Background and Aims. Activation of RAGE due to its increased expression in cancer cells or its stimulation by multiple ligands (AGEs, HMGB1, S100 proteins, etc.) may contribute to the proliferation, invasiveness of tumor cells and formation of distant metastases and also to the resistance of cancer to treatment. RAGE ligands could thus become both useful markers of disease severity and its outcome and, a potential therapeutic target.

Conclusions. Better understanding of the role of RAGE activation in different types of cancer may help to define the role of ligand/RAGE antagonists as promising cancer treatment.

Key words: RAGE, HMGB1, S100 proteins, advanced glycation end products, cancer, metastasis

Received: August 29, 2015; Accepted with revision: January 22, 2016; Available online: February 3, 2016

http://dx.doi.org/10.5507/bp.2016.003

INTRODUCTION

Mechanisms of innate immunity mediate reactions to exogenous pathogens. They can also mediate the response to dying or modified cells and may play an important role in the pathogenesis of cancer. Damaged necrotic cells release different damage-associated molecular patterns (DAMPs, alarmins) which are then recognized by different pattern-recognition receptors (PRRs), activate innate immunity and induce subsequent inflammation. The prototypic DAMP is the high mobility group box 1 (HMGB1), a DNA-binding nuclear protein released from the cells either passively during cell death, or actively on cytokine stimulation and activating PRRs (namely TLRs and RAGE). HMGB1 may associate with Toll-like receptor (TLR) ligands and activate cells through either TLR2 and TLR4, or the multiligand receptor called RAGE (receptor for advanced glycation end-products). Whereas TLRs are more often involved in detecting PAMPs (pathogen associated molecular patterns), they are also involved in recognizing endogenous molecules associated with tissue damage. Some ligands (e.g. HMGB1, S100A8/A9 and LPS) and signaling pathways may be shared by TLRs and RAGE and they may also cooperate in the innate immune response.

RAGE is a single-transmembrane, multiligand receptor, a member of the immunoglobulin superfamily and the gene for RAGE is located in the Class III region of the major histocompatibility complex. Apart from membrane-bound RAGE, there is also a soluble RAGE which may partly function as its natural antagonist. RAGE is constitutionally expressed only in the lungs, but its activation may be involved in tissue homeostasis, resolution of inflammation and tissue repair after acute injury in inflammation but under pathological condition, the activation of RAGE has also been demonstrated in diabetes, atherosclerosis, nephropathy, neurodegeneration and cancer. The effects of RAGE activation greatly depend on cell type and overall context but in the setting of limited nutrients or oxygenation, RAGE activation results in enhanced autophagy, diminished apoptosis, and (in the case of ATP depletion) necrosis with a potential role not only in inflammation but also in cancerogenesis and cancer progression.

Major RAGE ligands – AGEs, high mobility group B1 (HMGB1) and S100 proteins

RAGE may be bound by many ligands which include advanced glycation endproducts (AGEs), certain members of the S100/calgranulin family, extracellular HMGB1-ampothyerin, the integrin Mac-1, amyloid beta-peptide and amyloid fibrils. Serving as a counter-receptor for leukocyte integrins (β-2 integrins) RAGE may also play an important role in cell adhesion and clustering as well as recruitment of inflammatory cells. Other important ligands for RAGE may be glycosaminoglycans (including chondroitin sulfate, dermatan sulfate and heparan sulfate) which are frequently attached to proteoglycans on the surface of cancer cells and play an important role in the malignant transformation of the tumor and metastasis. Lysophosphatidic acid regulates proliferation, survival, motility and invasion of cells. Lysophosphatidic acid avidly binds to RAGE and RAGE is required for its signaling and phosphorylation of Akt and cyclin D, which may result in promoting carcinogenesis. Although these ligands are chemically very different they all share a negative charges on their surface and have a tendency to oligomerize.
AGEs are formed by nonenzymatic glycation and during oxidative and carbonyl stress and, they represent a heterogeneous group of compounds\(^1\). Highly reactive carbonyl compounds (e.g., methylglyoxal, glyoxal, 3-deoxyglucosone) whose increase is characteristic for carbonyl stress are generated via oxidative and non-oxidative pathways and are precursors of AGEs. Pentosidine, carboxymethyllysine (CML) are methylglyoxallysine dimer (MOLD) and are the best known AGE-products. AGEs modify proteins. Some of them cause crosslinking, and have toxic effects via binding to specific receptors, of which RAGE is the best known. Food and tobacco smoke represent exogenous sources of AGEs. AGEs may be degraded on their binding to RAGE (ref.\(^1\)) and some of their deleterious vascular effects may be also mediated by RAGE.

The high mobility group box 1 (HMGB1) protein is an abundant non-histone component of chromatin well-known for its two DNA binding domains, HMG box A and HMG box B. The main characteristics of the HMGB1 protein as an “architectural” factor, are its ability to recognize and bind with high affinity to distorted DNA and its ability to induce kinks in linear DNA fragments\(^1,16,17\). HMGB1 plays important intranuclear, cytosolic and extracellular roles in the regulation of autophagy\(^18\), a degradation of namely dysfunctional organelles and proteins to generate metabolic fuels during starvation.

Although loosely bound to chromatin, HMGB1 can be either passively released from necrotic, but not apoptotic cells, or actively secreted by activated macrophages in a partially tumor necrosis factor-dependent manner, or in response to inflammatory and angiogenic signals. HMGB1 interacts with RAGE on endothelial cells causing activation and leukocyte recruitment\(^16\). HMGB1 correlates with the inflammatory response and may also act as an endogenous pyrogen\(^1\) and may function as a cytokine, differentiation\(^16\) and proangiogenetic factor\(^18\).

HMGB1 can activate both TLRs (TLR2 and TLR4) and RAGE resulting in increased production of inflammatory mediators, but its main signaling pathway is activated through the interaction with RAGE (ref.\(^2\)) resulting in the activation of NF-κB further promoting inflammation\(^19\) in a positive feedback loop and contributing thus to sustaining inflammation and angiogenesis under different pathological conditions.

HMGB1 has been implicated in different disease states, including Alzheimer’s disease, sepsis, ischemia-reperfusion, arthritis, and cancer and targeting the HMGB1 ligand or its receptor may have important potential application in the treatment of these diverse pathological conditions.

The S100 protein family is the largest subgroup within the superfAMILY of proteins carrying the Ca\(^{2+}\)-binding EF-hand motif expressed only in vertebrates\(^21\) with a plethora of tissue-specific intra- and extracellular functions.

The S100 protein family consists of 24 members involved within the cells in the regulation of proliferation, differentiation, apoptosis, calcium homeostasis, energy metabolism, inflammation and migration/invasion interacting with a variety of target proteins, e.g. enzymes, cytoskeletal subunits, transcription factors and nucleic acids\(^22\). Some S100 proteins may be released or secreted and regulate cell functions in an autocrine and paracrine manner via different cell surface receptors (including RAGE and TLR4, or G-protein-coupled receptors) on many cell types (e.g. macrophages, endothelial and epithelial and vascular smooth muscle cells) and interact with heparan sulfate proteoglycans and N-glycans. S100A4 and S100B interact also with epidermal growth factor (EGF) and basic fibroblast growth factor (FGF2) enhancing the activity of their receptors. S100 proteins participate in regulating both innate and adaptive immunity, chemotaxis and cell migration, tissue development and repair and also leukocyte and tumor cell invasion. S100 proteins are thus implicated in the pathogenesis of many diseases, including inflammatory and neurodegenerative diseases and cancer. AGEs, their metabolism and cancer

Activation of RAGE by AGEs was shown to stimulate growth and/or migration of pancreatic cancer, melanoma and breast cancer cells\(^23\). In the case of breast cancer cells, this effect can be completely prevented by metformin\(^23\) (Table 1).

Elevated levels of Nε-carboxymethyllysine (CML) were associated with increased risk of pancreatic cancer but this relation was attenuated after adjustment for body mass index and smoking\(^24\). In a large study in a prospectively followed Finnish population CML-AGE and soluble RAGE (sRAGE) concentrations were inversely associated with liver cancer\(^25\). Unexpected negative association between CML-AGE and the risk of liver cancer is difficult to explain (CML-AGE may not be an optimal indicator of overall AGE production. Other RAGE ligands, e.g. HMGB1 may be more important in activating RAGE in this setting). In another large prospective cohort of the Finnish male smokers, low sRAGE, but not high CML-AGE was associated with increased risk of colorectal cancer\(^26\).

Serum levels of AGEs and also AOPP (advanced oxidation product proteins) were also increased in patients with breast cancer compared to controls\(^27,28\) even in the early stages (stage I and II) of the disease. Patients with advanced breast cancer (stage III and IV) had higher se-
ruin levels of both AGEs and AOPP not only compared to controls, but also to patients in early stages of the disease suggesting a role of carbonyl and oxidative stress in malignant transformation and progression of breast cancer.

In conclusion, although AGEs/RAGE interaction was shown to stimulate cancer cell proliferation available clinical data do not demonstrate AGEs (at least CML-AGEs) to be a good marker of the risk of either colorectal or liver cancer, although they may be related to the progression of breast cancer. The role of AGEs (and their activation of RAGE) remains to be established and the putative role of confounding factors (diabetes, impaired renal function) needs to be taken into consideration.

Impaired metabolism of AGEs may also play an important role in cancer. Methylglyoxal, a precursor of AGEs and potent inducer of apoptosis, may be degraded by the system of glutathione-dependent glyoxalases composed of glyoxalase I (Glo I) and glyoxalase II (Glo II) enzymes. Glyoxal and methylglyoxal bind to DNA and induce mutant base deletions and base-pair substitutions, mostly occurring at G:C sites (Table 1). Glyoxalase may be activated in chronic inflammatory diseases and in diabetes and uremia to counteract the increased carbonyl and oxidative stress with the overproduction of AGEs, ALEs and AGEs (ref. 29). Suppression of nucleotide glycation by glyoxalase inhibitors (e.g. polyphenols, PPARγ agonists) may be associated with clear cell renal cancer and with the genotype and allele frequencies of Ala111Glu glyoxalase I polymorphism were not significantly different for patients and controls. The Glu allele genotype, was, however, associated with the absence of progesterone receptor. No difference in allelic and genotype frequencies of glyoxalase I gene was found in patients with pancreatic cancer. Very recently in a large study, Ala111Glu glyoxalase polymorphism was shown to be associated with clear cell renal cancer and RAGE polymorphisms (-429T/C and 2184A/G) with its aggressiveness (ref. 40).

Impaired metabolism of AGEs may also play an important role in cancer. Methylglyoxal, a precursor of AGEs and potent inducer of apoptosis, may be degraded by the system of glutathione-dependent glyoxalases composed of glyoxalase I (Glo I) and glyoxalase II (Glo II) enzymes. Glyoxal and methylglyoxal bind to DNA and induce mutant base deletions and base-pair substitutions, mostly occurring at G:C sites (Table 1). Glyoxalase may be activated in chronic inflammatory diseases and in diabetes and uremia to counteract the increased carbonyl and oxidative stress with the overproduction of AGEs. ALEs and AOPP (ref. 14). S100A12, a RAGE ligand may decrease the expression of glyoxalase I and impair the degradation of AGEs.

Mutations arising from DNA glycation could explain the link of carbohydrate intake to incidence of colorectal cancer and increased risk of cancer in patients with diabetes. Suppression of nucleotide glycation by glyoxalase I protects DNA not only in normal, but also in cancer cells from damage and contributes to its recovery and experimental overexpression of glyoxalase I confers the cancer cells with resistance to drug-induced apoptosis. Small cell-permeable glyoxalase inhibitors were shown to counteract drug resistance in lung and prostate cancer.

Alterations in the expression of the glyoxalase genes have been reported in several human cancers. Amplification of glyoxalase I gene was demonstrated in some tumours (e.g. in invasive ovarian and breast cancer), especially drug-resistant tumour cell lines and increased glyoxalase I expression may be induced by malignant transformation and antitumour treatment, possibly supporting the viability of cancer cells with high glycolytic rates. In breast cancer, glyoxalase gene expression may be induced by 17βestradiol (probably due to the presence of estrogen response element in the glyoxalase gene) resulting in much higher activity of both glyoxalases I and II in cancer compared to normal breast tissue. Glyoxalase I was also overexpressed in the majority of patients with breast cancer and its upregulation correlated with advanced tumour grade.

The activity of glyoxalase I may be modulated by various polyphenols, e.g. curcumin is a strong competitive inhibitor of glyoxalase I with concomitant antiinflammatory activity. Curcumin was shown to inhibit the growth of breast and prostate cancer and brain astrocytoma and could be a promising anticancer compound. Resistance to doxorubicine in leukemia cells associated with increased glyoxalase I activity may be counteracted by the administration of thiazolidinedione troglitazone which downregulates glyoxalase I expression.

Some cancers are associated with high glyoxalase activity which may contribute to the progression and resistance to chemotherapy. Some small cell permeable glyoxalase I inhibitors (e.g. polyphenols, PPARγ agonists) could be a good adjunct treatment in different types of cancer.

Polymorphism of glyoxalase I was studied in several types of cancer. We studied the Glu111Ala polymorphism of glyoxalase I in patients with breast cancer and were able to show that the higher frequency of the mutated C allele was found in patients with negative estrogen receptors and in patients and more advanced disease (clinical stage III) compared to controls (P < 0.05) suggesting that the presence of the C allele could be a negative prognostic factor in breast cancer. In a large Italian study of patients with breast cancer, polymorphism of glyoxalase I was associated with breast cancer in univariate analysis but a number of confounding factors obfuscate the results. In another large study in Malaysian patients with breast cancer, the genotype and allele frequencies of Ala111Glu glyoxalase I polymorphism were not significantly different for patients and controls. The Glu allele genotype, was, however, associated with the absence of progesterone receptor. No difference in allelic and genotype frequencies of glyoxalase I gene was found in patients with pancreatic cancer. Very recently in a large study, Ala111Glu glyoxalase polymorphism was shown to be associated with clear cell renal cancer and RAGE polymorphisms (-429T/C and 2184A/G) with its aggressiveness.

The role of glyoxalase gene polymorphisms in cancer thus remain uncertain, but impaired glyoxalase activity may be associated with clear cell renal cancer and with outcome in breast cancer. Further studies are to elucidate the role of glyoxalase in cancer are therefore warranted.

HMGB1 and cancer

HMGB1 released from necrotic cancer cells (e.g. due to chemotherapy) may stimulate (through RAGE) the proliferation of the remnant cancer cells and metastasis contributing to the resistance to cancer therapy. In the mouse colon cancer model, lung and liver metastasis after doxorubicin treatment were abrogated by anti-HMGB1 treatment (Table 1). Tumor cells require ATP to support their proliferation. HMGB1-RAGE enhance tumor cell mitochondrial complex I activity, ATP production, tumor cell proliferation and migration. In the experimental setting, blockade of RAGE, or inhibition of HMGB1 release diminish ATP production and retard tumor growth. CpG oligodeoxynucleotides enhance the growth and invasive potential of lung cancer cells. Cpg oligodeoxynucleotides stimulate the secretion of HMGB1 and blockade of extracellular HMGB1 abrogated the CpG oligodeoxynucleotides-induced progression of cancer cells. Activation of both TL4 and RAGE was critical for the response to HMGB1 in this mode.
Table 1. Types of cancer associated with RAGE ligands and their putative effects.

<table>
<thead>
<tr>
<th>Type of cancer</th>
<th>RAGE ligand</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lung cancer</td>
<td>S100A4, S100A6 and RAGE expressed in lung cancer, S100P detected in lung cancer associated with malignant phenotype, hormone independence and resistance to chemotherapy, progression and metastasis possibly through autocrine RAGE-mediated signaling (ref.19,29,32)</td>
</tr>
<tr>
<td>Colorectal cancer</td>
<td>Highest quintile of CML-AGE not associated with an increased risk of colorectal cancer, HMGB1 and its receptors overexpressed in colon cancer and associated with the proliferation and metastasis, S100P detected in colon cancer associated with malignant phenotype, resistance to chemotherapy, progression and metastasis possibly through autocrine RAGE-mediated signaling, S100P may promote the development of colon cancer, S100A4 prognostic biomarker of the formation of distant metastases and reduced overall and metastasis-free survival (ref.19,26,57,54,60)</td>
</tr>
<tr>
<td>Hepatocellular cancer</td>
<td>CML-AGE (and sRAGE) concentrations inversely associated with liver cancer, HMGB1 levels increase after transarterial chemoembolization of liver cancer (ref.23,49)</td>
</tr>
<tr>
<td>Pancreatic cancer</td>
<td>AGEs shown to stimulate through RAGE growth and/or migration of pancreatic cancer, elevated prediagnostic levels of CML associated with increased pancreatic cancer risk, no difference in polymorphisms of GLO genes in pancreatic cancer compared to controls, S100P detected in pancreas cancer associated with malignant phenotype, hormone independence and resistance to chemotherapy, progression and metastasis possibly through autocrine RAGE-mediated signaling, S100 proteins (namely S100A11 and S100P) involved in the progression and metastases of pancreatic cancer and associated with poor outcome after surgical resection, expression of S100A4 and S100P associated with drug resistance, differentiation, metastasis and clinical outcome (ref.23,34,39,57)</td>
</tr>
<tr>
<td>Kidney cancer</td>
<td>EN-RAGE related to obesity status in renal cancer, upregulated in tumor tissue of clear cell renal cancer, especially in pts with poorer overall survival and related to obesity status, Ala111Glu polymorphism linked to clear cell renal cancer (ref.40,71)</td>
</tr>
<tr>
<td>Prostate cancer</td>
<td>HMGB1 co-expressed with RAGE in the prostatectomy specimens from a majority of pts with metastatic prostate cancer, S100P detected in breast, prostate, pancreas, lung and colon cancer associated with malignant phenotype, hormone independence and resistance to chemotherapy, progression and metastasis possibly through autocrine RAGE-mediated signaling, S100A4 expression related to progression of prostatic cancer in the mouse model, S100A8 and S100A9 (and RAGE) expression enhanced in human prostate cancer and both proteins secreted by prostate cancer cells, serum levels of S100A9 increased in prostate cancer compared to benign prostatic hyperplasia (ref.36,54,57,61,66)</td>
</tr>
<tr>
<td>Ovarian cancer</td>
<td>Glyoxalase I gene amplified in invasive ovarian cancer and glyoxalase 1 may play a role in multidrug resistance (ref.29)</td>
</tr>
<tr>
<td>Breast cancer</td>
<td>AGEs induced proliferation of breast cancer cells could have been completely prevented by metformin, Glyoxalase I gene amplified in breast cancer and glyoxalase 1 may play a role in multidrug resistance, glyoxalase expression influenced by 17β-estradiol, Glyoxalase I is overexpressed in breast cancer and its expression correlates with tumor grade, higher frequency of C allele in Glu111Ala polymorphism of glyoxalase I found in patients with breast cancer and negative estrogen receptors and patients with more advanced disease, no association of Glu111Ala polymorphism of glyoxalase I in Malaysian patients, polymorphism, however, associated with the absence of progesterone receptor, polymorphism of glyoxalase I associated with increased risk of breast cancer, HMGB1 and its receptors overexpressed in breast cancer and associated with the proliferation and metastasis, serum levels of HMGB1 decreased compared to controls in pts with metastatic breast cancer, pts with no response to neoadjuvant chemotherapy tended to have compared to pts achieving complete or partial remission higher HMGB1, S100A4, S100A6 and RAGE expressed in breast cancer, S100P detected in breast cancer associated with malignant phenotype, hormone independence and resistance to chemotherapy, progression and metastasis possibly through autocrine RAGE-mediated signaling, S100A7/psoriasin overexpressed in invasive, estrogen receptor negative breast cancer and may contribute to its proliferation, angiogenesis and metastasis, S1007 expressed already in high-grade ductal carcinoma in situ and high-grade comedo ductal carcinoma in situ with a higher risk of local recurrence, S100A8/A9 binding of RAGE may promote the invasion of breast cancer cells and associated with lymph node involvement and distant (lung) metastases (ref.19,23,32,33,36,38,46,52,57,63,66)</td>
</tr>
<tr>
<td>Melanoma</td>
<td>AGEs shown to stimulate through RAGE growth and/or migration of melanoma cells, HMGB1 and its receptors overexpressed in melanoma and associated with the proliferation and metastasis, S100A4 secreted by tumor-associated macrophages may contribute to the progression of melanoma and increase metastatic potential of melanoma cells, S100A8/S100A9 (calprotectin) may stimulate migration of melanoma cells by the RAGE-independent mechanism, S100B overexpressed in melanoma and reliable prognostic biomarker (ref.19,23,56,67,70)</td>
</tr>
</tbody>
</table>
HMGB1 and its receptors are widely overexpressed (and its protein levels increased) in virtually every examined type of cancer and its overexpression was (usually in parallel with the overexpression of RAGE) associated with the proliferation and metastasis of many tumor types, including breast, colon, melanoma, and others. On the other hand, data on the relation of HMGB1 to the histological grade of the tumor are very limited. In human malignant glioma cells, HMGB1 induced a dose-dependent increase in cell proliferation and cell migration. In glioma cells (as in other types of cancer) HMGB1 is predominantly bound in the nucleus and is released only by necrotic glioma cells (e.g. after chemotherapy or radiotherapy).

The HMGB1 protein has been correlated to cancer progression, especially invasion and metastasis in melanoma, colon, prostate, pancreatic and breast cancer. HMGB1 is co-expressed with RAGE in the prostatectomy specimens from a majority of patients with metastatic prostate cancer, but in only less than a quarter of non-metastatic prostate cancers. The invasive capacity of prostate cancer cells could have been suppressed in vitro with HMGB1 antisense S-oligodeoxynucleotide, but, on the other hand, HMGB1 secretion was induced by androgen deprivation. HMGB1 may be an important survival factor for malignant B cells in chronic lymphocytic leukemia (CLL). HMGB1 levels are increased in patients with CLL compared to controls and are associated with absolute lymphocyte cell count. CLL cells are able to passively release HMGB1 and this release is related to the differentiation of nurse-like cells (NLC), S100A8 promotes (through RAGE activation) autophagy of leukemia cells and contributes to the drug resistance of leukemia cells.

HMGB1 thus seems to play an important role in the stimulation of the proliferation of cancer cells, especially after previous chemotherapy. Except for competitive antagonists of HMGB1, HMGB1 may be also bound by a range of small natural or synthetic molecules, e.g. glycyrrhizin, or gabexate mesilate and neutralized by HMGB1-specific antibodies. The putative role of these interventions in the treatment of different types of cancer remains to be established.

S100 proteins and cancer

S100 proteins may play a role in different stages of tumorigenesis including cell differentiation, cell cycle regulation, cell growth, apoptosis, cell motility, migration, invasiveness and metastasis formation and also tumor microenvironment.

Expression of different S100 proteins was demonstrated in tumor cells and may be specific for different types of cancer. Using microarray technology to study S100 protein expression in tumor samples, S100A2 expression was observed in lymphoma biopsies, S100A4 and S100A6 (in parallel with RAGE) was abundant in breast and lung tumours. (Table 1). S100A4 was secreted by both tumor and stromal cells in melanoma xenograft model and supported (via RAGE) tumorigenesis and angiogenesis synergizing with vascular endothelial growth factor (VEGF) and also promoting endothelial cell migration by increasing MMP-9 activity. Endothelial cell migration, tumor growth and angiogenesis could have been abolished in this mode by the administration of anti-S100A4 monoclonal antibody. S100A4 increased cell migration and invasion also in colon cancer cells and this effect may have been counteracted by soluble RAGE and anti-RAGE antibodies. S100A4 also progressively increased in prostatic tissue with the progression of the disease in the mouse model of prostate cancer and S100A4 cancer cells grew more quickly (via RAGE and NFkB activation) than S100A4 negative cells. As heterozygously deleted S1004 mice exhibited an increased tumor latency period, reduced prostatic weights and no metastases S100A4 inhibition could be a promising therapeutic option to be tested in prostate cancer. S100A4 may be synthesized not only by tumor, but also by stroma cells. In melanoma, infiltrating tumor-associated macrophages may also secrete S100A4
Table 2. Putative interference with RAGE ligands and RAGE-mediated effect.

<table>
<thead>
<tr>
<th>Mechanism</th>
<th>Type of cancer</th>
<th>Effect</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Metformin</td>
<td>Breast cancer</td>
<td>Decreased incidence and better survival in diabetic pts with breast cancer treated with metformin</td>
<td>23, 75</td>
</tr>
<tr>
<td>Thiazolidinediones</td>
<td>Breast cancer</td>
<td>Better survival in diabetic pts with breast cancer treated with thiazolidinediones</td>
<td>75</td>
</tr>
<tr>
<td>PPARγ agonists</td>
<td>Not yet shown in cancer</td>
<td>Interference with the AGE-RAGE system</td>
<td>74</td>
</tr>
<tr>
<td>Cromolyn</td>
<td>Pancreatic cancer (ductal adenocarcinoma)</td>
<td>Inhibition of S100P-induced cell growth</td>
<td>76</td>
</tr>
<tr>
<td>Pravastatin</td>
<td>Not yet shown in cancer</td>
<td>Dose-dependent inhibition of AGE-mediated upregulation of RAGE</td>
<td>23</td>
</tr>
<tr>
<td>Irbesartan</td>
<td>Not yet shown in cancer</td>
<td>Inhibition of AGE-induced upregulation of RAGE</td>
<td>73</td>
</tr>
<tr>
<td>Heparin (O,3-O-desulfated heparin – ODSH)</td>
<td></td>
<td>Inhibition of RAGE interaction with CD11b/CD18 (Mac-1) and inhibition of its activation by many ligands (AGEs, HMGB1, S100 calgranulins), reduction of selectin-mediated lung metastasis of melanoma in mice</td>
<td>77</td>
</tr>
<tr>
<td>Antibodies against chondroitin sulfate (CS), or CS-E</td>
<td>Lung cancer</td>
<td>Inhibition of metastasis</td>
<td>78</td>
</tr>
<tr>
<td>Antibodies against RAGE</td>
<td>Lung cancer, melanoma cells</td>
<td>Inhibition of metastasis formation</td>
<td>12</td>
</tr>
<tr>
<td>Recombinant soluble RAGE</td>
<td>Hepatic cancer</td>
<td>sRAGE may protect against RAGE-induced tissue damage by neutralizing its ligands</td>
<td>79</td>
</tr>
<tr>
<td>Small S100P-derived RAGE antagonist (RAP)</td>
<td>Pancreatic cancer, glioma</td>
<td>RAP may block activation of RAGE by multiple ligands and inhibits interaction of S100P, S100A4 and HMGB1 with RAGE, administration of RAP results in reduction of growth and metastasis of pancreatic cancer and inhibition of glioma growth</td>
<td>76</td>
</tr>
<tr>
<td>RAGE-siRNA</td>
<td>Breast cancer</td>
<td>Decrease in proliferation of breast cancer cells</td>
<td>81</td>
</tr>
<tr>
<td>Anti-HMGB-1 antibodies,</td>
<td></td>
<td>Blocking extracellular effects of HMGB-1</td>
<td>80</td>
</tr>
<tr>
<td>HMGB-A box (competitive antagonist of HMGB1)</td>
<td></td>
<td>Blocking release of HMGB-1 from the nucleus</td>
<td>80</td>
</tr>
<tr>
<td>Anti-TLR2 antibody</td>
<td></td>
<td>Blocking HMGB1 signaling through TLR2</td>
<td>80</td>
</tr>
<tr>
<td>Soluble thrombomodulin</td>
<td></td>
<td>Modulation of HMGB-1 activity</td>
<td>80</td>
</tr>
</tbody>
</table>

and in this paracrine manner increased the metastatic potential of melanoma cells. Expression of another member of the S100 family, S100P, was detected in a spectrum of human tumor cell lines and tissues derived from breast, prostate, pancreas, lung and colon, where it was connected with malignant phenotype, hormone independence, resistance to chemotherapy and metastasis. S100P was shown to stimulate RAGE and increase proliferation and prolong survival of cancer cells in an autocrine manner. Its effects can be blocked by anti-RAGE antibodies. S100-P derived small antagonistic peptides could be potentially used to block tumor proliferation. In colon cancer cells elevated S100P stimulated RAGE, AP-1 and induced oncogenic miR-155 and S100P-induced proliferation, motility and invasion may have been blocked either by anti-RAGE antibodies, or miR-155 knockdown.

In pancreatic cancer, the expression of S100A4 and S100P was associated with drug resistance, differentiation, metastasis and clinical outcome and S100A11 and S100P, and possibly also S100A2 and S100A6 were related to the unfavorable outcome of the patients after surgical resection.

S100A7/psoriasin was highly expressed in high-grade ductal breast carcinoma in situ, high-grade comedo ductal carcinoma in situ with higher risk of local recurrence, and in invasive estrogen receptor negative breast cancer. Its expression was related to ductal hyperplasia, recruitment of tumor-associated macrophages, tumor growth, angiogenesis and metastasis. Tumor growth was inhib-
itted by the downregulation of psoriasin by short hairpin RNA (shRNA) resulting in decreased expression of vascular endothelial growth factor (VEGF) (ref. 62). Invasion of breast cancer may be also stimulated by S100A8/A9 via RAGE-mediated epithelial-mesenchymal transition through NF-κB mediated stabilization of Snail. In invasive ductal carcinoma S100A8/A9 binding is associated with lymph node involvement and distant (lung) metastases65.

Deregulated expression of different S100 proteins, e.g. S100A8 and S100A9, is associated with different neoplastic disorders. S100A8 and S100A9 expression is enhanced in human prostate cancer and are secreted by prostate cancer cells46. Extracellular S100A8 and S100A9 activate RAGE, induce the activation of NF-κB and increased phosphorylation of p38 and p44/42 MAP kinases and stimulate migration of benign prostatic cells in vitro. S100A8, S100A9 and RAGE are co-expressed and upregulated in prostatic intraepithelial neoplasia and preferentially in high-grade adenocarcinomas but not in benign prostatic tissue. Serum levels of S100A9 may help to distinguish between prostate cancer and benign prostatic hyperplasia66.

S100A8 and S100A9 may dimerize and form calprotectin. Cell surface glycoprotein EMMPRIN/BASIGIN (CD147/BSG) may serve as a receptor for calprotectin and stimulate migration of benign prostatic cells in vitro. S100A8, S100A9 and RAGE are co-expressed and upregulated in prostatic intraepithelial neoplasia and preferentially in high-grade adenocarcinomas but not in benign prostatic tissue. Serum levels of S100A9 may help to distinguish between prostate cancer and benign prostatic hyperplasia66.

S100A8 is elevated in drug-resistant leukemia cell lines and may play an important role in the drug resistance of leukemia cells by promoting autophagy. Adriamycin and vincristine increase S100A8 in human leukemia cells in parallel with the upregulation of autophagy68. Knockdown of S100A8 induced by RNA interference restored the chemosensitivity of leukemia cells. S100A8 could be thus a novel therapeutic target for improved drug sensitivity in leukemia therapy.

S100B is overexpressed by gliomas and its downregulation of S100B abrogates tumor growth in vivo and is related to higher infiltration with tumor-associated macrophages, stronger inflammatory response and increased vascularility. As RAGE ablation had no effect on the infiltration of gliomas with tumor-associated macrophages, other pathways (possibly CCL2 expression) may be involved and could be targeted68. S100B expression may also be a prognostic marker in malignant melanoma69.

S100A12 (ENRAGE) is related to obesity status in clear cell renal cancer (which is a risk factor of this type of cancer) and is overexpressed in tumor tissue, especially in patients with poorer overall survival. S10012 may serve through the activation of RAGE as an autocrine stimulator of the tumor growth71.

Targeting RAGE and its ligands – can it contribute to the treatment of breast cancer?

The activity of RAGE and its ligands may be suppressed in many possible way using both small molecules, monoclonal antibodies, or siRNAs (Table 2). Both statins72 and angiotensin receptor blockers73 inhibit RAGE signaling in diabetic nephropathy, but no data on their putative effect on RAGE activation in cancer are available. Metformin use may decrease the incidence and mortality in breast cancer by inhibition of AGEs induced proliferation of breast cancer cells73. Peroxisome proliferator-activated receptor-gamma (PPARgamma) agonists were also shown to interfere with the AGE-RAGE system74,75. Antiiallergic drug cromolyn and its derivatives may block the interaction of S100P with RAGE and high concentrations of cromolyn were shown to improve gemcitabine effectiveness in pancreatic ductal adenocarcinoma by inhibiting S100P-induced increase of NF-κB, cell growth and apoptosis and cromolyn derivatives may be promising drugs to block S100P in different types of cancer76.

Heparin and its low anti-coagulant derivative 2-0,3-0-desulfated heparin disrupt CD11b/CD18 mediated leukocyte adhesion to RAGE and inhibited activation of RAGE by AGEs, HMGB1 and S100 proteins. These heparins with low anti-coagulant activity were also able to prevent metastases77. Altered expression of chondroitin sulfate (CS - with higher proportion of E-disaccharide units) on the surface of tumor cells may contribute to malignant transformation and metastasis which can be inhibited in lung cancer cells by pre-administration of CS-E from squid cartilage rich in E units or antibodies against CS-E interfering with CS-RAGE signaling suggesting a putative role of these approaches in the treatment of pulmonary metastasis78.

AGEs induced proliferation of breast cancer cells could be completely prevented by anti-RAGE antibodies79. Antibodies against RAGE were also shown to inhibit metastasis of experimental lung cancer and melanoma cells80. Administration of recombinant soluble RAGE was shown to block RAGE signaling pathway in animal models, suggesting that the circulating sRAGE could protect the tissue against RAGE-induced tissue damage79 and sRAGE could serve also in cancer as a putative biomarker. HMGB-1 can be blocked in several different ways80: by anti-HMGB-1 antibodies, by the inhibition of HMGB-1 release from the nucleus into the extracellular space, by HMGB-A box, a competitive antagonist of HMGB-1, by blockage of RAGE-HMGB-1 signaling using RAGE antagonists, by blockage of TLR-HMGB-1 signaling using anti-TLR2 antibodies and by other molecules that modulate HMGB-1 activity using e.g. human soluble thrombomodulin.

The recently developed small S100P-derived RAGE antagonist peptide (RAP) blocking activation of RAGE by multiple ligands was shown to inhibit the interaction of S100P, S100A4, and HMGB-1 with RAGE at micromolar concentrations. Systemic in vivo administration of RAP reduced the growth and metastasis of pancreatic tumors and also inhibited glioma tumor growth76.

Decrease in the proliferation of different types of experimental breast cancer with increased RAGE expression (correlating with the severity of breast cancer) could have been induced by small interfering RNA against RAGE (RAGE siRNA) (ref. 81).
Overall specific modes of RAGE inhibition and its ligands could be promising not only in diabetes and other inflammatory and neurodegenerative disorders but also in cancer.

CONCLUSIONS

The activity of the RAGE ligand(s)/RAGE system appears to be involved in a number of cancers contributing to the proliferation of cancer cells, their invasiveness, metastasis and resistance to treatment. Serum levels and tissue expression of RAGE and RAGE ligands may thus be useful biomarkers of disease severity and disease outcome and potential therapeutic targets. We believe that the role RAGE and its ligands in cancer will be a fruitful area for further research and will help our understanding of the pathogenesis of cancer progression and metastasis.

Acknowledgement: Our research was supported by the research projects RVO-VFN64615 of the Ministry of Health and the research project of Charles University P25/LF1/2.

Author contributions: PT: clinical implications and interpretation of the data, MK and TZ: biochemistry of RAGE and interaction of RAGE with its ligands, VT: clinical implications of the data, literature search.

Conflict of interest statement: The authors declare that there are no conflicts of interest regarding the publication of this article.

REFERENCES

5. Schmidt AM, Stern DM. Receptor for age (RAGE) is a gene within the major histocompatibility class III region: implications for host response mechanisms in homeostasis and chronic disease. Front Biosci 2001;6:D1151-60.
10. Schmidt AM, Stern DM. Receptor for age (RAGE) is a gene within the major histocompatibility class III region: implications for host response mechanisms in homeostasis and chronic disease. Front Biosci 2001;6:D1151-60.
13.邵米工 S, 沙加拉 K. 谷酰胺酰二酰胺是功能性配体
18. 轻木木 K, 活森 KM, Zeh HJ, Zeh HJ, Loze MT, Tand D. 调味料的调节
19. 没有冲突的作者声明：我们声明没有利益冲突。


