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Claudin-14 promotes colorectal cancer progression via the PI3K/AKT/mTOR pathway

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Colorectal cancer is the third leading cancer in the world in terms of incidence and mortality. The role of differentially expressed Claudin-14 (CLDN14) in CRC has not been reported. We observed that CLDN14 was associated with the progression of CRC. Our functional studies have shown that CLDN14 promoted the proliferation of CRC cells. In addition, CLDN14 also increased the migration and invasion of CRC cells. *In vivo* experiments also showed that CLDN14 promoted the growth of colorectal cancer via the PI3K/AKT/mTOR. In summary, our research suggests that CLDN14 promotes the progression of colorectal cancer. Our findings may provide new strategies for clinical management and patient prognosis of CRC.

Key words: colorectal cancer, Claudin-14, PI3K/AKT/mTOR pathway

Colorectal cancer (CRC) is currently third-ranked cancer in both incidence and mortality, accounting for 10% of all cancer-related deaths worldwide [1]. Treatment regimens for CRC include surgery, radiotherapy, and chemotherapy. Surgical treatment is still the main treatment strategy [2]. And for patients with advanced CRC, chemotherapy has severe side effects and is mostly ineffective [3]. Therefore, in order to discover the prognostic biomarkers and therapeutic targets of colorectal cancer, more and more scholars are conducting research on the epigenetics of CRC and its underlying mechanisms [2, 4–7].

The claudins are members of the transmembrane tight junction family of proteins that interact with scaffold proteins and other claudins to form pores and barriers that regulate the permeability and selectivity of cellular bypass pathways [8]. Many studies have shown that Claudin-14 (CLDN14) is related to hearing loss [9, 10]. Studies have also shown that CLDN14 is a new prognostic biomarker in hepatocellular carcinoma [11]. However, the role of CLDN14 in CRC has never been reported.

PI3K/AKT/mTOR signaling pathways play a vital role in regulating cell proliferation, cell apoptosis, and glucose metabolism through various potential mechanisms [12]. PI3K stimulates the signal transduction cascade and promotes the activation of AKT [13]. AKT is an important signal regulator of cell growth by responding to various cellular stimuli. mTOR is an atypical serine/threonine kinase that exists in two distinct complexes, which are mainly regulated by PI3K/AKT/mTOR signal transduction pathway [14]. Currently, drugs targeting the PI3K/AKT/mTOR signaling pathway are also being developed and applied in clinics. However, the biological effects of CLDN14 on the PI3K/AKT/mTOR signaling pathway in CRC cells are still understood.

In this study, we first showed that CLDN14 was upregulated in the tumor tissues of patients with CRC and was related to the survival of patients. Functional analysis showed that CLDN14 can promote proliferation, migration, and invasion of CRC cells. *In vivo* experiments proved that CLDN14 promoted the progression of CRC through the PI3K/AKT/mTOR pathway. This study revealed that CLDN14 affects the progression of CRC, suggesting that CLDN14 may be a potential therapeutic target for patients with CRC.

Materials and methods

Cell lines and culture conditions. Human colorectal cancer cell line LOVO was purchased from the Shanghai Institutes for Biological Sciences (Shanghai, China). Human

normal colon epithelium cell line (NCM460) and human colorectal cancer cell lines (SW620, HT29, RKO, SW480, HCT116) were purchased from the American Type Culture Collection (ATCC, USA). HCT116 cell line was grown in McCoy's 5A medium (Kaiji Biotechnology, China). SW480 and SW620 cells were grown in Dulbecco's modified Eagle's medium (DMEM) medium (GIBCO, USA). NCM460, RKO, HT29, and LOVO cells were grown in RPMI (1640) medium (GIBCO, USA). All cells were cultured in a constant temperature and humidified incubator at 37 °C with 5% CO_2.

Cell transfection. The lentiviral vector encoding CLDN14, short hairpin RNAs, and empty vector were purchased from Wanlei (Shenyang, China). After lentiviral transfection, stable expression cell lines were obtained after 14 days of puromycin selection. See Supplementary Table S1 for the sequence of specific short interfering RNAs.

RNA extraction and quantitative PCR. The total RNA was extracted from the colorectal cancer cell lines by TRIzol (Invitrogen, Carlsbad, CA, USA), and then the cDNA was extracted and quantified by reverse transcription. The applied Biosystems 7500qpcr (Applied Biosystems, CA) was used to carry out quantitative PCR with SYBR Green reagent (Thermo Fisher Scientific, USA). See Supplementary Table S2 for primer sequences.

CCK-8 assay. The initial 5,000 cells/well were cultured in 96-well microplates (Corning, Life Sciences, USA) for 24, 48, 72, and 96 h. 10 µl of CCK-8 solution was added to the culture medium and incubated for 2 h to measure the optical density (OD) at 450 nm.

Colony formation. 1×10^3 cells were cultured in a 6-well plate (Corning, USA), and then 2 ml of the corresponding medium was supplemented with 10% FBS. After 14 days, cells were fixed with 4% paraformaldehyde for 15 min at room temperature, then washed three times with PBS, and finally stained with 0.1% crystal violet for 10 min. After drying, photos were taken and cell colonies were counted using ImageJ software.

Flow cytometry. The apoptosis kit (BD Biosciences, CA) was used for flow cytometry, and the Flowjo software was used for the analysis.

Migration and invasion assays. The wound-healing experiment was carried out on a six-well plate. After scratching with a 200 µl pipette tip, the degree of healing at 0 and 24 h was photographed, and then ImageJ software was used for the analysis.

For Transwell assays, we added 200 µl of serum-free medium to the upper layer and 600 µl of serum-containing medium to the lower layer of the chamber, then $1–2\times10^5$ cells were added to the upper layer, and then cultured in an incubator at 37 °C for 24–48 h before testing. After fixing with 4% paraformaldehyde for 15 min, it was stained with 0.1% crystal violet and then analyzed with ImageJ software.

Tumor growth in xenografts. This study was carried out with the approval of the Ethics Review Committee of the Second Affiliated Hospital of Harbin Medical University. All animal research has been approved by the Harbin Medical University and was conducted in accordance with international guidelines for the maintenance and care of laboratory animals. HCT116 cells transfected with shRNA were resuspended in McCoy's 5A medium and subcutaneously injected into BALB/c nude male mice (4 weeks old). The tumor volume was evaluated every 3 days using a caliper based on the following formula: $(A\times B^2)/2$, where A is the maximum diameter and B is the diameter perpendicular to A. After 4 weeks, the nude mice were sacrificed. Tumor xenografts were then harvested from dead nude mice and weighed. Tumor xenografts were fixed in 4% paraformaldehyde for subsequent immunohistochemical studies.

Western blotting assay and antibodies. Proteins were separated on a 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and transferred onto a nitrocellulose membrane. The blots were incubated with an antibody overnight at 4 °C. Following three washes, membranes were then incubated with a secondary antibody for 2 h at room temperature. Signals were visualized by ECL (Beyotime, China).

The following antibodies were used: AKT (Wanlei Bio, WL0003b), p-AKT (Wanlei Bio, WL02908), mTOR (Wanlei Bio, WL02477), p-mTOR (Wanlei Bio, WL03694), GAPDH (ZSGB-Bio, TA-8).

Immunohistochemistry. The tumor tissue was placed in 4% paraformaldehyde with 10–20 times volume, fixed for 24 h at room temperature, and then dehydrated and embedded. After the specimens were prepared into paraffin sections, antibody staining was performed. Then, tissue sections were incubated overnight at 4 °C with the primary antibodies including anti-CLDN14 (Abclonal Technology, A7787), anti-p-AKT (Wanlei Bio, WL02908), and anti-p-mTOR (Wanlei Bio, WLH3897). After 3 washing steps, the secondary antibody was added and incubated for 20 min. After washing, DAB solution (ZSGB-BIO, China) was added and then the slides were counterstained with hematoxylin.

Gene set enrichment analysis. A total of 380 primary samples of CRC were obtained from TCGA. The functional gene set data were obtained from MSigDB, C2 curated gene sets from online pathway databases, publications in PubMed, and knowledge of domain experts and C6 oncogenic signature gene sets defined directly from microarray gene expression data from cancer gene perturbations.

Statistical analysis. Statistical analysis of all data was performed using SPSS 25.0 software (IBM). Each experiment was performed independently at least three times, and the results were expressed as an average. An unpaired two-tailed Student's t-test between two groups was applied. A p-value <0.05 was considered to be significant.

Results

CLDN14 is associated with the prognosis of clinical CRC patients. In order to investigate the role of CLDN14

in patients with CRC, we first detected its expression in tumor tissues and normal tissues of patients with CRC in The Cancer Genome Atlas (TCGA) datasets. The results showed that the expression level of CLDN14 in human CRC tissues was significantly higher than that in normal tissues (Figure 1A). Moreover, the survival analysis showed that the overall survival (OS) of patients in the CLDN14 high expression group was shorter than that of the CLDN14 low expression group (p=0.031, Figure 1B). So, we hypothesized that CLDN14 might be associated with the progression of CRC. By detecting the expression of CLDN14 in various CRC cell lines and normal colon epithelial cell line NCM460, we found that the expression of CLDN14 in CRC cell lines increased (Figure 1C). Therefore, the data defined the potential value of CLDN14 in predicting the occurrence and development of CRC, suggesting that the expression of CLDN14 is associated with the prognosis of clinical CRC patients.

CLDN14 promotes the proliferation of CRC cells. In order to determine the specific functions of CLDN14 in CRC, we performed loss- and gain-of-function experiments by knockdown of CLDN14 in HCT116 cells (Figure 2A) and by overexpressing CLDN14 in RKO cells, respectively (Figure 2E). Next, Then, the CCK-8 and colony formation experiments were used to detect the growth of CRC cells. CLDN14 knockdown suppressed the cell growth and foci formation of HCT116 cells (Figures 2B, 2C), whereas CLDN14 overexpression promoted the cell proliferation and foci formation of RKO cells (Figures 2F, 2G). Then, we performed flow cytometry analysis to examine whether CLDN14 would affect apoptosis. It showed that CLDN14 knockdown increased the cell apoptosis (Figure 2D), whereas CLDN14 overexpression decreased the cell apoptosis (Figure 2H). All the above results demonstrated that CLDN14 was associated with the proliferation of CRC cells.

CLDN14 promotes CRC cell migration and invasion. Next, we explored whether the increased or decreased CLDN14 expression in CRC cell lines can affect cell viability and invasiveness. Migration and invasion assays showed that CLDN14 downregulation in HCT116 cells significantly reduced cell migration (Figure 3A) and invasion (Figure 3B), whereas CLDN14 upregulation in RKO cell line enhanced cell migration (Figure 3D) and invasion (Figure 3E). Wound-healing assays showed that CLDN14 knockdown reduced HCT116 cells' migration (Figure 3C) while CLDN14 overexpression accelerated RKO cells' migration (Figure 3F). These results indicated that CLDN14 was linked to cell migration and invasion of CRC.

CLDN14 promotes the proliferation of CRC *in vivo*. We established subcutaneous tumor models to verify the findings *in vivo*. Tumor growth was evidently decreased in HCT116 cells with stable CLDN14 knockdown relative to the control group (Figures 4A, 4B). The size and weight of the tumors were significantly reduced (Figures 4C, 4D). These results indicated that CLDN14 promoted the proliferation of CRC *in vivo*.

CLDN14 promotes the proliferation of CRC via the PI3K/AKT/mTOR pathway. Gene set enrichment analysis (GSEA) is a tool and method for gene enrichment, which is used by more and more scholars to predict downstream signaling pathways [15–17]. And the GSEA of the expression of CLDN14 showed a strong correlation with the AKT/mTOR pathway (Figure 5A). Western blot assay showed that the expression level of p-AKT and p-mTOR increased with the expression level of CLDN14 (Figures 5B, 5C). As expected, IHC analysis showed that the expression of CLDN14 in the knockdown group was significantly lower than that in the control group (Figure 5D) and the expression

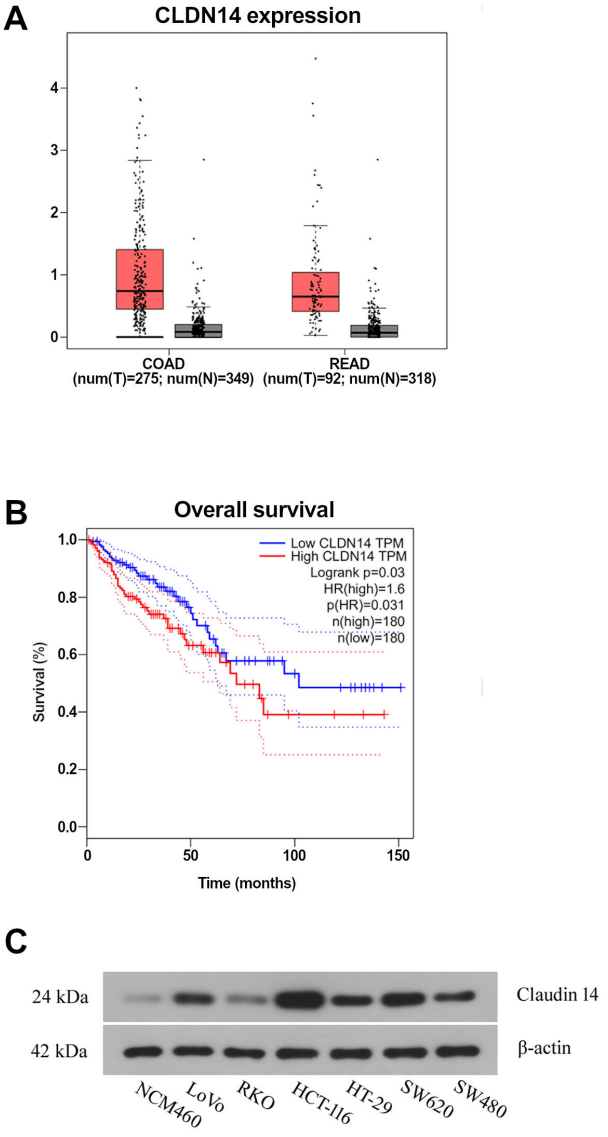

Figure 1. CLDN14 expression in CRC. A) Analysis of expression patterns of CLDN14 in cancer and normal tissues from TCGA dataset. B) Kaplan-Meier analysis of OS of CRC patients from TCGA dataset. C) Relative expression of CLDN14 in CRC cell lines and the normal colon epithelium cell line NCM460 by western blot.

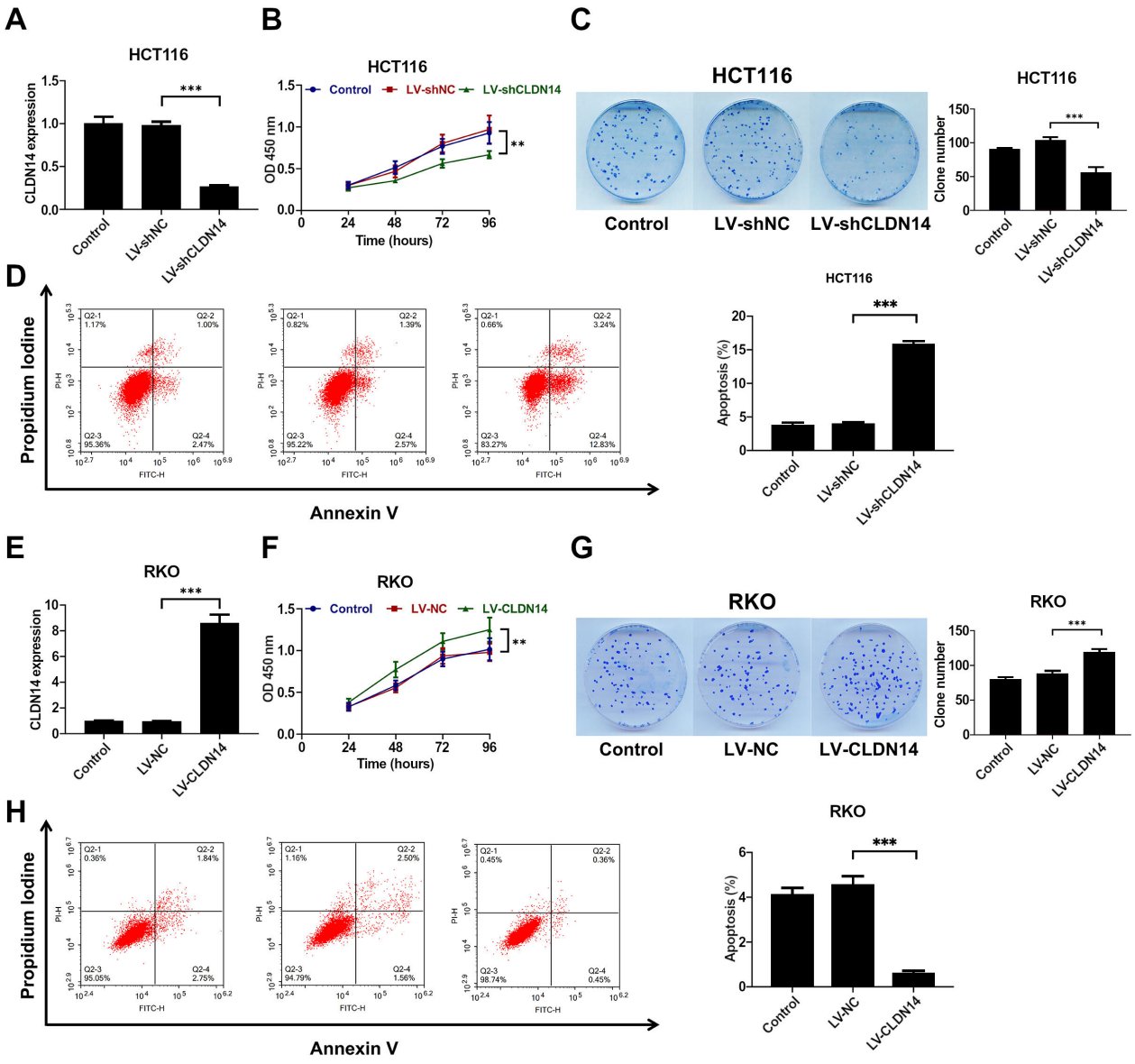

Figure 2. CLDN14 promotes proliferation and inhibits apoptosis of CRC cells. A, E) Validation of knockdown/overexpression efficacy of CLDN14 in CRC cell line by qRT-PCR. ***p<0.001. B, F) Growth curves were plotted by CCK-8 assay. **p<0.01. C, G) Representative images of colony formation assays and colony counts. ***p<0.001. E, F) Apoptosis was detected by flow cytometry.

level of p-AKT and p-mTOR in the knockdown group was significantly lower than that in the control group (Figures 5E, 5F). In order to further confirm that CLDN14 regulates the proliferation of CRC cells through the PI3K/AKT pathway, we treated the cell lines overexpressing CLDN14 with HS-173 (an inhibitor of PI3K) and PKI-402 (an inhibitor of PI3K and mTOR). We could clearly see that after inhibiting PI3K, the trend of cell proliferation mediated by CLDN14 was reversed (Figures 5G, 5H). This proved that CLDN14 affected the growth of CRC via the PI3K/AKT/mTOR pathway.

Discussion

Claudin is a kind of tight-junction protein, which is involved in maintaining tight-junction. Its abnormal expression can damage the structure of epithelial cells and endothelial cells, and lead to serious dysfunction [18–20]. A study has shown that the increase of CLDN1 expression may be related to the migration, invasion, and EMT of bronchial epithelial cells, and it may regulate migration and EMT through the Notch signaling pathway [21]. CLDN6

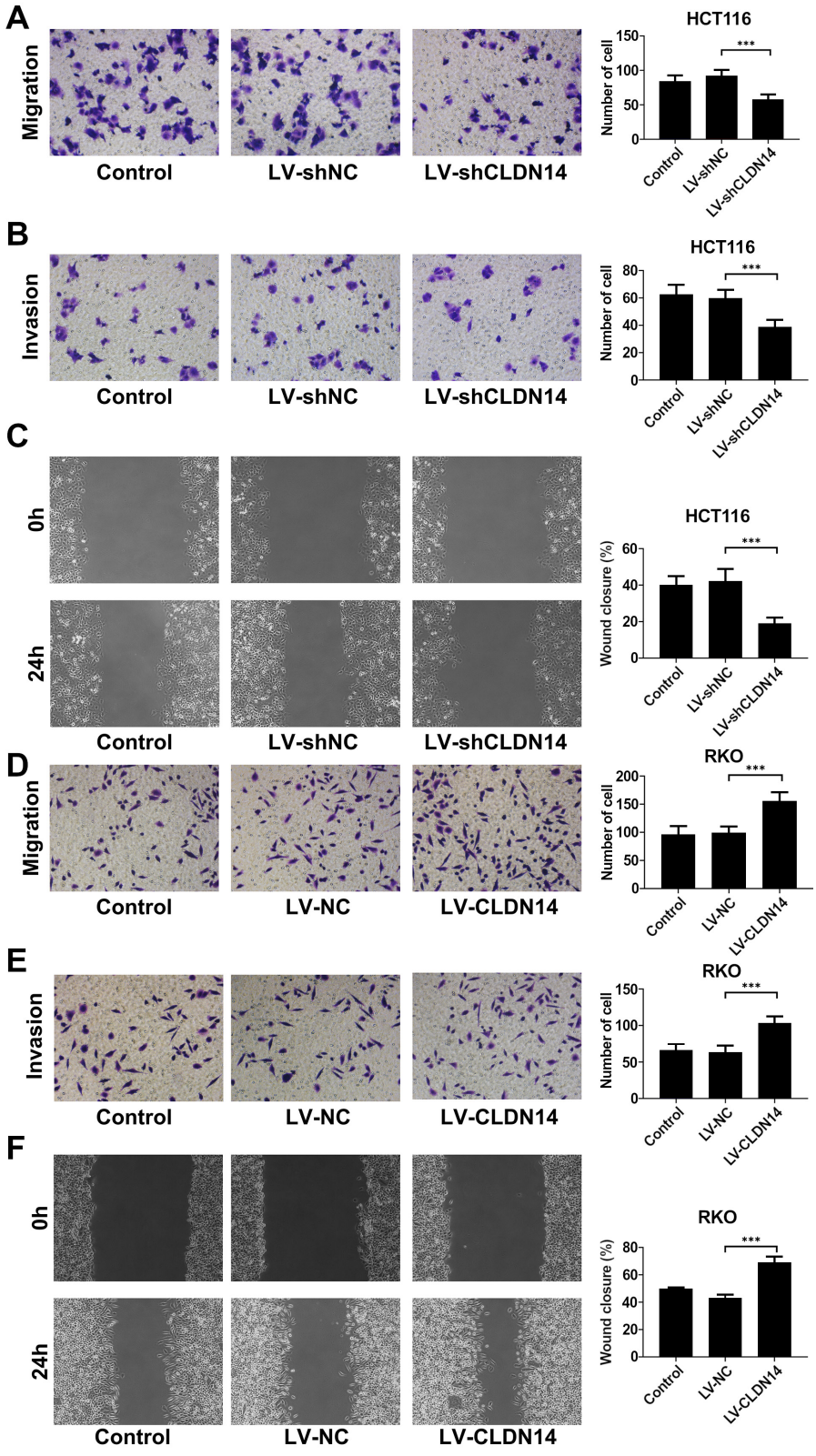

Figure 3. CLDN14 promotes the migration and invasion of CRC cells. A, D) Migration assays of the indicated cell lines. Scale bar = 100 μm. ***p<0.001. B, E) Invasion assays of the indicated cell lines. Scale bar = 100 μm. ***p<0.001. C, F) Wound-healing assays of the indicated cell lines. Scale bar = 200 μm. ***p<0.001

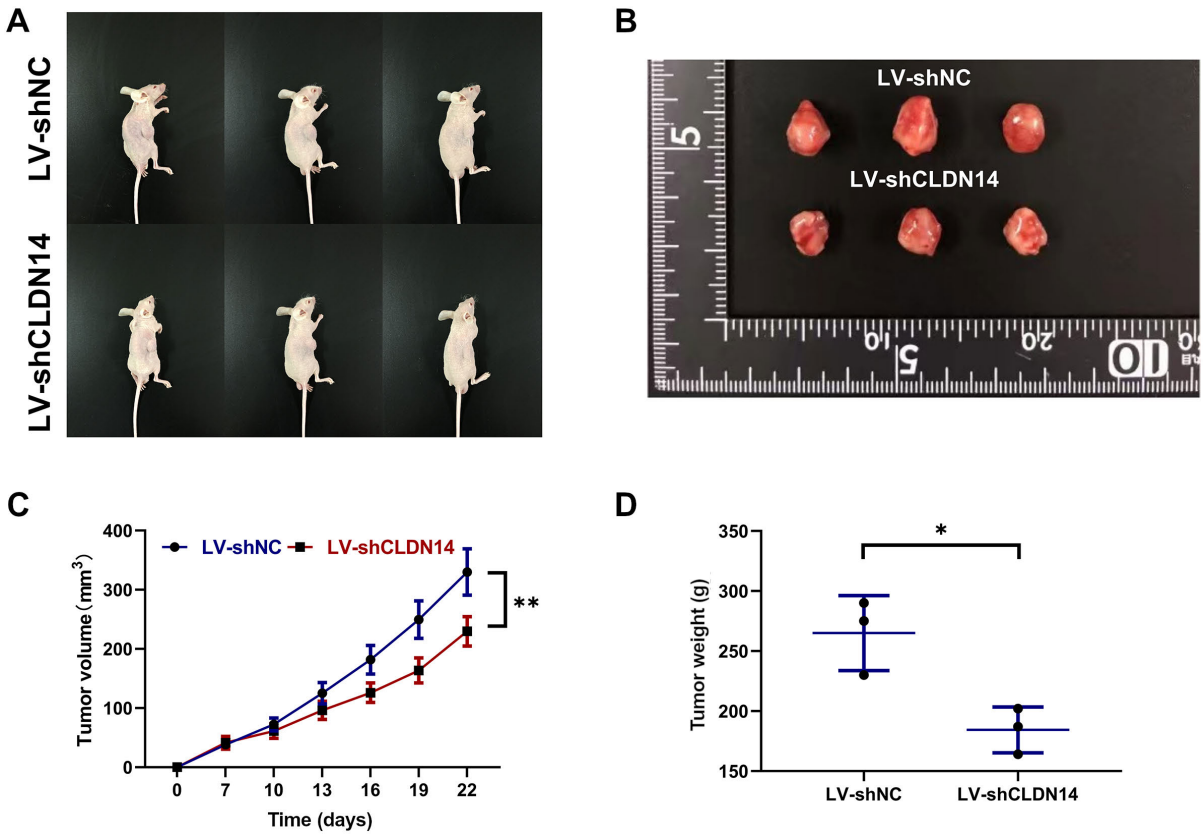

Figure 4. CLDN14 promotes CRC proliferation and growth *in vivo*. A, B) Images of subcutaneous xenograft tumors and nude mice of the indicated group (n=3/group). C, D) Subcutaneous xenograft tumor volumes and weights were measured for the different groups.

can also promote chemoresistance through GSTP1 in human breast cancer [22]. CLDN6 and CLDN18.2 were also found to be drug targets and markers of disease dynamics in non-small-cell lung cancer [23]. However, the function of CLDN14 in CRC is unclear. In this study, we explored the biological and underlying mechanism of CLDN14 in CRC. It has been reported that CLDN14 is overexpressed in hepatocellular carcinoma (HCC) and participates in tumorigenesis and metastasis [11]. Consistent with previous researches, we demonstrated that CLDN14 was overexpressed in CRC tissues and cell lines. Our results suggest that patients with high CLDN14 expression have a worse prognosis than patients with low CLDN14 expression. Our function experiments revealed that CLDN14 abnormal expression affected CRC proliferation, motility, and invasiveness. Therefore, CLDN14 is a candidate oncogene for CRC risk prognostication and therapy.

The PI3K/AKT/mTOR signaling pathway influenced the proliferation and is activated in various cancers including CRC [24, 25]. Our results indicated that CLDN14 mediated activation of the AKT signaling pathway and its downstream effects. *In vivo* experiments confirmed that the PI3K/AKT/mTOR signaling pathway activation contributed to the oncogenic effects of CLDN14 in CRC cells. We observed a positive correlation between CLDN14, p-AKT, and p-mTOR in xenografts. The clinical effect of the combined prognostic value of CLDN14, p-AKT, and p-mTOR needs to be verified by large size of samples. CLDN14 promotes CRC proliferation, at least in part, by activating the PI3K/AKT/mTOR signaling pathway.

What is more interesting is that we can see that CLDN14 not only promotes the proliferation of CRC cells but also promotes the migration and invasion of CRC cells. It might be due to the complex network function of the PI3K/AKT/mTOR pathway in tumor cells. Of course, other pathways, such as Wnt/β-catenin pathway, JAK/STAT3 pathway, or NF-κB pathway may also play an important role in this process.

There were still some limitations in our study. First of all, we only conducted experiments of proliferation *in vivo*. In order to get a more accurate conclusion, we should continue to construct the metastasis *in vivo*. Secondly, the mechanism of CLDN14 mediating the activation of the PI3K/AKT/mTOR pathway deserves further study.

In summary, our work shows that the expression of CLDN14 is upregulated in CRC tissues, correlated with

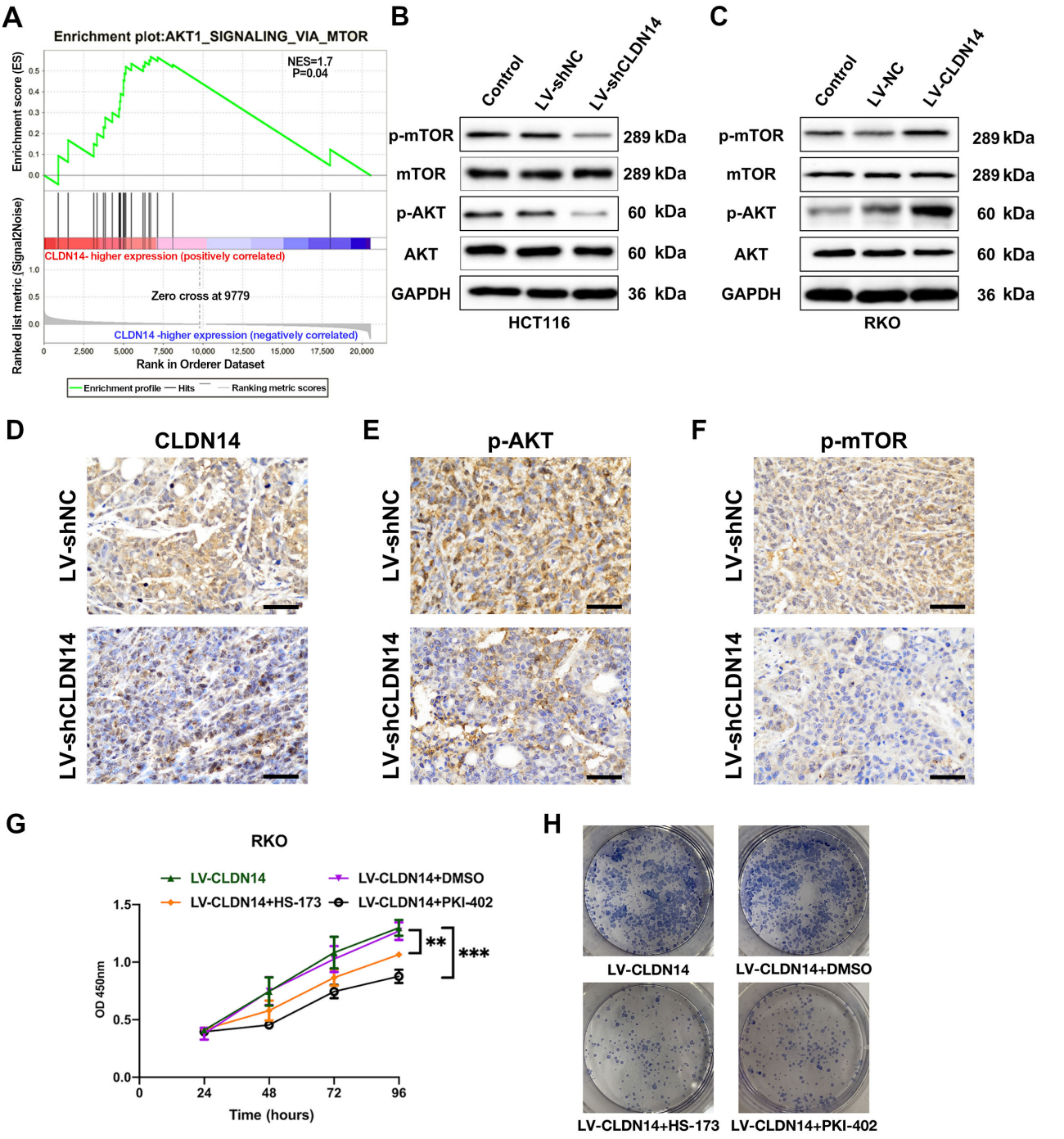

Figure 5. CLDN14 facilitates CRC proliferation by activating the PI3K/AKT/mTOR pathway. A) Gene set enrichment analysis of RNA-seq data of the different expressions of CLDN14. FDR values are provided. B, C) AKT, p-AKT, mTOR, and p-mTOR were detected by western blotting. D–F) Representative IHC staining of CLDN14 or p-AKT or p-mTOR in serial sections are shown. G) Growth curves were plotted by the CCK-8 assay after HS-173 (0.1 μM) or PKI-402 (33 nM) treatment. **p<0.01, ***p<0.001. H) Representative images of colony formation assays after HS-173 (0.1 μM) or PKI-402 (33 nM) treatment.

patients' survival, and may serve as a potential marker of clinical prognosis. Our findings revealed that CLDN14 can promote tumor migration and invasion. Moreover, CLDN14 can promote the proliferation of colorectal cancer cells via the AKT/mTOR signaling pathway. More meaningfully, we may use the expression level of CLDN14 to effectively predict the prognosis of patients and perform different clinical management of CRC patients.

Supplementary information is available in the online version of the paper.

References

[1] SIEGEL RL, MILLER KD, GODING SAUER A, FEDEWA SA, BUTTERLY LF et al. Colorectal cancer statistics, 2020. CA Cancer J Clin 2020; 70: 145–164. https://doi.org/10.3322/caac.21601

[2] DOW LE, O'ROURKE KP, SIMON J, TSCHAHARGANEH DF, VAN ES JH et al. Apc Restoration Promotes Cellular Differentiation and Reestablishes Crypt Homeostasis in Colorectal Cancer. Cell 2015; 161: 1539–1552. https://doi.org/10.1016/j.cell.2015.05.033

[3] GUSTAVSSON B, CARLSSON G, MACHOVER D, PETRELLI N, ROTH A et al. A review of the evolution of systemic chemotherapy in the management of colorectal cancer. Clin Colorectal Cancer 2015; 14: 1–10. https://doi.org/10.1016/j.clcc.2014.11.002

[4] ZHU Y, HU H, YUAN Z, ZHANG Q, XIONG H et al. LncRNA NEAT1 remodels chromatin to promote the 5-Fu resistance by maintaining colorectal cancer stemness. Cell Death Dis 2020; 11: 962. https://doi.org/10.1038/s41419-020-03164-8

[5] FU T, COULTER S, YOSHIHARA E, OH TG, FANG S et al. FXR Regulates Intestinal Cancer Stem Cell Proliferation. Cell 2019; 176: 1098–1112.e18. https://doi.org/10.1016/j.cell.2019.01.036

[6] JANNEY A, POWRIE F, MANN EH. Host-microbiota maladaptation in colorectal cancer. Nature 2020; 585: 509–517. https://doi.org/10.1038/s41586-020-2729-3

[7] ZAJAC O, RAINGEAUD J, LIBANJE F, LEFEBVRE C, SABINO D et al. Tumour spheres with inverted polarity drive the formation of peritoneal metastases in patients with hypermethylated colorectal carcinomas. Nat Cell Biol 2018; 20: 296–306. https://doi.org/10.1038/s41556-017-0027-6

[8] YU ASL. Claudins and the kidney. J Am Soc Nephrol 2015; 26: 11–19. https://doi.org/10.1681/ASN.2014030284

[9] LU Y, YAO J, WEI Q, XU J, XING G et al. Genetic analysis of CLDN14 in the Chinese population affected with non-syndromic hearing loss. Int J Pediatr Otorhinolaryngol 2018; 105: 6–11. https://doi.org/10.1016/j.ijporl.2017.11.016

[10] SHEARER AE, HILDEBRAND MS, SMITH RJH. Hereditary Hearing Loss and Deafness Overview. In: Adam MP, Ardinger HH, Pagon RA et al. (Eds.) Gene Reviews Seattle (WA), University of Washington, Seattle, 1999. ISSN: 2372-0697

[11] LI CP, CAI MY, JIANG LJ, MAI SJ, CHEN JW et al. CLDN14 is epigenetically silenced by EZH2-mediated H3K-27ME3 and is a novel prognostic biomarker in hepatocellular carcinoma. Carcinogenesis 2016; 37: 557–566. https://doi.org/10.1093/carcin/bgw036

[12] ENGELMAN JA, LUO J, CANTLEY LC. The evolution of phosphatidylinositol 3-kinases as regulators of growth and metabolism. Nat Rev Genet 2006; 7: 606–619. https://doi.org/10.1038/nrg1879

[13] SLOMOVITZ BM, COLEMAN RL. The PI3K/AKT/mTOR pathway as a therapeutic target in endometrial cancer. Clin Cancer Res 2012; 18: 5856–5864. https://doi.org/10.1158/1078-0432.CCR-12-0662

[14] LI Y, LU L, LUO N, WANG YQ, GAO HM. Inhibition of PI3K/AKt/mTOR signaling pathway protects against d-galactosamine/lipopolysaccharide-induced acute liver failure by chaperone-mediated autophagy in rats. Biomed Pharmacother 2017; 92: 544–553. https://doi.org/10.1016/j.biopha.2017.05.037

[15] REIMAND J, ISSERLIN R, VOISIN V, KUCERA M, TANNUS-LOPES C et al. Pathway enrichment analysis and visualization of omics data using g: Profiler, GSEA, Cytoscape and EnrichmentMap. Nat Protoc 2019; 14: 482–517. https://doi.org/10.1038/s41596-018-0103-9

[16] ZHANG C, ZHANG M, GE S, HUANG W, LIN X et al. Reduced m6A modification predicts malignant phenotypes and augmented Wnt/PI3K-Akt signaling in gastric cancer. Cancer Med 2019; 8: 4766–4781. https://doi.org/10.1002/cam4.2360

[17] HUANG R, MAO M, LU Y, YU Q, LIAO L. A novel immune-related genes prognosis biomarker for melanoma: associated with tumor microenvironment. Aging (Albany NY) 2020; 12: 6966–6980. https://doi.org/10.18632/aging.103054

[18] TSUKITA S, FURUSE M. Overcoming barriers in the study of tight junction functions: from occludin to claudin. Genes Cells 1998; 3: 569–573. https://doi.org/10.1046/j.1365-2443.1998.00212.x

[19] BEUTEL O, MARASPINI R, POMBO-GARCÍA K, MARTIN-LEMAITRE C, HONIGMANN A. Phase Separation of Zonula Occludens Proteins Drives Formation of Tight Junctions. Cell 2019; 179: 923–936. https://doi.org/10.1016/j.cell.2019.10.011

[20] TESSIER-CLOUTIER B, SOSLOW RA, STEWART CJR, KÖBEL M, LEE CH. Frequent loss of claudin-4 expression in dedifferentiated and undifferentiated endometrial carcinomas. Histopathology 2018; 73: 299–305. https://doi.org/10.1111/his.13525

[21] LV J, SUN B, MAI Z, JIANG M, DU J. CLDN-1 promoted the epithelial to migration and mesenchymal transition (EMT) in human bronchial epithelial cells via Notch pathway. Mol Cell Biochem 2017; 432: 91–98. https://doi.org/10.1007/s11010-017-3000-6

[22] YANG M, LI Y, SHEN X, RUAN Y, LU Y et al. CLDN6 promotes chemoresistance through GSTP1 in human breast cancer. J Exp Clin Cancer Res 2017; 36: 157. https://doi.org/10.1186/s13046-017-0627-9

[23] MICKE P, MATTSSON JSM, EDLUND K, LOHR M, JIRSTRÖM K et al. Aberrantly activated claudin 6 and 18.2 as potential therapy targets in non-small-cell lung cancer. Int J Cancer 2014; 135: 2206–2214. https://doi.org/10.1002/ijc.28857

[24] ZHANG T, MA Y, FANG J, LIU C, CHEN L. A Deregulated PI3K-AKT Signaling Pathway in Patients with Colorectal Cancer. J Gastrointest Cancer 2019; 50: 35–41. https://doi.org/10.1007/s12029-017-0024-9

[25] SONG W, MEI JZ, ZHANG M. Long Noncoding RNA PlncRNA-1 Promotes Colorectal Cancer Cell Progression by Regulating the PI3K/Akt Signaling Pathway. Oncol Res 2018; 26: 261–268. https://doi.org/10.3727/096504017X15031557924132

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Supplementary Information

Supplementary Table S1. Sequences of shRNA.

sgRNA	Sequence 5'-3'
CLDN14 shRNA	CCCCCTACCTGAAAGGGCTCTGGATTCAAGAGATCCAGAGCCCTTTCAGGTAGGTTTTT
scramble for shRNA	TTCTCCGAACGTGTCACGT

Supplementary Table S2. PCR primer sequences.

Primer name	Sequence 5'-3'
CLDN14 FW	CGGGATCCATGGCCAGCACGGCCGTGCAGCTTC
CLDN14 RW	GGGAATTCTCACACGTAGTCGTTCAGCCTGTAC