INVITED REVIEW

High-mobility group box 1 protein orchestrates responses to tissue damage via inflammation, innate and adaptive immunity, and tissue repair

Marco E. Bianchi | Massimo P. Crippa | Angelo A. Manfredi | Rosanna Mezzapelle | Patrizia Rovere Querini | Emilie Venereau

Summary
A single protein, HMGB1, directs the triggering of inflammation, innate and adaptive immune responses, and tissue healing after damage. HMGB1 is the best characterized damage-associated molecular pattern (DAMP), proteins that are normally inside the cell but are released after cell death, and allow the immune system to distinguish between antigens that are dangerous or not. Notably, cells undergoing severe stress actively secrete HMGB1 via a dedicated secretion pathway: HMGB1 is relocated from the nucleus to the cytoplasm and then to secretory lysosomes or directly to the extracellular space. Extracellular HMGB1 (either released or secreted) triggers inflammation and adaptive immunological responses by switching among multiple oxidation states, which direct the mutually exclusive choices of different binding partners and receptors. Immune cells are first recruited to the damaged tissue and then activated; thereafter, HMGB1 supports tissue repair and healing, by coordinating the switch of macrophages to a tissue-healing phenotype, activation and proliferation of stem cells, and neoangiogenesis. Inevitably, HMGB1 also orchestrates the support of stressed but illegitimate tissues: tumors. Concomitantly, HMGB1 enhances the immunogenicity of mutated proteins in the tumor (neoantigens), promoting anti-tumor responses and immunological memory. Tweaking the activities of HMGB1 in inflammation, immune responses and tissue repair could bring large rewards in the therapy of multiple medical conditions, including cancer.

KEYWORDS
adaptive immunity, damage associate molecular pattern (DAMP), immunogenic cell death (ICD), inflammation, innate immunity, tissue repair

1 | INTRODUCTION: WHAT CELLS VIEW AS DANGER

Multicellular animals have a sense of integrity: they must distinguish whether their cells are alive or dead, both to limit present damage and avoid it in the future, if possible. The management of damage due to viral and bacterial invasion is central to evolutionary success, and the adaptive immune system arguably exists to forestall successive onslaughts by the same or related pathogens. Despite being only a subsystem in the more basic and all-comprising system maintaining integrity (that comprises inflammation, coagulation, stem cells, and tissue repair, among others), the adaptive
immune system amazes us because of its sheer cleverness and utility. For decades, the overarching question was: how does the adaptive immune system recognize pathogens? The answer, for a long time, was that it discriminates between self and non-self, with the corollary that anything that belongs to the organism is non-antigenic and anything that does not is antigenic. That view was completely upended in the 1990s by two new concepts: pathogen-associated molecular pattern molecules (PAMPs) and damage-associated molecular pattern molecules (DAMPs).1,2

PAMPs are entire classes of molecules that are non-antigenic but are recognized by pattern recognition receptors (PRRs) with broad and often multiple specificities; examples are common components of bacteria (like peptidoglycan, lipoproteins, and lipopolysaccharide) and of viruses (like RNA unmodified by capping at its 5′ end).

DAMPs are molecules released by dead or dying cells and particularly by cells that face unscheduled death (as opposed to cells that undergo apoptosis). Like PAMPs, they activate PRRs, and sometimes the same PRR can be activated by both PAMPs and DAMPs. Indeed, both PAMPs and DAMPs elicit the same early responses, like inflammation. Both PAMPs and DAMPs alert antigen presenting cells of the immune system, and in particular dendritic cells (DCs), which then initiate an adaptive immune response.

Thus, the immune system does not distinguish between self and non-self, but simply whether PAMPs or DAMPs are present, and thus whether a present danger warrants a close examination of what is going on. PAMPs and DAMPs, in fact, are just telltales of danger, but from the immune system’s point of view, they are adjuvants: molecules that are not antigens, but prompt the immune system to react against any antigens that it can recognize.

This new paradigm of what is recognized by the immune system is in retrospect both simple and powerful, but in the early days, it had a major flaw: PAMPs had been identified galore, but DAMPs had no qualifying members. We were fortunate enough to recognize the first DAMP, High Mobility Group Box 1 (HMGB1) protein.2 Ironically, extracellular ATP now qualifies as a major DAMP, but in the 2000s was not considered one.

This review illustrates the role of HMGB1 as a DAMP, starting from the immune system, but framing it in the more general “system of integrity.” Indeed, our major interest is tissue repair, and how the immune system supports the healing of damaged tissues. Inevitably, how the immune system recognizes or does not recognize illegitimate new tissue—cancer—is the other face of the same coin. HMGB1 sticks a finger in all these pies.

2 | HMGB1 AS A DAMP

The defining characteristic of DAMPs was that they should be released by dead cells and then elicit immune responses. We had studied HMGB1 as a nuclear protein that acts as a DNA chaperone, by binding DNA transiently and bending it reversibly. What attracted our attention to HMGB1 as a DAMP was that, against all odds, it does elicit immune responses: Kevin Tracey and collaborators reported that HMGB1 is secreted by mouse macrophages challenged with LPS, both in vitro and vivo, and is a late mediator of endotoxin lethality.4 Macrophages do not die after secreting HMGB1, but we went on to test if dead cells could release it, which looked possible since HMGB1 is not tightly bound to DNA, unlike histones.5 Indeed, cells lysed with detergents, but also subjected to freeze/thawing or to metabolic poisoning, released HMGB1 in the extracellular medium, whereas apoptotic cells did not.3 Moreover, whereas lysed wildtype cells induced macrophages to secrete TNF, lysed Hmgb1−/− cells were almost inactive. This qualified HMGB1 as protein that is released by dead cells (Figure 1) and elicits immune responses, with the added specification that apoptotic cells, which are non-immunogenic, do not release HMGB1.

The second property of a DAMP is adjuvanticity, and we showed that exogenous HMGB1 activates DCs in vitro, and in vivo promotes the production of antibodies against a soluble antigen (ovalbumin) and boosts vaccination against poorly immunogenic cancer cells.6 Extracellular HMGB1 also controls the maturation of DCs and their migration to the closest draining lymph node and their interaction with T cells.7–9 Indeed, HMGB1 activation of antigen presenting cells and their signaling to T cells is so potent that countervailing suppressive mechanisms are required, including HMGB1 binding to the immunosuppressive receptors CD2410 and TIM-3.11

3 | HMGB1 AS A SECRETED ALARMIN

Although HMGB1 is passively released by dead cells, macrophages release it without dying,4 and therefore secrete it (Figure 1). HMGB1 does not have a secretory leader peptide, as befits a nuclear protein, and secretion does not involve the classical endoplasmic reticulum (ER)-Golgi secretory network, but a dedicated unconventional secretory pathway.12 HMGB1 has two nuclear localization signals (NLSs) and shuttles continually between nucleus and cytoplasm, although in normal conditions the vast majority of the protein is located in the nucleus. Acetylation of lysines or phosphorylation of serines in the NLSs13,14 preclude the re-entry of HMGB1 into the nucleus, and the protein accumulates in the cytoplasm. In hematopoietic cells, HMGB1 is then loaded into secretory lysosomes, which can disgorge their contents outside of the cell following specific signals.12 The mechanism for loading acetylated HMGB1 into secretory lysosomes, though, has not been elucidated. Non-hematopoietic cells can secrete HMGB1 as well, and the relocation from nucleus to cytoplasm appears to follow the same rules; however, eventual secretion does not appear to involve secretory lysosomes, but additional pathways have not been investigated. A possibility is that HMGB1 crosses the plasma membrane directly with the help of membrane transporters. In any case, acetylated HMGB1 is a biomarker for HMGB1 secretion, as opposed to passive release by dead cells (the possible phosphorylation of secreted HMGB1 has not been investigated). Unfortunately, specific antibodies against acetylated HMGB1 have not been developed, and the differentiation between acetylated and non-acetylated HMGB1 still depends on mass spectrometry.

The fact that HMGB1 can be actively secreted as well as passively released provides a way to emit the general alarm signal in the presence
of severe cell stress, but not necessarily cell damage, or in the presence of pathogens (or rather, PAMPs). An example of the former—severe cell stress—is ischemia. HMGB1 can be secreted during liver ischemia/reperfusion, for example. Reactive oxygen species (ROS) produced by hypoxic hepatocytes cause the rise of intracellular calcium, which activates calcium/calmodulin-dependent kinases (CaMKs); CaMK inhibition substantially decreased liver damage after I/R. An example of the latter—HMGB1 secretion in the presence of PAMPs—occurs when macrophages detect LPS (Figure 2). LPS binds TLR4, and signaling is initiated that activates NF-κB and phosphorylates IRFs. Both are transcription factors that, when activated, promote together the activation of interferon beta (IFNβ) transcription and eventual secretion. IFNβ binds to interferon receptors (IFNARs) on nearby cells, leading to activation of associated JAK kinases and STAT1 phosphorylation. Phospho-STAT1 dimers translocate to the nucleus where they promote the transcription of interferon-responsive genes. Phospho-STAT1 dimers also recruit histone acetylases, which acetylate HMGB1 and allow its eventual secretion. A similar cascade presumably occurs when cells respond to virus infection or when IFNγ binds to its receptor.

4 | FORMS AND FUNCTIONS OF EXTRACELLULAR HMGB1

When outside the cell, HMGB1 has many functions and many receptors. In fact, HMGB1 has posttranslational modifications that make it work as different ligands of different receptors. Whereas acetylation does not appear to alter the binding specificities and the activities of HMGB1, the redox status of its 3 cysteines—C23 and C45 within Box A, and C106 in Box B—plays essential roles. C23 and C45 are ideally placed to form an intramolecular disulfide bond while C106 remains unpaired. The cytosol and the nucleus have a strongly negative (reducing) redox potential, and intracellular HMGB1 is largely in the reduced state. However, the extracellular milieu is much more oxidizing in normal conditions, and even more so during inflammation, which favors the formation of the C23-C45 disulfide bond (Figure 2). The 3 cysteines can be further oxidized to sulfonates by ROS.

Fully reduced HMGB1 (also called all-thiol-HMGB1) forms a heterocomplex with the chemokine CXCL12, and the HMGB1-CXCL12 heterocomplex binds CXCR4, a 7-transmembrane G-protein-coupled

![Diagram](image-url)
receptor (GPCR). CXCR4 forms dimers, multimers, and heterodimers with other GPCR receptors and binds several ligands, including CXCL12, CXCL14, the HMGB1-CXCL12 heterocomplex, MIF, extracellular ubiquitin, and viral proteins. Different ligands induce different conformations of CXCR4, which determine the balance of several signaling pathways downstream, including G proteins and calcium pathways, beta-arrestins and JAK, GRK, MAPK, and PI3K kinases. Every ligand potentially activates different pathways to a different extent, an effect known as "ligand bias." Signaling and biological effects of CXCL12 and of the HMGB1-CXCL12 complex differ, but the details are scarcely known. Both CXCL12 and the HMGB1-CXCL12 complex promote cell migration, extravasation from vessels, and tissue invasion. Most cells that can move migrate toward the HMGB1-CXCL12 complex, which is most active at concentrations close to 1 nM, while peak activity of CXCL12 is at 30 to 100 nM.

The contribution of the HMGB1/TLR4 axis to inflammation and immune regulation has been demonstrated in a wide range of experimental models, such as liver and lung damage, stroke, cancer, and epilepsy, its contribution to pain perception is emerging. HMGB1 also binds to Toll-like receptors (TLRs). In complex with CpG-ODNs, HMGB1 binds to TLR9 and enhances cytokine production in macrophages, B cells, and plasmacytoid dendritic cells (DCs). When HMGB1 is bound to nucleosomes, it activates macrophages and DCs through TLR2. What is the redox state of HMGB1 in these interactions is not known.

The first receptor described for HMGB1 is the receptor for advanced glycation endproducts (RAGE), a multifunctional single-transmembrane protein of the immunoglobulin superfamily. RAGE recognizes multiple ligands, including various S100 proteins, the β2 integrin Mac-1, amyloid beta, fibrillar aggregates, and aspecifically carboxymethylated proteins (AGEs), in addition to HMGB1 (the disulfide form, most likely, but possibly also other forms). Upon binding of some of its ligands, RAGE is cleaved, generating a soluble form of RAGE that can function as a decoy receptor and a small cytoplasmic fragment that is essential for RAGE signaling. HMGB1 signaling through RAGE leads to activation of the NF-κB pathway, as well as to signal transduction through JNK, p38, and ERK MAP kinase pathways.
initially implicated as the receptor necessary for HMGB1-directed cell migration, but Rage^−/− MEFs are capable of migrating toward HMGB1, whereas Cxcr4^−/− MEFs are not (Schiraldi et al., 2012). However, the HMGB1/RAGE axis is directly responsible for inducing the expression of adhesion molecules such as VCAM-1 and ICAM-1 and secretion of chemokines, in particular CXCL12, which in turn forms the heterocomplex with HMGB1.

RAGE, though, is involved in many other HMGB1-dependent signaling pathways, including the ones that lead to thrombosis, migration of dendritic T cells to lymph nodes, T-cell activation, angiogenesis, and the spreading of brain damage after stroke.

5 | EXTRACELLULAR HMGB1 IN TISSUE DAMAGE AND HEALING

When a tissue is damaged, HMGB1 orchestrates two key events in inflammation, leukocyte recruitment and their induction to secrete inflammatory cytokines. We observed in a model of muscle injury induced by cardiotoxin (CTX) that different redox forms of HMGB1 are present sequentially: fully reduced HMGB1 is released first and becomes disulfide HMGB1 later. We proposed (Figure 2) that when released in an injured tissue by dead or severely stressed cells, HMGB1 will form a heterocomplex with the low concentrations of CXCL12 always present in extracellular fluids, and promote the production of more CXCL12 by binding the RAGE receptor on neighboring cells. A gradient of the HMGB1-CXCL12 heterocomplex will recruit leukocytes from the microcirculation. Incoming leukocytes will then be activated by disulfide HMGB1 derived by spontaneous or catalyzed oxidation of fully reduced HMGB1, and start producing cytokines, chemokines, more HMGB1, and ROS. ROS will initially convert HMGB1 into the disulfide form, potentiating the inflammatory response, but with time will further oxidize cysteines to sulfonates and inactivate HMGB1. Thus, HMGB1 would first recruit leukocytes, then would activate them, and eventually would be inactivated by them.

However, HMGB1 may also play an important role in the events that follow inflammation resolution: tissue repair and healing. Indeed, HMGB1 is important in muscle regeneration after injury. We envisage three reasons: HMGB1 recruits monocytes at the site of tissue damage, which eventually shift from an inflammatory to a tissue healing phenotype; it recruits local and mesenchimal stem cells (MSCs); and it promotes neoangiogenesis.

Besides recruiting them in the first place, HMGB1 stimulates macrophages to release proangiogenic cytokines, such as VEGF, TNF-α, and IL-8. HMGB1 secreted by leukocytes is important for the skeletal muscle to react to hypoxia and to initiate angiogenesis in response to injury.

HMGB1 released in the damaged tissue recruits local and bone-marrow-derived mesenchymal stem cells, which are essential or important players in the repair of bone, cartilage, muscle, bone marrow stroma, tendon, fat, and other connective tissues.

Finally, HMGB1 plays an important role in neovascularization of ischemic areas by recruiting endothelial progenitor cells through activation of integrins and inducing the migration and sprouting of endothelial cells in a RAGE-dependent manner.

6 | HMGB1 IN TUMOR BIOLOGY

Perhaps inevitably for a protein that is central in inflammation and injury, HMGB1 is deeply involved in tumor biology as well: inflammation is hallmark of cancer, and cancer is a full-blown illness that is highly stressed, if anything because it can overgrow its supply of nutrients and is deeply dependent on neoangiogenesis.

Exemplary is the involvement of HMGB1 in mesothelioma, an intractable tumor of mesothelial cells lining pleura and the peritoneum, which is highly associated to exposure to asbestos and other mineral fibers (Figure 3). Asbestos causes inflammation of the mesothelium, but the exact connection between inflammation and mesothelioma had remained obscure, until we discovered that asbestos induces the necrotic cell death of mesothelial cells, the release of HMGB1 into the extracellular space and the recruitment of inflammatory cells. The prolonged bio-persistence of asbestos fibers ensures that HMGB1-driven inflammation smolders chronically for many years; in fact, HMGB1 is elevated in the blood of people exposed to asbestos, and even more so in patients with diagnosed mesothelioma. How exactly chronic inflammation causes the tumor transformation of mesothelial cells is not clear, but a likely possibility is that macrophages favor the survival of cells that in normal conditions would simply die out. In fact, mesothelioma contains a striking abundance of macrophages with a tissue healing phenotype, and the mesothelioma cancer cells themselves contain a high level of HMGB1 and continually secrete it. Most mesotheliomas appear addicted to HMGB1, since targeting HMGB1 with antibodies or small molecules prolongs the survival of mice models of mesothelioma.

Similar to what happens in mesothelioma, macrophages recruited by secreted HMGB1 support the growth of colon carcinoma secondary lesions in the peritoneum. Melanoma and papilloma also can be initiated or supported through the HMGB1/TLR4 axis. Melanoma metastatization upon UV irradiation appears to depend on inflammation due to the HMGB1/TLR4 axis: neutrophils are attracted into the primary tumor and promote the migration of melanoma cells.

How general is the involvement of HMGB1 in different solid tumors is open to question; however, many cancer tissues overexpress HMGB1, including carcinomas of liver, breast, colon and prostate, adenocarcinomas, melanomas, and gastrointestinal stromal tumors, and many may secrete it, at least under some conditions (reviewed in ref. 67).

7 | HMGB1 IN TUMOR IMMUNOLOGY

Any condition that causes necrotic cell death in a tumor—including necrosis due to insufficient angiogenesis, chemotherapy, or radiation therapy—will cause HMGB1 release and the recruitment of macrophages and neutrophils into the tumor, which can help the tumor...
recover from damage inflicted to it or promote metastasis, as in melanoma. Viewed from this point of view, HMGB1 is abetting cancer and is a potential anti-cancer target (Figure 3B). However, an additional effect of HMGB1 may partially or completely counteract the help afforded by the innate immune system to the cancer tissue (Figure 3C). Some anti-cancer therapies cause a peculiar form of cell death—immunogenic cell death (ICD)—that greatly increases the immunogenicity of the cancer cells, and therefore unleashes an adaptive immune response against the tumor and immunological memory.

Cancer cells exposed to radiotherapy and to some currently used chemotherapeutics (including doxorubicin, mitoxantrone, oxaliplatin, and bortezomib, but not cisplatin) undergo ICD, as demonstrated by vaccination experiments in mice and the regression of non-irradiated metastases in humans (the abscopal effect).

ICD is a form of apoptotic death, and as such, it should be silent or even suppressive from an inflammatory and immunological point of view. However, ICD is characterized by the emission of calreticulin, HMGB1, and ATP. Notably, ATP is a "find-me" signal and calreticulin an "eat-me" signal for professional phagocytes, and both HMGB1 and ATP are deeply involved in inflammation at various levels. Their combination stimulates the cross presentation by DCs of neoantigens from cancer cells to the immune system and activation of CD4 T cells.

For obvious reasons, ICD can be experimentally studied only in mice, and therefore only syngeneic mouse tumors can be used. Work in mouse models has shown that siRNA-mediated downregulation of calreticulin or HMGB1 blunt immunological responses of ICD-inducing chemotherapy, and that Tlr4−/− or Myd88−/− mice do not become vaccinated against the tumor (MyD88 is a key adapter for TLR signaling), whereas Tlr2−/− and Ager−/− mice do develop immunological memory. Moreover, the loss of HMGB1 from malignant cells negatively affects prognosis in patients with breast cancer treated with anthracyclines as adjuvant chemotherapy.

Clearly, the well-choreographed emission of calreticulin, HMGB1, and ATP cannot occur by chance, nor can it derive from natural selection for efficacy of chemotherapeutic drugs. A likely possibility is that ICD is in fact an ancient response program to pathogens, as it activates response pathways that are similar to those activated by viruses.

What exactly triggers ICD in vivo is not known, but for sure calreticulin, a protein resident in the ER, is released in association with the triggering of ER stress, and detected via the low-density lipoprotein receptor-related protein 1 (LRP1), which plays multiple roles in intracellular signaling and endocytosis. PERK, a kinase activated during the unfolded protein response that is activated following ER

**FIGURE 3** Schematic representation of pro-tumor and anti-tumor activities of HMGB1. (A) Pathogenesis of malignant mesothelioma is discussed as an example. Mesothelial cells injured by asbestos undergo a programmed necrotic death, with HMGB1 release. Extracellular HMGB1 recruits and activates macrophages. (B) Pro-tumor activities of HMGB1. HMGB1 binding to TLR4 generates a status of chronic inflammation that leads to malignant transformation. Macrophages with a tissue healing phenotype are part of the mesothelial tissue, and HMGB1 is constitutively secreted by mesothelioma cells. (C) Anti-tumor activities of HMGB1. The question mark denotes that the involvement of these in mesothelioma has not been investigated and might not be relevant; however, the involvement of HMGB1 in anti-tumor activities against several tumors and in ICD has been extensively documented. HMGB1 secreted by cells undergoing ICD activates DCs to cross-present neoantigens (mutated tumor proteins) to lymphocytes, which mount B- and T-cell responses that kill tumor cells and establish anti-tumor immunological memory.
stress, is required for calreticulin release.\textsuperscript{77} Since most, if not all, ICD inducers induce the production of ROS, a search for procedures that would lead to ROS production that target the ER led to the identification of hypericin-based photodynamic therapy.\textsuperscript{77} This also suggests that ROS production that targets the ER is an upstream molecular event in ICD.

How and why HMGB1 is released during ICD is not clear, but certainly, it must be released prior to caspase 3 activation, since after that step, HMGB1 cannot be released and remains tightly associated to the apoptotic cell remnants.\textsuperscript{3,78} Likewise, what redox form(s) of HMGB1 are involved in ICD has not been formally tested, but the ICD-triggering production of ROS and the involvement of TLR4 suggest that disulfide HMGB1 is important. Notably, the complete oxidation of HMGB1 in apoptotic cells that have progressed past the activation of caspase 3 renders HMGB1 tolerogenic rather than inflammatory.\textsuperscript{79}

8 | CONCLUSION: FROM EVOLUTION TO THERAPIES

HMGB1, initially identified as a chromatin protein of unknown function,\textsuperscript{80} does function in the nucleus as a chaperone that facilitates DNA bending and nucleosome assembly.\textsuperscript{81} These functions are probably ancestral, as all eukaryotes contain proteins structurally related to HMGB1,\textsuperscript{82} and the yeast proteins NHP6A/B perform similar functions in chromatin.\textsuperscript{83,84} However, during evolution, HMGB1 has acquired an additional role as a DAMP that can signal death and stress consequent to an assault to the integrity of the cell; this new role must have preceded the animal-plant divergence, as Arabidopsis contains at least one HMGB protein that performs alarmin functions upon fungal infection.\textsuperscript{85} Notably, also a clam HMGB1 has been shown to activate innate immunity.\textsuperscript{86} Since HMGB1 functions as alarmin are so old, we have to assume that HMGB1 became one of the pillars of the innate immune system early on, and has been reused by the adaptive immune system since its evolutionary inception in teleost fish. Notably, the ancestral HMGB gene has acquired several paralogs in mammals,\textsuperscript{82} and possibly these closely related proteins (HMGB2 to 4) have related but subtly different functions when compared with HMGB1.

During such a long evolutionary time, HMGB1 and its family members have apparently been used in every biological process where any cell has an interest into the death or the possible death of a neighboring cell, for any reason from being alerted to pathogens, to supporting or rescuing the ailing cell, to the process of replacing it. Some of these processes are associated to pathologies, such as infection or degenerative diseases that cause cell death, but some may be completely physiological and related to the homeostasis of the organism: hundreds of millions of cells are replaced each day in our body. Perversely, some of these functions may not be completely aligned to each other, for example, when immune cells from the innate arm abet tumors, and immune cells from the adaptive arm try to eliminate them. The true step forward will come when we will be able to tweak the different functions of HMGB1 in order to favor one outcome over the opposite one: accelerating tissue healing must not accelerate tumor growth. Given the complexity of HMGB1 biology, a lot of work remains to be done, but the rewards in terms of innovative therapeutic approaches in a variety of clinical settings are fully worth the effort.

ACKNOWLEDGEMENTS

We thank Michele Carbone, Haining Yang, Angela Raucci, Anna Rubartelli, and all members of the Bianchi lab for valuable discussions on the content of this review. R. M. was supported by a research fellowship by Associazione Italiana Ricerca sul Cancro. This work was supported by the AIRC grant IG-18623 and the Fondazione CARIPLO grant 2015-0644 to M. E. B., and by grant GR-2011-02351814 from the Italian Ministry of Health to E. V.

CONFLICT OF INTEREST

M.E.B. is a co-inventor in multiple patents involving HMGB1 and is founder and part owner of HMBGbiotech, a company that provides goods and services related to HMGB proteins. E.V. is a co-inventor in a patent involving HMGB1.

REFERENCES

1. Janeway CA Jr, Medzhitov R. Innate immune recognition. Annu Rev Immunol. 2002;20:197-216.
2. Matzinger P. The danger model: A renewed sense of self. Science. 2002;296:301-305.
3. Scaffidi P, Misteli T, Bianchi ME. Release of chromatin protein HMGB1 by necrotic cells triggers inflammation. Nature. 2002;418:191-195.
4. Wang H, Bloom O, Zhang M, et al. HMG-1 as a late mediator of endotoxin lethality in mice. Science. 1999;285:248-251.
5. Falcioni L, Spada F, Calogero S, et al. High mobility group 1 (HMG1) protein is not stably associated with the chromosomes of somatic cells. J Cell Biol. 1997;137:19-26.
6. Rovere-Querini P, Capobianco A, Scaffidi P, et al. HMGB1 is an endogenous immune adjuvant released by necrotic cells. EMBO Rep. 2004;5:825-830.
7. Messmer D, Yang H, Telusma G, et al. High mobility group box protein 1: An endogenous signal for dendritic cell maturation and Th1 polarization. J Immunol. 2004;173:307-313.
8. Dumitriu IE, Barauh P, Valentinis B, et al. Release of High Mobility Group Box 1 by dendritic cells controls T cell activation via the receptor for advanced glycation end products. J Immunol. 2005;174:7506-7515.
9. Dumitriu IE, Bianchi ME, Bacci M, Manfredi AA, Rovere-Querini P. The secretion of HMGB1 is required for the migration of maturing dendritic cells. J Leukoc Biol. 2007;81:84-91.
10. Chen GY, Tang J, Zheng P, Liu Y. CD24 and Siglec-10 selectively repress tissue damage-induced immune responses. Science. 2009;323:1722-1725.
11. Chiba S, Baghdadi M, Akiba H, 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. 2012;3:832-842.
12. Gardella S, Andrei C, Ferrera D, et al. The nuclear protein HMGB1 is secreted by monocytes via a non-classical, vesicle-mediated secretary pathway. EMBO Rep. 2002;3:995-1001.
13. Bonaldi T, Talamo F, Scaffidi P, et al. Monocytic cells hyperacetylate chromatin protein HMGB1 to redirect it towards secretion. EMBO J. 2003;22:5551-5560.
14. Oh YJ, Youn JH, Ji Y, et al. HMGB1 is phosphorylated by classical protein kinase C and is secreted by a calcium-dependent mechanism. J Immunol. 2009;182:5800-5809.

15. Tsang A, Klune JR, Zhang X, 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. 2007;204:2913-2923.

16. Lu B, Antoine DK, Kwan K, et al. JAK/STAT1 signaling promotes HMGB1 hyperacetylation and nuclear translocation. Proc Natl Acad Sci USA. 2014;111:3068-3073.

17. Hoppe G, Talloc T, Khattamchary SK, Crab JW, Sears JE. Molecular basis for the redox control of nuclear transport of the structural chromatin protein Hmgb1. Exp Cell Res. 2006;312:3526-3538.

18. Venereau E, Casalgrandi M, Schiraldi M, et al. Mutually exclusive redox forms of HMGB1 promote cell recruitment or proinflammatory cytokine release. J Exp Med. 2012;209:1519-1528.

19. Schiraldi M, Raucci A, Munoz LM, et al. HMGB1 promotes recruitment of inflammatory cells to damaged tissues by forming a complex with CXCL12 and signaling via CXCR4. J Exp Med. 2012;209:551-563.

20. Pagw K, Klaseen C, Weber C, Bernhagen J, Noels H. Diversity and inter-connections in the CXCR4 chemokine receptor/ligand family. Molecular perspectives. Front Immunol. 2015;6:429.

21. Collins PJ, McCully ML, Martinez-Munoz L, et al. Epithelial chemokine CXCL14 synergizes with CXCL12 via allosteric modulation of CXCR4. FASEB J. 2017;31:3084-3097.

22. Yang H, Wang H, Ju Z, et al. MD-2 is required for disulfide HMGB1-dependent TLR4 signaling. J Exp Med. 2015;212:5-14.

23. Yang H, Hreghvidsdottir HS, Palmblad K, 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. 2010;107:11942-11947.

24. Abraham E, Arcaroli J, Carmody A, Wang H, Tracey KJ. HMG-1 as a mediator of acute lung inflammation. J Immunol. 2000;165:2950-2954.

25. Tsung A, Sahai R, Tanaka H, et al. The nuclear factor HMGB1 mediates hepatic injury after murine liver ischemia-reperfusion. J Exp Med. 2005;201:1135-1143.

26. Muhammad S, Barakat W, Stoyanov S, et al. Human mesenchymal stem cell death involves cytoprotection mediated by cell-free HMGB1. J Immunol. 2006;176:12-15.

27. Venereau E, Schiraldi M, Uguccioni M, Bianchi ME. HMGB1 and leukocyte migration during trauma and sterile inflammation. Mol Med. 2013;55:76-82.

28. Venereau E, Ceriotti C, Bianchi ME. DAMPs from cell death to new life. Front Immunol. 2015;6:422.

29. Dormoy-Raclet V, Cammas A, Celona B, et al. Cutting edge: Extracellular high mobility group box 1 protein is a proangiogenic cytokine. J Immunol. 2006;176:12-15.

30. van Beijnum JR, Dings RP, van der Linden E, et al. Gene expression of tumor angiogenesis dissected: Specific targeting of colon cancer angiogenic vasculature. Blood. 2006;108:2339-2348.

31. Campa A, Santarella F, Esposito A, et al. Leukocyte HMGB1 is required for vessel remodeling in regenerating muscles. J Immunol. 2014;192:5257-5264.

32. Palumbo R, Sampaolesi M, De Marchis F, et al. Extracellular HMGB1, a signal of tissue damage, induces mesoangioblast migration and proliferation. J Cell Biol. 2004;164:441-449.

33. Limana F, Germani A, Zacheo A, et al. Exogenous high-mobility group box 1 protein induces myocardial regeneration after infarction via enhanced cardiac C-kit+ cell proliferation and differentiation. Circ Res. 2005;97:e73-83.

34. Meng E, Guo Z, Wang H, et al. High mobility group box 1 protein inhibits the proliferation of human mesenchymal stem cells and promotes their migration and differentiation along osteoblastic pathway. Stem Cells Dev. 2008;17:805-813.

35. Sessa L, Gatti E, Zeni F, et al. The receptor for advanced glycation end-products (RAGE) is only present in mammals, and belongs to a family of cell adhesion molecules (CAMs). PLoS ONE. 2014;9:e86903.

36. Fritz G. RAGE: A single receptor fits multiple ligands. Trends Biochem Sci. 2011;36:625-632.

37. Raucci A, Cugusi S, Antonelli A, et al. A soluble form of the receptor for advanced glycation endproducts (RAGE) is produced by proteolytic cleavage of the membrane-bound form by the sheddase a disintegrin and metalloproteinase 10 (ADAM10). FASEB J. 2008;22:3716-3727.

38. Braley A, Kwak T, Jules J, Harja E, Landgraf R, Hudson BL. Regulation of receptor for advanced glycation end products (RAGE) ectodomain shedding and its role in cell function. J Biol Chem. 2016;291:12057-12073.

39. Kokkola R, Andersson A, Mullins G, et al. RAGE is the major receptor for the proinflammatory activity of HMGB1 in rodent macrophages. Scand J Immunol. 2005;61:1-9.

40. Fluza C, Bustin M, Talwar S, et al. Inflammatory promoting activity of HMGB1 on human microvascular endothelial cells. Blood. 2002;27:2652-2660.

41. Kew RR, Penzo M, Habil DM, Marcu KB. The IKKalpha-dependent NF-kappaB p52/RelB noncanonical pathway is essential to sustain a CXCL12 autocrine loop in cells migrating in response to HMGB1. J Immunol. 2012;188:2380-2386.

42. Vogel S, Bodenstein R, Chen Q, et al. Platelet-derived HMGB1 is a critical mediator of thrombosis. J Clin Invest. 2015;125:4638-4654.

43. Stark K, Philipp V, Stockhausen S, et al. Disulfide HMGB1 derived from platelets coordinates venous thrombosis in mice. Blood. 2016;128:2435-2449.

44. Mitola S, Belleri M, Uribini C, et al. Cutting edge: Extracellular high mobility group box 1 protein is a proangiogenic cytokine. J Immunol. 2006;176:12-15.

45. Venereau E, Schiraldi M, Uguccioni M, Bianchi ME. High mobility group box 1 promotes recruitment of inflammatory cells to damaged tissues by forming a complex with CXCL12 and signaling via CXCR4. J Exp Med. 2012;209:551-563.

46. Pagw K, Klaseen C, Weber C, Bernhagen J, Noels H. Diversity and inter-connections in the CXCR4 chemokine receptor/ligand family. Molecular perspectives. Front Immunol. 2015;6:429.

47. Collins PJ, McCully ML, Martinez-Munoz L, et al. Epithelial chemokine CXCL14 synergizes with CXCL12 via allosteric modulation of CXCR4. FASEB J. 2017;31:3084-3097.

48. Yang H, Wang H, Ju Z, et al. MD-2 is required for disulfide HMGB1-dependent TLR4 signaling. J Exp Med. 2015;212:5-14.

49. Abraham E, Arcaroli J, Carmody A, Wang H, Tracey KJ. HMG-1 as a mediator of acute lung inflammation. J Immunol. 2000;165:2950-2954.

50. Tsung A, Sahai R, Tanaka H, et al. The nuclear factor HMGB1 mediates hepatic injury after murine liver ischemia-reperfusion. J Exp Med. 2005;201:1135-1143.

51. Muhammad S, Barakat W, Stoyanov S, et al. The HMGB1 receptor RAGE mediates ischemic brain damage. J Neurosci. 2008;28:12023-12031.

52. Weng H, Deng Y, Xie Y, Liu H, Gong F. Expression and significance of HMGB1, TLR4 and NF-kappaB p65 in human epidermal tumors. BMC Cancer. 2013;13:311.

53. Maroso M, Baloso S, Ravizza T, et al. Toll-like receptor 4 and high mobility group box 1 are involved in icterogenesis and can be targeted to reduce seizures. Nat Med. 2010;16:413-419.

54. Agalave NM, Larsson M, Abdelmoaty S, et al. Spinal HMGB1 in- hibes TLR4-mediated anti-inflammatory responses to Cpg-DNA. Blood. 2008;22:3007-3018.

55. Parkkinen J, Raulo E, Merenemies J, et al. Amphoterin, the 30 kDa protein in a family of HMG1-type polypeptides. J Biol Chem. 1993;268:19726-19738.
Guo ZS, Liu Z, Bartlett DL, Tang D, Lotze MT. Life after death: Krysko DV, Garg AD, Kaczmarek A, Krysko O, Agostinis P, Casares N, Pequignot MO, Tesniere A, et al. Caspase-dependent immunogenic cell death of HMGB1-deficient tumors: Compensatory therapy with TLR4 agonists. Cell Death Differ. 2014;21:69-78.

Sistigu A, Yamazaki T, Vaccelli E, et al. Cancer cell-autonomous contribution of type I interferon signaling to the efficacy of chemother-apy. Nat Med. 2014;20:1301-1309.

Garg AD, Krysko DV, Verfaillie T, et al. A novel pathway combining calreticulin exposure and ATP secretion in immunogenic cancer cell death. EMBO J. 2012;31:1062-1079.

Trisciuglio L, Bianchi ME. Several nuclear events during apoptosis depend on caspase-3 activation but do not constitute a common pathway. PloS ONE. 2009;4:e6234.

Kazama H, Ricci JE, Herndon JM, Hoppe G, Green DR, Ferguson TA. Induction of immunological tolerance by apoptotic cells requires caspase-dependent oxidation of high-mobility group box 1 protein. Immunity. 2008;29:21-32.

Goodwin GH, Sanders C, Johns EW. A new group of chromatin-associated proteins with a high content of acidic and basic amino acids. Eur J Biochem. 1973;38:14-19.

Agreti A, Bianchi ME. HMGB proteins and gene expression. Curr Op Genet Develop. 2003;13:170-178.

Sessa L, Bianchi ME. The evolution of High Mobility Group Box (HMGB) chromatin proteins in multicellular animals. Gene. 2007;387:133-140.

Giavara S, Kosmidou E, Hande MP, et al. Yeast Nhp6A/B and mammalian Hmgb1 facilitate the maintenance of genome stability. Curr Biol. 2005;15:68-72.

Celona B, Weiner A, Di Felice F, et al. Substantial histone reduction modulates genomewide nucleosomal occupancy and global transcriptional output. PloS Biol. 2011;9:e1001086.

Choi HW, Manohar M, Manosalva P, Tian M, Moreau M, Klessig DF. Activation of plant innate immunity by extracellular high mobility group box 3 and its inhibition by salicylic acid. PloS Pathog. 2016;12:e1005518.

Li J, Zhang Y, Xiang Z, Xiao S, Yu F, Yu Z. High mobility group box 1 can enhance NF-κB activation and act as a pro-inflammatory molecule in the Pacific oyster, Crassostrea gigas. Fish Shellfish Immunol. 2013;35:63-70.

Venereau E, De Leo F, Mezzapelle R, Careccia G, Musco G, Bianchi ME. HMGB1 as biomarker and drug target. Pharmcol Res. 2016;111:534-544.

Bianchi ME, Manfredi AA. How macrophages ring the inflammation alarm. Proc Natl Acad Sci USA. 2014;111:2866-2867.

How to cite this article: Bianchi ME, Crippa MP, Manfredi AA, Mezzapelle R, Rovere Querini P, Venereau E. High-mobility group box 1 protein orchestrates responses to tissue damage via inflammation, innate and adaptive immunity, and tissue repair. Immuno-Rev. 2017;280:74-82. https://doi.org/10.1111/imr.12601