

CDK10 Regulates Gastric Cancer Metastasis By Inhibiting lncRNA-C5ORF42-5 Via Phosphorylation Level of AKT/ERK

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Research

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Abstract

Background: Gastric cancer is the fourth most common malignant disease. Both CDK10 and long noncoding RNAs (lncRNAs) have been found to exert biological functions in multiple cancers. However, it is still unclear whether CDK10 represses tumor progression in gastric cancer by reducing potential targeting lncRNAs.

Methods: The functions of CDK10 and lncRNA-C5ORF42-5 in proliferation, invasion and migration were assessed by MTS assays, colony formation assays, cell cycle and apoptosis assays, Transwell assays, wound healing assays and animal experiments. We used high-throughput sequencing to confirm the existence of lncRNA-C5ORF42-5 and quantitative real-time PCR was used to evaluate lncRNA expression. Then, with RNA-seq sequencing as well as GO function and KEGG enrichment analysis, we identified the signaling pathways in which lncRNA-C5ORF42-5 was involved in gastric cancer. Finally, western blotting was used to identify the genes regulated by lncRNA-C5ORF42-5.

Results: Our results showed that CDK10 is expressed at relatively low levels in gastric cancer cell lines and inhibits the progression of gastric cancer cells both in vitro and in vivo. Next, based on high-throughput sequencing, we identified a novel lncRNA, lncRNA-C5ORF42-5, in the stable CDK10-overexpressing cell line compared with the CDK-knockdown cell line and their controls. Additionally, we confirmed that lncRNA-C5ORF42-5 acts as an oncogene to promote metastasis in gastric cancer in vitro and in vivo. We then ascertained that lncRNA-C5ORF42-5 is a major contributor to the function of CDK10 in gastric cancer metastasis by upregulating lncRNA-C5ORF42-5 to reverse the effects of CDK10 overexpression. Finally, we explored the mechanism by which lncRNA-C5ORF42-5 overexpression affects gastric cancer cells to elucidate whether lncRNA-C5ORF42-5 may increase the activity of the SMAD pathway of BMP signaling and promote the expression of EMT-related proteins, such as E-cadherin. Additionally, overexpression of lncRNA-C5ORF42-5 affected the phosphorylation levels of AKT and ERK.

Conclusion: Our findings suggest that CDK10 overexpression represses gastric cancer tumor progression by reducing lncRNA-C5ORF42-5 and hindering activation of the related proteins in metastatic signaling pathways, which provides new insight into developing effective therapeutic strategies in the treatment of metastatic gastric cancer.

Background

Gastric cancer is the fourth most common malignant disease and the third leading cause of cancer-related death globally, and its incidence rates are generally highest in East Asia, especially in Korea, Mongolia, Japan and China^{1,2}. With the development of D2 gastrectomy and chemotherapy regimens, the prognosis of gastric cancer patients has improved³⁻⁵. However, gastric cancer is still a great threat to human health, resulting in low 5-year survival rates, especially for patients with locally advanced or metastatic diseases⁶. Therefore, identifying prognostic biomarkers and treatment targets for metastatic gastric cancer patients is of paramount importance.

Cyclin-dependent kinase (CDK) 10 is a CDC2-related kinase from a family of 20 serine/threonine protein kinases that play pivotal roles in the regulation of a variety of fundamental cellular processes⁷. It was first discovered in 1994 by sequence homology screening for CDK-related genes and revealed to play an essential role in the progression from G2 to M phase of the cell cycle⁸⁻¹⁰. However, it received little interest until it was identified as a major determinant of resistance to endocrine therapy for breast cancer in 2008¹¹. Several studies have shown that CDK10 is a putative tumor suppressor in multiple types of human cancers, such as hepatocellular carcinoma, biliary tract cancer, breast cancer and glioma¹²⁻¹⁷. In our previous study, we demonstrated that CDK10 may function as a prognostic marker in gastric cancer patients and that low CDK10 expression independently predicted poor overall survival¹⁸. However, further investigation of the molecular mechanisms involved in the regulation of CDK10 in gastric cancer is still needed.

Long noncoding RNAs (lncRNAs) are loosely defined as RNA transcripts that exceed 200 bases in length and have no apparent coding capacity¹⁹. It was found that lncRNAs could play an important role in regulating gene expression by different mechanisms, including chromatin modification and transcriptional and posttranscriptional processing^{20,21}. For example, lncRNA GLCC1 stabilizes the ubiquitination of the c-Myc transcription factor by directly interacting with the HSP90 chaperone in colorectal carcinogenesis²². Moreover, several studies have indicated that lncRNAs play important roles in the proliferation, migration, immune escape, apoptosis and drug resistance of gastric cancer²³⁻²⁵. For example, UCA1 could protect PDL1 expression from the repression of miRNAs and contribute to gastric cancer cell immune escape to serve as a potential novel therapeutic target for gastric cancer treatment²⁶. lncRNA MACC1-AS1, PTCSC3 and THOR could influence the stemness of gastric cancer cells²⁷⁻²⁹. Overexpression of HULC could induce patterns of autophagy to regulate apoptosis in gastric cancer cells³⁰. Mechanistically, lncRNAs are well known as competitive endogenous RNAs (ceRNAs) that regulate microRNAs (miRNAs) or competitively bind to transcription factor regions^{31,32}. Meanwhile, c-myc, TEAD4, and p53 could regulate the function of lncRNAs^{31,33,34}. Therefore, lncRNAs function by regulating or being regulated by corresponding factors in cancer progression. Considering lncRNAs' key biological role in cancer, lncRNAs are promising targets for gastric cancer treatment. However, the relationship between CDK10 and lncRNAs involved in gastric cancer remains unclear. Whether lncRNAs are major contributors to the function of CDK10 in gastric cancer needs further exploration. Therefore, a better understanding of the role of lncRNAs underlying gastric cancer progression will enrich the understanding of the molecular mechanisms of gastric cancer carcinogenesis and provide evidence for the diagnosis and treatment of gastric cancer.

In the present study, we explored the functions of CDK10 *in vivo* and *in vitro* and compared the expression of lncRNAs between cell lines with CDK10 overexpression or knockdown cell lines by using high-throughput sequencing. We further characterized one lncRNA named lncRNA-C5ORF42-5. The functions and mechanisms of lncRNA-C5ORF42-5 were explored to determine the mechanism by which CDK10 functions in gastric cancer cells.

Methods

Patients and samples

A total of 27 human samples in this study were obtained from patients who underwent resection of gastric cancer at The Sixth Affiliated Hospital of Sun Yet-sen University. This research was approved by the Ethics Committee of The Sixth Affiliated Hospital of Sun Yat-sen University. Written informed consent was obtained from all participants. The clinicopathological characteristics of the gastric cancer patients are summarized in Additional file 1: Table S1.

RNA isolation and quantitative RT-PCR

Total RNA was isolated by TRIzol Reagent (Thermo Fisher Scientific) following the manufacturer's protocol. The concentration of RNA was measured and reverse-transcribed to cDNA using a reverse transcription kit (Thermo Fisher Scientific). PCR analysis was performed using an ABI StepOne Real-time PCR System (Thermo Fisher Scientific). GAPDH was used as a control. We designed specific primers for CDK10 (sense: 5'-

TGGACAAGGAGAAGGATG-3'; antisense: 5'-CTGCTCACAGTAACCCATC-3'); lncRNA-C5ORF42-5 (sense: 5'-TTGTGGTCTGGGTGATGACT-3'; antisense: 5'-TTCACACCTGTTGATCCGAA-3'); and GAPDH (sense: 5'-CTCCTCCTGTTGACAGTCAGC-3'; antisense: 5'-CCCAATACGACCAAATCCGTT-3'). The results were collected and calculated using the $2^{-\Delta\Delta C_t}$ method.

Cell culture and transfection

Gastric cancer cell lines (SGC7901, AGS, HGC27, MKN45, BGC823 and MKN28) and the normal human gastric epithelial cell line GES-1 were obtained from Guangdong Institute of Gastroenterology in our hospital. SGC7901, AGS, HGC27, BGC823, MKN28, and GES-1 cells were cultured in Roswell Park Memorial Institute (RPMI) 1640 medium supplemented with 10% heat-inactivated fetal bovine serum (Sigma), 100 U/ml penicillin, and 100 µg/ml streptomycin (HyClone). MKN45 cells were cultured in RPMI 1640 with 20% FBS. All cells in the medium were placed in a humidified atmosphere containing 5% CO₂ at 37°C. The plasmid was transfected into gastric cancer cells using Lipofectamine 3000 according to the manufacturer's instructions (Invitrogen).

Western blotting analysis

Cells were collected and lysed in RIPA buffer (Thermo Scientific) plus complete protease inhibitor mixture (Roche Applied Science Indianapolis, USA). The concentration was determined using a bicinchoninic acid (BCA) protein assay kit (Pierce, Thermo Scientific). Immunoreactive bands were detected by using the Immobilon ECL substrate kit (Millipore, Merck KGaA, Germany). The images were acquired by using a BioSpectrum 600 Imaging System (UVP, CA, USA). E-cad (ab15148, Abcam Inc., Cambridge, MA, USA), BMP2 (ab14933, Abcam Inc., Cambridge, MA, USA), ABMP4 (#4680, Cell Signaling, Danvers, MA), AKT (ab8805, Abcam Inc., Cambridge, MA, USA), p-AKT (ab 18206, Abcam Inc., Cambridge, MA, USA),

ERK (#4370, Cell Signaling, Danvers, MA), and p-ERK (#4680, Cell Signaling, Danvers, MA) antibodies were used.

Cell proliferation, migration and invasion assays

Cell proliferation was analyzed using the 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium (MTS) assay (MTS, Promega, USA). Gastric cancer cells transfected with LV-CDK10, LV003 and LV-lncRNA-C5ORF42-5 were incubated in 96-well plates at a density of 5000 cells per well with RPMI 1640 medium supplemented with 10% FBS. Cells were treated with 10 μ l of MTS at 24 h, 48 h, and 72 h and subsequently incubated with 100 μ l of RPMI medium for 2 h. The color reaction was measured at 490 nm using an enzyme immunoassay analyzer (Bio-Rad, Hercules, CA).

Transwell filters were coated with Matrigel (3.9 μ g/ μ l, 60–80 μ l) on the upper surface of a polycarbonic membrane (diameter 6.5 mm, pore size 8 μ m). After incubation at 37°C for 30 min, Matrigel served as the extracellular matrix for the analysis of tumor cell invasion. For invasion and migration analysis, harvested cells (8×10^4) in 200 μ l of serum-free RPMI 1640 medium were added to the upper compartment of the chamber, while a total of 600 μ l of RPMI medium supplemented with 10% heat-inactivated fetal bovine serum (FBS) was placed in the bottom compartment of the chamber. After 24 h of incubation at 37°C with 5% CO₂, the medium was removed from the upper chamber. The cells that had migrated from the Matrigel into the pores of the inserted filter were fixed with 4% paraformaldehyde (PFA) for 15 min, stained with 1% crystal violet for 2 h, and dried at 65°C for 30 min. The number of cells invading through the Matrigel or migrating was counted in five randomly selected visual fields from the central and peripheral portions of the filter using an inverted microscope (200 \times magnification). Each assay was repeated three times.

Wound healing assay

Cells were cultivated in 6-well plates. Wounds were created with a 200- μ l plastic pipette tip when cells reached 100% confluence, and the medium was used to rinse several times to remove any floating cells. Fresh serum-free medium was added, and the wound-closing process was recorded with a microscope (200 \times magnification) at 0 h and 48 h after wounding.

Cell cycle and apoptosis assays

For the cell cycle analysis, the transfected cells were stained with propidium iodide by the Cycletest Plus DNA reagent kit (BD Biosciences, USA) and then measured by flow cytometry (BD Biosciences Accuri C6, USA). The ratios of cells in G0/G1, S, and G2 phases were counted and compared. To detect cell apoptosis, cells were stained using an annexin V-APC/7AAD apoptosis kit (BD Biosciences, USA) and analyzed using flow cytometry. The ratio of early apoptotic cells to late apoptotic cells was compared to the values obtained for the controls in each experiment.

Animal studies

The animal experiments were performed in the animal laboratory center of the Sixth Affiliated Hospital of Sun Yet-sen University and in accordance with the Guide for the Care and Use of Laboratory Animals (National Institutes of Health, Bethesda, MD, USA). For the in vivo tumor metastasis studies, gastric cancer cells stably transfected with Lv-sh-NC, Lv-sh-CDK10, Lv-003 and Lv-sh-LncRNA-C5ORF42-5 were injected into the tail vein for the pulmonary metastatic model. Mice were sacrificed at 6 weeks post injection and examined microscopically by H&E staining for the development of lung metastatic foci.

Immunohistochemistry assay

The tumor specimens were fixed with 4% PFA, embedded in paraffin, and incubated at 4°C overnight with primary antibodies against CDK10 (Abcam, USA, at a 1:500 dilution). The samples were then washed, incubated with secondary antibody for 30 min at room temperature, and stained with hematoxylin-eosin.

Statistical analysis

Data are presented as the mean \pm standard deviation, and $P < 0.05$ indicates statistical significance. Statistical analyses were performed using GraphPad Prism 5 software. The western blot band intensity was quantified by ImageJ software (National Institutes of Health).

Results

CDK10 is expressed at relatively low levels in gastric cancer cell lines and inhibits the progression of gastric cancer cells in vitro and in vivo

In a previous study, CDK10 was found to function as a prognostic marker in gastric cancer, and low CDK10 expression independently predicted poorer overall survival in patients¹⁸. To further investigate the molecular mechanisms of how CDK10 functions in gastric cancer, we first verified the expression level of CDK10 in several gastric cancer cell lines. By western blotting, relatively low expression of CDK10 was found in most of the gastric cancer cell lines, such as AGS, SGC7901, MGC803, BGC823, HGC27 and MKN28 cells, while in MKN-45 cells, CDK10 was relatively highly expressed (Fig. 1A).

To explore the function of CDK10 in gastric cancer cells, we transfected a CDK10 expression construct into AGS and SGC7901 cells and established stable transfectants. The expression of CDK10 was significantly increased in these stable transfectants (Fig. 1B). Functionally, MTS assays revealed that the proliferation of AGS and SGC7901 cell lines was decreased in the CDK10-overexpressing group compared with the vector group (Fig. 1C). Consequently, the colony numbers of CDK10-overexpressing cells were significantly lower than those of the vector group ($P < 0.05$, Fig. 1D). Moreover, wound healing assays and Transwell invasion assays indicated that the migration and invasion abilities of the AGS and SGC7901 cell lines were also suppressed by CDK10, which was in accordance with the wound healing assays ($P < 0.01$, Fig. 1E & F). To explore whether overexpression of CDK10 affects tumor metastasis in vivo, a pulmonary metastatic model was established by injecting AGS cells transfected with CDK10 and vector cells into the tail vein of nude mice. Six weeks later, the AGS-CDK10 group had significantly fewer lung

metastases than the AGS-vector control group. Meanwhile, hematoxylin and eosin (H&E) staining of dissected lungs further confirmed that overexpression of CDK10 could markedly inhibit lung metastasis (Fig. 1F). Moreover, our supposition was further confirmed by stably knocking down CDK10 in MKN-45 cells (Supplementary Fig. S1A-F). Taken together, these data indicate that CDK10 in gastric cancer is associated with growth and metastasis in vitro and in vivo.

Identification of lncRNAs by high-throughput sequencing analysis in stable CDK10-overexpressing and CDK10-knockdown cell lines

Since lncRNAs have been identified as playing key roles in cancer and considerable evidence has recently demonstrated that lncRNAs are crucial regulators of the development and progression of gastric cancer^{35,36}, we hypothesized that CDK10 may inhibit the growth and metastasis of gastric cancer by modulating lncRNAs. Based on high-throughput sequencing, we analyzed the expression level of lncRNAs by comparing the AGS cell line stably expressing CDK10 with the control and detected a series of differentially expressed lncRNAs. The total differentially expressed lncRNAs were displayed directly by hierarchical clustering analysis (Fig. 2A). We then selected the most differentially expressed lncRNA, C5ORF42-5, in CDK10-overexpressing cell lines and used clinical samples to verify the high-throughput sequencing results. lncRNA-C5ORF42-5 was successfully validated in gastric cancer tissues by quantitative reverse transcription PCR in 27 paired gastric cancer tissues and adjacent noncancerous tissues. It was higher in most (81.5%, 22/27) gastric cancer tissues than in normal gastric tissues (Fig. 2B), and the level of lncRNA-C5ORF42-5 negatively correlated with the level of CDK10 (Fig. 2C). In addition, we found lncRNA-C5ORF42-5 to be expressed at high levels in SGC7901 cells with CDK10 overexpression and at low levels in AGS and HGC27 cells with CDK10 knockdown by qRT-PCR analysis (Fig. 2D).

lncRNA-C5ORF42-5 promotes metastasis in gastric cancer cell lines

As shown in Fig. 3A, we used plasmid-mediated overexpression to exogenously manipulate the expression of lncRNA-C5ORF42-5 in AGS and HGC27 cells to further confirm its function. Transwell migration and invasion assays revealed that overexpression of lncRNA-C5ORF42-5 significantly promoted cell migration and invasion compared with the controls in both AGS and HGC27 cells (Fig. 3B and C). Next, flow cytometric analysis was performed to further examine whether lncRNA-C5ORF42-5 affected the cell cycle progression and apoptosis of gastric cancer cells. No significant differences were found in the cell cycle progression and apoptosis results between lncRNA-C5ORF42-5-overexpressing AGS cells and control HGC27 cells (Fig. 3E and F). Similarly, MTS assays showed that overexpression of lncRNA-C5ORF42-5 had no significant influence on cell proliferation compared with control cells (Fig. 3D). Nude mice were given intravenous injections of these cells, and we measured the formation of pulmonary metastases. Overexpression of lncRNA-C5ORF42-5 enhanced the ability of these cells to form lung metastases in mice (Fig. 3G).

To identify the functional interaction between CDK10 and lncRNA-C5ORF42-5, we designed rescue experiments in Transwell assays. We overexpressed lncRNA-C5ORF42-5 in CDK10 stable transfectants with exogenous plasmids. The results showed that CDK10 overexpression-induced suppression of migration and invasion could be rescued by lncRNA-C5ORF42-5 overexpression (Fig. 4A).

Taken together, these data indicate that lncRNA-C5ORF42-5 acts as an oncogene to promote metastasis in gastric cancer in vitro and in vivo. Importantly, lncRNA-C5ORF42-5 is a major contributor to the function of CDK10 in gastric cancer metastasis by upregulating lncRNA-C5ORF42-5 to reverse the effects of CDK10 overexpression.

lncRNA-C5ORF42-5 functions by modulating related proteins in the metastatic signaling pathway

To explore the mechanism of lncRNA-C5ORF42-5, we analyzed the results of RNA-seq sequencing by comparing lncRNA-C5ORF42-5-overexpressing cells with the control (Supplementary Fig. S2) and tried to determine the signaling pathways by which lncRNA-C5ORF42-5 is involved in gastric cancer. As shown in Fig. 5A and B, we found that lncRNA-C5ORF42-5 may function by modulating related proteins in metastasis pathways by GO function and KEGG enrichment analysis. Then, quantitative reverse transcription PCR and western blotting were used to verify the analysis results. The expression levels of E-Cad, BMP2 and BMP4 were upregulated, and a similar trend could be found in the phosphorylation levels of AKT and ERK (Fig. 5C and D). Therefore, these results further confirmed our supposition that lncRNA-C5ORF42-5 functions by modulating related proteins in the metastatic signaling pathway.

Discussion

Cyclin-dependent kinases (CDKs) form a protein family of 20 serine/threonine protein kinases, which are involved in the mechanisms of multiple biological processes, such as cell division, apoptosis, transcription, mRNA splicing, and metabolism⁷. They have been considered promising prognostic biomarkers as well as treatment targets in various human cancers¹²⁻¹⁷, while CDK10 was previously proven to function as a prognostic marker in gastric cancer, and reduced CDK10 expression independently predicts poor overall survival in patients¹⁸. Although massive efforts have been dedicated to the discovery and development of CDK small-molecule inhibitors as drug candidates, there have been few available small-molecule inhibitors specifically directed against CDK10³⁷. Therefore, in the present study, we further explored the functions and molecular mechanisms of CDK10 to determine its downstream molecules as treatment targets for metastatic gastric cancer patients.

In recent years, it has been found that lncRNAs are abnormally expressed in various human cancers and participate in all steps of carcinogenesis and tumor progression^{20,21,38}. Moreover, there is increasing evidence indicating that lncRNAs play an important role in the diagnosis and treatment of tumors, and they can be used as new markers for tumor prognosis²³⁻²⁶. Consequently, we analyzed the expression level of lncRNAs by comparing stable highly expressed CDK10 cell lines with the control based on high-throughput sequencing. The identified lncRNA-C5ORF42-5 is a 291-nucleotide sense transcript that

includes exon 1 of the C5ORF42 gene, whose functions and mechanisms are still unknown, especially in gastric cancer. In this study, we found that the expression of lncRNA-C5ORF42-5 was upregulated in gastric cancer tissue compared with matched normal tissue. We also confirmed that lncRNA-C5ORF42-5 acts as an oncogene to promote metastasis in gastric cancer in vitro and in vivo. Importantly, we then ascertained that lncRNA-C5ORF42-5 is a major contributor to the function of CDK10 in gastric cancer tumorigenesis by upregulating lncRNA-C5ORF42-5 to reverse the effects of CDK10 overexpression. These results illustrated that CDK10 promotes the tumor-suppressive effect in gastric cancer by inhibiting lncRNA-C5ORF42-5.

In our current work, we explored the mechanism by which lncRNA-C5ORF42-5 overexpression affects gastric cancer cells to elucidate whether lncRNA-C5ORF42-5 may increase the activity of the Smad pathway of BMP signaling and promote the expression of EMT-related proteins, such as E-cadherin. Additionally, overexpression of lncRNA-C5ORF42-5 affected the phosphorylation levels of AKT and ERK. However, further investigations into how CDK10 influences lncRNA-C5ORF42-5 and how lncRNA-C5ORF42-5 definitely affects downstream pathways are needed. One of the yet undetermined mechanisms not addressed in the current study is how lncRNA-C5ORF42-5 regulates the metastatic signaling pathway. Whether lncRNA-C5ORF42-5 can function as a competitive endogenous RNA (ceRNA) to regulate microRNAs (miRNAs) or competitively bind to transcription factor regions during metastatic gastric cancer development needs further exploration. Moreover, how to apply our genes to clinical treatment still needs further study, which is the focus of our future research.

Conclusion

In conclusion, we show that CDK10 overexpression represses tumor progression in gastric cancer by reducing lncRNA-C5ORF42-5 and hindering activation of the related proteins in metastatic signaling pathways. These findings shed light on novel molecular mechanisms of gastric cancer metastasis regulated by the CDK10-lncRNA-C5ORF42-5 axis and provide new insight into developing effective therapeutic strategies in the treatment of metastatic gastric cancer.

Abbreviations

CDK: Cyclin-dependent kinase; lncRNAs: Long noncoding RNAs; ceRNAs: competitive endogenous RNAs; miRNAs: microRNAs; BCA: bicinchoninic acid; RPMI: Roswell Park Memorial Institute; MTS: the 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium; FBS: fetal bovine serum; PFA: paraformaldehyde; qRT-PCR: quantitative real-time PCR; GO: Gene Ontology; KEGG: Kyoto Encyclopedia of Genes and Genomes; SMAD: drosophila mothers against decapentaplegic; EMT: Epithelial-mesenchymal transition; BMP: bone morphogenetic protein; ERK: extracellular regulated protein kinases; AKT: thymoma viral proto-oncogene; GAPDH: glyceraldehyde-3-phosphate dehydrogenase.

Declarations

Ethics approval and consent to participate

The animal experiments were performed in the animal laboratory center of the Sixth Affiliated Hospital of Sun Yet-sen University and in accordance with the Guide for the Care and Use of Laboratory Animals (National Institutes of Health, Bethesda, MD, USA). This study was approved by the Ethics Committee of the Sixth Affiliated Hospital of Sun Yat-Sen university.

Consent for publication

Not applicable.

Availability of data and materials

The data supporting results of this study are available within the article.

Competing interests

The authors have declared that no competing interest exists

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Authors' contributions

Shi Chen and Jun-Sheng Peng designed and supervised the study. Zi-jian Deng contributed to perform molecular biology experiments, interpret the results and write the paper. Dong-wen Chen contributed to vivo experiments and writing. Xi-jie Chen analyzed the data and prepare the figures and tables. Jia-Ming Fang and Liang Xv collected the tissue. Shi Chen guided the writing.

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Figures

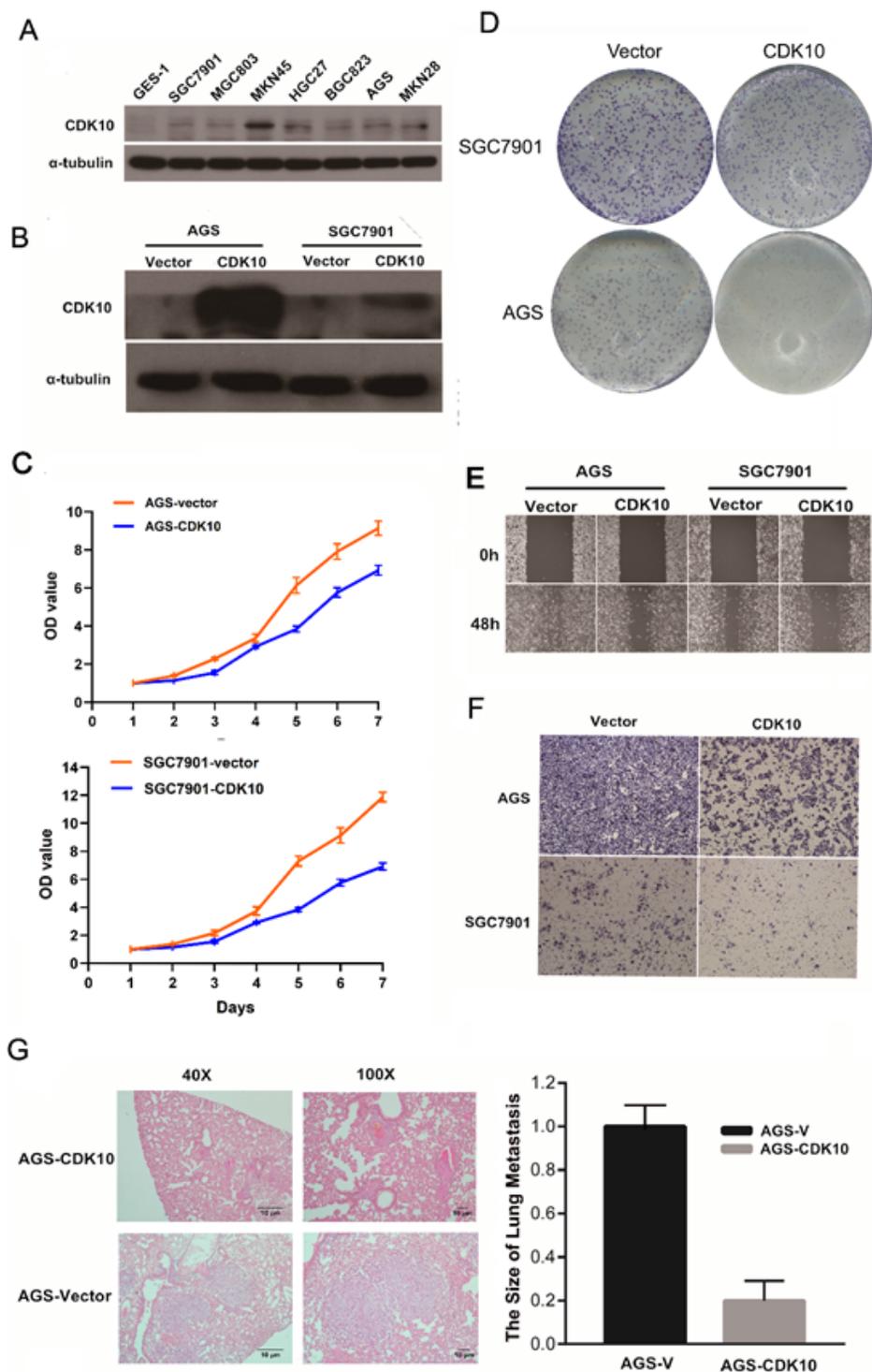


Figure 1

Overexpression of CDK10 inhibits the proliferation and metastasis of gastric cancer cells in vitro and in vivo. a) The expression of CDK10 in gastric cancer lines and normal gastric epithelial cells was detected by WB; b) overexpression of CDK10 in AGS and SGC7901 cells assessed by WB; c and d) MTS and colony formation assays were used to analyze the proliferation of AGS and SGC7901 cells after CDK10 overexpression; e) wound healing assays were used to assess the impact of CDK10 on the cell migration

ability; f) Transwell invasion assays were used to evaluate the cell invasion ability; g) H&E staining of the lung metastases was performed in the CDK10 overexpression and vector groups.

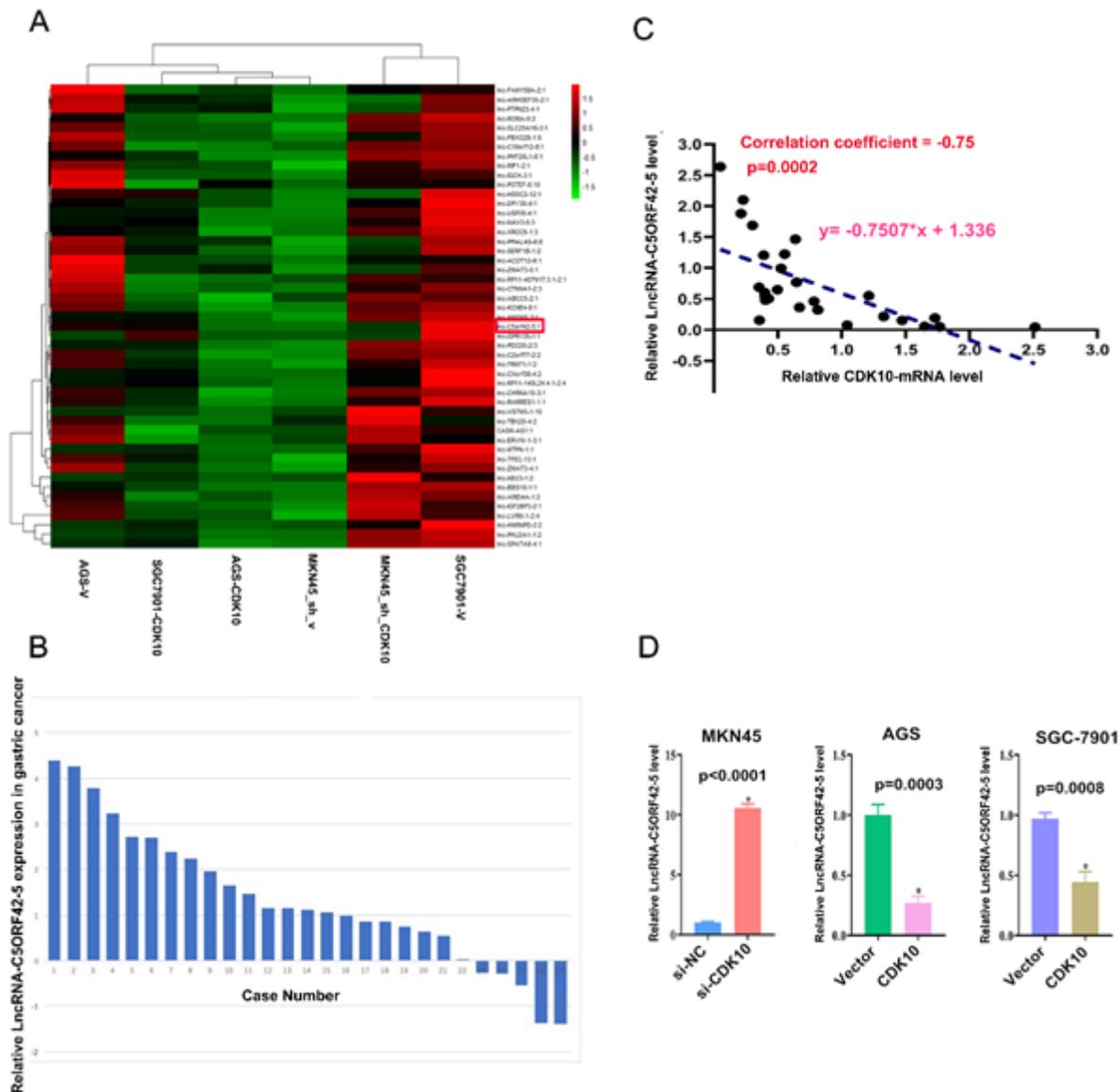


Figure 2

The relationship between CDK10 and lncRNA-C5ORF42-5 expression levels in gastric cancer tissues and gastric cancer cells. a) lncRNA-C5ORF42-5 was the most differentially expressed between CDK10-overexpressing and control group cell lines; b) the lncRNA-C5ORF42-5 expression level was significantly upregulated in 81.5% (22/27) of gastric cancer tissues compared with the adjacent normal tissues; c) lncRNA-C5ORF42-5 expression was negatively correlated with CDK10 in gastric cancer tissues; d) lncRNA-C5ORF42-5 expression was upregulated in CDK10-knockdown cells and downregulated in CDK10-overexpressing cells.

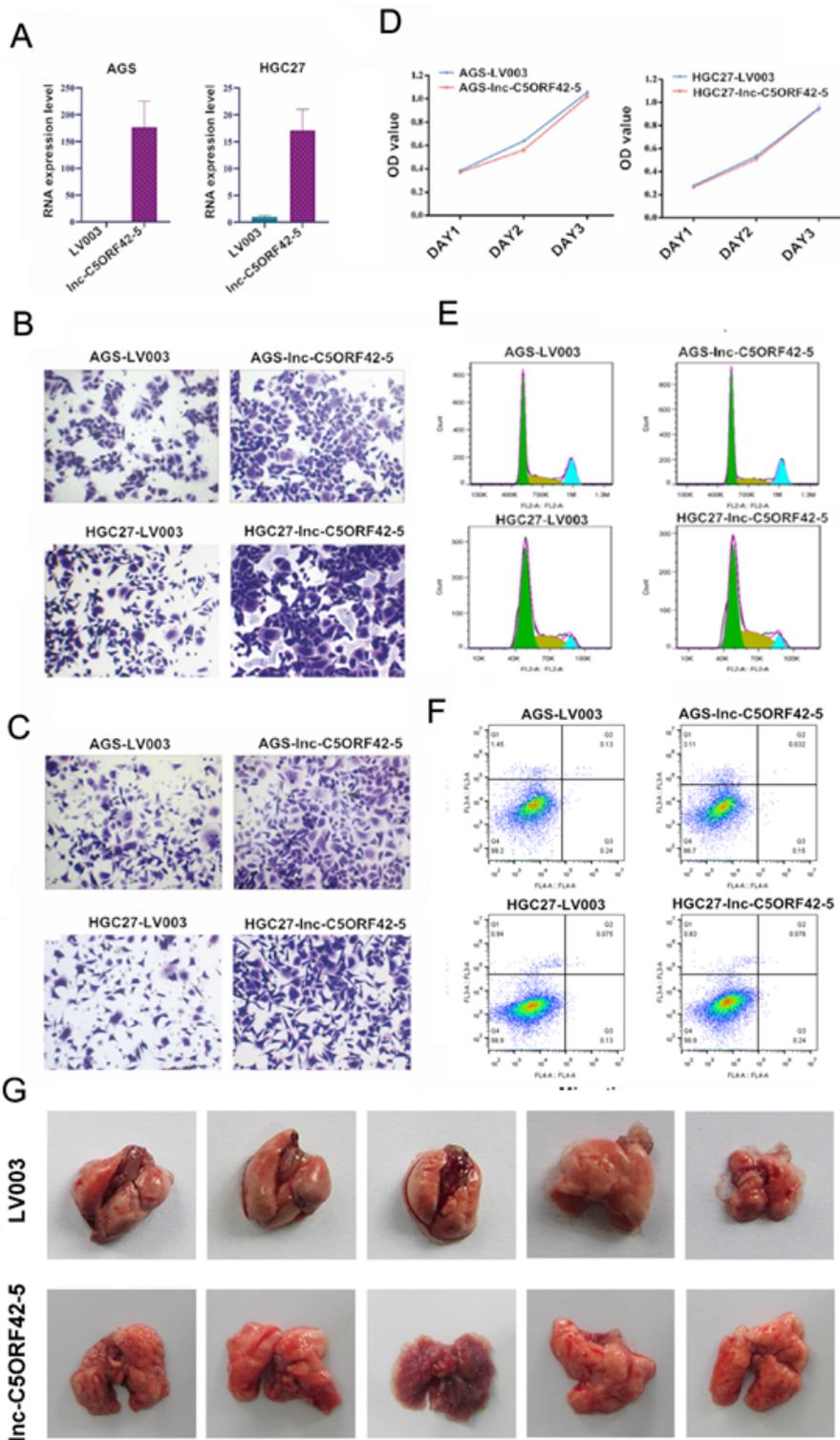


Figure 3

Overexpression of lncRNA-C5ORF42-5 promoted the metastasis of gastric cancer cells in vitro and in vivo. a) lncRNA-C5ORF42-5 was successfully overexpressed in AGS and HGC27 cells. b and c) The effects of lncRNA-C5ORF42-5 on migration and invasion were confirmed by Transwell assays. d-f) lncRNA-C5ORF42-5 had no effect on proliferation, cell cycle or apoptosis according to MTS assays and flow

cytometry. g) Comparison of the number of lung metastases induced by lncRNA-C5ORF42-5- or vector-transfected AGS cells.

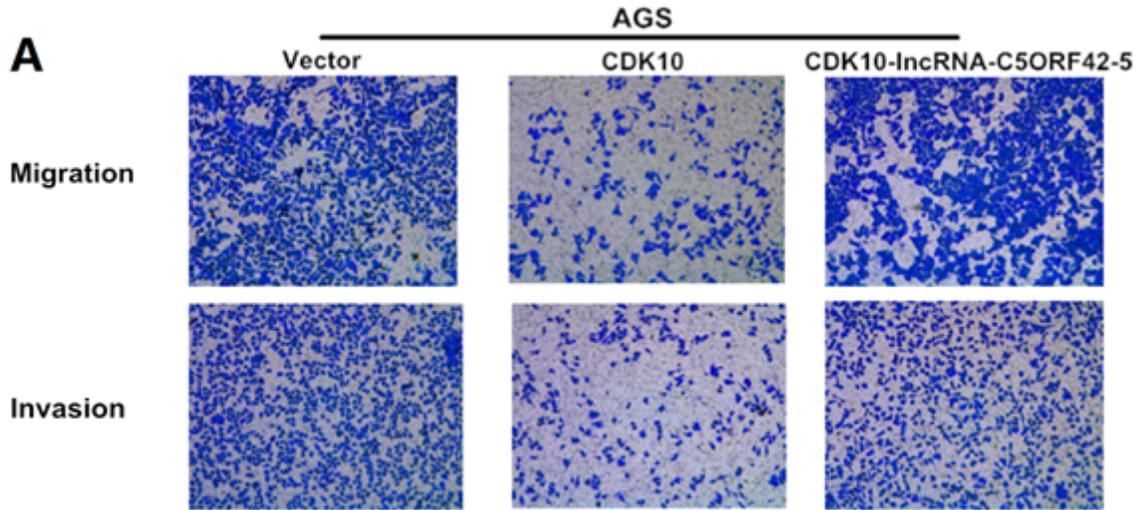


Figure 4

Transwell assays showed that lncRNA-C5ORF42-5 can rescue the migration and invasion suppressed by CDK10 in AGS cells.

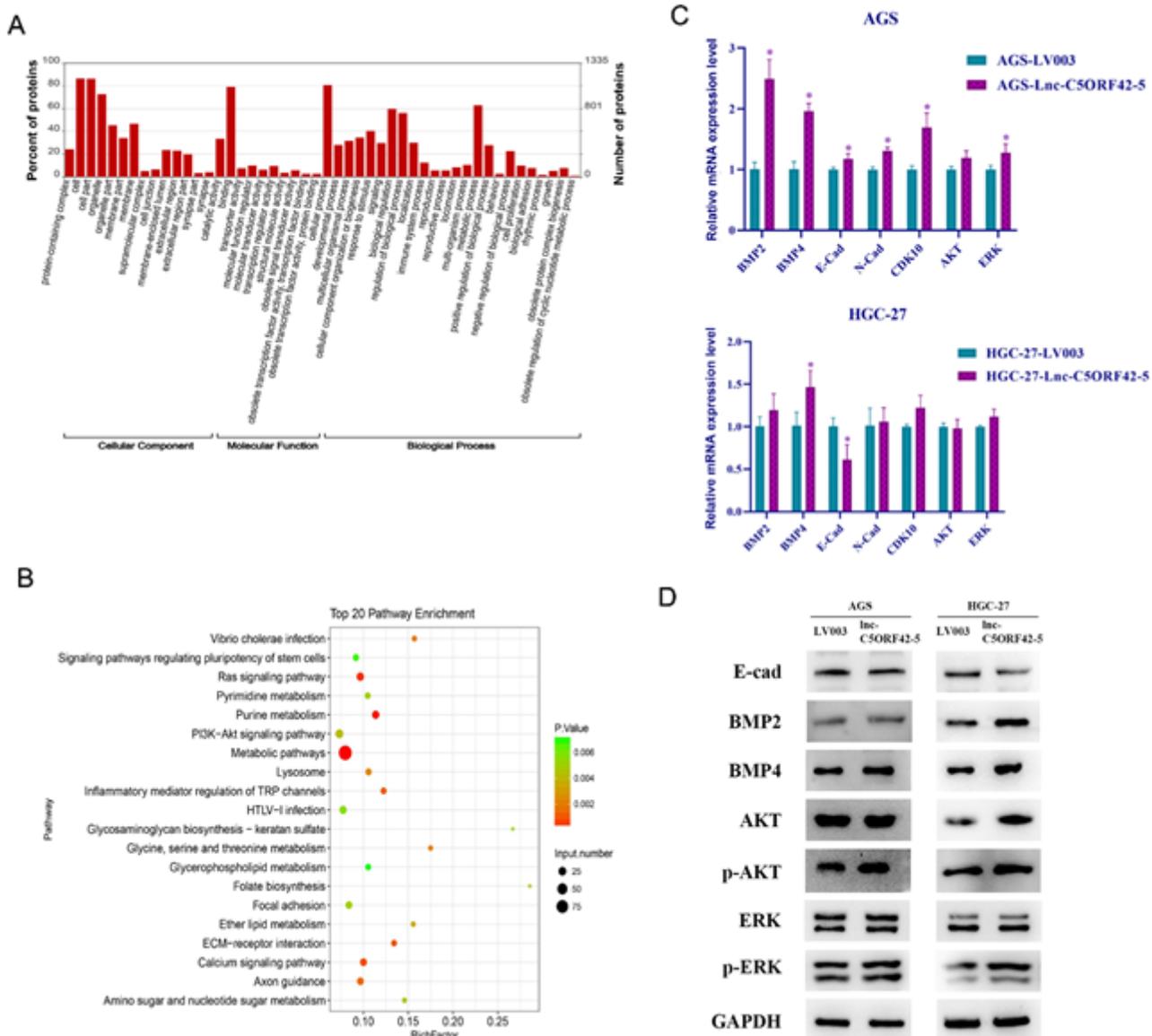


Figure 5

lncRNA-C5ORF42-5 regulated downstream metastasis-related signaling pathways. a and b) GO function and KEGG enrichment analysis revealed that lncRNA-C5ORF42-5 may function by modulating related proteins in metastasis pathways; c and d) RT-qPCR and WB successfully determined the expression of downstream metastasis-related proteins associated with lncRNA-C5ORF42-5 overexpression in AGS and HGC27 cells.

Supplementary Files

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- [TableS1.docx](#)

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