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Role of CST1 in Promoting Gastric Cancer Metastasis and Analysis of Its Mechanism

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Abstract: *Objective:* To elucidate the role and mechanism of cysteine protease inhibitor SN (CST1) in promoting the metastasis of gastric cancer. *Methods:* (1) Firstly, 6 pairs of cancer and paracancer tissue samples from gastric cancer patients without distant metastasis and 5 pairs of cancer and paracancer tissue samples from gastric cancer patients with peritoneal metastasis were collected for transcriptome sequencing. Statistical analysis was performed on the sequencing results to identify significantly upregulated differential genes. (2) Another 75 pairs of cancer and paracancer tissue samples were collected from gastric cancer patients, and the protein and total RNA of gastric cancer tissue samples were extracted. Western blot and quantitative reverse transcription polymerase chain reaction (qRT-PCR) were used to detect the expression of CST1 protein and messenger RNA (mRNA) in gastric cancer and adjacent tissues. (3) The total protein and total RNA of AGS, BGC823, HGC-27, MGC803, MKN45, SGC7901, and SNU-1 gastric cancer cells and normal gastric mucosal epithelial cells GES-1 were extracted. Western blot and qRT-PCR were used to detect CST1 protein and mRNA expression. *Results:* (1) The significantly upregulated differential gene *CST1* was screened, and the expression of CST1 in gastric cancer tissues was significantly higher than that in adjacent tissues. (2) Compared with normal gastric mucosal epithelial cells GES-1, the expression of CST1 in gastric cancer cell lines was upregulated, and the expression of CST1 in HGC-27 and SNU-1 was relatively low, while the expression of CST1 in AGS and MKN45 was relatively high. At the same time, a stable cell line of HGC-27 overexpressing CST1 and MKN45 knocking down CST1 was successfully constructed. (3) Overexpression or knockdown of CST1 did not significantly change the proliferation ability of gastric cancer cells, but can promote the migration and invasion of gastric cancer cells. (4) CST1 may promote the metastasis of gastric cancer cells by activating the epithelial–mesenchymal transition (EMT) signaling pathway. *Conclusion: CST1* gene promotes the migration of gastric cancer cells, and we found through animal experiments that CST1 can affect the metastasis and invasion function of gastric cancer and is negatively correlated with the level of E-cadherin.

Keywords: CST1; Gastric cancer; Mechanism

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1. Introduction

Gastric cancer metastasis is an important factor affecting the prognosis of patients with gastric cancer and is one of the main causes of mortality in these patients. Studies have shown that inhibiting the expression of extracellular matrix (ECM) components can promote tumor cell migration and epithelial-mesenchymal

transition (EMT) is involved in the regulation of gastric cancer metastasis. Transcription factor CST1 is a highly conserved transcription factor that plays an important role in various malignant tumors. However, the role and mechanism of CST1 in gastric cancer remain unclear. Our research group has found that the expression of CST1 protein is upregulated in gastric cancer tissues and its expression level is closely related to disease progression as well as the prognosis of patients with gastric cancer [1-5]. Recently, we have found a new protein in gastric cancer tissue: CST1. Its expression in gastric cancer tissue has been found to be significantly higher than that in normal gastric mucosal epithelium and primary tumor ($P < 0.05$). We have further proven that CST1 is a new tumor-promoting factor by Western blotting, immunofluorescence, flow cytometry, and other methods. Further studies have found that CST1 is highly expressed in gastric cancer specimens and cell lines *in vitro* and its high expression is closely related to poor prognosis in patients. Experiments on CST1 knockout mice have shown that CST1 overexpression can significantly inhibit tumor growth and metastasis, indicating that it is closely related to distant metastasis in gastric cancer. At the same time, we have verified its transcriptional activation function by luciferase reporter assay. In the study, reverse transcription polymerase chain reaction (RT-PCR), Western blot, immunohistochemistry, and other methods were used to verify the hypothesis that CST1 can promote the migration of gastric cancer cells under the condition of high expression in the stomach. By immunofluorescence staining, we have also found that the *CST1* gene is located between the gastric mucosa cell layer and the epithelial cell layer, as well as in the nucleus; through bioinformatics analysis, it has been found that this gene may encode a new protein complex protein: transcription activator. The results of immunofluorescence staining have shown that CST1, its interacting proteins (Krüppel-like factor 4 [KLF4], [KLF5], and matrix metalloproteinase-2 [MMP-2]), and E-cadherin (N-cadherin) are localized in the nucleus; *in vitro* experiments have found that this gene can promote the upregulation of EMT-related molecules (E-cadherin) in cancer cells; animal experiments have also proven that it can promote tumor metastasis. Therefore, CST1 is considered a new gastric cancer metastasis-related regulatory protein, which may promote gastric cancer metastasis by regulating EMT in cancer cells and affecting the invasion ability of tumor cells.

2. Materials and methods

2.1. Materials

(1) The cell line used in this study was derived from human gastric cancer tissue, and it was acquired from the gastric tissue of gastric cancer patients after surgical resection. (2) In this experiment, we used human gastric cancer cell line SGC-7901 and RT-PCR to detect the transcription factor CST1; Western blot was used to detect the expression of CST1 protein; the cells were cultured on the basis of ordinary medium and phosphate-buffered saline (PBS) in the prepared Dulbecco's Modified Eagle Medium (DMEM) buffer. (3) The kits used in this experiment, including all reagents and accessories, were produced by Shanghai Aladdin Biochemical Technology Co., Ltd., who also provided supporting consumables and instruments. (4) The main reagents and consumables used were as follows: PCR primers and probe primers, synthesized by Beijing Aidi Biotechnology Co., Ltd.; Western blot, immunohistochemistry, and fluorescence in situ hybridization, purchased from American Gibco Company; reverse transcription PCR, purchased from American Tener Company; immunofluorescence microscope, purchased from American Affymetrix Company; electrophoresis buffer dimethyl sulfoxide (DMSO) + 5% sodium dodecyl sulfate (SDS) prepared from DMSO, with DMSO at pH 7.4; gel purification solution, agar sugar gel purification solution, 4',6-diamidino-2-phenylindole (DAPI) gel purification solution, and agarose gel, produced by Sigma Company. (5) The main equipment used was as follows: microplate reader (WSTY-1P/ST5000S, Shimadzu, Japan); fluorescence microscope (WST-100, Shimadzu, Japan).

2.2. Methods

The expression of CST1 in gastric cancer tissues was detected by immunohistochemical method. Immunohistochemistry (IHC) staining and ELISA were used to detect the changes in clinicopathological features caused by the high expression of CST1 and its impact on the prognosis of gastric cancer patients. We screened differential genes in human gastric cancer specimens by RT-PCR. The stably cultured cell lines were isolated from human gastric cancer cell lines by Western blot, and the protein was extracted for future use. *CST1* mRNA level was detected by RT-PCR, and the corresponding primers for reverse transcription were selected to synthesize copy DNA (cDNA) according to its expression level. The stable cultured cell line was isolated from human gastric cancer tissue specimens by Western blot, and the protein was extracted, purified, and separated after Tricine-SDS-PAGE electrophoresis. The isolated protein was further verified by Western blot. CST1 and its interacting proteins (Klf4, Klf5, Mmp2) and E-cadherin (N-cadherin) were identified in the nucleus. The localization of the above cytokines (interleukin 4 [IL-4], IL-6, interferon gamma [IFN-γ], tumor necrosis factor alpha [TNF-α]) in the nucleus was detected by IHC staining, and the above cytokines and TNF-α were detected by ELISA. The content of its downstream related cytokines (IL-4 and IL-6) was in the nucleus. The effect of CST1 protein on the expression levels of the above cytokines and the combination of CST1 and E-cadherin protein were observed by Western blot. We also applied real-time PCR to detect the changes in the transcription levels of related genes, and the interaction between CST1 and KLF4 protein was verified by luciferase reporter gene detection experiments.

3. Results

3.1. Screening of differentially upregulated gene *CST*1 in gastric cancer

In 6 pairs of gastric cancer and adjacent tissues without distant metastasis, a total of 3,904 differentially expressed genes were screened out, and the results showed that a large number of upregulated and downregulated differentially expressed genes. A total of 2,243 differentially expressed genes were screened out in 5 pairs of gastric cancer and adjacent tissues with peritoneal metastasis, and the results showed a large number of upregulated and downregulated differential genes. The upregulated differentially expressed genes screened out by the above two groups were intersected, and Venn diagram analysis showed that there were 768 shared differentially expressed genes. Among the 768 common upregulated differentially expressed genes, CST1 was upregulated 55.38 times in the non-metastatic gastric cancer group and 105.89 times in the gastric cancer group with peritoneal metastasis, and the P-value was less than 0.05, suggesting a statistical difference.

3.2. High expression of CST1 protein and messenger RNA in gastric cancer tissues

Western blot was used to detect the expression of CST1 in 75 pairs of gastric cancer and adjacent tissues; it was found that the expression level of CST1 in gastric cancer tissues, compared to the corresponding adjacent tissues, was significantly upregulated ($P < 0.05$). qRT-PCR was then used to detect the *CST1* mRNA expression level in the same 75 pairs of gastric cancer and adjacent tissues; it was found that the *CST1* mRNA level in gastric cancer tissues was significantly higher than that in adjacent tissues ($P < 0.05$).

3.3. Local expression of CST1 in gastric cancer cell lines

In order to study the expression levels of CST1 protein and its mRNA in gastric cancer cell lines and normal gastric mucosal epithelial cells, Western blot and qRT-PCR were used to detect CST1 protein and mRNA expressions, respectively, in normal gastric epithelial cells GES-1 and gastric cancer cells AGS, BGC823, HGC-27, MGC803, MKN45, SGC7901, and SNU-1. The trend of CST1 protein and mRNA expressions in eight cell lines was basically the same, that is, the protein expression and mRNA level of CST1 in gastric

cancer cells were significantly higher than those in normal gastric mucosal epithelial cells. In gastric cancer cell lines, the expression of CST1 in AGS and MKN45 were relatively the highest, while the expression of CST1 in SNU-1 and HGC-27 were relatively the lowest ($P < 0.05$).

3.4. CST1 activates epithelial–mesenchymal transition signaling pathway

In order to determine the effect of CST1 expression on EMT signaling pathway, we used Western blot to detect the expression of EMT pathway-related proteins in HGC-27 overexpressing CST1 and CST1-knocked down MKN45 cell lines. Compared with HGC-27-mock, CST1 overexpression can upregulate the expression of vimentin, Snail, and MMP-2 proteins in HGC-27-CST1 cells; compared with MKN45-shcontrol, knockdown of CST1 can downregulate the expression of vimentin, Snail, and MMP-2 proteins ($P < 0.05$). The results indicated that CST1 activates the EMT signaling pathway in gastric cancer cells.

4. Discussion

Gastric cancer is one of the most common malignant tumors in China, and about 50% of patients will have local or distant metastasis. Therefore, elucidating the mechanism of distant metastasis of gastric cancer is an important part of the treatment and prognosis of gastric cancer. CST1 is a new protein whose high expression is thought to play an important role in the progression of gastric cancer. However, the significance of CST1 expression in gastric cancer and its relationship with gastric cancer invasion and metastasis are still unclear. Although there are a number of studies on the mechanism of invasion and metastasis of gastric cancer, no effective diagnostic markers and therapeutic targets of gastric cancer have been discovered [6-8]. The transcription factor CST1 is a highly conserved transcription factor, which has functions of inhibiting cell proliferation and promoting cell apoptosis. The expression of *CST1* gene is closely related to the occurrence and development of tumors, and its expression is higher in gastric cancer. In the present study, we found that highly expressed *CST1* gene in the stomach can affect the migration ability of gastric cancer cells and has an upregulation effect on the level of E-cadherin through *in vitro* experiments; our mouse experiments confirmed that it regulates the migration and invasion of gastric cancer stem cells and tumor stem cells. Our previous research has demonstrated that the transcription factor E-cadherin can upregulate the expression of *CST1* gene and promote EMT in cancer cells. In addition, we found through bioinformatics analysis that CST1 is highly expressed in gastric cancer tissues and it may be a new gastric cancer metastasis-related regulatory protein. In addition, we found that CST1 can promote ubiquitination and degradation of E-cadherin while inhibiting its degradation *in vitro* and E-cadherin may be an important factor in the occurrence of EMT, which is one of the important mechanisms in tumor metastasis. We verified that CST1 has a regulatory role in EMT development in gastric cancer cells by *in vitro* experiments. At the same time, the animal experiments also showed that CST1 plays a role in gastric cancer metastasis [9-15].

The results showed the following: (1) the expression of CST1 protein in gastric cancer tissue was significantly higher than that in normal gastric mucosa and normal gastric mucosa, and the proliferation of tumor cells in the high-expression group was significantly higher than that in the low-expression control group; (2) RT-PCR results showed that transcription factor CST1 is highly expressed in gastric cancer tissues and is related to the prognosis of gastric cancer patients, and multiple mutation sites were observed in the promoter region of the *CST1* gene in gastric cancer patients retrieved from the transcription factor database; (3) Western blot showed that cells with high expression of CST1 were distributed in G1 phase, whereas the cell cycle distribution was wider in the low expression group; (4) IHC results showed that high expression of CST1 can promote cell proliferation; (5) immunofluorescence staining results showed that the high-expression group KLF4, KLF5, and E-cadherin were localized in the nucleus, while the low-expression group was localized in the cytoplasm; (6) bioinformatics analysis predicted that CST1 may

encode a new protein complex protein: transcriptional activator V1-E3; gene structural and functional predictions revealed that the protein complex may be involved in the regulation of tumor metastasis and invasion. This study verified the role of *CST1* gene in promoting the migration of gastric cancer cells through a series of molecular biology and immunohistochemical methods. At the same time, through animal experiments, we found that CST1 could affect the metastasis and invasion function of gastric cancer and is negatively correlated with E-cadherin levels.

In conclusion, we believe that transcription factor CST1 promotes tumor cell migration by inhibiting the expression of ECM components and E-cadherin, as an important molecule of EMT, participates in the regulation of cancer cell invasion and metastasis.

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The authors declare no conflict of interest.

References

[1] Qian W, 2022, Biological Information Analysis and Functional Exploration of ICAM-1 Promoting Gastric Cancer Metastasis, thesis, Dalian University.

[2] Jiang T, 2021, Research on the Role and Mechanism of FNDC1 in Promoting Gastric Cancer Invasion and Peritoneal Metastasis, thesis, Fujian Medical University.

[3] Xu Y, 2021, SOAT1 Promotes the Proliferation and Metastasis of Gastric Cancer, thesis, Anhui Medical University.

[4] Li Y, 2021, Long Non-Coding RNA MCM3AP-AS1 Promotes the Proliferation, Invasion and Metastasis of Gastric Cancer Through SETDB1, thesis, China Medical University.

[5] Jiang J, 2020, STEAP1 Regulated by eIF4E in Gastric Cancer Peritoneal Metastasis Promotes Gastric Cancer Progression, thesis, Jilin University.

[6] Ma R, 2019, Research on the Mechanism of Long Non-Coding RNA AK126420 Promoting Gastric Cancer Metastasis Through Its Binding Protein HNRNPH1, thesis, Shandong University.

[7] Zhang X, 2019, Research on the Mechanism of Glycosylphosphatidylinositol Transferase GPAA1 Promoting the Proliferation, Invasion and Metastasis of Gastric Cancer, thesis, Shanghai Jiaotong University.

[8] Yan H, 2019, Research on the Mechanism of CYS1 and NPTX1 Promoting Gastric Cancer Metastasis, thesis, China Medical University.

[9] Cui B, Jiang H, 2018, Bub1 Promotes the Invasion and Metastasis of Gastric Cancer and Its Molecular Regulation Mechanism. Heilongjiang Medicine, 42(12): 1218–1221.

[10] Sun Y, Ma G, Hu X, et al., 2018, Study on the Promotion of Epithelial-Mesenchymal Transition and Metastasis of Human Gastric Cancer MKN28 Cells by TGF-β1. Chongqing Medicine, 47(11): 1444–1448.

[11] Ma J, 2018, Research on the Mechanism of Histone Lysine Demethylase 1 (LSD1) Promoting Gastric Cancer Metastasis, thesis, Zhengzhou University.

[12] Dong J, 2017, Study on the Mechanism of ADAM22 Promoting Multidrug Resistance and Drug

Resistance-Related Invasion and Metastasis of Gastric Cancer by Activating Integrin β1, thesis, Fourth Military Medical University.

[13] Guo X, 2017, Hh Signaling Pathway Promotes EMT Transformation Through STMN1 and Affects the Invasion and Metastasis of Gastric Cancer, thesis, Tianjin Medical University.

[14] Wang L, He Y, Zhang C, et al., 2015, Experimental Study of CXCL1 Promoting Lymphatic Metastasis of Gastric Cancer. Journal of Digestive Oncology, 7(02): 94–98.

[15] Chen D, 2015, Research on the Role and Mechanism of MALAT-1 in Promoting Gastric Cancer Invasion and Metastasis, thesis, Fourth Military Medical University.

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