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NEWLY IDENTIFIED MESSAGE FOR RESCUE AND REPAIR FROM NECROTIC CELL: BIOLOGY AND CLINICAL RELEVANCE OF "ENDOKINE, HMGB1"

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Abstract Responses to stimuli in cellar level are diverse and such hierarchical as secretion of stored factors, synthesis of lipid mediators and protein synthesis through genomic transcription. However, how can the cells respond in the case of necrosis? Recently a characteristic intranuclear protein, high-mobility group box 1 protein (HMGB1) is released from necrotic cells. The protein is an abundant nuclear protein with a dual function both inside and outside the cells. In physiological state, HMGB1 is present in the nucleus, and binds to DNA, playing a variety of crucial functions, including transcription and keeping the characteristic DNA architecture. However, the protein is released to extracellular space from most of necrotic cells, activated macrophages and dendritic cells. Out of the cells, HMGB1 acts as a signal of tissue damage and can promote inflammation, immune responses, and results tissue regeneration. During sepsis and/or disseminated intravascular coagulation (DIC), however, massive accumulation of HMGB1 in the systemic circulation will cause multiple organ failure (MOF) and subsequent lethal outcome. Thus HMGB1 in the systemic circulation has been considered as a lethal mediator of sepsis, and a promising therapeutic target for sepsis. Recently we identified that thrombomodulin (TM), a natural anticoagulant glycoprotein expressed on the surface of endothelial cells, plays an important role in sequestering HMGB1. TM may prevent HMGB1 from reaching remote organs, thereby restricting the spectrum of HMGB1 action in the site of injury. Here we review recent progress made in defining the physiological and pathological roles of HMGB1 and therapeutic strategies aimed at blocking circulatory HMGB1.

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Key words: sepsis; disseminated intravascular coagulation (DIC); thrombomodulin; alarmin; high-mobility group box 1 (HMGB1)

Introduction

During evolution, multi-organ lives with closed circulatory system have developed defense mechanisms to counteract life-threatening events, such as infection, trauma injury, and hemorrhage. To initiate and accomplish appropriate and rapid protective responses, our defense systems need to recognize potentially life-threatening events. So far, two theories have been proposed to explain how our defense systems detect such threatening: stranger hypothesis and danger hypothesis[1,2]. Immune cells possess molecular microdetectors, called pattern recognition receptors (PRRs), that recognize common molecular patterns of invading pathogens[3,4]. Such pathogen-associated molecular patterns (PAMPs) include component of bacterial cell walls, flagellar proteins, and viral nucleic acids, and immunocompetent cells can detect PAMPs as life-threatening strangers using PRRs[4-6]. Another strategy to detect life-threatening events is targeted on tissue damage. During trauma or microbial attack, the resulting cellular stress or tissue damage alerts our defense systems. In this case, instead of recognizing PAMPs, immunocompetent cells recognize molecules that are normally found only inside cells, unless released by damage. These

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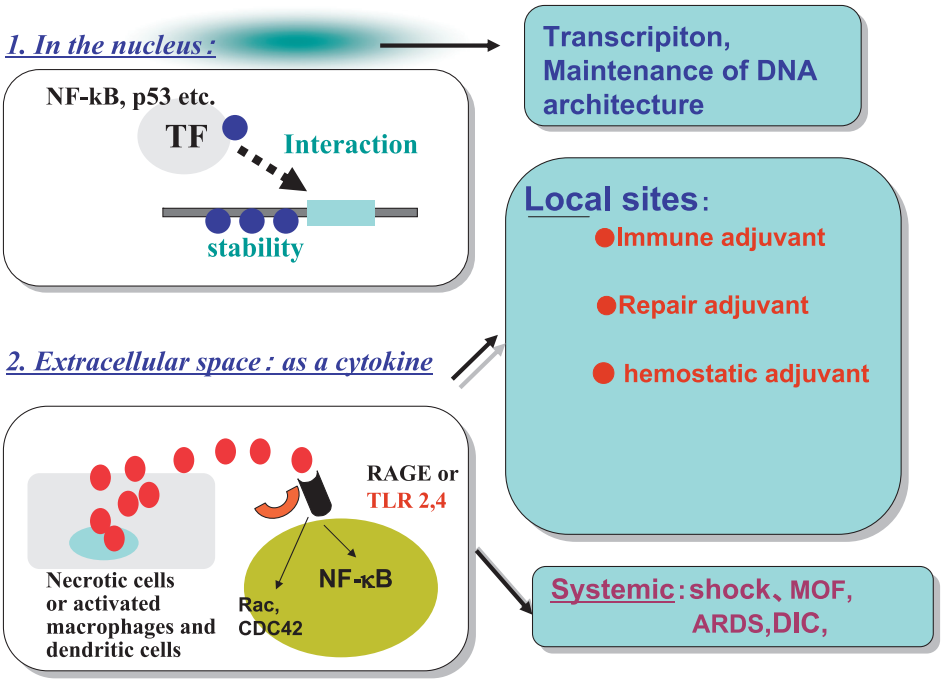

Figure 1 Intranuclear HMGB1 is release into outer cellular space by two manners. Nuclear protein HMGB1 is passively released from most of necrotic cells. The protein is also actively released from activated macrophages and dendritic cells. Extracellular HMGB1 acts as an adjuvant in immune, wound healing and hemostatic reaction in localized damaged tissue. However, circulatory systemic HMGB1 may metastasize inflammation and show remote effect resulting DIC, MOF and shock.

intracellular molecules include uric acid, RNA, high-mobility group box 1 protein (HMGB1), heat-shock proteins (HSPs), and S100 proteins, and the term damage-associated molecular patterns (DAMPs) or "alarmin" is proposed to categorize such endogenous molecules that signal tissue damage[2,7-9]. Thus, our defense systems scan for sings of both stranger and danger, and initiate protective responses, such as inflammation, immune response, hemostasis, and tissue repair.

Extracellular HMGB1 is a message for rescue from the necrotic cells

HMGB1 was previously thought to function only as a nuclear factor that enhances transcription. This protein is expressed in almost all eukaryotic cells, and is highly conserved through evolution[7,10]. HMGB1 is highly mobile and can shuttle between the nucleus and the cytoplasm[11]. HMGB1 binds to the minor groove of DNA, without sequence specificity, which causes a conformational change in the DNA that promotes its interaction with transcription factors such as NF-κB, steroid hormone receptors, and glucocorticoid receptors[12,13]. HMGB1 gene knock out mice die shortly after birth because of, at least in part, hypoglycemia caused by deficient glucocorticoid receptor function[14]. Besides its intracellular role, HMGB1 can be released into the extracellular milieu and plays quite distinct roles. There are two ways in which HMGB1 can be released: a passive release from necrotic cells and an active secretion from immunocompetent cells (Figure 1)[15-17].

Normally HMGB1 is bound loosely to chromatin, so if cells become necrotic or leaky, then HMGB1 diffuses out into the extracellular milieu[17]. Importantly, apoptotic cells do not release HMGB1 even after undergoing secondary necrosis and partial autolysis[17]. Thus, cells

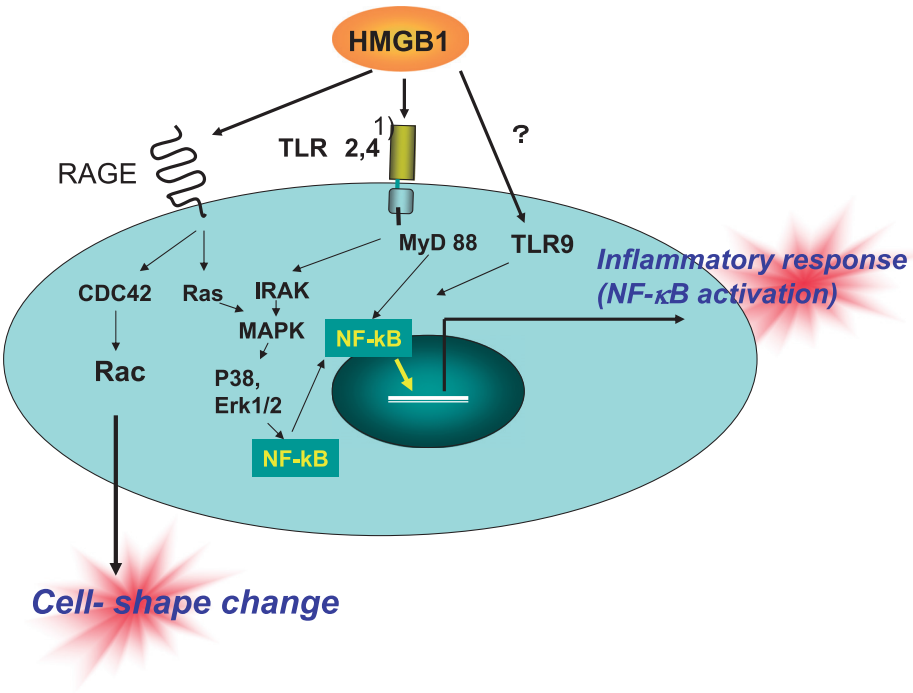

Figure 2 HMGB1 and its multiple receptor system. Extracellular HMGB1 acts on RAGE and Toll-like receptor-2 and -4, and results activations of NF κB and CDC2, Rac. These actions of HMGB1 play a crucial role in the development in DIC/MOF and septic shock.

undergoing apoptosis are programmed to withhold the signal, thereby preventing the inflammatory process during apoptosis. Several cell types, such as inflammatory cells, possess the ability to secrete HMGB1 actively, and thus produce a danger signal without dying. The active secretion depends on a relocalization of HMGB1 from the nucleus to specific cytoplasmic organelles, the secretory lysosomes[16]. Inflammatory stimuli lead to hyperacetylation of HMGB1, which causes an accumulation of HMGB1 in the cytoplasm and blocks a reentry to the nuclear compartment[18,19]. Cytoplasmic HMGB1 is taken up by secretory lysosomes, and then the HMGB1-containing secretory lysosomes are fused with the plasma membrane and are secreted, at least in part, in a specific ATP-binding cassette (ABC) transporter-dependent manner[9,16]. Once released into the extracellular space, HMGB1 acts as a signal of tissue damage and can promote inflammation through specific receptors including RAGE (Receptor for advanced glycation endproducts), Toll-Like receptor-2 (TLR2) and TLR4, and possibly other receptors[20-25] (Figure 2).

HMGB1 interacts with RAGE expressed on endothelial cells, inducing endothelial cell activation, with increased expression of vascular cell adhesion molecule 1 (VCAM1), intercellular adhesion molecule 1 (ICAM1), and E-selectin[26,27]. In addition, HMGB1 activates adhesive and migratory functions of neutrophils and monocytes in a AGER-dependent manner[28,29]. Engagement of RAGE therefore increases adhesion of neutrophils and monocytes to endothelial cells, and promotes the recruitment of leukocytes across endothelial barriers. HMGB1 also acts on leukocytes, inducing the expression of proinflammatory cytokines, such as tumor necrosis factor-α (TNF-α), interleukin-1 (IL-1), IL-6, and type I interferon (IFN)[15,30,31]. In this regard, HMGB1 and PAMPs synergistically

activate leukocytes, and it may contribute both to our defense systems against pathogen and to autoimmune pathogenesis[25]. Necrotic cells lacking HMGB1 have a greatly reduced ability to elicit the production of TNF-α by neighborhood monocytes, suggesting an important role of HMGB1 in promoting inflammation during necrotic cell death[17]. Extracellular HMGB1 also acts as an endogenous immune adjuvant. For the clonal expansion of naïve T cells, both specific antigen and co-stimulatory signals provided by the same antigen-presenting cell (APC) are required[32]. In bacterial infections, microbial components act as immune adjuvants and induce the expression of the co-stimulatory molecules on APCs[33]. However, there are some circumstances where immune responses are generated in the apparent absent of any microbial or other exogenous adjuvants. Such situations include immune responses to tumors, transplants, and possibly certain viruses. It has been postulated that in such conditions APCs can be activated by endogenous signals derived from cells that are stressed, virally infected, or killed necrotically, but not by healthy cells[34,35]. Now several actual and putative endogenous adjuvants, such as HSPs, uric acid, and HMGB1, have been identified, and there are others whose identities are not yet known (Figure 1b)[36-38]. In addition to its roles in inflammation and immune responses, extracellular HMGB1 has the ability to promote tissue repair. Compared to healthy muscle, dystrophic muscle contains more HMGB1, and exogenously added HMGB1 induces migration and proliferation of vessel-associated stem cells[39]. After myocardial infarction, exogenously added HMGB1 induces proliferation and differentiation of cardiac progenitor cells, thereby promoting cardiac regeneration[40]. Furthermore, cardiac performance in HMGB1-treated mice is significant improved compared to control mice, suggesting that HMGB1 can induce functionally relevant myocardial regeneration following myocardial infarction[40]. Although the role of endogenous HMGB1 in tissue regeneration remains to be clarified, exogenously added HMGB1 may be used to promote tissue regeneration. Taken together, HMGB1 released from damaged or activated cells seems to orchestrate post-injury responses: inflammation, immune responses, and subsequent tissue regeneration.

HMGB1 as a lethal mediator in sepsis and/or DIC

Sepsis is a life-threatening disorder that results from systemic inflammatory and coagulatory responses to infection[41]. Hyperactivation of the inflammatory system is the most important feature of sepsis, and has been the most common target of therapeutic strategies. So far, diverse therapies directed against proinflammatory mediators such as TNF-α and IL-1, have revealed dramatic effects in animal models of sepsis[42,43]. However, in humans, most of these strategies have not improved survival of septic patients[44,45]. It is in part because the classical proinflammatory mediators such as TNF-α and IL-1 are released within minutes after PAMPs exposure, then, even a minimal delay in treatment may result in treatment failure. HMGB1 is one promising therapeutic target for sepsis. In sepsis and/or disseminated intravascular coagulation (DIC), serum HMGB1 levels are elevated in both humans and animals[46-48] (Figure 3).

The accumulation of HMGB1 in the systemic circulation occurs considerably later than that of classically early proinflammatory mediators such as TNF-α and IL-1[46,49], and this delayed kinetics of HMGB1 makes it an attractive therapeutic target with a wider window of opportunity for the treatment of sepsis[50]. Indeed, blockade of HMGB1, even at later time points after onset of infection, has been shown to rescue animals from lethal sepsis[46,51-53]. Although data on higher species are not yet available, HMGB1 antagonism provide hope for new approaches that will be

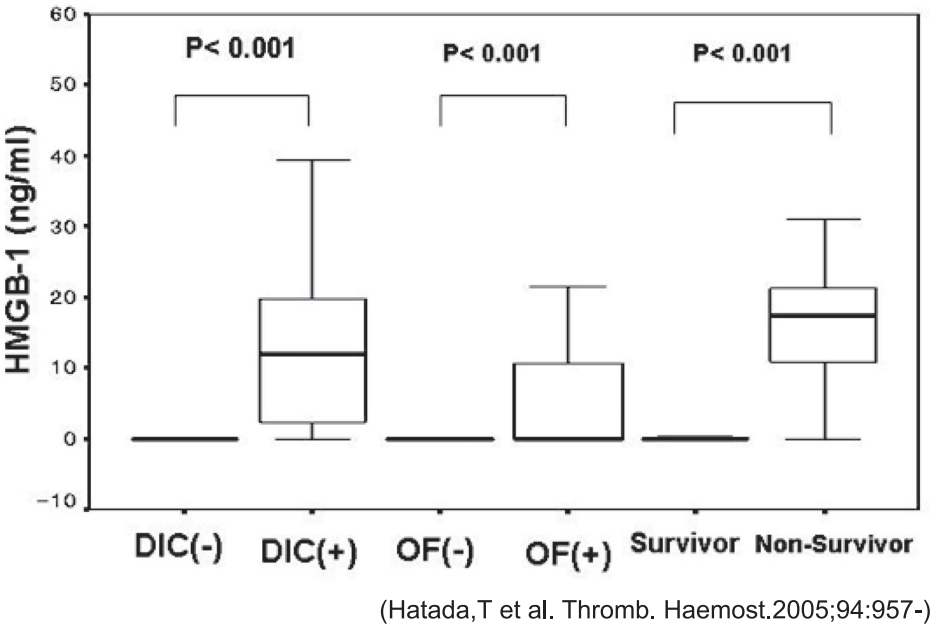

Figure 3 Elevated HMGB1 level in the plasma from the DIC patients. Among various infectious diseases, plasma HMGB1 was increased in the plasma from the patients complicated with DIC, MOF and lethality.

therapeutically effective in humans with sepsis. The mechanisms by which HMGB1 exerts its lethal effect in sepsis are not fully determined and seem to be multifactorial. Proinflammatory activity of HMGB1 is one possible mechanism. Extracellular HMGB1 triggers inflammation, and HMGB1 plays an important role in the pathogenesis of inflammatory diseases, including acute inflammatory lung injury, rheumatoid arthritis, and vasculitis[30,54-58]. Therefore, massive accumulation of HMGB1 in the systemic circulation will lead to systemic inflammatory response syndrome (SIRS), a major feature of sepsis. However, it is also reported that the roles of HMGB1 and TNF are distinguishable[7,50], and the lethal effects of HMGB1 in sepsis cannot be explained by its inflammatory activity only. HMGB1 may alter the procoagulant-anticoagulant balance, with an increase in procoagulant factors and a decrease in anticoagulant factors[59], and such alteration of the procoagulant-anticoagulant balance is another important aspect of sepsis[60]. HMGB1 induces tissue factor expression on monocytes, and inhibited anticoagulant protein C pathway mediated by thrombin-thrombomodulin (TM) complexes[59]. In thrombin-induced DIC model rats, HMGB1 accelerates microvascular thrombosis with irreversible renal and respiratory failure, thereby acting as a lethal mediator[59]. In patients with DIC, plasma HMGB1 levels are significantly increased and correlate with the DIC score and sepsis-related organ failure assessment (SOFA) score[48]. Given that DIC and concomitant multiple organ failure are strong predictors of mortality, procoagulant activity of HMGB1 may in part be responsible for its lethal effects. HMGB1 may act as a lethal mediator, by causing epithelial-cell barrier dysfunction[61,62]. Exposure of epithelial cells to HMGB1 causes downregulation of expression of cell surface molecules that are responsible for the tight adhesion between adjacent epithelial cells, leading

to epithelial hyperpermeability. When injected animals, HMGB1 B box increases ileal mucosal permeability and promotes bacterial translocation and hepatocellular injury. HMGB1 specific antibodies confer significant protection against the development of these epithelial abnormalities and subsequent organ dysfunction in animals with endotoxemia or sepsis, indicating that this might be a mechanism for how increased HMGB1 levels cause organ dysfunction[7,47].

Thrombomodulin as a security guard in blood vessels

In a recent clinical trial, recombinant human soluble thrombomodulin (rhsTM) significantly improved DIC[63]. Endothelial membrane-bound TM forms a high-affinity complex with thrombin, thereby inhibiting thrombin interaction with fibrinogen and protease-activated receptor-1 (PAR-1). In addition, TM enhances thrombin-mediated protein C (PC) activation by more than two orders of magnitude[64,65]. Like membrane-bound TM, the rhsTM, which is composed of the extracellular domain of TM, also binds to thrombin, thereby promoting PC activation. Activated PC (APC) has anti-inflammatory and anti-apoptotic activities that involve binding of APC to endothelial protein C receptor (EPCR) and cleavage of PAR-1[66]. APC exerts an antithrombotic effect by inactivating factors Va and VIIIa, limiting the generation of thrombin. As a result of decreased thrombin levels, the proinflammatory, procoagulant, and antifibrinolytic responses induced by thrombin are reduced[64,65,67]. In addition, TM itself has anti-inflammatory properties in its N-terminal lectin-

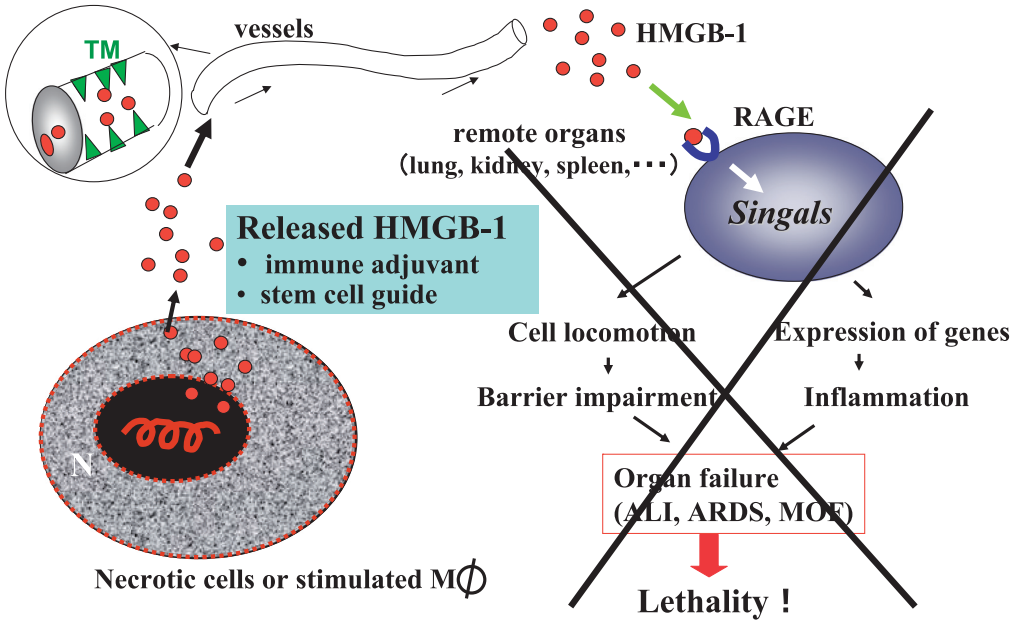

Figure 4 Endothelial thrombomodulin (TM) acts as a vascular safety guard. HMGB1 leased from necrotic cells or activated macrophages and dendritic cells acts as a adjuvant in innate immune, tissue regeneration and hemostatic system in the localized damaged tissues. Entry of the protein into the vessels and systemic circulation may be inhibited by endothelial TM by binding and subsequent degradation of the protein.

like domain[68] (Figure 4).

The target molecule of TM is not restricted to thrombin. TM can bind to HMGB1 as well as thrombin, thereby dampening the inflammatory and coagulatory responses[53,69]. TM also dampens the generation of reactive oxygen species (ROS) (unpublished observations). Under the conditions in which injured vessel locally loses the integrity of TM-bearing cells, HMGB1 and thrombin present in the circulation will propagate inflammatory and coagulatory responses[27,70]. However, once an adjacent portion of the vessel wall with intact endothelial cells is encountered, TM-bearing cells can sequester HMGB1 and thrombin, therefore preventing them from reaching remote organs (Figure 2). Thus, TM acts as a security guard in blood vessels. Since the expression of endothelial TM is systemically downregulated during septic conditions[71], replacement with rhsTM will offer therapeutic value in sepsis and/or DIC.

Concluding remarks

In the canonical model of sepsis, PAMPs are considered to be responsible for the development of sepsis. However, recent investigations suggest the importance of endogenous molecules as amplifiers of signals in the pathogenesis of sepsis[72,73]. Defining their functions during sepsis in greater detail will be necessary for the design of new therapeutic strategies in clinical sepsis.

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