Review

Emerging Role of High Mobility Group Box-1 in Thrombosis-Related Diseases

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Key Words

Coronary artery disease • Disseminated intravascular coagulation • HMGB-1 • Peripheral arterial disease • Stroke • Venous thrombosis

Abstract

High mobility group box-1 (HMGB-1), a typical damage-associated molecular pattern protein released from various cells, was first identified in 1973. It is usually stored in the nuclei of cells. Several modifications of HMGB-1 promote its translocation to the cytosol, and it is actively or passively released from cells. When outside of the cells, HMGB-1 is crucial in inflammation. It exerts its biological functions via interaction with its receptors, including receptor for advanced glycation end products (RAGE) and Toll-like receptor 4 (TLR4). A large number of studies showed a close link between inflammation and thrombosis. This review demonstrated the increased expression of HMGB-1 in thrombosis-related diseases, including coronary artery disease, stroke, peripheral arterial disease, disseminated intravascular coagulation, and venous thrombosis. Besides, it summarized the current understanding of the emerging link between HMGB-1 and thrombosis from three aspects: platelet, NETs, and coagulation and fibrinolysis factors. Finally, it explored the possible therapeutic strategies targeting HMGB-1 for treating thrombosis-related diseases.

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Structure of HMGB-1

A detailed overview of HMGB-1 has been extensively reviewed elsewhere [1]. Briefly, HMGB-1, a nonhistone protein, is constitutively present in nearly all types of cells. It is an evolutionarily conserved 30-kDa DNA-binding protein in the nucleus, including two internal repeats of positively charged domains (A box and B box) in the N-terminus and a continuous stretch of negatively charged residue in the C-terminus. HMGB-1 A box is usually considered as an antagonist of full-length HMGB-1 because it binds to the same site. Thus, HMGB-1 A box suppresses inflammatory response by competing with HMGB-1. However, HMGB-1 B box facilitates the affinity of HMGB-1 and its receptors, leading to inflammation (Fig. 1A).

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Redox states of HMGB-1

HMGB-1 is classified into three subtypes according to the modification of cysteine residues. It exerts its various effects via different isoforms [2]. HMGB-1 contains three critical cysteine residues (C23, C45, and C106), which are modified by redox signaling to form three HMGB-1 isoforms (all-thiol HMGB-1, disulfide HMGB-1, and oxidized HMGB-1) [3, 4]. When all the cysteine residues are fully reduced, the HMGB-1 is termed as all-thiol HMGB-1 (Fig. 1B). All-thiol HMGB-1 may promote leukocyte recruitment via binding to CXCL12 and CXCR4 [5, 6], suggesting that reduced forms of three cysteine residues contribute to its chemotactic activity. A recent study demonstrated that platelet-derived HMGB-1 promoted the recruitment of monocytes, with potential implications in thrombosis and inflammation [7]. However, two cysteine residues are oxidized to form a disulfide bond between C23

and C45, which is termed as disulfide HMGB-1 (Fig. 1C). Disulfide HMGB-1 could induce nuclear factor-kappaB (NF-κB) translocation and secretion of inflammatory factors via Toll-like receptor (TLR)4 in activated immune cells [3, 8, 9]. However, mutations of C23 or C45 could abolish HMGB-1 cytokine activity [2]. In line with these findings, another report indicated that disulfide HMGB-1 caused bladder pain through TLR4 activation [10]. Besides, it stimulated smooth muscle cells and macrophages to produce inflammatory cytokines via TLR4 [11, 12]. Recently, Frank et al. found that disulfide HMGB-1 potentiated neuroinflammatory response *in vivo*. However, all-thiol HMGB-1 failed to promote the microglial proinflammatory response to lipopolysaccharide (LPS) [13]. Consistent with the findings of others, a recent study showed that oxidative stress promoted the secretion of HMGB-1 and other proinflammatory factors, wherein HMGB-1 was partially oxidized [14, 15]. In a venous thrombosis model, disulfide HMGB-1, released from platelets and neutrophils, could promote neutrophil recruitment and neutrophil extracellular trap (NET) formation, and contributed to thrombosis via redundant pattern recognition receptors [16]. Thus, disulfide HMGB-1 might promote thrombosis via inflammation. The third form of HMGB-1 is oxidized HMGB-1, which is characterized by three fully oxidized cysteine residues (Fig. 1D). In contrast to all-thiol HMGB-1 and disulfide HMGB-1, oxidized HMGB-1 has no biological functions such as chemokine and proinflammatory activities [9, 17].

HMGB-1-mediated signaling pathways

HMGB-1 fundamentally resides in the nucleus under normal circumstances, stabilizing nucleosomes and facilitating gene transcription [18]. However, when it is hyperacetylated under some conditions, HMGB-1 translocates from the nucleus to the cytosol and is subsequently released from the stressed or activated cells [19]. Early reports demonstrated

Fig. 1. Structure and redox states of HMGB-1 (A) HMGB-1 consists of A box (anti-inflammatory domain), B box (pro-inflammatory domain), and acidic tail. Residues 89–108 were responsible for binding to TLR4, whereas residues 150–183 were responsible for binding to RAGE. TLR4, Toll-like receptor 4; RAGE, receptor for advanced glycation end products. (B) All-thiol HMGB-1 exhibited all reduced cysteine residues at C23, C45, and C106 (C) Disulfide HMGB-1 was formed by a disulfide bond between C23 and C45 (D) Oxidized HMGB-1 was termed as all three oxidized cysteines at C23, C45, and C106.

that HMGB-1 could be passively or actively released outside. On the one hand, HMGB-1 is passively diffused from various cells to the extracellular space during cellular necrosis or damage [20]. On the other hand, HMGB-1 is actively released outside of macrophages, monocytes, and dendritic cells (DCs) when the cells are activated [1].

Once in the extracellular space, HMGB-1 exerts its biological functions via interaction with its receptors, including receptor for advanced glycation end products (RAGE) [21, 22], TLR2 [23-26], TLR4 [27-30], and TLR9 [31]. The interaction between HMGB-1 and its receptors has been shown to induce cell adhesion [32], permeability [33, 34], chemotaxis [35], inflammation [36-38], autophagy [39-41], apoptosis [42-44], thrombosis [16, 45, 46], angiogenesis [47, 48], fibrosis [49, 50], and epithelial-mesenchymal transition (EMT) [51, 52]. Although the exact mechanisms involved in the progression still remain unclear, myeloid differentiation factor 88 (MyD88)-mediated pathways have been the most studied.

Recombinant HMGB-1 directly promoted the expression of MyD88, NF- κ B, tumor necrosis factor (TNF)- α , and interleukin (IL)-1 β in mouse primary cortical neurons, suggesting that HMGB-1 could induce proinflammatory factors via the MyD88-dependent pathway [53]. The deficiency of MyD88 inhibited HMGB-1-induced TNF- α release in macrophages [12]. However, an *in vivo* study demonstrated that MyD88-deficient mice exhibited significantly lower IL-6 levels after cold ischemia-reperfusion injury [54]. Several receptors have indicated the involvement of MyD88 in the HMGB-1-mediated pathway. HMGB-1 has been shown to promote antineutrophil cytoplasmic antibody-antigen translocation in neutrophils, which was attenuated by blocking TLR4, RAGE, MyD88, and NF- κ B [55]. Combined deficiency of TLR2/TLR4, or of MyD88 alone, attenuated HMGB-1-induced expression of MMP-3 and MMP-13 in chondrocytes [56]. In line with these findings, another study demonstrated that HMGB-1 treatment increased the levels of proinflammatory markers in the lungs of wild-type mice but not in TLR4-knockout mice [57]. An *in vitro* study demonstrated that blocking TLR4 or MyD88 inhibited HMGB-1-induced proinflammatory cytokine production in mouse tracheal epithelial cells [58]. Furthermore, Das et al. found that the interaction of HMGB-1 and TLR5 initiated NF- κ B activation via MyD88, resulting in proinflammatory cytokine production *in vivo* [59]. These findings indicated that HMGB-1 exhibited its proinflammatory effect via the MyD88-dependent pathway by binding to its receptors. As shown in Fig. 2, dozens of other molecules have also been implicated in HMGB-1-related signaling pathways.

Fig. 2. Signaling pathways of HMGB-1. The interaction between HMGB-1 and its receptors induced the activation of downstream signaling pathways during cell adhesion, permeability, chemotaxis, inflammation, immunization, autophagy, apoptosis, thrombosis, angiogenesis, fibrosis, and epithelial-mesenchymal transition (EMT).

Expression of HMGB-1 in coronary artery disease, stroke, peripheral arterial disease, disseminated intravascular coagulation, and venous thrombosis

A large number of reports in the last decade demonstrated the role of HMGB-1 in thrombosis-related diseases (Table 1). HMGB-1 has been considered as a critical mediator in cardiovascular diseases [1, 60]. Several investigations showed that serum HMGB-1 was higher in patients with acute coronary syndrome [61-65]. In line with these studies, the upregulated HMGB-1 level and thrombosis were found in patients with plaque rupture compared with volunteers [66]. Moreover, immunofluorescence staining showed abundant

expression of HMGB-1 in platelet-rich human coronary artery thrombi [46, 67]. Furthermore, plasma HMGB-1 level in coronary heart disease was significantly higher in patients with chest pain than in those with no plaque [66, 68-70], suggesting the value of HMGB-1 in the increased risk of coronary heart atherosclerosis [68, 69, 71]. Similar results were also observed in animal experiments showing that apoE^{-/-} mice exhibited increased expression of HMGB-1 in the aortic sinus [22, 72]. The occurrence of postprocedural myocardial injury was higher in the patients with elevated HMGB-1 levels [73]. However, HMGB-1 expression in circulating platelets was not associated with outcomes in symptomatic coronary artery disease [74]. Thus, serum /plasma HMGB-1 was considered as a potential and independent predictor of cardiovascular mortality in patients with acute coronary syndrome [61, 62, 64, 75].

A number of investigations indicated the link between HMGB-1 and ischemic stroke. Several studies showed that plasma HMGB-1 level was elevated following ischemic stroke for months [76, 77] and was correlated with the severity of stroke [76]. Furthermore, a recent study indicated that elevated HMGB-1 level was associated with poor functional outcome in ischemic stroke [78]. In animal experiments, ischemia mice/rats exhibited a higher expression of HMGB-1 in serum [79-81], plasma [82-84], cerebrospinal fluid (CSF) [85, 86], and brain tissue [21, 84, 87-91].

Accumulating data revealed a correlation between HMGB-1 and peripheral arterial disease (PAD). An earlier report demonstrated that the concentration of plasma HMGB-1 increased in patients with PAD than in healthy controls [92]. This notion was strongly supported by a recent study showing higher serum HMGB-1 levels in patients with type 2 diabetes affected by PAD [93]. Furthermore, the expression of HMGB-1 increased in diabetic foot atherogenesis and arteriosclerosis obliterans [94, 95].

Disseminated intravascular coagulation (DIC) is a clinical syndrome characterized by the activation of the coagulation system, and increasing evidence has supported the role

Table 1. Expression of HMGB-1 in thrombosis-related diseases

Disease	Sample	Detection method	Regulatory role	Ref.
CAD	Serum	ELISA	Increased preoperative HMGB-1 predicted myocardial injury post-PCI	[73]
CAD	Serum	ELISA	HMGB-1 levels were markedly increased in patients with CAD and were associated with plaque burden	[66, 69-71]
ACS	Serum	WB	Serum HMGB-1 levels were elevated in patients with myocardial ischemia	[65]
AMI	Serum/Plasma	ELISA	HMGB-1 levels were higher in patients with AMI and were associated with adverse clinical outcomes in the postinfarction healing process	[61-64, 75]
AMI	Thrombi tissue	IHC	Extracellular HMGB-1 was closely localized with platelets in the coronary thrombi	[46, 67]
Chest pain	Plasma	ELISA	Increased plasma HMGB-1 levels might be associated with increased risks of coronary atherosclerosis in young patients with chest pain	[68]
AS model	Aortic tissue	IHC	HMGB-1 was expressed within the aortic sinus of apoE ^{-/-} mice and restricted to cells within the intima	[22, 72]
Ischemic stroke	ELISA	Plasma	HMGB-1 levels were higher in patients with ischemic stroke than in controls	[76, 77]
Ischemic stroke	ELISA	Serum	Serum HMGB-1 levels increased in patients with ischemic stroke relative to healthy controls	[78]
MCAO model	ELISA/WB	Serum/Plasma	HMGB-1 levels were significantly increased in MCAO models	[79-84, 90]
MCAO model	WB	CSF	Expression of HMGB-1 in CSF was elevated in MCAO models	[85, 86, 90]
MCAO model	WB/IHC	Brain tissue	HMGB-1 translocated and was released into the extracellular space in postischemic brain	[21, 84, 87-90]
PAD	ELISA	Plasma	Concentration of HMGB-1 increased in patients with PAD	[92]
PAD	ELISA	Serum	HMGB-1 levels were higher in patients with T2D accompanied by PAD	[93]
PAD	IHC	Artery tissue	Expression of HMGB-1 increased in patients with diabetes accompanied by PAD	[94]
DIC	ELISA	Plasma	Plasma HMGB-1 levels were correlated with DIC	[98, 177]
DIC	ELISA	Serum/Plasma	HMGB-1 levels were higher in septic patients with DIC than in septic patients without DIC	[97, 98, 177]
DIC	ELISA	Serum	Patients with higher HMGB-1 levels developed DIC in hematological malignancies complicated with SIRS	[99]
DIC	ELISA	Serum	Platelet activation markers were correlated with serum HMGB-1 levels in DIC	[100]
VT	IF	Inferior vena cava	HMGB-1 was expressed in inferior vena cava	[16]
CVST	WB	Brain tissue	Expression of HMGB-1 protein increased in CVST	[101]

of HMGB-1 in DIC [96-98]. Serum HMGB-1 level was higher in patients with DIC than in those without DIC [96-98]. Plasma HMGB-1 level was demonstrated as an independent factor in the diagnosis of DIC with leukemia [96]. Moreover, patients with higher HMGB-1 in hematological malignancies might be complicated by DIC [99]. HMGB-1 was correlated with platelet activation markers in patients with DIC accompanied by hematologic malignancy [100]. Besides, patients with organ failure and nonsurvivors exhibited the highest HMGB-1 levels among patients with DIC [98]. This suggested that HMGB-1 level increased and was associated with organ failure in DIC [96, 98].

Two recent studies reported the role of HMGB-1 in venous thrombosis. The expression of HMGB-1 in brain tissue was higher in a cerebral venous sinus thrombosis (CVST) model compared with control animals [101]. Also, recombinant human soluble thrombomodulin protected against CVST via the inhibition of HMGB-1 [101]. Further, platelet-derived disulfide HMGB-1 contributed to venous thrombosis as a central mediator of the sterile inflammatory process [16].

Mechanisms of HMGB-1-mediated thrombosis

Numerous experimental and clinical studies have shed light on the role of HMGB-1 in thrombosis-related diseases. However, the underlying molecular mechanisms have not been fully elucidated. The present review aimed to discuss the mechanisms from three aspects: platelet, NETs, and coagulation and fibrinolysis factors.

HMGB-1 and platelets

Platelets, which are anucleated cells in blood, are believed to be centrally involved in thrombosis progression. Circulating platelets migrate to the sites and initiate a set of thrombosis-related processes upon vascular injury. The platelets aggregated and released granules through the receptor recognition of exposed subendothelium. These granules promoted the activation of platelets via a positive feedback and contributed to thrombosis formation.

Accumulating data suggested that inflammation induced thrombosis via the activation of platelets. Emerging studies in recent years have shed light on the functions of platelets induced by HMGB-1. In 2000, Rouhiainen et al. detected the expression of HMGB-1 in human platelets using Western blot and reverse transcription-polymerase chain reaction [102]. Moreover, HMGB-1 exhibited cytoplasmic localization in resting platelets and translocated into the surface of active platelets. Since then, a number of studies provided a close link between HMGB-1 and activation of platelets. Consistent with earlier reports, HMGB-1 was rarely detected in the plasma and was mainly an intracellular protein [103]. However, thrombin, collagen, C-reactive protein, or adenosine diphosphate induced translocation of HMGB-1 from the cytoplasm to the supernatant in platelets [104-106]. Interestingly, thrombin or collagen did not change the exosome secretion of purified platelets. However, cytoplasmic HMGB-1 could be released from thrombin- or collagen-induced platelets under conditions of high extracellular calcium, suggesting that extracellular calcium mediated thrombin/collagen-induced expression of HMGB-1 in platelets [104]. Thus, these findings indicated extracellular calcium-dependent HMGB-1 secretion in active platelets.

Once released outside of platelets, HMGB-1 could exhibit its effects on platelets. In a DIC model, HMGB-1-treated mice exhibited higher platelet concentrations compared with vehicle-treated mice [107]. Increasing evidence in the last decade suggested that HMGB-1 could recognize its receptor on platelets, contributing to platelet aggregation and secretion [46, 103]. Recently, HMGB-1 was shown to promote platelet activation, granule secretion, adhesion, and spreading *in vitro*. In animal experiments, platelet-specific deficiency of HMGB-1 induced increased bleeding times, reduced thrombus formation, and reduced

platelet aggregation [45]. Consistent with this study, another investigation indicated that HMGB-1 accelerated thrombin-induced coagulation *in vivo* [107], suggesting that HMGB-1 promoted coagulation via platelet activation. Many receptors exist for HMGB-1, the important ones being RAGE, TLR2, TLR4, and TLR9. Zhang et al. demonstrated that LPS-activated platelet aggregation required the TLR4 pathway [108]. The deletion of TLR4 gene resulted in the reduced expression of P-selectin in platelets and reduced aggregation ability on thrombin stimulation, indicating the role of TLR4 in platelet activation [109, 110]. Moreover, the enhanced expression of TLR4 on platelets in patients with acute coronary syndrome revealed the possible role of TLR4 in platelet aggregation [111]. Consistent with these studies, two recent investigations showed that the HMGB-1/TLR4-mediated signaling pathway contributed to platelet activation and thrombosis [45, 103]. Besides, they found that HMGB-1-induced platelet aggregation was attenuated in MyD88-deficient platelets, suggesting that the TLR4/MyD88 pathway contributed to HMGB-1-stimulated platelet aggregation [45]. Furthermore, extracellular regulated protein kinase (ERK) and guanylate cyclase (GC)/cyclic guanosine monophosphate (cGMP)/cGMP-dependent protein kinase I (cGKI) were found to be the downstream signaling pathways of TLR4 in HMGB-1-induced platelets [45]. This was in line with a previous report indicating that the TLR/MyD88- and cGMP-dependent protein kinase pathway collectively contributed to LPS-induced platelet activation [108].

Another well-characterized signaling mechanism by which TLR4 exhibits its effect on platelet is NF- κ B-mediated signaling pathway. Yang recently found that HMGB-1 triggered NF- κ B activation and regulated the expression of cGMP in platelets. Moreover, the inhibition of NF- κ B impaired HMGB-1-induced platelet activation and reduced I κ B α phosphorylation [103]. It meant that NF- κ B/I κ B α was involved in HMGB-1-induced platelet activation [103, 112]. Furthermore, the inhibition of NF- κ B reversed the decreased level of cGMP, indicating the role of cGMP in HMGB-1-induced platelet activation. These findings were consistent with a previous report showing that TLR4 agonists induced I κ B α degradation, p65 phosphorylation, and platelet aggregation [112]. Thus, HMGB-1 might induce the activation of platelets via the TLR4/NF- κ B/I κ B α /cGMP pathway.

Although the aforementioned evidence indicated that the TLR4-dependent signaling pathway (but not TLR2) mediated HMGB-1-induced platelet activation, divergent findings from other groups need to be taken into consideration. Ward et al. found that agonists of TLR4 were unable to modulate platelet activation. The discrepancy between these results might be due to different agonists with different dosages and durations [113].

Besides TLR4, engagement of platelet TLR9 by carboxy protein promoted platelet hyperreactivity and thrombosis [114]. However, whether HMGB-1 could induce the activation of platelets via TLR9 was still unclear. HMGB-1 could also bind to activated platelets by binding to RAGE and TLR4 [46]. However, whether HMGB/RAGE contributed to platelet activation remains debatable. Two recent studies demonstrated that HMGB-1 could not induce the activation of RAGE^{-/-} platelets [45, 103]. The different conditions of platelets involved in these experiments and different ways to detect platelet activation might explain the discrepancy among studies.

HMGB-1 and NETs

Sterile inflammation is a known critical factor in the development of thrombosis. The correlation between HMGB-1 and sterile inflammation was reviewed in 2014 [36]. However, increasing evidence in the last 3 years has supported the involvement of HMGB-1 in sterile inflammation [115-118]. Blockade of extracellular HMGB-1 attenuated inflammation and coagulation abnormalities in traumatic coagulopathy models [119], suggesting that HMGB-1 could promote thrombosis via sterile inflammation. Reduced HMGB-1 on the surface upon activation induced the recruitment of monocytes, and active leukocytes in turn promoted the

oxidation of HMGB-1, unleashing its prothrombotic activity [16]. Thus, sterile inflammation may be vital in HMGB-1-induced thrombosis.

NETs were produced during infection and created fibrous nets with high concentrations of antimicrobial proteins to trap and kill microbes in the extracellular environment. When the neutrophils were activated, granular enzymes such as neutrophil elastase translocated to the nucleus. Active enzymes promoted chromatin decondensation and released unwound chromatins outside the cells. Then, NETs were released from lysed cells, including extracellular DNA and histones [120, 121]. An accumulating body of evidence suggested that NETs contributed to thromboses such as venous thrombosis [122-124], DIC [125], and coronary thrombosis [126-129].

NETs may provide a reaction interface for von Willebrand factor (vWF), which facilitates the adhesion of platelets via platelet-surface receptor complex, contributing to platelet activation [130]. In addition to NET-platelet interactions, histones and neutrophil proteases in NETs also contributed to platelet activation. Semeraro and colleagues demonstrated that extracellular histones promoted the activation of platelets via TLR2 and TLR4 [131]. Besides, human neutrophil elastase promoted platelet aggregation via the activation of platelet receptors [132]. Thus, NETs promoted platelet adhesion, activation, and aggregation, contributing to thrombus formation when perfused with blood. In addition to white thrombus, NETs induced the formation of red thrombus via activating coagulation factors and trapping red blood cells and fibrin. Elevated NETs in patients with sepsis have been shown to promote the generation of thrombin and fibrin in normal plasma [133]. Gould et al. found that NETs enhanced thrombin generation regardless of platelets [134]. NETs from active neutrophils could bind and stimulate factor XII activation [123]. Another report suggested that serine proteases and extracellular nucleosomes induced tissue factor- and factor XII-dependent coagulation via the proteolytic inactivation of tissue factor pathway inhibitor (TFPI) [135].

In the last few years, much attention has been devoted to assessing the role of HMGB-1 in NET formation, which promoted coagulation and thrombosis formation [106]. Thus, the crosstalk between HMGB-1 and NETs was critical in thrombosis.

Oxidative stress in neutrophils was implicated in NET formation and led to thrombosis under inflammatory conditions. Treatment with H_2O_2 promoted the activation of neutrophils and monocytes. However, the stimulatory effects were reversed by catalase, suggesting that HMGB-1 might induce neutrophil activation via oxidative stress [136]. This notion was supported in endothelial progenitor cells, with HMGB-1 serving as a mediator for oxidative stress [15].

Nicotinamide adenine dinucleotide phosphate (NADPH) oxidase, a critical source of reactive oxygen species (ROS) genesis, was involved in the HMGB-1-induced activation of neutrophils [137, 138]. HMGB-1 exerted its effects on NET genesis via binding to its receptors including TLR2, TLR4, and RAGE, this process was dependent on NADPH oxidase [137]. In line with this study, Fan found that HMGB-1 promoted the activation of neutrophils via the TLR4/NADPH pathway. Furthermore, the TLR4-dependent activation of NADPH oxidase was mediated via the MyD88-(interleukin -1 receptor-associated kinase (IRAK)-(p38 mitogen activated protein kinase (p38MAPK)/Akt signaling pathway [139]. In contrast, another report showed that MyD88 did not apparently influence the ability of HMGB-1 for NET generation [106]. One possible explanation for this divergence might be the difference in models.

In addition to NADPH oxidase-dependent ROS genesis, NADPH oxidase-independent ROS generation also contributed to HMGB-1-induced oxidative stress. Active platelets induced by HMGB-1 expressed and released P-selectin, and then P-selectin/P-selectin glycoprotein ligand 1 (PSGL-1) interaction was crucial in oxidative stress in leukocytes [136]. Moreover, HMGB-1 induced activation of neutrophils via the activation of myeloperoxidase [136]. Moreover, blockade of the autophagic flux reverted HMGB-elicited NET generation, suggesting that HMGB-1 might promote NET formation via the activation of autophagy [106]. Thus, these studies collectively suggested that HMGB-1 induced thrombosis via NET formation.

HMGB-1 and coagulation and fibrinolysis factors

Tissue factor (TF), also known as coagulation factor III, was found to be involved in inflammation-related thrombosis. Also, HMGB-1 could upregulate the expressions of TF in monocytes [107]. This result was also confirmed by Lv et al., demonstrating that HMGB-1 induced expression and activation of TF in endothelial cells in a concentration- and time-dependent manner [140]. Its effect on TF activity induced by HMGB-1 was partly attenuated by three neutralizing antibodies (anti-TLR2, anti-TLR4, and anti-RAGE) and NF- κ B/early growth response 1 (Egr-1) interference, suggesting that TLR2/TLR4/RAGE and NF- κ B/Egr-1 pathway mediated the HMGB-1-induced expression of TF [140]. TF induced blood coagulation cascade via the formation of TF/FVIIa complex, which could be inhibited by TFPI [141]. Thus, TFPI was also detected in this study. It showed that the stimulation of endothelial cells with HMGB-1 did not regulate the expression of TFPI [140], suggesting that TFPI was not involved in the HMGB-1-induced expression of TF. Moreover, HMGB-1 could inhibit the activation of protein C [107], suggesting that the prothrombotic effects of HMGB-1 were mediated by increased coagulation factors and decreased anticoagulant factors.

Besides coagulation factors, the correlation between HMGB-1 and fibrinolysis has been indicated in several studies. Recently, Kules and colleagues found that HMGB-1, an endothelial activation marker, was increased in dogs with babesiosis in parallel with elevated soluble urokinase receptor of plasminogen activator (suPAR), suggesting a possible role of HMGB-1 in fibrinolysis [142]. Besides, HMGB-1 has been shown to induce the formation of plasminogen activator inhibitor-1/tissue plasminogen activator (PAI-1/tPA) complexes, suggesting that HMGB-1 might prevent fibrinolysis via increased PAI-tPA complexes in endothelial cells [143]. Collectively, these data provided evidence that coagulation and fibrinolysis factors were involved in HMGB-1-induced thrombosis.

Nitric oxide (NO), especially from endothelial NO synthase (eNOS), has been considered as an important antithrombotic factor [144]. Several studies demonstrated that HMGB-1 could regulate eNOS/NO axis in endothelial cells. A previous clinical study illustrated that the serum levels of HMGB-1 were correlated negatively with NO levels in patients with obstructive sleep apnea syndrome [145]. Moreover, cholesterol crystal markedly induced the expression of HMGB-1 and downregulated eNOS activity in coronary endothelial cells [146], and treatment with recombinant HMGB-1 directly induced endothelial dysfunction *in vitro* [146]. Recently, Tang et al. found that miR-126 upregulated eNOS activity by targeting HMGB-1, suggesting that HMGB-1 suppressed the activity of eNOS in endothelial cells [147]. Another recent *in vitro* study revealed that uric acid inhibited the expression of eNOS and NO production in endothelial cells and increased extracellular HMGB-1 secretion in parallel with elevated expression of RAGE, demonstrating that HMGB-1 could promote endothelial dysfunction via RAGE [148]. These data suggested the involvement of endothelial cell-derived NO in thrombosis, although the underlying mechanisms are not completely understood.

Therapeutic strategy for HMGB-1

Given the role of HMGB-1 in thrombosis, several studies have focused on drugs targeting HMGB-1 in thrombosis-related diseases. Although no drugs targeting HMGB-1 were used in clinical practice, some anti-HMGB-1 agents were shown to inhibit the expression of HMGB-1 in ischemic animal models, including glycyrrhizin [90, 149, 150], ethyl pyruvate (EP) [151, 152], tanshinone IIA [153, 154], fluvastatin [155], berberine [156], moclobemide [157], minocycline [158], probenecid [159], portulaca [160], apelin-13 [161], hesperidin [162] and bleacein [163]. Glycyrrhizin, a glycoconjugated triterpene present in licorice root, has been shown to exert protective effects in various diseases via suppressing HMGB-1. Glycyrrhizin administration inhibited HMGB-1 secretion in CSF and serum after stroke, suggesting that glycyrrhizin protected brain ischemic injury partly via the inhibition of HMGB-1 [90]. In line with the findings, Huang et al. found that glycyrrhizin alleviated the aggravation of infarct

volume in middle cerebral artery occlusion (MCAO) models [150]. Moreover, the infarct volume and release of HMGB-1 from the cerebral cortex into the serum were attenuated by glycyrrhizin in a focal cerebral ischemia-reperfusion model [149]. EP, a lipid derivative of pyruvate, has been considered as another drug targeting HMGB-1 in experimental models. In MCAO models, EP exerted its neuroprotective effect in the postischemic brain via reduced HMGB-1 release [151]. Furthermore, EP markedly reduced aortic sinus atherosclerosis lesions and inhibited the expression of HMGB-1 in apoE^{-/-} mice [152]. Tanshinone IIA was widely used as an antithrombotic drug in China. Recently, tanshinone IIA was shown to reduce infarct size and expression of HMGB-1 in MCAO models [153]. In addition, tanshinone IIA [154] but not IL-23 [164] dose-dependently improved the cardiac function and decreased HMGB-1 levels in myocardial injury induced by ischemia-reperfusion. A recent report by Filipek et al showed that oleacein could attenuate the destabilization of carotid plaque in hypertensive patients, suggesting its role in the reduction of the risk of ischemic stroke [163]. Several agents have protective effects on ischemic injury via inhibition of HMGB-1. However, whether HMGB-1-mediated thrombosis was involved in these processes remains poorly understood. Interestingly, several antithrombotic drugs have been shown to block HMGB-1. Nuclear magnetic resonance spectroscopic studies have shown the binding of salicylic acid, an active metabolite of aspirin, to HMGB-1 [165, 166]. Recently, Goetzel found that aspirin, a classical antiplatelet drug, significantly blocked thrombin- and collagen-induced HMGB-1 release in active platelets [104]. Besides, two other antiplatelet drugs (clopidogrel and cilostazol) attenuated the expression of HMGB-1 in septic shock [167, 168]. Thus, HMGB-1 might serve as a novel target for antiplatelet drugs in thrombotic diseases.

Besides their anticoagulant effects, low-molecular-weight heparins (LMWHs) decreased the expression of HMGB-1 in inflammation [169-171]. Recently, LMWH was shown to suppress the ability of human neutrophils to generate NETs and HMGB-1 [172], indicating that LMWHs might exert their anticoagulant effect by regulating HMGB-mediated NET formation. Besides, 2-O,3-O-desulfated heparin (ODSH) inhibited HMGB-1/RAGE-mediated airway inflammation [173]. In macrophages, ODSH blocked HMGB-1 secretion via the inhibition of the activity of acetyltransferase p300 [174]. Thus, HMGB-1 might be a new target for heparin during inflammation and thrombosis.

Similar to heparin, thrombomodulin is another potential drug for DIC. It exhibits its anti-inflammatory effect by regulating HMGB-1. Recombinant soluble thrombomodulin ameliorated cerebral ischemic injury via an HMGB-1 inhibitory mechanism in mice with MCAO [82] and rats with CVST [101]. In a rat septic model, recombinant thrombomodulin suppressed thrombus formation and HMGB-1 levels. Moreover, high-dose thrombomodulin tended to increase the bleeding events [175], suggesting that HMGB-1 was a target for the antithrombotic effect of thrombomodulin. As vascular endothelial cells are critical in the antithrombotic system, Bongoni explored the role of thrombomodulin in HMGB-1-induced endothelial cell activation. He demonstrated that transgenic expression of thrombomodulin inhibited the activation of endothelial cells via increased HMGB-1-induced cleavage [143, 176]. These findings indicated that HMGB-1 might serve as a potential target for anticoagulant drugs.

Conclusion

HMGB-1 could be actively or passively released from several cells. Once released into the extracellular space, HMGB-1 activated its down signaling via interaction with its receptors. Elevated HMGB-1 levels were associated with clinical prognosis in thrombosis-related diseases. HMGB-1 was critically involved in thrombosis via regulating platelet activation, NETs, coagulation factors, and fibrinolysis factors. Animal experiments indicated that several drugs targeting HMGB-1 exerted protective effects in thrombosis-related diseases. However, the effects of HMGB-1-targeted agents in thrombosis formation in these diseases need to be further explored.

Disclosure Statement

The authors declare to have no competing interests.

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