Review

Evangelos Marinakis*, Georgios Bagkos, Christina Piperi*, Paraskevi Roussou and Evanthia Diamanti-Kandarakis

Critical role of RAGE in lung physiology and tumorigenesis: a potential target of therapeutic intervention?

Abstract: Lung cancer is one of the most common malignancies in the world and one of the leading causes of death from cancer. In the search for molecules that may be involved in lung tumor induction and progression, the receptor of advanced glycation end products (RAGE) comes across as a critical regulator of lung physiology. RAGE is a multiligand receptor that presents a differential expression pattern in lung epithelial cells compared to other cell types being gradually increased from fetal to birth and adult life. Under stress conditions, RAGE expression and activation are rapidly elevated resulting in chronic inflammation, which, in turn, in many instances, promotes epithelial cell malignant transformation. RAGE overexpression in normal lung alveolar type I epithelial cells is followed by rapid downregulation upon malignant transformation, being associated with increased aggressiveness. This is a striking paradox, since in every other cell type the pattern of RAGE expression follows the opposite direction, suggesting the involvement of RAGE in the well-functioning of lung cells. Additionally, RAGE has been attributed with the role of adhesion molecule, since it can stabilize mature alveolar epithelial cells to their substrate (basal lamina) by interacting electrostatically with other molecules. However, the reduction of RAGE observed in lung tumorigenesis interrupts cell-to-cell and cell-to-substrate communication, which is a critical step for cancer cell induction, progression and migration. This review addresses the differential properties of RAGE in lung physiology and carcinogenesis, providing evidence of therapeutic possibilities.

Keywords: cancer; electrostatic charge; lung; receptor for advanced glycation end products.

Introduction

The receptor for advanced glycation end products (RAGE), also known as advanced glycosylation end product-specific receptor (AGER), is a transmembrane pattern recognition receptor of the immunoglobulin superfamily [1, 2]. Several RAGE isoforms have been recognized including a truncated circulating soluble form of RAGE lacking the transmembrane and the cytosolic domain (sRAGE), arising from receptor ectodomain shedding, as well as splice variant [endogenous secretory RAGE (esRAGE)] secretion [3, 4]. These isoforms represent naturally occurring competitive inhibitors of RAGE signaling pathways, being capable of counteracting RAGE-mediated pathogenesis and acting as potential biomarkers of endogenous protective response.

RAGE and its isoforms are encoded in the class III region of the major histocompatibility complex (chromosome 6, 6p21.3) along with other members of the innate immune system. RAGE structure consists of five domains: a short intracellular negatively charged C-terminal tail, a single transmembrane domain and three extracellular positively charged immunoglobulin-like domains (V, C1
and C2). The V domain binds most of the RAGE ligands, whereas the cytosolic tail is essential for downstream signal transduction [5–7].

RAGE was firstly identified in bovine endothelial cells and initially characterized by its ability to bind AGEs [1, 2], a complex group of heterogeneous compounds [8–12]. However, nowadays, the list of RAGE ligands has been significantly extended, with RAGE acting either as a receptor or as an adhesion molecule [13], being able to bind to amphoterin, S100 proteins, integrin Mac-1, collagen IV, amyloid-β and amyloid A peptides, glycosaminoglycans and others [5, 6, 13–20].

This property of RAGE to recognize and properly respond to myriads of diverse molecular ligands represents a challenge to the commonly accepted view of molecular recognition, where each receptor has one single ligand that binds to a specific area on the receptor’s surface [5]. Intensive research efforts are currently focused on the elucidation of multiligand recognition patterns by RAGE and the specificity of cellular response elicited by each of them.

Furthermore, the RAGE/ligand interaction activates several diverse cellular pathways. The type of cell response is signal-tissue specific and depends on the type of ligand, the particular cellular environment and the cell type. Thus, RAGE downstream signaling activates several diverse cell activities, such as differentiation, proliferation, adhesion and migration [23, 24, 21–24]. It is generally believed that RAGE-induced pathways are implicated in the development of several common diseases [15, 25–29]. This view is contradictory to data showing that the activation and upregulation of RAGE enhance cell survival in cases of cell dysfunction due to increased AGE levels, oxidative stress, etc. [16]. Thus, it seems highly likely that the observed RAGE upregulation in clinical disorders represents the cell defense response for dealing with the insulting cell damage. This is further supported by the fact that all known RAGE ligands are able to influence cell functioning independent of RAGE presence; for example, high-mobility group box 1 (HMGB1) can signal through Toll-like receptors (TLR) 2 and 4, bypassing RAGE [30, 31], whereas AGEs, among many other side effects, can inhibit the function of electron transport chain, reduce ATP synthesis and increase reactive oxygen species (ROS) levels [32].

In almost every cell type, RAGE expression is gradually decreased during the embryonic stages of development. Thus, it is usually downregulated in most mature cells, including endothelial, smooth muscle cells, fibroblasts, neurons and immune cells. RAGE has been functionally linked to almost all cell types involved in immune responses [23, 33–36]. In endothelial cells, RAGE acts just like an adhesion molecule interacting with leukocyte β2 integrin Mac-1 [13]. When activated, immune response cells enhance RAGE expression. Increased levels of RAGE have been observed in acute and chronic inflammatory diseases and cancer. Thus, the RAGE/ligand interaction represents the common causative mechanism that links chronic inflammation to cancer [6, 15, 16, 29, 37].

Regarding tumorigenesis, RAGE and its ligands are commonly overexpressed in most types of solid tumors [18, 38–48]. For example, both amphoterin and RAGE are overproduced in inflammatory states, as well as in many malignant tumors with high metastatic capacity [42–48]. However, a recent study showed that increased plasma AGE levels are associated with a protective effect over non-small cell lung cancer (NSCLC) tumor progression, presenting a simple predictor of patients’ outcome after curative surgery [49].

### The role of RAGE in lung physiology

Mature lung cells express higher levels of RAGE than other cell types. This suggests that RAGE has a specific role in lung cells (Table 1). Existing evidence shows that, in this case, RAGE is serving lung cells as an adhesion molecule rather than as a real receptor. The lung alveolar epithelium consists of two cell types: alveolar type I (ATI) and alveolar type II (ATII) epithelial cells. ATII cells are cuboidal in shape and, although more abundant, cover much less area of the alveolar surface. They are the primary sites of surfactant synthesis with a capacity to proliferate and differentiate into ATI cells. In contrast, mature ATI cells are thin and flat and cover more than 95% of the alveolar surface of the lung, being unable to differentiate. Their thin and flat morphology makes them ideal for normal bidirectional gas exchange in the lung [62]. Evidence shows that RAGE is mainly found at the basolateral membrane of ATI pneumocytes, establishing the contact between ATI cells and their substrate [22, 50, 65].

In the developing rat lung, RAGE expression is gradually increased from fetal to birth and adult lungs [51]. This pattern was demonstrated in both membrane and sRAGE protein expression, predominantly in ATI pneumocytes. Neonatal rat lung, which is not fully alveolarized, exhibits low RAGE expression, whereas the postnatal upregulation of RAGE reflects ongoing alveolarization characterized by an expansion of type I epithelial cell population. These observations are in accordance with the study of Demling et al. [22], where RAGE appears to be a highly selective...
Table 1: Differential pattern of RAGE expression: impact on lung physiology and cancer.

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<th>Normal RAGE expression</th>
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<td>Normal ATI cell differentiation and phenotype [22, 50, 51]</td>
<td>Normal: embryonic stages [50, 51]</td>
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<td>Normal ATI cell adhesion, spreading and stability [22]</td>
<td>Tumorigenesis: cancer cell regression to embryonic phenotypes [37, 52–58]</td>
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<td>Normal alveolar cell apoptosis</td>
<td>Decreased adhesiveness, increased migration, enhanced invasiveness [15, 22, 37, 59–61]</td>
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<td>Normal lung architecture [22, 64]</td>
<td>Malignant behavior [59, 60, 61, 63]</td>
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<td>Normal cell-to-cell and cell-to-matrix communication</td>
<td>Malignant tissue and tumor stroma reorganization and modification [15, 16, 29, 59–61]</td>
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differentiation marker that promotes adherence and spreading of human alveolar epithelial cells. Furthermore, the optimal regulation of RAGE expression is a prerequisite for normal lung development. Mice overexpressing RAGE during lung embryogenesis show altered respiratory epithelial cell differentiation, severe lung hypoplasia and exhibit 100% perinatal mortality rates [64]. These observations suggest that tight regulation of RAGE expression is a requirement for alveolar epithelial cell differentiation. Moreover, RAGE assists ATI cells in obtaining the flat and extended morphology, which is optimal for effective gas exchange [22].

At the same time, RAGE is one among many other receptors that assumes the role of an adhesion molecule. RAGE inhibition results in decreased adhesiveness and increased cell migration and invasiveness [15, 22, 37, 65–68]. Structural analysis revealed that the V domain of RAGE has features typical of I-set topology, which are characteristic of cell adhesion molecules [65–68]. The specific localization of RAGE between cells and their substrate strongly suggests that RAGE is the main, or even the only, adhesion molecule that stabilizes alveolar cells to their position. Lung epithelial cells, due to their role in gas exchange, have to be as thin and transparent as possible. RAGE is just the right choice, which ensures the optimal transparency and performance for these cells. The more complex molecular entities, which perform the same task, cannot be used because they will increase cell thickness, resulting in a reduced alveolar cell transparency and gas exchange efficiency.

It is well documented that electrostatic interactions play a key role in protein-protein recognition as well as in receptor/ligand recognition and binding [69–75]. Moreover, it is well known that many different cells are able to receive and respond to extremely weak electromagnetic fields emitted from different external sources [69–75]. In fact, due to the action of their receptors, cells are the only known entities that are able to receive extremely low frequency electromagnetic signals ranging from a few to 300 Hz [76]. This cannot be achieved, not even approximately, by most modern electromagnetic devices. Also, a large body of evidence shows that the living organisms from cells to humans possess a huge spectrum of receptors (photoreceptors) that are able to efficiently receive electromagnetic fields (signals). The V domain of RAGE, which is the major contributor to ligand “binding”, is positively charged [5, 77–80]. This positive charge extends far enough and renders RAGE an electrostatic trap for its negatively charged ligands [5, 77, 79–82]. Also, there is strong evidence that RAGE interacts with the negatively charged patches of AGE-modified proteins [5, 79, 80]. In addition, electrostatic charge modifications alter RAGE-ligand interactions and downstream cell signaling pathways, affecting major cell functions such as adhesion, migration and proliferation [81, 82]. The existing differences in individual electrostatic field physical properties of ligands may represent the characteristic electrostatic identity of each particular ligand. Given the evidence coming from different scientific fields, it is safe to assume that RAGE, just like many other receptors, is able to efficiently receive the extremely weak electrostatic field (signal) emitted from electrically charged ligands (Figure 1).

It has been shown that RAGE is present in cell membrane in the form of RAGE oligomers [16]. Oligomerization of RAGE provides a mechanism to increase the number of extracellular V-binding domains, which, in turn, enhance RAGE-ligand binding affinity [16]. Also, it is known that the clusters formed from receptor polymers or heteropolymers contain several different receptors and so acquire a higher sensitivity and accuracy. Thus, RAGE polymers are able to efficiently recognize the extremely weak electrostatic signals (fields) generated by their ligands and discriminate each one of them. The selective electrostatic interaction between RAGE and its ligands can explain the higher binding affinity that is observed when the multimers of RAGE interact with the multimers of ligands. It can also
explain the difference in binding affinity between different ligands, since each one of them possesses its own specific electrostatic signal.

Touré et al. showed that neutrophils exhibit enhanced adhesiveness and decreased migration on AGE-modified collagen in comparison to non-modified collagen [68]. Consequently, collagen glycoxidation may inhibit normal neutrophil migration in patients with diabetes and kidney disease, i.e., in conditions that are characterized by increased levels of AGES and RAGE. Furthermore, RAGE antibody inhibits neutrophil adhesion and enhances neutrophil migration on glycoxidated collagen [68]. Based on the electrostatic viewpoint, the increased adhesion of neutrophils to AGE-modified collagen can be explained by the AGE-modification process itself, during which negative charge is added to collagen and, thus, collagen acquires a higher electrostatic reactivity with RAGE.

Regarding the normal lung, RAGE mediates the adherence of alveolar cells on basal lamina (basal membrane). Alveolar basal lamina consists of numerous anionic (negatively charged) sites that contain heparan sulfate proteoglycans [83]. The heparin sulfate proteoglycan family includes, among others, collagen XVIII, which is abundant in the lung, although less than collagen IV [84]. The electronegativity enables heparin sulfate chains to interact with variable specificity with several growth factors and receptors and, thus, to affect many diverse cellular functions [85–91]. Heparan sulfate plays a crucial role in normal lung development. A decrease in heparan sulfate level is related to lung hypoplasia and other developmental malformations with increased perinatal mortality [92, 93]. In the lung, glycosaminoglycans and heparan sulfate bind strongly to RAGE and RAGE ligands such as HMGB1 [19, 20, 94]. Thus it is highly likely that the observed adhesion of lung epithelial cells with basal lamina is achieved by the electrostatic interactions exerted between RAGE and basal lamina glycosaminoglycans and heparin sulfate chains.

**Figure 1** RAGE as a sensor of ligand-specific electrostatic fields. RAGE senses ligand-specific electrostatic fields. V domain (black V shapes) is a positively charged electrostatic trap for negatively charged ligands (gray triangles). RAGE recognizes the individual electrostatic field emitted by a particular ligand. These electrostatic fields are ligand specific.

**RAGE expression is upregulated in most cancer tissues, except the lung**

RAGE and RAGE ligands are involved in cancer development and migration in most types of malignant diseases. Several detailed reviews and experimental reports clearly show that the multiligand-RAGE axis is implicated in the development of chronic inflammation, together with regional and systemic immune response deregulation, intercellular communication derangement and tumor promotion [6, 15–17, 29, 37, 38, 95].

RAGE activation modulates numerous intracellular factors, such as ROS, extracellular signal-regulated protein kinase (ERK1/2), p38 and stress-activated protein kinase/jun N-terminal kinase, mitogen activated protein kinases (MAPKs), p21ras and Rho GTPases (Rac1, Cdc42), phosphoinositol-J kinase, janus kinase/signal transducer and activator of transcription pathway, nuclear factor kappa B (NF-κB), activator protein-1 (AP-1) and activator of transcription 3 (STAT-3) [6, 15–17, 37, 38]. That is, RAGE activation has been considered to promote hypoxia resistance, angiogenesis of tumor vasculature, tumor growth and invasion, and modulation of the host immune response, contributing to the immunosuppressive state seen in cancer patients [15, 16, 29].

In most cancer types, a parallel increase in the levels of RAGE and RAGE ligands has been observed. RAGE ligands act in an autocrine fashion and induce direct activation of cancer cells by stimulating proliferation, invasion and metastasis. Moreover, they act in a paracrine manner over RAGE-positive cells within the tumor microenvironment. Thus, it is not clear whether the observed cellular responses are due to RAGE activation or to the direct influence of its ligands.

**Role of RAGE in lung cancer**

Lung cancer, one of the most invasive malignancies, expresses low levels of RAGE [37], which correlate positively to increased tumor growth and invasiveness [59, 60]. Furthermore, RAGE genes may have a role in the diagnosis of lung neoplasms, since they can be employed for the discrimination between normal and malignant lung tissues.
Investigation of the RAGE expression between normal lung and NSCLC tissues demonstrated a strongly reduced or absent expression in NSCLC tissues, both at the transcriptional and at the protein level, compared to normal [63]. Furthermore, a study of lung specimens from tumor and paired non-tumor tissues of 34 NSCLC patients, who underwent pulmonary resection surgery, showed that the reduced mRNA level of RAGE in NSCLC correlated with higher tumor stages [59]. Since the differences were even more prominent at the protein level, further post-transcriptional regulation must take place. The same reduction was also observed in squamous cell lung carcinomas and adenocarcinomas [59] as well as in benign lung neoplasms (hamartomas). Additionally, in metastatic lesions originating from the kidney and colon, the magnitude of RAGE reduction is comparable to the reduction observed in primary lung cancer specimens [59].

There is evidence that downregulation of RAGE in human NSCLC may contribute to the loss of normal cell differentiation and epithelial structure organization with concomitant oncogenic transformation (Table 1). Cultures of NCI-H358 cells on collagen layers exhibited a spreading epithelial-like growth of both RAGE- and ΔcytoRAGE-expressing cells (i.e., cells expressing RAGE without cytoplasmic domain), which was not seen in NCI-H358 control cells. In particular, RAGE-deficient lung cancer cells exhibited a multicellular-complexed proliferation pattern. Immunocytochemistry revealed a diffused membrane localization of RAGE in NCI-H358 single cells and the redistribution of the receptor towards intercellular contact sites at higher cell densities. Interestingly, ΔcytoRAGE-expressing cells, although lacking the cytosolic domain, exhibited preferential localization of the truncated receptor at cell-cell contact sites as well. Consequently, formation of intercellular contacts requires either the full-length or the truncated receptor, and RAGE localization is independent of the intracellular domain [59].

Regarding cell migration, it has been observed in ΔcytoRAGE transfection that overexpression of RAGE did not induce cell migration in vitro, suggesting that activation of RAGE per se does not contribute to tumor cell migration and metastases. In contrast, primary lung cancer and metastatic lesions demonstrate reduced RAGE expression, indicating that neither RAGE downregulation nor RAGE overexpression contributes to malignant phenotype behavior per se [59].

Co-cultivation of RAGE-deprived human lung cancer cells (H358, a NSCLC cell line) with lung fibroblasts (WI38) augments the proliferation of cancer cells, both in monolayer and in spheroid culture models. By contrast, the proliferative stimulus of fibroblasts is reduced in lung cancer cells overexpressing full-length RAGE [60]. These results show that the influence of fibroblasts in cancer cells is not mediated through RAGE but through other receptors like the Wnt, Notch and Hedgehog. Fibroblast-induced cancer cell proliferation was associated with increased activation of particular MAP kinases (p42/44) in lung cancer cells. The behavior of ΔcytoRAGE-expressing cells was investigated as well. Curiously, these cells often tended to a higher stimulation of p42/44 MAPKs (ERK1/2). Moreover, in three-dimensional cultures, they form the largest spheroids in response to fibroblasts impact, thus behaving like mock-transfected cells [60].

Whereas RAGE expression itself is downregulated in lung cancer, RAGE ligands are widely overexpressed [37]. Overexpression of S100 family members is related to worse prognosis and poor survival in patients with lung cancer [99–101]. Significant alterations in tissue and serum levels of HMGB1 (amphoterin) in lung cancer have also been reported [102–105].

The study of the RAGE variants and lung cancer relations provide interesting results. sRAGE is a RAGE isoform that lacks the transmembrane and cytoplasmic domains. Since it carries the V domain, sRAGE has the ability to bind ligands, acting as a decoy receptor that blocks RAGE signaling. Serum sRAGE levels are significantly decreased in lung cancer patients compared with healthy controls, and they are negatively correlated with lymph node involvement [98]. Similarly, the expression of cytoplasmic esRAGE, which is a splice variant of RAGE ubiquitously present in normal lung tissue, decreases or disappears in most NSCLC tissues. Lack of expression is related with poor histological differentiation, higher Ki67 index, increased tumor invasiveness and decreased survival after surgical resection in patients with stage I lung cancer [61]. However, esRAGE and RAGE expression may have different effects on cell proliferation and tumor growth, and may be independently regulated [61].

Malignancies share a number of major characteristics that include (a) cell dedifferentiation and acquisition of embryonic phenotype, (b) disruption of normal cell-to-cell and cell-to-matrix communication with concomitant cell isolation – autonomy and derangement of normal tissue architecture, (c) avoidance of apoptosis and (d) enhanced proliferation, invasiveness and metastatic potential. In order to acquire the above major characteristics, cancer cells accomplish a large number of structural, functional and metabolic changes. The outcome of these changes is the return of cells to embryonic stages. To this sense, lung cancer cells re-program the expression of numerous receptors,
such as RAGE and RAGE-ligands, epidermal growth factor receptor, vascular endothelial growth factor, β1 integrins, TLRs, Wnt, dishevelled, Notch receptors, connexins and others. In NSCLC, upregulation of expression has been reported for integrin α5β1, S100A8/A9, HMGB1, TLR4/9, dishevelled, Wnt-1, Wnt-2 and Notch 3 [52–55, 106, 107].

Interestingly, the same group of receptors is also overexpressed in embryonic stages of lung development. Their expression is suppressed in normal adult lung, only to be once again upregulated as part of the pathogenesis of malignant lung diseases. For example, Wnt, Notch and hedgehog receptors are normally expressed in embryonic lung cells and are downregulated in mature lung cells. Then, they are once more fully expressed in lung cancer cells [52, 53, 55, 106]. That is, the pattern of Wnt, Notch and hedgehog expression is linearly negatively correlated with the corresponding pattern of RAGE isoform expression. When one group of receptors is upregulated the other is downregulated. Thus, it is possible that, in lung cells, each one of these groups can efficiently substitute the other as signal receptors – although these receptors do not substitute RAGE in its role as an adhesion molecule.

In lung ATI cells, RAGE acts both as a receptor and as an adhesion molecule serving differentiation and adhesion. When these cells are malignantly transformed, RAGE presence is no longer required. Thus, lung cancer cells reduce RAGE production in order to break the intercellular and cell-matrix contacts and get ready to proliferate, migrate and invade. That is, downregulation of RAGE along with the concurrent upregulation of Wnt, Notch and hedgehog reflects the regression of lung cancer cells to embryonic phenotype (Figure 2).

**RAGE as a potential target of therapeutic intervention: evidence and concerns**

Animal models have demonstrated that blockade of RAGE signaling with various experimental methods, such as sRAGE antagonists, anti-RAGE antibodies and RAGE silencing, may be beneficial under certain circumstances. Potential therapeutic applications of RAGE-axis blockade include prevention of diabetic complications, treatment of acute or chronic inflammatory diseases and neurodegenerative diseases, management of myocardial and pulmonary ischemia-reperfusion injury, organ transplantations, acute lung injury and lung fibrosis therapy, as well as targeted cancer therapy and prevention of metastases formation to the lung [19, 26, 56–58, 61, 68, 107, 108–110].

However, in terms of RAGE-axis inhibition as a potential therapeutic multi-tool, one cannot draw a general conclusion that would be widely applicable. The view of RAGE as a receptor that mediates purely damaging effects and, consequently, the therapeutic approaches that aim in its blockade or inhibition present a rather oversimplified and misleading assumption [111]. Although there is evidence that RAGE-axis inhibition may be beneficial in certain occasions, experimental data exist that show RAGE blockade inducing deleterious side effects, whereas increased expression of RAGE or RAGE variants being beneficial [59, 60, 61, 66, 98].

According to evidence coming from in vitro and in vivo experimental and clinical studies on lung cancer (especially NSCLC), RAGE and RAGE variants are downregulated in lung cancer tissues. Nevertheless, this observation is not specific for lung malignancies, since RAGE expression is suppressed in both non-malignant lung tumors and lung metastatic disease from other primary sites [59].

Cell line and in vivo studies have demonstrated that RAGE overexpression has an inhibitory effect on the
proliferation and migration of lung cancer cells and tumor growth [59–61]. However, there are significant differences between observations seen in monolayer and spheroid cultures, as well as among particular studies, leading to the conclusion that RAGE re-expression per se does not inhibit tumor growth. A reasonable therapeutic approach would be the controlled upregulation of RAGE expression in lung cancer tissues; however, the precise and specific in vivo regulation of the expression of this ubiquitous receptor represents a great challenge. Moreover, although maintenance of RAGE expression appears to be a reasonable way of inhibiting lung tumorigenesis, it still remains theoretical. Consequently, considering the regulation of RAGE expression (and re-expression) as a potential therapeutic tool for lung cancer therapy is a very complicated issue. What may be more feasible as a therapeutic intervention is the prevention and treatment of lung metastatic disease arising from other primary tumors, such as melanoma. Both in vitro and in vivo studies demonstrate that ligand-RAGE axis inactivation restricts metastatic formation to the lung [19, 20, 112–114].

This observation applies to the inhibition of RAGE activation by classic ligands and several other molecules like glycosaminoglycans. In vivo studies have shown that glycosaminoglycan-RAGE interactions are promising targets in the prevention and management of pulmonary metastases [19, 20]. Administration of the derivative 2-O, 3-O-desulfated heparin significantly reduces the formation of lung metastasis after intravenous injection of melanoma cells [113]. The mechanism of protein inhibition by heparin and non-anticoagulant heparin derivatives is exerted by electrostatic interactions between heparin and positively charged proteins [115]. Thus the beneficial effects of heparin may also apply to RAGE interaction with these compounds, since the V-domain binding region of RAGE is characterized by its highly positive charge. RAGE/ligand binding inhibition by heparin and heparin derivatives can be explained by the competition between these compounds and the classic RAGE ligands for electrostatic binding within the cationic V-domain of RAGE.

Tissue and serum measurements of RAGE and RAGE variants in lung cancer patients could have diagnostic and prognostic significance, thus influencing indirectly treatment decisions [59, 61, 98, 116]. RAGE genetic polymorphisms were investigated in a prospective study with 562 patients with NSCLC and 764 healthy subjects. The single-nucleotide polymorphism G82S (rs 2070600) is located in RAGE gene exon 3, in a putative ligand binding site [117]. Patients with advanced NSCLC and the 82G/S polymorphisms showed significant differences in chemotherapy response, with responders being characterized by a remarkably lower 82SS and 82GS genotype than non-responders.

Another finding of the study was that 82GS and 82SS carriers had a significantly increased risk for NSCLC. Therefore, polymorphisms of the RAGE gene could be used as a screening diagnostic test to identify subjects at high risk for lung cancer, as well as prognostic markers that could modify treatment decisions in patients with NSCLC [116].

sRAGE and tissue esRAGE may prove to be useful biomarkers for the diagnosis of lung cancer, as well as for the assessment of prognosis [61, 98]. Serum concentration of sRAGE is markedly reduced in patients with lung cancer, independent of histologic type, and lower serum levels being correlated to lymph node infiltration [98]. Furthermore, immunohistochemical studies in 182 NSCLC surgical specimens demonstrated that downregulation of cytoplasmic esRAGE is an independent prognostic factor of survival, especially in patients with TNM stage I. Additionally, absence of esRAGE immunoreactivity was associated with significantly increased mortality, with a 5-year survival in patients with NSCLC stage I below 20% [61, 116].

Conclusions and perspective

RAGE occupies a central position in developmental and adult lung physiology and disease. Both as a receptor and as an adhesion molecule, RAGE is related to alveolar cell differentiation and preservation of normal lung architecture and function. The distinct property of RAGE to recognize and interact with numerous and diverse ligands can only be explained through an electrostatic viewpoint, according to which RAGE senses the electrostatic fields (signals) emitted by its ligands. This view may also apply to RAGE-mediated alveolar cell adhesion to alveolar basal lamina.

Deregulated RAGE expression contributes to lung morphogenic disorders, as well as to the development of inflammatory and neoplastic diseases in adult lung. RAGE is markedly downregulated in lung tumors, both at the mRNA and at the protein levels.

Lung cancer cells downregulate RAGE by detaching from the surroundings and then retaining a low differentiation state, whereas they interrupt normal intercellular and cell-matrix crosstalk in order to efficiently migrate and invade. Therefore, suppression of RAGE is considered as a critical step in lung tumorigenesis, occurring within the context of malignant tissue reorganization and cell regression to embryonic phenotype.
The regulation of RAGE expression as a potential therapeutic tool for lung cancer sounds reasonable. However, although RAGE downregulation is related to lung tumorigenesis, re-expression of RAGE is not per se inhibitory for lung cancer development. Moreover, the precise in vivo regulation of RAGE in lung tissue represents a great challenge. A more promising therapeutic approach can be the prevention and treatment of lung metastases derived from other primary sites. In this context, RAGE/ligand interactions could be potential therapeutic targets. Moreover, a new arrow in the quiver of treatment options for lung cancer could be the design of electrostatic inhibitors of RAGE/ligand interactions. These charged molecules could compete with classic RAGE ligands for electrostatic binding with the V domain, thus inhibiting RAGE-axis activation and preventing migration and metastasis.

Conflict of interest statement

Authors’ conflict of interest disclosure: The authors stated that there are no conflicts of interest regarding the publication of this article.

Research funding: None declared.

Employment or leadership: None declared.

Honorarium: None declared.

Received July 22, 2013; accepted August 20, 2013; previously published online October 9, 2013

References


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Download Date | 7/10/17 3:09 AM


94. Xu D, Young J, Song D, Esko JD. Heparan sulfate is essential for high mobility group protein 1 (HMGBl) signaling by the receptor for advanced glycation end products (RAGE). J Biol Chem 2011;286:41736–44.


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