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# Downregulation of CLDN7 due to promoter hypermethylation is associated with human clear cell renal cell carcinoma progression and poor prognosis

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**Introduction & Objectives:** Metastasis is the primary cause of death in renal cell carcinoma (RCC). Loss of cell-to-cell adhesion, including tight junctions (TJs) is the initial step in the process of metastasis. Claudin-7 (CLDN7) is a major component of TJs. However, the clinical significance and its regulation of kidney tumorigenesis remain poorly understood. In this study, we aimed to evaluate diagnostic and therapeutic value of CLDN7 in clear cell RCC (ccRCC).

**Materials & Methods:** A total of 120 fresh ccRCC specimens and 144 primary RCC and adjacent nonmalignant renal paraffin specimens were obtained from Department of Urology, Peking University First Hospital. Expression of CLDN7 in ccRCC tissues and cell lines were determined using bioinformatic data mining, quantitative real-time PCR (qRT-PCR), Western blotting and immunostaining. The clinical significance of CLDN7 expression and promoter DNA methylation status was analyzed in ccRCC patients from Peking University First Hospital and The Cancer Genome Atlas. Additionally, the methylation specific-PCR, bisulfite genomic sequencing and demethylation analysis of CLDN7 were performed. Biological functions of CLDN7 were investigated by examining cell proliferation using MTS assays and EdU incorporation assays, cell migration by *in vitro* wound healing assays and transwell migration assays, cell invasion by transwell invasion assays, and cell apoptosis by flow cytometry. Mouse model experiments were performed to evaluate the effects of CLDN7 on tumor growth and metastasis *in vivo*. The molecular mechanism of CLDN7 function was investigated using gene-set enrichment analysis (GSEA) and high-throughput cDNA sequencing (RNA-Seq) and confirmed by qRT-PCR, Western blot and immunostaining *in vitro* and *in vivo*.

**Results:** Our findings reveal that CLDN7 is frequently downregulated via hypermethylation of its promoter in ccRCC. CLDN7 can help predict aggressive tumor status and poor prognosis in ccRCC patients. Interestingly, hypermethylation of the CLDN7 promoter is related to advanced ccRCC status and poor prognosis. Moreover, overexpression of CLDN7 induces cell apoptosis, suppresses proliferation, migration and invasion abilities of ccRCC cells both *in vitro* and *in vivo*. Additionally, GSEA and RNA-Seq results show that CLDN7 has negative effects in cancer-associated signaling pathways and epithelial-mesenchymal transition (EMT)-related pathways and validated by qRT-PCR, Western blot and immunostaining.

**Conclusions:** We have demonstrated a previously undescribed role of CLDN7 as a ccRCC suppressor and suggest that loss of CLDN7 potentiates EMT and tumor progression. CLDN7 may serve as a potential biomarker and target in patients with ccRCC.