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Expert Opinion

Non-histone nuclear factor HMGB1 as a therapeutic target in colorectal cancer

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Introduction: High-motility group box (HMGB)-1 is the focus of recent cancer research. HMGB1 plays a critical role in cancer development, progression, and metastasis by activation of cancer cells, enhancement of tumor angiogenesis, and suppression of host anti-cancer immunity. HMGB1 is a relevant target for cancer treatment.

Areas covered: This review aims to overview the biological feature and diverses role in cancer of HMGB1. HMGB1 is a non-histone chromosomal protein, a secretory protein binding to the receptor for advanced glycation end products in cancer cells and monocyte-lineage immune cells, and a DNA presenting chaperon for toll-like receptors. HMGB1 enhances proliferation, motility, invasion and survival of cancer cells. In contrast, HMGB1 induces apoptosis in monocyte-lineage immune cells and inhibits tumor-infiltrating macrophages and dendritic cells, lymph node sinus macrophages and liver Kupffer cells to attenuate anti-cancer immune responses and anti-metastatic organ defense. Then the novel techniques for inhibiting HMGB1 are reviewed.

Expert opinion: Various techniques targeting HMGB1 are subjected to trial. HMGB1 targeting is a potential therapeutic techniqueagainst cancer development, progression, and especially metastasis. Technical breakthroughs in application of HMGB1 targeting to human diseases are now urgently required.

Keywords: AGE, colorectal cancer, HMGB1, macrophage, metastasis, RAGE

Expert Opin. Ther. Targets [Early Online]

1. Introduction

High motility group box (HMGB)-1 is a multifunctional protein possessing diverse biological activities in normal cells (**Tables 1** and 2). The roles of HMGB1 in cancer are also diverse and can be divided into two categories: its direct effect on cancer cells, and its effect on host immunity. HMGB1 provides pro-tumoral and anti-immune effects in cells expressing the receptor for advanced end glycation products (RAGE). Both cancer cells and monocyte-lineage cells express RAGE; however, the effect is completely different between the two cells. Essentially, HMGB1 accelerates the metastasis of cancer cells, which is the most common cause of cancer death. In this review article, we describe the roles of HMGB1 in cancer and immunity. The significant roles of HMGB1 in cancer suggest that HMGB1 is an excellent molecular target for cancer treatment, especially anti-metastatic therapeutics.

2. HMGB1 in cancer

2.1 HMGB1

HMGB1 is a product of the *HMGB1* gene, which encodes a non-histone chromosomal structural protein [1-3]. HMGB1 was isolated as a cytosolic 30-kDa

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Article highlights.

- HMGB1 is a multi-located protein with diverse functions.
- Nuclear HMGB1 is associated with transcription, replication, recombination, DNA repair and genomic stability.
- Cytoplasmic HMGB1 is associated with cell motility and autophagy.
- Secreted HMGB1 provides cancer cell activation, progression of severe inflammation, apoptosis of monocyte-lineage cells and innate immune system cells.
- HMGB1 targeting will suppress tumor progression and inflammatory exacerbation.
- Technical breakthroughs in application of HMGB1 targeting to human diseases are now urgently required.

This box summarizes key points contained in the article.

heparin-binding protein from growing brain tissue [4,5] and is associated with neurite outgrowth [2,3].

Diverse functions of HMGB1 are carried out in the nucleus, cytoplasm and extracellular milieu. As a nuclear protein, HMGB1 binds to non-B type DNA participating in multiple processes such as transcription, replication, recombination, DNA repair and genomic stability [6]. The role of HMGB1 in DNA repair provides drug resistance to platinum derivatives in cancer cells [7].

In the cytoplasm, HMGB1 is associated with cell motility as observed in outgrowing neurites. At the leading edge of the motile cell, HMGB1 accelerates formation of filopodia, adhesion, and detachment from the extracellular matrix, as well as actin-polymer formation [2]. The mechanism of HMGB1-dependent cell migration in cancer cells is considered to be similar to that of outgrowing neurites. HMGB1 is expressed in immature cells and malignant cells at high levels and it plays a major role in controlling cell migration activity [8]. HMGB1 plays an anti-apoptotic role in colon cancer by enhancing the expression of cellular inhibitor of apoptosis-2, which is a target gene of activated NF-κB, and interfering with apoptosomal caspase-9 activation [9]. Recently, endogenous HMGB1 was revealed to activate an autophagy signal, which promotes cell survival [10].

HMGB1 is secreted into the extracellular milieu as a cytokine. HMGB1 expression and secretion is upregulated in response to stimulation of cells by proinflammatory cytokines, endotoxin and oxidative stresses in macrophages [11-14]. Cancer cells also overexpress and secrete HMGB1 by stimulation of growth factors, cytokines and cellular stresses involving advanced glycation end products (AGE) and deoxycholic acid [15-18]. Secreted HMGB1 activates RAGE as a ligand to induce cell growth, motility, invasion and angiogenesis as described below. In the innate immune system, HMGB1 is the effective DNA sensor presenting the DNA to toll-like receptor (TLR) [19].

2.2 Receptor

The HMGB1 receptor, RAGE was purified from bovine lung endothelial extract as the receptor of AGE [20]. RAGE, which is a member of invasion-related genes, is a cell surface receptor belonging to the immunoglobulin superfamily [21-24]. Several reports on the relationship between RAGE expression and cancer have been published to date. RAGE is closely associated with cell growth, cell invasion through MAPK activation, and MMP-2/-9 expression in glioma cells [24]. RAGE upregulation is found in colon and oral carcinogenesis in rodents [18,25].

In clinical studies, since RAGE is a cell-surface receptor, the intracellular localization of RAGE is associated with its protumoral property. During progression from colonic adenoma to colonic adenocarcinoma, RAGE is relocated to the cytoplasmic membrane [21,22]. In contrast, high-grade cancer tends to show non-specific RAGE localization [21].

Many studies have focused on the pro-metastatic property of RAGE. The co-expression of HMGB1 and RAGE is one of the key systems for accelerating tumor metastasis and poor prognosis in glioma, gastric, colorectal and prostate cancer [17,24,26-28]. Gastric and colon cancer cells show concurrent expression of HMGB1 and RAGE, which is closely associated with the autocrine/paracrine regulation of cell motility and invasion of cancer cells [17,26-28]. Metastatic prostate cancer cases show HMGB1 induction in prostatic stromal cells. Concurrence of RAGE expression in tumor cells and HMGB1 expression in stromal cells accelerate cancer metastability [17]. Thus, the HMGB1–RAGE system facilitates a paracrine cancer–stromal cell interaction that plays an important role in the acceleration of the metastatic potential of cancer cells.

In contrast, some instances of the inverse correlation of RAGE with disease prognosis are reported. High-level expressions of RAGE and HMGB1 are found in normal lung tissue and NSCLC, which, in contrast with other cancers, is associated with tissue differentiation and good prognosis [29,30]. RAGE is also associated with myogenic differentiation of myoblasts and rhabdomyosarcoma, which is associated with reduction of malignant phenotypes of the disease [31,32]. RAGE expression is associated with good prognosis of esophageal cancer [33]. These findings suggest that the role of RAGE depends on the types of the cells and tissues involved.

2.3 Secretion

HMGB1 is overexpressed in various types of cancer with autocrine/paracrine networks involving other factors [24,26,27,34]. HMGB1 is released by both active and passive processes. HMGB1 is actively transported between the nucleus and cytoplasm following detachment from loosened chromosomes by histone acetylation [19]. The mechanism of cytoplasmic HMGB1 secretion is associated with an atypical endolysosomal-like secretory pathway in human monocytes [35]. Secretion of HMGB1 is enhanced by colon-cancer-related growth factors/cytokines such as

Table 1. Definition of abbreviations.

RAGE	Receptor for advanced glycation end products	HMGB1 receptor. Multi-ligand immunoglobulin superfamily protein. AGE, S100, amyloid β are also RAGE ligands
AGE	Advanced glycation end products	Modified substances by Amadori reactions, Schiff base reactions, and Maillard reactions, which is associated with diabetic complications
MAPK	Mitogen-activated protein kinase	Ser/Thr protein kinases of the phosphorylationh signaling pathways, which is associated with proliferation, differentiation, apoptosis and survival. Three isozymes, ERK1/2, p38 and JNK are involved in MAPK
iNOS	Inducible nitric oxide synthase	An enzyme catalyzing the production of NO from L-arginine. The expression is induced by NF-κB in immune cells and cancer
NF-κB	Nuclear factor kappa-light-chain-enhancer of activated B cells	An immune response-associated transcription factor, which is induced by cytokines, radicals etc
DCA	Deoxycholic acid	One of the secondary cholic acids, which is known to be a carcinogenic promoter of colon cancer
KC	Kupffer cell	Resident macrophage-lineage cells in the liver. They inhibit cancer cell embedding at the liver sinusoid by phagocytosis
TLR	Toll-like receptor	A membrane protein recognizing microbes-derived molecules, which plays a key role in the innate immune system
VEGF	Vascular endothelial cell growth factor	A protein enhancing proliferation and survival of endothelial cells. It consequently induces angiogenesis/lymphangiogenesis
MIA	Melanoma inhibitory activity	A pro-tumoral protein, which expression is transcriptionally enhanced by NF-κB and HMGB1

TGF-α, IL-15, and the colon cancer-related carcinogenic promoter deoxycholic acid (DCA) [16,19]. HMGB1 expression is induced by stress caused by androgen deprivation in human prostatic stromal cells, which show dedifferentiation from the fibroblastic phenotype [17]. Other cellular stresses such as hypoxia, glucose-deprivation, hyperglycemia, hyperlipidemia and formation of AGE also induce HMGB1 expression [18]. HMGB1 is released from necrotic cells by passive diffusion [36]. However, HMGB1 is not released from tightly packed nuclei of apoptotic cells.

2.4 Intracellular signals

The interaction of HMGB1 with RAGE also activates the intracellular signaling pathway of MAPK. Consequently, RAGE activates the small GTP-hydrolyzing proteins (GTPases), Ras, Cdc42, Rac, Rho, and MMP-2/-9 [3,24]. RAGE expression is associated with cell invasion into type IV collagen [26,28] and it is suggested that type IV collagenase activation may be one mechanism for enhancement of the invasive capacity of cancer cells. RAGE activation induces cell growth through MAP kinase signaling [24]. RAGE activation is also associated with induction of iNOS, NF-κB activation, and B cell leukaemia associated protein 2 (Bcl-2) production [37]. NF-κB activation is associated with HMGB1-dependent chemotaxis [38].

2.5 RAGE ligands

AGE, amyloid-β peptide, certain S100 and HMGB1 are the major ligands of RAGE [23,39]. AGE is associated with complications of diabetes and chronic renal failure and is the result of glycoxidation of tissue proteins such as albumin, collagen and lipids in response to long-term exposure to high concentrations of glucose and/or oxidative stresses. [4,40-42]. Increased AGE formation and the associated generation of reactive oxygen species suggest that AGE is responsible for diabetic microangiopathy involving diabetic nephropathy [4,41,42]. AGE is also associated with activation of NF-κB [43], and production of extracellular matrix proteinase [44]. Amyloid-β peptide causes the neural cell degeneration associated with Alzheimer's disease through RAGE-related tissue damage [45]. Differences in ligand activity between HMGB1 and AGE were examined using AGE–bovine serum albumin (BSA) and results revealed that AGE–BSA had limited effects on cell growth, migration and invasion in comparison with those of HMGB1 [28]. AGE–BSA had only partial effects on phosphorylation of extracellular signal-regulated kinase (ERK) 1/2, Rac1, and AKT, and production of MMP-9. In contrast, HMGB1 had partial effects on upregulation of iNOS and NF-κB p65 expression [28]. Both HMGB1 and AGE are reported to activate ERK1/2, p38 and JNK [24,46], whereas HMGB1 and AGE possess different abilities to activate ERK1/2.

2.6 Angiogenesis

Intracellular signaling pathways of RAGE induce VEGF expression and activate NF-κB in vascular endothelial cells [47]. Activated RAGE induces VEGF expression transcriptionally through activation of NF-κB, activator protein 1 (AP-1), and hypoxia-inducible factor (HIF)-1α [46,47], which is also associated with the complications in diabetes, such as diabetic retinopathy [48]. There is a difference in VEGF induction between AGE and HMGB1 [28]. AGE–BSA has a more pronounced effect on VEGF expression than HMGB1 in colorectal cancer cell lines. In our studies, HMGB1 induced the secretion of VEGF but not that of VEGF-C in human oral squamous cell carcinoma (OSCC) cell lines [34].

Table 2. Summary of the features of HMGB1.

Features	Ref.
Functions of HMGB1	
Neurite outgrowth	[2,3]
Cell and tissue differentiation of lung	[29]
Chromatin structural protein	[1-3]
DNA chaperon	[6]
Drug resistance	[7]
Cell migration	[3,8]
Inhibition of apoptosis	[9]
Autophagy	[10]
Mediation of TLR	[19,58,59]
Enhancement of VEGF expression	[46,47]
VEGF-C/-D induction via MIA by HMGB1	[34,50,52,53]
Apoptosis induction in macrophages	[61,62]
Inhibition of macrophage-associated anti-metastatic defense	[60,71,72]
Necrosis reporter to dendritic cells	[87]
HMGB1 receptor	
Receptor; RAGE	[20-24]
Anti-apoptotic effect	[16]
Carcinogenesis	[18,25]
Intracellular localization	[21,22]
Intracellular signals	[3,15,16,22,24, 26-28,61,62]
Differential roles of AGE from HMGB1	[28]
Expression and secretion of HMGB1	
Overexpression in cancer	[26,27,34,36]
HMGB1 release from nuclei by histone acetylation	[18]
Secretion by atypical endolysosomal-like secretory pathway	[35]
Induction of HMGB1 secretion associated with inflammation	[11-13]
Induction of HMGB1 secretion by stresses	[16,18]
Passive release of HMGB1 by necrosis	[36]

VEGF-C and VEGF-D are associated with lymph node metastasis [49]. Differential induction of VEGF from VEGF-C through activation of RAGE by HMGB1 may explain why RAGE expression is not associated with lymphangiogenesis. Lymph node metastasis of cancer is strongly associated with lymphangiogenesis [50].

Melanoma inhibitory activity (MIA) is an 11-kDa secretory protein isolated from supernatants of malignant melanoma cells [51]. MIA expression is regulated transcriptionally via the interaction of HMGB1 with NF-κB p65 [52], which concomitantly bind to a 30-bp region in the promoter region of the MIA gene designated as the highly conserved region (HCR). MIA expression is associated with VEGF-C and VEGF-D expression in OSCC cells [34,53]. The alteration of the signal balance between ERK1/2 and p38 might be associated with up-regulation of VEGF-C and VEGF-D expression by MIA [34,53].

These findings show that HMGB1 induces VEGF expression by RAGE activation and also induces expression of VEGF-C and VEGF-D by acting as a transcriptional co-factor of NF-κB. Thus, HMGB1 is associated with both angiogenesis and lymphangiogenesis in cancer.

3. HMGB1 in cancer immunity

HMGB1 plays a key role in the immune system by acting as a late inflammatory cytokine by activating macrophages in response to lipopolysaccharide [12]. This results in an increases in the secretion of the inflammatory cytokines IL-1β, IFN-γ, and TNF-α, which worsen septic shock, systemic inflammatory response syndrome, rheumatoid arthritis, brain injury and autoimmune diseases [12,54-57]. Recent research has shown HMGB1 to act as a mediator of TLR and to affect immune responses to transplanted grafts [58] and cytokine release from macrophages [59].

3.1 Apoptosis of macrophages

HMGB1 is associated with a significant reduction of intratumoral macrophage infiltration in metastatic colon cancer [60]. The number of phorbol-12-myristate-13-acetate (PMA)-induced U937 macrophages (PMA-U937) is markedly decreased when co-cultured with WiDr cells producing HMGB1 [61]. Recombinant human HMGB1 induces growth inhibition in thioglycollate-induced rat peritoneal macrophages, PMA-U937 cells, and human alveolar macrophages, and induces apoptotic death with phosphorylation of JNK and Rac1, and upregulation of caspase-3 and caspase-9 [61,62]. Rac1/Cdc42 is reported to be a major intracellular signaling pathway of RAGE [63] and is associated with activation of p38 and JNK to participate in cell survival and in apoptosis [64]. The bidirectional role of Rac1/Cdc42 depends on cell type. In U937 cells, a dominant-negative mutant of Rac1 abrogates TNF-α-induced apoptosis [65]. JNK is involved in Fas/TNF and ceramide-induced apoptotic pathways in macrophages [66]. In myoblasts or Rac1-overexpressing NIH3T3 cells, JNK/stress-activated protein kinase (SAPK) is associated with apoptotic signals transmitted by Rac1/Cdc42 [31,32,67].

Extremely high NO levels induce apoptosis and necrosis in macrophages [66]. Activated macrophages exert cytostatic and cytotoxic effects on tumor cells through the release of NO [68]. This activation of NO production by macrophage-iNOS has been shown to inhibit metastasis of K1735 melanoma cells [69]. In contrast, the nitrite concentration in the conditioned media was too low to induce apoptosis by macrophages in both PMA-U937 cells and thioglycollate-induced rat peritoneal macrophages, [61]. Inhibition of iNOS by NG-nitro-L-arginine methyl ester (L-NAME) does not reverse growth inhibition of these cells in response to HMGB1 [61]. In addition to NO and cholesterol accumulation, heavy metals, extracellular ATP and ceramide from cell membrane sphingomyelin induce apoptosis in macrophages [66,70]. In atherosclerosis, cholesterol accumulation in the endoplasmic reticulum induces an unfolded protein

response (UPR) in macrophages infiltrating the arterial intima [70]; this triggers the apoptotic process in macrophages and accelerates atherosclerosis. HMGB1 induces apoptosis not only in macrophages but also in monocyte-lineage cells such as Kupffer cells, and monocyte-dendritic cells, which express RAGE as an HMGB1 receptor [61,62,71,72].

3.2 Tumor-infiltrating macrophages

Tumor-associated macrophages also have anti-cancer effects [73]. In clinical studies, colon cancer patients with high-level macrophage infiltration show less invasion and metastasis than those with low-level macrophage infiltration [74]. Depletion of tumor-infiltrating macrophages is closely associated with advanced stages of human colon cancer and with metastatic ability in a mouse colon cancer model [60]. In *in vitro* studies, HMGB1 inhibits the infiltration of PMA-U937 cells into the KM12SM colon cancer cell layer in a dose-dependent manner [60]. Macrophage migration inhibitory factor (MIF) and macrophage inhibitory cytokine (MIC)-1 are reported to play a role in several human malignancies [75]; however, HMGB1 expression is more closely correlated with macrophage depletion in cancer than that of MIF or MIC-1 [60]. In clinical studies, examination of tumors with relevant macrophage infiltration revealed Dukes B cases with macrophage–cancer cell contact, whereas Dukes C cases showed no such contact. HMGB1 expression is associated with macrophage depletion in colon cancer tissues [60].

3.3 Lymph sinus macrophages

Lymph sinus macrophages and liver Kupffer cells (KCs) participate in the immune response of the organs against metastatic cancer cells. Sinus macrophages and KCs mediate the phagocytosis of cancer cells attached to the sinus wall in order to inhibit their metastasis [76,77]. In prostate cancer metastasis, a decrease in the number of nodal immune cells is associated with a reduction in the number of sinus macrophages in the lymph node. Sinus macrophages and KCs are also a part of an anti-cancer cytokine network involving TNF-α and IL-1β [78]. The genes expressed in the sinus macrophages in the lymph nodes and liver are distinct from those expressed in the splenic sinus macrophages, for example, the gene encoding sialoadhesin, CD36, CD163 and macrophage receptor with collagenous structure (MARCO) [78].

In clinical studies, macrophage numbers in the regional lymph nodes are decreased in both non-metastasized and metastasized nodes in Dukes C cases, whereas macrophage numbers in Dukes B nodes are higher [72]. Nodal HMGB1 concentration is higher in Dukes C nodes than that in Dukes B nodes; this is inversely correlated with macrophage numbers. Nodal HMGB1 concentration is correlated with the HMGB1 concentration and lymph vessel density found in the primary tumors [72]. High concentrations of HMGB1 are reported in effusions from cancer patients. These data indicate that HMGB1 secreted from primary tumors is delivered to the regional lymph nodes and decreases the number of macrophages to weaken the anti-metastatic defense of the lymph nodes in patients with CRCs.

3.4 Liver Kupffer cells

Liver metastasis is one of the critical conditions of colon cancer that determine the disease prognosis and the quality of patient's quality of life; one-third of colon cancer patients die from liver metastasis [79]. In a nude mouse liver metastasis model, the cecal administration of HMGB1 decreased the number of KCs and increased the embedment of colon cancer cells in a dose-dependent manner [71].

HMGB1 is secreted from primary tumors of colon cancer and delivered to the liver through portal blood flow. Following this, HMGB1 inhibits KCs to accelerate liver metastasis of colon cancer. In clinical studies, higher HMGB1 concentrations are found in the primary tumors and metastatic foci, and fewer KCs are found in Dukes D cases than in Dukes C cases. The portal blood HMGB1 concentration is higher in Dukes D cases than in Dukes C cases, and we have shown that the concentration of HMGB1 in the portal blood is strongly correlated with the concentration of HMGB1 in the primary tumors [71]. RAGE expressed in KCs is also activated by AGE, which is associated with liver disorders including diabetes [80].

As a result, HMGB1 affects the host immunity in the metastasis-target organs in a humoral manner. In endotoxic shock, HMGB1 is released into the blood circulation and affects various organs [14]. In hepatocellular carcinomas, serum HMGB1 levels are increased according to the disease progression [81], suggesting that large amounts of secreted HMGB1 can affect remote organs such as the target organs of metastases from CRCs.

3.5 Dendritic cells

Dendritic cells (DCs) play a crucial role in host immune response to various extrinsic microorganisms and also to cancer cells [82]. The dendritic cells infiltrating tumor tissues or lymph nodes are sentries against the cancer antigens, which are recognized by dendritic cells and presented to T lymphocytes [83]. In clinical studies, presence of dendritic cells is associated with improved survival rates of colon cancer [84]. Dendritic cells are also expected to enhance host responses to cancer vaccine [85]. Dendritic cell densities in primary tumors and metastatic tumors are suppressed [84]. Indeed, nodal-metastasis-positive colon cancer cases show higher HMGB1 concentrations in lymph node and primary tumor tissues and lower dendritic cell numbers [62]. HMGB1 produced by colon cancer cells resulted in a suppression of nodal dendritic cells to attenuate host anti-cancer immunity.

HMGB1 is released from the loosened chromatin in necrotic cells and diffuse into the extracellular milieu [36]. Studies have demonstrated that lysates of dying cells can induce the maturation of DCs [86]. HMGB1 is a nuclear DNA binding protein and has been shown to be a sensitive

probe of DNA for presenting to TLR [10]. HMGB1 bound to DNA-containing necrotic substances is recognized by RAGE expressed in dendritic cells [87].

HMGB1 results in activation of monocytes and dendritic cells; however, high concentrations of HMGB1 result in a death signal for dendritic cells, as found on macrophages [62]. Mouse peritoneal macrophage-derived dendritic cells (PMDDCs) treated with HMGB1 show a decrease in cell number in a dose-dependent manner. HMGB1-treated PMDDCs show apoptosis and increased levels of phosphorylated JNK, and intraperitoneal administration of HMGB1 decreased splenic dendritic cells in C57BL mice [62].

HMGB1 may provide cancer cells with the advantages of cancer progression and suppression of host immunity; therefore, further examination of the role of HMGB1-induced macrophage apoptosis in cancer may provide novel therapeutic targets against these diseases.

4. HMGB1-targeting therapy

As described above, HMGB1 participates in diverse processes promoting cancer development and progression as well as in various inflammatory diseases. HMGB1-targeting therapy is therefore an effective treatment for cancer (**Figure 1**).

4.1 Knockdown

We carried out *in vitro* experiments to target HMGB1 using antisense oligodeoxynucleic acid technology. Migration and *in vitro* invasion are inhibited by treatment with HMGB1 antisense oligodeoxynucleotides in cultured cancer cells [17,26,28,61]. Downregulation of HMGB1 expression by short hairpin RNAs results in the inhibition of cell growth and induction of apoptosis in LNCaP prostate cancer cells [88]. The bacterial lipoprotein (BLP) severely influences the occurrence of sepsis. Tolerance of BLP in mice leads to a downregulation of HMGB1 protein synthesis and release from macrophages and improves survival of lethal doses of BLP [89].

4.2 Neutralization

We used HMGB1-specific antibody to neutralize HMGB1. In an azoxymethane-induced rat colon cancer model, the colonic mucosal HMGB1 levels are increased in a time-dependent manner, and neutralization of HMGB1 abrogates colon cancer development [18]. In a mouse liver metastasis model, neutralization of HMGB1 abrogates the liver metastasis of colon cancer cells. HMGB1 antibody also inhibits TLR, especially TLR4, to suppress release of inflammatory cytokines in animal models of lethal endotoxemia/sepsis, collagen-induced arthritis, ischemia-reperfusion injury, and ischemic brain injury [90,91]. Soluble RAGE is an endogenous truncated form of RAGE consisting of the extracellular domain of RAGE [92], and binds to HMGB1 in the extracellular milieu. Overexpression of soluble RAGE affects the expressions of those genes critical in tumorigenesis and metastasis to inhibit tumor formation, cell invasion and angiogenesis [93]. The A box of HMG works as a competitive antagonist of HMGB1. A recombinant fusion protein of the A box with thrombomodulin or a truncated HMGB-1-derived A-box protein, effectively inhibits HMGB1-induced TNF-α secretion and reduces the severity of arthritis in animal models [54,94].

4.3 Adsorption

Hemoperfusion therapy using a cellulofine sulfate bead column that adsorbs HMGB1 reduces serum HMGB1 to decrease reperfusion injury of the rat liver [95]. Suda *et al.* also established an HMGB1 adsorption column using the heparin-binding nature of sulfate spheroids [96], which reduces serum levels of IL-4 and IL-8.

4.4 Chemicals

Some chemicals and bioactive substances affect HMGB1 expression. Molecular hydrogen, which is known as a strong antioxidant substance, reduces hydroxyl radicals to prevent tissue damage and reduces HMGB1 levels in serum and tissue [97]. Statins such as atorvastatin or tanshinone II A have been shown to prevent tissue damage in experimental brain ischemia by reducing the expression of HMGB1 and RAGE [98,99]. Dexamethasone, gold sodium thiomalate and chloroquine inhibit the extracellular release of HMGB1 from cultured activated macrophages in a dose-dependent manner and cause intracellular retention of HMGB1 [55]. Ethyl pyruvate reduces serum HMGB1 levels and increases survival in an endotoxemia mice model with inhibition of p38 and NFkB [94]. The pituitary adenylate cyclase-activating polypeptide (PACAP), an endogenous neuropeptide, significantly attenuates serum HMGB1 levels to increase survival in animals with endotoxemia [100].

4.5 RAGE inhibition

RAGE targeting is also effective in suppression of HMGB1. Antisense oligodeoxynucleic acids for RAGE also decrease the pro-tumoral effects of HMGB1 [26,28]. The p38 inhibitor SB239063, which suppresses RAGE intracellular signaling, decreases the angiogenic activity of HMGB1 via MIA [34,53]. In contrast, monocyte-lineage cells show suppression of growth inhibition or apoptosis by RAGE antisense treatment. JNK inhibitor (SP600125), which also suppresses RAGE intracellular signaling, decreases HMGB1-induced apoptosis (our unpublished data). RAGE expression in a rat tongue carcinogenesis model is suppressed by a selective COX-2 inhibitor, etodolac [25]. Recent studies have revealed TLR as an important partner of HMGB1; therefore, RAGE targeting may only provide a partial effect on HMGB1 inhibition.

5. Conclusion

The major cause of death from gastrointestinal cancer is the progressive growth of metastases that are resistant to therapy. Metastasis is a highly selective process that consists of a series

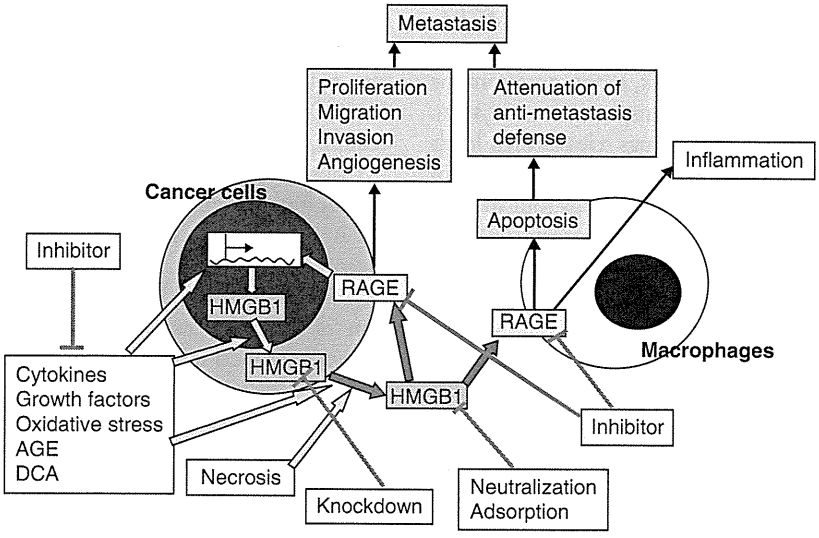

Figure 1. Scheme of HMGB1-RAGE system and the targeting. HMGB1 is released from cancer cells in responses to cellular stresses or by necrosis (uncoloured black-framed items). HMGB1 activates RAGE of cancer cells in a autocrine/paracrine manner to induce cancer progression (left items with yellow background). In contrast, HMGB1 induces apoptosis in macrophages through RAGE to attenuate anti-metastasis defense (right items with yellow background). Progression of cancer cells and inhibition of the host immunity enhances cancer metastasis. HMGB1 targeting methods are indicated by red-framed items.

of sequential and interrelated steps and HMGB1 participates in many steps of the metastatic process. HMGB1 targeting is a potential therapeutic against cancer development, progression, and especially metastasis. A technical breakthrough in the application of HMGB1 targeting to human diseases is now urgently required.

6. Expert opinion

In Section 4, various methods for HMGB1 targeting are summarized. For HMGB1 targeting, some problems should be considered. i) HMGB1 is expressed ubiquitously in all cell types and is responsible for diverse biological processes, as previously described. Therefore, HMGB1 inhibition may result in severe side effects, especially, since HMGB1 has a role in optimal DNA binding to TLR4 [10]. HMGB1 inhibition may also affect immune responses to virus infection. ii) HMGB1 has a dual role as a chromosomal protein and a cytokine [12], the function is linked to the intracellular location. iii) HMGB1 is a long-life protein trafficking between nucleus and cytoplasm using active nuclear transportation [35]. The protein is maintained at high levels, the reduction of which requires long-term treatment. iv) HMGB1 in the extracellular milieu is secreted from living cells or released from necrotic cells [36]. Inhibition of HMGB1 secretion tends to be insufficient in cancer cases. v) HMGB1 expression and secretion are affected by various factors including cytokines, growth factors, oxidative stress, cellular stresses, AGE and cytotoxic factors such as endotoxin [12-14,19,28]. Thus, the control of the expression and secretion of HMGB1 is complicated.

Knockdown of HMGB1 by antisense S-oligodeoxynucleic acid or siRNA is a common method for HMGB1 inhibition. Nucleic acid tends to accumulate in the reticuloendothelial system, therefore, an adequate *in vivo* gene delivery method, such as liposomes or polyplex micelles, is required in application of HMGB1 knockdown to cancer treatment. Since HMGB1 is an abundant nuclear protein with a long life, a continuous administration of antisense or siRNA is necessary to gain a reduction of HMGB1 protein levels. The cytotoxicity of nucleic acid must also be considered; inhibition of the roles of nuclear HMGB1 may cause insufficient transcription or DNA repair in normal cells [6].

Neutralization of HMGB1 in the extracellular fluid is achieved by HMGB1 binding proteins such as the HMGB1-specific antibody or soluble RAGE. As the cancer-associated role of HMGB1 is based on its cytokine-like property, this method is thought to be useful and has fewer side effects. It is also possible to deliver these proteins by standard methods or by using a specialized vehicle, such as liposomes or micelles. The soluble form of RAGE is a product of alternative splicing of the *RAGE* gene [93]. The gene can be administrated by an adequate vehicle involving a virus vector. The normal cells or cancer cells expressing soluble RAGE are expected to suppress HMGB1 expression over the long term, and the local delivery of the soluble RAGE vector will diminish the systemic side effects to a minimum.

Adsorption is a method for elimination of serum HMGB1 using a hemodialyzer and facilitates the reduction of HMGB1 function to a great extent. Taking into consideration the overall cost and the effect, this method is suitable

for resuscitation from severe inflammation or HMGB1-associated inflammation. HMGB1 adsorption may also be valuable in treatment of endotoxic shock, sepsis, autoimmune disease, brain injury, and organ transplantation [14,57,58]. Massive cell death due to anti-cancer drugs releases high levels of HMGB1 into the blood, which accelerates regrowth of the remnant cancer cells or dormant cancer cells (our unpublished data). A combination of HMGB1 adsorption with chemotherapy may enhance the anti-cancer effect.

The pro-tumoral and anti-immune effects of HMGB1 depend on RAGE activation; therefore, RAGE inhibition is another target for HMGB1 inhibition. To date, no effective RAGE inhibitor has been developed. RAGE is responsible for diabetic complications, and arterial sclerosis [4,41,42]. A RAGE inhibitor is therefore a relevant molecular target for cancer, diabetes or cardiovascular diseases. HMGB1 and RAGE expression is upregulated by AGE, making AGE inhibition an additional molecular target for HMGB1 inhibition. AGE formation is suppressed by losartan, and metformin (our unpublished data) and a COX-2 inhibitor also reduces AGE formation [25]. Several chemicals affecting HMGB1 expression or activity are described above and require further examination of their clinical applications.

Considering the essential role of HMGB1 in the innate immunity, systemic inhibition of HMGB1 might have too heavy deleterious consequences in terms of responses to invading organisms. In many experimental procedures, HMGB1 targeting is done by systemic situations. For application of HMGB1 targeting to human diseases to be safe, methods for local inhibition of HMGB1 need to be invented.

Declaration of interest

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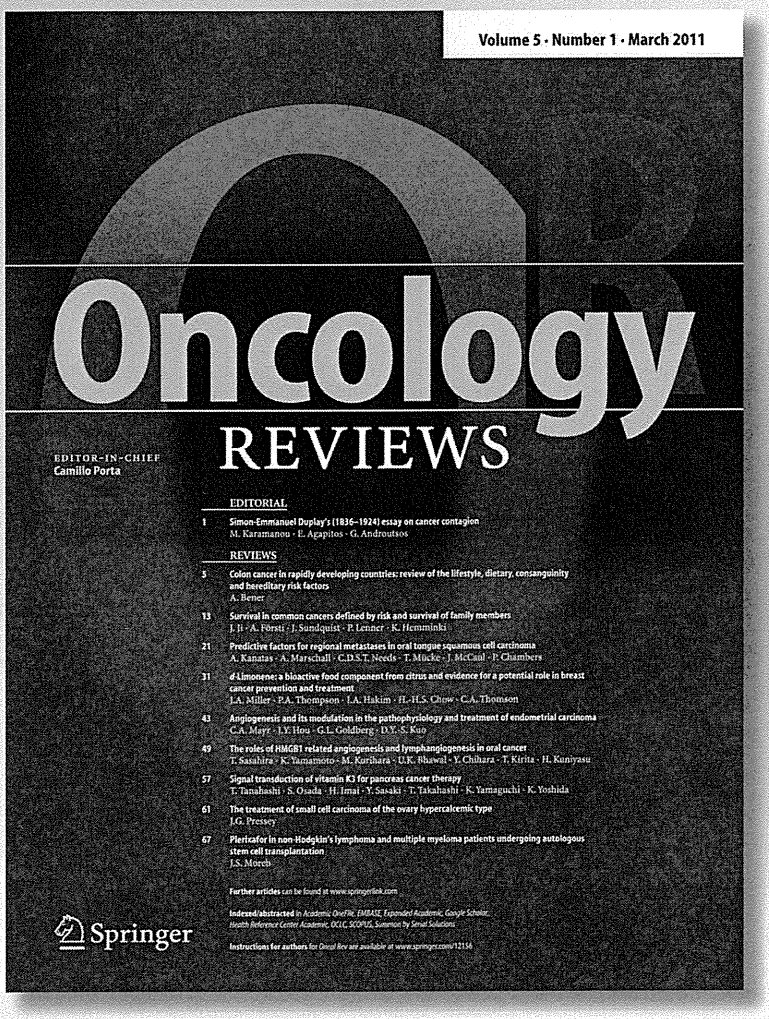

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REVIEW

The roles of HMGB1 related angiogenesis and lymphangiogenesis in oral cancer

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Abstract Angiogenesis and lymphangiogenesis are the vital factors in tumor progression and metastasis. In this review, we show that high-mobility group box 1 (HMGB1) is responsible for both angiogenesis and lymphangiogenesis in oral squamous cell carcinoma (OSCC). HMGB1 possesses the dual role as a cytokine and as a chromatin protein. HMGB1 as a cytokine is the ligand of receptor for advanced glycation end products (RAGE). RAGE activation upregulates secretion of vascular endothelial growth factor (VEGF). Then co-expression of HMGB1 and RAGE accelerates angiogenesis. In contrast, nuclear HMGB1 transcriptionally induces the expression of melanoma inhibitory activity (MIA) gene with NFkB. MIA upregulates expression of VEGF-C and VEGF-D via p38 phosphorylation by integrin activation. Then HMGB1 and MIA is associated with lymphangiogenesis. According to the differential roles of HMGB1 on angiogenesis and lymphangiogenesis, local invasion, disease recurrence, and poor prognosis are associated with HMGB1–RAGE system, whereas lymph node metastasis is associated with HMGB1–MIA system in OSCC.

Keywords Oral cancer · Angiogenesis · Lymphangiogenesis · VEGF-C · VEGF-D · MIA

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Introduction

Head and neck cancer is the sixth most common malignancy worldwide, accounting for 6.1% of all cancers, and the first leading cause of cancer death in South Asia [1]. Especially in India and Sri Lanka, OSCC is the commonest cancer, accounting for at least 40% of all malignancies [1, 2]. About 300,000 patients develop oral squamous cell carcinoma (OSCC) every year in the world [3, 4]. In Japan, mortality from oral squamous cell carcinoma (OSCC) is 3.7 per 100,000 people and gradually increasing [5]. The overall 5-year survival rate for patients with OSCC is the lowest among major cancers and has not changed during the past two decades [6]. Malignant potential of OSCC is closely associated with local expansion and lymph node metastasis [7], from which over 50% of patients died [8, 9]. More than 80% of stage I OSCCs can be cured by treatment, whereas more than 70% of stage III and IV OSCCs cannot [10]. Especially, local or nodal recurrence worsens prognosis of OSCC [11]. Extended local invasion involves surrounding important structures, which makes difficult to complete tumor resection and reduce cancer curability [12].

Angiogenesis is one of major factors in progression of various cancers involving OSCC [7, 13]. For angiogenesis, several angiogenic factors are recruited in OSCC: vascular endothelial growth factor (VEGF), basic fibroblast growth factor (bFGF), interleukin (IL)-8, and platelet-derived endothelial growth factor (PD-EGF) are reported for their roles on angiogenesis in OSCC [14–17]. These factors are significantly associated with tumor microvessel density (MVD); especially VEGF is relevant [15, 16].

We have reported that high-mobility group box 1 (HMGB1) and receptor for advanced glycation end products (RAGE) are the significant factors in cancer progression and

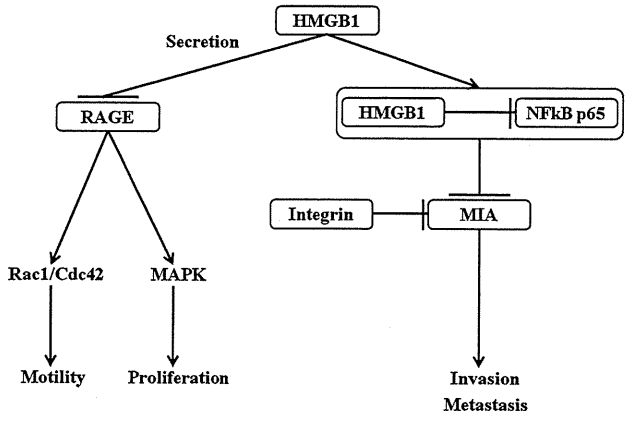

Fig. 1 Scheme of HMGB1–RAGE and HMGB1–MIA signals. Secreted HMGB1 bind to RAGE and promote cell motility and proliferation by activating Rac1/Cdc42 and MAPK in several cancer, respectively, while intracellular HMGB1 and NFkB p65 concurrently bind to a promoter region of MIA and stabilize MIA gene expression. MIA binds to some integrins ($\alpha 4\beta 1$ and $\alpha 5\beta 1$) and accelerates invasion and metastasis in malignant melanoma

metastasis [18–21]. HMGB1 secreted or released from cancer cells activates RAGE in autocrine and paracrine manners to accelerate growth, migration, and invasion of cancer cells. In OSCC, we have found that HMGB1 is closely associated with not only cancer cell proliferation but also angiogenesis and lymphangiogenesis by recruiting RAGE and melanoma inhibitory activity (MIA) as shown in the Fig. 1. In this review, we show the significance and the mechanism of angiogenesis and lymphangiogenesis enhanced by HMGB1, RAGE, and MIA in OSCC.

HMGB1

We first explain HMGB1 and the diverse roles in cancerous and non-cancerous situations. HMGB1 protein is originally identified as a chromatin protein that includes important processes such as transcription, DNA repair, differentiation, p53 function, and development [22–25]. HMGB1-knockout mice are lethal in early neonate due to insufficient transcriptional activity of glucocorticoid receptor and subsequent inability in glycogen usage [26]. HMGB1 is also designated as amphoterin, which is identified as a cell motility accelerating protein, which is deeply associated with organ development, especially central nervous system in acceleration of neurite outgrowth [27, 28]. HMGB1 was recently recognized as a cytokine that participates in cancer progression and inflammation [29–32]. Released HMGB1 into the extracellular milieu is associated with endotoxin-induced organ injury, macrophage-associated inflammation, and cancer progression [33, 34]. In inflammatory processes such as rheumatoid arthritis and sepsis, macrophages are responsible for HMGB1 secretion [35–37]. Lipopolysaccharide or endotoxin is a strong stimulant of HMGB1 secretion in macrophages [38]. In monocytes, HMGB1 secretion is induced by stimuli triggering lysosome exocytosis [39], and hyperacetylation of HMGB1 is associated with the secretion [40, 41]. HMGB1 secretion is triggered by lysophosphatidylcholine, generated later in the inflammation site in monocytes [39]. HMGB1 extracellular release is also activated by cell necrosis [42].

HMGB1 as a RAGE ligand

Many of HMGB1 functions depend on the activation of the specific receptor, the receptor for RAGE. RAGE is a multiligand cell-surface receptor that belongs to the immunoglobulin superfamily [43–45]. Advanced glycation end products (AGE), amyloid-beta peptide, S100, and HMGB1 are the major ligands of RAGE [27, 44, 45]. HMGB1 is produced in many types of neoplasms and immature cells [46], and its expression is highly correlated with cell migration and invasion induced by RAGE-associated intracellular signaling proteins, including GTPases, Cdc42, Rac1, MAPK, extracellular signal-regulated kinase (ERK)-1/2, p38, c-Jun N-terminal kinase (JNK), and nuclear factor kappa B (NFkB) [46, 47].

RAGE–HMGB1 system affects the host anti-cancer immunity. RAGE expressed in macrophages is associated with inhibition of macrophage infiltration into tumor tissues that produce HMGB1 [48, 49]. HMGB1 induces apoptosis in RAGE-expressing macrophages, particularly activated macrophages that show increased RAGE expression. Because TGF-alpha and IL-15 increase HMGB1 production, they can inhibit macrophage infiltration into tumor tissues [50]. Thus, RAGE is associated with both cancer progression and with host reaction against cancer.

RAGE expression appears to be closely associated with the invasion and metastasis of gastric cancer. Immunohistochemistry of gastric carcinomas showed 62 of the 96 cases (65%) to be RAGE-positive and that poorly differentiated adenocarcinomas preferentially expressed RAGE protein (38/42, 90%) [18]. Strong RAGE immunoreactivity was also correlated with the depth of invasion and with lymph node metastasis [18]. RAGE-positive cancer cells tended to distribute at the invasive front of primary tumors and were detected in all metastatic foci in lymph nodes. In contrast, HMGB1 was expressed in 82 of 96 samples (85%) regardless of histologic type and cancer progression [18]. When cancer cells expression RAGE and HMGB1 were exposed to antisense S-ODN for RAGE and/or HMGB1 showed that cell migration and invasion into type IV collagen-coated membranes were significantly suppressed [18, 20, 21]. Blockade of RAGE–HMGB1 system inhibits tumor cell

migration, invasion, and metastasis [18, 20, 27, 47]. RAGE is also implicated in disease advancement and poor outcome in cases of colon cancer [21], and disease advancement after androgen blockade in cases of prostate cancer [19, 51].

HMGB1–RAGE system in OSCC

RAGE is also associated with the progression and metastasis in OSCC. We first examine the RAGE expression in OSCCs. In the immunohistochemical analysis, 30 (40.5%) of 74 OSCCs show high RAGE expression [52]. A marked RAGE expression is found in invading cancer cells at the invasive front of OSCC tumors. In contrast, No RAGE immunoreactivity is observed in normal mucosal or glandular epithelia. High RAGE expression is associated with the depth of invasion and also the local recurrence of OSCC. Disease-free survival of all patients with high RAGE expression is significantly worse than that of patients with low RAGE expression. Even in stage I–II, patients with high RAGE expression show significantly worse prognosis than those with low RAGE expression in the same as stage III–IV patients. In comparison of RAGE expression with clinicopathological factors, RAGE expression is associated with invasive depth, nodal metastasis, T factor of TNM classification, and clinical stage. By multivariate analysis, RAGE expression is an independent prognostic factor for the disease-free survival of tumor invasion, nodal metastasis, T factor in TNM classification, and clinical stage [53].

The expression of RAGE is also associated with OSCC carcinogenesis [54]. RAGE expression in lesions developed during tongue carcinogenesis in Fischer 344 rat by 4-nitroquinoline 1-oxide (4-NQO). The tongue SCCs are induced in Fischer 344 rats given 20–30 ppm 4-NQO in their drinking water for 12 weeks. RAGE expression is detected in 4-NQO-induced SCCs and dysplasias. Importantly, a selective cyclooxygenase-2 inhibitor, etodolac significantly decreased RAGE expression in dysplasias and SCCs. Etodolac also significantly reduced the incidences of SCCs to 50% at doses of 300 ppm. Thus, RAGE is involved in rat tongue carcinogenesis by 4-NQO [54]. Oxidative stress enhances AGE formation in oral mucosa. Etodolac inhibits chronic inflammation and subsequently reduces oxidative stress.

HMGB1–RAGE system and angiogenesis

VEGF is one of key molecules of angiogenesis and blood vessel maintenance [55–58]. Many human cancers expressing high levels of VEGF show progression of stage, metastasis, and poor prognosis [57]. In 4 VEGF family members, VEGF-A and VEGF-B are commonly expressed with or without nodal metastasis, whereas VEGF-C and VEGF-D are associated with lymph node metastasis [59, 60]. Expression of VEGF and VEGF-C is significantly associated with MVD (mean density of CD34-positive vessels counted in immunostained specimens) and lymph vessel density (LVD; mean density of D2-40-positive vessels counted in immunostained specimens), respectively, in OSCC [61]. VEGF-C also participates relevantly in angiogenesis [62]. One of the suggested mechanisms is that binding affinity to the receptors of VEGF-C is different on the lymphoendothelial and vasculoendothelial cells for the proteolytic processing of VEGF-C [63].

RAGE is associated with abnormal neovascularization in diabetic retinopathy [64]. In that condition, AGE generated under hyperglycemic and oxidative conditions works as a RAGE ligand [64–66]. Activated RAGE induces VEGF expression transcriptionally through activation of NFkB, AP-1, and HIF-1alpha [67, 68]. Induction of VEGF-C is resulted by activation of p38 MAPK and NFkB [69]. Activation profile of MAPK might be associated with differential response to RAGE ligand on VEGF/VEGF-C induction. HMGB1 has partial effects on up-regulation of inducible nitric oxide synthase (iNOS), NFkBp65, and VEGF expressions compared with those of AGE [20]. We examine the angiogenesis or lymphangiogenesis in OSCC with reference to RAGE expression [52]. LVD and VEGF-C concentration are significantly associated with nodal metastasis and advanced disease stage. VEGF expression is significantly associated with MVD, whereas VEGF-C expression is associated with LVD. Concentrations of RAGE in the tumor tissues are correlated with MVD, but not with LVD. The RAGE concentration is also significantly associated with VEGF expression but not with VEGF-C expression [52]. When we examined the effect of hrHMGB1 on secretion of VEGF and VEGF-C in HSC3- and HSC-4 OSCC cells, secretion of VEGF but not VEGF-C is increased in a dose-dependent manner in both cells [52]. Inhibition of RAGE expression by RAGE *S*-oligodoxynucleotide (*S*-ODN) antisense abrogates increase of HMGB1-induced VEGF secretion in both cells. Thus, HMGB1–RAGE system is associated with angiogenesis by upregulation of VEGF secretion, but not with lymphangiogenesis in OSCCs. Differential induction of VEGF from VEGF-C by RAGE activation by HMGB1 might explain why RAGE expression is not associated with lymphangiogenesis. Therefore, HMGB1–RAGE system is responsible for local aggressiveness by enhancing angiogenesis, but not for lymphangiogenesis or lymph node metastasis in OSCCs (Fig. 2).

MIA

In the effect of HMGB1 on lymphangiogenesis, melanoma inhibitory activity (MIA) plays an important role. MIA is

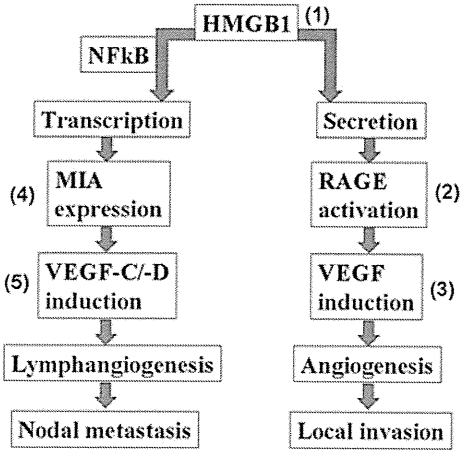

Fig. 2 HMGB1 activates RAGE as the ligand to induce VEGF expression. Intracellular HMGB1 is associated with MIA expression transcriptionally, which induces VEGF-C and -D expression. Thus, HMGB1 provides angiogenesis and lymphangiogenesis in OSCC. (*1*) Role of HMGB1, (*2*) RAGE activation, (*3*) VEGF induction by RAGE, (*4*) MIA induction, and (*5*) VEGF-C/-D induction are explained in sections "HMGB1", "HMGB1–RAGE system", "HMGB1–RAGE system and angiogenesis", "MIA", "MIA and angiogenesis"

an 11-kDa secretory protein isolated from supernatants of the HTZ-19 malignant melanoma cells [70, 71], whose gene locus is mapped to chromosome 19q13.32–13.33 [72]. Although previous reports indicated that MIA is correlated with invasion and metastasis in malignant melanoma [73–75], breast cancer [73], chondrosarcoma [76], glioma [77], and pancreatic cancer [78], the definite functions of MIA in the cancer cells are still unclear. MIA promotes cell detachment, migration, invasion, inhibits apoptosis of the cancer cells, and infiltration of lymphokine activated killer cells (LAK). MIA binds to fibronectin via SH3 domain-like structure and masks $\alpha 4\beta 1$ and $\alpha 5\beta 1$ integrins, which inhibits cell-to-stromal attachment [79, 80]. Further, MIA is able to bind to cell surface integrin $\alpha 4\beta 1$ and $\alpha 5\beta 1$, which suggests that MIA might play a role as a ligand for selected integrins [81]. Mitogen-activated protein kinase (MAPK) activity is reported to be affected by MIA [81].

Expression of MIA is detected in 48.4% of 62 cases [61]. Immunoreactivity for MIA is found in only cancer cells, but not in normal epithelium of oral mucosa. A significant association is found between MIA immunoreactivity and lymph node metastasis. The 17.4% cases without nodal metastasis expressed MIA, whereas the 66.7% cases with nodal metastasis expressed MIA. Marked MIA expression is also found in the nodal metastatic foci. However, no significant relation was found between MIA expression and T classification (extension of primary tumor), clinical stage, tumor recurrence, or disease-free survival. A significant correlation was observed between MIA immunoreactivity and LVD [61]. Thus, lymphanigiogenesis by MIA is thought to be associated with nodal metastasis.

MIA and angiogenesis

Several pathways are proposed to the mechanism of lymphangiogenesis by MIA. Expression of integrin $\alpha 5\beta 1$ in lymph vessel endothelial cells is associated with outgrowth of new lymphatic vessels [82]. MIA might stimulate lymphatic endothelial cells directly to induce lymphangiogenesis.

The expressions of VEGF-C or VEGF-D are significantly correlated with MIA expression or HMGB1 expression levels [61], suggesting that MIA is associated with lymph node metastasis of OSCC by upregulation of these lymphangiogenic factors. VEGF-C and -D are known as strong lymphangiogenic factors in various cancers [83]. Increased VEGF-C expression is associated with cervical lymph node metastasis in head and neck cancer [84]. Although there are still controversies about the role of VEGF-D in lymph node metastasis, VEGF-D expression is also associated with lymph node metastasis in animal model [85]. In HSC-3 OSCC cells, which express integrin $\alpha 5\beta 1$, neutralization of MIA by the specific antibody decreases VEGF-C and VEGF-D expression in association with decreased p38 phosphorylation, but not ERK1/2 [61]. Transcriptional regulation of VEGF-C and VEGF-D is different from that of VEGF, which is associated with hypoxic and/or acidic conditions [86]. VEGF-C expression is inhibited by p38 inhibitor but not by ERK1/2 inhibitor [87]. p38 activation up-regulates VEGF-D as well as VEGF-C in HSC3 cells [61].

Recently, nuclear HMGB1 was revealed to interact with nuclear factor kB (NFkB) p65 to accelerate MIA expression [88, 89]. HMGB1 and NFkB p65 concurrently bind to a 30-bp region in the promoter region of the MIA gene designated as the highly conserved region (HCR) [88]. Therefore, MIA is suspected to play an important role in HMGB1-overexpressing cancers. In OSCCs, MIA expression is significantly correlated with HMGB1 nuclear immunostaining. Extrinsic HMGB1 treatment does not alter MIA expression, whereas HMGB1 siRNA treatment decreases MIA expression. A physical association between HMGB1 and NFkB p65 is associated with MIA expression in OSCCs [61]. These findings show that HMGB1 might induce MIA expression acting as a nuclear protein but not as a secreted cytokine.

Malignant potential of OSCC

HSC3 cells are tongue squamous cell carcinoma-derived metastatic cell lines, which provide many sublines with