REVIEW

HMGB1 and its physiological and pathological roles

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Abstract: HMGB1 has been formerly known for its intracellular function – as the intranuclear non-histone DNA binding protein, which contributes to stabilization of nucleosomes, mediation of DNA bending and is regarded to have an essential position in DNA repair. Lately, its participation in innate and specific immune responses has been revealed. Passively released from necrotic cells or actively produced by various cell types it acts as an alarmin and is responsible for production of pro-inflammatory cytokines. HMGB1 is able to interact with RAGE and TLRs, receptors that belong into family of pattern recognition receptors and are involved in activation of pathways leading to production of pro-inflammatory cytokines. Its key role has been revealed in mediation of sepsis and as it is released later than other pro-inflammatory cytokines it became known as a “late mediator of sepsis”. HMGB1 also contributes to the development of atherosclerosis and autoimmune diseases, e.g. its association with immunopathogenesis of SLE and RA has been suggested. Beside its negative function, HMGB1 protein seems to be able to attract stem cells to the area of inflammation and thus promotes regeneration processes. This paradoxical function of HMGB1 protein has also been revealed in growth and spread of many types of tumours. HMGB1 represents a potential target in therapy of various disorders related to inflammation (Fig. 2, Ref. 137). Full Text in PDF www.elis.sk.

Key words: atherosclerosis, autoimmune diseases, cancer, HMGB1, inflammation, sepsis.

The high mobility group (HMG) nuclear proteins were discovered in 1973 in an attempt to find a better explanation how gene expression is regulated (1). Since then this family of non-histone, chromatin-associated proteins have been considered involved in DNA organization and regulation of transcription. This group of proteins has common structural characteristics unusual to other chromosomal proteins. Long AT-rich 3’ untranslated regions as well as highly negatively charged carboxy-terminated regions belong to these different characteristics (2).

The high mobility group box-1 protein (HMGB1), a member of HMG family, was first isolated from perinatal rat brain in 1987. It was found in central neurons, where this heparin-binding protein could support neurite outgrowth (3) Belonging to group of highly evolutionarily conserved and ubiquitous proteins, HMGB1 in mouse has amino acid sequence 100 % identical to amino acid sequence in rat and there is nearly a 99 % identity between rodents and humans (4–6). While in mice HMGB1 gene lies on chromosome 5 (7), in humans the gene is localized on chromosome 13 (8).

HMGB1 was originally found in the nucleus. It was characterized as a 215 amino acid DNA-binding non-histone chromosomal protein, which consists of two positively charged DNA binding domains named HMGB box A and box B, and a negatively charged C-terminal domain that contains 30 repetitive glutamic and aspartic acid residues. Box A is responsible for anti-inflammatory activity of HMGB1 protein and also forms the region for binding of heparin and proteoglycans. Box B except of its pro-inflammatory, migration-enhancing and cell differentiating activity carries also a binding region for RAGE.

C-terminal domain that contains 30 repetitive glutamic and aspartic acid residues (Fig. 1) (9, 10). HMGB1 can bind without sequence specificity to double-stranded, single-stranded as well as misshapen (deformed, distorted) DNA (2, 10–13). Highly affinity interactions of HMGB1 with nucleosomes lead to the stabilization of their structure and mediation of DNA bending. These interactions also facilitate the binding of transcription factors along with steroid hormone receptors (14), steroid/nuclear hormones progesterone (15) and oestrogen (16, 17), HOX proteins (18) and transcription factor IIB (19). HMGB1 is regarded to be a master DNA repair mechanic due to its specific ability to bind to distorted and dam-
Necrotic and damaged cells can passively secrete HMGB1, while apoptotic cells can’t, thus giving a distinct signal to organism, so it can recognize these two types of cell death. Passive release of HMGB1 to extracellular fluid represents a good intracellular signal of tissue injury and a very good „necrotic marker”, and it results in immunostimulatory, inflammatory and reparative responses. HMGB1 belongs to the group of alarmins - endogenous signals of threat to organism called also damaged associated molecular patterns (DAMPs). It promotes the recruitment of mononuclear cells that clear cellular debris and protects against infection (24, 89, 90).

In contrast to necrotic cells, in apoptotic cells on the basis of cell death, HMGB1 can not be released to extracellular fluid. Responsible for this distinction between apoptotic and necrotic cells is the chromatin binding affinity of HMGB1. During a whole cell cycle, HMGB1 is not bound to the chromatin of living cells in a tight manner, and association and dissociation can proceed easily. This also allows a passive release of HMGB1 after damage or necrosis of the cell. The intracellular retention of HMGB1 in apoptotic cells is the result of an irreversible association of HMGB1 with one or more hypoacetylated components of chromatin. So after apoptosis, HMGB1 can induce only negligible inflammation in the surrounding tissue (24, 88, 91).

**Active production of HMGB1**

Apart from a passive releasing from necrotic or damaged cells, HMGB1 can be also secreted actively. As a consequence of exogenous bacterial stimulation, e.g. lipopolysaccharide (LPS) or stimulation coming from pro-inflammatory cytokines, such as TNF, IL-1 and also HMGB1 itself, HMGB1 is actively secreted from macrophages, monocytes, dendritic cells and many other components of innate immune system. Activated leucocytes secrete HMGB1 actively through processes that are routed differently from classical pathways of secretion, i.e. through endoplasmatic reticulum or Golgi apparatus. Specific process differs in the way of stimulation. While stimulating cells by TNF leads to releasing of HMGB1 through phosphorylation, stimulation by lipopolysaccharide results in HMGB1 releasing dependent on hyper-acetylation of its own lysine residues. This hyper-acetylation processes cause the changes in structure of HMGB1 protein that directly contribute to gathering of HMGB1 in cell’s cytoplasm and do not allow its returning into the nucleus of the cell (25, 26). HMGB1 is then absorbed into secretory lysosomes in a specific manner. This process is followed by a specific secretion of HMGB1 that depends on extracellular stimulation of leukocyte by lys-phospatidylcholine (LPC). LPC is produced in the area of inflammation later than other contributors of inflammatory response, e.g. IL-1. After all, secretory lysosomes can fuse with cell membrane and HMGB1 is secreted into the extracellular fluid (27).

There are some of the non-immune cells that HMGB1 can be released from, e.g. pituicytes stimulated with IL-1 or TNF (28). Also enterocytes can produce HMGB1 after the cytokine stimulation (29). In addition to stimulation by cytokines, cells like hepatocytes can secrete HMGB1 under hypoxic conditions or during oxidative stress and this secretion is based on changes in calcium level in a cell (30).

HMGB1 like a membrane-bound protein was found also to promote neurite outgrowth and to be involved in migration of some tumour cell lines when situated at the advancing plasma membrane or filopodia (31–33). HMGB1 is also involved in generation of active plasmin and matrix metalloproteinases, that facilitate migration by degrading extracellular matrix components, by binding plasminogen and tissue plasminogen activator (tPA) (34, 35).
**HMGB1 receptors RAGE**

HMGB1 can perform its extracellular roles through two types of receptors: Receptor for Advanced Glycation End-products (RAGE) and Toll-Like Receptors (TLR) 2, 4 and 9.

RAGE – the receptor for advanced glycation end-products (AGEs) was initially found in diabetes, renal impairment and disorders that lead to local or systemic oxidative stress. Encoded in the Class III region of the major histocompatibility complex (MHC), RAGE belongs to the immunoglobulin super-family. As it also functions as a pattern recognition receptor (PRR), it is able to recognize a 3-D structure of proteins instead of their amino acid sequence.

RAGE is lowly expressed on endothelial cells, epithelial cells, smooth-muscle cells, neurons. A significantly higher expression of RAGE is found on mature lung type-I pneumocytes than on other differentiated adult cells. Expression in high levels is also easily noticeable on embryonic cells (39, 40).

RAGE can interact with many ligands, e.g. AGEs, amyloid β-peptide as well as with HMGB1, and is capable of binding HMGB1 seven-times tightly than AGEs (41). Two major pathways are activated after ligands have been bound to RAGE – CDC42/Racl and various MAPKs, both leading to NFκB-dependent transcriptional activity. It was shown that RAGE and HMGB1 interaction in the developing nervous system contributes to neurite outgrowth and cell migration, and that was the first time when RAGE was determined to be the receptor for HMGB1 (42, 43, 92). CDC42/Racl pathway activated by HMGB1 is connected with migratory phenotype of neurites and various cancer cells and results in changes in cytoskeleton of the cell (43). As an alternative, there is another way of RAGE signalling that leads through activation of 38 MAPK (mitogen-associated protein-kinase) and Erk1/2. Thereafter, this interactions result in phosphorylation and degradation of IκB, and in activation of gene expression mediated by NF-κB (35, 41, 93). This gene expression contributes to production of molecules that are involved in inflammatory response, i.e. adhesion molecules (ICAM1, VCAM1) and cytokines like TNF, IL-1 and IL-8. Therefore, RAGE activation by HMGB1 is responsible for both initiation and also sustaining of pro-inflammatory phenotype (44, 45, 93).

Expression of RAGE has been found to be raised in many states due to acute or chronic inflammatory conditions, e.g. chronic renal failure, sepsis, rheumatoid arthritis, inflammatory bowel disease, arteriosclerosis, vasculitis and late diabetic complications (46, 94). Many findings confirm RAGE’s important role in innate and adaptive immune system processes (47, 48). At first, RAGE was found on the surface of various immune cells like monocytes/macrophages, neutrophils, dendritic cells, and T and B lymphocytes (49–51). RAGE can interact through β2 integrin Mac-1 on leukocytes with endothelial cells, on which it is also expressed like an adhesion receptor and so contributes to recruitment of leukocytes in mouse models of inflammation (52, 53). Also numerous extracellular RAGE’s ligands are connected with acute or chronic inflammatory responses (54–56). Finally, there is an activation of transcriptional factor NF-κB and variety of its downstream genes, which are also responsible for the regulation of innate and adaptive immune system (57, 94).

**Toll-like receptors (TLRs)**

As a member of pattern recognition receptors (PRRs) family, the group of TLRs can recognize damage associated molecular pattern molecules (DAMPs) and microbial molecular patterns (PAMPs – pathogen-associated molecular patterns) and are able to trigger inflammatory immune responses directly against pathogens that carry these molecular patterns (58, 61, 62).

TLRs are expressed on various cells of the innate immune system including neutrophils, macrophages and dendritic cells, and are also expressed on the surface of endothelial cells and mucosal epithelial cells (63).

Each receptor interaction is specific, i.e. double-stranded RNA is recognized by TLR3, TLR4 can recognize LPS from gram-negative bacteria and TLR5 is activated by bacterial flagellin. Furthermore, single-stranded RNA is recognized by TLR7 and un-methylated CpG motifs in DNA by TLR9. Finally, microbial peptidoglycans, lipoarabinomannan, lipoproteins, lipoteichoic acid and zymosan are able to activate TLR1, TLR2 and TLR6. Due to their function, some of TLRs are localized in endolysosomal compartments of the cell, like TLR3, TLR7 and TLR9 that can recognize viral nucleic acids, contrary to those that recognize bacterial protein and lipid ligands directly on the cell surface (59, 60, 95). All TLRs, except TLR3, can signal through myeloid differentiation factor 88 (MyD88) pathway that leads to the activation of MAPKs, extracellular signal regulated kinases and NF-κB (59, 60).

HMGB1 is ligand for TLR2, TLR4 and furthermore for TLR9 (64, 68). After HMGB1 signalling, TLR pathways are responsible for the activation of NF-κB and later production of pro-inflammatory cytokines in macrophages in the area of inflammation, and consequently for recruitment of neutrophils in response to releasing of these cytokines in vivo (65). Anti-tumour T-cell immunity is induced through TLR4 pathway by HMGB1 that comes from cells after dead from chemotherapy (66, 95).

Many studies have been speculating about HMGB1 protein’s ability to trigger activation of receptors in complexes with DNA. Tian et al. demonstrated that activation of TLR9 pathway is possible after complex of HMGB1 and DNA has been bound to it (68, 96). TLR9 activation by HMGB1 is likely to be mediated rather by complexes of HMGB1 with DNA than by HMGB1 itself. In this way, TLR9 pathway leads in immune cells to maturation and production of cytokines (68, 69). Other researches indicated that in some other cell types HMGB1-DNA complexes are able to cause the suppression of immune response (70). In addition to signalling by HMGB1-DNA complexes, HMGB1 bounded to nucleosomes derived from apoptotic cells can interact with TLR2 and is able to induce production of anti-dsDNA and anti-histone IgG antibodies (67, 95). Increased pro-inflammatory activity is noticed also after signalling HMGB1 in complexes with other cytokines like IL-1, IFN-γ, and TNF, in comparison to HMGB1 signalling alone (71).

It is still questionable if other HMGB1 complexes or its modifications are necessary for activation of PRR’s pathways (96).
Physiological and pathological roles of HMGB1

HMGB1, formerly known as non-histone DNA binding protein, was regarded to have an important function in structural stabilization of nucleosomes and proper regulation of transcription in somatic cell. In addition to its intracellular activities, HMGB1 also acts as a cytokine that activates many different receptors and through multiple downstream pathways induces various specific responses in numerous cell types (96, 97).

Effects of HMGB1 on individual cells

 Cultures of monocytes and macrophages were revealed to produce pro-inflammatory cytokines, e.g. TNF, IL-1, IL-1Ra, IL-6, IL-8, MIP-1, but not IL-10 or IL-12 after recombinant HMGB1 had been added (72). Besides, LPS induction lead to monophasic TNF response, while HMGB1 stimulated TNF production seemed to be biphasic with peaks in 4th and 10th hour (73, 92). Also an increase of the adhesive capacity occurred after HMGB1 stimulation and this appears to be augmented by other pro-inflammatory cytokines (74, 75).

In contrast to monocytes, in LPS, activated neutrophils induced the TNF release in 4hrs, HMGB1 lead to TNF peaking just in 60 min (77, 92). Stimulation by HMGB1 also lead to an increased interaction between MAC-1 and RAGE that resulted in activation of adhesive and migratory phenotype of neutrophils (53). Under the influence of HMGB1, NAD-(P)-H oxidase and NF-xB were activated, and thus neutrophils were stimulated to produce reactive oxygen species and also production of the pro-inflammatory cytokines was increased (64, 76, 77).

HMGB1 in dendritic cells lead to higher expression of cell surface markers and increased releasing of pro-inflammatory cytokines as well, so it may play a role in maturation of these cells (78, 79, 96).

T-cells appeared to increase proliferation, survival and cytokine production as a result of HMGB1 stimulation. Furthermore, Th-1 polarization was evident (78, 80, 96).

HMGB1 functions as a stimulator of expression of RAGE in endothelial cells. Also pro-inflammatory cytokines (TNF, IL-8 and MCP-1) and regulators of fibrinolytic activity (tPA and PAI) are released from endothelial cells under the effect of HMGB1. Furthermore, the expression of vascular adhesion molecules (ICAM-1 and VCAM-1) was increased on the cell’s surface, thereby endothelial cells were able to attract cells that played role in inflammatory response as well as to promote their transition into the area of inflammation. In addition to participation of HMGB1 in the processes of inflammation, there was also local TNF production that contributed to amplification of pro-inflammatory effect of this protein on endothelial cells (83, 92).

The expression of inducible nitric oxide synthase was also increased by HMGB1 and so barrier function of epithelial cells was damaged. It was revealed that systemic administration of HMGB1 could impair the barrier function of gut-epithelial cells in mice, resulting in raised permeability of ileal mucosa and in subsequent bacterial invasion into mesenteric lymph nodes (81, 92).

HMGB1 is at least as strong stimulator of smooth-muscle cell migration as bFGF is. It also induced cytoskeleton reorganization and thus enabled shape changes of these cells (88, 92).

HMGB1, reparative process and regeneration

Besides its role in the inflammatory process, this protein is further implicated in regeneration that this is another interesting feature of this molecule. Stem cells tend to move towards the area of inflammation under the influence of HMGB1. In this way, HGB1 contributes to reparative and regenerative tissue changes (53, 84). HMGB1 lead to increased myogenesis and angiogenesis in skeletal muscle (85, 96). While in normal mice wound healing has been slowed-down as a consequence of inhibition in HMGB1 signalling, topical application of HMGB1 caused an acceleration of this process in diabetic mice, so HMGB1 might also take an important part in diabetic wound healing (86). Moreover, an exogenous HMGB1 directly injected to peri-infarcted area contributed to an increased amount of myocytes inside the area of infarcted cardiomyocytes that went along with an improved outcome confirmed by structural and functional measures (87, 96). These examples support an effect of HMGB1 in the regenerative processes.

HMGB1 and atherosclerosis

An injury of endothelium is essential for the initiation of atherosclerosis as it leads to the attraction of macrophages. Progression of atherosclerosis goes along with prolonged pro-inflammatory response (98). It was revealed that HMGB1 and RAGE were expressed in endothelial cells, smooth muscle cells, and macrophages of atherosclerotic lesions (99). Therefore, up-regulation and secretion of HMGB1 may lead to the intensification of inflammatory response in endothelium lesions and thus promote further atherosclerotic changes (93).

HMGB1 in ischemic and reperfusion injury

Many factors have been revealed to be involved in pathogenesis of ischemic and reperfusion (IRI) including nitric oxide or plenty of cytokines released under pro-inflammatory conditions in the afflicted area in many organs i.e. heart, brain, kidney or liver (108, 109). Recent studies suggested a potential implication of HMGB1 signalling in IRI (110). Ischemia lead to tissue damage and due to these changes high levels of HMGB1 protein were released around the central ischemic area (111). It was demonstrated that HMGB1 was able to stimulate releasing of glutamate and while glutamate-excitotoxicity contributed to pathogenesis of stroke, it is possible that HMGB1 played a substantial role in stroke. Also RAGE has been lately identified as an important receptor implicated in stroke, giving another suggestion about function of HMGB1 protein in this condition (112).

HMGB1 and its role in sepsis and infectious inflammation

Despite advances in antibiotic therapy and intensive care, sepsis remains the most common cause of death in the intensive care units.
SIRS (Systemic Inflammatory Response Syndrome) is a systemic inflammatory state triggered by a huge variety of stimuli, i.e. infection, trauma, ischemia, hemorrhage, burns and pancreatitis. SIRS is commonly complicated by multiple organ dysfunction syndrome (MODS), which is a result of SIRS-induced hypotension and disseminated intravascular coagulopathy, and finally can lead to death. The sepsis is a SIRS, in which an infection is the triggering stimulus. Its mortality is close to 30% (100, 92).

In 1999, Wang and colleagues using an animal model revealed that HMGB1 functioned as a mediator of endotoxin-induced lethality (73). Afterwards, Yang with his group described the same increased levels of HMGB1 also in animal CLP-induced (cecal ligation and puncture) model of sepsis as a cause of mortality (101, 102).

Participation of HMGB1 in sepsis is interesting because of its later releasing and peaking during inflammatory response in opposite to other pro-inflammatory cytokines, e.g. TNF or IL-1. This was the reason why HMGB1 was known as “late mediator of sepsis”. Its levels are raising in 16hrs and remain significantly elevated for 32hrs after induction with LPS in mice model (73).

There were also studies, in which HMGB1 was revealed to be significantly elevated in serum of septic patients and patients with MODS induced by sepsis. Comparing surviving patients with those who did not survive, a significantly higher serum levels of HMGB1 were in coincidence with a higher mortality in septic patients (73). Furthermore, increased levels of HMGB1 were found in plasma of patients after TNF or other classical pro-inflammatory cytokines have been secreted in early phase of inflammation, it is likely that TNF together with damaged and dying cells were stimuli for release of HMGB1 (101, 103).

The exact mechanism of tissue injuring by HMGB1 is not yet completely elucidated. High plasma levels of HMGB1 are regarded to be responsible for epithelial leakage after impairing an intestinal barrier. This is mediated through RAGE pathway (104, 105). Furthermore, the production of ROS (reactive oxygen species) after activation of NAD(P)H-oxidase in neutrophils through TLR4 signalling represents another mechanism of HMGB1 activity in tissue injury (106, 107). However, there are still many details that remain to be explained.

**HMGB1 and autoimmune diseases**

Pro-inflammatory and immune-stimulatory function of HMGB1 indicates its association with autoimmune diseases, e.g. rheumatoid arthritis (RA) or systemic lupus erythematosus (SLE). HMGB1 levels have been found to be elevated in animal models of experimental arthritis (113) and in synovial fluid of patients with RA, where levels adequate to promote maturation of dendritic cells have been detected (78, 88, 115, 116). In contrast to synovial fluid, HMGB1 levels had no tendency to be elevated in serum or plasma of patients with RA. This finding was most likely caused by interaction of HMGB1 with serum components, which resulted in creation of complexes that restrain HMGB1 to be detected by ELISA (114). Complexes consist of HMGB1 and IgG class anti-HMGB1 antibodies, high levels of which have been detected in patients with RA (117).

SLE is another autoimmune disease, in which HMGB1 is implicated. E. Voll with his colleagues (118) suggested a model of immunopathogenesis of SLE, in which impaired phagocytosis of dead cells in patients with SLE (119, 120) was accompanied by secondary necrosis of apoptotic cells. This process leads to the release of HMGB1 in complexes with nucleosomes (HMGB1 is tightly bound to the chromatin of apoptotic cells). These complexes are later able to activate dendritic cells and macrophages and consequently the immunological tolerance to nucleosomes and dsDNA is disrupted.

**HMGB1 in cancer and metastatic processes**

HMGB1 has been revealed to regulate transcription of a few genes that contribute to growth and spread of tumours, e.g. TNF, BRCA or E-selectin (121–123). Also, HMGB1/RAGE-signalling has been found to be implicated in various cancer diseases (33) like colon cancer (124, 125) or prostate cancer (126). Lately, HMGB1 overexpression in tumour endothelial cells has been suggested to be associated with pro-angiogenic and metastatic potential of tumour mass (127). Furthermore, high levels of HMGB1 were accompanied with low differentiation of tumour cells (128, 129). Recent studies revealed paradoxical dual effect of HMGB1: in addition to its negative contribution in tumour neo-angiogenesis it also triggered protective anti-neoplastic T-cell responses (130). These findings support the theory that HMGB1 plays an important role in tumour growth and metastatic process. Therefore, further investigation may offer an important target in therapy of cancer diseases.

**Therapeutic approach**

There are few approaches in blockage of pathological activities of HMGB1 protein. The variety of antibodies have been used to restrain or reduce cytokine function of HMGB1 (132). Beside these biological agents there is another group – the cytokine-release inhibitory drugs (CRIDs) – that consists of small-molecule compounds like ethyl pyruvate, cholinergic agonists – nicotine and acetylcholine, stearoyl lysophosphatidylcholine and steroid-like pigment tanshinone IIA. These are able to react directly with HMGB1 and thus inhibit its release out of the cells, but they have no influence on individual systemic activities of HMGB1 (133–136). Glycyrrhizin have also been revealed to inhibit cytokine function of extracellular HMGB1 (132, 137). It does not affect HMGB1 releasing, once HMGB1 has been released out of the cell, glycyrrhizin directly binding to HMGB1 created complexes with it and blocked this way the chemotactic and mitogenic activities (24, 132, 137).

Due to pro-inflammatory effects of HMGB1, it is important to talk about its inhibition. To do this, a wide range of chemical substances have been studied experimentally. It was found that HSP72, which also plays a role of alarmin by itself, could interestingly inhibit releasing of HMGB1. Original studies revealed that heat shock in macrophages, which were previously stimulated by LPS, could inhibit HMGB1 releasing (36). Furthermore, HSP72 over-expression resulted in inhibited HMGB1 releasing.
from LPS-, TNF-, or oxidative stress-stimulated macrophages. The intracellular cooperation between HMG1 and HSP72 was likely the reason for this inhibition (37, 38).

Also endogenous neuroptides, vasoactive intestinal peptide and uroctin were revealed to decrease levels of HMG1 and thus to increase survival when administered in animal model of lethal sepsis (131, 103).

References


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