What's the importance of peptidases in cancer?

Qual é a importância das peptidases em câncer?

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ABSTRACT

A synonym for a successful tumor spread is a productive invasive cell migration, a process by which the extracellular matrix plays the role of substrate for cells to move and reach a secondary site. Peptidases participate actively in this process to degrade the extracellular matrix. The activity of these enzymes is regulated by inhibitors, activators and receptors. However, cancer occurs in a breach of the balance of proteolytic-antiproteolytic activity. The peptidases, enzymes that hydrolyze peptide bonds of proteins, can act directly by degrading the components of the extracellular matrix or indirectly by activating other peptidases, in a process that may also generate bioactive fragments, interact with cell surface receptors, and be involved in the angiogenic process. The modification and remodeling of the extracellular matrix caused by peptidases modify the anchoring mediated by integrins, focal adhesion and architecture of the cytoskeleton, and direct signaling molecules that can affect gene expression and influence some behavioral aspects, such as proliferation, survival, differentiation and mobility. Recently, some studies showed an inverse correlation between the low expression of peptidases and increased potential for tumor development. Thus, despite offering an excellent alternative of a more effective and targeted cancer treatment, protease inhibitors should be specific, administered at the correct time with the aid of biomarkers and act locally, and finally, their activity should not be prolonged to the point of interfering with the activity of peptidases when they are, for example, being used in a process of remodeling.

Keywords: Peptide hydrolases; Protease inhibitors; Neoplasms; Neoplasm metastasis; Neoplasm invasiveness; Extracellular matrix

RESUMO

Um sinônimo para o sucesso da disseminação do tumor é uma produtiva migração celular invasiva, um processo pelo qual a matriz extracelular possui papel de substrato para as células se moverem e atingirem um sítio secundário. Para degradar a matriz extracelular, as peptidases participam ativamente deste processo. A atividade destas enzimas é regulada por inibidores, ativadores e receptores. Entretanto, no câncer ocorre uma quebra do balanço da atividade proteolítica-antiproteolítica. As peptidases, enzimas que clivam ligações peptídicas, podem atuar de forma direta ao degradar componentes da matriz extracelular ou de forma indireta, ao ativar outras peptidases a gerar fragmentos bioativos, interagir com receptores da superfície celular, e participar no processo angiogênico. A modificação e o remodelamento da matriz extracelular causadas por peptidases modificam a ancoragem mediada por integrinas, a adesão focal e a arquitetura do citoesqueleto direcionam moléculas de sinalização que podem afetar a expressão gênica e influenciar no comportamento como proliferação, sobrevivência, diferenciação, e mobilidade. Recentemente, alguns trabalhos demonstraram uma correlação inversa entre a baixa expressão de peptidases e o aumento do potencial do desenvolvimento do tumor. Desta forma, apesar de oferecerem uma excelente alternativa mais efetiva e direcionada para o tratamento do câncer, os inibidores de peptidases devem ser específicos, administrados no tempo correto com o auxílio de biomarcadores e atuar localizadamente.

Descritores: Peptídeo hidrolases; Inibidores de proteases; Neoplasias; Metástase neoplásica; Invasividade neoplásica; Matriz extracelular

INTRODUCTION

Peptidases – proteins that hydrolyze peptide bonds – occur in all organisms and represent approximately 2% of total number of proteins present in all types of organisms. They are synthesized as zymogens (inactive form) and are transformed into the active form by other peptidases or may convert themselves autocatalytically. Using bioinformatic analysis of mouse and human genomes, at least 500 to 600 peptidases have been identified, many of which are orthologous[1].

Two generic groups comprise the peptidase family: endopeptidases (that attack internal peptide bonds) and exopeptidases (that remove terminal amino acids). Based on the catalytic mechanisms of hydrolysis, on the active site and on the three-dimensional structure, the endopeptidases are divided into subclasses on the

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basis of statistically significant similarities in the amino acid sequence, metallopeptidases, serine peptidases, cysteine peptidases, aspartic peptidases, and threonine peptidases[2]. Some peptidases are involved in the intra- and extracellular digestion of proteins, while others have functions in more specialized biological processes such as zymogen activation, tissue remodeling, release of active peptides, receptor activation, embryonic development, organ morphogenesis, angiogenesis, apoptosis, and others[3]. Activity of these enzymes is regulated by zymogen activation, limited proteolysis, activators, inhibitors, cell receptors and genetic control[3]. Our view of the proteolytic agent has expanded considerably after the recognition that, beyond their nonspecific functions in protein catabolism, peptidases act as processing enzymes that carry out highly selective cleavage of specific substrates and influence cell behavior, survival, and death[4]. The equilibrium between peptidases and inhibitors is unregulated in many pathophysiological processes, like inflammation, infection, hemorrhage and cancer.

Peptidases participate in several key protumorigenic processes: angiogenesis, adhesion, proliferation, invasion, and metastasis, and through extensive degradation they can modify and remodel the extracellular matrix (ECM). This remodeling associated with cancer progression influences cellular behavior and phenotype, and can lead to the release of bioactive fragments (cytokines) and to autocrine and paracrine shedding of growth factors[5], resulting in modified cell-cell, cell-extracellular matrix interaction and cell signaling, events involved in tumor development. These processes can be multiplied by direct or indirect actions resulting in a proteolytic cascade. The enzymes are either secreted by the tumor cells or by cells in the surrounding stromal compartment recruited by the tumor cells[6]. Although peptidases stimulate cell migration on several occasions, current evidence suggests that protease activity, per se, is not always essential. In this review, it will be focused on the action of two subfamilies: metallopeptidases and serine peptidases.

Metallopeptidases

The matrixins (MMP) are capable of degrading virtually all ECM components, and malignant cells produce a spectrum of MMP known traditionally as substrates of ECM either at the primary or the metastatic site, which can provide a beneficial and protective effect in some situations of multiple cancer progression stages. In the MEROPS database, matrix metallopeptidases or MMP are the members of metzicins – M10. The metzincin subfamily of metallopeptidases is characterized by a three-histidine zinc-binding motif and a preserved methionine turn following the active site.

More than 25 members comprise the MMP, 23 of which are found in man, and they contain a signal peptide sequence peptide which is removed to generate pro-MMP during translation and are secreted or anchored to the cell surface. The propeptides have the "cysteine switch" motif, PRCGXPD, which has about 80 amino acids and is responsible for the latency of the enzyme. The MMP are distinguished from other metzincins in the primary structures of the catalytic domains. The catalytic domain contains the Zn^{2+} binding motif, HEXXHXXGXXH, and the methionine forming 'Met-turn'. The majority of MMP members contain a flexible proline-rich hinge region and a hemopexin-like C-terminal domain of about 200 amino acids. Exceptions to this are MMP-7 (matrilysin 1), MMP-26 (matrilysin 2), and MMP-23; they lack the linker peptide and the Hpx domain, and MMP-23 has a unique cysteine-rich domain and an immunoglobulin-like domain after the metallopeptidase domain. Two family members, MMP-2 (gelatinase A) and MMP-9 (gelatinase B), have a gelatin-binding domain that contains three fibronectin-type II repeats inserted into the catalytic domain[7]. Based on their structure and substrate specificity, the MMP can be subdivided into six evolutionary groups: collagenases, gelatinases, stromelysins, membrane-type metallopeptidases (MT-MMP), and others, including seven MMP not classified in the five abovementioned subgroups.

The three-dimensional structure of a number of MMP has been determined by X-ray crystallography and nuclear magnetic resonance (NMR); although the domains' primary structure showed little homology, the polypeptide folds of MMP catalytic domains are almost superimposable. The MMP perform highly selective and limited cleavage of specific substrates, including growth factors and their receptors, cell adhesion molecules, cytokines, chemokines, apoptotic ligands, and angiogenic factors. During normal tissue homeostasis, expression and activation of MMP are limited and tightly regulated. A certain level of MMP expression is seen in any repair or remodeling process, in any diseased or inflamed tissue, and in any cell type grown in culture[7]. Nevertheless, only MMP7 appears to be unique in its restricted expression in tumor cells[8]. The expression and activity of these enzymes are highly regulated in a multistep process that includes transcription, translation, protein stability, compartmentalization, proenzyme and enzyme inactivation. For example, the activity of MMP is regulated by endogenous inhibitors such as α_2-macroglobulin and tissue inhibitors of

metallopeptidases (TIMP), considered the major inhibitors in tissues, comprised of a glycoprotein family of four members (TIMP-1 to TIMP-4) that show different specificities. The TIMP comprise 184 to 194 amino acid residues, forming 1:1 enzyme-inhibitor complexes, and are important for decreasing tumor angiogenesis, growth, migration, and metastasis in experimental models[7].

MMP have several roles in cancer

The equilibrium between TIMP and MMP is disrupted in many pathological conditions including cancer. These peptidases can affect cell proliferation, adhesion, migration, and invasion, as well as angiogenesis and metastasis, not only by degradation of the ECM, but also by the release of sequestered growth factors or the generation of bioactive fragments and degradation of functional proteins involved in cell-cell and cell-ECM interactions[9]. Through receptor-mediated signaling, MMP have the capacity of activating other peptidases that contribute to tumor formation, or influence the cellular microenvironment, tumor initiation and progression through release of bioactive fragments, following degradation of ECM or latent extracellular growth factors and their receptors[10]. MMP have also been found to cleave intracellular targets, inducing mitotic abnormalities and genomic instability. Various studies correlate the high expression of MMP with the metastatic potential of tumors and poor survival in lung, prostate, stomach, colon, breast, ovary, pancreatic, oral squamous cell cancers, and other cancers. The overexpression of MMP-7, determined by immunohistochemistry, was associated with tumor proliferation and a poor prognosis in non-small cell lung cancer[11].

More recently, MMP have been related to induced epithelial-mesenchymal transition (EMT), a process in which alterations occur in the cell-cell and cell-ECM interactions resulting in the mobile phenotype of mesenchymal cells. The combination between the actions of locally expressed growth factors and proteolytic degradation can promote EMT. In malignant tumors, the result from the acquisition of the mesenchymal-like phenotype facilitates the process involved in metastasis[12]. MMP-28 (epilysin) can induce stable EMT when overexpressed in epithelial lung carcinoma cells, indicating that transient activity of epilysin is sufficient to induce a coordinated TGF-β-dependent program leading to the loss of the epithelial phenotype and to the gain of characteristics of invasive cancer cells[13]. MMP-3 (stromelysin-1) and MMP-7 (matrilysin) were able to induce EMT in culture cell lines[12]. On the other hand, there is emerging evidence of a protective role of some MMP in tumor progression, despite rare examples suggesting otherwise. Studies with an experimental model of squamous cell carcinoma (SCC), in MMP-3 null, and wild-type animals indicated a correlation of MMP-3 expression with slower-growing tumors and slower disease progression[14]. Down-regulation (RNA silencing, antisense DNA constructs) and depletion of MMP-8, MMP-9 and MMP-12 showed an inverse correlation, with an increased expression of MMP resulting in decreased tumorigenic events[15].

Serine peptidases

The catalytic action of serine peptidases depends on the interplay among a nucleophile, a general base and an acid. For example, the serine-histidine-aspartate catalytic triad is found in the trypsin-like family (clan S1)[2]. These enzymes participate in many important physiological processes, including digestion (trypsin, chymotrypsin), immune responses (complement factors B, C, D), blood coagulation (factors VIIa, IXa, Xa, XIIa), fibrinolysis (urokinase, tissue plasminogen activator, plasmin, kallikrein) and reproduction (acrosin). Serine Peptidase Inhibitors (serpins) consist of approximately 350 to 500 amino acids and Mr 38 to 70 kDa. Serpins can also be post-translationally modified by glycosylation, sulfation, phosphorylation and oxidation to alter their function. Around 90% of characterized serpins inhibit chymotrypsin-like serine peptidases[16]. The similarity, based on primary structure is around 30%, but it can reach 70% when compared to the hydrophobic sequences. Serpins are irreversible inhibitors because of a mechanism in which the minimalist kinetic scheme is composed of two steps: the formation of the encounter complex or Michaelis complex, in which the sequence of the reactive site loop is recognized by the protease as a substrate; and the formation of a final covalent complex in which the protease is trapped in an inactive state[17]. The serine peptidases and serpins have a role in several events in cancer. In the next topics we will discuss the members involved in cell migration and invasion, and angiogenesis.

Human tissue kallikreins

Human tissue kallikreins (KLK), the largest group of serine peptidases in the human genome, are acidic glycoproteins with approximately 25 to 30 kDa, high specificity for the cleavage of substrate, and are localized in tandem on human chromosome 19q13.4[18]. The KLK are expressed in various tissues such as pancreas, kidneys, brains, pituitary gland, placenta, among others.

Kallikreins mean pancreas in Greek, and it received this name because in 1930 it was identified as the most abundant protease in the pancreas. Kallistatin and α_1-antitrypsin are endogenous inhibitors of human tissue kallikreins. According to the official nomenclature, symbols KLK and hK are used for genes and proteins from human tissue kallikrein, respectively. These genes are expressed as single-chain preproenzymes of approximately 30 to 40 kDa. Zymogen activation is generated by limited hydrolysis of the pro-peptide, inducing a change in conformation of the active site and specificity to the substrate. This process may occur intra and extracellularly by other trypsin-like family members or through self-activation[19]. Once active, KLK promote kinin generation from kininogen, for example, bradykinin (BK), a peptide that dilates blood vessels and is involved in the release of nitric oxide. Pharmacological studies show that kinins promote their biological activity through the activation of two different types of receptors, B1 and B2. The B1 receptor is expressed in healthy tissues in very low concentrations, but is driven by pro-inflammatory cytokines such as IL-1β, TNF-α, and IL-8[20]. The B2 receptor is expressed constitutively and is highly expressed in endothelial cells. BK and kallidin are potent agonists of these receptors. Additionally, receptor antagonists of B1 and B2 have arisen with the development of peptides based on the structures of their agonists, such as HOE140 (B2), [Leu8] des-Arg9-BK (B1) among others, and non-peptide antagonists have been developed for studies offering advances in the understanding of different diseases. BK is involved in angiogenesis by the binding to receptors B1 and B2 in endothelial cells[21]. The use of these receptor antagonists' has shown that blocking this connection reduces vascular permeability, activation of nitric oxide, growth, and progression of cancer. The B2 receptor has been detected in different tumor tissues and in strains of mice[22].

In the last five years, several members of the KLK family have been reported as potential cancer biomarkers. The most well-known member is KLK3 or prostate-specific antigen (PSA), used as a marker for diagnosis and monitoring of prostate cancer. However, the ability to distinguish benign prostatic hyperplasia from prostate cancer based on PSA levels is limited[23]. Due to the structural similarity between members of the KLK's family, other members are being explored in combination with PSA in an attempt of improving specificity[24]. The concentration of Zn^{2+} is decreased in prostate cancer, which consequently increases enzymatic activity of multiple kallikreins (KLK2, KLK3, KLK4, KLK5, KLK8, and KLK14). This overexpression can contribute to the degradation of ECM. There are many studies correlating the expression of KLK with survival and tumor grade across a variety of cancer types, e.g., breast, testicular, renal, esophageal, gastric, pancreatic, colon, lung and salivary gland cancers, squamous cell carcinomas, intracranial cancers, and acute lymphoblastic leukemia[25].

uPA/plasmin system

The uPA/plasmin system is comprised of plasminogen activated proteolytically by urokinase-type plasminogen activator (uPA) and tissue-type plasminogen activator (tPA), two plasminogen activator inhibitors (PAI 1 and PAI 2), uPA receptors (uPAR), and plasmin. Plasminogen is activated by plasma kallikrein and coagulation factors XIa and XIIa, is continually produced in the liver, adrenals, kidneys, brain, heart, liver, uterus, among other organs[26]. uPA correlates with increased cell proliferation and migration, invasion, and metastasis as a result of the dissolution of ECM, including fibrin, fibronectin, proteoglycans, and, as the main molecules in basement membranes, laminin and collagen IV. Once activated, uPA binds its receptor, uPAR, a cysteine-rich glycoprotein attached to the plasma membrane via its carboxy-terminal to a glycosylphosphatidylinositol anchor. This receptor has only been found to physically and functionally associate with β1, β2, and αV integrins. Therefore, the complexes (uPAR-integrin) can contribute to multiple cancer-related events, including adhesion, migration, proliferation, chemotaxis, and possibly tumor-induced immune suppression from immature myeloid cells. Plasmid-mediated RNAi targeting uPAR and uPA inhibited intracranial tumor growth in nude mice by approximately 70 and 55%, respectively [27]. Plasmin is involved in various biological processes such as ovulation, cell migration, wound healing, development of tumors, formation of aneurysms, among others. Plasmin linked to the membrane and protected from circulating inhibitors degrades the components of ECM, activates MMP-2, MMP-3, MMP-9, MMP-12 and MMP-13[28], and due to the proteolytic cleavage of laminin, fibrin, fibronectin, von Willebrand factor, thrombospondin, growth factors, and chemokines, induces tumor development. Angiostatin, a large internal fragment of plasminogen with the ability to inhibit angiogenesis, can maintain metastases in a dormant state in laboratory animals when administered exogenously[29].

The primary inhibitor of plasmin is α_2-anti-plasmin, forming an irreversible complex. PAI-1 and PAI-2 belong to the serpins family. PAI-1 glycoprotein of approximately Mr 52 kDa reacts quickly with uPA and tPA, while PAI-2 has two forms, non-glycosylated

(47 kDa) and glycosylated (60 kDa), inhibits the two activators, but reacts more slowly than PAI-1. Currently, PAI-1 and PAI-2 are targets of tumor models *in vitro* and *in vivo*. Surprisingly, the loss of PAI-1 has been related to reduced tumor growth, invasion, metastasis and poor prognosis. PAI-2 is a predictor of good prognosis for breast cancer, non-small cell lung cancer, and adenocarcinoma, but is associated with aggressive disease in some studies of ovarian, colorectal, colon, and endometrial cancer[30].

CONCLUSION

Metastasis depends on a complex interplay between promoters and suppressors of cell migration, in which the balance between peptidases-inhibitors has an important role. The first signal of peptidase activity can be amplified through proteolytic cascades, which can result in the degradation of components of ECM. Consequently, the collapse of this physical barrier can generate bioactive fragments, activate growth factors, and produce cytokines and chemokines, and changes in cell signaling, among others. Among peptidases, metallopetidases and serine peptidases deserve special attention as targets for drug development and application in diagnostics.

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