activation of TLRs in endosomes would be of particular interest.

Box 1

Active secretion of HMGB1

The mechanism by which pathogen-associated molecular patterns induce cells to secrete high-mobility group box 1 (HMGB1) is different from the secretion of most cytokines having classical signal peptides. In contrast to the endoplasmic reticulum–Golgi-mediated conventional secretory pathway, the intracellular trafficking and secretion of HMGB1 involve alternative pathways that vary by cell type and depend on the specific signalling context. The current widely accepted model for HMGB1 secretion was originally established in lipopolysaccharide-treated monocytes and consists of three steps[180]. First, HMGB1 is acetylated by lysine acetyltransferase 2B (KAT2B, also known as PCAF), CREB binding protein (CREBBP; also known as CBP) and E1A binding protein P300 (EP300) at two lysine-rich nuclear localization sequences, which leads to the dissociation of HMGB1 from the chromosome. Second, exportin 1 (XPO1; also known as CRM1) mediates the nuclear export of HMGB1 to the cytosol, where it can carry out various cytosolic functions. Third, cytosolic HMGB1 can aggregate into lysosomes and then reaches the extracellular environment in extracellular vesicles through lysosomal exocytosis[181,182]. Other studies have provided evidence that other modifications of HMGB1 (such as phosphorylation[183] and ADP-ribosylation[184]), second messenger molecules (such as reactive oxygen species[91] and calcium[80]), secretory autophagy[46,47] and metabolic reprogramming[185] also contribute to the nucleocytosolic translocation of HMGB1 and its extracellular release. These findings establish that HMGB1 secretion is a complex dynamic process; the crosstalk and feedback mechanisms underlying these events are only partially understood.

Membrane HMGB1

HMGB1 is not commonly found in the membrane of most cells, with the exception of its involvement in neurite outgrowth and platelet activation[60]. During infection, HMGB1 can translocate to the membrane of platelets in response to oxidative stress signals and bind to TLR4 or AGER on neutrophils to promote the formation of neutrophil extracellular traps (NETs)[61,62]. NETs induced by HMGB1 exacerbate inflammatory damage within the brain, lung and heart through various mechanisms[63,64]. Furthermore, HMGB1 released following platelet activation sustains neutrophil autophagy, leading to the generation of NETs, in systemic sclerosis[65]. Platelet-derived HMGB1 is also a driver of thrombosis through interaction with TLR4 in the platelet plasma membrane[66].

Thus, similar to extracellular HMGB1, membrane HMGB1 may modulate communication between various cell types in response to inflammatory stimuli.

HMGB1 in pathological immune responses

HMGB1 has broad roles in inflammatory and immune responses. Here, we focus on HMGB1 release and signalling in the contexts of infection, sterile inflammation, ageing and tumour immunity — four major types of immune response involved in human health and disease.

Pathogen infection

Various pathogen-associated molecular patterns, such as LPS and poly(I:C), can induce immune cells to secrete HMGB1 (refs. 28,67). Furthermore, the infection-induced production of endogenous host DAMPs (such as ATP and DNA) or cytokines (such as tumour necrosis factor (TNF) and IFNs) can further promote HMGB1 secretion[30,68]. In turn, increased levels of extracellular HMGB1 bind receptors such as TLR4 and AGER on immune cells to trigger the production of cytokines, leading to a systemic inflammatory response[69] (Fig. 2). HMGB1 directly binds to other pro-inflammatory molecules (such as DNA, RNA, histones, nucleosomes, CXC-chemokine ligand 12 (CXCL12), IL-1α and IL-1β) or microbial components (such as LPS) to enhance their immunostimulatory activity in immune cells (particularly myeloid cells), often by promoting their interaction with cognate receptors[31,35,70,71] (Fig. 2). In terms of the adaptive immune response, HMGB1 may enhance the migration of B cells in response to CXCL12, leading to the production of

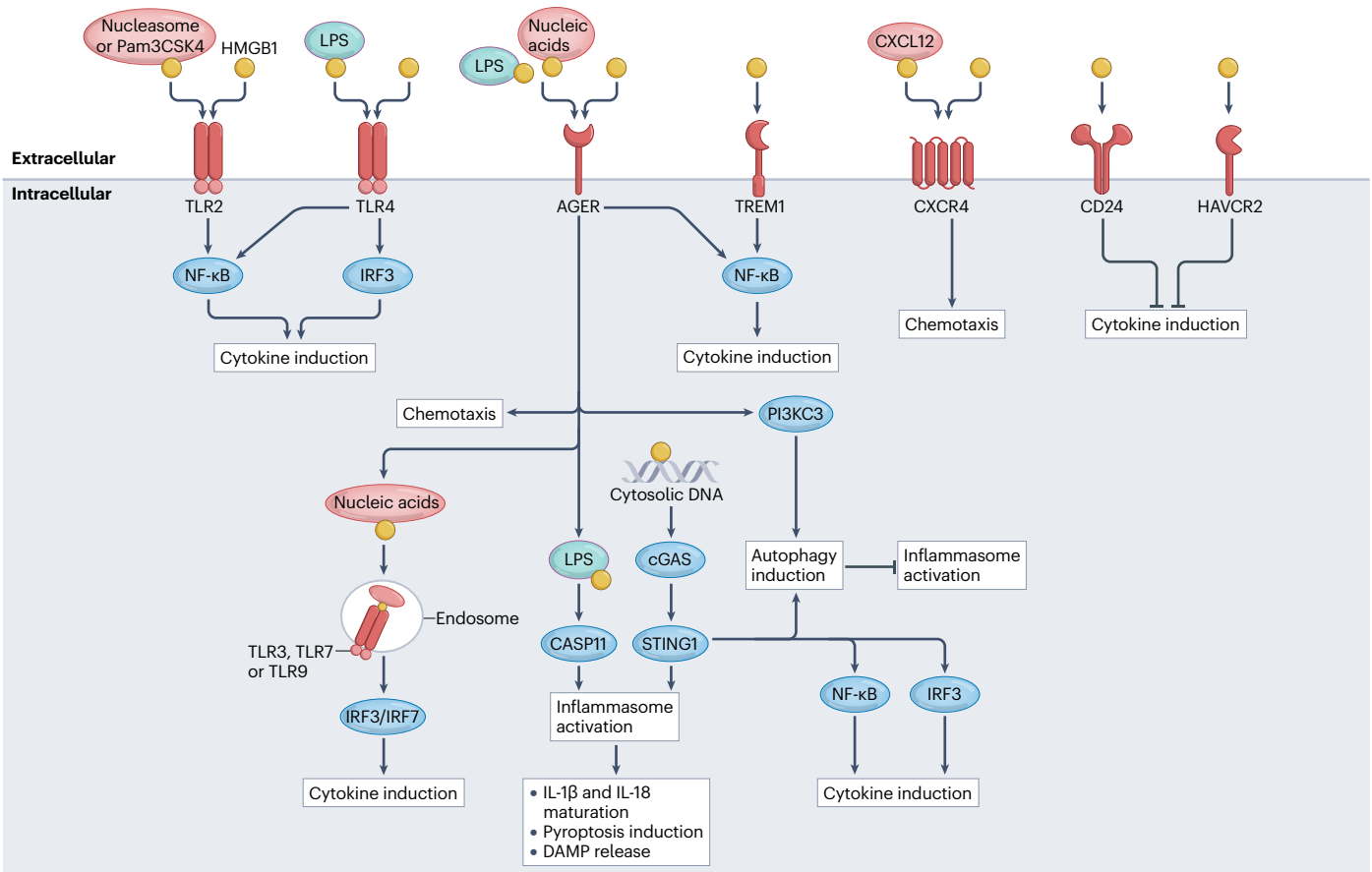


Fig. 2 | Signalling pathways of extracellular HMGB1. Once released from a cell, high-mobility group box 1 (HMGB1) can activate various receptors, either alone or in combination with different immune mediators, in a context-dependent manner. Toll-like receptor 4 (TLR4) and advanced glycosylation end product-specific receptor (AGER) are two of the most well-studied receptors for HMGB1, which mediate its activity in multiple immune cells through activation of the nuclear factor-κB (NF-κB) and interferon regulatory factor 3 (IRF3) pathways, leading to the production of pro-inflammatory cytokines and chemokines. HMGB1 binding to triggering receptor expressed on myeloid cells 1 (TREM1) in macrophages and neutrophils also induces cytokine production through NF-κB, whereas CD24 and hepatitis A virus cellular receptor 2 (HAVCR2; best known as TIM3) can attenuate HMGB1-induced cytokine production. CXC-chemokine receptor 4 (CXCR4) has a specific role in mediating cell migration induced by the complex of HMGB1 with CXC-chemokine ligand 12 (CXCL12). HMGB1 can bind nucleic acids from pathogens or host cells to activate various DNA-sensing pathways or receptors, including TLR3, TLR7 and TLR9 in endosomes and cyclic GMP–AMP synthase (cGAS)–stimulator of interferon response cGAMP interactor 1 (STING1) in the cytosol, also leading to cytokine production. HMGB1-mediated delivery of lipopolysaccharide (LPS) to the cytosol through AGER activates the caspase 11 (CASP11)-dependent inflammasome, leading to the maturation of IL-1 family cytokines (IL-1β and IL-18), pyroptosis and the release of damage-associated molecular patterns (DAMPs; such as HMGB1, tissue factor and sequestosome 1). Furthermore, AGER is required for extracellular HMGB1-induced autophagy through activation of the phosphatidylinositol 3-kinase catalytic subunit type 3 (PI3KC3) pathway. In addition to mediating the production of type 1 interferons and pro-inflammatory cytokines, STING1 is a conserved promoter of autophagy. Autophagy can negatively regulate inflammasome activation by selectively degrading components of the inflammasome complex.

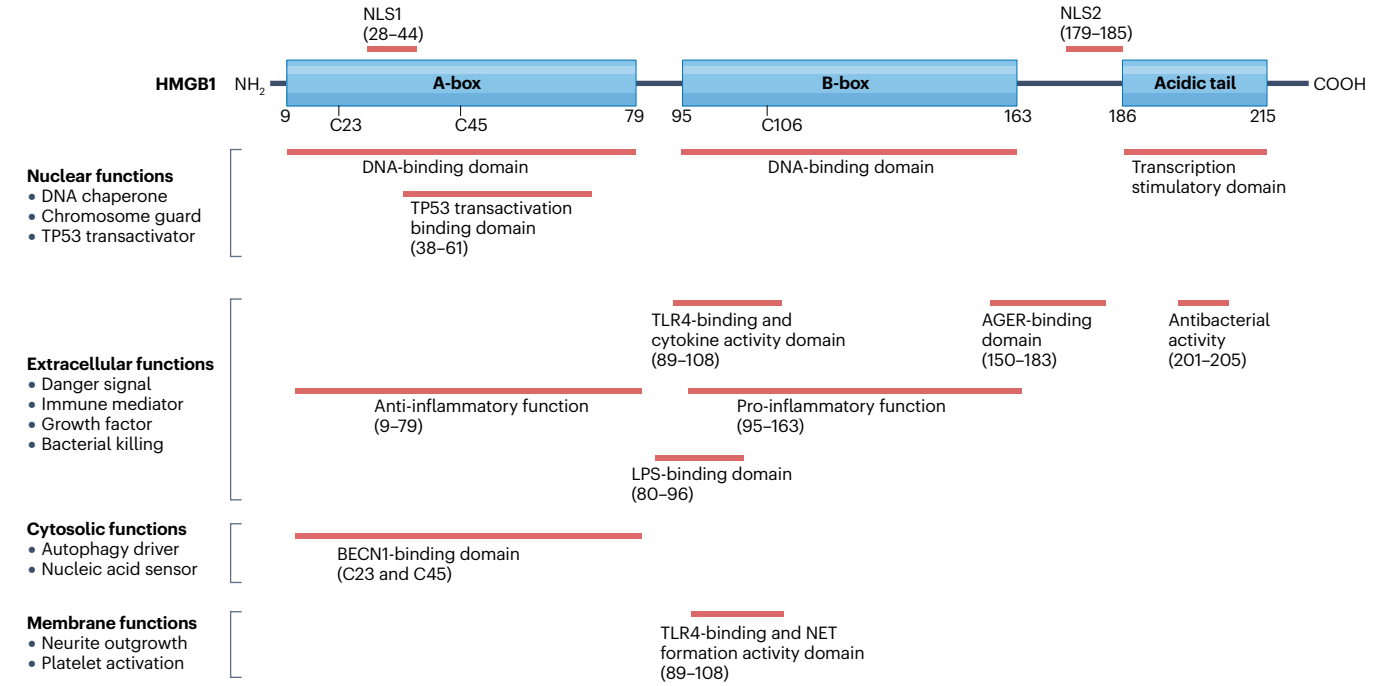


Fig. 3 | Structure and function of HMGB1. Human high-mobility group box 1 (HMGB1) is a 215-amino-acid protein of approximately 30 kDa. Structurally, the HMGB1 protein consists of three domains: two DNA-binding domains (A-box and B-box) and a negatively charged C terminus (acidic tail). HMGB1 contains three redox-sensitive cysteine moieties (C23, C45 and C106). The functions of HMGB1 depend on its subcellular location. Under normal physiological conditions, HMGB1 is found mainly in the nucleus owing to its two nuclear localization signals (NLS1 and NLS2). Nuclear HMGB1 is a DNA chaperone that has an important role in maintaining chromosome structure and functions. Furthermore, the A-box (residues 38–61) of HMGB1 interacts with tumour protein p53 (TP53) to enhance its transcriptional activity. Extracellular HMGB1 functions as a damage-associated molecular pattern or danger signal under conditions of stress and mediates immune responses through interacting with various receptors, including Toll-like receptor 4 (TLR4) and advanced glycosylation end product-specific receptor (AGER). In addition, extracellular HMGB1 has activities that promote cell growth and tissue regeneration and, in some cases, limit bacterial proliferation. Cytosolic HMGB1 drives autophagy by binding to the autophagy core protein BECN1. Cytosolic HMGB1 also enhances nucleic acid-induced immune responses owing to its DNA-binding activity. Membrane HMGB1 may mediate neurite growth. In addition, translocation of HMGB1 to the cell membrane of platelets and subsequent release are involved in neutrophil extracellular trap (NET) formation during infection or tissue damage. LPS, lipopolysaccharide.

IgA in the gut and strengthening of intestinal mucosal defences[72]. However, HMGB1 can promote immunosuppression and immune paralysis later in infection. For example, binding of extracellular HMGB1 to AGER on macrophages triggers dynamin-dependent endocytosis of HMGB1, which in turn initiates the activation of cathepsin B and its release from ruptured lysosomes, followed by inflammasome activation[73]. This leads to HMGB1-induced pyroptosis, which not only results in the release of IL-1β but also impairs the ability of macrophages to clear bacteria[73,74].

Inhibition of HMGB1 reduces the systemic inflammatory response and improves survival in various experimental infection models. For example, blocking antibodies to HMGB1 prevent lung injury in mice infected with *Mycobacterium tuberculosis*[75], and mice that receive exogenous HMGB1 protein via intratracheal administration have a markedly increased bacterial burden 1 week after infection[75]. In a model of lethal polymicrobial sepsis resulting from caecal ligation and puncture, mice are rescued in a dose-dependent manner by administration of HMGB1-specific neutralizing antibodies or the antimalarial medicine chloroquine (which inhibits HMGB1 release) beginning 24 hours following the onset of sepsis[76,77]. Thus, the therapeutic window for targeting HMGB1 is larger than that for pro-inflammatory cytokines, such as TNF, which are involved at earlier stages of disease.

HMGB1 also has bactericidal activity against many pathogens (such as *Haemophilus influenzae*, *Burkholderia cenocepacia*, Enterobacteriaceae, *Staphylococcus aureus*, *Klebsiella pneunomiae*, *Acinetobacter baumanii*, *Pseudomonas aeruginosa* and *Enterococcus faecium*) by promoting NET formation[78,79]. The presence of HMGB1 within bacterial biofilms promotes recruitment of the NET components neutrophil elastase and histone H3. Furthermore, HMGB1 binds to DNA, which is responsible in part for its role in disrupting the DNA-dependent matrix of bacterial biofilms[78]. Therefore, HMGB1 has a dual function in pathogen infection — both being bactericidal and promoting tissue damage — depending on the immunopathological stage and nature of the pathogen.

Extracellular HMGB1 modulates the production of cytokines and chemokines in response to pathogen infection through receptors with immunostimulatory or immunoinhibitory activity (Fig. 2). TLR4 (refs. 80,81) and AGER[37,82,83] mediate the immunostimulatory activities of HMGB1. By contrast, CD24 (ref. 84) and hepatitis A virus cellular receptor 2 (HAVCR2; also known as TIM3)[85], receptors for HMGB1

expressed on splenocytes or DCs, have immunoinhibitory effects in animal models of liver injury and tumour immunity. Moreover, HMGB1-mediated DNA uptake through an endocytic process can inhibit HAVCR2 function, thereby restoring STING1-dependent type I IFN production in DCs[86]. CD24 on DCs presents HMGB1 to AGER on responding $CD8^+$ T cells, thereby enhancing their activation during influenza A virus

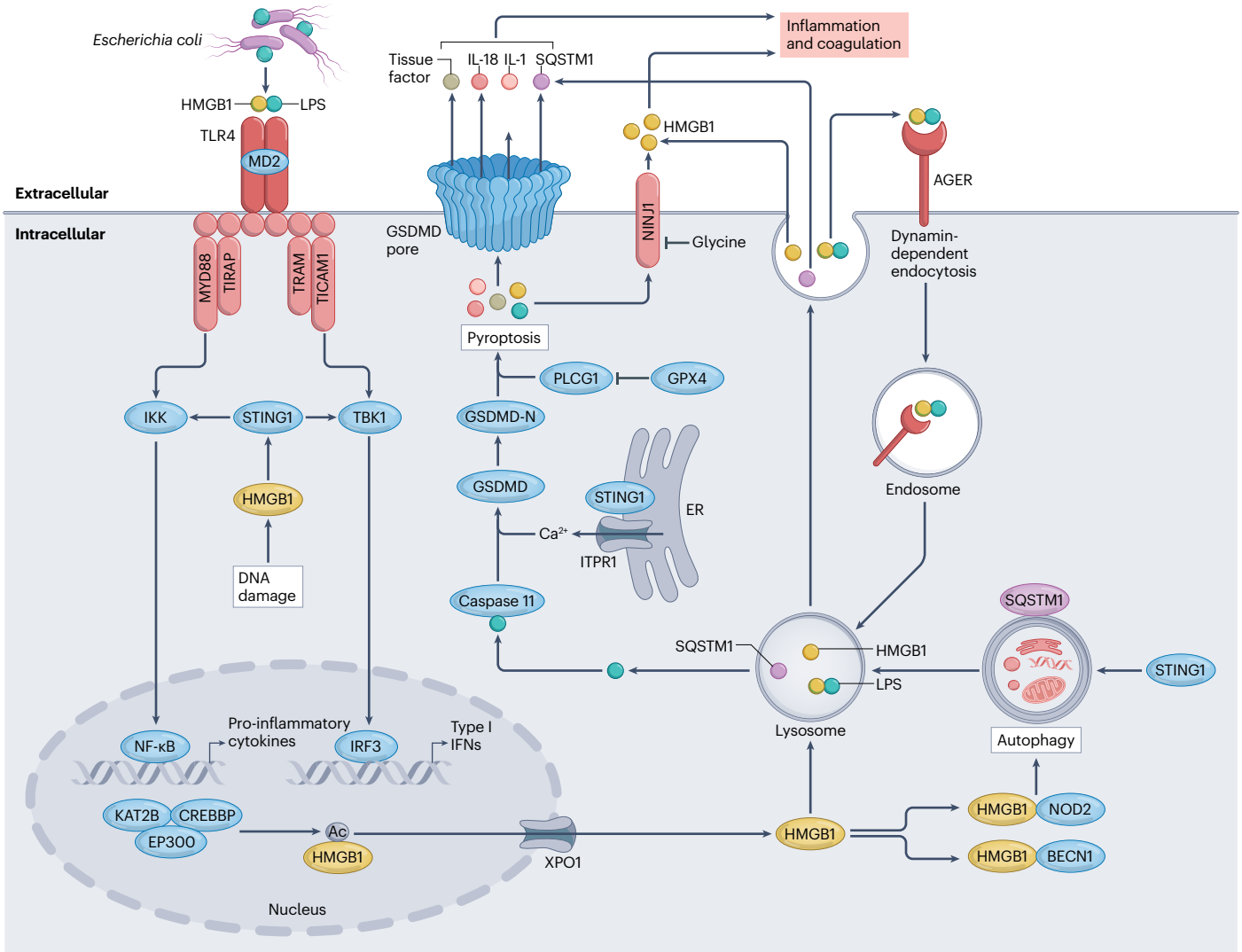


Fig. 4 | Interactions between lipopolysaccharide and HMGB1 in inflammation and coagulation. The Toll-like receptor 4 (TLR4)–myeloid differentiation protein 2 (MD2) complex recognizes lipopolysaccharide (LPS) from Gram-negative bacteria, resulting in activation of the TIR domain containing adaptor protein (TIRAP)–MYD88–IκB kinase (IKK)–nuclear factor-κB (NF-κB) pathway and the translocation-associated membrane protein (TRAM)–TIR domain containing adaptor molecule 1 (TICAM1)–TANK binding kinase 1 (TBK1)–interferon regulatory factor 3 (IRF3) pathway. These pathways lead to the production of pro-inflammatory cytokines (such as IL-1 and tumour necrosis factor) and type I interferons (IFNs) in macrophages. The combined effects of LPS, cytokines and IFNs promote the acetylation (Ac) of nuclear high-mobility group box 1 (HMGB1) mediated by acetyltransferase 2B (KAT2B), CREB binding protein (CREBBP) and E1A binding protein P300 (EP300), resulting in its translocation from the nucleus to the cytoplasm via exportin 1 (XPO1). Cytosolic HMGB1 binds beclin 1 (BECN1) or nucleotide binding oligomerization domain containing 2 (NOD2) to promote autophagosome formation, which regulates cargo degradation and protein secretion. HMGB1 also senses host DNA damage to induce stimulator of interferon response cGAMP interactor 1 (STING1)-dependent cytokine and IFN production. HMGB1 is actively secreted extracellularly by lysosomes, forming an extracellular HMGB1–LPS complex that binds the advanced glycosylation end-product specific receptor (AGER) in macrophages and reaches endosomes via dynamin-dependent endocytosis. Extracellular LPS taken into cells in this manner can be released into the cytosol via the endosome–lysosome system. Cytosolic LPS binds caspase 11 (caspase 4 and caspase 5 in humans), leading to gasdermin D (GSDMD) cleavage to generate N-terminal GSDMD (GSDMD-N), a pyroptotic effector protein. This process is driven by calcium ions released by the STING1–inositol 1,4,5-trisphosphate receptor type 1 (ITPR1) complex in the endoplasmic reticulum (ER). GSDMD-N-mediated membrane oxidative damage is enhanced by phospholipase Cγ1 (PLCG1) and inhibited by glutathione peroxidase 4 (GPX4). Release of DAMPs (such as IL-1, IL-18, sequestosome 1 (SQSTM1), tissue factor and HMGB1) associated with cell membrane damage is regulated by ninjurin 1 (NINJ1) or the GSDMD pore. Intracellular SQSTM1, an autophagy receptor for cargo degradation, is also secreted by lysosomes in LPS-activated macrophages. Together, the interactions between signalling pathways activated by LPS and HMGB1 aggravate inflammation and blood coagulation, leading to sepsis and even death.

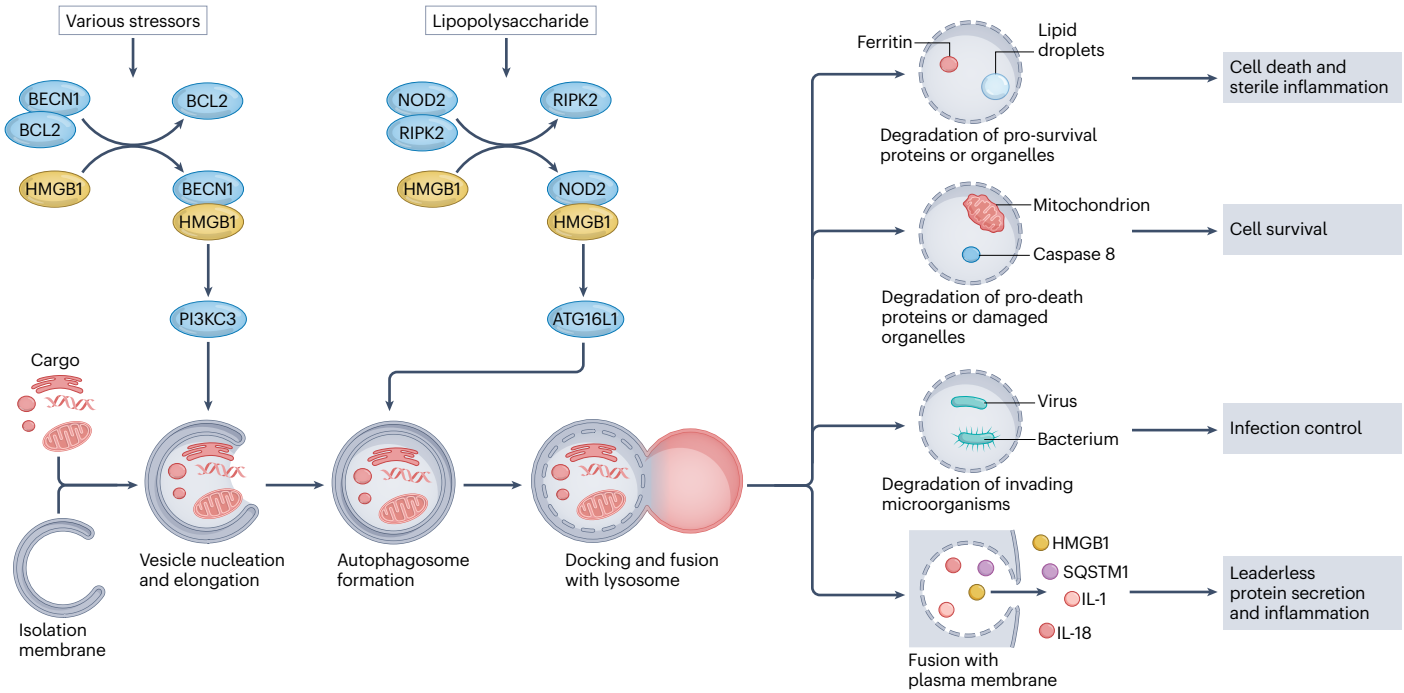


Fig. 5 | Interplay between HMGB1 and autophagy in cell death and inflammation. Autophagy is a dynamic process involving the formation of membrane vesicles that engulf cargo to form autophagosomes, which subsequently fuse with lysosomes. Cytosolic high-mobility group box 1 (HMGB1) can promote autophagosome formation through two pathways, dependent on the nature of the stimuli. On the one hand, cytosolic HMGB1 can compete with B cell lymphoma 2 (BCL-2) for binding to beclin 1 (BECN1) through C23 and C45 residues in the HMGB1 A-box, leading to activation of the phosphatidylinositol 3-kinase catalytic subunit type 3 (PI3KC3) pathway, which enables vesicle nucleation and elongation in immune and non-immune cells. On the other hand, cytosolic HMGB1 can bind to nucleotide binding oligomerization domain containing 2 (NOD2) to disrupt the NOD2–receptor-interacting serine/threonine kinase 2 (RIPK2) complex, leading to autophagy-related 16-like 1 (ATG16L1)-mediated maturation and formation of autophagosomes in macrophages. ATG16L1 and NOD2 are components of an autophagy-mediated antibacterial pathway that is altered in a cell-specific manner. The induction of autophagy can mediate at least four pathways that may affect inflammatory responses. In many cases, increased autophagy is an important defence mechanism that promotes cell survival and infection control by selectively degrading pro-death proteins (such as activated caspase 8), organelles (such as damaged mitochondria) or invading microorganisms. However, excessive autophagy can cause cell death, which leads to the release of damage-associated molecular patterns (including HMGB1) and inflammation. For example, there is increasing evidence that iron-dependent ferroptosis is an autophagy-dependent form of cell death that occurs as a result of the autophagic degradation of anti-ferroptotic factors, such as ferritin and lipid droplets. In addition to their well-characterized degradation functions, autolysosomes can fuse with the plasma membrane to mediate the secretion of leaderless proteins, including HMGB1, sequestosome 1 (SQSTM1) and members of the IL-1 family, which promote inflammation. The autophagic degradation of components of immune signalling pathways can also affect inflammatory responses (not shown).

infection within the lung[87]. Thus, various receptors for HMGB1 are involved in determining the immune response to infection, although the dynamics and feedback of this process remain poorly understood.

The extracellular functions of HMGB1 during pathogen infection also depend on its form (reduced or oxidized, single or complexed with other protein and nonprotein molecules) (Fig. 2), which complicates an assessment of the induced immune response. For example, extracellular HMGB1 can deliver LPS into the cytosol through AGER-mediated endocytosis to trigger caspase-11-dependent pyroptosis[31] and pyroptosis-mediated coagulation by releasing tissue factor, an important initiator of the coagulation cascade[88] (Fig. 4). Phospholipase Cγ1 is essential for the gasdermin D-mediated formation of pyroptotic membrane pores and release of HMGB1 in macrophages during bacterial infection, and this process can be hindered by glutathione peroxidase 4 (GPX4)[89].

The redox status of HMGB1 is determined by the level of oxidative stress during inflammatory responses and tissue repair, with the exchange between reduced and disulfide forms of HMGB1 being reversible. Reduced all-thiol HMGB1 has chemokine-like activity, whereas disulfide HMGB1 has only cytokine-like activity[21]. Fully reduced extracellular HMGB1 can bind to the chemokine CXCL12 and enhance the chemotaxis of human monocytes through CXC-chemokine receptor 4 (ref. 21). Atypical chemokine receptor 3 and CXCR7 can also bind to CXCL12 (ref. 90), although the role of HMGB1 in this binding is unclear. By contrast, oxidized extracellular HMGB1 has no immunostimulatory activity owing to its inability to activate TLR4 in macrophages and DCs[11,57,81].

Sterile inflammation

Various sterile stimuli, such as trauma, ischaemia, toxins, foreign bodies, chemicals, antigens and organ transplantation, can also trigger inflammation through the release of DAMPs. Although, in theory, any endogenous protein could become a DAMP, HMGB1 is of particular importance in this regard for several reasons. HMGB1 is an abundant

protein expressed in nearly every tissue, and HMGB1 is released during various types of cell death and sterile tissue injury (Box 2). HMGB1 is released earlier than other DAMPs during tissue injury in response to early damage signals (such as reactive oxygen species)[91]. Also, HMGB1 has a relatively long half-life owing to its ability to bind to other molecules to form large complexes, which stabilize it in the circulation. The loss of intracellular HMGB1 in tissues as a result of extracellular secretion in turn results in nuclear stress and the release of other nuclear DAMPs (such as histones and genomic DNA), which recruit and activate immune cells that secrete additional HMGB1 (ref. 25). Furthermore, HMGB1 can enhance DNA-sensing pathways — for example, those involving STING1, TLR3, TLR7, TLR9 or absent in melanoma 2 (AIM2) — induced by host or pathogenic nucleic acids through a synergistic effect or by increasing the interaction of DNA with its sensor[5,58,83]. Excess HMGB1 production in response to sterile stimuli promotes inflammatory mediator release and complement activation, which exacerbate tissue damage and increase the risk of subsequent secondary pathogen infection. However, extracellular HMGB1 may also exert growth factor activity to initiate healing and tissue regeneration by recruiting resident stem cells[92]. HMGB1 signalling through TLR4 and AGER can lead to both systemic inflammation and wound healing. Therefore, the effects of targeting HMGB1 in sterile inflammation may depend on the pathological stage.

The common forms of regulated cell death (apoptosis[93], pyroptosis[94,95], necroptosis[96,97] and ferroptosis[44]) can all lead to the release of HMGB1, thereby triggering sterile inflammation (Box 2). This release of HMGB1 from dead and dying cells in the late stages of tissue injury has traditionally been considered an unregulated passive process. However, recent studies have shown that plasma membrane rupture and DAMP release, including of HMGB1, during the final stages of lytic cell death (such as pyroptosis) are active processes mediated by the oligomerization of ninjurin 1 (NINJ1)[98], which can be inhibited by glycine[99] (Fig. 4). Interestingly, *Ninj1*$^{-/-}$ mice are more susceptible to infection with *Citrobacter rodentium* than wild-type mice, suggesting a role for DAMP release mediated by NINJ1 in host defence[98]. If apoptotic cells are not scavenged within a day or two, they may progress to a lytic and inflammatory phase known as secondary necrosis, which is also an active process that involves the release of HMGB1 (refs. 93,100).

HMGB1 is well documented as a mediator of sterile inflammation in diseases such as tissue I/R injury, autoimmunity, cardiovascular and neurodegenerative diseases and metabolic disorders. For example, HMGB1 levels are increased during experimental I/R injury of liver; inhibition of HMGB1 with neutralizing antibodies decreases liver damage following I/R, whereas administration of recombinant HMGB1 aggravates liver I/R injury[7]. Levels of extracellular HMGB1 in blood, synovial tissue and synovial fluid are increased in patients with rheumatoid arthritis, with HMGB1 having a role in disease pathogenesis[101]. Blocking HMGB1 activity ameliorates disease course in a mouse model of spontaneous arthritis, leading to reduced joint destruction[102]. HMGB1 expression is increased in plasma and myocardial biopsies of patients with myocarditis, and inhibition of HMGB1 by glycyrrhizin or neutralizing antibodies decreases myocardial inflammation[103]. Monoclonal antibodies to HMGB1 inhibit neuroinflammation and prevent brain damage in experimental models of Parkinson disease, epilepsy and Alzheimer disease[104,105]. HMGB1 is also implicated in the development of diabetes, hyperglycaemia and various associated complications in the brain, lung, kidney and bones, perhaps in part through AGER signalling. The development of streptozotocin-induced type 1 diabetes in mice is suppressed by treatment with ethyl pyruvate, an HMGB1 inhibitor[106]. Further understanding of the secretory pathways used by HMGB1 during cell death, and mechanisms of HMGB1-mediated sterile inflammation and its role in disease pathogenesis,

Box 2

Release of HMGB1 during cell death

The release of high-mobility group box 1 (HMGB1) occurs in various models of cell death and injury, highlighting the crucial role of HMGB1 in mediating cell death-induced inflammation. HMGB1 is also actively secreted by macrophages following the phagocytosis of dead cells; although HMGB1 induces differentiation to a pro-inflammatory macrophage state, HMGB1 and complement component C1q together induce differentiation to an anti-inflammatory state[161].

Apoptosis is the most well-studied form of regulated cell death and is usually mediated by the activation of caspases. Apoptosis is immunologically well tolerated and generally leads to an anti-inflammatory response at the tissue level. However, late apoptotic cells release HMGB1 in a caspase 3 (CASP3)-dependent manner, which can lead to either immunological tolerance[57] or immunogenic cell death[133] depending on the redox state of HMGB1.

The association of pyroptosis with innate immunity and inflammation has received increasing attention. Pyroptosis in macrophages is mainly induced by canonical CASP1-mediated and atypical CASP11-mediated inflammasome activation, followed by membrane rupture mediated by gasdermin family proteins. This process involves the maturation and release of pro-inflammatory cytokines of the IL-1 family (such as IL-1β and IL-18) as well as damage-associated molecular patterns (such as HMGB1 (ref. 68) and sequestosome 1 (ref. 48)). In addition to CASP1 and CASP11, CASP8 can also promote gasdermin-dependent pyroptosis with HMGB1 release in infection[186].

Necroptosis is considered as a back-up mechanism of apoptosis because it is mainly induced in the absence of caspase activation in combination with the activation of receptor interacting serine/threonine kinase 1 (RIPK1) and RIPK3. Inhibition of RIPK1 and/or RIPK3 or depletion of the necroptotic effector mixed-lineage kinase domain-like pseudokinase blocks HMGB1 release under various inflammatory conditions[187].

Ferroptosis, a lipid peroxidation-mediated form of regulated necrosis controlled by various antioxidant systems, as well as the autophagic machinery, is also associated with the release of HMGB1 (refs. 123,188). The inhibition of ferroptosis reduces HMGB1 release and subsequent advanced glycosylation end product-specific receptor (AGER)-dependent inflammation[44].

could lead to the development of novel therapies for these diseases. A panel of biomarkers, including but not limited to HMGB1, should be monitored to detect more complex patterns of cell death changes and the activation of immunosensor pathways at individual stages of tissue damage[107].

Ageing and senescence

Ageing increases vulnerability to most diseases in humans, particularly cancer, cardiovascular disease and neurodegeneration. One of the major changes that occurs during ageing is the emergence of a state of chronic, low-grade systemic inflammation[108]. Aberrant HMGB1 expression and release are involved in this process. For example, the expression of HMGB1 in mouse brain gradually decreases during ageing, which contributes to the accumulation of DNA damage and triggers the release of nuclear DAMPs including HMGB1 and neuroinflammation[109,110]. Inhibiting HMGB1 release from damaged neurons prevents neuroinflammation during ageing. Studies have also established a role for extracellular HMGB1 in pathological ageing processes and inflammation in the heart, by sustaining the polarization of macrophages to a pro-inflammatory phenotype through TLR2 and TLR4 signalling[111]. Human umbilical vein endothelial cells tend to have decreased nuclear HMGB1 levels and increased extracellular HMGB1 levels as they age, which drives transcriptional changes associated with ageing[112].

At the cellular level, increased oxidative damage associated with ageing is a major factor in inducing cellular senescence, a terminal stage of cell differentiation and non-proliferation. Senescent cells have a specialized senescence-associated secretory phenotype (SASP) that includes secretion of HMGB1 in an autocrine and/or paracrine manner. Furthermore, HMGB1 is actively secreted by irradiation-induced senescent cells before the development of the SASP in a manner dependent on HMGB1 acetylation and TP53 (as identified in mouse fibroblasts or a human colorectal adenocarcinoma cell line)[113]. Decreasing HMGB1 levels in the nucleus of senescent cells lead to a TP53-dependent cell-cycle arrest[113]. This interplay between HMGB1 and TP53 is also observed during autophagy in mouse fibroblasts or human colorectal adenocarcinoma cell lines[40], supporting the existence of a conserved HMGB1–TP53-dependent mechanism that is involved in regulating disparate processes including protein degradation and that contributes to ageing.

HMGB1 can also enter the circulatory system to regulate the activities of distant organs, which promotes age-related immunopathology including tumorigenesis and neurodegenerative diseases. For example, depletion of fructose bisphosphatase 1 specifically in mouse hepatocytes can trigger the activation and senescence of hepatic stellate cells and lead to tumorigenesis through the release of HMGB1 (ref. 114). The release of HMGB1 is also a crucial factor in the senescence of primary astrocytes from patients with Alzheimer disease or frontotemporal dementia induced by tau oligomers. HMGB1 release inhibitors, such as ethyl pyruvate and glycyrrhizic acid, improve tau pathology, reduce the release of inflammatory cytokines and prevent cognitive decline in a transgenic mouse model of tau-dependent neurodegenerative disease[115].

HMGB1 release during senescence can also activate innate immune pathways responsible for detecting DNA damage, such as the cGAS–STING1 pathway[116]. HMGB1 suppresses expression of the E3 ligase tripartite motif protein 30α, which in turn stabilizes STING1 protein. STING1 signalling induces expression of cyclin-dependent kinase inhibitor 1A (best known as p21), which is facilitated by STAT6 signalling in response to genotoxic stress caused by the drug doxorubicin, thus leading to inhibition of tumour growth[116].

Further studies are clearly needed to better understand the varying composition of the SASP in vivo and how individual DAMPs, such as HMGB1, can remodel tissues and associated immune responses during ageing. Although senescent cells are expected to release oxidized HMGB1 (ref. 113), it is not well understood how the various redox forms of HMGB1 might function in mediating immune cell clearance of senescent cells. Obtaining a deeper understanding of the interplay between immune cells and senescent cells could potentially lead to the development of novel therapeutic approaches for ageing, tissue repair and cancer.

Tumour immunity

Acute inflammation can initially activate antitumour immune responses, particularly in the context of cancer therapy, whereas chronic inflammation caused by infection or other carcinogens increases cancer risk and stimulates malignant progression. Similarly, HMGB1 has diverse roles in tumorigenesis and response to cancer

Glossary

Autophagy
A lysosome-dependent cellular process by which cells break down and recycle their own cellular components, including aged or dysfunctional proteins and organelles, as well as invading microorganisms.

Cytokine storm
A severe and excessive release of cytokines, which can result in a systemic and potentially life-threatening inflammatory response.

Damage-associated molecular pattern
(DAMP). Part of a group of endogenous molecules that are released in response to cellular injury or damage to alert the immune system.

Disseminated intravascular coagulation
A medical condition in which abnormal blood clotting occurs throughout the small blood vessels of the body.

Endotoxin lethality
The ability of bacterial lipopolysaccharides, also known as endotoxins, to cause death in organisms.

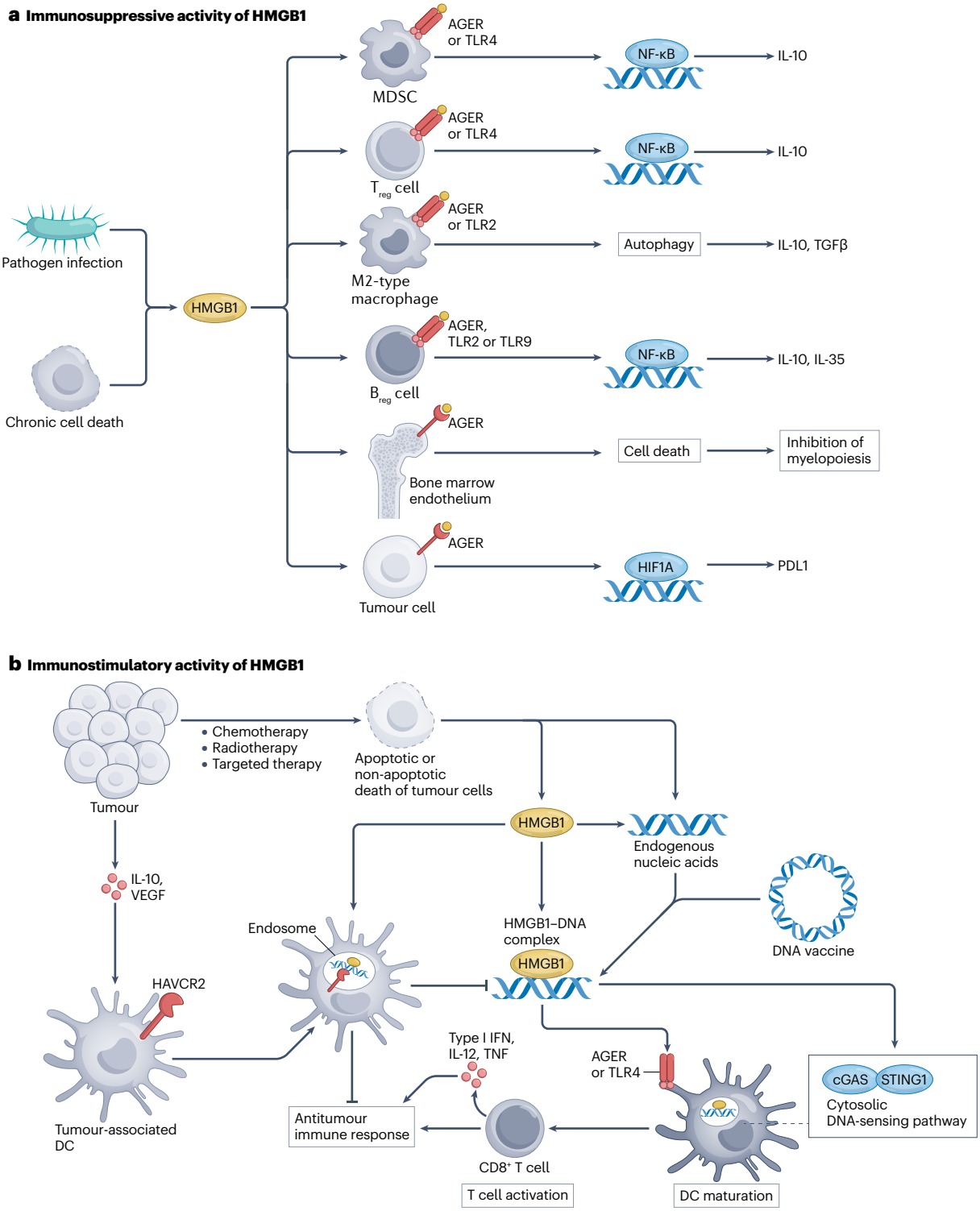
Endotoxin tolerance
The adaptive process by which the immune system becomes less responsive to the effects of bacterial lipopolysaccharides, also known as endotoxins.

Immunogenic cell death
(ICD). A type of cell death that is characterized by the release of intracellular components, such as DAMPs, into the surrounding environment to trigger an immune response.

Neutrophil extracellular traps
(NETs). A type of cellular defence mechanism used by neutrophils to capture and eliminate invading microorganisms such as bacteria and fungi.

Sequential organ failure assessment
A standardized tool used to assess the severity of organ dysfunction (respiratory, cardiovascular, liver, coagulation, kidney and nervous system) in critically ill patients.

Sterile inflammation
A type of immune response that occurs in response to damage or injury to cells or tissues, without the presence of a pathogenic microorganism.



therapy. HMGB1 overexpression is commonly observed in multiple forms of cancer and has oncogenic effects, by enhancing anti-apoptotic mechanisms, inflammation, immune system evasion and aerobic glycolysis[117,118]. By contrast, in the setting of pancreatic cancer, intracellular HMGB1 functions as a tumour suppressor[119]. Using mice with conditional knockout of *Hmgb1* in the pancreas, we found that the loss of *Hmgb1* leads to the release of nucleosomes in pancreatic cells and a subsequent AGER-dependent inflammatory response in macrophages, thereby accelerating oncogenic *KRAS*-driven pancreatic tumorigenesis in mice[119]. Thus, it is important to look at changes in the localization

Fig. 6 | The role of HMGB1 in cancer biology and tumour immunity. a, The immunosuppressive activity of extracellular high-mobility group box 1 (HMGB1) in tumour immunity. Pathogen infection or chronic cell death can result in the release of HMGB1, which activates myeloid-derived suppressor cells (MDSCs), regulatory T (T_{reg}) cells, anti-inflammatory (M2-type) macrophages and regulatory B (B_{reg}) cells through advanced glycosylation end product-specific receptor (AGER) or Toll-like receptors (TLRs), resulting in the activation of the nuclear factor-κB (NF-κB) pathway or autophagy. HMGB1 also impairs the bone marrow endothelial cell niche by inducing non-pyroptotic cell death, and can promote the expression of immune checkpoint molecules (such as PDL1) by AGER-dependent hypoxia inducible factor 1 subunit α (HIF1A) transcriptional activity in tumour cells to drive an immunosuppressive tumour microenvironment. **b**, The immunostimulatory activity of extracellular HMGB1 in tumour immunity. Chemotherapy or radiotherapy leads to apoptotic or non-apoptotic death of tumour cells, which promotes the release of HMGB1 and other damage-associated molecular patterns (such as DNA). Extracellular HMGB1 promotes dendritic cell (DC) maturation, alone or in combination with DNA from multiple endogenous sources or exogenous sources (such as DNA vaccines), to activate cytotoxic $CD8^+$ T cells to release type I interferon (IFN), IL-12 and tumour necrosis factor (TNF). This process is mediated by the HMGB1 receptors TLR4 and AGER and can be enhanced by the cytoplasmic cyclic GMP–AMP synthase (cGAS)–stimulator of interferon response cGAMP interactor 1 (STING1) DNA-sensing pathway. Conversely, the expression of hepatitis A virus cellular receptor 2 (HAVCR2; best known as TIM3) on tumour-associated DCs, which are induced by IL-10 and vascular endothelial growth factor (VEGF) produced by tumour cells, limits the immunostimulatory activity of HMGB1 in promoting tumour immunity. HAVCR2 actively competes with HMGB1 for nucleic acid binding and inhibits the response to HMGB1–nucleic acid in endosomes. TGFβ, transforming growth factor-β.

of HMGB1 within the tumour tissue, as well as its expression level, to determine its role in distinct tumour stages.

Extracellular HMGB1 within the tumour microenvironment promotes tumour development in multiple ways (Fig. 6a). Both cell-death-induced sterile inflammation and microbial infection in mice can contribute to the development of a pro-inflammatory microenvironment that includes the accumulation of HMGB1, leading to tumour initiation and development. For example, blocking the HMGB1–AGER pathway can reduce the growth and metastasis of implanted lung carcinoma cells in mice, as well as limit the spontaneous development of tumours induced by *v-Ha-ras* oncogene in susceptible mice[82]. In addition, the inflammatory response of neutrophils induced by ultraviolet light stimulates angiogenesis and facilitates migration of melanoma cells towards endothelial cells in a HMGB1–TLR4-dependent manner[120]. Extracellular HMGB1 recruits and activates immunosuppressive MDSCs and regulatory T cells through AGER or TLR4 to produce IL-10, thereby limiting DC maturation[121,122]. In mice with liver or pancreatic tumours deficient in the antioxidant enzyme *Gpx4*, the release of DAMPs including HMGB1 as a result of ferroptosis can induce immune suppression through MDSC activation or the polarization of macrophages towards an anti-inflammatory phenotype[123,124]. HMGB1 also promotes the expression of immune checkpoint proteins through the AGER-dependent hypoxia-inducible factor 1α transcriptional pathway in pancreatic cancer cells[125]. It also disrupts the bone marrow endothelial cell niche by inducing non-pyroptotic death through an unknown mechanism[126], thereby inhibiting myelopoiesis and promoting an immunosuppressive tumour microenvironment in both humans and mice. The expression of TIR domain containing adaptor protein, which is a component of the TLR4 signalling pathway, leads to an increase in the expression of IFNγ, resulting in the release of HMGB1 and disruption of the bone marrow endothelial niche[126]. In addition to supporting immune cell development, bone marrow endothelial cells also regulate immune cell trafficking between the bone marrow and other tissues, including tumours. Therefore, understanding the role of the bone marrow endothelial cell niche, including the effect of HMGB1, in tumour immunity is crucial for developing new strategies to enhance antitumour immune responses.

Release of HMGB1 from cancer-derived exosomes induces the differentiation of regulatory B cells that promote immune evasion in hepatocellular carcinoma[127]. It also polarizes B cells towards a phenotype that mediates vascular endothelial growth factor-dependent angiogenesis in the setting of oesophageal cancer[128]. Although AGER, TLR9 and TLR2 are known to be receptors for HMGB1 in B cells in the context of autoimmune disease[37,129], further studies are needed to define the receptors involved in HMGB1-induced activation of pro-tumorigenic B cells. HMGB1 decreases the diversity of γδ T cells, which enables tumour immune escape[130]. Through the induction of autophagy, HMGB1 also induces the differentiation of pro-tumorigenic, anti-inflammatory macrophages in gastric cancer and hepatocellular carcinoma by activating AGER or TLR2, leading to the production of IL-10 and transforming growth factor-β[131,132]. Given these roles of HMGB1 in the tumour microenvironment, reducing HMGB1 release and/or activity could enhance tumour immune surveillance.

In the context of cancer therapy, extracellular HMGB1 (together with ATP and type I IFN) can mediate immunogenic cell death (ICD) and enhance antitumour immunity. Although the concept of ICD was originally reported in the context of radiation therapy and apoptosis induced by certain chemotherapeutic agents (such as anthracyclines, cyclophosphamide or oxaliplatin)[133], ICD is now thought to occur for various types of cell death (including apoptotic and non-apoptotic forms). HMGB1 mediates immune activation in response to cell death by promoting the maturation of DCs and subsequent $CD8^+$ cytotoxic T cell responses to cancer cells, as shown in multiple acute xenograft or vaccine tumour models (Fig. 6b). Both TLR4 and AGER signalling contribute to HMGB1-dependent ICD, as shown by various genetic and pharmacological approaches in colorectal or lung cancer models[133–135]. However, as HMGB1 is also released upon immune-tolerant cell death (such as some cases of apoptosis[57]), the release of HMGB1 alone cannot be interpreted as a reliable marker of ICD. The distinction between immune-tolerant cell death and ICD may be determined by the redox state of HMGB1.

HMGB1 can also activate or enhance antitumour immunity through other mechanisms. For example, natural killer cell-derived HMGB1 eliminates colon cancer cells by inhibiting mitochondrial respiration and inducing subsequent metabolic cell death[136]. Autophagy-mediated unconventional secretion of HMGB1 promotes the pro-inflammatory polarization of tumour-infiltrating macrophages, thereby enhancing the anticancer activity of the DNA-methylating chemotherapeutic temozolomide in glioblastoma[137]. DNA damage and HMGB1 release induced by topoisomerase II inhibitor therapy enhance the efficacy of immune checkpoint blockade (anti-PD1) therapy by activating STING1-dependent type I IFN signalling and DC activation in a mouse model of colorectal cancer[138]. By contrast, HAVCR2 (also known as TIM3) expressed on tumour-associated DCs limits responses to HMGB1-complexed nucleic acids in endosomes, resulting in impaired ICD

Box 3

HMGB1 as a disease biomarker

Aberrant expression and secretion of high-mobility group box 1 (HMGB1) are associated with several diseases, including infectious diseases (bacterial and viral infections[189]), deficiency diseases (such as vitamin D deficiency[190]), hereditary diseases (such as sickle cell disease[191] and muscular dystrophy[192]) and chronic inflammatory diseases (such as cancer, cardiovascular disease and autoimmune disease). Although these associations indicate the therapeutic potential of targeting HMGB1 signalling, further clinical studies are needed to clarify whether HMGB1 is indeed a cause of pathology in these conditions in humans. Biomarkers can be diagnostic (determining the presence and type of disease), prognostic (providing information on the expected outcome of treatment) or predictive (identifying patients who are more likely to respond to treatment). A large number of clinical studies have analysed the use of HMGB1 as a biomarker in patients with various diseases (summarized in Supplementary Table 2). Most of these have shown that serum levels of HMGB1 are increased in inflammatory diseases and positively correlate with disease progression.

For example, serum HMGB1 levels are increased in patients with severe COVID-19 compared with non-severe COVID-19 and healthy controls[167,193]. Subsequent functional studies showed that advanced glycosylation end product-specific receptor (AGER), but not Toll-like receptor 4, was required for HMGB1-induced expression of the SARS-CoV-2 receptor angiotensin-converting enzyme 2 in lung endothelial cells[167]. In the case of sepsis, circulating HMGB1 levels correlate with scores for disseminated intravascular coagulation and sequential organ failure assessment, two important measures for monitoring sepsis severity in the intensive care unit (ICU)[194]. HMGB1 levels are increased in almost all patients with community-acquired pneumonia, and higher circulating levels of HMGB1 have been associated with increased mortality[195]. In a clinical study of 185 individuals, higher levels of HMGB1 were found in infected patients compared with healthy controls and in patients with severe sepsis compared with less severe sepsis[196]. Autoantibodies to HMGB1 are associated with favourable outcomes in patients with septic shock[197]. However, in a study of 631 patients, the serum HMGB1 level was found not to be sensitive or specific for the diagnosis of sepsis in ICU settings with relatively heterogeneous and younger populations[198]. To gain a deeper understanding of the importance of serum HMGB1 levels in ICU patients, it will be crucial to consider not only the type of infection but also the underlying conditions, pre-existing diseases and age of patients.

HMGB1 expression correlates with worse prognosis in some cancer types, such as cervical cancer and breast cancer[199,200], but it is positively correlated with patient survival in pancreatic cancer[119]. The cytosolic HMGB1 level can predict disease recurrence and poor survival in patients with hepatitis B virus-associated hepatocellular carcinoma[201]. According to a clinical study of 202 patients with advanced solid cancer, a low baseline level of serum HMGB1 was found to be an independent positive prognostic and predictive factor for the efficacy of oncolytic adenovirus therapy[202]. The challenges of HMGB1-related clinical cancer research include the complexity and heterogeneity of genetic data, the need for large sample sizes and the importance of standardization and reproducibility of results. Overcoming these challenges will be crucial to advancing our understanding of HMGB1 as a biomarker for both tumour diagnosis and treatment, as well as long-term follow up for disease control or progression.

during tumour therapy[85]. How to maintain the immune-stimulatory, adjuvant activity of HMGB1 while inhibiting its immunosuppressive activity remains a crucial challenge during immunotherapy. Also, the role of HMGB1 in chronic cancer-bearing states has been less well studied than in an acute context.

Therapeutic targeting of HMGB1

Owing to its involvement in inflammation and its release in response to cellular stress, HMGB1 has been extensively studied as a potential biomarker of disease (Box 3). Blocking the release and activity of HMGB1 has been used with great success in a wide range of preclinical inflammatory disease models (for example, endotoxaemia and polymicrobial sepsis), confirming that HMGB1 is an attractive therapeutic target for acute infectious and sterile inflammatory conditions. Strategies to inhibit HMGB1 can be divided into direct inhibition and indirect inhibition. Direct inhibition involves chemicals or neutralizing antibodies that bind to HMGB1 to interfere with its immune activity. Indirect inhibition is mainly accomplished by disrupting the translocation and release of HMGB1 through pharmaceutical or non-pharmaceutical methods. Indirect inhibition is more likely to have off-target effects, although some off-target effects may promote an anti-inflammatory outcome. Here, we discuss examples of different types of HMGB1 inhibitors and their efficacy (see Supplementary Table 1 for further details).

Direct small-molecule inhibitors

Glycyrrhizin — a natural triterpene found in the roots and rhizomes of the licorice plant — is the first reported direct inhibitor of HMGB1. It binds directly to both HMG boxes of HMGB1, thereby inhibiting the interaction of HMGB1 with AGER and subsequent chemokine-like activity of HMGB1 (ref. 139). Glycyrrhizin has broad anti-inflammatory properties and protects against sepsis and I/R injury in animal models. HMGB1 administration reverses the protective effect of glycyrrhizin on thrombin-induced injury in the brain[140]. Epigallocatechin gallate, a green tea extract, also directly binds to HMGB1 and delivers it to lysosomes for degradation in an autophagy-dependent manner in both immune cells and non-immune cells[141]. Metformin, a prescription drug used to control hyperglycaemia in type 2 diabetes, directly binds to the C-terminal domain of HMGB1 to inhibit the extracellular pro-inflammatory effects of HMGB1 on mouse macrophages (such as the activation of mitogen-activated protein kinase 14 and the production of TNF)[142]. Both epigallocatechin gallate and metformin delay lethal endotoxaemia in mice[141,142]. These findings could lead to the

development of more potent, direct HMGB1 inhibitors with higher specificity.

Indirect small-molecule inhibitors

Another large class of compounds can inhibit HMGB1 release. Such inhibitors can be derived from natural sources (for example, tanshinone IIA[143], quercetin[144], lycopene[145], resveratrol[146], chloroquine[77], danggui[147] and danshen[148]) or can be chemically synthesized (for example, ethyl pyruvate[149], statins[150], oxaliplatin[151] and cisplatin[152]). Among them, ethyl pyruvate is commonly used to inhibit the release of HMGB1 experimentally. For example, ethyl pyruvate treatment initiated 4 hours after the onset of endotoxaemia, at which point clinical signs of LPS-induced toxicity are already evident, can inhibit HMGB1 release and protect against death of mice[149]. By inhibiting HMGB1 release, ethyl pyruvate inhibits pro-inflammatory pathways in mouse models of experimental sepsis or sterile tissue damage (such as liver I/R injury[153] and pancreatitis[154]). However, ethyl pyruvate and other indirect small-molecule inhibitors often have broad and unspecified antioxidant and anti-inflammatory activities (such as inhibiting the NF-κB pathway[149]), which indicates that HMGB1 may only be one of their targets. The effectiveness of these inhibitors for the prevention and treatment of systemic inflammation is owing to their multitarget effects on cytokine responses, making them a better choice than specific inhibitors of HMGB1.

Direct antibody-mediated inhibition

The use of antibodies to HMGB1 has provided specific evidence that extracellular HMGB1 is involved in various immunopathological conditions, although this has yet to be translated to clinical practice. For example, the mouse monoclonal antibody 2G7 is protective in certain inflammatory disease models, such as those for sepsis, haemorrhagic shock, arthritis, pancreatitis and autoimmune myocarditis[155], and a humanized version is being tested in experimental models of osteoarthritis[156]. 2G7 binds to an epitope in the A-box of HMGB1, which affects the interaction of HMGB1 with AGER and TLR4. Another extensively studied monoclonal antibody to HMGB1, #10–22, which recognizes an epitope in the C-terminal sequence of HMGB1 (ref. 157), prevents inflammatory diseases associated with nerve and brain injury in mouse models[157]. The humanized monoclonal antibody IA-4, which recognizes mouse and human HMGB1, blocks HMGB1-induced IL-6 release, which might have therapeutic benefit for the treatment of lupus nephritis[158]. However, treatment with 2G7 does not affect lupus nephritis in MRL/lpr mice[159], which argues against a pathological role of HMGB1 in this model. Commercially available antibodies, in particular chicken HMGB1-neutralizing polyclonal antibodies, provide additional evidence that HMGB1 is a mediator of inflammatory disease[160].

Peptide and protein inhibitors

A-box proteins bind to the B-box of endogenous HMGB1 and have been established as potential anti-inflammatory agents in mouse models of experimental arthritis, sepsis, stroke, I/R injury and pancreatitis[76]. Furthermore, several endogenous molecules can bind to HMGB1 and regulate its pro-inflammatory activity in experimental sepsis or endotoxaemia. However, the structural basis of HMGB1-binding proteins in shaping HMGB1 activity remains poorly understood, and producing peptides and proteins for clinical use can be expensive and challenging.

Examples of endogenous HMGB1 inhibitors include complement component C1q, which forms a complex with HMGB1 in lipid rafts that promotes the differentiation of human monocytes into anti-inflammatory macrophages through AGER signalling[161]. Thrombomodulin is a transmembrane protein normally expressed in endothelial

Box 4

Remaining questions in HMGB1 research

In the future, interdisciplinary cooperation and application of new technologies should be used to answer the following questions regarding high-mobility group box 1 (HMGB1). First, how can we distinguish the specific contribution of each HMGB1 receptor in shaping the immune response? The currently reported HMGB1 receptors seem to be widely expressed by various cell types, including immune cells, endothelial cells and fibroblasts. Although some in vitro studies indicate that HMGB1 binds to Toll-like receptor 4 with higher affinity than advanced glycosylation end product-specific receptor (AGER)[8,9], the ability of HMGB1 to bind these receptors in vivo remains unknown, as does its time-dependent and cell-dependent selectivity. Extracellular HMGB1-mediated transport of pathogen-associated or damage-associated molecular patterns into cells suggests that intrinsic pathways, involving additional and as-yet-unknown HMGB1-binding proteins, may fine-tune HMGB1 activity. The biological relevance of the dynamics of HMGB1 translocation from intracellular to extracellular environments and back in response to the same or similar inflammatory stimuli requires further investigation.

Second, does HMGB1 release during various types of cell death stimulated by different cell death signals mediate the same immune responses? Dead or dying cells release not only HMGB1 but also other non-immunogenic and immunogenic molecules. In contrast to the reported synergistic effects of HMGB1 and other DAMPs, the effect of non-immunogenic DAMPs generated by cell death on the inflammatory regulatory function of HMGB1 remains poorly understood. Further identification of DAMP networks and signalling in response to injury in individual tissues may facilitate our understanding of the inflammatory phase of tissue injury and the simultaneous initiation of responses to resolve it.

Third, highly effective and selective HMGB1 inhibitors are still lacking. The design of HMGB1 inhibitors may consider the selective inhibition of particular intracellular or extracellular functions. Overall, the selective inhibition of autophagy and the nuclear homeostasis functions of intracellular HMGB1 may be beneficial for tumour therapy, and interfering with extracellular HMGB1 activity may contribute to the treatment of inflammatory diseases. HMGB1 has several binding partners, and designing an inhibitor that disrupts the interaction of HMGB1 with one binding partner without affecting its interaction with others can be quite challenging.

cells that binds to thrombin to form an anticoagulant complex. Soluble thrombomodulin can also bind to HMGB1, thereby facilitating its proteolytic cleavage by thrombin and inhibiting HMGB1 activity[162]. Heparin, which also has anticoagulant properties, can inhibit the HMGB1–LPS interaction and prevent the degradation of the macrophage glycocalyx by endogenous heparanase, thereby blocking the HMGB1-mediated cytosolic delivery of LPS to activate the caspase-11-dependent inflammasome[163]. Binding of HMGB1 to haptoglobin can also trigger an anti-inflammatory response through the receptor CD163 in macrophages[164].

In the context of virus infection, adenovirus core protein VII can bind to host HMGB1 to inhibit its release in lung cells in vitro or in mice, thereby inhibiting host immune responses[165]. The C-type lectin domain family 3 member B (CLEC3B; also known as tetranectin), which binds to plasminogen in plasma and the extracellular matrix to enhance proteolytic processes, was recently found also to bind to extracellular HMGB1. CLEC3B brings HMGB1 into cells through endocytosis, resulting in inflammatory pyroptosis in macrophages and monocytes[74]. Humanized antibodies to CLEC3B prevent HMGB1-mediated polymicrobial sepsis and endotoxaemia in mice[74]. Given that anti-CLEC3B also recognizes angiotensin-converting enzyme 2 (the main cell entry receptor for SARS-CoV-2)[166] and that HMGB1 is a mediator of severe SARS-CoV-2 pathology[167], this antibody should be tested to see whether it is protective against COVID-19.

DNA-based inhibitors

Several structure-based oligonucleotides have been designed to inhibit HMGB1 activity in immune cells and non-immune cells. For example, kinked hairpin–loop DNA, ISM ODN (a non-immunogenic CpG-B oligodeoxynucleotide analogue that carries GpG or GpC instead of CpG) or bent oligonucleotide duplexes inhibit HMGB1-induced cell migration and cytokine production in a concentration-dependent manner in bovine aortic endothelial cells, fibroblasts or macrophages[168–170]. However, the effects of DNA-based drugs on HMGB1 inhibition in vivo and potential immunological side effects on DNA-sensing pathways in inflammatory disease models require further investigation. One of the biggest challenges in using DNA-based inhibitors is delivering them to target cells effectively and selectively. The oligonucleotides are typically too large to cross the cell membrane and must be delivered through various chemical modifications or encapsulated in nanoparticles.

Neurotransmitter-based inhibition

The cholinergic anti-inflammatory pathway is mediated by vagal nerve stimulation through the activation of cholinergic receptor nicotinic α7 subunit (CHRNA7; also known as α7nAchR)[171,172]. Similarly to vagal nerve stimulation[173], the CHRNA7 agonist GTS-21 protects against hyperoxia-induced acute inflammatory lung injury, lethal endotoxaemia or experimental sepsis in mice by attenuating the accumulation of HMGB1 in the circulation[174,175]. In addition, release of the neurotransmitter dopamine protects against HMGB1 release in experimental sepsis by inhibiting inflammasome activation[176,177]. Whether the vagal–adrenal anti-inflammatory axis directly affects HMGB1 release under pathological conditions remains to be investigated[178].

Conclusions and perspectives

The evolutionarily conserved protein HMGB1 has distinct functions inside and outside cells. Although early studies focused on the role of nuclear HMGB1 as a DNA chaperone in maintaining chromatin structure and function, studies over the past 20 years have focused more on identifying the functions of extracellular and cytosolic HMGB1 in cellular stress and immune responses. In addition to the active secretion of HMGB1 induced by inflammatory stimuli, various cell death pathways can lead to the release of HMGB1 following tissue injury or microbial infection[179]. During the early stages of inflammation, the release of HMGB1 may stimulate immune and inflammatory responses that contribute to microbial clearance and wound healing. However, chronic inflammation and HMGB1 release can trigger a cytokine storm followed by immune cell death and immunosuppression, leading to multi-organ failure and even death of the organism. HMGB1 antagonists have shown great success in a wide range of preclinical disease models, particularly infectious and sterile inflammatory diseases, indicating that HMGB1 is an attractive therapeutic target awaiting full clinical realization. It will be important to further explore strategies for combining HMGB1 with other targets to treat critical illness. Despite, and perhaps because of the many challenges in HMGB1 research, we look forward to a new era of HMGB1 discovery that addresses its multiple functions in multiple compartments within and outside the cell (Box 4).

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References

1. 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).
2. Sharman, A. C., Hay-Schmidt, A. & Holland, P. W. Cloning and analysis of an HMG gene from the lamprey Lampetra fluviatilis: gene duplication in vertebrate evolution. *Gene* **184**, 99–105 (1997).
3. Calogero, S. et al. The lack of chromosomal protein Hmg1 does not disrupt cell growth but causes lethal hypoglycaemia in newborn mice. *Nat. Genet.* **22**, 276–280 (1999).
4. Aikawa, S. et al. Uterine deficiency of high-mobility group box-1 (HMGB1) protein causes implantation defects and adverse pregnancy outcomes. *Cell Death Differ.* **27**, 1489–1504 (2020).
5. Yanai, H. et al. HMGB proteins function as universal sentinels for nucleic-acid-mediated innate immune responses. *Nature* **462**, 99–103 (2009).
This study reports that cytosolic HMGB1 is a mediator of nucleic-acid-induced immune responses.
6. Tang, D. et al. Endogenous HMGB1 regulates autophagy. *J. Cell Biol.* **190**, 881–892 (2010).
This study reports that cytosolic HMGB1 is a mediator of autophagy.
7. Tsung, A. et al. The nuclear factor HMGB1 mediates hepatic injury after murine liver ischemia–reperfusion. *J. Exp. Med.* **201**, 1135–1143 (2005).
This study reports that HMGB1 is a mediator of sterile inflammation.
8. Ling, Y. et al. Heparin changes the conformation of high-mobility group protein 1 and decreases its affinity toward receptor for advanced glycation endproducts in vitro. *Int. Immunopharmacol.* **11**, 187–193 (2011).
9. Liu, R. et al. Establishment of in vitro binding assay of high mobility group box-1 and S100A12 to receptor for advanced glycation endproducts: heparin's effect on binding. *Acta Med. Okayama* **63**, 203–211 (2009).
10. He, M., Bianchi, M. E., Coleman, T. R., Tracey, K. J. & Al-Abed, Y. Exploring the biological functional mechanism of the HMGB1/TLR4/MD-2 complex by surface plasmon resonance. *Mol. Med.* **24**, 21 (2018).
11. Yang, H. et al. A critical cysteine is required for HMGB1 binding to toll-like receptor 4 and activation of macrophage cytokine release. *Proc. Natl Acad. Sci. USA* **107**, 11942–11947 (2010).
12. Huang, J. et al. DAMPs, ageing, and cancer: the 'DAMP hypothesis'. *Ageing Res. Rev.* **24**, 3–16 (2015).
13. Kleinschmidt, J. A., Seiter, A. & Zentgraf, H. Nucleosome assembly in vitro: separate histone transfer and synergistic interaction of native histone complexes purified from nuclei of *Xenopus laevis* oocytes. *EMBO J.* **9**, 1309–1318 (1990).
14. Bianchi, M. E., Beltrame, M. & Paonessa, G. Specific recognition of cruciform DNA by nuclear protein HMG1. *Science* **243**, 1056–1059 (1989).
15. Pil, P. M. & Lippard, S. J. Specific binding of chromosomal protein HMG1 to DNA damaged by the anticancer drug cisplatin. *Science* **256**, 234–237 (1992).
16. Ru, H. et al. Molecular mechanism of V(D)J recombination from synaptic RAG1–RAG2 complex structures. *Cell* **163**, 1138–1152 (2015).
17. Zhang, Y. et al. Reconstitution of 5′-directed human mismatch repair in a purified system. *Cell* **122**, 693–705 (2005).
18. Wang, X. et al. Dynamic autoinhibition of the HMGB1 protein via electrostatic fuzzy interactions of intrinsically disordered regions. *J. Mol. Biol.* **433**, 167122 (2021).
19. Mensah, M. A. et al. Aberrant phase separation and nucleolar dysfunction in rare genetic diseases. *Nature* **614**, 564–571 (2023).

20. Uguen, K. et al. Heterozygous HMGB1 loss-of-function variants are associated with developmental delay and microcephaly. *Clin. Genet.* **100**, 386–395 (2021).
21. Venereau, E. et al. Mutually exclusive redox forms of HMGB1 promote cell recruitment or proinflammatory cytokine release. *J. Exp. Med.* **209**, 1519–1528 (2012).
22. Park, S. & Lippard, S. J. Redox state-dependent interaction of HMGB1 and cisplatin-modified DNA. *Biochemistry* **50**, 2567–2574 (2011).
23. El Gazzar, M. et al. Chromatin-specific remodeling by HMGB1 and linker histone H1 silences proinflammatory genes during endotoxin tolerance. *Mol. Cell. Biol.* **29**, 1959–1971 (2009).
24. Huang, H. et al. Hepatocyte specific HMGB1 deletion worsens the injury in liver ischemia/reperfusion: a role for intracellular HMGB1 in cellular protection. *Hepatology* **59**, 1984–1997 (2014).
25. Kang, R. et al. Intracellular Hmgb1 inhibits inflammatory nucleosome release and limits acute pancreatitis in mice. *Gastroenterology* **146**, 1097–1107 (2014).
26. Chen, Q. et al. Circulating nucleosomes as a predictor of sepsis and organ dysfunction in critically ill patients. *Int. J. Infect. Dis.* **16**, e558–e564 (2012).
27. Cavalier, E. et al. Circulating nucleosomes as potential markers to monitor COVID-19 disease progression. *Front. Mol. Biosci.* **8**, 600881 (2021).
28. Wang, H. et al. HMG-1 as a late mediator of endotoxin lethality in mice. *Science* **285**, 248–251 (1999).
This study reports that HMGB1 can be secreted into the extracellular space to mediate inflammation.
29. Gardella, S. et al. The nuclear protein HMGB1 is secreted by monocytes via a non-classical, vesicle-mediated secretory pathway. *EMBO Rep.* **3**, 995–1001 (2002).
This study reports the mechanism of HMGB1 secretion.
30. Rendon-Mitchell, B. et al. IFN-gamma induces high mobility group box 1 protein release partly through a TNF-dependent mechanism. *J. Immunol.* **170**, 3890–3897 (2003).
31. Deng, M. et al. The endotoxin delivery protein HMGB1 mediates caspase-11-dependent lethality in sepsis. *Immunity* **49**, 740–753.e7 (2018).
32. Yang, H. et al. HMGB1 released from nociceptors mediates inflammation. *Proc. Natl Acad. Sci. USA* **118**, e2102034118 (2021).
33. Scaffidi, P., Misteli, T. & Bianchi, M. E. Release of chromatin protein HMGB1 by necrotic cells triggers inflammation. *Nature* **418**, 191–195 (2002).
This study reports that HMGB1 functions as a DAMP during necrosis.
34. Kang, R. et al. HMGB1 in health and disease. *Mol. Asp. Med.* **40**, 1–116 (2014).
35. Urbonaviciute, V. et al. Induction of inflammatory and immune responses by HMGB1-nucleosome complexes: implications for the pathogenesis of SLE. *J. Exp. Med.* **205**, 3007–3018 (2008).
36. Ivanov, S. et al. A novel role for HMGB1 in TLR9-mediated inflammatory responses to CpG-DNA. *Blood* **110**, 1970–1981 (2007).
37. Tian, J. et al. Toll-like receptor 9-dependent activation by DNA-containing immune complexes is mediated by HMGB1 and RAGE. *Nat. Immunol.* **8**, 487–496 (2007).
38. Rouhiainen, A., Tumova, S., Valmu, L., Kalkkinen, N. & Rauvala, H. Pivotal advance: analysis of proinflammatory activity of highly purified eukaryotic recombinant HMGB1 (amphoterin). *J. Leukoc. Biol.* **81**, 49–58 (2007).
39. Iachettini, S. et al. The telomeric protein TERF2/TRF2 impairs HMGB1-driven autophagy. *Autophagy* https://doi.org/10.1080/15548627.2022.2138687 (2022).
40. Livesey, K. M. et al. p53/HMGB1 complexes regulate autophagy and apoptosis. *Cancer Res.* **72**, 1996–2005 (2012).
41. Kim, S. W., Oh, S. A., Seol, S. I., Davaanyam, D. & Lee, J. K. Cytosolic HMGB1 mediates LPS-induced autophagy in microglia by interacting with NOD2 and suppresses its proinflammatory function. *Cells* **11**, 2410 (2022).
42. Huebener, P. et al. High-mobility group box 1 is dispensable for autophagy, mitochondrial quality control, and organ function in vivo. *Cell Metab.* **19**, 539–547 (2014).
43. Deretic, V., Saitoh, T. & Akira, S. Autophagy in infection, inflammation and immunity. *Nat. Rev. Immunol.* **13**, 722–737 (2013).
44. 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).
45. Dupont, N. et al. Autophagy-based unconventional secretory pathway for extracellular delivery of IL-1beta. *EMBO J.* **30**, 4701–4711 (2011).
46. Wang, Z. et al. Autophagy-based unconventional secretion of HMGB1 by keratinocytes plays a pivotal role in psoriatic skin inflammation. *Autophagy* **17**, 529–552 (2021).
47. Kim, Y. H. et al. Secretory autophagy machinery and vesicular trafficking are involved in HMGB1 secretion. *Autophagy* **17**, 2345–2362 (2021).
48. Zhou, B. et al. Extracellular SQSTM1 mediates bacterial septic death in mice through insulin receptor signalling. *Nat. Microbiol.* **5**, 1576–1587 (2020).
49. Zhu, X. et al. Cytosolic HMGB1 controls the cellular autophagy/apoptosis checkpoint during inflammation. *J. Clin. Invest.* **125**, 1098–1110 (2015).
50. Zeng, X., Liu, F., Liu, K., Xin, J. & Chen, J. HMGB1 could restrict 1,3-beta-glucan induced mice lung inflammation by affecting beclin1 and Bcl2 interaction and promoting the autophagy of epithelial cells. *Ecotoxicol. Environ. Saf.* **222**, 112460 (2021).
51. Zhang, Y. G. et al. Intestinal epithelial HMGB1 inhibits bacterial infection via STAT3 regulation of autophagy. *Autophagy* **15**, 1935–1953 (2019).
52. Yanai, H. et al. Conditional ablation of HMGB1 in mice reveals its protective function against endotoxemia and bacterial infection. *Proc. Natl Acad. Sci. USA* **110**, 20699–20704 (2013).
53. Parker, K. H., Horn, L. A. & Ostrand-Rosenberg, S. High-mobility group box protein 1 promotes the survival of myeloid-derived suppressor cells by inducing autophagy. *J. Leukoc. Biol.* **100**, 463–470 (2016).
54. Xue, J. et al. Asbestos induces mesothelial cell transformation via HMGB1-driven autophagy. *Proc. Natl Acad. Sci. USA* **117**, 25543–25552 (2020).
55. Li, J. et al. HMGB1 promotes resistance to doxorubicin in human hepatocellular carcinoma cells by inducing autophagy via the AMPK/mTOR signaling pathway. *Front. Oncol.* **11**, 739145 (2021).
56. Tang, D. et al. HMGB1 release and redox regulates autophagy and apoptosis in cancer cells. *Oncogene* **29**, 5299–5310 (2010).
57. 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).
58. Andreeva, L. et al. cGAS senses long and HMGB/TFAM-bound U-turn DNA by forming protein–DNA ladders. *Nature* **549**, 394–398 (2017).
59. Razi, M., Chan, E. Y. & Tooze, S. A. Early endosomes and endosomal coatomer are required for autophagy. *J. Cell Biol.* **185**, 305–321 (2009).
60. Merenmies, J., Pihlaskari, R., Laitinen, J., Wartiovaara, J. & Rauvala, H. 30-kDa heparin-binding protein of brain (amphoterin) involved in neurite outgrowth. Amino acid sequence and localization in the filopodia of the advancing plasma membrane. *J. Biol. Chem.* **266**, 16722–16729 (1991).
61. Tadie, J. M. et al. HMGB1 promotes neutrophil extracellular trap formation through interactions with toll-like receptor 4. *Am. J. Physiol. Lung Cell Mol. Physiol.* **304**, L342–L349 (2013).
62. Ahrens, I. et al. HMGB1 binds to activated platelets via the receptor for advanced glycation end products and is present in platelet rich human coronary artery thrombi. *Thromb. Haemost.* **114**, 994–1003 (2015).
63. Zhan, Y. et al. HMGB1-mediated neutrophil extracellular trap formation exacerbates intestinal ischemia/reperfusion-induced acute lung injury. *J. Immunol.* **208**, 968–978 (2022).
64. Zhang, X. L. et al. HMGB1-promoted neutrophil extracellular traps contribute to cardiac diastolic dysfunction in mice. *J. Am. Heart Assoc.* **11**, e023800 (2022).
65. Maugeri, N. et al. Platelet microparticles sustain autophagy-associated activation of neutrophils in systemic sclerosis. *Sci. Transl. Med.* **10**, eaao3089 (2018).
66. Vogel, S. et al. Platelet-derived HMGB1 is a critical mediator of thrombosis. *J. Clin. Invest.* **125**, 4638–4654 (2015).
67. Jiang, W., Li, J., Gallowitsch-Puerta, M., Tracey, K. J. & Pisetsky, D. S. The effects of CpG DNA on HMGB1 release by murine macrophage cell lines. *J. Leukoc. Biol.* **78**, 930–936 (2005).
68. Lamkanfi, M. et al. Inflammasome-dependent release of the alarmin HMGB1 in endotoxemia. *J. Immunol.* **185**, 4385–4392 (2010).
69. Andersson, U. et al. High mobility group 1 protein (HMG-1) stimulates proinflammatory cytokine synthesis in human monocytes. *J. Exp. Med.* **192**, 565–570 (2000).
This study reports that HMGB1 can induce cytokine production.
70. 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).
This study reports that the activity of HMGB1 is regulated by its redox status.
71. Hartl, J. et al. Autoantibody-mediated impairment of DNASE1L3 activity in sporadic systemic lupus erythematosus. *J. Exp. Med.* **218**, e20201138 (2021).
72. Spagnuolo, L. et al. HMGB1 promotes CXCL12-dependent egress of murine B cells from Peyer's patches in homeostasis. *Eur. J. Immunol.* **51**, 1980–1991 (2021).
73. Xu, J. et al. Macrophage endocytosis of high-mobility group box 1 triggers pyroptosis. *Cell Death Differ.* **21**, 1229–1239 (2014).
74. Chen, W. et al. Identification of tetranectin-targeting monoclonal antibodies to treat potentially lethal sepsis. *Sci. Transl. Med.* **12**, eaaz3833 (2020).
75. Hernandez-Pando, R. et al. The role of high mobility group box 1 protein (HMGB1) in the immunopathology of experimental pulmonary tuberculosis. *PLoS ONE* **10**, e0133200 (2015).
76. Yang, H. et al. Reversing established sepsis with antagonists of endogenous high-mobility group box 1. *Proc. Natl Acad. Sci. USA* **101**, 296–301 (2004).
77. Yang, M. et al. Chloroquine inhibits HMGB1 inflammatory signaling and protects mice from lethal sepsis. *Biochem. Pharmacol.* **86**, 410–418 (2013).
78. Devaraj, A. et al. The extracellular innate-immune effector HMGB1 limits pathogenic bacterial biofilm proliferation. *J. Clin. Invest.* **131**, e140527 (2021).
79. Zhou, H. et al. Platelet HMGB1 is required for efficient bacterial clearance in intra-abdominal bacterial sepsis in mice. *Blood Adv.* **2**, 638–648 (2018).
80. Tsung, A. et al. HMGB1 release induced by liver ischemia involves toll-like receptor 4 dependent reactive oxygen species production and calcium-mediated signaling. *J. Exp. Med.* **204**, 2913–2923 (2007).
81. Yang, H. et al. MD-2 is required for disulfide HMGB1-dependent TLR4 signaling. *J. Exp. Med.* **212**, 5–14 (2015).
82. Taguchi, A. et al. Blockade of RAGE-amphoterin signalling suppresses tumour growth and metastases. *Nature* **405**, 354–360 (2000).
This study reports the oncogenic role of HMGB1.
83. Wang, J. et al. HMGB1 participates in LPS-induced acute lung injury by activating the AIM2 inflammasome in macrophages and inducing polarization of M1 macrophages via TLR2, TLR4, and RAGE/NF-kappaB signaling pathways. *Int. J. Mol. Med.* **45**, 61–80 (2020).
84. Chen, G. Y., Tang, J., Zheng, P. & Liu, Y. CD24 and Siglec-10 selectively repress tissue damage-induced immune responses. *Science* **323**, 1722–1725 (2009).
85. 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).

86. de Mingo Pulido, A. et al. The inhibitory receptor TIM-3 limits activation of the cGAS–STING pathway in intra-tumoral dendritic cells by suppressing extracellular DNA uptake. *Immunity* **54**, 1154–1167.e7 (2021).
87. Kim, T. S., Gorski, S. A., Hahn, S., Murphy, K. M. & Braciale, T. J. Distinct dendritic cell subsets dictate the fate decision between effector and memory CD8(+) T cell differentiation by a CD24-dependent mechanism. *Immunity* **40**, 400–413 (2014).
88. Zhang, H. et al. TMEM173 drives lethal coagulation in sepsis. *Cell Host Microbe* **27**, 556–570.e6 (2020).
89. Kang, R. et al. Lipid peroxidation drives gasdermin D-mediated pyroptosis in lethal polymicrobial sepsis. *Cell Host. Microbe* **24**, 97–108.e4 (2018).
90. Yen, Y. C. et al. Structures of atypical chemokine receptor 3 reveal the basis for its promiscuity and signaling bias. *Sci. Adv.* **8**, eabn8063 (2022).
91. Tang, D. et al. Hydrogen peroxide stimulates macrophages and monocytes to actively release HMGB1. *J. Leukoc. Biol.* **81**, 741–747 (2007).
92. Tirone, M. et al. High mobility group box 1 orchestrates tissue regeneration via CXCR4. *J. Exp. Med.* **215**, 303–318 (2018).
93. Bell, C. W., Jiang, W., Reich, C. F. III & Pisetsky, D. S. The extracellular release of HMGB1 during apoptotic cell death. *Am. J. Physiol. Cell Physiol.* **291**, C1318–C1325 (2006).
94. Volchuk, A., Ye, A., Chi, L., Steinberg, B. E. & Goldenberg, N. M. Indirect regulation of HMGB1 release by gasdermin D. *Nat. Commun.* **11**, 4561 (2020).
95. Lu, B. et al. Novel role of PKR in inflammasome activation and HMGB1 release. *Nature* **488**, 670–674 (2012).
96. Kamiya, M. et al. Targeting necroptosis in muscle fibers ameliorates inflammatory myopathies. *Nat. Commun.* **13**, 166 (2022).
97. Zou, J. et al. Poly IC triggers a cathepsin D- and IPS-1-dependent pathway to enhance cytokine production and mediate dendritic cell necroptosis. *Immunity* **38**, 717–728 (2013).
98. Kayagaki, N. et al. NINJ1 mediates plasma membrane rupture during lytic cell death. *Nature* **591**, 131–136 (2021).
99. Borges, J. P. et al. Glycine inhibits NINJ1 membrane clustering to suppress plasma membrane rupture in cell death. *eLife* **11**, e78609 (2022).
100. Rogers, C. et al. Cleavage of DFNA5 by caspase-3 during apoptosis mediates progression to secondary necrotic/pyroptotic cell death. *Nat. Commun.* **8**, 14128 (2017).
101. Taniguchi, N. et al. High mobility group box chromosomal protein 1 plays a role in the pathogenesis of rheumatoid arthritis as a novel cytokine. *Arthritis Rheum.* **48**, 971–981 (2003).
102. Ostberg, T. et al. Protective targeting of high mobility group box chromosomal protein 1 in a spontaneous arthritis model. *Arthritis Rheum.* **62**, 2963–2972 (2010).
103. Bangert, A. et al. Critical role of RAGE and HMGB1 in inflammatory heart disease. *Proc. Natl Acad. Sci. USA* **113**, E155–E164 (2016).
104. Fu, L. et al. Therapeutic effects of anti-HMGB1 monoclonal antibody on pilocarpine-induced status epilepticus in mice. *Sci. Rep.* **7**, 1179 (2017).
105. 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).
106. Koprivica, I., Vujicic, M., Gajic, D., Saksida, T. & Stojanovic, I. Ethyl pyruvate stimulates regulatory T cells and ameliorates type 1 diabetes development in mice. *Front. Immunol.* **9**, 3130 (2018).
107. Soloff, A. C. & Lotze, M. T. A peaceful death orchestrates immune balance in a chaotic environment. *Proc. Natl Acad. Sci. USA* **116**, 22901–22903 (2019).
108. Mogilenko, D. A., Shchukina, I. & Artyomov, M. N. Immune ageing at single-cell resolution. *Nat. Rev. Immunol.* **22**, 484–498 (2022).
109. Enokido, Y., Yoshitake, A., Ito, H. & Okazawa, H. Age-dependent change of HMGB1 and DNA double-strand break accumulation in mouse brain. *Biochem. Biophys. Res. Commun.* **376**, 128–133 (2008).
110. Gao, H. M. et al. HMGB1 acts on microglia Mac1 to mediate chronic neuroinflammation that drives progressive neurodegeneration. *J. Neurosci.* **31**, 1081–1092 (2011).
111. Karuppagounder, V. et al. Modulation of macrophage polarization and HMGB1-TLR2/TLR4 cascade plays a crucial role for cardiac remodeling in senescence-accelerated prone mice. *PLoS ONE* **11**, e0152922 (2016).
112. Sofiadis, K. et al. HMGB1 coordinates SASP-related chromatin folding and RNA homeostasis on the path to senescence. *Mol. Syst. Biol.* **17**, e9760 (2021).
113. Davalos, A. R. et al. p53-dependent release of Alarmin HMGB1 is a central mediator of senescent phenotypes. *J. Cell. Biol.* **201**, 613–629 (2013).
114. Li, F. et al. FBP1 loss disrupts liver metabolism and promotes tumorigenesis through a hepatic stellate cell senescence secretome. *Nat. Cell Biol.* **22**, 728–739 (2020).
115. Gaikwad, S. et al. Tau oligomer induced HMGB1 release contributes to cellular senescence and neuropathology linked to Alzheimer's disease and frontotemporal dementia. *Cell Rep.* **36**, 109419 (2021).
116. Lee, J. J. et al. HMGB1 orchestrates STING-mediated senescence via TRIM30alpha modulation in cancer cells. *Cell Death Discov.* **7**, 28 (2021).
117. Hernandez, C. et al. HMGB1 links chronic liver injury to progenitor responses and hepatocarcinogenesis. *J. Clin. Invest.* **128**, 2436–2451 (2018).
118. Chen, R. et al. High mobility group protein B1 controls liver cancer initiation through yes-associated protein-dependent aerobic glycolysis. *Hepatology* **67**, 1823–1841 (2018).
119. Kang, R. et al. Intracellular HMGB1 as a novel tumor suppressor of pancreatic cancer. *Cell Res.* **27**, 916–932 (2017).
This study reports the tumour suppressor role of HMGB1.
120. Bald, T. et al. Ultraviolet-radiation-induced inflammation promotes angiotropism and metastasis in melanoma. *Nature* **507**, 109–113 (2014).
121. Parker, K. H. et al. HMGB1 enhances immune suppression by facilitating the differentiation and suppressive activity of myeloid-derived suppressor cells. *Cancer Res.* **74**, 5723–5733 (2014).
122. 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).
123. Conche, C. et al. Combining ferroptosis induction with MDSC blockade renders primary tumours and metastases in liver sensitive to immune checkpoint blockade. *Gut* https://doi.org/10.1136/gutjnl-2022-327909 (2023).
124. Dai, E. et al. Ferroptotic damage promotes pancreatic tumorigenesis through a TMEM173/STING-dependent DNA sensor pathway. *Nat. Commun.* **11**, 6339 (2020).
125. Li, C. et al. PINK1 and PARK2 suppress pancreatic tumorigenesis through control of mitochondrial iron-mediated immunometabolism. *Dev. Cell* **46**, 441–455 e448 (2018).
126. Gopal, A. et al. TIRAP drives myelosuppression through an Ifngamma-Hmgb1 axis that disrupts the endothelial niche in mice. *J. Exp. Med.* **219**, e20200731 (2022).
127. Ye, L. et al. Tumor-derived exosomal HMGB1 fosters hepatocellular carcinoma immune evasion by promoting TIM-1(+) regulatory B cell expansion. *J. Immunother. Cancer* **6**, 145 (2018).
128. Kam, N. W. et al. Peritumoral B cells drive proangiogenic responses in HMGB1-enriched esophageal squamous cell carcinoma. *Angiogenesis* **25**, 181–203 (2022).
129. Liu, M. et al. The HMGB1 (C106A) mutation inhibits IL-10-producing CD19(hi) FcgammaRIIb(hi) B cell expansion by suppressing STAT3 activation in mice. *Front. Immunol.* **13**, 975551 (2022).
130. Soloff, A. C. et al. HMGB1 promotes myeloid egress and limits lymphatic clearance of malignant pleural effusions. *Front. Immunol.* **11**, 2027 (2020).
131. Rojas, A. et al. HMGB1 enhances the protumoral activities of M2 macrophages by a RAGE-dependent mechanism. *Tumour Biol.* **37**, 3321–3329 (2016).
132. Shiau, D. J. et al. Hepatocellular carcinoma-derived high mobility group box 1 triggers M2 macrophage polarization via a TLR2/NOX2/autophagy axis. *Sci. Rep.* **10**, 13582 (2020).
133. Apetoh, L. et al. Toll-like receptor 4-dependent contribution of the immune system to anticancer chemotherapy and radiotherapy. *Nat. Med.* **13**, 1050–1059 (2007).
This study reports that HMGB1 is a mediator of immunogenic cell death.
134. Solari, J. I. G. et al. Damage-associated molecular patterns (DAMPs) related to immunogenic cell death are differentially triggered by clinically relevant chemotherapeutics in lung adenocarcinoma cells. *BMC Cancer* **20**, 474 (2020).
135. Fahmueller, Y. N. et al. Immunogenic cell death biomarkers HMGB1, RAGE, and DNAse indicate response to radioembolization therapy and prognosis in colorectal cancer patients. *Int. J. Cancer* **132**, 2349–2358 (2013).
136. Gdynia, G. et al. The HMGB1 protein induces a metabolic type of tumour cell death by blocking aerobic respiration. *Nat. Commun.* **7**, 10764 (2016).
137. Li, Z. et al. Autophagy-based unconventional secretion of HMGB1 in glioblastoma promotes chemosensitivity to temozolomide through macrophage M1-like polarization. *J. Exp. Clin. Cancer Res.* **41**, 74 (2022).
138. Wang, Z. et al. cGAS/STING axis mediates a topoisomerase II inhibitor-induced tumor immunogenicity. *J. Clin. Invest.* **129**, 4850–4862 (2019).
139. Mollica, L. et al. Glycyrrhizin binds to high-mobility group box 1 protein and inhibits its cytokine activities. *Chem. Biol.* **14**, 431–441 (2007).
140. Ohnishi, M. et al. HMGB1 inhibitor glycyrrhizin attenuates intracerebral hemorrhage-induced injury in rats. *Neuropharmacology* **61**, 975–980 (2011).
141. Li, W. et al. EGCG stimulates autophagy and reduces cytoplasmic HMGB1 levels in endotoxin-stimulated macrophages. *Biochem. Pharmacol.* **81**, 1152–1163 (2011).
142. Horiuchi, T. et al. Metformin directly binds the alarmin HMGB1 and inhibits its proinflammatory activity. *J. Biol. Chem.* **292**, 8436–8446 (2017).
143. Zhang, Y. et al. Tanshinone IIA sodium sulfonate facilitates endocytic HMGB1 uptake. *Biochem. Pharmacol.* **84**, 1492–1500 (2012).
144. Tang, D. et al. Quercetin prevents LPS-induced high-mobility group box 1 release and proinflammatory function. *Am. J. Respir. Cell Mol. Biol.* **41**, 651–660 (2009).
145. Lee, W., Ku, S. K., Bae, J. W. & Bae, J. S. Inhibitory effects of lycopene on HMGB1-mediated pro-inflammatory responses in both cellular and animal models. *Food Chem. Toxicol.* **50**, 1826–1833 (2012).
146. Zainal, N. et al. Resveratrol treatment reveals a novel role for HMGB1 in regulation of the type 1 interferon response in dengue virus infection. *Sci. Rep.* **7**, 42998 (2017).
147. Wang, H. et al. The aqueous extract of a popular herbal nutrient supplement, *Angelica sinensis*, protects mice against lethal endotoxemia and sepsis. *J. Nutr.* **136**, 360–365 (2006).
148. Li, W. et al. A cardiovascular drug rescues mice from lethal sepsis by selectively attenuating a late-acting proinflammatory mediator, high mobility group box 1. *J. Immunol.* **178**, 3856–3864 (2007).
149. Ulloa, L. et al. Ethyl pyruvate prevents lethality in mice with established lethal sepsis and systemic inflammation. *Proc. Natl Acad. Sci. USA* **99**, 12351–12356 (2002).
150. Jin, D. et al. Atorvastatin reduces serum HMGB1 levels in patients with hyperlipidemia. *Exp. Ther. Med.* **4**, 1124–1126 (2012).
151. Ostberg, T. et al. Oxaliplatin retains HMGB1 intranuclearly and ameliorates collagen type II-induced arthritis. *Arthritis Res. Ther.* **10**, R1 (2008).
152. Pan, P. et al. Low-dose cisplatin administration in murine cecal ligation and puncture prevents the systemic release of HMGB1 and attenuates lethality. *J. Leukoc. Biol.* **86**, 625–632 (2009).

153. Tsung, A. et al. Ethyl pyruvate ameliorates liver ischemia–reperfusion injury by decreasing hepatic necrosis and apoptosis. *Transplantation* **79**, 196–204 (2005).
154. Turkyilmaz, S. et al. Ethyl pyruvate treatment ameliorates pancreatic damage: evidence from a rat model of acute necrotizing pancreatitis. *Arch. Med. Sci.* **15**, 232–239 (2019).
155. Schierbeck, H. et al. Monoclonal anti-HMGB1 (high mobility group box chromosomal protein 1) antibody protection in two experimental arthritis models. *Mol. Med.* **17**, 1039–1044 (2011).
156. Aulin, C., Lassacher, T., Palmblad, K. & Erlandsson Harris, H. Early stage blockade of the alarmin HMGB1 reduces cartilage destruction in experimental OA. *Osteoarthr. Cartil.* **28**, 698–707 (2020).
157. Nishibori, M., Mori, S. & Takahashi, H. K. Anti-HMGB1 monoclonal antibody therapy for a wide range of CNS and PNS diseases. *J. Pharmacol. Sci.* **140**, 94–101 (2019).
158. Zickert, A. et al. Renal expression and serum levels of high mobility group box 1 protein in lupus nephritis. *Arthritis Res. Ther.* **14**, R36 (2012).
159. Schaper, F. et al. Treatment with anti-HMGB1 monoclonal antibody does not affect lupus nephritis in MRL/lpr mice. *Mol. Med.* **22**, 12–21 (2016).
160. Muire, P. J., Avila, J. J., Lofgren, A. L. & Wenke, J. C. Neutralization of HMGB1 improves fracture healing and gammadelta T lymphocyte counts at the fracture site in a polytrauma rat model. *J. Exp. Orthop.* **9**, 21 (2022).
161. Son, M. et al. C1q and HMGB1 reciprocally regulate human macrophage polarization. *Blood* **128**, 2218–2228 (2016).
162. Ito, T. et al. Proteolytic cleavage of high mobility group box 1 protein by thrombin–thrombomodulin complexes. *Arterioscler. Thromb. Vasc. Biol.* **28**, 1825–1830 (2008).
163. Tang, Y. et al. Heparin prevents caspase-11-dependent septic lethality independent of anticoagulant properties. *Immunity* **54**, 454–467.e6 (2021).
164. Yang, H. et al. Identification of CD163 as an antiinflammatory receptor for HMGB1–haptoglobin complexes. *JCI Insight* **1**, e85375 (2016).
165. Avgousti, D. C. et al. A core viral protein binds host nucleosomes to sequester immune danger signals. *Nature* **535**, 173–177 (2016).
166. Qiang, X. et al. Monoclonal antibodies capable of binding SARS-CoV-2 spike protein receptor-binding motif specifically prevent GM-CSF induction. *J. Leukoc. Biol.* **111**, 261–267 (2022).
167. Chen, R. et al. HMGB1 as a potential biomarker and therapeutic target for severe COVID-19. *Heliyon* **6**, e05672 (2020).
168. Musumeci, D. et al. DNA-based strategies for blocking HMGB1 cytokine activity: design, synthesis and preliminary in vitro/in vivo assays of DNA and DNA-like duplexes. *Mol. Biosyst.* **7**, 1742–1752 (2011).
169. Yanai, H. et al. Suppression of immune responses by nonimmunogenic oligodeoxynucleotides with high affinity for high-mobility group box proteins (HMGBs). *Proc. Natl Acad. Sci. USA* **108**, 11542–11547 (2011).
170. Ju, Z. et al. Sequestering HMGB1 via DNA-conjugated beads ameliorates murine colitis. *PLoS ONE* **9**, e103992 (2014).
171. Wang, H. et al. Nicotinic acetylcholine receptor alpha7 subunit is an essential regulator of inflammation. *Nature* **421**, 384–388 (2003).
172. Rosas-Ballina, M. et al. Acetylcholine-synthesizing T cells relay neural signals in a vagus nerve circuit. *Science* **334**, 98–101 (2011).
173. Huston, J. M. et al. Transcutaneous vagus nerve stimulation reduces serum high mobility group box 1 levels and improves survival in murine sepsis. *Crit. Care Med.* **35**, 2762–2768 (2007).
174. Sitapara, R. A. et al. The alpha7 nicotinic acetylcholine receptor agonist, GTS-21, attenuates hyperoxia-induced acute inflammatory lung injury by alleviating the accumulation of HMGB1 in the airways and the circulation. *Mol. Med.* **26**, 63 (2020).
175. Pavlov, V. A. et al. Selective alpha7-nicotinic acetylcholine receptor agonist GTS-21 improves survival in murine endotoxemia and severe sepsis. *Crit. Care Med.* **35**, 1139–1144 (2007).
176. Kang, R. et al. A novel PINK1- and PARK2-dependent protective neuroimmune pathway in lethal sepsis. *Autophagy* **12**, 2374–2385 (2016).
177. Yan, Y. et al. Dopamine controls systemic inflammation through inhibition of NLRP3 inflammasome. *Cell* **160**, 62–73 (2015).
178. Liu, S. et al. A neuroanatomical basis for electroacupuncture to drive the vagal-adrenal axis. *Nature* **598**, 641–645 (2021).
179. Chen, R., Kang, R. & Tang, D. The mechanism of HMGB1 secretion and release. *Exp. Mol. Med.* **54**, 91–102 (2022).
180. Bonaldi, T. et al. Monocytic cells hyperacetylate chromatin protein HMGB1 to redirect it towards secretion. *EMBO J.* **22**, 5551–5560 (2003).
181. Qian, W. et al. Hypoxic ASCs-derived exosomes attenuate colitis by regulating macrophage polarization via miR-216a-5p/HMGB1 axis. *Inflamm. Bowel Dis.* **29**, 602–619 (2023).
182. Zhao, Y. et al. Cardiopulmonary bypass-derived plasma exosomal HMGB1 contributes to alveolar epithelial cell necroptosis via mtDNA/cGAS/STING pathway. *Shock* **58**, 534–541 (2022).
183. 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).
184. Yang, Z. et al. PARP-1 mediates LPS-induced HMGB1 release by macrophages through regulation of HMGB1 acetylation. *J. Immunol.* **193**, 6114–6123 (2014).
185. Xie, M. et al. PKM2-dependent glycolysis promotes NLRP3 and AIM2 inflammasome activation. *Nat. Commun.* **7**, 13280 (2016).
186. Orning, P. et al. Pathogen blockade of TAK1 triggers caspase-8-dependent cleavage of gasdermin D and cell death. *Science* **362**, 1064–1069 (2018).
187. Chen, H. et al. RIPK3 collaborates with GSDMD to drive tissue injury in lethal polymicrobial sepsis. *Cell Death Differ.* **27**, 2568–2585 (2020).
188. Turubanova, V. D. et al. Immunogenic cell death induced by a new photodynamic therapy based on photosens and photodithazine. *J. Immunother. Cancer* **7**, 350 (2019).
189. Wei, J. et al. Genome-wide CRISPR screens reveal host factors critical for SARS-CoV-2 infection. *Cell* **184**, 76–91.e13 (2021).
190. Satish, M., Gunasekar, P., Asensio, J. A. & Agrawal, D. K. Vitamin D attenuates HMGB1-mediated neointimal hyperplasia after percutaneous coronary intervention in swine. *Mol. Cell. Biochem.* **474**, 219–228 (2020).
191. Vogel, S. et al. The platelet NLRP3 inflammasome is upregulated in sickle cell disease via HMGB1/TLR4 and Bruton tyrosine kinase. *Blood Adv.* **2**, 2672–2680 (2018).
192. Careccia, G. et al. Rebalancing expression of HMGB1 redox isoforms to counteract muscular dystrophy. *Sci. Transl. Med.* **13**, eaay8416 (2021).
193. Chen, L. et al. Elevated serum levels of S100A8/A9 and HMGB1 at hospital admission are correlated with inferior clinical outcomes in COVID-19 patients. *Cell Mol. Immunol.* **17**, 992–994 (2020).
194. Cai, J. & Lin, Z. Correlation of blood high mobility group box-1 protein with mortality of patients with sepsis: a meta-analysis. *Heart Lung* **50**, 885–892 (2021).
195. Angus, D. C. et al. Circulating high-mobility group box 1 (HMGB1) concentrations are elevated in both uncomplicated pneumonia and pneumonia with severe sepsis. *Crit. Care Med.* **35**, 1061–1067 (2007).
196. Gaini, S., Koldkjaer, O. G., Moller, H. J., Pedersen, C. & Pedersen, S. S. A comparison of high-mobility group-box 1 protein, lipopolysaccharide-binding protein and procalcitonin in severe community-acquired infections and bacteraemia: a prospective study. *Crit. Care* **11**, R76 (2007).
197. Barnay-Verdier, S. et al. Emergence of autoantibodies to HMGB1 is associated with survival in patients with septic shock. *Intensive Care Med.* **37**, 957–962 (2011).
198. Gamez-Diaz, L. Y. et al. Diagnostic accuracy of HMGB-1, sTREM-1, and CD64 as markers of sepsis in patients recently admitted to the emergency department. *Acad. Emerg. Med.* **18**, 807–815 (2011).
199. Pang, X. et al. Expression and effects of high-mobility group box 1 in cervical cancer. *Int. J. Mol. Sci.* **15**, 8699–8712 (2014).
200. Sun, S. et al. High mobility group box-1 and its clinical value in breast cancer. *Onco Targets Ther.* **8**, 413–419 (2015).
201. Chen, S. et al. Hepatitis B virus X protein stimulates high mobility group box 1 secretion and enhances hepatocellular carcinoma metastasis. *Cancer Lett.* **394**, 22–32 (2017).
202. Liikanen, I. et al. Serum HMGB1 is a predictive and prognostic biomarker for oncolytic immunotherapy. *Oncoimmunology* **4**, e989771 (2015).

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