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YAP/TAZ activation, induced by disruption of E-cadherin-mediated cell-to-cell contact, promotes the cadherin switch by facilitating nuclear translocation of Slug in human oral squamous cell carcinoma HSC-4 cells

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Abstract : Epithelial-to-mesenchymal transition (EMT) is closely related to the development of malignancy in human oral squamous cell carcinoma (hOSCC) cells. “Cadherin switch” refers to expression changes from E-cadherin to N-cadherin that occur during the malignant transformation of a cancer cell accompanying the EMT. The molecular mechanisms underlying the cadherin switch in hOSCC have not yet been fully elucidated. The Hippo pathway is known to transmit extracellular mechanical cues induced by changes in cell density, which in turn affect gene expression. Here, we found that the cadherin switch is induced at a low but not high cell density in the hOSCC cell line HSC-4. We also found that knockdown of YAP/TAZ, which is part of the Hippo pathway, increased E-cadherin expression but decreased N-cadherin expression in HSC-4 cells. The nuclear translocation of EMT-related transcription factor Slug was observed in HSC-4 cells at low cell density. In addition, low cell density-induced Slug nuclear translocation was inhibited by knockdown of YAP. Moreover, disruption of cell-to-cell adhesion with anti E-cadherin antibody promoted nuclear translocation of YAP, resulting in the cadherin switch in HSC-4 cells. These results suggested that YAP/TAZ mediates the low cell density-induced cadherin switch through nuclear translocation of Slug and that intercellular contact mediated by E-cadherin inhibits the cadherin switch through negative modulation of the Hippo pathway. Thus, disruption of E-cadherin-

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mediated cell-to-cell contact-induced YAP/TAZ activation promotes cadherin switch through promotion of EMT-related transcription factor Slug nuclear translocation in hOSCC HSC-4 cells.

Keywords: cadherin switch; Hippo pathway; YAP/TAZ; Slug; E-cadherin

Epithelial-to-mesenchymal transition (EMT) is closely related to the development of malignancy in human oral squamous cell carcinoma (hOSCC) cells¹⁾. It is known that advancement of EMT increases the invasive and metastatic potential of hOSCC cells, while mesenchymal-to-epithelial transition (MET) decreases the invasive and metastatic potential of hOSCC cells²⁾. Therefore, balance between EMT and MET in hOSCC cells modulates the status of cancer invasion and metastasis. Elucidation of the molecular mechanisms underlying the switch between EMT and MET, which could help the establishment of novel therapeutic methods to control hOSCC invasion and metastasis, has not been fully accomplished yet.

Cadherin switch, the change in gene expression from E-cadherin to N-cadherin accompanying EMT, is known to be important for the malignant transformation of cancer cells^{3), 4)} and has also been reported in the clinical analysis of the cancer⁵⁾. In addition, it has been reported that the cadherin switch is induced by Slug, an EMT-related transcription factor^{6), 7)}. EMT-related transcription factors play important roles in the promotion of EMT in various kinds of SCC cells: Snail, Slug, Twist and ZEB promote expression of mesenchymal marker N-cadherin but suppress E-cadherin, resulting in EMT progression⁸⁾. We previously reported that transforming growth factor- β 1 (TGF- β 1) suppresses the expression of E-cadherin in hOSCC cell line HSC-4, resulting in the promotion of EMT in this cell

line^{9), 10)}. TGF- β 1-induced expression of Slug did not affect the expression of N-cadherin in HSC-4 cells. It is unknown whether other EMT-related transcription factors lead to upregulation of N-cadherin in hOSCCs.

The Hippo pathway is known to include molecular mechanisms that relay extracellular signals stimulated by changes in cell density which then affect gene expression within the cells¹¹⁾. In the Hippo pathway, transcriptional coactivator with PDZ-binding motif (TAZ) binds to phosphorylated Smad2/3-Smad4 complex in the cytoplasm, which promotes the nuclear translocation of the TAZ-Smad2/3-Smad4 complex¹²⁾. Yes-associated protein (YAP) and TAZ interacted with TEAD or Runx2 to upregulate their transcriptional activity^{13), 14)}. YAP/TAZ regulate cell proliferation and cell differentiation^{14), 15)}. The Hippo pathway positively regulates the progression of EMT in breast cancer cells¹⁶⁾. Several molecules of the cell membrane act by sensing extracellular mechanical stress, i.e., Fat1¹⁷⁾, Crumbs complex¹⁸⁾, Scribble complex¹⁶⁾, or E-cadherin¹⁹⁾, and relay signals to the Hippo pathway. However, it remains to be clarified whether the Hippo pathway positively regulates the progression of EMT in hOSCC cells.

Here, we examined how the status of cell-to-cell contact affects the activity of extracellular mechanical stress transmitters YAP and TAZ in the Hippo pathway in HSC-4 cells. In addition, we evaluated how YAP and TAZ affect the activity of EMT-related transcription

factor Slug. Thus, we aimed to investigate whether YAP and TAZ play important roles in the promotion of EMT in HSC-4 cells.

Materials and Methods

Materials

hOSCC HSC-4 cell lines were obtained from the Human Science Resource Cell Bank (Osaka, Japan). hOSCC LMF-4 cell line was gifted by Dr. F. Momose of Tokyo Medical and Dental University²⁰⁾. Protease inhibitor cocktail for use in mammalian cell and tissue extracts and phosphatase inhibitor cocktail 1 and 2 were purchased from Merck (St. Louis, MO, USA). All other reagents were analytical grade.

Cell culture

All cell lines were grown at 37°C and 5% CO₂. Human HSC-4 squamous cell carcinoma cells were cultured in Eagle's minimum essential medium (MEM, Merck) supplemented with 10% fetal bovine serum (FBS, Thermo Fisher Scientific, Rockville, MD, USA). LMF-4 cells were cultured in DMEM (Thermo Fisher Scientific) containing 10% FBS.

Suppression of gene expression by small interfering RNAs (siRNAs)

Human siRNAs of Slug (MISSION siRNA, Hs_SNAIS_9785, Merck), YAP siRNA (MISSION esiRNA, HU-11302-1, Merck), and TAZ siRNA (MISSION esiRNA, HU-08032-1, Merck) were used for gene knockdown analysis. Cells were seeded at a density of 1×10^5 cells per well in 24 well-plates and transfected with 10 nM siRNA using Lipofectamine RNAiMAX (Life Technologies, Carlsbad, CA) according to the manufacturer's instructions. Forty-eight hours after transfection, cells were used for RT-qPCR analysis of gene expressions or

immunofluorescence analysis, as described below. siControl (MISSION[®] siRNA Universal Negative Control #1, SIC-001, Merck), which does not possess significant homology to vertebrate gene sequences, was used as a negative control. Suppression of gene expression by siRNA was evaluated by RT-qPCR for the target genes.

Quantitative real time RT-PCR (RT-qPCR)

For total RNA preparation, 1.0 or 5.0×10^5 cells were cultured in 12-well dishes. Total RNA was isolated using the ISOGEN reagent (Nippon Gene, Toyama, Japan) according to manufacturer's instructions. We reverse transcribed total RNA to first-strand cDNA using the poly-A primer provided in the RT-PCR System Kit (Takara Bio Inc., Shiga, Japan). RT-qPCR was performed on a Thermal Cycler Dice Real Time System (Takara Bio) using SYBR Premix Ex Taq II (Takara Bio) with gene-specific primers (listed in Table I). The mRNA expression levels of target genes were normalized to that of the endogenous reference gene β -actin or GAPDH and expressed as fold increase or decrease relative to levels in the control sample²¹⁾.

Immunofluorescence analysis of cultured cells

For immunofluorescence analysis of cultured cells, cells were sub-cultured in individual wells on 8-chamber slides at a density of 1.0 or 5.0×10^4 cells/well (BD Biosciences, NJ, USA) and maintained in MEM or DMEM supplemented with 10% FBS for 2 days. Cells were then fixed in 4% paraformaldehyde for 30 min and permeabilized with 0.2% Triton X-100 in PBS. After blocking with 2% (w/v)

Table I. Sequence of primers for RT-qPCR

Target mRNA	Oligonucleotide sequence (5' - 3')	Predict size (bp)
E-Cadherin	(F) TACTACTGCCAGGAGCCAGA	103
	(R) TGGCACCAGTGTCCGGATTA	
N-Cadherin	(F) CGAATGGATGAAAGACCCATCC	171
	(R) GCCACTGCCTTCATAGTCAAACACT	
Slug	(F) TGTTGCAGTGAGGGCAAGAA	158
	(R) GACCCTGGTTGCTTCAAGGA	
YAP	(F) GGGTGGCAATATTCAGTGCTTAAC	122
	(R) GTTATGATAGGCACACCCACAATTC	
TAZ	(F) GCTGAGCGTACAGGCAGACA	95
	(R) AGGAACATAAACCATGGGTCCTT	
GAPDH	(F) GCACCGTCAAGGCTGAGAAC	138
	(R) TGGTGAAGACGCCAGTGGA	
β -Actin	(F) GGAGATTACTGCCCTGGCTCCTA	89
	(R) GACTCATCGTACTCCTGCTTGCTG	

bovine serum albumin in PBS, cells were labelled with mouse anti-E-cadherin (1:200, #610181, BD Biosciences), mouse anti-N-cadherin (1:200, #610920, BD Biosciences), mouse anti-Slug (1:200, sc-166476, Santa Cruz Biotechnology, Santa Cruz, CA, USA), rabbit anti-YAP (1:200, #4912 Cell Signaling Technology, Beverly, MA, USA), or mouse anti-TAZ (1:300, #560235, BD Biosciences) antibody and incubated at room temperature for 1 h and then at 4° C overnight. After being washed with 0.2% Triton X-100 in PBS to remove excess primary antibody, cells were incubated with Alexa Fluor® 488 or 568-conjugated goat anti-mouse IgG (1:1000, Molecular Probes, Thermo Fisher Scientific), or with Alexa Fluor® 488 or 568-conjugated goat anti-rabbit IgG (1:1000, Thermo Fisher Scientific) for anti-Slug antibody. Cells were incubated in this mixture for 60 min at room temperature. After washing with 0.2% Triton

X-100 in PBS to remove excess secondary antibody, cells were stained with DAPI (1:500, KPL, Gaithersburg, MD, USA). The slides were washed, and a fluorescent signal was detected by confocal microscopy (C1 si, Nikon, Tokyo, Japan) (Fig. 1A) or fluorescence microscope (IX70, Olympus, Tokyo, Japan).

Calcium Depletion Experiment

HSC-4 cells (1.0 or 5.0×10^4 cells/well in 8-chamber slide) were cultured in MEM without FBS medium, which was then changed to either DMEM without FBS (control) or to SMEM, containing Ca^{2+} and Mg^{2+} free minimal essential medium, without FBS and containing $30 \mu\text{M}$ CaCl_2 (Ca^{2+} Depletion), and then incubated for 4 h. Cells were then fixed for immunofluorescence analysis accordingly as described by Varelas, X. *et al.*¹⁸⁾.

Contact inhibition assay

Briefly, 1×10^5 cells in 12-well dishes were treated with 10 $\mu\text{g/ml}$ anti-human E-cadherin mouse antibody (Clone SHE78-7, Takara Bio Inc., Shiga, Japan) or normal mouse IgG (sc-2025, Santa Cruz) for 24 h under serum-free conditions. Antibody-treated HSC-4 cells were then stained with anti-YAP antibody and then analyzed by a fluorescence microscope. Expression levels of E- and N-cadherin mRNAs were examined by RT-qPCR analysis, as

described above.

Statistical analysis

All experiments were performed at least 3 times independently. Results are expressed as the mean \pm standard deviation (SD). Differences between high- and low-cell density groups were analyzed using the unpaired Student t-test, while differences among multiple samples were analyzed using the Tukey's multiple comparison

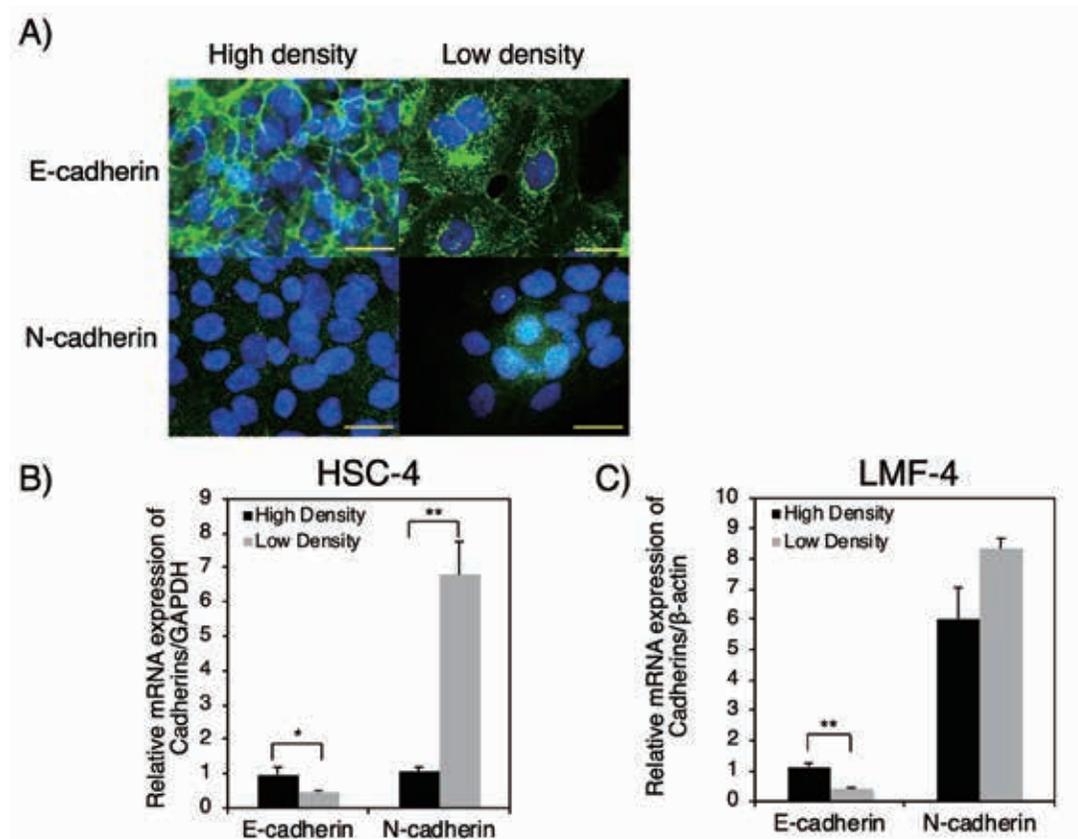


Figure. 1 : Expression levels of cadherins are regulated by cell density status in HSC-4 culture

(A) The localization of E- and N-cadherin was determined using confocal microscopy. Cells were cultured at high or low cell density for 48 h. Cells were then immunostained with the indicated antibodies as well as DAPI to detect nuclei and observed by confocal microscopy, as described in Materials and Methods. Scale bars represent 25 μm . mRNA expression levels of cadherins in HSC-4 cells (B) or LMF-4 cells (C) cultured at low or high density for 48 h were analyzed by using RT-qPCR. Primers for RT-qPCR are indicated in Table I. The expression levels of E-cadherin (black bars) and N-cadherin (gray bars) were examined. Values have been normalized to β -actin mRNA levels. Data are the mean \pm SD of triplicate experiments (* $P < 0.05$; ** $P < 0.01$).

test with IBM SPSS Statistics 24 software. For all statistical analyses, P value <0.05 was considered to be statistically significant, and all tests were two-tailed.

Results

Expression levels of E-cadherin and N-cadherin are reciprocally regulated by the status of HSC-4 cell density

We examined whether the expression of E- and N-cadherin were affected by hOSCC cell

density. E-cadherin was strongly expressed and localized around the cell membrane in HSC-4 cells at a high cell density (5.0×10^4 cells/well in 8-chamber slide) (Fig. 1A, upper panels). In contrast, E-cadherin was detected as punctate dots, and its localization was frequently observed in the cytoplasmic region in HSC-4 cells at low cell density (1.0×10^4 cells/well in 8-chamber slide). Furthermore, N-cadherin expression was not observed in HSC-4 cells at a high cell density (Fig. 1A, left

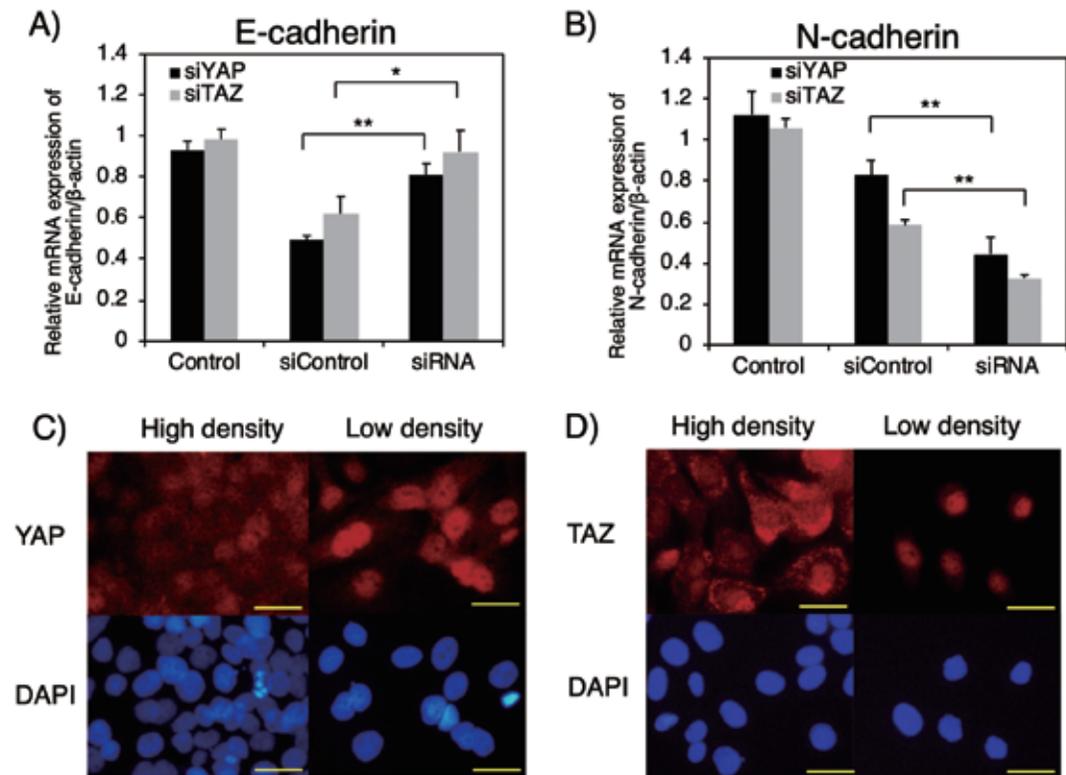


Figure. 2 : YAP/TAZ positively regulates cadherin switch in HSC-4 cells

(A and B) The cells were transfected with YAP or TAZ siRNA (siYAP or siTAZ) or negative control siRNA (siControl) as described in the Materials and Methods section. E-cadherin (A) and N-cadherin (B) expression were detected by RT-qPCR analysis in HSC-4 cells. Values have been normalized to β -actin mRNA levels. Data are expressed as the mean \pm SD of triplicate experiments ($*P < 0.05$; $**P < 0.01$). The localization of YAP (C) and TAZ (D) in HSC-4 cells at low and high cell density was evaluated by fluorescence immunostaining. Cells were immunostained with anti-YAP (C) or anti-TAZ (D) antibodies and with DAPI to detect nuclei. Scale bars represent 25 μ m.

lower panel), but a slight expression of N-cadherin was observed at low cell density (Fig. 1A, right lower panel). Next, the expression levels of E- and N-cadherin in HSC-4 cells were analyzed by RT-qPCR. In HSC-4 cells, a low cell density culture induced N-cadherin expression 6.5 times higher than in a high cell density culture (Fig. 1B, right graph). In contrast, a low cell density culture reduced expression of E-cadherin by 2 times compared to the high cell density culture in HSC-4 cells (Fig. 1B, left graph). Thus, a low cell density in HSC-4 cell culture induced the cadherin switch. A low cell density culture induced a 2.5 times lower E-cadherin expression than a high cell density culture in the other hOSCC cell line LMF-4 than in HSC-4 cells (Fig. 1C, left graph). In addition, a low cell density culture increased N-cadherin expression, although statistical significance was not reached (Fig. 1C, right graph). Moreover, western blot analysis revealed that the protein expression levels of E-cadherin at low cell density were lower than that at a high cell density in HSC-4 cells (data not shown). In addition, while protein expression levels of N-cadherin were not detectable by Western blot at high cell density in HSC-4 cells, N-cadherin protein expression levels were detectable at low cell density, (data not shown). These results indicate that the expression of E- and N-cadherin are reciprocally regulated by the levels of cell density in hOSCC cells.

Hippo signaling targets YAP/TAZ regulate the cadherin switch in HSC-4 cells

We examined whether the expression levels of E- and N-cadherin were affected by gene knockdown of YAP/TAZ, respectively. RT-qPCR analysis revealed that both siYAP and

siTAZ led to upregulation of E-cadherin expression and downregulation of N-cadherin expression in HSC-4 cells (Fig. 2A and B). In addition, nuclear translocation of YAP/TAZ from the cytoplasm was observed at low cell density in HSC-4 cells (Fig. 2C and D).

Nuclear translocation of EMT-related transcription factor Slug is positively regulated by the Hippo signaling target YAP

We examined the effect of EMT-related transcription factor Slug, known to be involved in EMT, on the cadherin switch. RT-qPCR analysis revealed that Slug siRNA led to upregulation of E-cadherin (Fig. 3A). In addition, while Slug was observed to localize to the nucleus of HSC-4 cells at low cell density, it localized to the cytoplasmic region at high cell density (Fig. 3B). However, expression levels of Slug were not influenced by high-, and low-cell densities in HSC-4 cells (Fig. 3C). In addition, administration of YAP siRNA inhibited the nuclear localization of Slug (Fig. 3D). These data indicate that nuclear translocation of EMT-related transcription factor Slug is positively regulated by the Hippo signaling target YAP, resulting in Slug-mediated suppression of E-cadherin expression.

Elucidating factors upstream of Hippo signaling which regulate YAP/TAZ in HSC-4 cells

We aimed to elucidate the upstream factors that regulate Hippo signaling targets YAP/TAZ which induce a Slug-mediated cadherin switch. Firstly, we examined the contribution of the Ca²⁺-dependent adhesion protein(s) at the cell surface by using a Ca²⁺-depletion treatment of the culture media¹⁸⁾. The rationale for this approach is that various

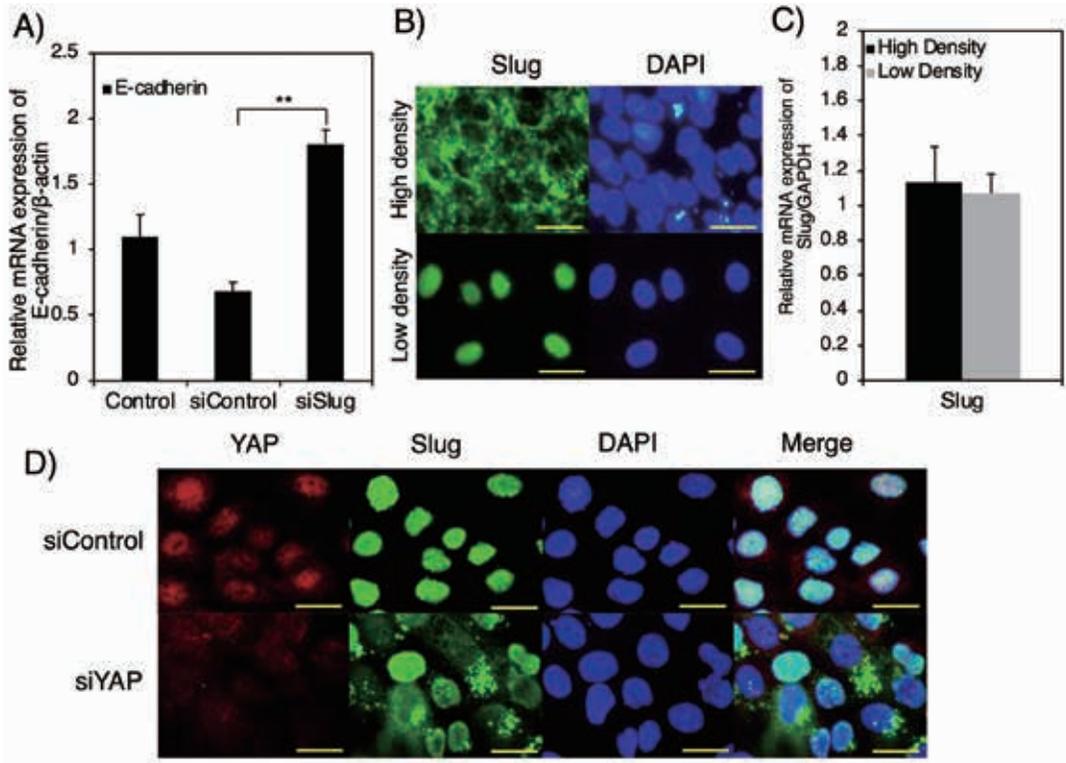


Figure. 3 : Nuclear translocation of Slug is positively regulated by YAP

(A) Expression levels of E-cadherin in HSC-4 cells with Slug knockdown were examined. Cells were transfected with Slug siRNA (siSlug) or negative control siRNA (siControl), as described in the Materials and Methods section. The expression of Slug mRNA was examined by RT-qPCR. Primers for RT-qPCR are indicated in Table I. The values have been normalized to β -actin mRNA levels. Data are expressed as mean \pm SD of triplicate experiments (** $P < 0.01$). (B) The localization of Slug was observed using fluorescent microscopy. Cells were immunostained with anti-Slug antibody, and stained with DAPI to detect nuclei. (C) The mRNA expression levels of Slug in HSC-4 cells cultured at low or high cell density were analyzed using RT-qPCR. Values have been normalized to GAPDH mRNA levels. Data are expressed as the mean \pm SD of triplicate experiments. Scale bars represent 25 μ m. (D) Localization of E- and N-cadherin was observed using fluorescent microscopy. Cells were transfected with YAP siRNA (si-YAP) or negative control siRNA (siControl) for 48 h, and then immunostained with the anti-YAP (red) and anti-Slug (green) antibodies, and DAPI (blue). Scale bars represent 25 μ m.

types of cell-to-cell adhesive molecules, including E-cadherin, need Ca^{2+} for their adhesive functions²⁰. The nuclear localization of YAP was observed following Ca^{2+} -depletion treatment (30 μ M) but not observed under non- Ca^{2+} -depleted culture conditions (1,800 μ M) in HSC-4 cells (Fig. 4A). Then, we

examined how the disruption of E-cadherin-mediated cell-to-cell adhesion affects nuclear localization of YAP. As shown in Fig. 4B, the E-cadherin neutralizing antibody inhibited the nuclear localization of YAP in HSC-4 cells and also induced the cadherin switch as opposed to control antibody in HSC-4 cells (Fig. 4C). We

also found that the degree of cadherin switch induced by the neutralizing antibody against E-cadherin was weaker at high cell density than at low cell density in HSC-4 cells (data not shown). These results indicate that the cadherin switch is induced by disruption of intercellular bonds between E-cadherins in a Hippo pathway-dependent manner.

Discussion

Our findings show that a low cell density culture increases expression of N-cadherin while decreasing expression of E-cadherin, whereas a high cell density culture decreases expression of N-cadherin and increases expression of E-cadherin in hOSCC cells (Fig. 1). LMF-4 cells have higher invasive and metastatic characteristics than HSC-4 cells²⁰. Expression levels of E-cadherin at high cell density were higher than that at low cell

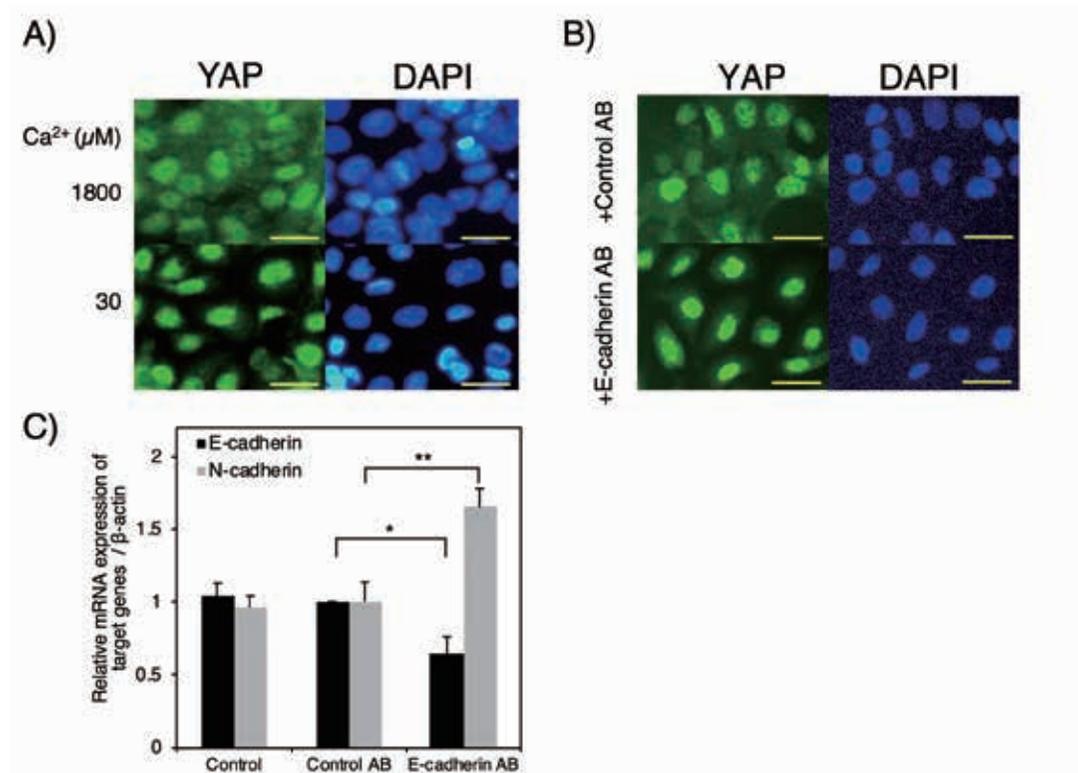


Figure. 4 : Intercellular contact mediated by E-cadherin inhibits the cadherin switch through negative modulation of Hippo pathway

(A) HSC-4 cells were cultured in low Ca²⁺ (30 μM) medium in SMEM or in standard Ca²⁺ concentration (1,800 μM) in DMEM for 4 h. Cells were immunostained with anti-YAP antibody, and with DAPI to detect nuclei. The localization of Slug was observed using fluorescent microscopy. Scale bars represent 25 μm. (B and C) HSC-4 cells were incubated with an anti-human E-cadherin neutralizing antibody (Clone SHE78-7) (E-cadherin AB) or with control IgG (Control AB) for 24 h under serum-free conditions. (B) The localization of YAP in HSC-4 cells treated with E-cadherin AB was observed using fluorescent microscopy. Cells were immunostained with an anti-YAP antibody, and stained with DAPI to detect nuclei. Scale bars represent 25 μm. (C) The expression levels of E-cadherin (black bars) and N-cadherin (gray bars) were examined by RT-qPCR. Values have been normalized to the β-actin mRNA levels. Data are expressed as the mean ± SD of triplicate experiments (**P* < 0.05; ***P* < 0.01).

density in LMF-4 cells; however, expression levels of N-cadherin at low cell density were almost the same as those at high cell density (Fig. 1C), suggesting that N-cadherin expression might not be affected by the status of cell density in hOSCC cells with highly invasive and metastatic characters.

siYAP and siTAZ upregulated E-cadherin expression but downregulated N-cadherin expression in HSC-4 cells (Fig. 2A and B). In addition, the nuclear translocation of YAP/TAZ from the cytoplasmic region was observed at low cell density in HSC-4 cells (Fig. 2C and D). Taken together, these results strongly suggest that Hippo signaling targets YAP/TAZ play an important role in the low cell density-induced cadherin switch in HSC-4 cells.

Slug is an important EMT-related factor for downregulation of E-cadherin gene expression²³⁾. Slug contributes to activation of the cadherin switch in bladder cancer⁷⁾. We demonstrated that a low cell density culture promotes nuclear translocations of Slug (Fig. 3C), and YAP/TAZ (Fig. 2C and D). The nuclear translocation of Slug was inhibited by gene knockdown of YAP in HSC-4 cells (Fig. 3D). Tang Y *et al.* reported that Slug/Snail bound to YAP/TAZ²⁴⁾ and Serrano I, *et al.* showed that nuclear translocation of Slug is promoted by TGF- β 1, resulting in EMT²⁵⁾. TAZ regulates the nuclear translocation of the TGF- β 1-provided transcription factors complex Smad2/3/4¹²⁾. These results suggest that YAP/TAZ in the Hippo pathway play important roles in the cadherin switch through induction of nuclear translocation of EMT-related transcription factors in hOSCC cells.

On the other hand, Serrano I, *et al.* showed that nuclear translocation of Slug is promoted by TGF- β 1, resulting in EMT²⁵⁾. TAZ regulates the nuclear translocation of the TGF- β 1-provided

transcription factors complex Smad2/3/4¹²⁾. These results suggested that YAP/TAZ in the Hippo pathway play important roles on the cadherin switch through induction of nuclear translocation of EMT-related transcription factors in hOSCC cells. On the other hand, Snail is one of the most widely studied EMT-related transcription factors, which regulates the cadherin switch in EMT³⁾. Snail positively regulates the TGF- β 1-induced cadherin switch in human lung carcinoma A549 cells²⁶⁾. In addition, Snail protein has been shown to increase and accumulate in nuclei through mechanical signals by YAP²⁷⁾ and to bind to YAP/TAZ²⁴⁾. We previously found that the expression levels of Snail were upregulated during the TGF- β 1-induced EMT in HSC-4 cells²⁸⁾. However, it remains to be clarified whether YAP played an important role in the nuclear translocation of Snail in TGF- β 1-stimulated HSC-4 cells.

We examined what kind of factors were involved in the activation of YAP/TAZ through the Hippo pathway in HSC-4 cells. We first examined the contribution of the Ca²⁺-dependent adhesion proteins on the cell surface by using low calcium treatments of the culture media¹⁸⁾. Cadherin family proteins involving E-cadherin require Ca²⁺ for cadherin-cadherin interaction on the cell surface²²⁾. We found that the nuclear localization of YAP occurred following low calcium treatment (Fig. 4A). We also found that the localization of E-cadherin was observed in the cytoplasmic region at low cell density (Fig. 1A). Kim *et al.* reported that the cell-to-cell contact mediated by E-cadherin negatively regulates the Hippo signaling pathway through nuclear exclusion of YAP in breast cancer¹⁹⁾. Therefore, we examined whether cell-to-cell interaction mediated by E-cadherin suppressed nuclear localization of

YAP by using a neutralizing antibody against E-cadherin. The neutralizing antibody against E-cadherin expectedly suppressed nuclear localization of YAP (Fig. 4B), resulting in inhibition of the cadherin switch in HSC-4 cells (Fig. 4C). This data suggests that the disruption of E-cadherin-mediated adherens junction might induce deactivation of Hippo pathway, which in turn activates YAP/TAZ and promotes its nuclear translocation.

Scribble (SCRIB) is a cell polarity protein, which is localized to adherens junctions, constituting the Scribble complex and resulting in maintenance of epithelial cell polarity and the suppression of tumorigenesis²⁹⁾. SCRIB depletion disrupts cell-to-cell interaction mediated by E-cadherin³⁰⁾. In addition, loss of E-cadherin leads to mislocalization of SCRIB from adherens junctions³¹⁾. Also, SCRIB binds to TAZ, inhibiting TAZ-promoted EMT progression in epithelial cells¹⁶⁾. These results suggest that the cadherin switch was inhibited by E-cadherin/SCRIB axis which suppresses the activity of TAZ in epithelial cells. However, it is unknown whether SCRIB plays an important role in the high density culture-induced inhibition of the cadherin switch in HSC-4 cells.

Here, we demonstrated that YAP/TAZ mediates the low cell density-induced cadherin switch through nuclear translocation of Slug and that intercellular contact mediated by E-cadherin inhibits the cadherin switch through negative modulation of the Hippo pathway. Thus, disruption of E-cadherin-mediated cell-to-cell contact induces YAP/TAZ activation and promotes cadherin switch by facilitating nuclear translocation of the EMT-related transcription factor Slug in hOSCC HSC-4 cells.

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Conflicts of Interest

The authors declare that they have no competing interests.

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