Rab25 suppresses colon cancer cell invasion through upregulating claudin-7 expression

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Abstract

Purpose

Ras-related protein 25 (Rab25) is a member of small GTPase and implicated in various cancer cell progression. Growing evidence suggests the context-dependent roles of Rab25 in cancer invasiveness. Claudin-7 is a tight junction protein and has been known to suppress cancer cell invasion. Although Rab25 was reported to repress cancer aggressiveness through recycling β1 integrin to the plasma membrane, the detailed underlying mechanism remain unanswered. In the present study, we identify the critical role of claudin-7 in Rab25-induced suppression of colon cancer invasion.

Methods

To define the role of Rab25 and claudin-7 in colon cancer cells, we performed plasmid transfection and analyzed cancer cell invasion by utilizing 2D and 3D Matrigel invasion chambers. Immunoblotting, immunofluorescence and ELISA assay were used to identify the level of protein expression and pathways implicated in Rab25-induced colon cancer cell invasion.

Results

Enforced expression of Rab25 attenuates colon cancer cell invasion. In addition, Rab25 inactivated epidermal growth factor receptor (EGFR) and increased E-cadherin expression. Unexpectedly, we observed that Rab25 induces claudin-7 expression through protein stabilization. Moreover, ectopic expression of claudin-7 reduced EGFR activity and Snail expression as well as colon cancer cell invasion. However, silencing of claudin-7 expression reversed the tumor suppressive role of Rab25, thereby increasing colon cancer cell invasiveness.

Conclusion

Collectively, the present data indicate that Rab25 inactivates EGFR and colon cancer cell invasion through upregulating claudin-7 expression.

1. Introduction

Rab25 is a member of the RAS-oncogene superfamily of small GTPase and implicated in various cancer cell invasion and metastasis [1–3]. This small GTPase is exclusively expressed in epithelial cells and arbitrates recycling of proteins from the late endosome to the plasma membrane to maintain cellular polarity and cell signaling [4]. Emerging evidence indicates the context-dependent characteristics of Rab25 on cancer cell progression. While Rab25 promotes cancer cell invasion in ER-positive breast, ovarian and gastric cancers [1, 5, 6], Rab25 in colon and triple negative breast cancer is under expressed
and suppresses invasion and metastasis of these cancers [7, 8]. In addition, the level of Rab25 expression is inversely correlated with colorectal patient survival, reinforce the tumor suppressive role of Rab25 in colon cancer.

Claudins are major components of tight junction and maintain cellular polarity. Disruption of claudins is associated with tumorigenesis. Among 27 known claudin families, claudin-7 distributed in both apical and basolateral membranes in epithelial cells and tissues. In addition, this tight junction protein is critical for maintaining of epithelial cell-matrix communications and intestinal equilibrium [9]. A plethora of studies suggest the pivotal role of claudin-7 in suppressing colon cancer progression. Claudin-7 expression is downregulated in various cancers including colorectal cancer [10]. Moreover, low expression of claudin-7 leads to poor outcome of colon cancer patients [11–13]. Mechanistically, claudin-7 was known to co-localize with β1 integrin, and loss of claudin-7 downregulates β1 integrin expression, leading to promoting lung cancer cell invasion [14]. Furthermore, claudin-7 was shown to suppress colon cancer cell tumorigenesis in a Rab25-dependent manner [15].

Previously, we have shown that Rab25 induces cancer cell endothelial-mesenchymal transition (EMT) and invasiveness through the β1 integrin/EGFR/VEGF1/Snail signaling cascades [16]. In present study, we for the first time demonstrate that Rab25 suppresses colon cancer cell invasion through upregulating claudin-7 expression.

2. Materials and Methods

2.1. Reagents

Chloroquine (CQ), cycloheximide (CHX) and MG132 were purchased from Sigma-Aldrich (St. Louis, MO, USA). All other reagents were of the purest grade available.

2.2. Cell culture

HCT-116 (CCL-247) cells were purchased from the American Type Culture Collection (Manassas, VA, USA). Caco-2 cells were obtained from Korean Cell Line Bank (Seoul, South Korea). HCT-116 and Caco-2 cells were cultured in RPMI-1640 and Minimal Essential Medium (MEM), respectively, supplemented with 10% fetal bovine serum and 1% penicillin/streptomycin.

2.3. Plasmid transfection

The colon cancer cells were transiently transfected according to the manufacturer's instructions with Lipofectamine 3000 (Invitrogen, Carlsbad, CA, USA). Transfection was performed as previously described [15]. The Rab25 cDNA was subcloned into a pcDNA3 vector, and an empty pcDNA3 vector was used as a negative control. RAS-V12 constructs were kindly provided by Dr. A.R Moon (Duksung University, Seoul). Claudin-7 constructs were purchased from Origene (Rockville, MD, USA). siRNAs against Rab25 (SASI_Hs01_00216284), claudin-7 #1 (SASI_Hs01_00214821), claudin-7 #2 (SASI_Hs01_00214822) were
purchased from Sigma-Aldrich (St Louis, MI, USA). Control scrambled siRNA was from Invitrogen (Carlsbad, CA, USA).

2.4. Quantitative RT-PCR (qRT-PCR)

Isolation of total RNA and subsequent analysis were performed as previously [17]. The cDNA complex was amplified using an iQ5 Real-Time PCR Detection System (Bio-Rad Laboratories, Hercules, CA) with the following primer sets: K-RAS, 5’-TGT TCA CAA AGG TTT TGT CTC C-3’ (forward) and 5’-CCT TAT AAT AGT TTC CAT TGC CTT G-3’ (reverse); CLDN1, 5’-TTT ACT CCT ATG CCG GCG AC-3’ (forward) and 5’-GAG GAT GCC AAC CAC CAT CA-3’ (reverse); CLDN7, 5’-AGT TAG GAG CCT TGA TGC CG-3’ (forward) and 5’- GCA CAG GGA GTA GGA TAC GC-3’ (reverse); RAB25, 5’-CCA TCA CCT CGG CGT ACT AT-3’ (forward) and 5’- TTT GTT ACC CAC GAG CAT GA-3’ (reverse); β-actin, 5’-AGA GCT ACG AGC TGC CTG AC-3’ (forward) and 5’- AGC ATC GTG TTG GCG TAC AG-3’ (reverse). The β-actin gene was used as a control for calculation of dCt value. The qRT-PCR data were analyzed using the 2-(ddCt) method.

2.5. Immunoblotting

Immunoblotting was performed as previously described [18]. E-cadherin antibody (610182) was purchased from BD Biosciences (San Jose, CA, USA). Antibodies for Rab25 (4314), Snail (3879), Twist (46702), Ras (3965), p-EGFR (3777) and EGFR (2085) were obtained from Cell Signaling Inc. (Danvers, MA, USA). Antibodies for β-actin (47778) claudin-1 (sc-166338) and claudin-7 (sc-17670) were purchased from Santa Cruz Biotechnology (Texas, CA, USA). K-Ras antibody (ab180772) was purchased from Abcam (Cambridge, UK). All primary antibodies were used at 1:1000 dilutions. Secondary antibodies for anti-rabbit and anti-mouse were from Thermo Fisher Scientific (1:3000, Waltham, MA, USA), while an anti-goat secondary antibody was purchased from Santa Cruz Biotechnology (1:3000 dilutions).

2.6. Immunofluorescence

Immunofluorescence was performed as previously described [19]. Briefly, antibodies of E-cadherin (610182, 1:500; BD Bioscience), Snail (sc-271977, 1:500; Santa Cruz Biotechnology), and claudin-7 (ab207300, 1:500; Abcam) were used at a dilution of 1:500. The cells were reacted with Cy2-conjugated goat anti-mouse IgG (111-223-003, 1:500; Jackson ImmunoResearch, PA, USA) and Cy3-conjugated goat anti-rabbit IgG (111-156-003, 1:500; Jackson ImmunoResearch, PA, USA). The nuclei of the cells were stained with 4’, 6’-diamidino-2-phenylindole dihydrochloride (62249, 1:1000, Thermo Fisher Scientific, Waltham, MA, USA) at room temperature for 5 min. Fluorescence images were taken by confocal microscopy (LSM710; Carl Zeiss, Jena, Germany).

2.7. Wound healing assay

Wound healing assay was performed as previously reported [20]. The cells were cultured to confluence in 35 mm dish and transfected for 24 h. The cells were scraped with a micropipette tip. Photographic images were captured immediately after scraping and after 24 h, 36 h in the same locations. Wound closure rate was determined through the image J after 3 repeated experiments, and the average value was shown.
2.8. \textit{In vitro} migration and invasion assay

\textit{In vitro} migration assay was performed in triplicates using a 48-well chemotaxis chamber as described previously [17]. 1% FBS medium was added to the each well of the lower chamber, and type 1 collagen-coated 8 µm or 12 µm pore membranes (Neuro Probe, Gaithersburg, MD, USA) were added. The transfected cell suspension, the volume of $2 \times 10^6$ cells/well, was placed to each well of the upper chamber. After incubation for 6 h at 37°C, invaded cells were fixed and stained with Diff-Quik reagents (Dade Behring Inc., Newark, DE, USA). The average numbers of three random microscopic fields ($\times 200$) of invasion filters were counted in each experiment. \textit{In vitro} invasion assay was used in triplicates using 48-well chemotaxis chamber as previously [21]. 1% FBS medium was added to each well of the lower chamber, and Matrigel-coated 8 µm or 12 µm membrane was added. The transfected cell suspension, the volume of $2 \times 10^6$ cells/well, was placed to each well of the upper chamber. After incubation for 16 ~ 18 h at 37°C, invaded cells were fixed and stained with Diff-Quik reagents (Dade Behring Inc., Newark, DE, USA). The average numbers of three random microscopic fields ($\times 200$) of invasion filters were counted in each experiment.

2.9. Three-dimensional (3D) Matrigel invasion assay

3D Matrigel invasion assay was performed as described previously [22–25]. Cancer cells were labeled with Dil (Thermo fisher Scientific, Waltham, MA, USA). The mixture was prepared by mixing of 20% type I collagen (Nitta Gelatin Inc, Cell matrix Type I-P, Japan) and Matrigel (Corning, Acton, MA, USA) and solidify on the in 3 µm pore size transwell inserts (Corning, Acton, MA, USA). $5 \times 10^4$ cells were mixed in 200 µl of medium supplemented with 0.2% FBS and plated on the gels. The 24-well plate filled with culture medium or serum-free medium. After 3 ~ 5 days, the embedded gel was sectioned without fixation, and the cells were analyzed by a fluorescence confocal microscopy. In these images, the distance of invaded cells was measured from five different positions and calculated by the ZEN blue edition program of Carl Zeiss Microscopy GmbH. The distance in µm was calculated as described previously [26].

2.10. Three-dimensional (3D) Matrigel culture

3D cultures were observed as described previously [18]. Total $3 \times 10^4$ cells/ml cells were suspended in 400 µL medium supplemented with 2% Matrigel and seeded over a layer of the 100% Matrigel in 8-well culture slide (#345108; Corning, Acton, MA, USA). Cells were grown for 7 days and media was changed every 2 days. Cells were monitored every day and examined using a light microscope.

2.11. Measurement of RAS activation using ELISA assay

The supernatants were removed and determined RAS activation using a RAS activation ELISA assay kit (#17–497, Millipore, Temecula, CA, USA) according to the manufacturer's instructions. The level of activated RAS compared with the vector levels of activated RAS. The results represent triplicated experiments.

2.12. Ras-GTP pull down assay
The cell lysates were according to the manufacturer's recommendation (Cytoskeleton Inc., #BK008, Denver, Co, USA). The lysates were incubated with Raf-RBD beads for 1 h at 4°C, and then eluted with 2X sample buffer. Samples were resolved by SDS-PAGE. After that, procedures were same immunoblotting. Antibodies for Pan-Ras (3395, Cell Signaling Inc.), K-Ras (sc-30, Santa Cruz Biotechnology), GST (sc-138, Santa Cruz Biotechnology) were used at 1:1000 dilutions.

2.13. Statistical analyses

Data are shown as the means ± standard deviation (s.d.). Statistical analysis was assessed with SigmaPlot software (SYSTAT Software, San Jose, CA) using the Student's t-test. A P value less than 0.05 was reflected statistically significant (*P < 0.05; **P < 0.01; ***P < 0.001; ns; no statistically significant difference). Differences among three or more groups were estimated by analysis of variance followed by Bonferroni multiple comparison tests.

3. Results

3.1. Rab25 suppresses colon cancer cell invasion

In order to determine the role of Rab25 in colon cancer cell invasion, we transfected the cells with a vector of control or Rab25 in colon cancer Caco-2 and HCT-116 cells and observed that the ectopic expression of Rab25 (Supplementary Fig. S1) significantly attenuates the invasiveness (Fig. 1a) and the wound closure rates (Fig. 1b) of these colon cancer cells. Consistently, Rab25 markedly reduced HCT-116 cancer cell invasion (Fig. 1c) and spike formation (Fig. 1d) in 3D Matrigel system. To confirm the Rab25-induced inhibition of colon cancer cell invasion, we transfected the cells with Rab25 siRNA and noted that silencing of Rab25 (Supplementary figure S2) significantly induces colon cancer cell invasion (Fig. 1e). Therefore, these data clearly indicate that Rab25 suppresses colon cancer cell invasion and migration.

3.2. Rab25 inhibits colon cancer cell EMT

Given that an EMT transcription factor, Snail, is closely associated with Rab25-induced various cancer cell invasiveness [16], we next determined whether Rab25 regulates the expression of EMT factors. Immunoblotting data showed that Rab25 upregulates E-cadherin expression, while Snail expression was reduced by Rab25 (Fig. 2a). Consistently, immunofluorescence results demonstrated the upregulation of E-cadherin and downregulation of Snail expression by Rab25 in colon cancer cells (Fig. 2b). Furthermore, ectopic expression of Snail recovered colon cancer cell invasion repressed by Rab25 (Fig. 2c). Therefore, these data imply that Rab25 inhibits colon cancer cell EMT through downregulation of Snail expression, leading to suppression of colon cancer cell invasion.

3.3. Rab25 inactivates EGFR

Previous data show that EGFR plays a critical upstream governor of Snail in Rab25-induced cancer cell invasion [16]. In addition, EGFR has been closely associated with Rab25-induced cancer progression [16, 27]. Therefore, we analyzed the involvement of EGFR in Rab25-induced suppression of colon cancer cell
invasion. Intriguingly, Rab25 profoundly reduced the levels of p-EGFR expression (Fig. 3a and 3b), suggesting that Rab25 inactivates EGFR and subsequent Snail expression for colon cancer cell invasion. Furthermore, we also observed that Rab25 reduces Pan-Ras and K-Ras activities in colon cancer cells (Fig. 3c and 3d). Moreover, ectopic expression of constitutively active KRAS (KRAS-V12) rescued Rab25-induced HCT-116 cell invasion (Fig. 3e), consolidate the involvement of Ras in Rab25-induced suppression of colon cancer cell invasion. Overall, these data indicate that Rab25 inhibits the EGFR/Ras/Snail signaling cascade to attenuate colon cancer cell invasion.

3.4. Rab25 induces claudin-7 expression

Given that claudin-7 induces Rab25 expression and suppresses colon cancer cell growth in a Rab25-dependent manner [15], we questioned in the opposite direction; whether claudin-7 is implicated in Rab25-induced colon cancer cell invasion. Notably, Rab25 markedly increased claudin-7 expression (Fig. 4a and b). However, we could not observe that Rab25 induces claudin-7 transcript (Fig. 4c). Instead, Rab25 maintained the level of claudin-7 expression in the presence of CHX (Fig. 4d). Furthermore, treatment of the cells with pharmacological inhibitors of proteasome (MG-132, Fig. 4e) and lysosome (CQ, Fig. 4f) increased Rab25-induced claudin-7 expression. Collectively, these results suggest that Rab25 induces claudin-7 expression through stabilizing its protein.

3.5. Claudin-7 mediates Rab25-induced suppression of colon cancer cell invasion

We next explore the role of claudin-7 in Rab25-induced colon cancer cell invasion. Ectopic expression of claudin-7 reduced the expression of p-EGFR and Snail (Fig. 5a). In addition, claudin-7 upregulated E-cadherin expression (Supplementary Fig. S3) and attenuated colon cancer cell invasion, which was reversed by enforced expression of Snail (Fig. 5b). Consistently, silencing of claudin-7 expression increased colon cancer cell invasion (Fig. 5c) and migration (Fig. 5d). Furthermore, claudin-7 siRNA recovered Rab25-induced EGFR inactivation and suppression of Snail expression (Fig. 5e). In addition, we observed that claudin-7 significantly reduces Pan-Ras and K-Ras activities in colon cancer cells (Fig. 5f) and that ectopic expression of constitutively active KRAS (KRAS-V12) rescued claudin-7-induced suppression of HCT-116 cell invasion (Fig. 5g). Therefore, these data imply that claudin-7 mediates Rab25-induced attenuation of colon cancer cell invasion.

4. Discussion

Accumulating evidence suggests the context-dependent role of Rab25 in cancer cell progression. We previously showed that Rab25 aggravates cancer cell invasion through the β1 integrin/EGFR/Snail signaling axis [16]. In the present study, we demonstrate that Rab25 suppresses colon cancer cell invasion via upregulating claudin-7 expression. Both Rab25 and claudin-7 inhibit EGFR activation and EMT. In addition, these proteins attenuate colon cancer cell invasion. Importantly, our data show that Rab25 induces claudin-7 expression via protein stabilization, uncovering the critical role of these proteins in the regulation of colon cancer cell invasion.
Rab25 was demonstrated to induce cancer cell invasion through upregulating β1 integrin and subsequent activation of the EGFR/VEGF/Snail signaling axis in ovarian, stomach and estrogen receptor-positive breast cancer [16]. However, little has been known how Rab25 suppresses invasiveness of various cancer cells including colon cancer except hypothesizing the role of upregulated β1 integrin in Rab25-induced alleviation of colon cancer cell invasiveness [7, 28–30]. In the present study, we identify the critical role of claudin-7 in Rab25-induced suppression of colon cancer cell invasion. First, Rab25 induces claudin-7 expression through protein stabilization. Second, Rab25 inactivates EGFR in a claudin-7-dependent manner. Silencing of claudin-7 rescued Rab25-induced EGFR inactivation. In addition, Rab25 downregulated Snail expression which is important for colon cancer cell EMT and invasiveness. Furthermore, claudin-7 by itself reduced Snail expression and consequently attenuated colon cancer cell invasion.

Claudin-7 is one of family of tight junction proteins and implicated in suppression of proliferation and invasion of various cancers including colon cancer [14, 15, 31]. Congruently, claudin-7 was shown to induce Rab25 expression to suppress colon cancer cell tumorigenesis and invasion [15]. In the present study, we observed that Rab25 in turn increases claudin-7 protein expression. Although claudin-7 was shown to induce Rab25 expression through transcriptional activation [15], our present data demonstrate that Rab25 increases claudin-7 protein expression without any effect on mRNA expression. At present, we do not know how Rab25 protects claudin-7 protein from proteosomal degradation. Since our present data show that CQ maintains the level of claudin-7 protein (Fig. 4f), Rab25 might recycle claudin-7 in the late endosome to the plasma membrane, preventing its lysosomal degradation, similar to what occurs with β1 integrin. Interestingly, previous papers suggest that claudin-7 forms a protein complex with β1 integrin in lung cancer cells [14], reinforce the hypothesis that Rab25 protects both β1 integrin and claudin-7 from lysosomal degradation through recycling endosomes to plasma membrane and subsequent inactivation of EGFR/Snail axis for colon cancer cell invasion. Further detailed mechanistic study is currently underway. To illustrate our results schematically, we provide model (Fig. 6) in which Rab25 salvages claudin-7 from lysosomal degradation and thereby inhibits the EGFR/Ras/Snail signaling axis to attenuate colon cancer cell invasion. Collectively, our present study demonstrates a novel role of claudin-7 in Rab25-induced suppression of colon cancer cell invasion through inactivating EGFR/Snail axis, providing crucial biomarkers and prospective therapeutic armament for colon cancer.

Declarations

Supporting Information The online version contains supplementary material available at [website].

Author Contributions Conception and design of the study: Hoi Young Lee, Chang Gyo Park; Data curation: Su Jin Cho, Bo Young Jeong; Formal analysis: Su Jin Cho, Investigation: Su Jin Cho, Bo Young Jeong, Methodology: Su Jin Cho, Bo Young Jeong; Supervision: Hoi Young Lee, Chang Gyo Park; Writing original draft: Hoi Young Lee; Writing editing: Hoi Young Lee, Su Jin Cho. All authors discussed the results and commented on the manuscript.
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Data Availability The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests The authors declare no competing interests.

References


Rab25 suppresses colon cancer cell invasion. **a-d** Cancer cells were transfected with indicated vectors for 24 h. **a** Invasion was analyzed by utilizing a 48-well chemotaxis chamber (**P < 0.01 versus control vector, ***P < 0.001 versus control vector). **b** Wound healing analysis for detecting cell migration (*P <
0.05 versus control vector). c 3D Matrigel invasion analysis was performed 3D Matrigel-coated transwell chambers for 5 days. The distance between the invaded HCT-116 cells was measured in five different positions and then calculated (**P < 0.001 versus control vector). d 3D Matrigel culture was analyzed counting spike of transfected HCT-116 cells with indicated vectors on Matrigel for 7 days (**P < 0.01 versus control vector). e Cancer cells were transfected with indicated vectors or siRNAs for 24 h, and invasion was analyzed by utilizing a 48-well chemotaxis chamber (**P < 0.01 versus scrambled siRNA, ***P < 0.001 versus control vector). Representative results of at least three independent experiments with similar results.

Figure 2

a

b

C
Rab25 inhibits EMT. a-b Cancer cells were transfected with indicated vectors for 24 h. a The cell lysates were analyzed by immunoblotting with indicated antibodies. b The expressions of E-cadherin and Snail were visualized by immunofluorescence. Original magnification, ×400; scale bar, 20 μm. c HCT-116 cells were co-transfected with indicated vectors for 24 h. Invasion was analyzed by utilizing a 48-well chemotaxis chamber (**P < 0.001 versus control vector and ###P < 0.001 versus Rab25 transfection only). The cell lysates were analyzed by immunoblotting with indicated antibodies. Representative results of at least three independent experiments with similar results.

Figure 3
Rab25 inactivates EGFR. a-d Cancer cells were transfected with indicated vectors for 24 h. a The cell lysates were analyzed by immunoblotting with indicated antibodies. b The expression of p-EGFR was visualized by immunofluorescence. Original magnification, ×400; scale bar, 20 µm. c Ras activity was analyzed by Ras-GTP pull down assay. Total protein expressions were analyzed by immunoblotting with indicated antibodies. d Ras activity was analyzed by Ras activation ELISA kit (***P < 0.001 versus control vector). e HCT-116 cells were co-transfected with indicated vectors for 24 h. Invasion was analyzed by utilizing a 48-well chemotaxis chamber (**P < 0.01 versus control vector, ***P < 0.001 versus control vector and ####P < 0.001 versus Rab25 transfection only). Representative results of at least three independent experiments with similar results.
Figure 4

Rab25 induces claudin-7 expression. **a-c** Cancer cells were transfected with indicated vectors for 24 h. **a** The cell lysates were analyzed by immunoblotting with indicated antibodies. **b** The expression of claudin-7 was visualized by immunofluorescence. Original magnification, ×400; scale bar, 20 μm. **c** qRT-PCR. **d** HCT-116 cells were transfected with indicated vector for 24 h, serum-starved and treated with CHX for 6 h. The cell lysates were analyzed by immunoblotting with indicated antibodies (*P < 0.05 versus control).
vector). 

HCT-116 cells were transfected with indicated vector for 24 h, serum-starved and treated with MG132 for 6 h. The cell lysates were analyzed by immunoblotting with indicated antibodies (*$P < 0.05$ versus control vector, #$P < 0.05$ versus Rab25 transfection only).

HCT-116 cells were transfected with indicated vector, serum-starved and treated with CQ with 12 h. The cell lysates were analyzed by immunoblotting with indicated antibodies. Representative results of at least three independent experiments with similar results.

**Figure 5**

**a**

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**Vector**

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- + - + -

**Claudin-7**

- + + + +
- + + + +

**b**

Field change in cell invasion normalized to vector

**Vector**

- + - + -
- + - + -

**Claudin-7**

- + + + +
- + + + +

**Snail**

- + - + -
- + - + -

**Claudin-7**

- + + + +
- + + + +

**β-actin**

- + + + +
- + + + +

**c**

Field change in cell invasion normalized to vector

**Scrambled siRNA**

- + - + -
- + - + -

**Claudin-7 siRNA**

- - + + +
- - + + +

**d**

Field change in cell invasion normalized to vector

**Scrambled siRNA**

- + - + -
- + - + -

**Claudin-7 siRNA**

- - + + +
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**e**

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**Vector**

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**Claudin-7**

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**Rab25**

- + - + -
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**Claudin-7**

- + + + +
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**β-actin**

- + + + +
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**f**

**Vector**

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- + - + -

**Claudin-7**

- + + + +
- + + + +

**Pan-Ras**

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**K-Ras**

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**GST**

- + - + -
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**Ras**

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- + - + -

**Rab25**

- + - + -
- + - + -

**β-actin**

- + + + +
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**g**

Field change in cell invasion normalized to vector

**Vector**

- + - + -
- + - + -

**Claudin-7**

- + - + -
- + - + -

**HRAS-V12**

- + - + -
- + - + -
Figure 5

Claudin-7 mediates Rab-25-suppressed cancer cell invasiveness. **a** Cancer cells were transfected with indicated vectors for 24 h. The cell lysates were analyzed by immunoblotting with indicated antibodies. **b** HCT-116 cells were co-transfected with indicated vectors for 24 h. Invasion was analyzed by utilizing a 48-well chemotaxis chamber (*P* < 0.05 versus control vector and ###*P* < 0.001 versus claudin-7 transfection only). The cell lysates were analyzed by immunoblotting with indicated antibodies. **c-d** The cancer cells were co-transfected with indicated vector and siRNA for 24 h. Invasion and migration were analyzed by utilizing a 48-well chemotaxis chamber (*P* < 0.05 versus control vector, **P* < 0.01 versus control vector, #*P* < 0.05 versus Rab25 transfection only, ###*P* < 0.01 versus Rab25 transfection only and ####*P* < 0.001 versus Rab25 transfection only). **e** HCT-116 cells were transfected with indicated vector and siRNA for 24 h. The cell lysates were analyzed by immunoblotting with indicated antibodies. **f** Ras activity was analyzed by Ras-GTP pull down assay. Total protein expressions were analyzed by immunoblotting with indicated antibodies. **g** HCT-116 cells were co-transfected with indicated vectors for 24 h. Invasion was analyzed by utilizing a 48-well chemotaxis chamber (***P* < 0.01 versus control vector, ####*P* < 0.001 versus claudin-7 transfection only). Representative results of at least three independent experiments with similar results.

Figure 6

A model demonstrating the critical role of claudin-7 in Rab25 induced suppression of colon cancer cell invasion and EMT.
Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- SupplementaryInformation.docx