

High-mobility group box-1 and its role in angiogenesis

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ABSTRACT

HMGB1 is an architectural chromatin-binding protein that can be released actively by activated cells or passively by dying cells and can serve as a DAMP molecule to drive the pathogenesis of inflammatory and angiogenic diseases. Through TLR4 and RAGE signaling pathways, HMGB1 could regulate vascular growth in vivo and in vitro through diverse mechanisms, including induction of proangiogenic cytokine release and activation of ECs, macrophages, EPCs, and mesoangioblasts, all of which could contribute to vessel formation. Accordingly, HMGB1 plays a significant role in many angiogenesis-related conditions, such as tumors, PDR, wound-healing, and ischemia-induced angiogenesis. In this review, we focus on the regulatory role of HMGB1 in angiogenesis and recent progress in therapeutic strategies targeting HMGB1. *J. Leukoc. Biol.* 95: 563–574; 2014.

Introduction

HMGB1 is a nonhistone DNA-binding protein and serves as a structural component to facilitate the assembly of nucleoprotein complexes in the nucleus [1, 2]. Chromatin-associated HMGB1 plays multiple roles in the regulation of genome replication, recombination, mRNA transcription, and DNA repair [1–3]. As it possesses such essential functions in maintaining genomic stability, HMGB1 is highly conserved in all mammals and is expressed consistently in the nucleus of almost all cell types examined [4]. Besides its functions in nucleus, HMGB1 can also be passively released by injured or necrotic cells or actively secreted by immunologically competent cells [5, 6]. In this way, it serves as a DAMP and mediates various physical or

pathological processes, including inflammatory cytokine release, cell migration, and angiogenesis, mainly through TLR2, TLR4, and RAGE [7–9]. Thus, it has not only been demonstrated as a late mediator of infection but also has been implicated as a putative danger signal involved in the pathogenesis of a variety of noninfectious, inflammatory conditions, including autoimmunity [10], cancer [11], trauma [12], and IR injury [13]. In some of these conditions, angiogenesis, the formation of new blood vessels driven by ECs, EPCs, as well as activated macrophages, is importantly involved in the pathogenic process. Indeed, as a DAMP molecule, HMGB1 gets more and more attention as a proangiogenic factor that could modulate the function of these angiogenesis-related cells. Up-regulation of HMGB1 has been reported in many angiogenesis-related conditions, including cancer [7], cornea neovascularization [14], PDR [15], wound-healing [16], and ischemia-induced angiogenesis [17]. Here, we describe recent progress on how HMGB1 regulates angiogenesis in physiological or pathological status and discuss the therapeutic potential of targeting HMGB1 in angiogenesis-related conditions.

MOLECULAR BIOLOGY OF HMGB1

HMGB1 is a nuclear, 215-aa protein composed of three distinct domains: two tandem DNA-binding domains, termed A box and B box, and a 30-aa-long acidic C-terminal tail. In the nucleus, HMGB1 binds linear DNA without sequence specificity and acts as a structural protein of chromatin and a transcriptional regulator. In the cytosol, HMGB1 regulates autophagy, a self-eating process crucial for cell survival during stress [18, 19]. Cytosolic HMGB1 was also found to be involved in the nucleic acid-sensing system and promotes the DNA- or RNA-induced immune response [20, 21].

Besides its essential roles in nucleus and cytosol, HMGB1 gains more and more attention as a secreted protein capable of inducing cytokine release, macrophage maturation, and EC activation and thus, plays a crucial role in mediating infectious and sterile inflammation (Table 1). The site of proinflammation

Abbreviations: ACS=acute coronary syndrome, AFP= α fetal protein, CAM=chicken embryo chorioallantoic membrane, CDC42=cell division cycle 42, CRC=colorectal cancer, CSCC=cervical squamous cell carcinoma, DAMP=damage-associated molecular pattern, EC=endothelial cell, EPC=endothelial progenitor cell, FISH=fluorescence in situ hybridization, HCC=hepatocellular carcinoma, HMGB1=high-mobility group box-1, IHC=immunohistochemistry, IR=ischemia-reperfusion, Mac-1=macrophage antigen-1, MCAO=middle cerebral artery occlusion, MD-2=myeloid differentiation 2, MI=myocardial infarction, MMP=matrix metalloproteinase, PDR=proliferative diabetic retinopathy, RAGE=receptor for advanced glycation end-product, siRNA=small interfering RNA, TIM-3=T cell Ig mucin-3, TNM=tumor size, node status, metastasis, WB=Western blot

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TABLE 1. Biological Activity and Pathophysiological Effects of HMGB1

Molecular and cellular biology	
Cell-specific expression	Ubiquitous expressed in almost all cell types and actively secreted from multiple cell types, including macrophages, monocytes, NK cells, DCs, ECs, and platelets
Complex with other molecules	LPS, CXCL12, IL-1 β , DNA, nucleosomes, palmitoyl-3-cysteine-serine-lysine-4
Receptors	TLR2, TLR4, TLR9, RAGE, Mac-1, syndecan-3, CXCR4, CXCR12, Tim-3, and CD24-Siglec-10
Target cells	Macrophages/monocytes, DCs, neutrophils, platelets, T lymphocytes, B lymphocytes, epithelial cells, ECs, smooth muscle cells, mesoangioblasts, EPCs, cardiomyocytes, osteoclasts, neurons, astrocytes, microglial cells, tumor cells
Signaling responses to HMGB1	MAPK, NF- κ B, PI3K/Akt, CDC42/Rac
Regulation	Migration, proliferation, differentiation, cytokine release, angiogenesis
Pathological processes	Sepsis, sterile inflammation, autoimmunity, cancer, trauma, ischemia, wound-healing

tory activity of the HMGB1 position is located in the B box, whereas the truncated A box antagonizes this activity and induces immune tolerance [8]. There are two major pathways by which HMGB1 is released into the extracellular milieu; one is a passive process, initiated instantly in an apoptotic or a necrotic cell, and the other is active secretion, induced by signal transduction in activated, immunologically competent cells (Fig. 1). During necrosis, HMGB1 is released as a result of loss of cellular integrity and may be accompanied with other DAMPs, such as hyaluronic acid, uric acid, hepatoma-derived growth factor, ATP, and DNA. For cells undergoing apoptosis, HMGB1 is retained initially in the nucleus and can be passively released during the late apoptosis (secondary necrosis) as a result of increased cell permeability, as well as nucleosomal degradation [7]. The active secretion of HMGB1 by macrophages, monocytes, NK cells, ECs, platelets, and other immunological-competent cells is triggered by apoptotic cell bodies, PAMPs, and inflammatory stimuli, such as IFN- γ , LPS, and IL-1 β . Upon stimulation, HMGB1 translocates from the nucleus to the cytosol and accumulates in specialized intracellular secretory vesicles, which fuse with the cell membrane and release into extracellular space [4, 22–24]. In addition, HMGB1 can be actively secreted by dying cells undergoing pyroptosis and autophagy, as well as by cells stressed by hypoxic conditions, irradiation, and antitumor drugs [25–30].

CYTOKINE ACTIVITY OF HMGB1

As a secreted protein, HMGB1 serves its proinflammatory function through RAGE, TLR2, TLR4, TLR9, Mac-1, CXCR4, CXCR12, TIM-3, and CD24-Siglec-10 (Siglec-G in mice) and activates a series of signaling pathways, including MAPKs, PI3K/Akt, and NF- κ B, all of which play significant roles in inflammation and angiogenesis (Table 1) [8, 31–38]. Importantly, the activity of HMGB1 can be modulated by post-translational modifications, such as oxidation, acetylation, phosphorylation, and methylation [39–43].

RAGE and TLR4

RAGE and TLR4 are the most important and well-known receptors for HMGB1 to mediate proinflammatory and proangiogenic responses. RAGE is the first reported receptor of HMGB1 [31], which is a cell surface, transmembrane, multiligand receptor con-

sisting of three Ig-like regions expressed in ECs, vascular smooth muscle cells, macrophages, monocytes, DCs, and EPCs [44–46]. HMGB1 physically binds to RAGE and activates two major signaling pathways. One is the Rho-family small GTPase CDC42/Rac,

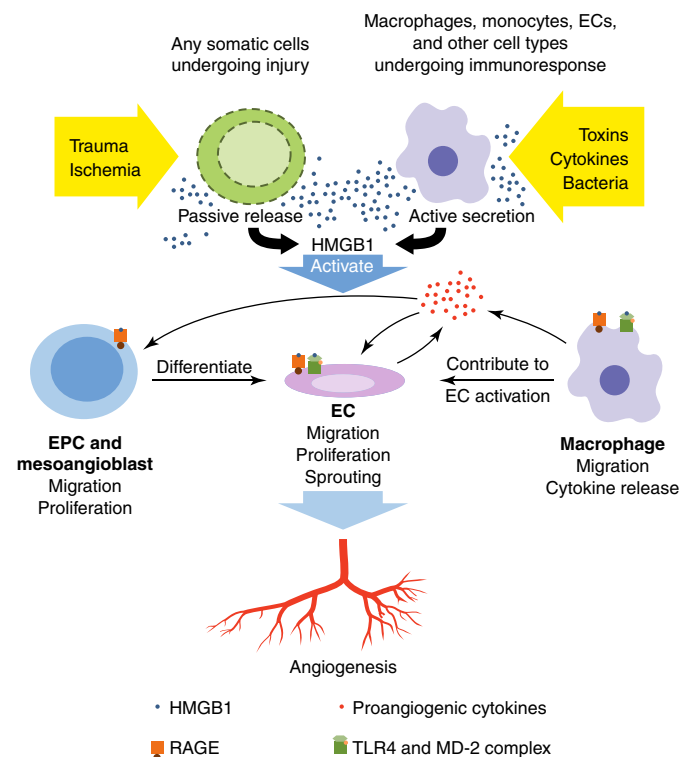


Figure 1. HMGB1 contributes to angiogenesis. HMGB1 could be rapidly released by dying cells or actively secreted by immunocompetent cells in response to inflammatory stimulation. Extracellular HMGB1 binds to RAGE physically or TLR4 in a MD-2-dependent manner and regulates the angiogenic process directly or indirectly in different types of cells. HMGB1 directly contributes to vessel formation through promoting EC proliferation, migration, and sprouting. Besides, HMGB1 could promote the release of proangiogenic cytokines from ECs and macrophages, as well as trigger the activation of macrophages, EPCs, and mesoangioblasts. The macrophage could physically interact with the sprouting vasculature to contribute to the formation of vessel-tube networks. EPCs and mesoangioblasts could differentiate into ECs and incorporate into the newly formed vessel.

which is well known as a regulator of cell remodeling and motility [47, 48]. The other one is diverse MAPKs, including ERK and JNK, which finally lead to activation of NF- κ B [49, 50]. Through these pathways, the HMGB1-RAGE axis modulates cell migration, proliferation, differentiation, and adhesion and up-regulates expression of cell surface receptors, including TLR4 and RAGE (Table 1) [51–55].

TLR4, a typical PRR recognizing LPS, could also recognize HMGB1. The interaction between TLR4 and LPS strictly requires an extracellular adaptor protein MD-2 and a cell surface protein CD14, which transfers LPS to the TLR4/MD-2 complex [56]. Recent studies found that just like LPS, the HMGB1-TLR4 interaction also requires MD-2 and CD14. HMGB1 binds directly to the MD-2 component of the TLR4/MD-2 complex to induce TLR4-dependent cytokine production [40, 57]. The coreceptor CD14 is required for the clustering of the TLR4/MD-2/CD14 complex in the lipid raft, which is crucial for HMGB1-TLR4 signaling [58]. Upon recognition of HMGB1, TLR4 activates IKK- β and IKK- α and leads to the nuclear translocation of activated NF- κ B, leading to the release of various proinflammatory and proangiogenic cytokines, including TNF- α , IL-1, IL-6, IL-8, VEGF, and b-FGF, all of which are involved in macrophages and EC activation and may lead to vessel formation [14, 32, 59].

Redox modification and binding specificity of HMGB1

The cytokine activity of HMGB1 is strictly dependent on the redox state of three conserved cysteine residues at Positions 23, 45, and 106. Initial studies suggested that only the reduced form of Cys 106 is required for triggering inflammation, whereas HMGB1 with an oxidized Cys 106 could only promote immune tolerance rather than trigger inflammation [60]. Furthermore, for TLR4 activation, only with reduced C106 (thiol) and a disulfide bond between C23 and C45 at the same time could HMGB1 bind directly to MD-2 in the TLR4/MD-2 complex [39, 40]. Recently, an all-thiol form was found to be required for the formation of the HMGB1-CXCL12 complex, which is a more potent inducer for CXCR4 activation [36, 61, 62]. As HMGB1 could bind to other putative receptors, such as RAGE, TLR2, and TLR9, further studies are needed to clarify the redox form required for HMGB1 to interact with these receptors.

HMGB1 activities are dictated by accompanying bound molecules

In addition to its well-documented role as an extracellular DAMP, HMGB1 could act in concert with other proinflammatory ligands, including PAMPs, nucleosomes, and cytokines, to augment or modify its activity (Table 1) [29, 63]. For example, extracellular HMGB1-ssDNA complexes could act alone or bind to autoantibodies to enhance cytokine production by DCs and macrophages via TLR9 [33, 64]. In a liver IR injury model, it was found that endogenous DNA and HMGB1, derived from necrotic cells, could mediate inflammatory responses in a coordinate manner through TLR9 [65]. It was reported that HMGB1 could bind to LPS to destabilize catalytically LPS from the membrane and presents LPS monomers to CD14, thus enhancing the production of

TNF- α via TLR4 [66]. In the case of nucleosomes, the HMGB1/nucleosome complex, derived from apoptotic cells, stimulates inflammatory and immunostimulatory responses via TLR2, thus inducing the production of autoantibodies, including anti-DNA antibody and anti-histone antibody, both of which are the hallmarks of systemic lupus erythematosus [67]. Furthermore, the complex of HMGB1 and IL-1 β could induce a more potent proinflammatory response through IL-1R [68]. More recently, it was found that HMGB1 and CXCL12 could form a heterocomplex, which activates CXCR4 rather than other HMGB1 receptors. Compared with CXCL12 alone, the HMGB1/CXCL12 complex is more potent to induce the migration of inflammatory cells [36, 38, 61, 62].

HMGB1 signaling may sustain positive-feedback loops

To sustain its role in inflammation and the angiogenesis cascade, HMGB1 itself promotes a positive-feedback loop to amplify its signaling through autocrine and paracrine manners. HMGB1 stimulates monocytes, macrophages, and ECs to release TNF- α [40, 69, 70], which in turn, induces more HMGB1 release from macrophages and ECs [23, 71, 72]. Meanwhile, HMGB1 could induce the expression of proangiogenic cytokines and their receptors, including VEGFA, VEGFR1, and VEGFR2 [72]. Furthermore, a positive-feedback loop exists between HMGB1 and its receptors. In vitro, endogenous or exogenous HMGB1 induces up-regulation of RAGE and TLR4 in ECs [72]. In turn, interfering TLR4 and RAGE with siRNA or antibodies against TLR4 or RAGE significantly reduced HMGB1 mRNA levels in activated ECs [72]. Overexpression of TLR4 in hypoxic hepatocytes leads to up-regulation of endogenous HMGB1 through increased ROS production. Knockout of TLR4 leads to decreased HMGB1 production in hypoxic hepatocytes and in a mouse model of liver IR injury [73, 74].

HMGB1 CONTRIBUTES TO ANGIOGENESIS

Pathological or physiological angiogenesis, unlike vasculogenesis or arteriogenesis, is the process of developing new blood vessels from pre-existing ones in normal or abnormal tissues to promote organ restoration and support the growth of proliferative tissue. Dysregulation of angiogenesis contributes to a variety of pathological conditions, including tumor growth and metastasis, wound-healing, and ischemia, under which, cell death and chronic inflammation lead to significant release of HMGB1. Accordingly, HMGB1 was shown to be up-regulated in the serum or tissue specimen of patients with cancer [72] and proliferative retina disease [15] and patients undergoing wound-healing [75] and ischemic injury (see Table 2) [76]. Under these conditions, as a DAMP molecule, HMGB1 not only shows its proinflammatory activity but also acts as a potent proangiogenic factor. Through RAGE and TLR4, HMGB1 mediates EC activation, angiogenic cytokine release, and other proangiogenic cells, including macrophages, mesoangioblasts, and EPC activation, thus to promote angiogenesis directly and indirectly (Fig. 1).

HMGB1 promotes angiogenesis directly

In recent years, more and more studies indicated that HMGB1 could promote EC functions directly in vitro and in vivo. In 2005, Schluter et al. [77] first reported that HMGB1 could induce EC sprouting in a collagen gel. As the HMGB1-RAGE axis could activate CDC42/Rac and MAPK-NF- κ B signalings, both of which are involved in EC activation and angiogenesis [78–81], HMGB1 is speculated to induce the angiogenic response of ECs via RAGE. Indeed, Mitola and colleagues [78] found that rHMGB1 promotes EC mitosis and sprouting in a three-dimensional fibrin gel. HMGB1 promotes EC migration in a Boyden chamber assay and a monolayer wound-closure assay. Inhibition of RAGE by neutralizing antibodies impairs the HMGB1-mediated mitogenic response and membrane-ruffle formation at the edge of the wounded monolayer in ECs. These results indicate that HMGB1 mediates RAGE-dependent EC proliferation and migration, both of which are important processes for angiogenesis [78]. In vivo, HMGB1 was shown to promote angiogenesis on the top of CAM. RAGE is expressed on the surface of ECs, as shown by immunostaining of CAM sections. Blockade of RAGE with anti-RAGE antibodies inhibits the HMGB1-mediated angiogenesis in the CAM model, indicating that RAGE is important for HMGB1-mediated angiogenesis [78]. Furthermore, De Mori et al. [82] found that HMGB1 stimulates EC sprouting via ERK and JNK signaling pathways. These results strongly suggest that HMGB1 could act as a proangiogenic cytokine.

Not only does exogenous HMGB1 have the effect to promote EC activation, but also, endogenous HMGB1, released by stressed ECs, could exert its proangiogenic function. Initial studies indicated that stimulation of ECs with LPS or TNF- α led to release of HMGB1 [71]. Lately, Sachdev et al. [25] found that the HMGB1 released by ECs could also be regulated by hypoxia or starvation-induced autophagy. The endogenous HMGB1 released by stressed ECs could also induce EC sprouting. Blockade of endogenous HMGB1 significantly impaired tube formation. Besides, inhibition of autophagy abrogates the tube-formation process of stressed ECs, whereas enhance autophagy with rapamycin promotes HMGB1 translocation and reinforces EC sprouting [25]. Recently, van Beijnum et al. [72] extended these findings and found that the expression of HMGB1 parallels EC activation and that the exposure of ECs to exogenous HMGB1 could increase the expression of HMGB1 receptors, including TLR4 and RAGE, as well as induce the production of endogenous HMGB1. The increased expression of HMGB1, as well as its receptors, in turn, promotes EC sprouting and migration [72]. Thus, the function of HMGB1 in ECs is involved in an autocrine loop to drive angiogenesis.

HMGB1 promotes angiogenesis indirectly

HMGB1 could also trigger angiogenesis in multiple ways, including promoting release of proangiogenic cytokines and activating other cells that could contribute to vessel formation. Several studies found that HMGB1 could promote the production of proangiogenic cytokines, including VEGF, TNF- α , and IL-8 from ECs and macrophages [72, 83]. Furthermore, treat-

ment of ECs with HMGB1 induces elevated expression of VEGFR1 and VEGFR2 and increased activity of MMPs and integrins, leading to stronger proangiogenic signaling and enhanced interaction between ECs and extracellular matrix, both of which are important processes during angiogenesis [72]. In vivo, in a hindlimb-ischemia model, Biscetti et al. [16] found that exogenous HMGB1 could promote the secretion of VEGF, leading to a reinforced angiogenesis response, and accelerate tissue regeneration.

Besides its effect on inducing cytokine release, HMGB1 could also stimulate the recruitment and activation of macrophages, which could release proangiogenic cytokines, as well as physically interact with the sprouting vasculature to contribute to the formation of vessel-tube networks [84, 85]. Although initial studies indicated that RAGE is the only receptor mediating HMGB1-induced angiogenesis, using an alkali-induced cornea neovascularization model, Lin et al. [14] first found that TLR4 is also capable of mediating HMGB1-induced angiogenesis. In this model, exogenous HMGB1 could promote macrophage recruitment and exacerbate neovascularization in a TLR4-dependent manner, and administration of an HMGB1 antagonist, truncated HMGB1 A box, could potentially reduce macrophage infiltration and alleviate the severity of cornea neovascularization (see Table 5) [14].

Other interesting target cells of HMGB1 during angiogenesis are EPCs and mesoangioblasts. EPCs, derived from the bone marrow, could be activated and recruited to the sites of neoangiogenesis, where they differentiate to ECs and incorporate into the newly formed vessel [86]. Mesoangioblasts, which originally come from embryonic dorsal aorta, could differentiate into ECs, as well as different types of solid mesoderm [87, 88]. HMGB1 could promote the proliferation and migration of these two cell types toward the site of tissue injury and tumors to promote angiogenesis, possibly through RAGE signaling pathways [46, 89–91]. Interestingly, RAGE is sufficient but not necessary for HMGB1-induced mesoangioblasts homing, indicating possible coexistence of other pathways [89]. Different than mesoangioblasts, Chavakis et al. [46] found that HMGB1-induced EPC migration is dependent on RAGE rather than other HMGB1 receptors. With the ability to enhance integrin affinity and mediate integrin polarization, as well as activate the small GTPase Ras-related protein 1, HMGB1 potentially induces EPC adhesion and migration. Moreover, prestimulate EPCs with HMGB1 promote the adhesion and homing of EPCs to the tumor tissue in vivo [46]. Later on, Hayakawa et al. [90, 91] also found that HMGB1 could promote EPC proliferation, migration, and sprouting through RAGE and ERK signaling pathways in vitro, and by using antibodies targeting RAGE in experimental models of transient focal cerebral ischemia and focal demyelination, HMGB1 was suggested to attract EPCs to promote recovery after neural injury in a RAGE-dependent manner in vivo. Recently, Schiraldi et al. [36, 61] found that the heterocomplex of HMGB1/CXCL12 is more potent than CXCL12 alone to induce the migration of inflammatory cells through CXCR4 [38]. As CXCL12 has already been strongly suggested to play a pivotal role to drive angiogenesis through activating CXCR4⁺ proangiogenic cells, such as EPCs [92–95], HMGB1 may exert its proangiogenic role by

recruiting proangiogenic cells, including EPCs and macrophages through the CXCL12-CXCR4 axis.

DETECTION OF HMGB1 IN ANGIOGENESIS-RELATED CONDITIONS

Quantitation of the HMGB1 level in tissue samples or fluids is essential to investigate its role in angiogenesis-related conditions, as well as other pathophysiological processes. WB is commonly used to detect the expression of HMGB1 in cultured cells, tissue samples, and serum from patients and experimental models [72, 75, 96–98]. ELISA is most commonly used in the measurement of HMGB1 levels in biological fluids, such as serum from cancer or ACS patients, vitreous fluid from PDR patients, and supernatant of cell cultures and lysates [15, 99]. IHC and immunofluorescence are used to detect the expression level as well as the localization (intranuclear, cytoplasmic, or extracellular) of HMGB1 from tissue samples in experimental models or cancer specimen from patients (see Tables 2 and 3) [96, 100, 101]. Other quantitative methods, such as real-time PCR and FISH, can also be used for detection of the HMGB1 level in tissue samples [102–104]. How and where HMGB1 levels are detected in clinical studies and animal models are summarized (see Tables 2 and 3).

HMGB1 AND ANGIOGENESIS-RELATED CONDITIONS

HMGB1 and cancer

Cancer is a typical pathological angiogenesis-associated disease, as neovascularization is an essential process to provide enough nutrition to support tumor formation and growth. Accompanied with the rapid growth of tumor tissue, the microvessel density inside the tumor is reduced, thus resulting in chronic

hypoxia and cell necrosis, which induce macrophage infiltration and blood-vessel formation [105]. Both macrophages activated by hypoxic condition and necrotic cells, as a result of ischemia, serve to release a considerable amount of HMGB1. Also, the hypoxic condition helps to keep the reduced state of HMGB1, thus maintaining its proinflammatory and proangiogenesis activity [77].

As reviewed by Tang et al. [11], the elevated HMGB1 level is correlated with many hallmarks of cancer, including unlimited replicative potential, apoptosis, self-sufficiency in growth signals, insensitivity to inhibitors of growth, inflammation, tissue invasion, and metastasis. Here, we emphasize the regulatory effect of HMGB1 on angiogenesis in tumor growth. Accordingly, recent studies show that the HMGB1 level does increase in the serum or tissues of patients suffering various tumors, including CRC [106], HCC [96, 107], gastric cancer [99, 108], breast cancer [109], lung cancer [97, 110], prostate cancer [102], and cervical cancer [100], as detected by ELISA, WB, and RT-PCR (see Table 2). In addition, HMGB1 is highly expressed in metastatic foci or LNs in advanced CRC, as detected by ELISA and IHC [111, 112]. The expression level of HMGB1 also positively correlates with the tumor invasion, metastasis, and TNM stage of cancer patients (Table 2) [96, 97, 99, 101, 107, 113]. As angiogenesis is an essential process for energy and oxygen supply in expanding and metastasizing tumor tissue, the abundant source of active HMGB1 is indispensable for the angiogenic process during tumor progression. Accordingly, initial studies indicated that blockade of the HMGB1-RAGE axis inhibits the growth and metastasis of implanted and spontaneous tumors in susceptible mice [116].

Indeed, accumulating evidences have suggested the involvement of HMGB1 in tumor-vessel formation. van Beijnum et al. [72, 83] found that the level of HMGB1 is elevated in endothelial cells derived from colorectal tumor tissue but not nor-

TABLE 2. HMGB1 Levels in Angiogenesis-Related Diseases of Patients

Diseases	Detection methods	Clinical samples	HMGB1 level (references)
Lung cancer	WB	Serum	Increased and correlates with tumor metastasis and TNM stage ([97])
Prostate cancer	RT-PCR	Prostate tissue	Increased ([102])
	FISH	Prostate tissue	Higher in metastatic cases than nonmetastatic ones ([103])
CSCC	RT-PCR, IHC, WB	Cervical tissue	Increased and correlates with tumor metastasis and stage ([104])
	IHC, ELISA	Cervical tissue and serum	Higher in recurrent cases than nonrecurrent ones and controls and inversely correlates with survival ([100])
Gastric cancer	ELISA	Serum	Correlates with tumor metastasis ([99])
HCC	RT-PCR, WB, IHC	Liver tissue	Increased and correlates with tumor stage and vascular invasion ([96])
	WB	Serum	Increased and correlates with AFP levels and tumor stage ([107])
CRC	IHC	Colon tissue	Overexpressed in most cases and associates with tumor invasion, metastasis, and Duke's stage ([101])
PDR	ELISA	Vitreous sample	Increased and correlates with the severity of neovascularization ([15])
ACS	ELISA	Serum	Higher in STEMI patients who died than surviving patients and controls ([114])
	WB	Serum	Increased ([115])
Cerebral ischemia	WB	Serum	Increased ([115])

STEMI, ST-segment elevation MI.

mal colon tissue, as detected by RT-PCR and flow cytometry. Antibodies targeting HMGB1 inhibit tumor angiogenesis in a model of CAM inoculated with human tumor cell spheroids [72]. Moreover, administration of exogenous HMGB1 to ECs in vitro leads to up-regulation of HMGB1 receptors and HMGB1 itself; enhancement of the expression of a series of proteins involved in proangiogenic signaling cascades, including VEGFA, VEGFR1, and VEGFR2; as well as activation of MMPs and integrins. All of these molecules are crucial for tumor angiogenesis [72]. Thus, HMGB1 is indicated to be involved in an autocrine mechanism to enforce tumor angiogenesis. Correspondently, Sasahira and coworkers [117] found that in human oral squamous cell carcinoma cell lines, HMGB1 promotes VEGF secretion in a RAGE-dependent manner. In a xenograft glioma model, prestimulation of EPCs with HMGB1 significantly increases the ability of EPC homing to tumor tissue [46]. In a malignant mesothelioma xenograft model and a colitis-associated cancer model, blockade of HMGB1 significantly inhibited tumor growth [118, 119]. Moreover, In a CRC cell liver metastasis model, HMGB1-neutralizing antibody inhibits tumor metastasis [111]. Hence, these studies suggested that HMGB1 contributes to tumor angiogenesis and might be a promising therapeutic target in cancer therapy.

HMGB1 and PDR

In diabetic patients, hypoxia-induced pathological growth of new blood vessels is one of the hallmarks in the development of PDR. Pachydaki and coworkers [120] first reported that the level of HMGB1 and its receptor RAGE are increased in the vitreous cavity and epiretinal membranes of PDR patients. Later on, Abu El-Asrar et al. [15, 121–123] found an elevated level of HMGB1 in the retina of diabetic mice, indicating that HMGB1 may be the promoter of the angiogenesis process in PDR. Studies from the same group further suggested that the levels of HMGB1 in vitreous of PDR patients are positively correlated with the severity of the neovascularization and the expression levels of VEGF and soluble vascular endothelial-cadherin [15, 121–123]. These facts strongly suggest that HMGB1

may play a critical role in PDR, indicating that HMGB1 might be a promising target for treatment and prevention of PDR.

HMGB1 and wound-healing

Wound-healing of the skin represents a dynamic process involving re-epithelialization, fibroplasias, and neovascularization [124]. Accompanied with cell death and sterile or infectious inflammation during tissue injury, HMGB1 is massively released and may act as a proangiogenic cytokine to promote wound closure. Indeed, in 2008, Straino et al. [75] uncovered the angiogenic role of HMGB1 in wound-healing. They found that the expression of HMGB1 in diabetic human and mouse skin wounds is reduced, as detected by WB (Table 3), which indicates that the delayed wound-healing in diabetic patients is probably associated with low levels of HMGB1. Topical application of HMGB1 increases vessel density and accelerates skin wound-healing in diabetic mice but not normoglycemic mice (Table 4). The antagonizing of endogenous HMGB1 by the truncated A box significantly impairs wound-healing in normoglycemic mice but does not affect diabetic mice. These results suggest that both exogenous and endogenous HMGB1 is sufficient for optimal angiogenesis and wound closure [75]. In the mouse model of embryonic wound-healing, a high level of HMGB1 also positively correlates with increased vessel formation and macrophage infiltration [127]. Besides, HMGB1 also affects fibroblasts activation and lymphangiogenesis, indicating the complexity of wound-healing and the diverse physiological effects of HMGB1. Thus, accurate modulation of HMGB1 activity in wound-healing is necessary.

HMGB1 and ischemia-induced angiogenesis

In ischemic injury, blood-flow deficits result in deprivation of oxygen and nutrition. In response to the lethal threats for cell viability, proangiogenic factors are up-regulated, and proangiogenic cells are recruited to induce neovascularization, an essential process for tissue restoration after ischemic injury [129, 130]. Notably, this “ischemia-induced angiogenesis” mainly occurred in ischemic limb, heart, and brain [130–133]. As a typical DAMP molecule, HMGB1 is released as a result of cell

TABLE 3. HMGB1 Levels in Experimental Models of Angiogenesis-Related Conditions

Experimental models	Detection methods	Samples	HMGB1 level (references)
Skin wound	WB	Wounded skin tissue	Decreased in diabetes mice ([75])
Corneal neovascularization	RT-PCR	Cornea	Increased in alkali-induced corneal neovascularization mouse model ([14])
Hindlimb ischemia	WB, IHC, and IF	Skeletal muscle	Increased after ischemia ([82]) Decreased in diabetic mice ([16])
Myocardial infarction	WB, RT-PCR, IHC	Myocardial	Increased ([125])
	ELISA	Serum	Increased ([125])
MCAO	WB	Cerebrospinal fluid	Increased ([98, 126])
	RT-PCR	Infarct regions	Increased ([98])
	IHC	Infarct regions	Translocates into cytoplasm of neurons immediately after MCAO/reperfusion; increased in reactive microglia, astrocytes, and blood vessel-associated cells, 2 days later ([98])

IF, Immunofluorescence.

TABLE 4. Use of HMGB1 to Promote Angiogenesis in Tissue Regeneration

Experimental model	Administration routes	Effects (references)
Skin wound in diabetic mice	Topical application	Increases vessel density and accelerates wound-healing ([75])
Skin wound in fetal mice	s.c. Injection	Increases vessel density and macrophage infiltration ([127])
Hindlimb ischemia	Intramuscular injection	Promotes perfusion recovery and increases EC density ([16, 25, 82])
Left anterior-descending coronary artery ligation	Use of HMGB1 transgenic mice	Enhances angiogenesis and restores cardiac function ([128])

death and hypoxia during the ischemic process [25, 73, 90]. Accumulating evidences have shown that HMGB1 also plays a significant role in ischemia-induced angiogenesis and contributes to tissue recovery [16, 90, 128].

HMGB1 and ischemia-induced angiogenesis in limbs. In a hindlimb ischemia mouse model, the level of HMGB1 starts to increase at Day 3 and reaches the apex at Day 7, and the elevated HMGB1 contributes to myoblast and EC functions to promote skeletal muscle regeneration (Table 3) [82]. Accordingly, as ischemia is always accompanied with hypoxia and depletion of nutrition, Sachdev et al. [25] found that autophagy, initiated by hypoxia and serum depletion, leads to significant release of HMGB1, which promotes the angiogenic behavior of ECs. In an in vivo mouse model of hindlimb ischemia, exogenous HMGB1 could promote perfusion recovery and increase EC density [25]. Blockade of HMGB1 activity or TLR4 signaling leads to exacerbated muscle necrosis [17]. Surprisingly, compared with control C57B6 mice, TLR4- and RAGE-incompetent mice show more blood flow in ischemic limbs, which suggests that the proangiogenic effect of HMGB1 might be regulated by many complicated factors in vivo [17]. In diabetic mice, the HMGB1 level in ischemic hindlimbs is lower than normoglycemic mice, leading to an attenuated perfusion recovery after ischemic injury. Exogenous HMGB1 administration increases vessel density and promotes recovery of blood flow in the ischemic hindlimbs of diabetic mice by up-regulating the expression of VEGF [16]. Besides its effect on ECs, HMGB1 also promotes the homing of EPCs to ischemic muscles, which may also contribute to angiogenesis and tissue recovery [46]. Therefore, HMGB1 is indicated to act as a proangiogenic factor in ischemic limb injury, and the administration of HMGB1 may be a novel, therapeutic agent to protect ischemic muscles.

HMGB1 and ischemia-induced angiogenesis in neural tissue. In ischemic stroke patients, the serum level of HMGB1 is elevated as the result of neuronal injury up to 14 days after the ischemic events, as detected by ELISA (Table 2) [115, 134]. In experimental models, passively released HMGB1 is increased 1–2 h after MCAO and starts to decrease after 3 h (Table 3) [98, 126]. Two days later, the expression of HMGB1 in the brain starts to increase in activated microglia, astrocytes, and microvascular structures and remained elevated for several days [98].

In the early phase after in ischemic neural injury, HMGB1 could trigger inflammatory cascade through RAGE and TLR4

and promote brain edema and infarction after ischemic injury [59, 76]. Thus, antagonizing HMGB1 or its receptors shows protective effects on neural injury (Table 5) [135–142]. In the late phase after ischemic neural injury, optimal vascular repair is a restorative process for neural tissue recovery. In contrast to the acute phase after neural ischemia, Hayakawa et al. [90] found that HMGB1 promotes neurovascular remodeling in the late phase by activating EPCs. In vivo, HMGB1 is up-regulated in reactive astrocytes, which leads to increased EPC accumulation in the peri-infarct cortex from Day 3 to Day 14 in the experimental mice model of MCAO. Administration of HMGB1 siRNA leads to a significant reduction in EPC accumulation [90]. These facts indicate that reactive astrocytes that produced HMGB1 could promote neurovascular repair in the late phase of neural ischemic injury.

HMGB1 and ischemia-induced angiogenesis in the heart. In clinical studies of ACS, the serum HMGB1 level could be detected by ELISA or WB. Compared with the control group, the HMGB1 level in patients suffering from ACS is increased significantly (Table 2) [115]. In rodent models of MI induced by left anterior descending coronary artery ligation, the expression of HMGB1 in the infarcted area is increased on Day 3, reaches the apex on Day 7, and sustains up to Day 14, as detected by WB [125]. Four weeks after MI, mice overexpressing HMGB1 exhibit enhanced vessel formation, improved cardiac function, and increased survival rate compared with that of WT mice [128]. Hence, HMGB1-mediated angiogenesis may play a protective role in cardiac function restoration after MI. Besides, HMGB1 blockade impairs the postinfarction healing process, leading to worsening of left-ventricle remodeling [125]. However, if the ischemic myocardium suffers from a following reperfusion injury, then the elevated serum HMGB1 level mediates recruitment of the neutrophil and promotes the expression of a series of proinflammatory cytokines to promote inflammatory injury, which leads to enhanced myocardial injury [143–149].

CLINICAL RESEARCH ON HMGB1 IN ANGIOGENESIS-RELATED CONDITIONS

Table 2 summarized the clinical research on HMGB1 and its potential role in angiogenesis-related conditions. Clinical research has found that the serum HMGB1 level is up-regulated in patients with lung cancer, CSCC, gastric cancer, and HCC and positively correlates with tumor progression and poor

TABLE 5. Use of HMGB1 Antagonist in Angiogenesis-Related Conditions

HMGB1 antagonist	Mechanism	Disease models	Routes of administration	Effects (references)
Truncated HMGB1 A box	Competitive, antagonizes HMGB1	Corneal neovascularization	Topical use	Alleviates cornea neovascularization ([14])
		MCAO	i.p. Injection	Ameliorates neural ischemic damage ([76])
HMGB1 neutralizing antibody	Neutralizes the activity of HMGB1	Malignant mesothelioma xenografts	i.p. Injection	Reduces tumor growth ([82])
		Colitis-associated cancer model	i.p. Injection	Decreases tumor incidence and size ([119])
		CRC cells liver metastasis model	i.p. Injection	Inhibits tumor metastasis ([118])
		MCAO	i.p. or i.v. Injection	Ameliorates neural ischemic injury ([111])
		Hindlimb ischemia	i.p. Injection	Leads to muscle necrosis ([17])
		MI	s.c. Injection	Aggravates MI ([125])
Glycyrrhizic acid	Directly inhibits the activity of HMGB1	MCAO	i.v. Injection	Ameliorates neural ischemic injury ([125])
siRNA	Specific knockdown of HMGB1 mRNA	MCAO	Intranasal delivery	Ameliorates neural ischemic injury ([135])
		Focal cerebral ischemia	Intracerebroventricular injection	Exacerbates neurologic outcomes ([137])
HMGB1-binding heptamer peptide	Directly binds with HMGB1	MCAO	Intranasal delivery	Ameliorates neuronal ischemic injury ([136])

prognosis (Table 2) [97, 99, 100, 104, 107]. HMGB1 is a more specific and sensitive marker than carcinoembryonic antigen in diagnosing early gastric cancer [99]. HMGB1 is positively correlated with AFP level in HCC patients [107]. The HMGB1 level (detected by RT-PCR, WB, or IHC) in CRC, prostate cancer, CSCC, and HCC samples is higher than normal tissues or tissues around tumors [96, 101–104] (Table 2). In vitreous samples of PDR patients, the HMGB1 level is significantly higher than control and is correlated with the severity of the neovascularization [15, 121–123] (Table 2). These results indicated great potential of HMGB1 as a diagnosis and prognostic marker in patients with cancer or PDR, in which pathological angiogenesis acts as a key factor.

In myocardial and cerebral ischemia, serum HMGB1 expression is increased, as detected by ELISA [114, 115] (Table 2). As mentioned above, HMGB1 is a double-edged sword, which could promote inflammation and angiogenesis in myocardial and cerebral ischemia. In myocardial ischemia patients, the levels of HMGB1 are related to infarct size, residual ejection fraction, and mortality, indicating the potential of HMGB1 as a prognostic biomarker [114, 150].

POTENTIAL THERAPIES TARGETING HMGB1 IN ANGIOGENESIS-RELATED CONDITIONS

Given the important role of HMGB1 in inflammation and angiogenesis, in response to tissue injury or tumor, HMGB1-targeted therapies were developed in many experimental models,

although these therapeutic effects still have not been examined in patients. Overexpression of HMGB1 or administration of exogenous HMGB1 significantly increases vessel density and promotes recovery in skin-wound, ischemic hindlimb, or myocardium (Table 4) [16, 25, 75, 82, 127, 128], whereas inhibition of HMGB1 with the HMGB1-neutralizing antibody, HMGB1 truncated A box, glycyrrhizic acid, HMGB1 siRNA, and HMGB1-binding heptamer peptide could reverse its proinflammatory or proangiogenic effects (Table 5). Blockade of HMGB1 leads to reduced pathological angiogenesis and inhibits tumor growth and metastasis (Table 5) [111, 118, 119]. In the settings of tissue injuries, such as hindlimb, myocardium ischemia, and skin-wound, neutralizing the activities of HMGB1 leads to decreased vessel density and impaired tissue recovery (Table 5) [17, 82, 125]. In brain ischemic injury, blockade of HMGB1 alleviates inflammation in the acute phase and inhibits neural vascular remodeling in the late phase (Table 5) [76, 90, 135–137, 141, 151, 152]. Future studies are necessary to optimize HMGB1 activity in different angiogenesis-related conditions and to translate the results of animal studies into clinical applications.

CONCLUDING REMARKS

HMGB1 is a ubiquitous nuclear protein in almost every cell type and acts as a prototype alarmin to mediate immune response and angiogenesis when released extracellularly. To promote angiogenesis, HMGB1 signals through TLR4 and RAGE and exerts multiple proangiogenic effects. It could act as a

proangiogenic cytokine and activate ECs, thus promoting vessel formation directly. HMGB1 could also promote the secretion of other proangiogenic cytokines from ECs and macrophages, as well as activate macrophages and progenitor cells, including EPCs and mesoangioblasts, all of which participate in the process of vessel formation. Besides, HMGB1 could sustain a proangiogenic regulatory feedback loop to reinforce its effect on angiogenesis. The participation of HMGB1 has been implicated in many angiogenesis-related conditions, including cancer, cornea neovascularization, PDR, wound-healing, and ischemic injury, and the use of HMGB1 in experimental models has opened the door on the future therapy of angiogenesis-related conditions.

Although much has already been learned about HMGB1 in angiogenesis, the molecular mechanism by which HMGB1 regulates angiogenesis is far from complete. For example, the receptors and signaling cascades mediating the angiogenic effect of HMGB1 in ECs and EPCs need to be characterized further. Furthermore, it is unclear whether the post-translational modification, such as redox state of HMGB1, could affect its angiogenic activity. Also, it is still unknown whether the angiogenic activity of HMGB1 could be modulated by binding molecules, such as LPS, CXCL12, and IL-1 β . Importantly, the impact of HMGB1 on angiogenesis-related diseases also needs to be determined precisely, considering its synergistic functions. The answers to these and other questions will further extend our understanding of HMGB1 and its role in the orchestration of angiogenic and immunological activity in pathological conditions.

AUTHORSHIP

S.Y. and L.X. wrote the draft of the manuscript. T.Y. and F.W. supervised this project and contributed to the revision and final edition of this manuscript. All authors read and approved the final manuscript.

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DISCLOSURES

The authors declare no conflict of interest.

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