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Chapter 18

HMGB1 in Cell Death

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Abstract

High mobility group box 1 (HMGB1) is named for its electrophoretic mobility on polyacrylamide gels when it was first identified in calf thymus in 1973. HMGB1 plays a critical role in the stress response not only inside the cell as a DNA chaperone and cell death regulator, but also outside the cell as the prototypic damage-associated molecular pattern molecule. The physiological and pathological role of HMGB1 in health and disease has been widely studied for years. In this chapter, we will focus on the release and function of HMGB1 in cell death types such as apoptosis, autophagy, and necrosis.

Keywords: hmgb1, autophagy, necrosis, apoptosis

1. Introduction

Cell death is the cell's process of losing its biological ability to carry out all the essential life processes. The Nomenclature Committee on Cell Death proposes several cell death classification criteria. According to morphological appearance, cell death is divided into apoptosis (type I), autophagy (type II), and necrosis (type III) [1, 2]. According to enzymological qualities, cell death is divided into several subtypes depending on the involvement or noninvolvement of nuclei or distinct protease classes such as caspases, calpains, cathepsins, and transglutaminases. According to immunological characteristics, cell death is divided into immunogenic or tolerogenic cell death [3]. For example, apoptosis is generally considered nonimmunogenic cell death, whereas necrosis is considered immunogenic cell death. In addition, cell death can be classified into regulated or accidental cell death based on functional aspects [4]. Accidental cell death is caused by unexpected and accidental cell damage (e.g., ischemic and trauma), whereas regulated cell death is mediated by an expected program in response to different stimuli. The list of regulated cell death subtypes is rapidly increasing and includes anoikis, autophagic cell death, apoptosis, cornification, entosis, ferroptosis, mitotic catastrophe,

necroptosis, netosis, parthanatos, and pyroptosis [4]. Cell death is essential for a plethora of physiological processes, and its deregulation is implicated in several human diseases such as infections, neurodegeneration, cancer, autoimmunity, and ischemic disease [5-7]. During the past few decades, a number of important concepts regarding the regulation of cell death and its roles in human health and disease have arisen. Understanding the molecular mechanisms and signaling pathways of cell death is crucial for identifying new diagnostic and therapeutic targets.

Compared to pathogen-associated molecular pattern molecules (PAMPs), which are generated from the components of foreign pathogens such as bacteria and viruses, damage-associated molecular pattern molecules (DAMPs) are endogenous or self-molecules that are secreted, released, or undergo surface exposure by dead, dying, or injured cells [8-12]. Both PAMPs and DAMPs are mainly recognized by pattern recognition receptors such as receptor for advanced glycation end products (RAGE) and toll-like receptors (TLRs) to mediate the inflammatory, immunity, and metabolism response. The release and activity of DAMPs during cell death can determine whether cell death is immunogenic or tolerogenic [13]. Thus, DAMPs are suitable emergent targets for cell-death-associated immune therapy.

High mobility group box 1 (HMGB1) is named for its electrophoretic mobility on polyacrylamide gels when it was first identified in calf thymus in 1973 [14]. As an extremely conserved protein, HMGB1 originated before the divergence of the protostomes and deuterostomes, approximately 525 million years ago [15]. HMGB1 shares 100% amino acid sequence identity between mice and rats, and a 99% homology between rodents and humans [16-18]. The homolog of mammalian HMGB1 has been reported for several species such as *Nhp6A*/*B* in yeast and *HMG-D* and *DSP1* in *Drosophila* [19-21]. HMGB1 plays a critical role in the stress response not only inside the cell as a DNA chaperone and cell death regulator, but also outside the cell as the prototypic DAMP. The physiological and pathological role of HMGB1 in health and disease has been widely studied for years [22]. In this chapter, we will focus on the release and function of HMGB1 in cell death types such as apoptosis, autophagy, and necrosis.

2. HMGB1 structure and function

2.1. Structure

Human HMGB1 consists of 215 amino acid residues and has two L-shaped DNA-binding domains (HMG A box [9-79aa], HMG B box [95-163aa]) and a shorter C-terminal tail (186-215aa) [23]. Both A- and B-box domains are necessary for efficient DNA bending and flexure. HMGB1 binds to DNA without apparent sequence specificity. HMGB1 normally locates in the nucleus due to two nuclear-localization signals (NLS): NLS1 (28-44aa) and NLS2 (179-185aa) [24]. In contrast, HMGB1 contains nuclear-emigration signals in DNA-binding domains, which contributes to extranuclear HMGB1 during stress in a nuclear exportin chromosome-region maintenance 1-dependent manner. In addition to DNA, HMGB1 can bind a number of proteins involved in multiple biologic processes. For example, HMGB1 binds to RAGE, TLR4, and p53 by residues 150-183, 89-108, and 7-74, which mediates cell migration

[25], cytokine production [26], and gene transcription [27], respectively. The recombinant B box protein exhibits proinflammatory activity, whereas the recombinant A box protein displays anti-inflammatory activity [28], although the potential mechanism remains unknown. The C terminus is composed of 30 acidic amino acid residues and is able to regulate DNA binding/bending by intramolecular interaction with the A- and B- box [29, 30] or by intermolecular interaction with histones (e.g., H1 and H3) [31, 32]. Additionally, residues 201-205 in the C-terminal acidic tail region contribute to the antibacterial activity of recombinant HMGB1 [33]. Hence, the structural basis of HMGB1 determines its biological function.

2.2. Intracellular HMGB1

2.2.1. Nuclear HMGB1

HMGB1 translocates between the cytoplasm and the nucleus, but normally stays in the nucleus in most cells and tissues. Nuclear HMGB1 is the structural protein of chromatin and orchestrates a number of nuclear events by its DNA chaperone activity as follows: (1) Nucleosome stability and sliding. As basic unit of chromatin, nucleosome contains a short length of DNA wrapped around a core of histone proteins. HMGB1 and histone H1 can bind to linker DNA between successive nucleosomes in the chromatin fiber [34]. H1 stabilizes nucleosome with less mobility, whereas HMGB1 relaxes nucleosome and makes chromatin more accessible at the distorted site [35, 36]. (2) Nucleosome number and genome chromatinization. Loss of HMGB1 in mammalian and yeast cells leads to 20-30% less histones and nucleosomes and more RNA transcripts [37]. (3) Nuclear catastrophe and nucleosome release. Conditional knockout of HMGB1 in the pancreas causes nuclear oxidative injury and proinflammatory nucleosome release, which mediates sterile inflammation [38]. (4) DNA bending and binding. HMGB1 binds to DNA with structure-specificity, but not sequence-specificity [39]. After binding DNA, the major function of HMGB1 is to bend and change DNA conformation by unwinding [40], looping [41], or compacting DNA [42]. This DNA chaperone activity of HMGB1 is implicated in the regulation of gene transcription [43], DNA repair [44], DNA replication [45], V(D)J recombination [46], gene delivery [47], and gene transfer [48]. (5) Telomere homeostasis. Loss of HMGB1 in yeast and mammalian cells inhibits telomerase activity, decreases telomere length, and increases DNA damage and chromosomal instability [49].

2.2.2. Cytosolic HMGB1

Several cell types (e.g., fibroblasts [50], thymocytes [51]), and tissue types (e.g., liver, kidney, heart, and lung) [52] have normal cytosolic HMGB1 expression. The ratio of nuclear to cytoplasmic HMGB1 is about 30:1 [52]. Importantly, the translation of HMGB1 from the nucleus to the cytosol, including mitochondria and lysosomes, are observed in response to various stressors (e.g., cytokines, chemokines, heat, hypoxia, oxidative stress, and oncogenes). Although the function of cytosolic HMGB1 still remains poorly studied, HMGB1 may act as a positive regulator of mitochondrial quality in an autophagy-dependent and autophagy-independent manner [53, 54], which will be discussed later in the "Autophagy" section. In addition to autophagy, cytosolic HMGB1 is involved in the regulation of unconventional

secretory pathways based on mass spectrometry-mediated binding partner analysis [55]. In one study, several HMGB1-binding partners in nuclear and cytosol fraction were identified in several cancer cells [55]. Among them, nine of the cytosolic HMGB1-binding proteins were related to protein translocation and secretion. In particular, immunoprecipitation analysis further confirmed four cytosolic HMGB1-binding proteins, including annexin A2, myosin IC isoform a, myosin-9, and Ras-related protein Rab10 [55]. These proteins are directly implicated in the process of unconventional protein secretion. Further studies are needed to define the function of cytosolic HMGB1 in unconventional protein secretion. In addition to nuclear and cytosolic HMGB1, intracellular HMGB1 presents on cell surface membranes and regulates neurite outgrowth [56], platelet activation [57, 58], cell differentiation [59], erythroid maturation [60], adhesion [61], and innate immunity [62].

2.3. Extracellular HMGB1

HMGB1 is released in two different ways. On the one hand, HMGB1 can be actively secreted by normal cells, especially immune and endothelial cells [63, 64]. On the other hand, HMGB1 can be passively released by dead, dying, or injured cells in response to autophagic cell death [65], apoptosis [66, 67], necrosis [68], necroptosis [69, 70], netosis [71], and pyroptosis [72]. Oxidative stress refers to elevated intracellular levels of reactive oxygen species (ROS) that play a central role in the regulation of HMGB1 secretion and release, although the actual mechanism of action remains ambiguous [73]. Once released, HMGB1 acts as a cytokine, chemokine, and growth factor that is implicated in multiple biological processes including inflammation, immunity, migration, invasion, metabolism, proliferation, differentiation, antimicrobial defense, angiogenesis, tissue regeneration, death, autophagy, senescence, and efferocytosis. Extracellular HMGB1 plays important roles in the pathogenesis of human disease and is a potential therapy target in infection and sterile inflammation [74-76]. Several factors can affect HMGB1 activity in different experimental settings. For example, RAGE [77] and TLRs [78, 79] are positive receptors in macrophages and fibroblasts, whereas CD24 [80] and T cell immunoglobulin mucin 3 [81, 82] are negative receptors of HMGB1-mediated signaling in macrophages and dendritic cells (DCs). In addition to receptors, HMGB1 can be directly taken up and mediate the inflammatory and metabolism response [83, 84]. Ultra-pure HMGB1 (free from contaminating bacterial proteins and nucleic acids) exhibits very low immune activity in macrophages. In contrast, extracellular HMGB1 is in fact a "sticky" protein and a synergistic immune effect is observed between HMGB1 and PAMPs (e.g., lipopolysaccharide), DAMPs (e.g., DNA), and other molecules (e.g., cytokines, chemokine, and IgG) in multiple cells [85]. Thus, serum and plasma components (e.g., immunoglobulins, phospholipids, thrombomodulin, and proteoglycans) can interfere with HMGB1 detection by enzyme-linked immunosorbent assay [86]. Another important factor affecting HMGB1 activity is its redox status [87]. HMGB1 contains three conserved redox-sensitive cysteine residues: C23, C45, and C106. Reduced all-thiol-HMGB1 only exhibits chemokine activity, whereas disulfide-HMGB1 displays only cytokine activity, and oxidized HMGB1 has neither in immune cells [88]. In addition, reduced HMGB1 induces autophagy, whereas oxidized HMGB1 triggers apoptosis in cancer cells [89]. This redox status of HMGB1 also affects the affinity between HMGB1 and its receptors [26]. A recent study demonstrates that HMGB1 is specifically cleaved

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by caspase-1 but not other caspases during inflammasome activation [90]. Collectively, the release and activity of HMGB1 is context-dependent.

3. HMGB1 regulates cell death

3.1. Mechanism of HMGB1-mediated autophagy regulation

Autophagy, including macroautophagy, microautophagy, and chaperone-mediated autophagy, is a highly conserved degradation process in organisms from yeasts to plants and animals [91]. The well-studied form of autophagy is macroautophagy (hereafter referred to as autophagy). As a complex dynamic process, autophagy is composed of the formation and maturation of three major membrane structures: the phagophore, autophagosome, and autolysosome [92]. Briefly, the phagophore originates from multiple membrane resources and engulfs the cytosolic materials, which leads to the formation of a closed autophagosome with a double membrane. Of note, microtubule-associated protein light chain 3 (LC3)-II is a widely used autophagosome marker [93]. Finally, autophagosomes fuse with lysosomes to form autolysosomes, which results in degradation of the engulfed material, including LC3-II, by lysosomal enzymes into elementary pieces that can be used for protein synthesis and energy production. Thus, autophagy is a programmed cell survival pathway in response to intracellular and extracellular stress [94]. However, excessive or impaired autophagy can cause cell death, indicating a dual role of autophagy in cell survival and cell death. In particular, autosis is an Na^+, K^+-ATPase-dependent form of cell death triggered by autophagy-inducing peptides, starvation, and hypoxia–ischemia [95]. The process of autophagy is controlled by multiple posttranslational modifications of the autophagy-related gene (Atg) family and shares regulators derived from other cell death pathways [96].

HMGB1 promotes autophagy in a location- and modification-dependent manner. Nuclear HMGB1 regulates heat shock protein β-1 (HSPB1) expression at a transactional level [54]. The protein expression of HSPB1, but not other heat shock proteins, is significantly inhibited in HMGB1 knockout or knockdown cells. Both HMGB1 and HSPB1 regulate mitochondrial selective autophagy, namely mitophagy, following mitochondrial injury [54]. Like other ATGs, it was recently suggested that HMGB1-independent autophagy exists in the regulation of mitochondrial quality, including the mitochondrial DNA damage response [53]. Cytosolic HMGB1 is a Beclin-1 binding protein [97]. HMGB1 C23S and C45S mutants lose their ability to bind Beclin-1 and therefore cannot promote autophagy [97]. The binding of HMGB1 with Beclin-1 is positively regulated by unc-51-like kinase 1 [98] mitogen-activated kinase-like protein [99], and nucleus accumbens-1 [100]. In contrast, p53 [101], SNCA/α-synuclein [102], lysosomal thiol reductase [103], miR34A [104], and miR22 [105] negatively regulate HMGB1-mediated autophagy by disrupting HMGB1-Beclin-1 complex formation. Moreover, activation of poly [ADP-ribose] polymerase 1 (PARP1) is required for tumor necrosis factor (TNF)-related apoptosis-inducing ligand (TRAIL)-induced ADP-ribosylation of HMGB1 and subsequent HMGB1-Beclin-1 complex formation in cancer cells [106]. Extracellular reduced HMGB1, but not oxidized HMGB1, significantly induced autophagy in cancer cells in a RAGE-dependent manner [89]. This process may sustain anaerobic energy

production during tumor growth and development [107]. Collectively, these findings suggest an HMGB1-dependent autophagic pathway at multiple levels in response to stress. However, HMGB1-independent autophagy may exist in several organs, although the underlying mechanism of its action remains obscure [108].

3.2. Mechanism of HMGB1-mediated apoptosis regulation

Apoptosis is the process of programmed cell death and includes classical "extrinsic" and "intrinsic" pathways and nonclassical T and natural killer cell-mediated cytolytic pathways. The extrinsic pathway is primarily mediated by the binding of a ligand to a transmembrane death receptor (DR). DRs are members of the TNF receptor gene superfamily, including FasR, TNFR1, lymphotoxin receptor, DR3, and DR4/DR5 [109]. In addition to DRs, dependence receptors mediate apoptosis by monitoring the absence of certain trophic factors or the presence of anti-trophic factors [110]. The intrinsic pathway for apoptosis involves activation of a mitochondrial pathway including altering mitochondrial permeability and subsequent release of mitochondrial proteins such as cytochrome c and second mitochondrial-derived activator of caspases [111]. The process of apoptosis is tightly regulated by the Bcl-2, caspase, and nuclease families [112-114]. Caspases are a family of endoproteases linking inflammation and cell death. Initiator caspases (e.g., caspases-8 and -9) activate executioner caspases (e.g., caspases-3, -6, and -7) that mediate the cleavage of key structural proteins such as PARP1. However, caspase-independent apoptosis may exist by translocation of apoptosis-inducing factor [115, 116] and endonuclease G [117] from the mitochondria to the nucleus, or activation of Omi/HTRA2 (a mitochondrial serine protease) [118]. Remarkably, several caspases (e.g., caspase-1, -4, -5, and -12 in humans; caspase-1, -11, and -12 in mice) are critical mediators of innate immune responses partly by activation of inflammasome, but not activation of the apoptosis pathway.

Intracellular HMGB1 is generally an anti-apoptotic protein in response to several apoptotic stimuli such as ultraviolet radiation, CD95, TRAIL, caspase-8, and Bax [119]. Knockdown of HMGB1 increases drug sensitivity in cancer cells [120]. Mechanically, HMGB1 plays transcriptional-dependent (e.g., regulation of Bcl-2 family protein expression) and transcriptional-independent roles (e.g., regulation of autophagy and p53 location) in the regulation of apoptosis. For example, inhibition of HMGB1-mediated autophagy can increase caspase activity [121]. In addition to caspases, several non-caspase proteases such as calpain (Ca^{2+}-dependent proteases) may play a role in the execution of apoptosis. Interestingly, HMGB1 deletion can enhance calpain activity and trigger cleavage of Beclin-1 and ATG5 [122]. Thus, HMGB1 is an important regulator of the cross talk between apoptosis and autophagy. *In vivo*, conditional knockout of HMGB1 in pancreas, liver, intestinal epithelial and myeloid cells enhances sterile inflammation and infection partly through inhibition of autophagy and induction of apoptosis [38, 122-124]. In some cases, overexpression of HMGB1 renders cells sensitive to apoptosis in response to chemotherapy agents [125]. In addition, extracellular oxidized HMGB1 can induce caspase-dependent apoptosis in cancer cells [89]. These findings suggest that HMGB1 plays dual roles in the regulation of apoptosis.

3.3. Mechanism of HMGB1-mediated necrosis regulation

Necrosis includes accidental and regulated necrosis [2]. Partially, the term "necroptosis" has recently been used to describe regulated necrosis when cells lack the capacity to activate caspase [126]. Necroptosis is mediated by a signaling complex called necrosome, containing receptor-interacting protein (RIP)1, RIP3, and mixed-lineage kinase domain-like (MLKL) [127, 128], and can be inhibited by small molecule inhibitors necrostatin 1 and necrosulfonamide [129, 130] [126]. The fundamental causes of necrosis include calcium overload, ROS generation, cellular energy depletion, and membrane lipid injury [131]. PARP is a protein family involved in a number of cellular processes such as DNA repair and programmed cell death. Induced overactivation of PARP1 can lead to adenosine triphosphate (ATP) depletion and subsequent necrosis [132]. The process of necrosis ends with the leaking out of enzymes from lysosomes to digest cell components that are associated with HMGB1 release. *In vivo*, loss of HMGB1 in the pancreas increases L-arginine-induced apoptosis and necrosis due to oxidative injury [38]. However, the role of HMGB1 in necroptosis remains undefined.

4. HMGB1 release in cell death

4.1. Mechanism of HMGB1 release in autophagy

Autophagic cell death is not only a morphologic notion such as cell death associated with autophagosomes and autolysosomes, but also a functional description that excessive autophagy can cause cell death. Induction of autophagy facilitates both active secretion and passive release of HMGB1. For example, the release of HMGB1 is significantly increased in response to epidermal growth factor (EGF) receptor-targeted diphtheria toxin (DT-EGF)-induced autophagic cell death [65]. In contrast, suppression of ATG5, ATG7, or ATG12 expression by RNA interference (RNAi) inhibits autophagy and subsequent HMGB1 release after treatment with DT-EGF in cancer cells [65]. In addition, ATG5-dependent autophagy promotes HMGB1 secretion in fibroblasts and macrophages after treatment with Hank's balanced salt solution and lipopolysaccharide [97, 133]. Antioxidant (e.g., N-acetyl-L-cysteine) inhibits the cytosolic translocation and release of HMGB1 in starvation-induced autophagy [97]. In contrast, ROS and knockdown of superoxide dismutases (SOD)-1 and SOD2 by RNAi promotes cytosolic HMGB1 expression and extracellular release [134]. These findings suggest that oxidative stress is involved in autophagy-mediated HMGB1 release.

4.2. Mechanism of HMGB1 release in apoptosis

An early study indicated that HMGB1 is released only by necrotic cells, but not apoptotic cells [68]. However, recent studies demonstrated that activation of caspases and deoxyribonuclease (DNase) in apoptosis regulates HMGB1 release and activity in apoptosis. Caspase-3 and caspase-7 are important executioner caspases in apoptosis through amplified initiation signals from caspase-8 and caspase-9. Activation of caspase-3 and -7 induces mitochondrial complex 1 protein p75 NDUFS1 cleavage, which results in mitochondrial ROS production and subse-

quent HMGB1 release during apoptosis in DCs [135]. Interestingly, the activity of released HMGB1 in apoptosis is impaired, which promotes immunological resistance due to its oxidized form [135]. In addition to caspase-3 and -7, caspase-1 is responsible for HMGB1 cleavage and release in the response to pyroptosis in immune cells [72, 136, 137]. This caspase-1-mediated HMGB1 fragment can rescue apoptosis-induced immune tolerance in a RAGE-dependent manner [137]. Thus, different caspases can determine HMGB1 release and action in apoptosis and pyroptosis.

DNase is responsible for DNA fragmentation during cell death. Activation of DNA endonuclease (DNase-gamma) contributes to the degradation of DNA into nucleosomal units in apoptosis, whereas activation of DNase I and II facilitates degradation of DNA in necrosis [138]. The release of HMGB1 in apoptosis is triggered by DNase-gamma-mediated nucleosomal DNA fragmentation [139, 140]. Thus, inhibition of DNase gamma activity by small molecular compound DR396 can significantly diminish HMGB1 release in response to apoptotic stimuli [139, 140].

4.3. Mechanism of HMGB1 release in necrosis

The nuclear enzyme PARP1, which catalyzes the synthesis of the biopolymer poly(ADP-ribose), exhibits an essential role in the DNA damage response and genomic stability. However, overactivation of PARP1 may deplete the stores of cellular NAD+, which results in ATP depletion and subsequent necrosis [141]. In fact, HMGB1 release in necrosis is regulated by PARP1. Genetic and pharmacologic inhibition of PARP1 inhibits alkylating DNA damage agent-mediated necrosis as well as HMGB1 release [142]. In addition to necrosis, activation of PARP1 also contributes to HMGB1 translocation and release in autophagy and inflammation [106, 143]. Interestingly, loss of HMGB1 in tissue and cells accelerates DNA damage that results in PARP1 overactivation [144]. These findings suggest interplay between HMGB1 and PARP1 in response to cell death.

The RIP3-mediated signaling pathway is responsible for HMGB1 release in necroptosis. Upregulation of RIP3 expression *in vitro* triggers necroptosis, whereas suppression of RIP3 expression by RNAi *in vitro* or *in vivo* significantly inhibits inflammatory stimuli-induced necroptosis. RIP3-deficient mice exhibit resistance to sepsis and donor kidney inflammatory injury. This anti-inflammatory function of RIP3 is due partly to inhibition of HMGB1 and release of other DAMPs [145]. Additionally, RIP3-mediated necroptosis also contributes to dsRNA/poly (I:C)-induced HMGB1 release [146]. This process promotes retinal degeneration and triggers an inflammatory response in the mouse retina [146]. In addition to RIP3, interferon-β promoter stimulator 1 (an adaptor molecule for RIG-I-like receptors) may be critical for poly (I:C)-induced HMGB1 release in necroptosis in DCs.

Cysteine cathepsins are lysosomal proteases with housekeeping functions that also initiate a specific cell death pathway termed lysosomal cell death. This type of cell death includes morphological features of necrosis and apoptosis [147]. Cathepsin B, a critical lysosomal cysteine protease, mediates HMGB1 release following *L. pneumophila*-induced lysosomal cell death [148]. Mechanically, cathepsin B can translocate from the lysosome to the nucleus, where it interacts with HMGB1 and inhibits its cytosolic translocation. In addition to lysosomal cell

death, cathepsin B is also important for HMGB1 release during inflammasome activation [149, 150]. In contrast, cathepsin D may facilitate HMGB1 release in necroptosis in DCs. The function of other cathepsins in the regulation of HMGB1 release remains unknown.

5. Concluding remarks

HMGB1 is a member of family containing the evolutionarily conserved HMG box domains. The function of HMGB1 depends on its cellular location. Besides its functions in the nucleus and cytosol, HMGB1 plays a critical role in extracellular signaling associated with multiple biological processes. Both intracellular and extracellular HMGB1 are involved in the regulation of types of cell death such as apoptosis, necrosis, and autophagy. Intracellular HMGB1 regulates cell death in both transactional-dependent or transactional-independent manners. In many cases, HMGB1 is a negative regulator of apoptosis and necrosis, but a positive regulator of autophagy. In addition, the release and activity of HMGB1 in cell death is context-dependent, which may cause immunogenic cell death or tolerogenic cell death. Future studies are needed to define the upstream and downstream signaling of HMGB1 in the regulation of cell death; clarify the interplay and cooperative role of HMGB1 and other DAMPs in the cell-death-associated microenvironment; and develop new therapeutic strategies for targeting HMGB1 in cell-death-associated disorders.

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