Fueling inflammation at tumor microenvironment: the role of multiligand/RAGE axis

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The receptor for advanced glycation end products (RAGE), firstly described in 1992, is a single-transmembrane and multiligand member of the immunoglobulin protein family. RAGE engagement produces activation of multiple intracellular signaling mechanisms involved in several inflammation-associated clinical entities, such as diabetes, cancer, renal and heart failures, as well as neurodegenerative diseases. Although RAGE expression has been extensively reported in many cancer types, it is now emerging as a relevant element that can continuously fuel an inflammatory milieu at the tumor microenvironment, thus changing our perception of its contribution to cancer biology. In this review, we will discuss the role of multiligand/RAGE axis, particularly at the multicellular cross talk established in the inflammatory tumor microenvironment. A better understanding of its contribution may provide new targets for tumor management and risk assessment.

Introduction

In the 19th century, Rudolph Virchow first launched the idea about a putative connection between inflammation and cancer. At present, resurgent research interests on this topic have raised a growing body of evidences supporting the contribution of chronic inflammation to the development of malignancies, as well as the positive association between the usage of non-steroidal anti-inflammatory agents and protection against formation of various tumor types (1,2).

It is estimated that almost 25% of all cancers are somehow associated with chronic infection and inflammation (3,4). Accordingly, several evidences derived from both epidemiological studies and basic research have shown that organ-specific carcinogenesis is linked to the development of a chronic local inflammatory milieu, as reported in pathological states (23). The mature 382 aa RAGE expression is also markedly augmented by increased levels of AGE formation of advanced glycation end products. AGE, advanced glycation end products; EPCs, endothelial progenitor cells; HMGBl, high-mobility group box 1; MDSC, myeloid-derived suppressor cell; NF-kB, nuclear factor-kappaB; NK, natural killer; NO, nitric oxide; NOS, nitric oxide synthase; PGE2, prostaglandin E2; RAGE, receptor for advanced glycation end products; TLR, Toll-like receptor; Tregs, T-regulatory cells.

Abbreviations: AGE, advanced glycation end products; EPCs, endothelial progenitor cells; HMGBl, high-mobility group box 1; MDSC, myeloid-derived suppressor cell; NF-kB, nuclear factor-kappaB; NK, natural killer; NO, nitric oxide; NOS, nitric oxide synthase; PGE2, prostaglandin E2; RAGE, receptor for advanced glycation end products; TLR, Toll-like receptor; Tregs, T-regulatory cells.

The RAGE

RAGE is a member of the immunoglobulin protein family of cell surface molecules (17) and shares structural homology with other immunoglobulin-like receptors (18), see Figure 1. Firstly described in 1992, RAGE has attracted increasing attention due to its diverse ligand repertoire and its involvement in several pathophysiological processes associated with inflammation, such as diabetes, cancer, renal and heart failure, as well as neurodegenerative diseases (19,20).

The RAGE gene is localized on chromosome 6 in the vicinity of the major histocompatibility complex class III region in humans and mice and in close proximity to both, the homeobox gene HOX12 and the human counterpart of the mouse mammary tumor gene int-3 (21,22).

RAGE is highly expressed during development, especially in the brain, but its expression level decreases in adult tissues. However, RAGE expression is also markedly augmented by increased levels of ligands, as observed in pathological states (23). The mature 382 aa long RAGE is composed of an extracellular domain (85 aa), a single-transmembrane spanning helix (27 aa) and a short cytosolic region (41 aa) (24). The extracellular domain of RAGE contains one variable-like V-domain (and two constant-like C-type domains frequently referred to as C1 and C2 domains. Recent studies suggest that RAGE forms oligomers at the cell surface (25). RAGE possesses two N-glycosylation sites, one adjacent to the V-domain and the second one located within the V-domain (26).

Recently, RAGE splice variants were classified and renamed according to the Human Gene Nomenclature Committee (27), and many of them appear to be more abundant under various pathological conditions. At DNA level, the RAGE gene consists of 11 introns/exons that can alternatively be spliced into the different variants. In terms of prevalence, the three major isoforms appear to be the full-length RAGE, a secreted form RAGE_v1 (previously named as sRAGE, secretory C-truncated RAGE, esRAGE, hRAGEsec or sRAGE1/2/3) and an N-terminally truncated isoform RAGE_v2 (previously named...
In addition to AGEs, RAGE ligands include members of the S100/calgranulins family, HMGB1, amyloid-β and amyloid A peptides. Some of these ligands (in bold) are particularly relevant in tumor biology. RAGE-v1-spliced version may function as a decoy receptor, preventing ligand interaction with full-length RAGE.

RAGE as a multiligand receptor

AGEs were the first identified RAGE ligands, particularly N-carboxymethyl lysine-modified proteins (29).

The formation of AGEs is based on the non-enzymatic reaction of the reactive aldehyde moiety of glucose with the amino groups of proteins, forming slowly reversible Amadori products. Rearrangement reactions then occur to produce a chemically related group of moieties, termed AGEs, which remain irreversibly bound to proteins (30). The presence of AGEs has been detected in human cancer tissues and their expression showed significant variation between different types of tumors (31).

It is becoming apparent that exogenous sources of AGEs, such as diet, may have significant impact on disease mechanisms as well. Diet is the major source of exogenous AGEs, showing the highest content in cooked foods, particularly those rich in carbohydrates, proteins and fats. The existence in the diet of AGEs commonly found in vivo, such as methylglyoxal and carboxymethyl lysine, has long been recognized by food chemists (32). However, their pathogenic significance has not been seriously considered.

In addition to AGEs, other molecules have been identified as RAGE ligands, as demonstrated for amyloid-β peptide and amyloid A, which are relevant to the physiopathology of Alzheimer’s disease and systemic amyloidosis, respectively (33,34). Of particular importance in tumor biology, S100/calgranulins and high-mobility group box 1 (HMGB1) have also been identified as ligands of this promiscuous receptor.

S100/calgranulin protein family comprises several members of non-ubiquitous Ca-binding proteins of the EF-hand type that have both intracellular and extracellular functions. At intracellular level, S100 proteins are responsible for different roles in cell cycle, cell differentiation and cell motility. However, some members of the family have additional relevant extracellular roles, particularly at sites of chronic inflammation where they are able to activate via RAGE endothelial cells, macrophages and peripheral blood mononuclear cells, including T lymphocytes (35).

The DNA-binding protein HMGB1 stabilizes nucleosome function and acts as a transcription factor that regulates the expression of several genes (36). HMGB1 belongs to the so-called damage-associated molecular pattern molecules or alarmins, which are released in response to infection or inflammatory stimuli, especially during tissue damage (37).

Both, S100/calgranulins and HMGB1 ligands are expressed and secreted by cancer cells (38,39). These ligands can interact, in an autocrine manner, to induce direct activation of cancer cells, thereby stimulating proliferation, invasion and metastasis. RAGE ligands derived from cancer cells can also influence a variety of important cell types within the tumor microenvironment, including fibroblasts, leukocytes and vascular cells, by RAGE-mediated mechanisms.

Most of the cancer-promoting effects of RAGE ligands are the result of their interaction with RAGE. Last year, two elegant works clearly demonstrated the relevance of RAGE signaling to tumor-promoting inflammation (40,41).

As already mentioned, cancer cells express and release RAGE ligands, which then can act not only in an autocrine fashion but also in a paracrine manner over many RAGE-positive cells at tumor-host interface.

A key consequence of RAGE engagement is the activation of multiple signaling pathways (Figure 2), including reactive oxygen species, p21ras, erk1/2 (p44/p42) mitogen-activated protein kinases, p38 and SAPK/JNK mitogen-activated protein kinases, rhoGTPases, phosphoinositol-3 kinase and JAK/STAT pathway, with important downstream inflammatory consequences, such as activation of nuclear factor-kappaB (NF-kB), AP-1 and Stat-3 (42–44).

At present, the role of NF-kB activation as a molecular bridge between inflammation and cancer is well documented, favoring the induction of several proinflammatory genes not only in tumor cells but also in tumor-associated cells, as well as surrounding host tissues (45). In this context, NF-kB activation promotes the transactivation of target proinflammatory mediators, such as COX-2, iNOS, TNF-α, IL-1 and IL-6, as well as antiapoptotic signals (XIAP, Bcl-2 Bcl-X) and proangiogenic factors (VEGF). Furthermore, NF-kB downregulates apoptosis-promoting genes (p53, Bax and Bad) (46).

NF-kB is tightly controlled on the basis of the right balance between activators [RAGE ligands, proinflammatory cytokines, Toll-like receptors (TLRs)] and inhibitors such as Toll IL-1R8, a member of the IL-1 receptor family (47). Because RAGE engagement triggers a sustained period of cellular activation and inflammatory signals amplification, it is tempting to speculate that RAGE may work by controlling a differential balance between activators and inhibitors, rather than triggering only activation signals. At present, no data are available about the putative effect of RAGE activation on Toll IL-1R8 expression.

Hypoxia is a common feature of the tumor microenvironment. It is associated with tumor progression, increased aggressiveness, enhanced metastatic potential and poor prognosis (48). There is an increasing body of both in vitro and in vivo evidences supporting the functional interconnection between NF-kB and HIF-1α since NF-kB is a critical transcriptional activator of HIF-1α and that basal NF-kB activity is required for HIF-1α protein accumulation under hypoxia (49). Strikingly, increased RAGE expression has been shown to confer cell resistance to a hypoxic milieu through the acquisition of a hypoxia-resistant phenotype in hepatocellular carcinoma cells (50). Hypoxia has also been reported to induce upregulation of the
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![Diagram of RAGE signaling](https://academic.oup.com/carcin/article-abstract/31/3/334/2477274)

**Fig. 2.** RAGE signaling. Ligation of RAGE initiates a series of cell-signaling events, such as reactive oxygen species production and activation of several kinases, leading to different inflammatory consequences, such as the activation of NF-κB, AP-1 and Stat-3. Intermediate events in these cascades are under active investigation. Interestingly, there is a convergence between RAGE- and TLR-2/4-signaling cascades, when activated by HMGB1. Based on shared functions, TLRs and RAGE may even cooperate to strengthen inflammatory response through the recruitment and/or assembly of homo- and hetero-oligomers; abbreviations used: Akt, a member of serine-threonine kinase family; AP-1, activator protein 1; Cdc 42, cell division cyle 42; Erk1/2, extracellular signal-regulated kinases 1/2; IRAKs, IL-1R associated kinases; JAK, Janus kinase; JNK, Jun N-terminal kinase; IRAKs, IL-1R associated kinases; JAK, Janus kinase; JNK, Jun N-terminal kinase; MMK, mitogen-activated kinase kinase; MyD88, myeloid differentiation primary response protein 88; NADPH, reduced nicotinamide adenine dinucleotide phosphate; PI3K, phosphoinositide 3-kinase; p38, p38 MAP kinase; Rac 1, Ras-related C3 botulinum toxin substrate 1; Ras, Ras GTPases; Stat-3, signal transducer and activator of transcription; TRAF, tumor necrosis factor receptor-associated factor.

HMGB1 is a first line of defense of the immune system, leading to the activation and recruitment of different types of immune cells to sites of infection and malignant cell growth (58).

Accumulating evidence suggests that the neoplastic process may sabotage TLRs signaling pathways to favor cancer progression (59). TLRs on tumor cells may facilitate their evasion from immune surveillance. Blockade of TLR-4 pathway reversed tumor-mediated suppression of T-cell proliferation and NK cell activity, delaying tumor growth and prolonging survival of tumor-bearing mice (60).

RAGE has the ability to interact with a wide range of endogenous ligands as well as with surface molecules on bacteria, prions and leukocytes through recognition of three-dimensional structures, rather than specific amino acid sequences (61). Noteworthy, ligand oligomerization not only may strengthen proinflammatory signaling upon RAGE stimulation but also can promote RAGE recruitment and dimerization (62). This process has been established as a general mechanism for the initiation of signal transduction, as demonstrated for cell surface receptors such as TLRs (63,64). Therefore, RAGE is thought to function as a pattern-recognition receptor, able to generate signaling pathways to incite and perpetuate an inflammatory condition.

Noteworthy, the ligand HMGB1 may signal through both RAGE and TLRs (TLR2 and TLR4), see Figure 2. Stimulation of these receptors results in the activation of NF-κB, AP-1 and Stat-3, thereby promoting inflammation (65). Based on these findings, as well as on the similarities between TLRs and RAGE signaling, RAGE has been considered to be a non-canonical Toll receptor (66). Thus, it is tempting to speculate that both TLRs and RAGE may cooperate as essential partners through the recruitment and assembly of homo- and hetero-oligomers in order to strengthen the inflammatory response.

The myeloid differentiation factor 88 (MyD88) plays a relevant role in inflammation-driven tumorigenesis (3,67). This factor is a critical downstream signaling molecule shared by TLRs, pattern-recognizing receptor and IL-1 and IL-18 receptors. Strikingly, HMGB1 has been reported as an essential component of DNA-containing immune complexes that stimulates cytokine production through a TLR9–MyD88 pathway involving the multivalent receptor RAGE (68).

One of the major obstacles to cancer immunotherapy is the immunosuppression that is frequently associated with cancer progression. This immunosuppressive state in cancer patients is characterized by downregulation of ζ chain of the T-cell receptor and NK-cell activating receptors, leading to T-cell and NK-cell dysfunction (69,70). Furthermore, several evidences suggest that myeloid suppressor cells recruited into lymphoid organs, as a result of chronic inflammation, may play an important role in the observed ζ chain downregulation in cancer diseases (71,72).

It has been suggested that the release of putative endogenous TLRs ligands during cancer progression may cause chronic inflammation, leading to the recruitment of myeloid suppressor cells and downregulation of T-cell and NK-cell receptor ζ chain resulting in T-cell and NK-cell dysfunction. Furthermore, it has been reported that a reduced inflammatory milieu in the tumor microenvironment markedly delays the accumulation of myeloid suppressor cells and limits tumor progression (73).

Just recently, it has been reported that the RAGE ligands S100A8/A9 proteins contribute to the recruitment and retention of myeloid suppressor cells through a mechanism mediated, at least in part, by the binding to carboxylated N-glycans expressed on RAGE and the subsequent activation of NF-κB signaling pathway (74).

Interestingly, AGEs can also downregulate *in vitro* the ability of dendritic cells to express costimulatory signals and to activate T cells (75). Similar results have been described after blockade of the autoimmune condition of HMGB1 and the inhibition of RAGE activation (76,77).

Under normal physiological conditions, T-regulatory cells (Tregs) have a beneficial role in preventing autoimmunity (78). A growing body of evidences indicates that Tregs within the tumor microenvironment play a key role in restraining antitumor immunity. From the mechanistic point of view, the suppression of antitumor immune response has been associated not only with the capacity of Tregs to
secrete IL-10, transforming growth factor β and prostaglandin E2 (PGE2) but also to cell–cell contact dependent inhibition (79,80). Additionally, Tregs at tumor microenvironment further suppress the CD4+–mediated induction of effective antitumor response by cytotoxic CD8+ T cells required for the control of tumor growth (81).

Of importance, HMGB1, which is extensively released not only at necrotic zones within tumors but also by chemotherapy, may stimulate CD4+CD25+Treg activity via RAGE binding on the surface of Tregs and triggering a shift of Th1 to Th2 pattern with suppression of T-lymphocyte immune function (82).

COX-2-derived PGE2 is associated with cellular immune tolerance during the process of tumorigenesis (83). PGE2 has been involved in the immunosuppressive milieu at tumor microenvironment, either by the accumulation and retention of myeloid-derived suppressor cells (MDSCs) (84) or the induction of FOXP3 expression and T regulatory functions in CD4+ T cells (85,86). Additionally, the enzyme indoleamine 2,3-dioxygenase, which exerts well-established immunosuppressive functions in tumorigenesis, is also linked to COX-2-derived PGE2 since the antitumor effects of COX-2 inhibitors correlate with the inhibition of indoleamine 2,3-dioxygenase and Tregs activity (87).

Indoleamine 2,3-dioxygenase is expressed within the tumor itself as well as by antigen-presenting cells in tumor-draining lymph nodes, where it promotes the establishment of peripheral immune tolerance to tumor antigens and the recruitment, expansion and activation of Tregs (88). In this particular context, a very interesting cross talk has been recently reported between RAGE activation and COX-2 regulation. RAGE ligation by S100b produces an increased stabilization of COX-2 messenger RNA by the opposite actions of movement of key DNA/RNA-binding protein (hnRNK) from the COX-2 promoter to the 3′ untranslated region and a marked reduction in microRNA binding (miR-16) to the same region, thereby rendering stabilized transcripts of relevance in the immunosuppressive tumor microenvironment (89).

All these data are highly suggestive that RAGE activation contributes to a tumor-promoting milieu as depicted in Figure 3.

Angiogenesis

Since the pioneering report by Folkman in 1971, compelling evidences support the role of angiogenesis as a key player in tumor growth, invasion and metastasis (90,91). In order to stimulate angiogenesis, tumor cells and surrounding stromal cells produce several proangiogenic factors (92).

In recent years, a growing body of evidence supports the role of multiligand/RAGE axis in angiogenesis. Upon RAGE activation, profound effects are reported in endothelial cells, including upregulation of vascular endothelial growth factor and metalloproteinase-2, as well as the disruption of VE-cadherin–catenins complex, thereby favoring capillary tube formation (93–95). Additionally, RAGE activation also increases endothelial permeability to macromolecules, a condition very common in tumor microvasculature (96).

Although many aspects on differentiation, mobilization and recruitment of endothelial progenitor cells (EPCs) remain controversial, it has been reported that the levels of peripheral blood EPCs have been shown to be increased in certain malignant states (97). HMGB1 increased EPCs adhesion to the immobilized integrin ligands intercellular adhesion molecule-1 and fibronectin, in a RAGE-dependent manner, and thus stimulating EPCs homing to ischemic tissues (98).

It is known that Epstein-Barr virus oncproteins, such as latent membrane protein 1, contribute to the metastasis of nasopharyngeal carcinoma by inducing factors to promote tumor invasion and angiogenesis (99). As recently reported, promotion of angiogenesis in lymph node metastasis by latent membrane protein 1 is associated with an increased expression of RAGE (100).

Besides promoting T-cell and NK-cell dysfunction, MDSCs are now considered as tumor-associated cells that could be involved in the promotion of angiogenesis. These cells stimulate angiogenesis by releasing soluble factors such as matrix metalloproteinase 9 and vascular endothelial growth factor, as well as directly by differentiat-
oxide synthase (NOS) II is expressed in neoplasias of various species, including human tumors of diverse tissue origins. Some studies have found that NOS II is tumor suppressive, whereas others have provided evidence that NOS II promotes tumor growth (108). Recent studies using NOS II−/− animals and tumor cells have shown that NO produced by host stromal cells can be sufficient to execute antitumor activity (109). Accordingly, NO production by NOS II is upregulated by RAGE activation by different mechanisms, as reported by several authors (110,111).

The putative contribution of endothelial NOS III has also called the attention of various research groups. Experimental approaches using NOS III-deficient mice demonstrated that animals developed liver tumors more frequently in response to carcinogens compared with controls. On the contrary, NOS III overexpression in the tumor microenvironment attenuated both the number and size of tumor implants (112). We and others have demonstrated that RAGE engagement on endothelial cells produce a marked reduction in NO synthesis by downregulating NOS III expression (113,114).

However, the real impact of RAGE activation on l-arginine:NO pathway in tumor microenvironment remains to be fully understood, mainly due to the coexistence of two enzymatic systems competing for the same substrate as do arginase and NOS, which can directly activate several biochemical circuits that negatively regulate each other (115).

Invasion and metastasis
The invasive activity of cancer cells is controlled by several independent but coordinated processes. Cell adhesion, cell motility and production of matrix metalloproteinases are essential for cell invasion. The role of RAGE and its ligand HMGBl on cell motility was initially reported in neurite outgrowth in the normal developing brain, where RAGE engagement accelerates the formation of filopodia at the leading edge of motile cells, as well as adhesion and detachment from extracellular matrix (116). In 2000, a seminal report on the contribution of multiligand/RAGE axis on invasion and metastasis demonstrated that blockade of RAGE-HMGBl-derived signaling decreased growth and metastases of both implanted tumors and tumors developing spontaneously in susceptible mice (117).

Similar results have confirmed the role of RAGE in both invasion and metastasis. Prevention of tumor formation of melanoma cell xenografts in athymic mice was achieved by treatment with anti-RAGE neutralizing antibodies. Additionally, in tumor-bearing mice, survival rates were prolonged, and spontaneous pulmonary metastases were inhibited by anti-RAGE neutralizing antibodies (118).

Moreover, in squamous cell carcinoma cell lines, both metastatic and invasive activities were significantly reduced by RAGE antisense S-oligodeoxynucleotide treatment (14). Contrarily, RAGE expression was negatively correlated with depth of tissue and venous invasion in patients with esophageal squamous carcinoma (119).

Although NO can also enhance migration, invasion and metastasis by activating mitogen-activated protein kinase-signaling dependent process, as observed in breast and colon cancer (120), the real contribution of NO remains to be fully understood and limited by the complex functional cross talk between both arginase and NOS pathways, as already mentioned.

The usage of non-steroidal anti-inflammatory agents has been correlated with protection against the development of various tumor types. It is widely accepted that deregulation of COX-2 leads to increased levels of PGE2, and both factors are thought to play crucial roles not only in the genesis of colorectal cancer but also as necessary for sustained angiogenesis and cancer cells dissemination to other organs (83). The finding that RAGE activation in many cell types including human monocytes can significantly increase both COX-2 messenger RNA and protein expression, together with a rise in PGE2 levels, highlights the importance of RAGE in promoting neoplastic outcomes (121).

Finally, many gene polymorphisms have been assessed for their effects on cancer risk in population studies (122,123). Very recently, some RAGE polymorphisms have been reported to confer increased risk of gastric and breast cancers (124,125). Hereafter, RAGE polymorphisms should be taken into consideration in our efforts to pinpoint the contribution of multiligand/RAGE axis in human cancer.

Conclusion
At present, compelling evidences demonstrate that fueling inflammation at the tumor microenvironment creates a tumor-promoting milieu, which in turn favors proliferation and survival of cancer cells. In this context, emerging experimental data suggest that the multiligand/RAGE axis may be an important contributor to inflammation-related tumorigenesis through different signaling mechanisms. These may include the activation of key processes that might promote resistance to apoptotic insults and hypoxia, interfering with antitumor immunity, stimulating angiogenesis and supporting invasiveness.

However, many questions remain to be addressed to fully understand the role of this promiscuous receptor in tumor–host cell interactions, particularly those involved in the gradual switching of macrophage polarization toward an M2 phenotype, the recruitment and stimulation of MDSCs and Tregs and the cross talk between RAGE and TLRs.

Given the importance of the acquisition of a hypoxic-resistant phenotype to poorly oxygenated milieu present at tumor microenvironment, the putative cross talk between RAGE and many other elements also relevant to cell survival in hypoxic conditions should be further studied, specially with reference to glucose transporters, glycolytic enzymes as well as erythropoietic and angiogenic factors.

Further insights aimed to clarify the role of the multiligand/RAGE axis in the generation of an immunosuppressive microenvironment that jeopardizes antitumor immunity is a fascinating challenge for the coming years. This is particularly attractive since nowadays many current therapeutic options are focused on targeting tumor microenvironment interactions, rather than tumor cell itself, and many of them are directed to block tumor-induced immune suppression. Furthermore, the development and success of therapeutic cancer vaccines are also highly impacted by the immunosuppressive milieu within tumor microenvironment.

In this complex scenario, multiligand/RAGE axis has emerged as a very attractive target for pharmacological interventions directed to block RAGE–ligand interactions at the receptor level. However, it is also possible to envisage strategies oriented to interfere downstream post-receptor signaling. These actions represent distinct potential therapeutic alternatives that may disrupt the inflammatory milieu at tumor microenvironment, thereby detaining tumor progression.

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