Cathepsin S as a target in gastric cancer (Review)

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Abstract. Cathepsin S (Cat S) is a protein expressed in some epithelial cells, which appears to be associated with cancer metastasis and recurrence. The abnormal expression of Cat S has been reported to be associated with the progression of certain types of gastrointestinal neoplasms, including gastric cancer (GC). There is a need to identify novel biomarkers and therapeutic targets associated with the growth, invasion and migration of GC cells, in order to develop non-invasive diagnostic and prognostic procedures and design new therapeutic approaches. The aim of the present study was to assess the association between Cat S and oncogenic processes implicated in the development of GC, focusing on the diagnostic and therapeutic potential of this molecule in GC. A search was conducted through the PubMed and Cochrane Central Register of Controlled Trials electronic databases for relevant literature published between 2003 and 2018, using the mesh terms 'cathepsin S' and 'cancer' and 'gastric cancer'.

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1. Introduction

Proteases represent approximately 2.8% of the proteins expressed by the human genome. They are commonly involved in degradation pathways eliminating unwanted and defective proteins. Researchers have been studying the effects of proteases on cancer development, aiming to develop new anticancer therapies (1-3). Proteases are a large group of enzymes that catalyze the cleavage of peptide bonds. Cathepsin S (Cat S) is a cysteine protease found more frequently in lysosomes of hematopoietic cells, but it may also be segregated in the extracellular environment (4-7).

Studies have demonstrated that the inhibition of Cat S results not only in attenuation of angiogenesis, but also in increased apoptosis and reduction of tumor volume and invasion. These effects indicate a potential relevant role for Cat S in tumor growth and progression, and this molecule may be a possible target for cancer treatment (3,4,8).

The literature on the association between Cat S and gastrointestinal neoplasms is scarce, with even fewer studies assessing specifically its association with GC. The aim of the present study was to review the currently available information regarding Cat S and the occurrence and progression of GC, with the purpose of developing future perspectives for using Cat S as a possible therapeutic target for this malignancy.

2. Gastric cancer

GC is the fifth most frequently diagnosed cancer and the third leading cause of cancer-related mortality worldwide (9). The incidence of GC varies according to different geographic regions. Approximately 60% of gastric neoplasms occur in developing countries. The highest incidence is observed in Eastern Asia, Eastern Europe and the Andean region of South America, whereas the lowest rates are encountered in North America, Northern Europe, Southeast Asia and the majority of African countries (10,11).

Statistical data have been demonstrating a declining incidence of GC, particularly in USA, England, and other developed countries (11). However, an increasing incidence is observed in patients <30 years of age, with the possible implication of different molecules, pathways and epigenetic mechanisms (12).

The GC-related mortality rates are considerably high worldwide, with a mean 5-year survival rate of 21% in Europe and 18% in the USA. The highest rates are reported in Osaka, Japan, with a mean 5-year survival rate of 47% (10).

The majority of GCs occur sporadically due to complex interactions between environmental and ethnic factors. However, approximately 1-3% display an inherited familial component. Patients with germline mutations in tumor protein *p53* (Li-Fraumeni syndrome), breast cancer 2 gene and cadherin-1, in particular, are at an increased risk of developing GC (13).

Infection by *H. pylori* is considered the most important risk factor for the development of GC, particularly gastric adenocarcinoma (14). Although it is clear that *H. pylori* is the most frequent predisposing agent for GC, the precise molecular mechanisms underlying the development of this neoplasm in reaction to *H. pylori* infection have not been clearly determined. However, the increased cellular replication and the constant attraction of polymorphonuclear leukocytes are well known events that appear to exert carcinogenic effects (15,16). Amedei *et al* (17) reported that the secreted peptidyl prolyl cis, trans-isomerase of *H. pylori* is able to drive gastric Th17 response in patients with distal GC. Therefore, they inferred that *H. pylori* may be linked to GC through the pro-inflammatory low cytotoxic response, matrix degradation and pro-angiogenic pathways (17).

Several oncogenes, tumor suppressor genes and metastasis-related genes have been implicated in GC (18). Some of the dysregulated genes in GC, including *p53,* Kirsten rat sarcoma 2 viral oncogene homolog, transforming growth factor receptor-2 and catenin β-1, may be implicated in the development of a wide variety of other tumors. Other genes, such as fibroblast growth factor receptor-2 and MET proto-oncogene, are more specific to GC. Among the mutated genes or those with increased expression in GC, some, such as human epidermal growth factor receptor-2 (*HER2/NEU*), cyclooxygenase-2 (*COX2*) and epidermal growth factor receptor (*EGFR*), have been investigated as possible targets for GC therapy (19,20). It has been demonstrated that trastuzumab, an inhibitor of *HER2/NEU*, can affect the growth of *HER2/NEU*-overexpressing GC cells (21). GC with *COX2* overexpression has been associated with lymphatic metastasis, and the use of *COX2*-specific inhibitors, such as celecoxib, are under investigation as a possible intervention for advanced disease (19). Regarding *EGFR*, it has been observed that increased expression of this receptor is associated with worse prognosis in GC. Its selective inhibition by gefitinib has shown promising results for metastatic disease (22).

3. Cathepsin S and carcinogenesis

During the development and progression of tumors, one of the most important events is local invasion, which is mediated by the degradation of the extracellular matrix (ECM), and the proteases involved in this process are being increasingly identified (23). Invasive tumor cells and their microenvironment are enriched with a wide range of proteases (4).

New therapeutic strategies and biomarkers have been identified for the treatment and diagnosis of malignant tumors. Among these, a group of proteases, the lysosomal cysteine cathepsins (e.g., Cat S), appear to be of extreme relevance. The inhibition of Cat S using a selective monoclonal antibody (Fsn0503) has been shown to be beneficial, acting against colorectal, prostatic and breast carcinoma invasion, and it has also achieved attenuation of angiogenesis in several types of tumors (24). The *in vivo* inhibition of Cat S by Fsn0503 has also achieved a significant decrease in colorectal tumor growth in murine models, not only as an isolated agent (24), but also in combination with chemotherapy (25,26).

The involvement of Cat S in carcinogenesis appears to be related to apoptosis, autophagy, angiogenesis, and cell migration and invasion.

Apoptosis. Lysosomes are essential organelles in the process of apoptosis, and cathepsins are important executors of lysosome-mediated apoptosis. Cat S assists with the essential mediation of apoptotic signaling to release cathepsins to the cytosol. Apoptosis induced by Cat S occurs through different apoptotic pathways, including the intrinsic pathway (mitochondrial death) and the extrinsic pathway (death receptor). The former is controlled by members of the B-cell lymphoma-2 (Bcl-2) family, such as Bcl-2 and Bcl-2-associated death promoter. In the latter, death receptors on the plasma membrane activate the tumor necrosis factor receptor 1 and Fas/CD95. However, the specific molecular mechanisms implicated in lung cancer, GC and prostate cancer are unclear (27,28).

Autophagy. Cat S is associated with autophagy in cancer cells. This may be explained by the association between lysosomes and Cat S. Targeting Cat S may induce autophagy in cancer cells, such as nasopharyngeal cancer, colon adenocarcinoma, oral-epidermoid carcinoma, alveolar basal epithelial and human squamous carcinoma cells. Therefore, the inhibition and induction of autophagy mediated by Cat S is not cell-specific, and targeting Cat S may induce autophagy in GC (27,29).

Angiogenesis. It has been observed that Cat S plays an important role in angiogenesis, which is a crucial part of tumor development and a fundamental step in the transition of tumors from a benign to a malignant state (27).

In an experiment on human umbilical vein endothelial cells (HUVECs), it was observed that the vascular endothelial growth factor (VEGF) stimulated HUVEC capillary tube formation, whereas the addition of three specific Cat S inhibitors suppresses the proteolytic activity of Cat S, resulting in significant reduction of VEGF-induced capillary-like tube development (30).

In another experiment, suppressed VEGF secretion and restrained HUVEC tube formation in human hepatocellular carcinoma was achieved through targeting Cat S by small interfering RNA (28). However, the precise molecular mechanisms through which Cat S interferes with angiogenesis remain elusive (27).

Invasion and migration. Cat S is of paramount importance in cell migration and invasion. It has been observed that silencing Cat S by specific siRNAs leads to inhibition of GC cell invasion (31). The same was observed for other cancer cells, such as hepatocellular carcinoma, lung adenocarcinoma, and skin melanoma cells. Therefore, Cat S may be an important factor for containing malignant cell invasion and migration (27,28).

The expression of Cat S was found to be increased in several types of cancer, including GC. One of its main sources are tumor-associated macrophages (27,32). Therefore, Cat S may be of value not only as a therapeutic target, but also as a prognostic marker, as it is closely associated with the occurrence of metastasis (3,32).

4. Cathepsin S and gastric cancer

The results previously reported in the literature regarding the inhibition of Cat S in different gastrointestinal neoplasms are summarized in Table I. The data on the experimental use of Cat S inhibitors and its outcomes, either *in vivo* or *in vitro*, for gastrointestinal cancer development are summarized. Of note, only a few studies have assessed the role of this molecule in gastrointestinal cancers, and even fewer in GC, which is the main objective of the present study.

Regarding the occurrence of GC, Cat S is associated with one of the hallmarks of tumor development, namely local invasion. This process occurs due to the fact that Cat S is able to degrade the ECM, modulate inflammation and the immune response, as well as regulate other carcinogenic factors (27,33).

Cat S acts as a regulator of signaling pathways including: i) Tyrosine kinase receptors, such as c-Met and AXL (33). Cat S may be necessary for the proteolytic maturation and secretion of c-Met into the extracellular compartment of GC cells; ii) peptidases, including members of the cathepsin family (e.g., Cat D), matrix metallopeptidases and kallikrein families (33). Cat D and matrix metallopeptidases are associated with cancer metastasis and recurrence (33), and they have the ability to digest the ECM that otherwise impedes cancer cell invasion; iii) chemokines/cytokines, such as IL-11 and CXCL16. CXCL16 is a molecule essential for the development of peritoneal carcinomatosis in GC (33), whereas IL-11 is required for GC development; iv) Cytoskeletal proteins and adhesion molecules, such as cortactin (*CTTN*) and integrins, respectively (33). CTTN and integrin α6β4 play important roles in cell-cell and focal adhesion, thereby affecting cell migration (33). The inhibition of Cat S leads to diminished expression of these molecules; v) proteins from *HFE145*, *MKN7* and *MKN45* (33). When compared with normal cells, Cat S expression is increased in the GC cells MKN7 and MKN45 (33).

The members of the cysteine cathepsin family displaying increased expression in GC are Cat B, E, K, L, S, X and Z. Cat S is the only one among the cysteine cathepsins the expression of which is associated with antigen-presenting cells. Therefore, as regards protein processing in the extracellular microenvironment, Cat S is of higher physiological importance compared with the remaining family members (34-36).

Invasive methods, such as endoscopy and biopsy, are used for GC diagnosis. Certain tumor markers may also prove useful in the diagnosis and follow-up of this disease,

Table I. Cathepsin S as an inhibitor of gastrointestinal malignant neoplasms.

Tumor site	Testing	Role	Cell line	Results of cathepsin S inhibitors	Author (Refs)
Colorectal	*In vitro*	Increased expression, associated with tumor growth and invasion	Fsn0503h, MC38	Cytotoxic effect (antibody-dependent cytotoxicity) in 22% of the tumor cells, in addition to the inhibition of invasion and angiogenesis	Wilkinson *et al* (7)
	In vivo				Burden *et al* (25)
					Burden *et al* (26)
					Kwok *et al* (32)
					Small *et al* (30)
Pancreatic	*In vivo, in vitro*	Increased expression, associated with tumor growth	RIP1-Tag2	Inhibition of tumor growth and angiogenesis	Wang *et al* (37)
Hepatocellular	*In vivo*	Induced apoptosis Increased expression, associated with growth, invasion and metastasis	MHCC97-H	CTSS silencing with lentivirus-mediated RNAi can induce significant apoptosis and chemosensitivity in MHCC97-H cells	Lee *et al* (38)
	In vitro				Wang *et al* (39)
Gastric	*In vitro*	Increased expression, associated with tumor invasion and metastasis	MFEN45 and MKN7	CTSS inhibitors may be able to impede invasion and migration of gastric cancer cells	Yang *et al* (33)
					Liu *et al* (31)

CTSS, cathepsin S.

such as carcinoembryonic antigen (CEA), carbohydrate antigen (CA) 72-4 and CA 19-9. However, their sensitivity is not sufficient to enable diagnosis at the early stages (31). Therefore, there is a pressing need for biomarkers with higher specificity and sensitivity to enable early, non-invasive diagnosis, and to monitor the progression of GC (3).

The diagnostic value of serum Cat S has been found to have a specificity similar to the conventionally used markers CEA, CA 72-4 and CA 19-9, but with a higher sensitivity. The combination of serum Cat S and these traditional markers has demonstrated the best results, with specificity and sensitivity of 91.2 and 72.6%, respectively (31).

5. Conclusion

As regards the occurrence of GC, increased expression of Cat S, which is a member of a large group of extracellular proteases, was observed; in addition, the inhibition of Cat S was shown to suppress migration and invasion of gastric neoplastic cells. Therefore, it may be inferred that Cat S may hold promise as a biomarker for the diagnosis and prognosis of GC, in addition to being a possible therapeutic target to control disease progression.

However, due to the scarcity of the currently available literature on the association between Cat S and GC, more studies, particularly clinical trials and prospective studies, are required to provide more solid data and elucidate the true value of Cat S, not only as a diagnostic and prognostic marker, but also as an effective therapeutic target, in the hope of achieving better disease control and improving the life quality and survival rate of GC patients.

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Authors' contributions

ACDC, AABF and JLF were involved in the initial design of the study and conception of the project. FSC, LARM, MARA and CRM helped with the literature research and the writing of the manuscript. ACDC and AABF helped draft the manuscript. AABF and JLF supervised the study. FSC, LARM, JLF were responsible for the final revision. All authors read and approved the final manuscript.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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