Clin. Lab. 2026;72:XXX-XXX
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REVIEW ARTICLE

Clinical Application of Cystatins in Gastric Cancer: a Review

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ABSTRACT

Background: **Cystatins are protease inhibitors, and their imbalance relative to target enzymes is an important factor in cancer development. Cystatins play a significant role in the development of gastric cancer (GC). This article reviews current research on cystatins in GC and provides a comprehensive reference for future studies in the field.**

Methods: **A comprehensive literature search was conducted in PubMed using the key terms: cystatins, gastric cancer, cancer, and tumor. The search strategy was limited to English-language publications, including original articles, review articles, case reports, and clinical trials, with a cutoff date of September 2025. Non-peer-reviewed publications, such as conference abstracts and news reports, were excluded. Additionally, studies that were not directly relevant to the primary research topic were excluded to ensure the pertinence and credibility of the final dataset. Ultimately, 82 high-quality, highly relevant articles were included in the final analysis. The primary aim of this review is to comprehensively summarize the current evidence and recent advances regarding the role of cystatins in gastric cancer.**

Results: **This paper traces the biological origin, biological characteristics, structure, and function of cystatins, as well as their roles in the occurrence and development of GC. The results indicate that cystatin detection, either alone or in combination with other biomarkers, exhibits high sensitivity and specificity for the diagnosis of GC. Cystatins promote the initiation and progression of GC by activating or inhibiting signaling pathways. Studies have shown that cystatins can be used to evaluate the prognosis of GC, and targeting the cystatin/signaling pathway axis may be a promising therapeutic strategy for GC.**

Conclusions: **Cystatins play a pivotal role in the diagnosis of GC, the evaluation of prognosis, and the facilitation of targeted therapy. Cystatins have the advantages of convenience, ease of acquisition, and relatively low cost. If more research is devoted to elucidating the mechanisms of interaction among signaling pathways, cystatins will undoubtedly gain greater clinical utility in the diagnosis and treatment of GC.**

(Clin. Lab. 2026;72:xx-xx. DOI: 10.7754/Clin.Lab.2026.251250)

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Review Article accepted April 12, 2026

KEYWORDS

cystatins, gastric cancer, diagnosis, treatment, prognosis, biomarker

LIST OF ABBREVIATIONS

GC - gastric cancer
CSTA - cystatin A/ stefin A
CSTB - cystatin B/ stefin B
CST1 - cystatin SN
CST2 - cystatin SA

CST3 - cystatin C
CST4 - cystatin S
CST5 - cystatin D
CST6 - cystatin E/M
CST7 - cystatin F
HK - high molecular weight kininogen
LK - low molecular weight kininogen
GPX4 - glutathione peroxidase 4
UVB - ultraviolet B irradiation
EPM1 - myoclonic epilepsy 1
SPHK1 - sphingosine kinases type 1
MHC - II - major histocompatibility class II
NK - natural killer
CRP - c-reactive protein
GSEA - gene set enrichment analysis
OS - overall survival
RFS - recurrence-free survival
KNG1 - kininogen 1
BK - bradykinin
CAS - contact activation system
KKS - kallikrein-kinin system
LPS - lipopolysaccharides
MMP - matrix metalloproteinases
VEGF - vascular endothelial growth factor
PLGF2 - placental growth factor 2
CLL - chronic lymphocytic leukemia
TPI1 - triosephosphate isomerase 1
DMBT1 - deleted in malignant brain tumors 1
ROS - reactive oxygen species
EMT - epithelial-mesenchymal transition
ELFN2 - extracellular leucine rich repeat and fibronectin type III domain containing 2
CCR - creatinine/cystatin C ratio
Scr - serum creatinine
SMI - skeletal muscle index
AUC - area under the receiver operating characteristic curve
TGF-β1 - transforming growth factor β1

INTRODUCTION

Cystatins constitute a superfamily of cysteine protease inhibitors that are widely distributed in body fluids and tissues [1,2]. Based on similarities in amino acid sequence and three-dimensional structure, the cystatin family is classified into three subfamilies: type I, type II, and type III [3]. Type I cystatins encompass stefin A (cystatin A/CSTA) and stefin B (cystatin B/CSTB); Type II cystatins include cystatin SN (CST1), cystatin SA (CST2), cystatin C (CST3), cystatin S (CST4), cystatin D (CST5), cystatin E/M (CST6), and cystatin F (CST7); Type III cystatins comprise both high and low molecular weight kininogens (HK, LK). The core function of cystatins is to modulate the activity of cysteine peptidases via complex formation, thereby maintaining normal intracellular and extracellular physiological processes [3,4]. Meanwhile, individual members of this superfamily exhibit diverse functions, including tumor growth, immune responses, and apoptosis, as well as modulation of signaling pathways [2,5,6].

As a highly prevalent and lethal malignancy worldwide, gastric cancer (GC) is associated with low early diagnosis rates, limited treatment options for advanced stages, and prominent issues of recurrence, metastasis, and chemoresistance, all contributing to poor patient survival and unfavorable prognosis [7-9]. In recent years, the potential of cystatins as novel biomarkers for GC has gradually come to the fore [2]. This review focuses on the biological origins, structures, functions, and roles of cystatins in GC development. It explores their potential as biomarkers for the diagnosis, treatment, and prognosis of GC, providing a reference for related studies on GC.

Introduction of the cystatin family

The biological origin of the cystatin family

The cystatin family originated in plants [10], and phylogenetic analyses indicate that a common ancestor may have been lost in some algal lineages, including the prasinophytes. In contrast, it is maintained in other groups, such as the chlorophyceae [11]. Muller-Esterl et al. demonstrated that the mammalian cystatin superfamily comprises at least three distinct families: stefins, cystatins, and kininogens, which evolved from a common ancestor [12]. The evolutionary model of mammalian cysteine protease inhibitors, based on sequence homology, suggests that diversity emerged from two ancestral units that gave rise to the stefin and cystatin structural units, with gene triplication of the prototype inhibitor leading to the kininogen heavy chain, which contains three copies of the cystatin sequence [12]. The identification and characterization of the first cysteine protease inhibitor were reported in egg white [13]. The name “cystatin” was assigned by Barrett (1981) to a protein from chicken egg white that inhibits various cysteine proteinases [14]. The concept of the “Cystatin superfamily” emerged in the early 1980s from observations of multiple sequences resembling cystatins in kininogen and stefins, which were found to be associated with repetitive sequences [15]. The cystatin superfamily is widely distributed across organisms, including animals, plants, fungi, and protozoa [16]. So far, the superfamily comprises at least 11 distinct members, including CSTA and CST1 (Figure 1).

The structure and function of the cystatin family

The structure and function of type I cystatins

Type I cystatins are acidic and neutral cysteine protease inhibitors that act intracellularly and lack disulfide bonds or sugar groups. They are 12 kDa proteins that function as single-chain inhibitors of papain and of cathepsins B, H, and L [17,18].

CSTA possesses anti-apoptotic properties [18]. Caspase-3 is a crucial cysteine-aspartic protease involved in apoptosis. CSTA inhibits ultraviolet B irradiation (UVB)-induced apoptosis in keratinocytes by suppressing caspase-3 activation [19]. Furthermore, CSTA can

impede tumor cell growth, angiogenesis, invasion, and metastasis by suppressing cathepsin activity [18, 20]. CSTA also serves other biological functions, including key roles in the development and maintenance of the epidermis [21]. CSTB differs from CSTA in several aspects: structurally, CSTB has a free cysteine residue at the NH_2-terminal of the protease binding domain, while CSTA lacks this feature [17]; on the cell type being expressed, CSTA is mainly expressed in keratinocytes, and CSTB is expressed in mononuclear-macrophage cell lines [17, 22]; in intracellular localization, CSTB's intracellular localization depends on the cellular differentiation state, localizing in the nucleus in proliferating cells and both in the nucleus and the cytoplasm in differentiated cells [17]. However, CSTA is a component of the keratinized cell envelope of keratin-forming cells [21]. Besides, CSTB is involved in cell proliferation and neuronal differentiation, and loss-of-function mutations in the CSTB gene cause progressive myoclonic epilepsy 1 (EPM1) [23]. This association may be attributable to the critical role of CSTB in brain physiology and synaptic plasticity [24]. In addition, CSTB helps prevent apoptosis of brain cells and protects neurons from oxidative stress [25] (Table 1).

The structure and function of type II cystatins

Type II cystatins are 13 kDa secreted proteins featuring two disulfide bonds at their COOH-terminal [17]. The proteins encoded by different genes exhibit distinct inhibition profiles towards cysteine proteases and play crucial roles in both physiological and pathological processes. Additionally, cystatins demonstrate dual effects on tumors.

CST3 is a classical inhibitor of cysteine proteases and is among the most extensively studied members of the cystatin family [26]. CST3 is expressed at high levels in all nucleated cells and in most organs, and is detectable in all body fluids [27]. The non-specific expression of CST3 may be related to the structure of the 5' flanking region [28]. CST3 is freely filtered in the glomerulus, is almost entirely reabsorbed and metabolized by proximal tubular cells, and is cleared by the kidney, which is the primary organ responsible for its clearance. Therefore, CST3 showed higher accuracy than serum creatinine (Scr) in assessing kidney function [29]. CST3 plays various physiological roles, including inhibition of cathepsins B, H, K, L, and S [30] and involvement in cell proliferation, differentiation, migration, immunomodulation, defense against microbial and viral infections, neuroprotection, and bone remodeling [27]. Moreover, CST3 exerts either inhibitory or promoting effects on tumor growth, primarily by modulating the activity of cysteine proteases [27]. The anti-cancer function of CST3 is predominantly associated with its inhibitory activity against lysosomal cysteine proteases, which are frequently upregulated in tumor cells and tissues and are implicated in several critical steps in tumor progression, such as cell proliferation, invasion, angiogenesis, and metastasis [31-34]. The precise mechanism underlying the pro-tumor effects of CST3 remains unclear. Nevertheless, lysosomal cysteine proteinases have been shown to induce various forms of cell death in malignant cells *in vitro*, suggesting that CST3 may indirectly contribute to cancer growth and dissemination [27].

CST6 is considered an atypical cystatin due to its ability to inhibit not only lysosomal cysteine proteases, such as cathepsin L, V, and B, but also legumain [35]. CST6 is predominantly expressed in the mammary epithelium, the epidermal granular layer of the skin, sweat glands, hair follicles, and nails, and serves as a selective inhibitor of cathepsins and legumain [36]. CST6 shares structural similarities with other type II cystatins, encompassing a signal peptide and two intrachain disulfide bonds, and possesses glycoprotein properties [37]. Furthermore, this cysteine protease inhibitor has been implicated in regulating epidermal homeostasis and promoting fetal and placental development [38]. CST6 exerts dual effects that are highly microenvironment-dependent during tumor progression. On the one hand, CST6 upregulates the hydrolytic substrate sphingosine kinase type 1 (SPHK1) by inhibiting cathepsin B activity, thereby suppressing p38 activation and osteoclast generation, ultimately hindering breast cancer bone metastasis [39]. This mechanism is supported by a coherent evidence chain spanning cellular assays, animal models, and preclinical pharmacological studies, rendering the conclusion well-substantiated [40]. On the other hand, paracrine CST6 facilitates tumor immune evasion by inhibiting protease-dependent presentation of major histocompatibility complex class II (MHC-II) molecules on the cell surface and by preventing T-cell lysosomal protease-mediated cell death [36]. However, direct experimental evidence for this immunomodulatory function remains relatively scarce, and such inferences are largely based on the established functions of the cystatin family. CST7 has been identified as a critical subunit of type II cystatins through survival analysis [41]. CST7 exhibits distinct characteristics. For instance, human CST7 possesses a third disulfide bond that stabilizes the N-terminal of the molecule [42]. CST7 effectively inhibits papain, cathepsin L, and cathepsin H [43, 44]. The limited inhibition spectrum can be attributed to its dimeric structural features [45]. Analogous to CST3, the N-terminally processed monomeric form of CST7 also inhibits cathepsin C and exhibits enhanced protease inhibitory activity [45,46]. Initially produced as an inactive dimer, CST7 can be targeted to endosomes/lysosomes or secreted, but its main function is intracellular [47, 48]. CST7 is expressed in natural killer (NK) cells and $CD8^+$T cells under steady-state conditions [49]. Modulating glycosylation patterns can enhance NK cell cytotoxicity, modulate immune responses, and improve therapeutic efficacy in cancer by altering the subcellular localization of CST7 [50]. CST7 plays a crucial role in modulating the differentiation and maturation of immune cells during the inflammatory response, thereby exerting a pro-inflammatory effect [51]. The upregulation of CST7 during inflammatory re-

Table 1. The structure of cystatins and their cysteine protease inhibition spectrum.

Type	Coding gene	Structure	Target enzyme
Type I cystatins		12 kDa, lacking disulfide bonds or sugar groups [17]	
Stefin A	CSTA		Papain, Cathepsin B, H, L [18]
Stefin B	CSTB		Papain, Cathepsin B, H, L [17,18]
Type II cystatins		13 kDa, COOH end has two disulfide bonds [17]	
Cystatin SN	CST1		Papain [56]
Cystatin SA	CST2		Papain [56]
Cystatin C	CST3		Cathepsin B, H, L, K, S [30]
Cystatin S	CST4		Papain [56]
Cystatin D	CST5		Cathepsin H, L, S [57]
Cystatin E/M	CST6		Legumain, Cathepsin B, L, V [35] Papain, Cathepsin H, L [43,44]
Cystatin F	CST7		
Type III cystatins			
HK	-	60 - 120 kDa, various structural domain protein glycosylation [38]	Papain [63]
LK	-		Papain [63]

HK: high molecular weight kininogen, LK: low molecular weight kininogen.

Table 2. Research on the diagnosis of cystatins in gastric cancer.

Study	Year	Type	Up or down regulation
Li et al. [2]	2023	CST1	up
Chen et al. [70]	2023	CST4	up
Dou et al. [71]	2018	CST4	up
Zhang et al. [73]	2017	CST4	up
Koopaie et al. [74]	2022	CSTB (Saliva)	down
Xiao et al. [75]	2016	CSTB (Saliva)	-

CST1 - cystatin SN, CST4 - cystatin S, CSTB - cystatin B/stefin B.

Table 3. Research on the therapeutic targets of cystatins in gastric cancer.

Study	Year	Type	Up or down regulation
Chen et al. [76]	2021	CST1	up
Li et al. [2]	2023	CST1	up
Zhang et al. [77]	2020	CST2	up
Zhang et al. [73]	2017	CST4	up

CST1 - cystatin SN, CST2 - cystatin SA, CST4 - cystatin S.

Table 4. Research on the prognosis of cystatins in gastric cancer.

Study	Year	Type	Up or down regulation
Chen et al. [76]	2021	CST1	up
Choi et al. [72]	2009	CST1	up
Li et al. [2]	2023	CST1	up
Zhang et al. [77]	2020	CST2	up
Wang et al. [78]	2017	CST2	-
Chen et al. [70]	2023	CST4	up
Sun et al. [79]	2022	Scr/cystatin C ratio	-
Gao et al. [80]	2021	preoperative CCR	-

CST1 - cystatin SN, CST2 - cystatin SA, CST4 - cystatin S, Scr - serum creatinine, CCR - creatinine/cystatin C ratio.

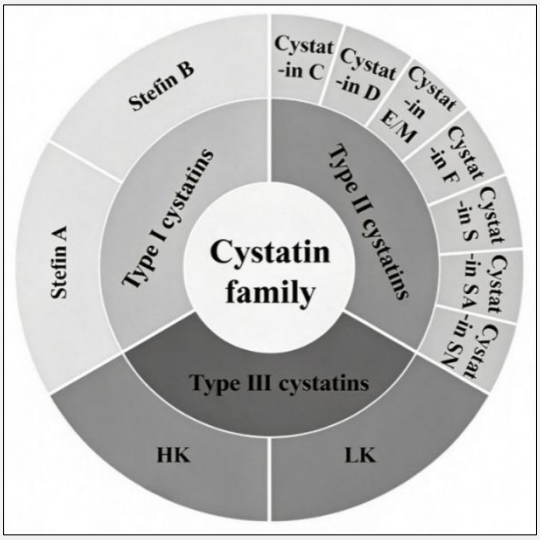


Figure 1. Classification of cystatin family.

The cystatin family includes three types: type I, type II, and type III. Type I cystatins: stefin A and stefin B; Type II cystatins: cystatin C, cystatin D, cystatin E/M, cystatin F, cystatin S, cystatin SA and cystatin SN; Type III cystatins: high and low molecular weight kininogen (HK, LK).
HK - high molecular weight kininogen, LK - low molecular weight kininogen.

sponses is specific to neutrophils [49]. CST7, either alone or in combination with C-reactive protein (CRP), could serve as a biomarker of acute inflammation [49]. CST7 is also closely associated with tumorigenesis. In a gene set enrichment analysis (GSEA) conducted by Yang et al., CST7 expression in patients with early-stage pancreatic ductal adenocarcinoma was associated with regulation of immune cell activity [41]. Furthermore, the role of CST7 in tumor progression is highly context-dependent. Existing studies indicate that neutrophil-derived secretory CST7 may indirectly promote tumor progression by inhibiting anti-tumor immune responses [49,52]. In contrast, intracellular CST7 in tumor cells can directly impair tumor cell invasion by inhibiting cathepsin L [47]. This contradiction underscores the necessity of rigorously distinguishing the cellular origin, subcellular localization, and specific microenvironment in which the molecule exerts its effects in

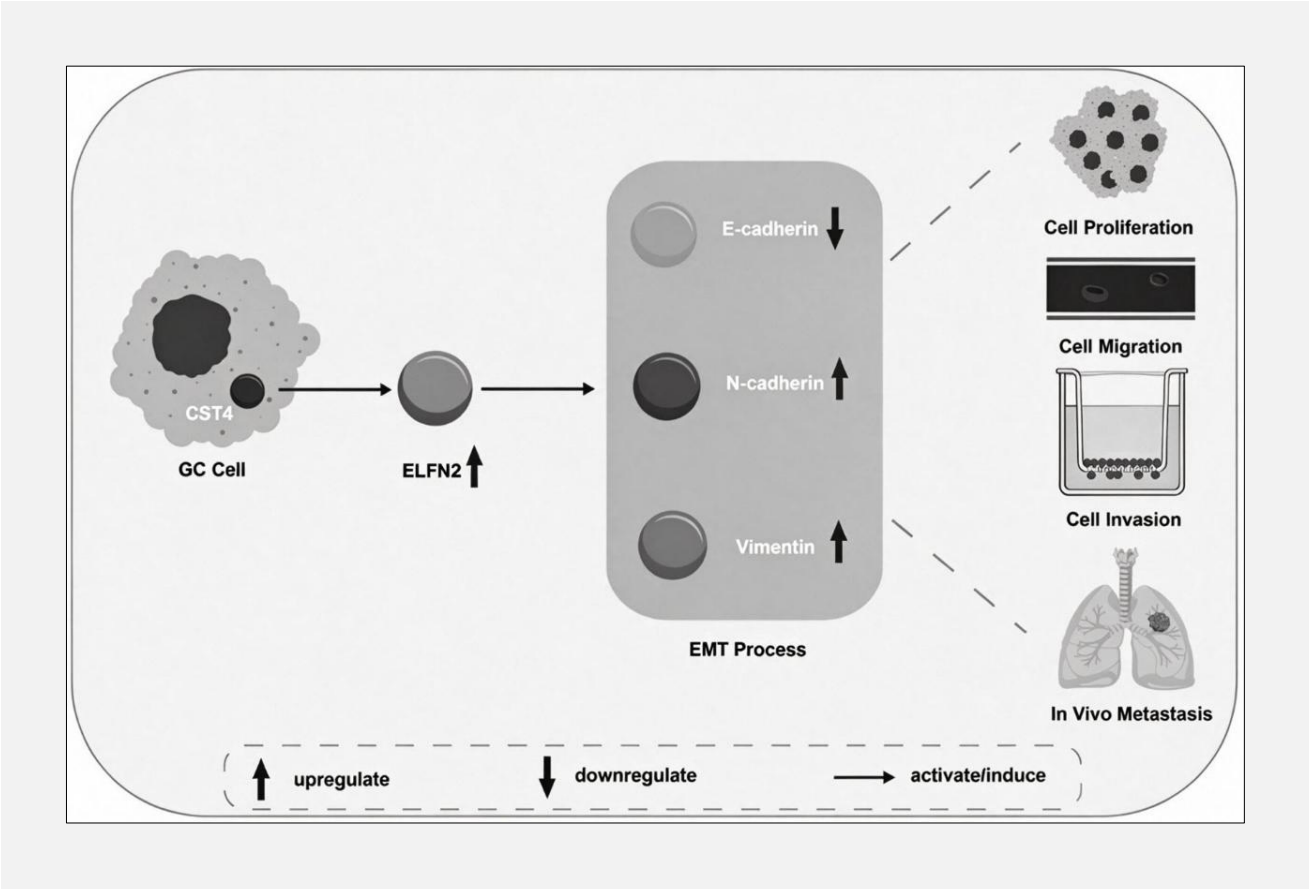


Figure 2. Schematic diagram of the CST4/ELFN2/EMT signaling axis in gastric cancer.

In GC cells, high intracellular CST4 expression upregulates its downstream target ELFN2. This upregulation induces EMT, characterized by downregulation of E-cadherin and upregulation of N-cadherin and Vimentin. Consequently, these molecular changes promote GC cell proliferation, migration, invasion, and in vivo metastasis. Created with BioGDP.com [82]. GC - gastric cancer, CST4 - cystatin S, ELFN2 – extracellular leucine rich repeat and fibronectin type III domain containing 2, EMT - epithelial-mesenchymal transition.

tumor research, to fully elucidate the complex, potentially bidirectionally modulatory biological functions of CST7. Future investigations should conduct separate validation and integrative analysis of these two distinct pathways in specific experimental models.

CST1, CST2, CST4 (S-type cystatins), and CST5 (D-type cystatins) share homology with CST3 and have been shown to inhibit cysteine protease activity through peptide sequencing of human salivary proteins; they are also referred to as salivary cystatins (SD-type cystatins) [53]. CST1, CST2, and CST4 are detectable in tears, urine, and seminal plasma [1]. The tissue distribution of CST5 is more restricted, with primary expression in salivary and lacrimal glands [54]. The inhibitory efficacy of SD-type cystatins on cysteine proteases is comparatively lower than that of CST3 [55]. S-type cystatins universally inhibit papain, with CST1 and CST2 demonstrating greater potency [56]. However, D-type cystatins can inhibit cathepsins H, S, and L [57]. Salivary cystatins play a crucial role in oral homeostasis [55]. Their weak inhibitory activity against cathepsins and strong inhibition of parasite cysteine proteases suggest that they help control oral microorganisms rather than prevent periodontal disease [58]. They also participate in host defense mechanisms against viral infections by interfering with viral replication processes [58]. In recent years, several studies have demonstrated the importance of SD-type cystatins for tumor prognosis, treatment, and as biomarkers. A significant association between upregulation of CST1 expression and decreased overall survival (OS) and recurrence-free survival (RFS) in postoperative hepatocellular carcinoma patients has been observed [59]. Additionally, CST1 has been shown to stimulate cell proliferation by increasing the expression of proteins associated with pancreatic cancer [60]. The study presents, for the first time, the potential utility of CST1 as a biomarker for early pancreatic cancer detection [60]. CST5, a potential tumor suppressor in colorectal cancer, is induced by 1,25-$(OH)_2D_3$ [57]. Furthermore, CST5 has been identified as

a direct target gene of the tumor suppressor protein p53 [61]. Consequently, the activation of CST5 through activating p53 and/or dietary intake of vitamin D_3 may hold significant therapeutic potential for the treatment of colorectal cancer [61] (Table 1).

The structure and function of type III cystatins

The type III cystatins are kininogens [3]. Kininogen is a glycosylated protein, ranging in molecular weight from 60 to 120 kDa, and characterized by multiple structural domains [38]. It consists of an N-terminal heavy chain and a C-terminal light chain interconnected through disulfide bonds [38]. Kininogen is present in various biological fluids (plasma, urine, sperm, synovium, amniotic fluid), organs (liver, lung, spleen), and leukocytes [38]. It is predominantly synthesized by hepatocytes [38].

Kininogens are categorized into high-molecular-weight kininogen (HK, approximately 76 kDa) and low-molecular-weight kininogen (LK, approximately 48 kDa) based on their respective molecular weights. These two proteins are products of alternative splicing of the kininogen 1 (KNG1) gene's messenger RNA [62]. HK is composed of six distinct domains (D1-D6) [63]. D1-D3 serve the function of cysteine protease inhibitors, which effectively suppress the activity of papain [63]. Bradykinin (BK) is released from high molecular weight kininogen after cleavage at two specific sites within the D4 and is integral to various physiopathological processes, including smooth muscle contraction, inflammation, pain, and the promotion of mitosis [63,64]; D5 and D6 are essential for the contact pathway of coagulation [63]. HK functions as both a carrier factor and a cofactor in the contact activation system (CAS)/kallikrein-kinin system (KKS) [63]. As a cofactor, HK binds to negatively charged surfaces to facilitate activation of the system, thereby playing a crucial role in thrombosis and embolization [63]. In contrast, a deficiency in HK can prevent thrombosis, blood-brain barrier disruption, and inflammation by influencing the formation of BK and fibrin [65]. Additionally, activation of KKS is implicated in endotoxemia, in which HK serves as a significant carrier of lipopolysaccharide (LPS) [65,66]. In this context, HK contributes to LPS catabolism and enhances the immune response [65]. This duality highlights the pivotal role of HK in regulating coagulation processes and modulating immune defense mechanisms. Regarding tumors, HK may impede tumor growth by influencing various cell types within the tumor microenvironment, including by inhibiting matrix metalloproteinase (MMP) expression and vascular endothelial growth factor (VEGF) and placental growth factor 2 (PLGF2) expression [67]. Differently, LK possesses five distinct structural domains, which share the same structural D1-4 as HK [63]. LK is not a coagulation factor but is an inhibitor of platelet aggregation [63]. LK, in conjunction with HK, can be cleaved by kinin-releasing enzymes to generate BK [68]. Furthermore, LK expression is upregulated in chronic lymphocytic leukemia (CLL) samples following B-cell receptor stimulation, with 71% of CLL B-lymphocytes exhibiting basal LK protein levels [69]. In contrast, normal B cells do not express the LK protein, suggesting that LK may serve as a biomarker for CLL [69]. In summary, while HK exhibits multiple biological activities, the functional characteristics of LK remain incompletely understood (Table 1).

The cystatins in gastric cancer

The role of cystatins in the diagnosis of gastric cancer

Cystatins play an important role in the diagnosis of GC. In GC tissues and cell lines, the mRNA and protein levels of CST1, CST2, and CST4 were found to be upregulated [70,71]. Choi et al. demonstrated that the mRNA level of CST1 in GC tissues was more than 6-fold higher than that in normal tissues [72]. Moreover, preliminary findings indicate that CST1 may promote gastric carcinogenesis by enhancing cell proliferation and inhibiting cathepsin activity [72]. However, its functional validation has relied primarily on a single AGS cell line; thus, its clinical translational potential warrants further verification via in vivo models and mechanistic studies. Future research could focus on the cell-type-specific roles of CST1 in the GC microenvironment and the feasibility of its development as a therapeutic target. Additionally, Li et al. identified upregulation of CST1 expression in peritoneal metastatic GC tissues using RNA sequencing [2]. This finding suggests that CST1 may also serve as a novel oncological marker for the diagnosis of gastric metastasis [2]. Dou et al. developed a CST4 antibody sandwich ELISA detection system, which showed a sensitivity of 69.0% and a specificity of 85.6% for diagnosing GC with a cutoff value of 1.0107×10^{-4} mg/L, and the area under the receiver operating characteristic curve (AUC) was 0.841 [71]. The positive detection rate for GC with this system was 53% higher than that with the best traditional marker, CA19-9 [71]. This study provides solid methodological and clinical evidence supporting CST4 as a potential GC biomarker, as confirmed by Zhang et al. [71,73]. Nevertheless, its clinical translational potential still warrants further validation in multicenter prospective studies. Furthermore, the presence of cystatins in saliva contributes to the diagnosis of GC. Koopaie et al. demonstrated that salivary CSTB was downregulated in GC. It exhibited a diagnostic sensitivity of 83.87%, a specificity of 70.97%, and an AUC of 0.728 for GC, indicating moderate diagnostic value, although its performance was limited when used alone [74]. Future multicenter prospective cohorts are warranted to optimize the cutoff value and explore its combination with biomarkers such as deleted in malignant brain tumors 1 (DMBT1) to improve clinical utility. Xiao et al. identified, via quantitative proteomics and TMT (tandem mass tag) technology, that the combined diagnostic sensitivity and specificity of three salivary proteins, namely CSTB, triosephosphate isomerase 1 (TPI1), and DMBT1, for GC could reach as high as 85% and 80%, respectively

[75]. This offers a new perspective on early screening for GC. The noninvasive and readily obtainable characteristics of saliva make CSTB a crucial biomarker for large-scale screening and early detection of GC. In conclusion, the detection of cystatin levels can screen individuals at risk of GC to help diagnose GC. Additionally, combined detection has a higher diagnostic value (Table 2).

The role of cystatins in the treatment of gastric cancer

Cystatins can serve as therapeutic targets for GC by modulating signaling pathways. Almost all cystatins expressed in GC tissues and cell lines are elevated. Results from Chen et al. demonstrated that high CST1 expression was significantly associated with Wnt pathway activation (NES > 1.5, P < 0.05) [76]. This suggests that targeting the CST1-Wnt axis may represent a novel therapeutic strategy for GC. Nevertheless, this study was limited by its sample size and the completeness of statistical reporting. Further rigorous preclinical and clinical studies are therefore warranted to consolidate its translational foundation. In metastatic GC, CST1 upregulation has been shown to enhance the migratory, invasive, and metastatic properties of GC cells in nude mice [2]. This effect is mediated by the stabilization of glutathione peroxidase 4 (GPX4) and the reduction of intracellular reactive oxygen species (ROS) through the recruitment of the deubiquitinating enzyme OTUB1 ultimately suppressing ferroptosis and promoting epithelial-mesenchymal transition (EMT) and metastasis in GC cells [2]. Therefore, targeting CST1 may represent a promising therapeutic strategy for the management of metastatic GC. EMT is a crucial biological process associated with the migration and invasion abilities of malignant tumor cells. Upregulation of CST2 enhances the growth, migration, and invasion of GC cells by promoting EMT and transforming growth factor β1 (TGF-β1) signaling pathways [77]. This suggests that the CST2/TGF-β1 axis may be a therapeutic target for GC. Furthermore, a study by Zhang et al. demonstrated that CST4 expression is positively correlated with extracellular leucine rich repeat and fibronectin type III domain containing 2 (ELFN2) expression and that CST4 activates the EMT process by upregulating ELFN2, thereby promoting GC progression [73] (Figure 2). These results suggest that targeting the CST4/ELFN2 signaling axis could be a promising therapeutic approach for GC. This study conducted multi-level validation by integrating clinical specimens, cell models, and animal experiments. However, its clinical sample size was relatively small, and further validation in multicenter, large-sample cohorts is warranted. Notably, members of the cystatin family not only exhibit regulatory potential at the individual molecular level but also demonstrate promising clinical application prospects in combinatorial models. For instance, a 9-gene model that includes CST2 may aid in monitoring the long-term treatment of GC [78]. In a word, cystatins may serve as potential targets for GC and serve as biomarkers to monitor treatment response (Table 3).

The role of cystatins in the prognosis of gastric cancer

Indeed, the prognostic role of cystatins has been extensively investigated in GC. Mounting lines of evidence consistently demonstrate that high expression of CST1 is associated with more advanced disease progression and poorer prognosis in patients with gastric cancer. For example, Choi et al. performed immunohistochemical staining on surgical specimens from 77 cases of gastric adenocarcinoma and found that high CST1 expression was significantly associated with advanced pTNM stage [72]. This clinical association is functionally supported at the mechanistic level: Chen et al. (2021) confirmed, using Transwell assays, that overexpression of CST1 increased SNU-5 cell migration by 2.1-fold and invasion by 1.8-fold [76]. Furthermore, another study has further indicated that CST1 is upregulated in metastatic gastric cancer tissues and is closely associated with poor patient prognosis [2]. Collectively, multi-level evidence from clinical observations to functional experiments demonstrates that CST1 acts as a pro-tumorigenic factor in gastric cancer progression, particularly in metastatic processes. Although the underlying mechanisms have been rigorously investigated with statistically significant results, their potential clinical translational value requires further validation through in-depth studies. Similarly, Zhang et al. demonstrated that CST2 promotes GC migration and invasion by activating the TGF-β1/EMT axis, and that its high expression is significantly correlated with malignant tumor progression [77]. CST2 is expected to serve as a prognostic biomarker for GC, whereas further in vivo studies and targeted intervention experiments are warranted to validate its translational value. Wang et al. developed a 9-gene model that includes CST2, demonstrating its efficacy as a prognostic tool for predicting survival outcomes in GC patients [78]. The study demonstrated that the AUC of the 9-gene signature model was 0.741 using multivariate survival analysis, indicating high predictive accuracy [78]. The impact of other cystatins on patient prognosis varies. Chen et al. (2023) conducted an integrated analysis of multicenter databases (including TCGA and KM Plotter) and a clinical cohort (n = 115). They found that patients with high CST4 expression had a median survival time of 18 months shorter than those with low CST4 expression [70]. More importantly, multivariate analysis confirmed that CST4 is an independent prognostic factor for GC (HR = 1.76, p = 0.015) [70]. This study had a large sample size and reproducible results, but lacked functional experiments to validate the direct signaling pathway of CST4. Sarcopenia has been identified as an adverse factor impacting surgical outcomes and long-term survival among GC patients [79]. The serum creatinine-to-cystatin C ratio (CCR) has been proposed as a potential surrogate marker for sarcopenia [79]. A study has shown that lower preoperative CCR levels in GC patients are linked to a higher risk of postoperative complications, underscoring the potential utility of CCR in predicting these complications and supporting clinicians in preoperative risk

assessment [80]. Two additional studies have corroborated the findings that GC patients with higher CCRs have higher overall survival rates than those with lower ratios [79,81]. Conversely, lower serum CCR is associated with poorer recurrence-free survival in patients with gastrointestinal stromal tumors [81]. Moreover, combining the CCR with the skeletal muscle index (SMI) improves prognostic accuracy in patients with GC [79]. These collective results indicate that serum CCR is a valuable prognostic predictor in patients with GC. Cystatins have demonstrated utility as significant biomarkers for evaluating the prognosis of GC, offering pertinent insights for clinical applications. Utilizing cystatins as pivotal biomarkers for prognostic evaluation in GC can provide valuable guidance for clinical decision-making. Through vigilant monitoring of cystatins and related parameters, healthcare providers can enhance their ability to assess patient condition and prognosis, facilitating the formulation of personalized treatment strategies (Table 4).

CONCLUSIONS AND PERSPECTIVES

The involvement of cystatins in GC has emerged as a focal point of significant research interest. This article offers an insightful overview of the biological origins and molecular architecture of cystatins, along with a comprehensive exploration of their pivotal functions and the latest research on GC. Based on an analysis of the relevant literature, it can be concluded that cystatins, as important bioactive peptides, not only regulate proliferation, apoptosis, invasion, and metastasis in GC cells but also are closely associated with clinical manifestations and prognosis.

Consequently, cystatins emerge as critical factors in the diagnostic, therapeutic, and prognostic landscape of GC.

However, the mechanisms of cystatins within the GC landscape remain largely unresolved. For example, the precise molecular mechanism underlying cystatin receptor activity in GC remains incompletely elucidated. There is a pressing need for additional research to elucidate the intricate interplay between cystatins and cellular signaling pathways, which is crucial for understanding their role in GC progression. Furthermore, in the therapeutic approach for patients with GC, optimizing the utilization of cystatins and their associated signaling pathways for personalized treatment represents a key direction for future research.

Given the significant regulatory role of cystatins in GC and their potential clinical value, cystatin-related research and translation have become an important area in the precision diagnosis and treatment of GC. Moving forward, it is essential to prioritize standardized research to advance in-depth exploration and translate cystatin-based strategies into clinical practice. On the one hand, multicenter, large-sample clinical trials are needed to establish detection standards and cutoff values for key cystatin subtypes, explore their combined application with traditional clinical indicators, and validate their clinical utility through prospective cohort studies. This will facilitate their incorporation into routine clinical workflows for early diagnosis, pathological subtyping, treatment response evaluation, and recurrence monitoring in GC, thereby providing a precise reference for individualized treatment decision-making. On the other hand, it is crucial to investigate in depth the synergistic mechanisms between cystatins and various treatment modalities, such as chemotherapy, radiotherapy, and immunotherapy, and to develop targeted therapeutic strategies that focus on cystatins and their receptors. Fully leveraging their potential as critical targets to activate or inhibit relevant signaling pathways and precisely regulate the proliferation and metastasis of GC cells will aid in the development of targeted drugs and combination therapies, enabling precise intervention against tumor cells. Additionally, attention should be given to the influence of cystatin functional status on the sensitivity of GC cells to conventional chemotherapeutic agents, offering new guidance for clinical chemotherapy regimens. However, critical knowledge gaps remain in this field. Current basic and clinical studies are gradually addressing these shortcomings, laying the groundwork for subsequent mechanistic elucidation and clinical translation. At the clinical research level, efforts are primarily focused on analyzing the correlation between cystatin expression levels and diagnostic efficacy and prognostic outcomes in patients with GC. At the basic research level, studies aim to clarify the molecular mechanisms of cystatin receptor activation, their regulatory relationships with downstream signaling pathways, and their specific roles within the tumor microenvironment. Concurrently, research is delving into the dynamic changes and functional heterogeneity of cystatins during the progression of GC. Overall, there remains substantial scope for exploration and significant potential for development in both the mechanistic understanding of cystatins in GC and their clinical translation. As research in this area continues to deepen, cystatins are expected to transition from biomarkers to precision therapeutic targets, opening new dimensions for the advancement of personalized medicine in GC.

Source of Funds:
The Chengguan District Science and Technology Project in Lanzhou City funded this work. (Grant No. 2021SHFZ0002).

Declaration of Generative AI in Scientific Writing:
During the preparation of this work, the authors used ChatGPT to improve the language. After using this tool, the authors reviewed and edited the content as needed and assumed full responsibility for the publication.

Declaration of Interest:
The authors have no conflicts of interest to declare.

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