Inhibition of hepatocellular carcinoma invasion by suppression of claudin-10 in HLE cells

Ying Chi Ip, Siu Tim Cheung, Yuk Ting Lee, Jenny C. Ho, and Sheung Tat Fan
Department of Surgery, The University of Hong Kong, Pokfulam, Hong Kong SAR, China

Abstract
Previously, we showed that down-regulation of claudin-10 (CLDN-10) in hepatocellular carcinoma is associated with prolonged disease-free survival after curative surgery. Claudins are important tight junction components. Increasing evidence shows that claudins are involved in cancer progression but each member of claudins is specifically expressed in a variety of malignancies. The biological role of CLDN-10 in hepatocellular carcinoma is unexplored. In the current study, we investigated the CLDN-10 function in two different hepatocellular carcinoma cell lines by *in vitro* assays with the CLDN-10 overexpression and small interfering RNA–mediated knockdown transfectants. We observed that overexpression of CLDN-10 conferred malignant phenotypes to hepatocellular carcinoma cells, Hep3B, which lack CLDN-10 expression, by promoting cancer cell survival, motility, and invasiveness. More importantly, matrix metalloproteinase 2 (MMP2) was up-regulated. Increase in mRNA transcription and protein expression of membrane type 1-MMP (MT1-MMP) was also observed in the CLDN-10 transfectants, where MT1-MMP was a protease shown to promote intrahepatic metastasis in hepatocellular carcinoma in our earlier study. In addition, CLDN-1, CLDN-2, and CLDN-4 was up-regulated in CLDN-10 overexpression transfectants, indicating that the expression of CLDN-10 in cancer cells might affect the expression levels of its family members. On the contrary, small interfering RNA–based knockdown of CLDN-10 in HLE, an invasive cell line with high level of CLDN-10 expression, abolished invasion and strongly decreased activation of MMPs and claudin members expression.

These findings showed that CLDN-10 is functionally involved in hepatocellular carcinoma invasion and is a potential target for hepatocellular carcinoma therapy. [Mol Cancer Ther 2007;6(11):2858–67]

Introduction
Hepatocellular carcinoma is among the five leading causes of cancer death globally. The 5-year recurrence-free survival rate after curative liver resection is 20% (1). Recurrence due to intrahepatic metastatic spread remains a major obstacle to long-term survival. Recently, we found that the claudin-10 (CLDN-10) expression is associated with recurrence after curative hepatectomy (2). CLDN-10 is not well characterized (3). In particular, the function of CLDN-10 in cancer development remains unexplored. The association of CLDN-10 expression has been shown only in hepatocellular carcinoma recurrence (2) and papillary thyroid carcinoma (4), suggesting that CLDN-10 would contribute functionally to cancer progression.

Claudins are components of tight junctions (5). They have critical functions during normal development and cellular homeostasis. It is increasingly clear that abnormalities in tight junction contribute to human pathogenesis. In particular, tight junction structure and function were modulated in epithelial tumorigenesis (6, 7). Aberrant claudin expression was found in >12 cancer types (8), including hepatocellular carcinoma (9). CLDN-4 was down-regulated in invasive high-grade bladder carcinoma (10), but significantly up-regulated in cirrhotic nodules, hepatocellular carcinoma (11), pancreatic cancer (12), and ovarian cancer (13). In colorectal cancer, CLDN-4 was overexpressed along with CLDN-1 and CLDN-3 (14). Likewise, loss of CLDN-1 and CLDN-7 expression was observed in melanocytic neoplasms (15) and invasive ductal breast carcinoma (16), but increased expression was found during the progression of cervical neoplasia (17). Thus, the expression pattern and dysregulation of individual claudin is stage dependent and cancer type specific.

On the other hand, the claudin proteins consist of a PDZ-binding motif in their cytoplasmic domains. The PDZ-binding domains are able to interact with various adapter molecules in claudin/tight junction–dependent signaling pathway that leads to the alteration of tissue-specific gene expression (18). This may represent a novel mechanism to regulate the cellular fate of cancer cells. In addition, claudins are expressed at the cell surface and might be more accessible in tumors (8). Hence, claudins have been proposed to be a good target for cancer therapy (8, 19).

To gain better insights of CLDN-10 functions in hepatocellular carcinoma, we examined the biological role of CLDN-10 by modulating the CLDN-10 expression levels in two hepatocellular carcinoma cell lines. We showed for the...
first time that hepatocellular carcinoma cells expressing CLDN-10 were endowed with malignant phenotypes, including increased survival ability in anchorage-dependent and anchorage-independent environments, enhanced invasion, and migration in low invasive hepatocellular carcinoma cells, Hep3B. Furthermore, CLDN-10 overexpression resulted in up-regulation of matrix metalloproteinases (MMP) and other members of the claudin family. On the contrary, knockdown experiments by CLDN-10–specific small interfering RNA (siRNA) in an invasive hepatocellular carcinoma cell line, HLE, abolished invasion and decreased MMP and claudin expression. The current study provided the first clear evidence that CLDN-10 is functionally important in hepatocellular carcinoma and may serve as a therapeutic target for hepatocellular carcinoma.

Materials and Methods

Antibodies
Antibodies against occludin, CLDN-1, CLDN-2, CLDN-3, CLDN-4, and CLDN-10 were from Zymed. Antibody against β-actin was from Santa Cruz Biotechnology. Antibodies against MT1-MMP and Na+,K+-ATPase were from Chemicon.

Cell Lines
Eight human hepatocellular carcinoma cell lines, including PLC, Hep3B, HepG2, SNU449, SNU475 (American Type Culture Collection), Huh-7, HLE, and HLF (Human Sciences Research Resources Bank, Tokyo, Japan), and two liver cell lines, MIHA and Hs399Li (American Type Culture Collection), were examined for CLDN-10 expression level by semiquantitative reverse transcription-PCR (RT-PCR).

CLDN-10 Overexpression
Human CLDN-10 was amplified according to the published sequence (accession number NM 006984) using specific primers (forward: 5'-ATAAGCTTATGGCTAGCAGGCTTCGGAGATCAT-3'; reverse: 5'-ATTCTAGATTGCCAGGCTCTTTAGAC-3'; HindIII and XbaI sites were underlined) and cloned into pcDNA3.0 (Invitrogen). After confirmation by nucleotide sequencing, the construct was transfected into Hep3B using FuGene 6 (Roche) following manufacturer’s instructions. Stable transfectants were selected in complete DMEM with 400 μg/mL genetin (U.S. Biochemical) for 3 weeks. Hep3B cells stably transfected with empty pcDNA3.0 vector were used as control for all experiments. Expression levels of CLDN-10 in the transfectants were compared by real-time quantitative PCR and Western blotting.

Quantitative Real-time RT-PCR
Transcript was quantified by quantitative real-time RT-PCR as described previously (2). Briefly, the C_T value represented the PCR cycle at which an increase in reporter fluorescence above a baseline signal could first be detected. The relative amount of RNA, after normalization with calibrator and adjustment for plate-to-plate variation, presented the fold difference (log 2 base scale) relative to baseline level. The amount relative to vector control was expressed as ddCT, where ddCT = [dCT (vector control) – dCT (test sample)].

Knockdown of CLDN-10 by siRNA
Three human CLDN-10–specific Stealth siRNA oligonucleotides and two Stealth-negative controls with high GC content (control 1) and low GC content (control 2) were obtained from Invitrogen. An invasive hepatocellular carcinoma cell line, HLE (20), was transfected with 50 pmol siRNA or control oligonucleotide together with pSV-β-galactosidase vector (Promega) using Fugene 6 (Roche) according to the manufacturer’s instructions. After 48 h, the cells and medium were collected for real-time PCR and assays. β-Galactosidase activity was assayed by β-galactosidase Enzyme Assay System (Promega) for each transfection to examine the transfection efficiency.

Protein Extraction and Western Blotting
Whole-cell lysate was extracted with urea buffer [9 mol/L urea, 50 mmol/L Tris-Cl (pH 7.5)] in the presence of Complete protease inhibitor cocktail (Roche) and analyzed with 0.5 μg/mL CLDN-10 polyclonal antibody (Zymed). Subcellular protein fractions were extracted by Proteo Extract subcellular proteome extraction kit (Merck). Immunoreactive bands were visualized with Enhanced Chemiluminescence Western Blotting Detection Kit (Amersham Biosciences) with β-actin or Na+,K+-ATPase antibody as loading control.

Immunocytochemistry
Cells seeded on glass slides were rinsed with PBS, fixed, and permeabilized in 0.18% Triton X-100/1% bovine serum albumin in PBS and blocked with 3% bovine serum albumin/PBS for 1 h. Cells were then incubated with 3 μg/mL CLDN-10 antibody (Zymed) at 4°C overnight followed by 30-min incubation with Alexa Fluor 488 goat anti-rabbit IgG (Invitrogen) and then counterstained by 0.5 μg/mL 4,6-diamidino-2-phenylindole for the nuclei.

Cell Proliferation Assay, Soft Agar Assay, and Clonogenic Assay
Cell proliferation were assessed using conversion of 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (Sigma-Aldrich) to formazan product at 24-h intervals as described previously (21). For soft agar assay, 6,000 cells

1 Supplementary material for this article is available at Molecular Cancer Therapeutics Online (http://mct.aacrjournals.org/).
were resuspended in 2 mL 0.8% agarose/DMEM/10% fetal bovine serum, overlaid onto 2 mL solidified 1% agarose/DMEM/10% fetal bovine serum. Medium on top was changed every 3 to 4 days. After 5 weeks, colonies were counted at ×200 magnification for 10 microscopic fields. For clonogenic assay, Hep3B cells cotransfected with pSV40 zeo2 vector (Invitrogen) were plated at equal density in 90-mm culture dishes and selected with 400 μg/mL G418 and 10 μg/mL Zeocin for 14 days. The cells were rinsed with PBS before staining with 0.25% crystal violet/20% ethanol for 5 min and the number of colonies was counted manually.

In vitro Invasion Assay
Invasion assay were done using 5 × 10^4 Hep3B or 2 × 10^4 HLE cells in BD BioCoat Matrigel Invasion Chambers (Becton Dickinson) according to the manufacturer’s instruction. Invaded cells were counted under the microscope at ×200 magnification for 10 random fields.

Cell Adhesion Assay
Fifty thousand cells were seeded in 100 μL DMEM in fibronectin or collagen I–coated plates for 1 h. After extensive washes, adherent cells were fixed and stained with crystal violet in 10% ethanol. Following washing with PBS, the incorporated dye was extracted with a 1:1 mixture of 100 mmol/L sodium phosphate (pH 4.5) and 50% ethanol. Absorbance was read at 540 nm.

Cell Migration Assay and Wound-Healing Assay
Fifty thousand cells, resuspended in 0.5 mL DMEM, were loaded into cell culture insert. The lower chamber was filled with 0.5 mL DMEM/10% fetal bovine serum. After 8 h, cells at the membrane undersurface were fixed, stained, and counted at ×200 magnification for four microscopic fields. For wound-healing assay, 10^6 cells were seeded in 35-mm dish overnight. The cell monolayer was scratched with a single pass of a pipette tip, washed twice with PBS, and incubated in serum-free DMEM. Marked wounds were photographed at indicated time points.

Gelatin Zymography
Cells, at 80% confluence, were plated at equal density in serum-free medium for 24 h. The conditioned medium was then collected and concentrated using Centricon-50 (Millipore). The protein concentration was determined using detergent-compatible protein assay (Bio-Rad). Equal amounts of proteins were separated in nonreducing 6% zymogram gel containing 0.1% gelatin. After electrophoresis, gels were incubated in 2.5% Triton X-100 for 30 min followed by an overnight incubation at 37°C in 50 mmol/L Tris-Cl (pH 7.8), 0.2 mol/L NaCl, 5 mmol/L CaCl_2, and 0.02% Brij 35 (Sigma). Gels were then stained in Coomassie blue stain and destained. Human MMP2 (Chemicon) was used as positive control. Clear bands that appeared on the stained background were determined as the areas of

![Figure 1](https://example.com/figure1.png)

**Figure 1.** CLDN-10 overexpression study. RT-PCR (A, ve, negative PCR control) and Western blotting (B) showed overexpression of CLDN-10 in Hep3B transfectants. C, immunofluorescence staining showed CLDN-10 protein was overexpressed and localized at the cell-cell boundary in CLDN-10 transfectant C4, whereas control V2 was CLDN-10 negative.
gelatinolytic activity and quantified by densitometric scanning using a gel-imaging system and analyzed by GeneTools (Synoptics). Experiments were carried out with three independent lots of culture medium.

Statistics
Each data point represented results from at least three independent experiments done in duplicates and was presented with mean value (SD). Comparison between groups was made with the Student’s t test and considered statistically significant when the P value was <0.05. Correlation analysis of continuous variables was assessed by Spearman correlation test. Statistical analysis was carried out using statistical software (SPSS version 14.0 for Windows; SPSS).

Results
Overexpression of CLDN-10
CLDN-10 was overexpressed in CLDN-10–negative human hepatocellular carcinoma cell line, Hep3B. CLDN-10 mRNA was highly expressed in clones 4 and 6 (C4 and C6), low in clones 2 and 5 (C2 and C5), and undetectable in vector control (V2) by semiquantitative RT-PCR (Fig. 1A). By real-time quantitative RT-PCR, the fold increase for C2, C4, C5, and C6 were 4.1, 9.1, 2.9, and 10.1, respectively, relative to V2. CLDN-10 protein levels, as examined by Western blotting, were coherent with the mRNA levels (Fig. 1B). Immunofluorescent staining of C4 indicated that CLDN-10 protein localize at the cell-cell boundary (Fig. 1C). To examine the biological function of the cancer cells under differential CLDN-10 expression level, two high CLDN-10 expression clones (C4 and C6) and one low expression clone (C5) were compared with the vector control clone (V2) in subsequent functional studies.

CLDN-10 Did Not Alter Cell Proliferation but Promoted Survival in Anchorage-Dependent and Anchorage-Independent Environment
3-[4,5-Dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide assay revealed that transfectants, with or without CLDN-10 expression, had no significant difference in their growth rate (Fig. 2A), showing that CLDN-10 had no significant effect on cell proliferation. In clonogenic assays, cells transfected with CLDN-10–expressing construct or empty vector construct together with a Zeocin-expressing construct for transfection efficiency determination were respectively plated out at low density. After 14-day culture in selection medium, the number of colonies that arise from surviving transfectants and that attach to the substratum would reflect the survival efficiency at low cell density. The CLDN-10 transfectants showed increased colony formation ability by 253% compared with the vector control (307.3 ± 44.2 and 87.0 ± 11.1, respectively; P = 0.010; Fig. 2B). The result indicated that CLDN-10 promotes cell survival in an anchorage-dependent environment. When cells were deprived of anchorage in soft agar assay, high CLDN-10 expression transfectants yielded significantly more colonies (C4, 5.3 ± 0.2; C6, 4.9 ± 0.1) compared with low CLDN-10 expression transfectants (C5, 2.4 ± 0.5) and the control transfectants (V2, 1.5 ± 0.1; P < 0.001; Fig. 2C). These results showed that expression of CLDN-10 did not alter cell proliferation rate but conferred survival advantage in both anchorage-dependent and anchorage-independent environment.
CLDN-10 Enhanced Cell Migration and Invasion without Affecting Matrix Adhesion Efficiency

The adhesion of hepatocellular carcinoma cells onto common matrix components was investigated. CLDN-10 overexpression did not show a significant influence on the cell adhesion efficiency onto fibronectin or collagen I (Fig. 3A). However, wound-healing assay showed that high CLDN-10 expression promoted migration. In serum-free conditions, high CLDN-10 expression transfectants (C4 and C6) closed the wound completely in 22 h after the start of the experiment, whereas gaps remained in low CLDN-10 expression transfectants (C5) and control (V2; Fig. 3B). The same trend was found in the migration assay done under attraction of 10% serum. High CLDN-10 expression significantly increased the number of migrated cells through the chamber (C4, 25.5 ± 3.3 and C6, 13.3 ± 2.0)

Figure 3. CLDN-10 enhanced cell migration and invasion without affecting matrix adhesion. A, adhesion assay showed that CLDN-10 did not alter cell adhesion ability in hepatocellular carcinoma on collagen I (left) or fibronectin (right). B, in wound-healing assay, uniform scratches (arrows) created in the confluent cultures were sealed in high expression transfectants (C4 and C6) after 22 h, whereas gaps (small arrows) still remained in the low expression transfectants (C5) and control (V2). Migration assay (C) and invasion assay (D) showed that CLDN-10 conferred hepatocellular carcinoma cells with significantly higher cell motility and invasion ability. **, P < 0.001; *, P < 0.05.
compared with low expression (C5, 4.6 ± 0.7) and the vector control (V2, 4.8 ± 0.7, P < 0.001; Fig. 3C). The invasion ability of the transfectants across matrix was tested using the standard Boyden chamber. Again, enhanced cell invasion was observed in the high CLDN-10 expression transfectants (C4, 27.5 ± 9.2; C6, 12.4 ± 2.5), compared with the low CLDN-10 expression transfectants (C5, 4.0 ± 1.7) and the control cells (V2, 2.2 ± 0.5; P < 0.001; Fig. 3D). Taken together, CLDN-10 enhanced cell migration and cell invasion across matrix without affecting adhesion efficiency.

**CLDN-10 Increased Active MMP2 Level and MT1-MMP Expression**

In light of considerable increase in invasiveness, MMP activities were evaluated by gelatin zymography. All transfectants expressed and secreted MMP2 and MMP9 into the medium (Fig. 4A), but no change in the pro-MMP9 and active MMP9 level was observed. Importantly, the amount of active MMP2 was significantly increased in the high CLDN-10 expression transfectants (C4, P < 0.01 and C6, P < 0.05).

Quantitative real-time PCR validated that the mRNA levels of MT1-MMP, the major activator of MMP2, were significantly correlated to CLDN-10 mRNA levels in hepatocellular carcinoma clinical specimens (r = 0.370; P = 0.017, data not shown). To see if the enhanced MMP2 activation was related to MT1-MMP, equal amount of membrane fractions of the transfectants were analyzed by Western blotting (Fig. 4B, top). Increase of MT1-MMP protein was seen in high CLDN-10 expression transfectants (C4 and C6). RT-PCR (Fig. 4B, middle) showed the increase of MT1-MMP was at transcriptional level. These data indicated that CLDN-10 expression in hepatocellular carcinoma could lead to enhanced MMP2 activation and elevation of MT1-MMP mRNA and protein levels.

**CLDN-10 Modified Expression of CLDN-1, CLDN-2, and CLDN-4**

During normal development, expression of claudin family members is finely tuned. To find out if the balance of claudins in cancer cells is affected upon overexpression of CLDN-10, the expression of CLDN-1 to CLDN-5 and occludin were examined. As shown in Fig. 5A, CLDN-1, CLDN-2, and CLDN-4 were barely detectable in vector transfectant (V2) and low expression transfectant (C5), but drastic increase of these claudins were observed as the CLDN-10 expression levels increased (C4 and C6). No apparent change in CLDN-3 and occludin was observed in the transfectant regardless of their CLDN-10 levels. CLDN-5 was not detected in any of the transfectants (data not shown). The increase of these claudin members was confirmed by RT-PCR (Fig. 5B), suggesting that increased CLDN-10 expression up-regulated CLDN-1, CLDN-2, and CLDN-4 at mRNA and protein levels.

**Knockdown of CLDN-10 by siRNA**

To complement the overexpression studies, knockdown experiments were done using three human CLDN-10–specific siRNA (siRNA1, siRNA2, and siRNA3) in HLE, which is a highly invasive hepatocellular carcinoma cell line (20) with high levels of CLDN-10 expression. Effective

---

**Figure 4.** CLDN-10 overexpression up-regulated the MMP expressions. A, gelatin zymogram of the conditioned medium from CLDN-10 transfectants. Gelatin lysis bands appeared as clear area against the stained background. Human MMP2 was used as positive control (+ve). Quantification of active MMP2 showed that the increase in high expression clones (C4 and C6) was significant compared with vector control. B, MT1-MMP expression in Hep3B transfectants. Equal amount of membrane fraction protein was analyzed by Western blotting. Na⁺,K⁺-ATPase is the loading control. Densitometric quantification indicated significant elevation of the MT1-MMP protein (left) and mRNA (right) levels in high expression clones (C4 and C6). –ve, negative PCR control; **, P < 0.01; *, P < 0.05.
inhibition of CLDN-10 was shown by Western blotting and semiquantitative RT-PCR (Fig. 6A, top and middle, respectively). Real-time quantitative RT-PCR indicated that the siRNA2 and siRNA3 reduced CLDN-10 mRNA levels to 40% and 24%, respectively, compared with the controls (mean level of the two nonspecific RNA controls), whereas no change was observed by siRNA1.

Following effective inhibition of CLDN-10, invasion were abolished upon introduction of siRNA2 (19.27 ± 3.6) and siRNA3 (17.13 ± 0.3) compared with control RNAs (control 1, 47.1 ± 4.3; control 2, 42.8 ± 2.3; P < 0.001) and mock transfection control (42.3 ± 2.8; Fig. 6A, bottom). Introduction of siRNA1 with minimal effect on modulation of CLDN-10 level resulted in no significant effect on invasion (51.2 ± 10.1). In addition, in transfectants with efficient suppression of CLDN-10, the level of active MMP2 was significantly lowered in the gelatin zymogram (Fig. 6B), whereas MT1-MMP was suppressed on protein and mRNA levels (Fig. 6C, top and middle, respectively). Furthermore, expression of CLDN-1, CLDN-2, CLDN-4, protein, and mRNA was inhibited upon knockdown of CLDN-10 in HLE cells (Fig. 6D, top and bottom, respectively). Taken together, suppression of CLDN-10 inhibited cell invasion through down-regulation of MMP2 and MT1-MMP, and the expression of its family members CLDN-1, CLDN-2, and CLDN-4.

Discussion

We previously reported (2) that down-regulation of CLDN-10 in hepatocellular carcinoma was associated with prolonged disease-free survival after curative liver resection and validated CLDN-10 as a prognostic marker for hepatocellular carcinoma. To elucidate the functional relevance of CLDN-10 in hepatocellular carcinoma recurrence, we did functional assays and found that CLDN-10 conferred hepatocellular carcinoma with malignant phenotypes and is a potential therapeutic target for hepatocellular carcinoma.
Figure 6. Knockdown study of CLDN-10 by siRNA in HLE cells. A, successful knockdown of CLDN-10 was shown by Western blotting (top) and RT-PCR (middle). Invasion after CLDN-10 siRNA knockdown was significantly decreased compared with control cells (control 1 and control 2) and mock transfection (bottom). B, gelatin zymogram showed that active MMP2 was reduced by CLDN-10 siRNA2 and siRNA3 compared with the controls (high GC control and low GC control) and mock transfection (**, \( P < 0.01 \)). C, both MT1-MMP protein (top) and mRNA (middle) levels was reduced upon siRNA-mediated knockdown of CLDN-10. Na⁺, K⁺-ATPase is the loading control. D, CLDN-1, CLDN-2, and CLDN-4 protein (top) and mRNA (bottom) levels were decreased upon efficient suppression of CLDN-10 by siRNA.
Surgical resection is the mainstay for curative treatment of hepatocellular carcinoma (22). However, the long-term prognosis remains unsatisfactory due to high incidence of recurrence. Most patients with tumor-node-metastasis stage II still have only a 50% 5-year survival, and patient death is usually caused by recurrence (23). Therefore, effective treatment of recurrence is important in prolonging survival after resection of hepatocellular carcinoma. Unfortunately, due to the tumor heterogeneity within patients, targeted therapy against the entire population of patients may become less effective. It has been proposed (24) that if appropriate patient selections are pursued, it could allow suitable treatment and improve outcome. Thus, stratifying hepatocellular carcinoma patients with different risk of disease recurrence will be more beneficial to patients and could allow identification of the responders to targeted therapy. CLDN-10 is an independent predictor of hepatocellular carcinoma recurrence after curative hepatectomy (2). Using CLDN-10 as prognostic marker could allow more rational basis for patient selection. Now, given the functional involvement of CLDN-10 in hepatocellular carcinoma invasion and the specific aberrant expression of CLDN-10 in hepatocellular carcinoma (2), CLDN-10 may also represent an opportunity for hepatocellular carcinoma–targeted therapy.

CLDN-10 promotes malignant behaviors of hepatocellular carcinoma. CLDN-10 conferred survival advantage in clonogenic assay without affecting cell proliferation, which was in concordance with a study on its family member CLDN-3 and CLDN-4 (25). Overexpression of CLDN-10 enhanced anchorage-independent survival, cell invasiveness, and motility, which are typical changes during malignant progression of cancers. On the contrary, the inhibition of CLDN-10 abolished hepatocellular carcinoma invasion. More importantly, knockdown of CLDN-10 could decrease MMP2 activation and MT1-MMP expression. Clinically, there is also a significant correlation of CLDN-10 and MT1-MMP expression. MT1-MMP is a protease that strongly promotes invasion, intrahepatic metastasis, and aggressive features in hepatocellular carcinoma (21, 26, 27). Intrahepatic metastasis is an important mechanism of early recurrence after resection of hepatocellular carcinoma (28). Adjuvant therapy aiming at suppression of intrahepatic metastasis may help prevent recurrence after resection. Therefore, the down-regulation of MT1-MMP expression by CLDN-10 knockdown is clinically important. The suppression of CLDN-10 expression represents a potential strategy for therapeutic intervention of hepatocellular carcinoma recurrence after curative hepatectomy. CLDN-10 is transmembrane protein expressed on hepatocellular carcinoma tumor cell surface (2); it could serve as a good target for antibody-based therapy. Development of CLDN-10 antibody can be a good method to treat hepatocellular carcinoma.

The modulation of CLDN-10 levels caused changes in the expression of other claudins. In the current setting, we have not further delineated if the enhanced invasiveness was related to the up-regulated claudins. Despite this, the suppression of CLDN-10 abolished invasion and decreased the expression of other claudin members that might involve in hepatocellular carcinoma metastasis. In fact, considering that CLDN-1 and CLDN-4 could promote metastatic transformation (29) and invasion (25), it is pertinent to propose that CLDN-10 may work in concert with other claudins. There might be even coordinated expression of claudin family members during cancer progression to promote metastasis. Future studies will be needed to explore the possibility of interactions of claudins in hepatocellular carcinoma cancer development and progression. On the other hand, we speculate that there might be posttranscriptional regulatory mechanism modulating the claudins protein expressions because it was observed that the change in claudins protein levels did not exactly correspond with the respective RNA levels in the CLDN-10 overexpression clones.

We provided the first clear evidence that CLDN-10 enhance hepatocellular carcinoma invasiveness, and up-regulated MT1-MMP expression and activation of MMP2. The down-regulation of CLDN-10 abolished invasion; reduced MMP activation showed that CLDN-10 may represent a new target for hepatocellular carcinoma therapy. Our results support and strengthen the view of using CLDN-10 as prognostic marker for hepatocellular carcinoma recurrence. Further application of the knowledge can help to identify patients at high risk for hepatocellular carcinoma recurrence so that better disease management, for example, targeted therapy, can be provided.

References
Molecular Cancer Therapeutics

Inhibition of hepatocellular carcinoma invasion by suppression of claudin-10 in HLE cells

Ying Chi Ip, Siu Tim Cheung, Yuk Ting Lee, et al.


Updated version
Access the most recent version of this article at:
http://mct.aacrjournals.org/content/6/11/2858

Cited articles
This article cites 29 articles, 11 of which you can access for free at:
http://mct.aacrjournals.org/content/6/11/2858.full.html#ref-list-1

Citing articles
This article has been cited by 3 HighWire-hosted articles. Access the articles at:
/content/6/11/2858.full.html#related-urls

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.