Int J Clin Exp Pathol 2018;11(12):5792-5800
www.ijcep.com /ISSN:1936-2625/IJCEP0087348

Original Article

Up-regulation of E-cadherin by saRNA inhibits the migration and invasion of renal carcinoma cells

Jun Dai[1], Hongchao He[1], Dengqiang Lin[2], Chenghe Wang[1], Yu Zhu[1], Danfeng Xu[1]

[1]Department of Urology, Ruijin Hospital, Shanghai Jiao Tong University School of Medicine, Shanghai, China; [2]Department of Urology, Xiamen Hospital of Zhongshan Hospital, Fudan University, Shanghai, China

Received October 25, 2018; Accepted November 23, 2018; Epub December 1, 2018; Published December 15, 2018

Abstract: Previous studies have reported that double stranded RNAs (dsRNAs) have a potent ability to induce gene expression by targeting its promoter in cancer cells, which is called RNA activation (RNAa). In the present study, we have identified that a candidate dsRNA (dsEcad-215) could stimulate E-cadherin mRNA and protein expression via RNAa in renal cell carcinoma (RCC). Because the expression level of E-cadherin was down-regulated in RCC tissues compared to adjacent non-tumor tissues, dsEcad-215 was subsequently transfected into the RCC cell lines ACHN and 786-O. Expectedly, our results indicated that transfection of dsEcad-215 readily inhibited cell migration and invasion. In addition, several critical EMT-promoting genes (ZEB-1 and Vimentin) were down-regulated, while the anti-EMT gene β-catenin was up-regulated both at the mRNA and protein levels after dsEcad-215 transfection, suggesting that an enhanced E-cadherin level by dsEcad-215 suppressed EMT to inhibit cell motility. Collectively, our findings provide a potential effective therapeutic strategy for RCC, and dsEcad-215 might act as an alternative anti-cancer metastasis drug.

Keywords: saRNA, E-cadherin, renal cell carcinoma, RNA activation, metastasis

Introduction

Renal cell carcinoma (RCC) is one of the most common malignant tumors in the urinary system, and clear cell RCC represents the main histological type. In recent years, the incidence and the mortality rates of renal cell carcinoma have increased gradually, with an expected 66,800 newly-diagnosed cases and 23,400 deaths in China in 2015 [1]. Currently, nearly 40% of all RCC patients die from advanced stages with local or systematic metastasis [2]. Although the tumor can be completely removed by surgery, it commonly recurs during follow-up. As RCC is resistant to current treatment regimens, patients with metastatic RCC have poor prognoses [3]. Therefore, it is urgent to identify novel therapeutic targets, including non-coding RNAs (ncRNAs) in RCC, in order to develop more effective treatment options for this fatal disease.

Previous studies have reported that E-cadherin was low expressed in RCC [4]. Moreover, the deregulation of cell-to-cell adhesion molecules facilitates RCC invasion and metastasis [5]. However, as a vital cell adhesion molecule, E-cadherin maintains the integrity of epithelial cells. Reduced E-cadherin expression or mutation results in a functional loss of the cadherin complex, and leads to the metastasis of epithelial malignancies [6, 7]. Furthermore, the epithelial-to-mesenchymal transition (EMT) is a process that plays crucial roles in cancer metastases and invasiveness at an early stage [8, 9]. Thus, the reactivation of E-cadherin in RCC may contribute to RCC cell suppression.

As we know, RNA activation (RNAa) is a recently discovered method to up-regulate specific gene expressions [10, 11]. Because of its unique ability to enhance endogenous gene expression, RNAa may act as a potential novel therapeutic strategy for RCC. Inspiringly, it has been shown that a candidate small activating RNA (saRNA) (dsEcad-215) possesses a potent capacity to stimulate E-cadherin expression by targeting its promoter at sequence positions at -215 relative to the transcription start site (TSS) in prostate cancer and bladder cancer cells [10,

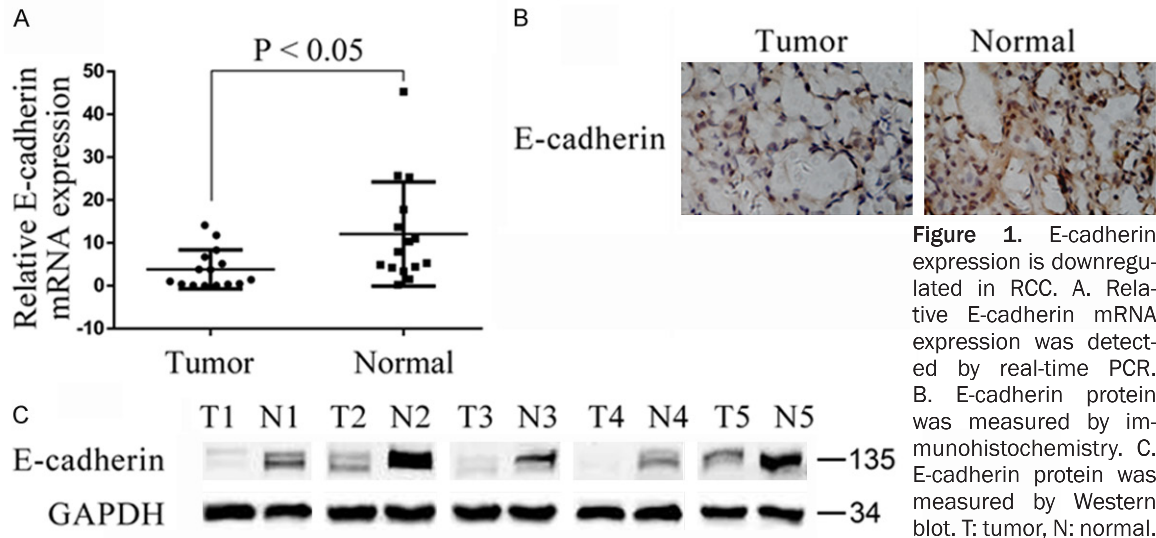

Figure 1. E-cadherin expression is downregulated in RCC. A. Relative E-cadherin mRNA expression was detected by real-time PCR. B. E-cadherin protein was measured by immunohistochemistry. C. E-cadherin protein was measured by Western blot. T: tumor, N: normal.

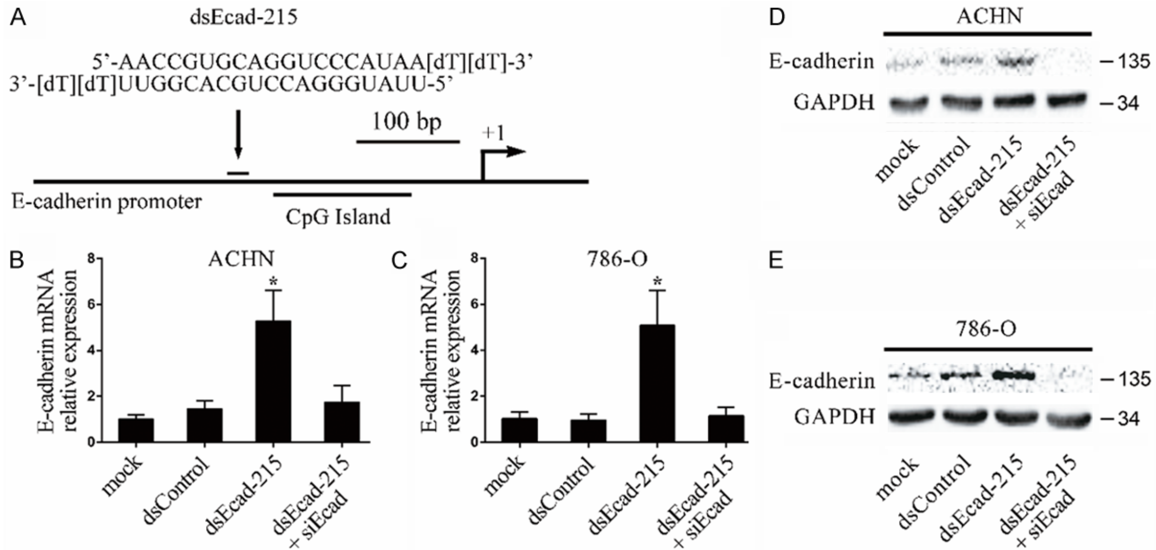

Figure 2. dsEcad-215 induces E-cadherin expression by targeting promoter in human RCC cells. ACHN and 786-O cells were transfected with 50 nM of the indicated dsRNAs for 72 h. GAPDH levels were detected and served as a loading control. A. Schematic representation of E-cadherin promoter and dsEcad-215 target location relative to TSS. B and C. Expression of E-cadherin mRNA levels was assessed by real-time PCR. *$P < 0.05$ compared to other three groups. D and E. Expression of E-cadherin protein was detected by Western blot analysis.

12] (**Figure 2A**). However, whether it can up-regulate E-cadherin in RCC cell lines and then inhibit cell invasion and migration remains unclear.

In the present study, we first examined E-cadherin expression in RCC tissues, and then transfected dsEcad-215 into RCC cells to examine its effects *in vitro*. Our results showed that E-cadherin was decreased in RCC tissues. Furthermore, dsEcad-215 had a potent ability to induce E-cadherin expression by targeting its promoter region, and inhibiting RCC cells migration and invasion.

Materials and methods

Tissue samples

From August 2016 to November 2016, paired RCC and adjacent normal tissue specimens were collected from 15 renal cancer patients who were operated on in the Department of Urology in the Ruijin Hospital affiliated to the

Table 1. Sequences for dsRNAs used in present study

RNAs	Sequences (5'-3')
dsControl Sense	ACU ACU GAG UGA CAG UAG A [dT] [dT]
dsControl Antisense	UCU ACU GUC ACU CAG UAG U [dT] [dT]
dsEcad-215 Sense	AAC CGU GCA GGU CCC AUA A [dTdT]
dsEcad-215 Antisense	UUA UGG GAC CUG CAC GGU U [dTdT]
siEcad Sense	GGC CUG AAG UGA CUC GUA A [dT] [dT]
siEcad Antisense	UUA CGA GUC ACU UCA GGC C [dT] [dT]

Table 2. Sequences for real time quantitative PCR primers used in present study

Primers	Sequences (5'-3')
GAPDH (F)	TCCCATCACCATCTTCCA
GAPDH (R)	CATCACGCCACAGTTTCC
E-cadherin (F)	ACCAGAATAAAGACCAAGTGACCA
E-cadherin (R)	AGCAAGAGCAGCAGAATCAGAAT
β-catenin (F)	GGAAGATGGGATCAAACCTG
β-catenin (R)	TCCGTCTCCGACCTGGAA
ZEB1 (F)	ACTCTGATTCTACACCGC
ZEB1 (R)	TGTCACATTGATAGGGCTT
Vimentin (F)	GATGCGTGAGATGGAAGAGA
Vimentin (R)	GGCCATGTTAACATTGAGCA

F, forward; R, reverse; GAPDH, glyceraldehyde-3-phosphate dehydrogenase. CDK4/6, cyclin-dependent kinase 4/6.

Medical School of Shanghai Jiaotong University (Shanghai, China). The samples were obtained from patients undergoing RCC resection. All cases were confirmed RCC by surgical pathology. Each sample for the experiment was immediately obtained from the operative specimen and frozen in liquid nitrogen and stored at -80°C until use. Following approval by the institutional ethics review board in Ruijin Hospital and after getting written informed consent from the patients, their specimens were obtained.

dsRNAs

In this study, all the RNA duplexes which possess 2-nucleotide 3' overhangs were chemically synthesized by RiboBio Co., Ltd. (Guangzhou, China). A small interfering RNA (siEcad) purchased from RiboBio (Guangzhou, China) was used to silence E-cadherin expression. We used a nonspecific control dsRNA (dsControl) which lacks significant homology to all known human sequences for a negative control [10, 13]. The sequences of all the custom RNAs are listed in **Table 1**.

Cell culture and transfection

Under a humidified atmosphere with 5% CO_2 at 37°C, human renal cancer cell lines ACHN and 786-O (ATCC, Maryland, USA) was cultured with RPMI 1640 medium (Hyclone, Los Angeles, USA) comprising 10% fetal bovine serum (FBS, Gibco, New York, USA). Cells were trypsinized and plated in a 6-well plate without any antibiotic before transfection. Cells at a densityof 50-60% were transfected with 50 nM dsRNAs and Lipofectamine RNAiMax (Invitrogen, Massachusetts, USA) according to the manufacturer's instructions. Moreover, dsRNA was displaced by MEM (Invitrogen, Massachusetts, USA) in a mock transfection.

RNA isolation and quantitative real-time PCR

By using TRIzol reagent (Invitrogen, Massachusetts, USA) according to the manufacturer's instructions, total cellular RNA was extracted from the RCC cells. 500 ng RNA was reversely transcribed into cDNA according to the directions offered by the Takara reverse transcription kit (Takara, Liaoning, China). Then the cDNA was amplified by SYBR Premix Ex Taq II (Takara, Liaoning, China) conducted on the Mx3000P instrument (Stratagene, California, USA). All the primers included in this study were offered by Invitrogen (Shanghai, China) and listed in **Table 2**. The $2^{-\Delta\Delta Ct}$ method was used to calculate the relative expression of the target genes' mRNA; GAPDH was used as an internal control. All the experiments were repeated three times.

Protein extraction and Western blotting analysis

Using a RIPA lysis buffer supplemented with protease inhibitor cocktail (Roche, Basel, Switzerland), all the cells were gathered, and the total proteins were extracted. After the protein concentrations were calculated, 50 μg protein samples were separated by 10% sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and then transferred to polyvinylidene fluoride (PVDF) membranes. 5% bovine serum albumin (BSA) (Sigma-Aldrich, California, USA) was used to block the nonspecific binding for 2 h at room temperature. The membrane was then incubated with primary

antibodies at 4°C overnight, which included GAPDH (1/500) (Boster, Wuhan, China), E-cadherin (1/1000) (BD Biosciences, New York, USA), β-catenin (1/500) (Boster, Wuhan, China), Vimentin (1/500) (Boster, Wuhan, China) and ZEB1 (1/1000) (Cell Signaling Technology, Boston, USA). After a few washes, the membranes were incubated with a relevant secondary antibody and tested using an enhanced chemiluminescence (ECL) assay kit (Millipore, Massachusetts, USA).

Immunohistochemistry

Paraffin-embedded tissue sections (5 μm-thick) were prepared, deparaffinized, and rehydrated. To quench endogenous peroxidase activity, the sections were incubated with 3% hydrogen peroxide for 10 min at room temperature. A 0.01M sodium citrate buffer (pH 6.0) was used to carry out antigen retrieval at 95°C for 45 minutes, and then incubated in 5% BSA in a phosphate-buffered saline (PBS) for 10 minutes. After this, the slides were incubated overnight with the primary anti-E-cadherin antibody (Cell Signaling Technology, Boston, USA) at a dilution of 1:300 at 4°C. The next day, the sections were incubated with the secondary antibody for 20 minutes at room temperature and coated using an Envision Dual Labeled Polymer kit (BioGenex, San Ramon, CA) according to the manufacturer's directions. Haematoxylin was used for nuclear staining.

Wound healing assay

The cells were trypsinized and counted after 72 hours' transfection. The cells were then reseeded in a new 6-well plate. The monolayer cells were scratched manually with a plastic pipette tip, and after two washes with PBS, the wounded cellular monolayer was allowed to heal for 24 h in a serum-free medium. Photographs of the central wound edges per condition were taken at 0 h, 12 h and 24 h after they were scratched using the inverted microscope (Olympus, Tokyo, Japan). Three random non-overlapping areas in each well were pictured.

Migration and invasion assay

Cell migration and invasion was evaluated using a transwell assay. For the cell migration assay, 24 h after transfection, cells at a concentration of 2 × 10^4 were seeded in the top chamber with the non-coated membrane transwell (8 μm pore size inserts, Corning, New York, USA). For the cell invasion assay, matrigel (BD Biosciences, New Jersey, USA) was polymerized in the transwell inserts for 45 min at 37°C. For both assays, the cells were plated in the top chamber in the medium without FBS, while the lower chamber was filled with 10% FBS. The cells were further incubated for 24 h, and the cells that did not migrate or invade through the pores were removed by a cotton swab. Cells on the lower surface of the membrane were stained with 0.5% crystal violet (Sigma, California, USA) and counted under a microscope at 200 × magnification for 5 visual fields.

Statistical analysis

The statistical analyses were performed using SPSS version 13.0 software (SPSS Inc., Chicago, IL, USA). Student's *t*-test was used to analyze variables and all the data were presented as the mean ± standard deviation (SD) for these experiments. A P-value < 0.05 was considered to be statistically significant. All experiments were repeated three times.

Results

E-cadherin expression was reduced in RCC tissues compared with normal tissues

To identify the expression patterns of E-cadherin in clinical RCC tissues, quantitative real-time PCR was performed to assess its level compared with adjacent non-tumor tissues. Results showed that expression level of E-cadherin in tumorous tissues was significantly lower than the corresponding normal tissues (**Figure 1A**). Immunohistochemical and Western blot analysis (**Figure 1B** and **1C**) further confirmed these results. So, E-cadherin may act as a tumor suppressor in RCC.

dsEcad-215 activated E-cadherin expression by targeting promoter

According to the previous study, we speculated that this dsEcad-215 may also activate E-cadherin expression in human RCC cells. Therefore, we transfected synthetic dsEcad-215 into ACHN and 786-O cells, and measured the E-cadherin expression 72 h later. Compared with the mock and dsControl groups, dsEcad-215 caused a significant increase in E-cadherin mRNA (**Figure 2B** and **2C**). This

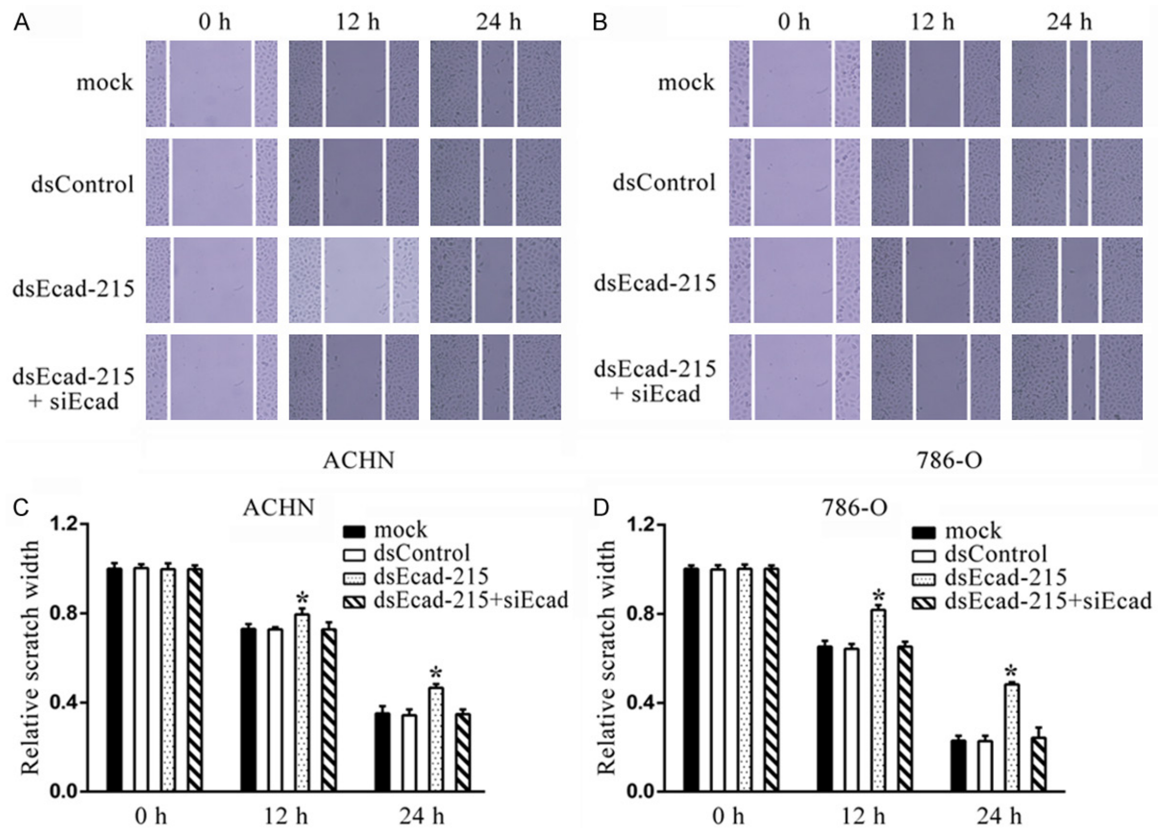

Figure 3. dsEcad-215 inhibits RCC cells migration ability by enhancing E-cadherin. ACHN and 786-O cells were transfected with 50 nM of the indicated dsRNAs for 72 h. A and B. Representative wound healing images were pictured at 0, 12 and 24 h. C and D. The relative distances between wound edges of RCC cells at 0, 12 and 24 h. $^*P < 0.05$ compared to other groups.

induction was further verified by protein analysis (**Figure 2D** and **2E**).

dsEcad-215 inhibited RCC cells migration and invasion depended on the up-regulation of E-cadherin expression

Next, we examined the potential roles of dsEcad-215 on the migration and invasion capacities of RCC cells. The transfection of dsEcad-215 led to retarded wound closing compared with the control groups from 12 h in both ACHN and 786-O cells (**Figure 3A** and **3B**). Then the wound widths of each group were measured and normalized to a corresponding mock baseline. After the ACHN and 786-O cells were scratched at 12 h, the relative distances between the wound edges in the dsEcad-215 groups were significantly shorter than the matched groups (**Figure 3C** and **3D**). Additionally, wound healing in the group co-transfected with siEcad and dsEcad-215 was faster within 24 h compared to the group transfected with dsEcad-215 alone (**Figure 3A** and **3B**). Further quantitative analysis of wound width manifested relative scratch widths in the co-treatment group were remarkably less than the dsEcad-215 transfection groups from 12 h (**Figure 3C** and **3D**).

Next, the transwell assay was conducted to further assess the cells' migration and invasion abilities. Compared with the mock and dsControl groups, dsEcad-215 had a potent inhibiting effect on the migration of the both ACHN and 786-O cells (**Figure 4A**). Likewise, the invasion capability of RCC cells transfected with dsEcad-215 was measured using a matrigel invasion chamber assay. As we expected, dsEcad-215 could significantly inhibit their invasion ability compared to the matched groups (**Figure 4C**). At the same time, the depletion of E-cadherin remarkably restored the cells' migration capacity in response to ds-

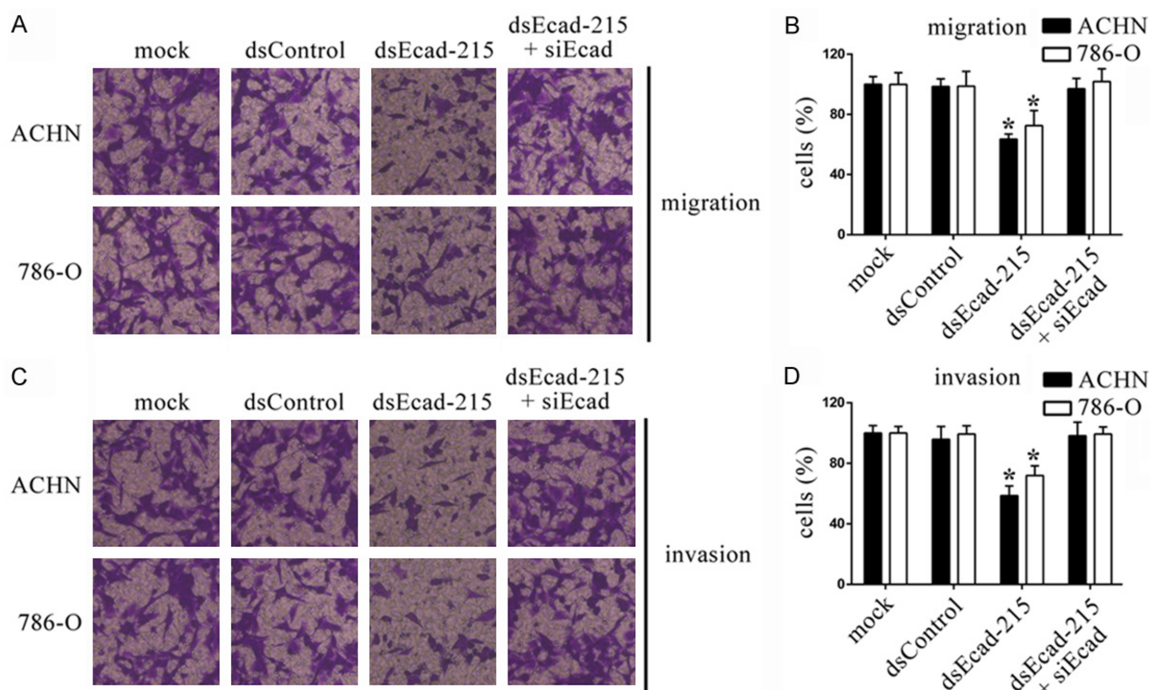

Figure 4. dsEcad-215 suppresses RCC cells migration and invasion largely depended on manipulating E-cadherin expression. ACHN and 786-O cells were transfected with 50 nM of the indicated dsRNAs for 72 h. A and C. Representative photographs of transwell assay (× 200). B and D. Number of migrated and invaded cells was quantified in 5 random images from each treatment group. Results are plotted as percent (%) relative to mock group. *P < 0.05 compared to other groups.

Ecad-215 treatment (**Figure 4A** and **4B**). Consistent with that, more cells in co-transfection group (dsEcad-215+siEcad) migrated the membranes pre-coated with matrigel compared to the dsEcad-215 group (**Figure 4C** and **4D**). Together, these findings strongly implied that dsEcad-215-inhibited RCC cell migration and invasion was totally dependent on the up-regulation of E-cadherin.

dsEcad-215 regulated EMT-associated genes mainly by enforcing E-cadherin expression

We then assessed the effects of dsEcad-215 transfection on the expression of some essential genes associated with the EMT process in RCC cells. Compared to the mock and dsControl groups, dsEcad-215 significantly up-regulated β-catenin expression, and down-regulated ZEB1 and Vimentin expressions in both ACHN and 786-O cells at the transcriptional level, respectively (**Figure 5A** and **5B**). Moreover, Western blot analysis also revealed a potent increase in β-catenin, and a decrease in ZEB1 and Vimentin protein levels of the two kind cells after the dsEcad-215 transfection (**Figure 5C**). These data indicated that following the up-regulation of E-cadherin, dsEcad-215 could also affect the EMT-associated gene expression of RCC cells.

Next, to investigate whether E-cadherin was mainly responsible for tumor suppression after dsEcad-215 transfection, we used RNA interference technology to knockout the expression of E-cadherin in the RCC cells (**Figure 2B-E**). Compared to the dsEcad-215 group, the mRNA of β-catenin was down-regulated, whereas ZEB1 and Vimentin mRNA were up-regulated after the co-treatment of siEcad for 72 h in both cell lines (**Figure 5A** and **5B**). Additionally, an immunoblot analysis further proved those findings (**Figure 5C**). Taken together, these data confirmed that dsEcad-215 manipulated EMT-associated genes largely via stimulating E-cadherin expression.

Discussion

Gene activation mediated by small ncRNAs, called RNAa, is a promising discovery, which has been identified in many cancer cell lines [10, 14, 15]. As a potently positive gene regulation approach, it can reactivate a functional

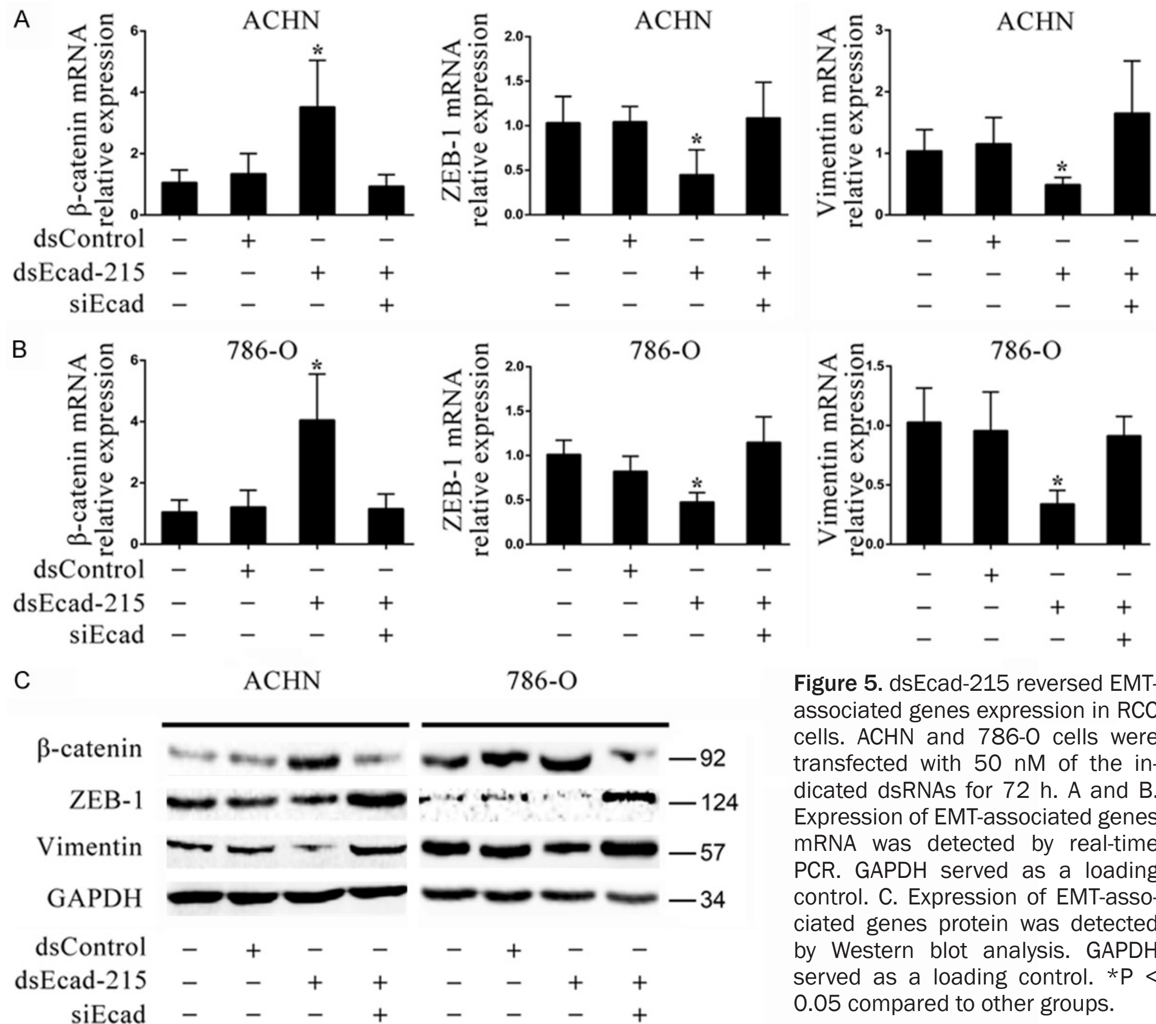

Figure 5. dsEcad-215 reversed EMT-associated genes expression in RCC cells. ACHN and 786-O cells were transfected with 50 nM of the indicated dsRNAs for 72 h. A and B. Expression of EMT-associated genes mRNA was detected by real-time PCR. GAPDH served as a loading control. C. Expression of EMT-associated genes protein was detected by Western blot analysis. GAPDH served as a loading control. *P < 0.05 compared to other groups.

silenced or mutant tumor suppressor in cancer patients [16]. With a greater understanding of RNAa's molecular mechanisms, RNAa-based research tools and therapeutic strategies have been applied [17, 18].

In the present study, we initially found that the anti-oncogene E-cadherin was decreased in human RCC tissues compared with paired normal renal tissues. Subsequently, we found that dsEcad-215, a synthetic dsRNA, could induce E-cadherin expression by interacting with a promoter in human RCC ACHN and 786-O cells. Moreover, cell migration and invasion abilities were inhibited after the transfection of dsEcad-215. By this mechanism, several critical EMT-promoting genes (ZEB-1 and Vimentin) were down-regulated, while the anti-EMT genes β-catenin was up-regulated after dsEcad-215 treatments. Most importantly, a significant difference of function and protein levels were reversed after co-transfection of siEcad, indicating that dsEcad-215's antitumor effects were dependent on the up-regulation of E-cadherin expression.

The most common characteristic of EMT is that the tumor cells undergo morphological changes and gain migratory and invasive properties [19]. Here, the transfection of dsEcad-215 could suppress EMT via increasing the expression level of E-cadherin and β-catenin, as well as decreasing ZEB-1 and Vimentin. The complex of E-cadherin/β-catenin can regulate cell adhesion [20]. Loss function of the complex led to reduced cell adhesion and elevated tumor metastasis [21]. As we know, the ZEB-1 is a transcriptional repressor of E-cadherin [22] and acts as a crucial contributor to EMT [23]. But the exact relationship among dsEcad-215, E-cadherin and ZEB-1 remains unknown. As a type III intermediate filament protein, vimentin

is mainly found in mesenchymal cells and migratory epithelial cells [24]. The overexpression of vimentin thought to be a typical phenotype for EMT, and metastasis was seen in RCC [25]. Based on our study, dsEcad-215 could profoundly inhibit the EMT process, helping to retard tumor progression.

At present, cancer metastases frequently occur in RCC patients, and the treatment of RCC has become increasingly difficult. It has been reported that reduced expression of E-cadherin may be an important biological marker for the invasion and metastasis of RCC [26]. The E-cadherin down-regulation finally results in the tumor cells breaking away from the original site and the subsequent occurrence of tumor invasion and metastasis [27]. So, we believe that reactivation of E-cadherin by RNAa would undoubtedly help to suppress RCC metastasis.

Taken together, this research demonstrated that the activation of E-cadherin by a promoter-targeted E-cadherin saRNA significantly inhibited RCC cell migration and invasion. Our study showed that targeted activation of E-cadherin is a promising therapeutic method for human RCC and should be paid more attention in a preclinical setting.

Acknowledgements

This work was supported by the National Natural Science Foundation of China [grant number 81602215, China]. The funders had no role in the study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Disclosure of conflict of interest

None.

Address correspondence to: Drs. Chenghe Wang and Yu Zhu, Department of Urology, Ruijin Hospital, Shanghai Jiao Tong University School of Medicine, Shanghai 200025, China. Tel: (86) 21-64370045; E-mail: wangch8603@163.com (CHW); zhuyuruijin@163.com (YZ)

References

[1] Chen W, Zheng R, Baade PD, Zhang S, Zeng H, Bray F, Jemal A, Yu XQ, He J. Cancer statistics in China, 2015. CA Cancer J Clin 2016; 66: 115-132.

[2] Ko JJ, Xie W, Kroeger N, Lee JL, Rini BI, Knox JJ, Bjarnason GA, Srinivas S, Pal SK, Yuasa T, Smoragiewicz M, Donskov F, Kanesvaran R, Wood L, Ernst DS, Agarwal N, Vaishampayan UN, Rha SY, Choueiri TK, Heng DY. The international metastatic renal cell carcinoma database consortium model as a prognostic tool in patients with metastatic renal cell carcinoma previously treated with first-line targeted therapy: a population-based study. Lancet Oncol 2015; 16: 293-300.

[3] Motzer RJ, Michaelson MD, Redman BG, Hudes GR, Wilding G, Figlin RA, Ginsberg MS, Kim ST, Baum CM, DePrimo SE, Li JZ, Bello CL, Theuer CP, George DJ, Rini BI. Activity of SU11248, a multitargeted inhibitor of vascular endothelial growth factor receptor and platelet-derived growth factor receptor, in patients with metastatic renal cell carcinoma. J Clin Oncol 2006; 24: 16-24.

[4] Zhang X, Yang M, Shi H, Hu J, Wang Y, Sun Z, Xu S. Reduced E-cadherin facilitates renal cell carcinoma progression by WNT/β-catenin signaling activation. Oncotarget 2017; 8: 19566-19576.

[5] Mikami S, Katsube K, Oya M, Ishida M, Kosaka T, Mizuno R, Mukai M, Okada Y. Expression of snail and slug in renal cell carcinoma: E-cadherin repressor snail is associated with cancer invasion and prognosis. Lab Invest 2011; 91: 1443-1458.

[6] Khawam K, Giron-Michel J, Gu Y, Perier A, Giuliani M, Caignard A, Devocelle A, Ferrini S, Fabbi M, Charpentier B, Ludwig A, Chouaib S, Azzarone B, Eid P. Human renal cancer cells express a novel membrane-bound interleukin-15 that induces, in response to the soluble interleukin-15 receptor alpha chain, epithelial-to-mesenchymal transition. Cancer Res 2009; 69: 1561-1569.

[7] Katagiri A, Watanabe R and Tomita Y. E-cadherin expression in renal cell cancer and its significance in metastasis and survival. Br J Cancer 1995; 71: 376-379.

[8] Rhim AD, Mirek ET, Aiello NM, Maitra A, Bailey JM, McAllister F, Reichert M, Beatty GL, Rustgi AK, Vonderheide RH, Leach SD, Stanger BZ. EMT and dissemination precede pancreatic tumor formation. Cell 2012; 148: 349-361.

[9] Chen D, Gassenmaier M, Maruschke M, Riesenberg R, Pohla H, Stief CG, Zimmermann W, Buchner A. Expression and prognostic significance of a comprehensive epithelial-mesenchymal transition gene set in renal cell carcinoma. J Urol 2014; 191: 479-486.

[10] Li LC, Okino ST, Zhao H, Pookot D, Place RF, Urakami S, Enokida H, Dahiya R. Small dsRNAs induce transcriptional activation in human cells. Proc Natl Acad Sci U S A 2006; 103: 17337-17342.

[11] Place RF, Li LC, Pookot D, Noonan EJ, Dahiya R. MicroRNA-373 induces expression of genes with complementary promoter sequences. Proc Natl Acad Sci U S A 2008; 105: 1608-1613.
[12] Mao Q, Li Y, Zheng X, Yang K, Shen H, Qin J, Bai Y, Kong D, Jia X, Xie L. Up-regulation of E-cadherin by small activating RNA inhibits cell invasion and migration in 5637 human bladder cancer cells. Biochem Biophys Res Commun 2008; 375: 566-570.
[13] Srsen V, Gnadt N, Dammermann A, Merdes A. Inhibition of centrosome protein assembly leads to p53-dependent exit from the cell cycle. J Cell Biol 2006; 174: 625-630.
[14] Ren S, Kang MR, Wang J, Huang V, Place RF, Sun Y, Li LC. Targeted induction of endogenous NKX3-1 by small activating RNA inhibits prostate tumor growth. Prostate 2013; 73: 1591-1601.
[15] Chen Z, Place RF, Jia ZJ, Pookot D, Dahiya R, Li LC. Antitumor effect of dsRNA-induced p21 (WAF1/CIP1) gene activation in human bladder cancer cells. Mol Cancer Ther 2008; 7: 698-703.
[16] Wang C, Ge Q, Zhang Q, Chen Z, Hu J, Li F, Ye Z. Targeted p53 activation by saRNA suppresses human bladder cancer cells growth and metastasis. J Exp Clin Cancer Res 2016; 35: 53.
[17] Kang MR, Yang G, Place RF, Charisse K, Epstein-Barash H, Manoharan M, Li LC. Intravesical delivery of small activating RNA formulated into lipid nanoparticles inhibits orthotopic bladder tumor growth. Cancer Res 2012; 72: 5069-5079.
[18] Place RF, Wang J, Noonan EJ, Meyers R, Manoharan M, Charisse K, Duncan R, Huang V, Wang X, Li LC. Formulation of small activating RNA into lipidoid nanoparticles inhibits xenograft prostate tumor growth by inducing p21 expression. Mol Ther Nucleic Acids 2012; 1: e15.
[19] Kalluri R and Weinberg RA. The basics of epithelial-mesenchymal transition. J Clin Invest 2009; 119: 1420-1428.
[20] Tian X, Liu Z, Niu B, Zhang J, Tan TK, Lee SR, Zhao Y, Harris DC, Zheng G. E-cadherin/beta-catenin complex and the epithelial barrier. J Biomed Biotechnol 2011; 2011: 567305.
[21] Gottardi CJ, Wong E and Gumbiner BM. E-cadherin suppresses cellular transformation by inhibiting beta-catenin signaling in an adhesion-independent manner. J Cell Biol 2001; 153: 1049-1060.
[22] Guaita S, Puig I, Franci C, Garrido M, Dominguez D, Batlle E, Sancho E, Dedhar S, De Herreros AG, Baulida J. Snail induction of epithelial to mesenchymal transition in tumor cells is accompanied by MUC1 repression and ZEB1 expression. J Biol Chem 2002; 277: 39209-39216.
[23] Larsen JE, Nathan V, Osborne JK, Farrow RK, Deb D, Sullivan JP, Dospoy PD, Augustyn A, Hight SK, Sato M, Girard L, Behrens C, Wistuba II, Gazdar AF, Hayward NK, Minna JD. ZEB1 drives epithelial-to-mesenchymal transition in lung cancer. J Clin Invest 2016; 126: 3219-3235.
[24] Liu LK, Jiang XY, Zhou XX, Wang DM, Song XL, Jiang HB. Upregulation of vimentin and aberrant expression of E-cadherin/beta-catenin complex in oral squamous cell carcinomas: correlation with the clinicopathological features and patient outcome. Mod Pathol 2010; 23: 213-224.
[25] Zhang Y, Sun B, Zhao X, Sun H, Cui W, Liu Z, Yao X, Dong X. Spheres derived from the human SN12C renal cell carcinoma cell line are enriched in tumor initiating cells. J Exp Clin Cancer Res 2016; 35: 163.
[26] Cai JQ. Roles of transcriptional factor Snail and adhesion factor E-cadherin in clear cell renal cell carcinoma. Exp Ther Med 2013; 6: 1489-1493.
[27] Rosivatz E, Becker I, Specht K, Fricke E, Luber B, Busch R, Höfler H, Becker KF. Differential expression of the epithelial-mesenchymal transition regulators snail, SIP1, and twist in gastric cancer. Am J Pathol 2002; 161: 1881-1891.