REVIEW

Research progress of cystatin SN in cancer

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Abstract: Cystatin SN, belonging to the type 2 cystatin superfamily, is widely expressed and distributed in mammals. Cystatin SN is involved in inflammation, cell cycle, cellular senescence, tumorigenesis, and metastasis. Cystatin SN is also known to participate in signaling pathways like Wnt signaling pathway, GSK3 signaling pathway, AKT signaling pathway, and IL-6 signaling pathway. Cystatin SN was found to be highly expressed in peritumoral normal tissues in esophageal squamous cell carcinoma (ESCC); however, low cystatin SN expression was found in ESCC cancer tissues. Conversely, in other cancer types such as lung cancer, breast cancer, gastric cancer, pancreatic cancer, and colorectal cancer, high cystatin SN expression in cancer tissues but low cystatin SN expression in peritumoral normal tissues was found. Survival analyses showed that high cystatin SN expression benefited ESCC patients but did harm to other types of cancer patients. Univariate and multivariate analyses indicated that cystatin SN possibly acts as a marker for cancer prognosis. Here, we provide a brief introduction about the role of cystatin SN in cancer and discuss the different prognostic effects of cystatin SN on different tumors. Cystatin SN might be a potential marker for cancer prognosis and a target for cancer therapy.
Keywords: cystatin, cancers, signaling pathway, inflammation

Introduction

Cystatin belongs to a class of protease inhibitors, and in human cells and tissues, the number of cystatin forms appear to be 12.[1] In the center of their sequence is present a highly conserved segment called "cystatin motif", which is crucial for their functions as protease inhibitors.[1] According to differences in the distribution of cystatin in the organism, 12 kinds of cystatin are identified and divided into three types: type 1, type 2, and type 3.[2] Type 1 cystatin is an intracellular type and includes cystatin A and cystatin B, with the simplest structure among the three different types of cystatins.[2] Type 1 cystatin is a single-chain protein containing 100 amino acid residues, with no disulfide bond and sugar chain.[3] Type 2 cystatin is extracellular and includes cystatin C, cystatin D, cystatin E/M, cystatin F, cystatin G, cystatin S, cystatin SN, and cystatin SA.[3] Type 2 cystatin is composed of non-glycosylated proteins which may be phosphorylated.[2,3] Type 3 cystatin comprises intravascular inhibitors and includes L-kininogen and H-kininogen; this is also a multi-domain protein containing three cysteine protease inhibitors that exhibit different activities and structures.[2,3]

The type 2 cystatin superfamily was widely expressed and distributed in animals and microorganisms.[1,4] Among animals, type 2 cystatin is found in body fluids and tissue fluids.[5] All forms of human type 2 cystatin presented two conserved disulfide bridges at the C-terminal end of the sequence.[6] Cystatin SN belongs to the type 2 cystatin superfamily, and it is a type of secretory protein consisting of 121 amino acids and with a relative molecular mass of ~14.5 kDa.[6] Cystatin SN is encoded by CST1 gene which contains three exons and two introns and is located on chromosome 20p11.2.[6]

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With regard to the function of cystatin SN, it is widely involved in determining cell fate and disease development, such as cell cycle, cellular senescence, tumorigenesis, and metastasis.[7–14] Also, cystatin SN is involved in inflammation and functioned in signaling pathways like Wnt signaling pathway, GSK3 signaling pathway, AKT signaling pathway, and IL-6 signaling pathway.[18,19,27,36] Cystatin SN was found to be an independent prognostic factor through univariate and multivariate analyses and was greatly related to survival of cancer patients.[7,8,18,19,26,27,36] In this study, we are going to provide a detailed review of the function and role of cystatin SN in different cancers.

Function of cystatin SN and its role in non-neoplastic disease

Cystatin SN in human saliva has the function of antimicrobial activity and plays a crucial role in maintaining the homeostasis of the oral cavity.[8] In healthy individuals, cystatin SN acts as the inhibitor of cysteine proteases in the oral environment and is involved in the control of the proteolytic events such as inhibiting the activity of papain in vivo.[15,16]

Studies showed that the expression of cystatin SN was related to the sensitivity of individual oral cavity to caffeine bitterness.[14] For individuals who were not sensitive to caffeine bitterness, the expression level of cystatin SN was increased, while the sensitive individuals showed a decreased expression of cystatin SN.[14] Studies also showed that caffeine intake may increase cystatin SN abundance and caused possible consequences on taste sensitivity.[15]

Cystatin SN was upregulated in patients with allergic rhinitis, and cystatin SN could amplify inflammation and may contribute to the severity and recurrence of intractable chronic rhinosinusitis with nasal polyps.[10,16,17]

Cystatin SN and inflammation

Cystatin SN is also involved in inflammation.[10,16–18] In patients with seasonal allergic rhinitis, cystatin SN expression in the nasal mucosa was upregulated by allergen exposure through activation acquired Th2-type immune responses.[10] Another study showed increased expression of cystatin SN in seasonal allergic rhinitis patients and that cystatin SN may contribute to inactivation of protease allergens and help to re-establish homeostasis of the nasal membranes through inhibiting histamine release.[16] Cystatin SN could amplify eosinophilic infiltration and T-helper cell type 2 inflammation by interacting with epithelial-derived cytokines and fibroblasts on nasal polyps.[17] IL-6 is an important inflammatory factor related to immune response.[12] In MDA-MB-231 and SW480 cancer cells, CST1

knockdown lead to IL-6 accumulation,[18] which affected the growth of cancer cells. Abovementioned studies showed that cystatin SN is a potential inflammation-related factor.

Cystatin SN and cell cycle

In SW480 colon cancer cells and MDA-MB-231 breast cancer cells, CST1 knockdown exhibited G1-phase cell cycle arrest, which was caused by the expression of cell cycle inhibitors p21 and p16 and the downregulation of cyclin D1 and p-Rb.[18] In pancreatic cancer cell lines, *CST1* mRNA overexpression contributed to the expression of cell cycle associated proteins like cyclin D1, cyclin A2, and cyclin E.[19] All these studies showed that cystatin SN played critical roles in cell cycle.[18,19]

Cystatin SN and cellular senescence

Most healthy human tissues expressed little or no detectable levels of cystatin SN; however, significant amounts of cystatin SN were detected inside the senescent cells, which could make it an excellent candidate biomarker for identifying senescent cells in vivo.[20]

One study showed that during the progress of cellular senescence, human fibroblasts exhibited consistent and strong upregulation of cystatin SN, which showed cystatin SN was greatly related to cellular senescence.[20] However, another study showed CST1 knockdown also contributed to cellular senescence and inhibited cancer cell proliferation and tumor growth.[18]

Cystatin SN and tumor

Cystatin SN was originally isolated as a neutral salivary cystatin,[21] and in vitro studies suggested that cystatin SN might restrict the proteolytic activities of cysteine proteases.[22–25] Cystatin SN is expressed differently in cancer, as shown in Figure 1. In esophageal squamous cell carcinoma (ESCC), cystatin SN showed higher expression in peritumoral tissues than in the ESCC tissues; however, in other types of cancer, tumor tissues usually showed higher cystatin SN expression than the normal tissues.[7,9,13,19,26,27] Numerous studies have implied that cystatin SN played important roles in the development of several kinds of human cancer, such as pancreatic malignant neoplasm, gastric cancer, ESCC, and colorectal cancer (CRC).[11,19,26,27] In different types of cancers, cystatin SN functioned in various ways, and its function mechanisms in cancer types are summarized in Table 1. Univariate and multivariate analyses of cystatin SN expression indicated that cystatin SN was an independent prognostic factor in several types of cancers, as shown in Table 2. In ESCC, high
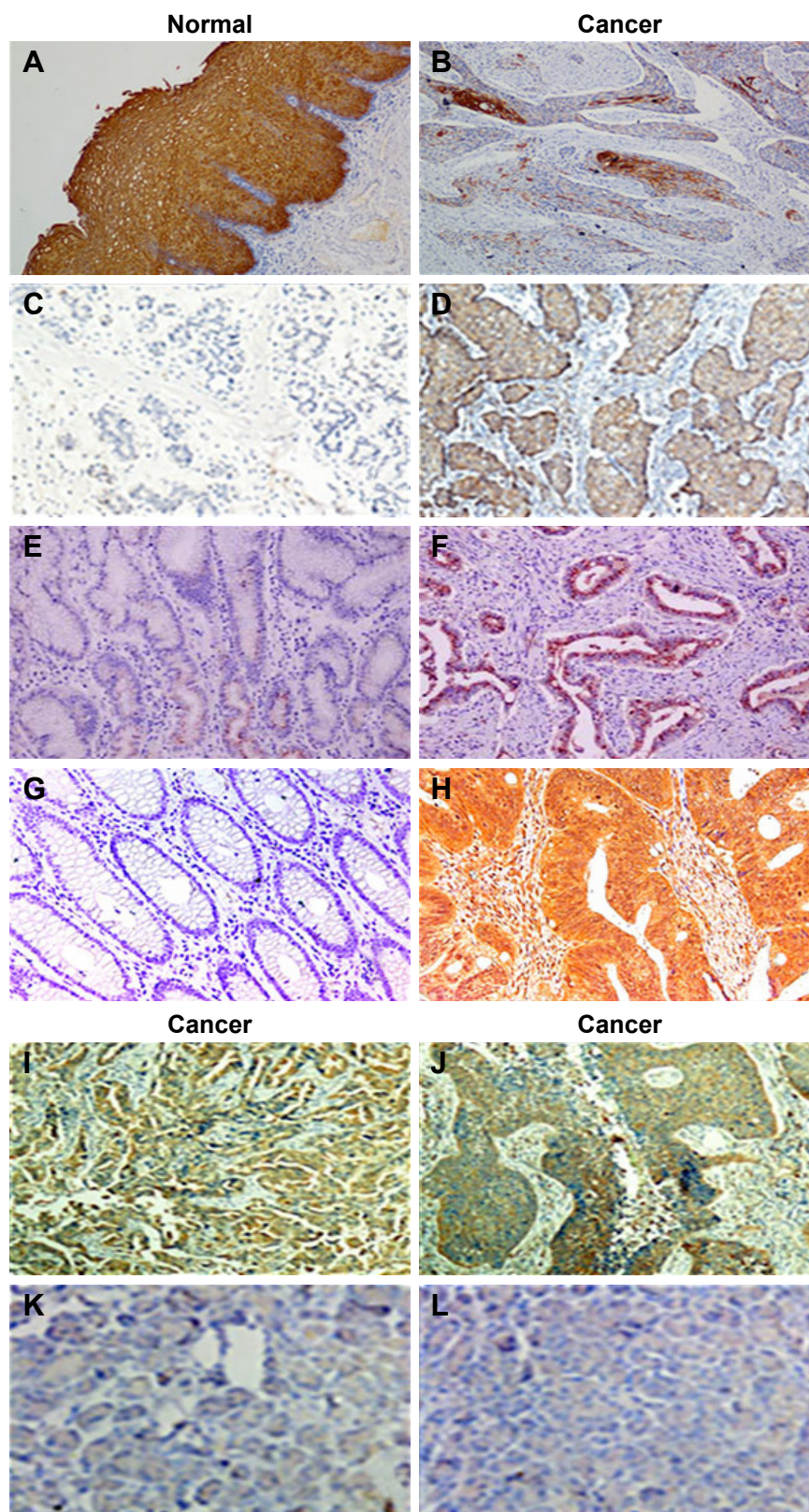
Figure 1 Representative immunohistochemistry of cystatin SN in cancers.
Notes: Peritumoral normal tissues (**A**, **C**, **E**, **G**) and tumor tissues (**B**, **D**, **F**, **H**, **I**, **J**, **K**, **L**). Normal and tumor tissues in ESCC (**A** and **B**), normal and tumor tissues in breast cancer (**C** and **D**), normal and tumor tissues in gastric cancer (**E** and **F**), normal and tumor tissues in CRC (**G** and **H**), tumor tissues in NSCLC (**I** and **J**), tumor tissues in pancreatic cancer (**K** and **L**). (**A** and **B**) adapted from Chen et al,[26] (**C** and **D**) from Dai et al,[7] (**E** and **F**) Reprinted from *Clin Chim Acta*, 406(1–2), Choi EH, Kim JT, Kim JH, et al, Upregulation of the cysteine protease inhibitor, cystatin SN, contributes to cell proliferation and cathepsin inhibition in gastric cancer, 45–51, copyright 2009, with permission from Elsevier,[27] (**G** and **H**) from Li et al,[13] (**I** and **J**) from Cao et al,[9] (**K** and **L**) Reprinted by permission from Springer Nature: *Tumour Biol.* Jiang J, Liu HL, Liu ZH, Tan SW, Wu B. Identification of cystatin SN as a novel biomarker for pancreatic cancer. 2015;36(5):3903–3910. Copyright 2015.[19]
Abbreviations: ESCC, esophageal squamous cell carcinoma; NSCLC, non-small-cell lung cancer; CRC, colorectal cancer.

Table 1 Cystatin SN and its functional mechanism in cancer types

Cancer types	Cystatin SN	Mechanism	References
Esophageal squamous cell carcinoma	Increased in peritumoral tissues	Through analysis of a large patient cohort	26
Lung cancer	Increased in cancer tissues	The promoter region of CST1 presents hypomethylation	9, 28
Breast cancer	Increased in cancer tissues and in cell lines BT-474, MDA-MB-468, and MDA-MB-231	By affecting cellular senescence	7, 18
Gastric cancer	Increased in cancer tissues and in cell lines SNU-620 and AGS	Through T-cell factor-mediated proliferative signaling	27
Pancreatic cancer	Increased in cancer tissues and in cell lines BXPC-3, PANC-1, and SW1990	By increasing malignancy-associated proteins such as PCNA, cyclin D1, cyclin A2, and cyclin E	19
Colorectal cancer	Increased in cancer tissues and in cell lines HCT116, SW480, and HT-29	By neutralizing the inhibition of CTSB proteolytic activity by CST3; by regulating autophagy induction and ROS production; and by affecting let-7d/CST1/p65 pathway	13, 18, 30, 31

expression of cystatin SN was a profitable survival factor; however, other cancer types showed completely opposite results. The results of the survival analyses of cystatin SN expression in patients are presented in Figure 2.

ESCC

Peritumoral tissues showed higher cystatin SN expression than ESCC tissues (Figure 1A and B). Patients with high level of cystatin SN expression had more favorable survival time after surgical treatment than those with low expression, (Figure 2A and B). Besides, cystatin SN was believed to be an independent predictor of the 5-year survival rate of patients with surgically resected ESCC, based on the analysis of a large patient cohort.[26] In this study, univariate and multivariate analyses demonstrated that cystatin SN expression was an independent prognostic factor, as shown in Table 2.

Lung cancer

In lung cancer, the promoter region of CST1 presents hypomethylation, which played an important role in the upregulation of cystatin SN.[28] High expression level of cystatin SN was observed in non-small-cell lung cancer (NSCLC) patients (Figure 1), and patients with high level of cystatin SN showed more serious recurrence and poorer survival times compared to those with lower expression (Figure 2C and D). Through univariate and multivariate analyses, we could infer that high expression of cystatin SN was a significant prognostic indicator for a higher rate of recurrence, metastatic risk, and poor survival in patients with surgically resected NSCLCs,[9] as summarized in Table 2.

Breast cancer

CST1 was generally upregulated in breast cancer at both mRNA and protein levels and played an important role in breast cancer

patients (Figure 1C and D). Studies showed cystatin SN could promote cell proliferation and clone formation, migration, and invasion in BT-549 and MDA-MB-415 cells.[7] Survival curves showed that the low cystatin SN expression group appeared to have higher survival probability than the high expression group, which indicated that the expression level of cystatin SN was closely correlated to the survival risk in breast cancer patients (Figure 2E and F). Table 2 shows that by univariate and multivariate analyses, CST1 expression was demonstrated as an independent prognostic factor. CST1 knockdown in BT-474 and MDA-MB-468 cells significantly inhibited cell migration capability.[7] In MDA-MB-231 cells, CST1 knockdown increased phosphorylation of glycogen synthase kinase 3β at serine 9, leading to induction of glycogen accumulation associated with cellular senescence and inhibition of tumor proliferation,[18] which indicated cystatin SN might affect the growth of cancer cells by affecting the GSK3 signaling pathway (Figure 3A). Furthermore, CST1 knockdown suppressed cancer cell proliferation and tumor growth in a xenograft model and led to IL-6 accumulation,[18] which indicated that cystatin SN might also affect the IL-6 signaling pathway (Figure 3A). Another study in MDA-231 cell lines showed that the expression of CST1 gene could be suppressed significantly by CAPC, a tumor suppressor gene that played important roles in the suppression of tumor growth and metastasis.[29]

Gastric cancer

Cystatin SN expression was found to be upregulated in gastric tumor tissues (Figure 1E and F) and cell lines (SNU-620 and AGS). Upregulation of cystatin SN contributed to cell proliferation in gastric cancer.[27] Classical Wnt signaling pathway was widely involved in a variety of cancers and played diverse roles in governing cell fate, proliferation, migration, polarity, and death.[33–35] In AGS and SNU638 gastric cancer
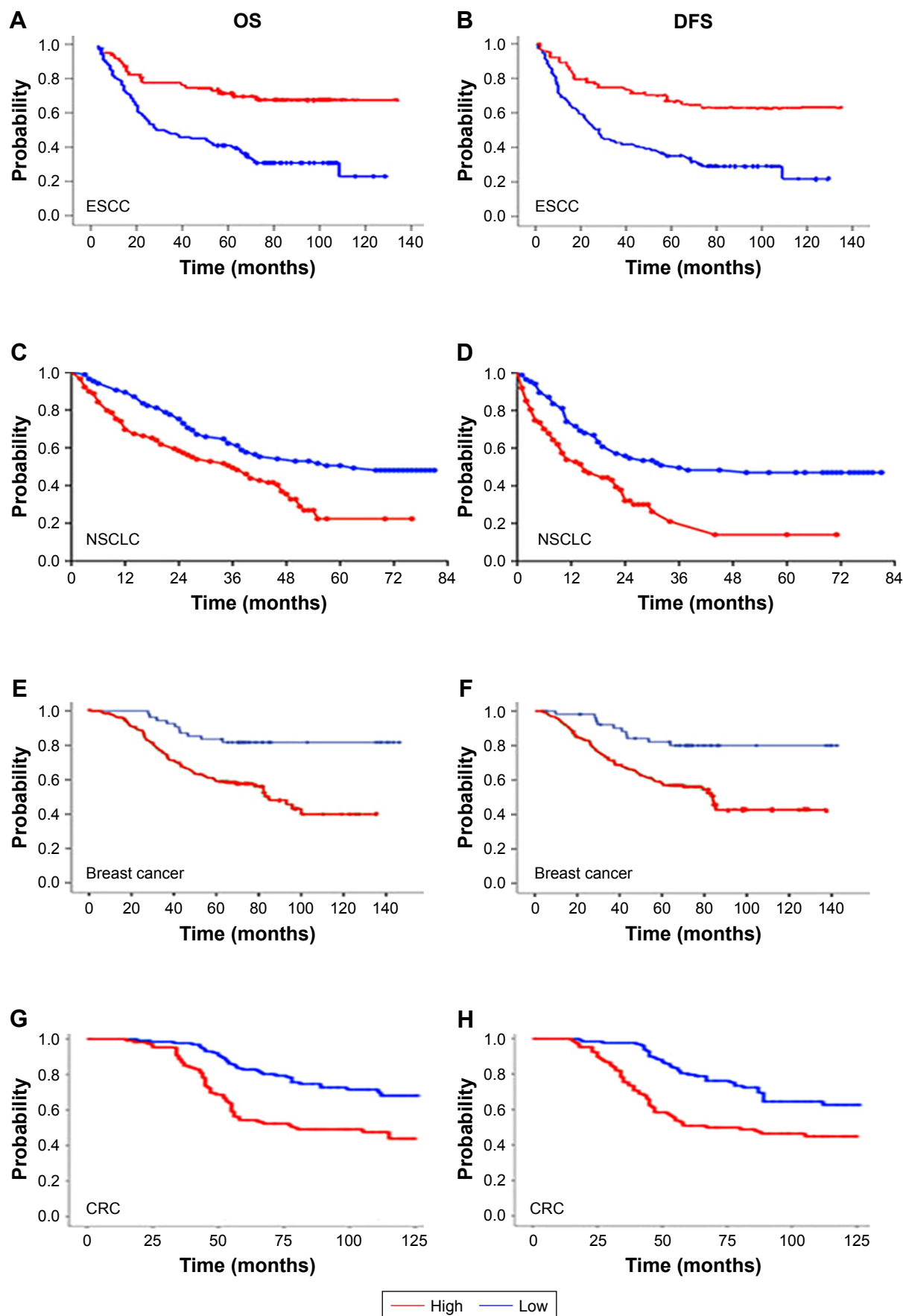
Table 2 Univariate and multivariate analyses of cystatin SN expression

Cancer types	Cases	Cystatin SN expression IHC	DFS						OS						Reference
			Univariate analysis			Multivariate analysis			Univariate analysis			Multivariate analysis			
			HR	95% CI	P-value	HR	95% CI	P-value	HR	95% CI	P-value	HR	95% CI	P-value	
ESCC	209	Higher in peritumoral normal tissues	0.431	0.271–0.685	<0.001	0.426	0.270–0.672	<0.001	0.377	0.231–0.616	<0.001	0.378	0.233–0.614	<0.001	26
NSCLC	174	Higher in cancer	2.099	1.412–3.121	<0.001	2.473	1.457–4.074	0.001	1.906	1.284–2.830	0.001	2.048	1.231–3.406	0.006	9
Breast cancer	244	Higher in cancer	5.083	2.219–11.64	<0.001	2.971	1.258–7.01	0.013	5.611	2.443–12.88	<0.001	3.390	1.428–8.05	0.006	7
Gastric cancer	77	Higher in cancer	–	–	–	–	–	–	1.69	1.42–2.02	<0.001	–	–	–	27
Pancreatic cancer	6	Higher in cancer	–	–	–	–	–	–	–	–	–	–	–	–	19
CRC	375	Higher in cancer	2.143	1.441–3.187	0.0002	2.118	1.1410–3.182	0.0003	2.386	1.566–3.636	<0.001	2.252	1.466–3.461	0.0002	13

Abbreviations: ESCC, esophageal squamous cell carcinoma; NSCLC, non-small-cell lung cancers; CRC, colorectal cancer; OS, overall survival; DFS, disease-free survival; IHC, immunohistochemistry.

cells, cystatin SN contributed to the proliferation of cancer cells by affecting the expression of two main important Wnt signaling pathway molecules, β-catenin and TCF,[27] which indicated CST1, as a target of TCF,[36] may regulate the proliferation of cancer cells through affecting Wnt signaling pathway (Figure 3B). Study also showed that cystatin SN was significantly correlated with pTNM stage.[27] Survival analyses from the online tool Kaplan–Meier plotter (http://kmplot.com/analysis/index.php?p=service&cancer=gastric) indicated that patients with high cystatin SN expression showed worse survival probabilities than those with low cystatin SN expression (Figure 2). Univariate analyses indicated that cystatin SN may be an independent prognostic factor for gastric cancer patients, the results for which are summarized in Table 2.

Pancreatic cancer

Cystatin SN was a potential biomarker for early diagnosis of malignant pancreatic neoplasms. Immunohistochemistry confirmed that cystatin SN was upregulated in tumor tissues (Figure 1). In several pancreatic cancer cell lines (BXPC-3, PANC-1, SW1990), CST1 was upregulated at both protein and mRNA levels, and was correlated closely with malignancy-associated proteins such as PCNA, cyclin D1, cyclin A2, and cyclin E. Consistent with this finding, CST1 knockdown reduced the expression of the proliferation-related proteins *p*-AKT and PCNA significantly, as well as colony formation and xenograft development in vitro.[19] In conclusion, cystatin SN possibly contributed to the proliferation of cancer cells by participating in the AKT signaling pathway (Figure 3A).

Colorectal cancer

Cystatin SN was highly expressed in colon tumor tissues (Figure 1),[13,18,30–32] and cystatin SN overexpressing cell line HCT116 exhibited increased tumor growth and tumor metastasis in vitro. Survival analysis has demonstrated that high cystatin SN expression was closely associated with poor clinical status in colon cancer patients (Figure 2).[13] Cystatin SN acted by neutralizing the inhibition of CTSB proteolytic activity by CST3, which might be involved in colorectal tumorigenesis and poor prognosis.[31] In human SW480 cancer cells, cystatin SN enhanced tumor development by affecting the GSK3 signaling pathway,[18] which was similar to the mechanism observed in MDA-MB-231 cells (Figure 3A). Furthermore, CST1 knockdown suppressed tumor growth in vivo and led to IL-6 accumulation,[18] which indicated cystatin SN might affect the IL-6 signaling pathway (Figure 3A). In HCT116 CRC cells, the let-7d/CST1/
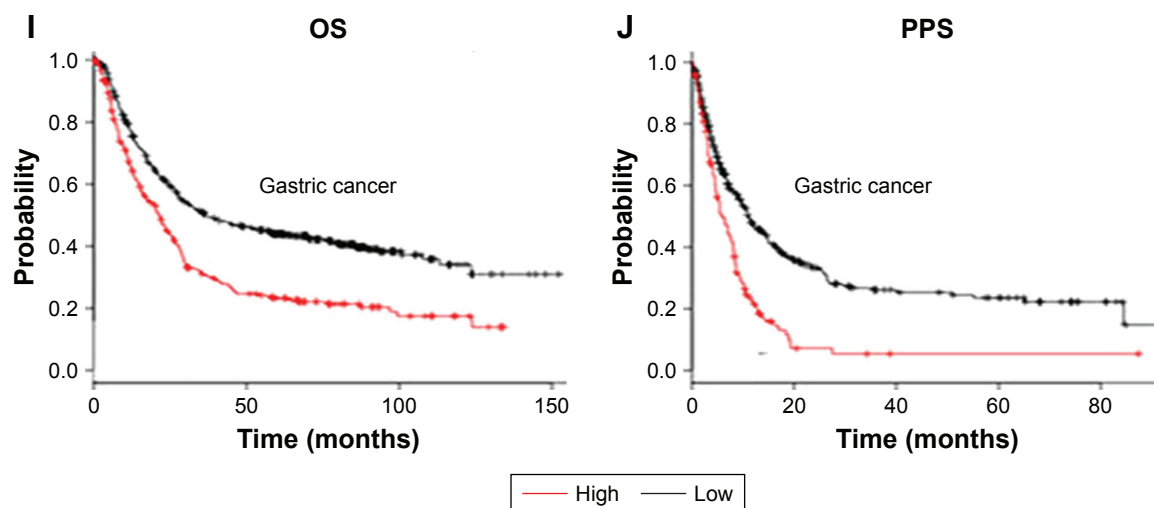
Figure 2 (*Continued*)

Figure 2 Survival analyses of cystatin SN in cancers.
Notes: ESCC patients (**A** and **B**, $P<0.001$), NSCLC patients (**C** and **D**, $P<0.01$), breast cancer patients (**E** and **F**, $P<0.01$), CRC patients (**G** and **H**, $P<0.001$), gastric cancer patients (**I** and **J**, $P<0.001$). (**A** and **B**) from Chen et al,[26] (**C** and **D**) from Cao et al,[9] (**E** and **F**) from Dai et al,[7] (**G** and **H**) from Li et al,[13] (**I** and **J**) from the online tool Kaplan–Meier Plotter.
Abbreviations: ESCC, esophageal squamous cell carcinoma; NSCLC, non-small-cell lung cancer; CRC, colorectal cancer; OS, overall survival; DFS, disease-free survival; PPS, post-progression survival.
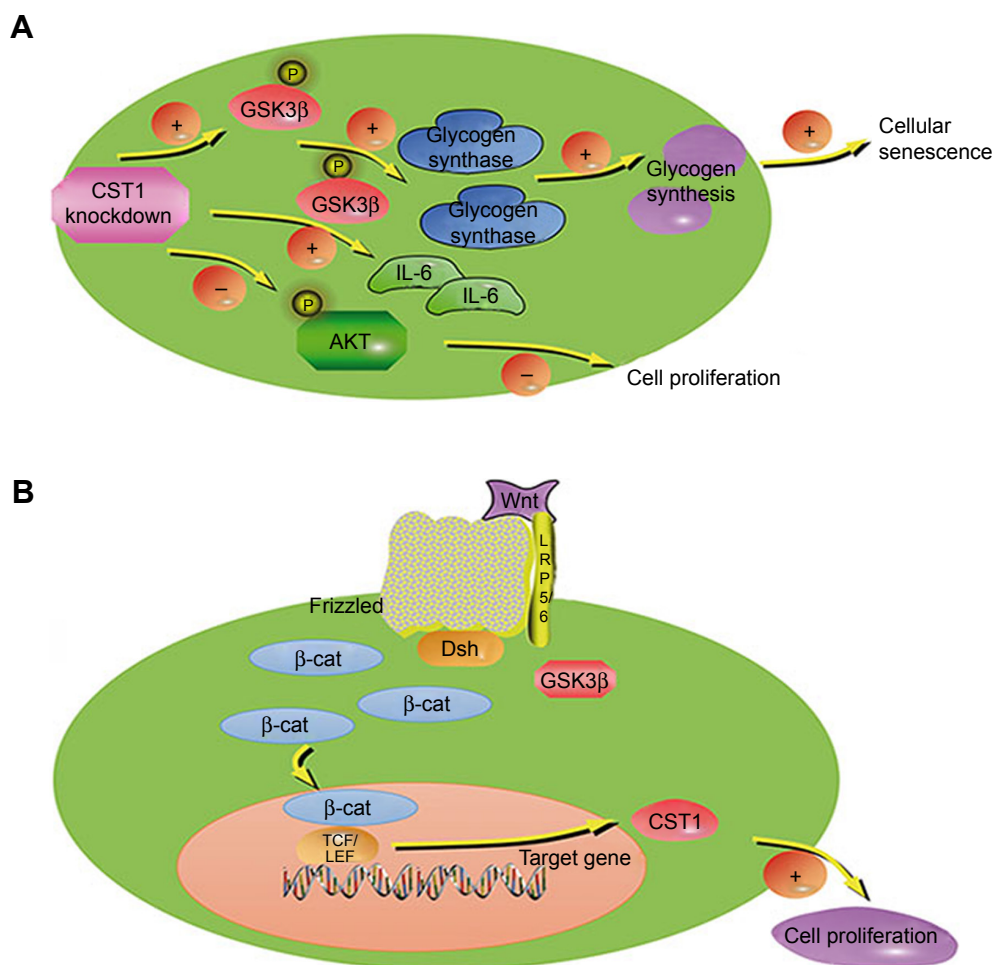
Figure 3 Proposed models of CST1 participating in the signaling pathways.
Notes: CST1 was involved in GSK3, AKT, and IL-6 pathways. CST1 knockdown increased phosphorylation of GSK3, leading to induction of glycogen accumulation associated with cellular senescence and inhibition of tumor proliferation. CST1 knockdown reduced phosphorylation of AKT and inhibited cancer cell proliferation. CST1 knockdown led to IL-6 accumulation and regulated cell growth and proliferation (**A**). CST1 was involved in Wnt pathway as a target of TCF and contributed to cell proliferation (**B**).

p65 pathway modulated CRC cell proliferation, and also, the let-7d/CST1/p65 pathway may prove to be useful in the prevention and treatment of CRC.[32] A previous study in HT-29 and SW480 cells showed that cystatin SN was highly expressed at both the mRNA and protein levels.[30] Increased cystatin SN expression inhibited auranofin-induced cell death by regulating autophagy induction and ROS production.[30] Univariate and multivariate analyses demonstrated that cystatin SN possibly acted as an independent prognostic factor for CRC prognosis,[13,18,30–32] as shown in Table 2.

Conclusion

Studies showed that the expression level of cystatin SN was related to the survival of cancer patients.[7,9,13,26] High expression of cystatin SN was closely related to poor prognosis such as recurrence and metastasis in several cancer types like gastric cancer, CRC, pancreatic cancer, and lung cancer; however, in ESCC, high cystatin SN expression patients showed more favorable survival times after surgical treatment. The conclusion was presented based on the analysis of a large patient cohort and the different roles that cystatin SN plays in these several types of cancers. We concluded that the possible causes for functional differences among cancers may be related to factors such as tissue specificity and pathological types. As we all know esophageal cancer mainly includes esophageal adenocarcinoma and ESCC, and the later accounts for the majority of esophageal cancers. At present, cystatin SN is mainly studied in ESCC, but rarely studied in esophageal adenocarcinoma. There may be completely opposite expression level of cystatin SN in the two types of esophageal cancer. In other types of cancer, similar phenomenon may also exist among different pathological types. Therefore, basic research in different pathological types of tumors is necessary, which will help to clearly clarify the function of cystatin SN in cancer.

In different types of cancers and cell lines, cystatin SN functions in various ways. In gastric cancer, cystatin SN contributed to gastric tumorigenesis through T-cell factor-mediated proliferative signaling. In pancreatic cancer, cystatin SN acted by increasing the expression of malignancy-associated proteins such as PCNA, cyclin D1, cyclin A2, and cyclin E. In CRC, cystatin SN was found to play a vital role in cell proliferation by regulating autophagy and by affecting let-7d/CST1/p65 pathway. Though some basic studies have been done in this regard, the reasons why cystatin SN functions in different ways among different cancers is still not very clear. Univariate and multivariate analyses of cystatin SN expression indicated that cystatin

SN was an independent prognostic factor in several types of cancers. In ESCC, high expression of cystatin SN was a profitable prognostic factor; however, in other types of cancers like gastric cancer, CRC, pancreatic cancer, and lung cancer, high expression of cystatin SN was a poor prognostic factor, which indicated cystatin SN could be a potential target in therapy of cancer patients. In terms of its different prognosis in different cancers, upregulating cystatin SN in ESCC and downregulating cystatin SN in other cancers may be a good approach for treatment. Keeping in mind the strong relationship between expression of cystatin SN and survival probability, as well as generally observed high expression of cystatin SN in cancers, cystatin SN may be taken as a diagnostic factor.

Cystatin SN is expected to become a biomarker for tumor diagnosis and prognosis prediction. Cystatin SN regulates proliferation of cancer cells by affecting signaling pathways like Wnt signaling pathway, GSK3 signaling pathway, AKT signaling pathway, and IL-6 signaling pathway. In the process of studying cystatin SN, trying to figure out why cystatin SN functions so differently between ESCC and other cancers and determine the specific molecular mechanisms involved in normal and cancer tissues would be exciting and challenging research goals. If the mechanisms are well researched, specific targeted intervention could be made to treat cancer patients.

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Disclosure

The authors report no conflicts of interest in this work.

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