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ECT2 promotes proliferation and metastasis of esophageal squamous cell carcinoma *via* the RhoA-ERK signaling pathway

B.-Y. SUN[1], Q.-Q. WEI[1], C.-X. LIU[1], L. ZHANG[1], G. LUO[1], T. LI[2], M.-H. LÜ[1,3]

[1]Department of Gastroenterology, The Affiliated Hospital of Southwest Medical University, Luzhou City, Sichuan Province, China
[2]Department of Anesthesiology, West China Hospital, Sichuan University, Chengdu City, Sichuan Province, China
[3]Nuclear Medicine and Molecular Imaging Key Laboratory of Sichuan Province, the Affiliated Hospital of Southwest Medical University, Luzhou City, Sichuan Province, China

Binyu Sun and QiongQiong Wei contribute equally to this work

Abstract. – OBJECTIVE: In this study, the effect of epithelial cell transformation sequence 2 (ECT2) on the proliferation, invasion and migration of esophageal squamous cell carcinoma (ESCC) was investigated by interfering the expression of ECT2.

PATIENTS AND METHODS: Interfering with the expression level of ECT2 in human squamous cell carcinomas KYSE140 and EC9706 cell lines, the changes of KYSE140 and EC9706 cell proliferation, invasion, and migration were measured using the CCK-8 method, transwell test, and scratch test, respectively. The effects of ECT2 on the Ras homolog gene family, member A-extracellular regulated protein kinases (RhoA-ERK) signaling pathway were also observed.

RESULTS: Compared with the control group, the proliferation, migration, and invasion ability of EC9706 and KYSE140 cells after ECT2 knockout were significantly reduced (p <0.05). The knockdown of ECT2 expression in ESCC cell lines suppressed the activation of RhoA-ERK signaling pathway and protein expression of VEGF and MMP9.

CONCLUSIONS: ECT2 could regulated the expression of VEGF and MMP9 to inhibit cells proliferation, invasion, migration and tumor development through RhoA-ERK signaling pathway. Therefore, ECT2 could be an available marker, and provide a new theoretical basis for the treatment of ESCC.

Key Words:
Esophageal squamous cell carcinoma (ESCC), Epithelial cell transformation sequence 2 (ECT2), RhoA-ERK signaling pathway, Proliferation, Migration

Abbreviations

ECT2, epithelial cell transformation sequence 2; ERK, extracellular regulated protein kinases; VEGF, vascular endothelial grown factor; MMP9, matrix metalloproteinase-9; RhoA, RAS homolog gene family, member A; ESCC, esophageal squamous cell carcinoma; MAPK, mitogen-activated protein kinase; JNK, c-Jun N-terminal kinase; siRNA, small interfering RNA; shRNA, short hairpin RNA; GTP, guanosine triphosphate; GDP, guanosine diphosphate.

Introduction

Esophageal cancer (EC) is one of the most common malignant tumors in the world. The morbidity and mortality of EC rank eighth and sixth, respectively. China is a high incidence area of EC, where EC ranks fourth among all cancers in men and sixth among women, and more than half of all cases and deaths of EC worldwide occur in China[1]. The most common pathological type of esophageal cancer in European and American countries is adenocarcinoma[2], while in China, squamous cell carcinoma is the main type. These types of EC have different pathogenesis and biological characteristics. So, the diagnosis and treatment methods are different. Gene mutation, amplification, and deletion have been widely observed in the occurrence and development of esophageal squamous cell carcinoma (ESCC)[3]. Gene targeting therapy improves the prognosis of patients at different stages of ESCC[4]. However, the molecular mechanism of ESCC development is still unclear, which prompts us to further study. Epithelial cell transformation sequence 2 (ECT2) is a guanine nucleotide exchange factor. It can transform inactive Rho GTPase protein family members (CDC42-GDPase, Rac1-GDPase, Rho-GDPase) into active forms (CDC42-GTPase, Rac1-GTPase, Rho-GTPase), which mediates

Corresponding Author: Muhan Lü, MD; e-mail: lvmuhan@swmu.edu.cn

subsequent biochemical processes by catalyzing GTPase activity of guanine nucleotide exchange factor[5-7]. ECT2 was initially reported to be an oncogene that transformed NIH-3T3 cells, while the absence of the N-terminal domain of ECT2 was reported to lead to malignant transformation of NIH-3T3 cells and activation of MAPK pathways, including JNK, p38 and ERK[8]. Overexpression of ECT2 and its downstream molecules had also been reported to mediate cancer proliferation or metastasis in a variety of human cancers, including lung cancer, breast cancer, primary hepatocellular carcinoma, colon cancer, osteosarcoma and glioblastoma[9-15]. The molecular mechanism of ECT2 mediating the occurrence and development of ESCC is less studied.

In our research, we extracted RNA from the ESCC tissue and adjacent tissues of 40 patients and confirmed by qRT-PCR that ECT2 was over expressed in ESCC. According to the relevant literature, we hypothesized that ECT2 promoted the proliferation and metastasis of ESCC through the RhoA-ERK signaling pathway. Next, we knocked down the expression of ECT2 in EC9706 and KYSE140 cell lines by transfection with siRNAs and treated each cell line with pathway inhibitors. The corresponding data from Western blot, cell proliferation, invasion, migration assays, and other experiments confirmed that ECT2 can indeed promote the proliferation and metastasis of ESCC cells through the RhoA-ERK signaling axis.

Patients and Methods

Clinical Samples

All esophageal squamous cell carcinoma tissue and adjacent tissue for this study were provided by the Affiliated Hospital of Southwest Medical University. All the specimens were immediately snap-frozen and stored in liquid nitrogen then transferred to a refrigerator at -80°C until used. All tissues were collected with the consent of the patient and the patient's family members, and the collection was certified by the Ethics Committee of the Affiliated Hospital of Southwest Medical University.

Cell Lines and Culture

Human squamous cell carcinomas KYSE140 and EC9706 cell lines were provided by Shanghai Shenggong Inc. (Shanghai, China). The cells were grown in Dulbecco's Modified Eagle's Medium (DMEM; HyClone Laboratories, Inc., South Logan, UT, USA) containing 10% fetal bovine serum (FBS; Invitrogen, Carlsbad, CA, USA), and the cells were maintained at 37°C in humidified air with 5% CO_2.

Reverse Transcription-Coupled to Quantitative PCR (qRT-PCR)

Total cell RNA was extracted from all cells and tissues using TRIzol reagent (Tiangen Biotech Co, Beijing, China). A total of 800 ng of mRNA in 10 µl aliquots from each sample were reverse transcribed to generate single-stranded cDNAs using a random primer mix (TOYOBO, Tokyo, Japan). To examine the mRNA levels, Real Time-PCR was performed, and the fluorescence signal was detected with the StepOnePlus™ Real Time-PCR System (Applied Biosystems, Foster City, CA, USA). Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as an internal reference to calculate the relative expression of ECT2. The relative expression levels of each mRNA were calculated using the $2^{-\Delta\Delta Ct}$ (CT, cycle threshold) method[16]. The primer sequences are ECT2-F: 5'-GCGTTTTCAAGATCTAGCATGTG-3'; ECT2-R: 5'-CAATTTTCCCATGGTCTTATCC-3'; GAPDH-F: 5'-CTGACTTCAACAGCGACACC-3'; GAPDH-R: 5'-TGCTGTAGCCAAATTCGTTGT-3'.

Western Blot Analysis

Protein extraction was performed using radio immunoprecipitation assay (RIPA) lysis buffer (Beyotime, Guangzhou, Guangdong, China). A bicinchoninic acid assay (BCA) protein concentration assay kit (Beyotime, Guangzhou, Guangdong, China) was used to determine the protein concentration of the samples. Extracted total proteins were separated by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and transferred to polyvinylidene difluoride (PVDF) membranes (Bio-Rad, Foster City, CA, USA). After blocking with 5% non-fat milk in Tris-Buffered Saline and Tween-20 (TBST) for 1 h, the membranes were incubated with a rabbit polyclonal antihuman antibody (Abcam, Cambridge, MA, USA) for 1 h at room temperature. Horseradish peroxidase (HRP)-conjugated secondary antibodies (Abcam, Cambridge, MA, USA) were then added for incubations at room temperature for 1 h. After washing with TBST for 3 times, the membranes were exposed to enhanced chemiluminescence (ECL) reagents (Merck KGaA, Darmstadt, Germany) for visualization of immune zone.

Cell Transfection

Small interfering RNA (siRNA) duplexes were generated against the target gene (ECT2). The target sequences of the synthetic oligonucleotides for RNA interference were as follows: control (scramble), chloroplast *Euglena gracilis* gene coding for 5S and 16S (rRNAs), 5'-GCGCGC-UUUGUAGGAUUCG-3'; si-ECT2, 5'-CAGAG-GAGAUUAAGACUAU-3'. A total of 5×10^4 cells were seeded into a 24-well plate and incubated for 24 h. Confluence was approximately 30% at the time of transfection, and the total volume of medium before transfection was 200 µl. Transfection was performed according to the siRNA manufacturer's directions (Ruibo Biotech, Guangzhou, Guangdong, China) at a concentration of 70 nmol/µl. SiScramble was used as a negative control. Cell transfection was conducted using Lipofectamine 2000 transfection reagent (Invitrogen, Carlsbad, CA, USA) and Opti-MEM (Invitrogen, Carlsbad, CA, USA) following the manufacturer's instructions.

Cell Proliferation Assay

Cell proliferation after transfection was analyzed using CCK-8 assays. Briefly, 1×10^3 cells were seeded into a 96-well plate and incubated for 24 h, 48 h, 72 h, or 96 h. At the indicated time point, 20 µL of CCK-8 (5 mg/mL; Sigma-Aldrich, St. Louis, MO, USA) was added to each well and incubated for 2 h. Then, the OD values of each well were measured at 450 nm with Varioskan software (Thermo Fisher Scientific Inc., Waltham, MA, USA).

Wound-Healing Assay

To examine cell migration *in vitro*, a wound-healing assay was conducted. Briefly, transfected cells were seeded in 6-well tissue culture plates and grown to a density of 70-80%. Cells were scratched using 200 µL pipette tips to create streaks in the monolayer after 12 h culture. The wound closure was observed, and the amount of closure was calculated at 24 h using a light microscope (Olympus, Tokyo, Japan) as described by others.

Transwell Assay

0.8×10^4 cells were seeded in a transwell chamber (Corning, Midland, MI, USA) with Matrigel coating. After the cells were incubated for 12 h, we changed the medium in the upper chamber with serum-free medium, and medium supplemented with 20% FBS was added to the lower compartment. After incubating the cells for 18-24 h at 37°C in 5% CO_2, cells that invaded through the membrane were fixed with 4% formaldehyde and stained with 0.1% crystal violet. Cells adhering to the lower surface were counted under a microscope in 5 randomly selected visual fields.

ESCC Xenograft Model

The entire animal experiment followed the National Institutes of Health (NIH) Animal Use Guidelines and passed the Animal Ethics Committee of Southwestern Medical University. Female nude mice were provided by the Animal Experimental Center of Southwestern Medical University (approximately 3 weeks in size). We injected approximately 1×10^6 EC9706 cells into the bilateral axilla of every mouse and measured the tumor size every week for 4 weeks. After 4 weeks, the nude mice were sacrificed, and the tumor tissues were taken for immunohistochemical analysis. All animal studies followed a blind randomized animal study protocol.

Immunohistochemistry

The tissue was first fixed in 4% paraformaldehyde phosphate buffer, dehydrated with ethanol, embedded in paraffin, and sectioned. Then, antibodies against ECT2 (NO. Ab51494), MMP9 (NO. Ab38898), VEGF (NO. Ab1316), and Ki-67 (NO. Ab15580) (Abcam, Cambridge, MA, USA) were incubated with the sections. A ubiquitin-peroxidase kit was used to detect immunoreactivity, followed by restaining of the sections with hematoxylin, sealing the slides, and examining the slides under a microscope.

H&E Staining

Tissues were fixed with 4% paraformaldehyde at room temperature for 48 h, embedded in paraffin and sliced into 4 µm-thick sections for H&E staining and immunohistochemistry. Sections were counterstained with hematoxylin and eosin at room temperature for 4 min to observe the morphological changes of cells.

Statistical Analysis

The data were statistically analyzed with SPSS Statistics 23. Statistical differences among groups were evaluated using either Student's *t*-test (for two groups) or chi-square test (and Fisher's exact test). *p*-values less than 0.05 were considered significant. Significance for statistical analysis was

defined as $p<0.05$ (*), $p<0.01$ (**) and $p<0.001$ (***). The results are expressed as the mean ± standard deviation from at least three independent experiments.

Results

ECT2 Is High Expressed In Esophageal Squamous Cell Carcinoma Tissue, and the Expression of ECT2 Correlates With the Clinical Characteristics of ESCC Patients

To clarify the correlation between ECT2 and ESCC, we first performed HE staining in three pairs of ESCC cancer tissues and adjacent tissues (Figure 1A). Then, we compared the expression of ECT2 in 40 pairs of ESCC tissues and adjacent tissues by qRT-PCR. ECT2 expression was significantly higher in the ESCC tissues than in the adjacent tissue ($p<0.05$; Figure 1B). To further clarify the correlation between high and low ECT2 expression groups and clinicopathological factors, we found that the expression level of ECT2 was not correlated with age, gender, tumor size, histological grade, and pT stage. However, ECT2 expression was negatively correlated with TNM stage (Table I). Taken together, these data confirmed that ECT2 may be a prognostic factor for ESCC.

ECT2 can Promote Proliferation, Invasion and Migration of ESCC In Vitro

To confirm the mechanistic role of ECT2 in ESCC, we carried out related experiments with siRNA-targeting ECT2 for functional study. Knockdown of ECT2 expression in KYSE140 and EC9706 cells by siRNA significantly attenuated proliferation ($p<0.05$; Figure 2A). To further evaluate the ECT2-mediated ESCC migratory and invasive ability, we silenced the expression of ECT2 in EC9706 and KYSE140 cells by siRNA, and then we performed wound-healing assays and transwell assays. These assays showed that the invasive ability ($p<0.05$; Figure 2B) and migratory ability ($p<0.05$; Figure 2C) of EC9706 and KYSE140 cells following stable knockdown of ECT2 was significantly attenuated compared with the control groups. Taken together, these results showed that ECT2 can promote the proliferation, invasion and migration of ESCC cells *in vitro*, which suggested that ECT2 can confer a strong oncogenic ability to ESCC.

ECT2 can Promote the Proliferation a nd Metastasis of ESCC Through the RhoA-ERK Signaling Pathway

Figure 3A showed the RhoA-ERK signaling pathway in ESCC regulated by ECT2. We compared the expression levels of total-RhoA, GTP-RhoA, ERK, p-ERK, MMP9, and VEGF between siRNA-ECT2 and siRNA-ctrl cells with Western blotting after knocking down the expression of ECT2 in two cell lines by transfection. The results suggested that knocking down ECT2 expression in ESCC cell lines inhibited the activation of the RhoA-ERK signaling pathway and the protein expression of VEGF and MMP9 (Figure 3B). To further verify the integrity of the ECT2-RhoA-ERK-VEGF/MMP9 signaling axis, which can promote the growth, tumorigenesis and metastasis of ESCC cells, we compared the expression

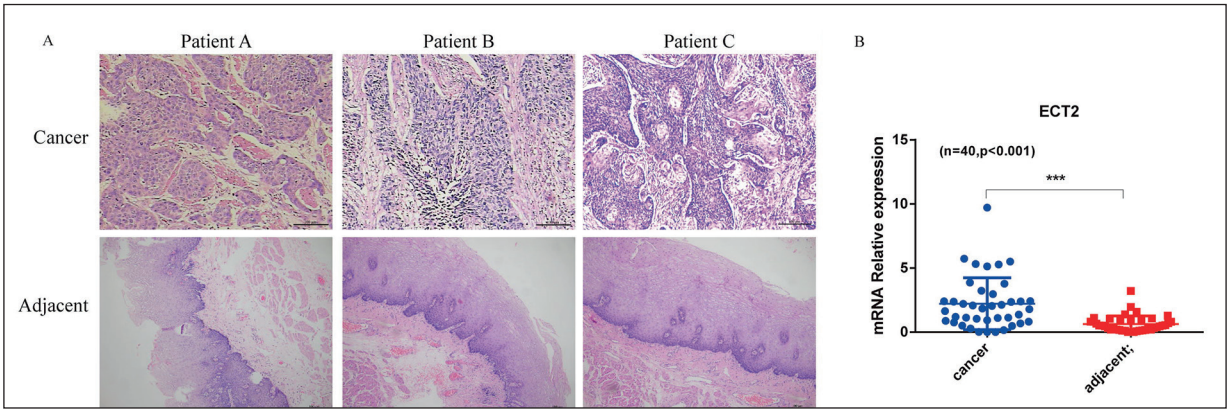

Figure 1. ECT2 is expressed at higher levels in ESCC than in adjacent tissues. **A**, Pathology images of cancerous and adjacent tissue from three patients with ESCC (×100). **B**, Analysis of ECT2 expression in 40 pairs of ESCC cancer tissues and corresponding adjacent tissues by qRT-PCR. Then, GraphPad Prism software was used to process these data.

Table I. Association between the expression of ECT2 in ESCC tissue and the characteristics of patients.

Characteristics	No. of patients (%)	Low expression of ECT2	High expression of ECT2	*p*-value
All cases	46	23	23	
Age				0.743①
< 60	13 (39.39%)	6	7	
≥ 60	33 (60.61%)	17	16	
Gender				0.187②
Male	40 (86.96%)	22	18	
Female	6 (13.04%)	1	5	
Tumor size (cm^3)				0.930③
Vmean ± SD	46	15.86 ± 13.56	16.95 ± 13.89	
Histological grade				0.268④
Low	11 (23.91%)	9	2	
Middle	25 (54.35%)	8	17	
High	10 (21.74%)	6	4	
pT stage				0.405⑤
T1	1 (2.17%)	1	0	
T2	21 (45.66%)	11	10	
T3	23 (50%)	11	12	
T4	1 (2.17%)	0	1	
TNM				0.028*⑥
I	3 (6.52%)	3	0	
II	20 (43.48%)	12	8	
III	11 (23.91%)	5	6	
IV	12 (26.09%)	3	9	

①Chi-square test; ②Fisher's exact test; ③, ④, ⑤, ⑥, Rank sum test; $p<0.05$*.

of downstream proteins in ESCC cells treated with an ERK1/2 inhibitor (VX-11e) or a RhoA inhibitor and DMSO (control) by Western blot. These results confirmed that ECT2 can indeed activate the RhoA-ERK-VEGF/MMP signaling axis (Figure 3C and F). To determine whether ECT2 can promote the development of ESCC by activating this signaling pathway, we carried out corresponding functional experiments. We found that VX-11e and Rho inhibitors inhibited the proliferation and invasion of ESCC cells in two ESCC cell lines (Figure 3D/3E/3G/3H). These results confirmed that ECT2 can promote the proliferation, metastasis and invasion of ESCC cells by participating in a signaling axis (ECT2-RhoA-ERK-VEGF/MMP9) with downstream related proteins.

ECT2 can Promote the Tumorigenic Ability of ESCC In Vivo

We further conducted an *in vitro* experiment to confirm that ECT2 promotes the carcinogenicity of ESCC cells. Therefore, we next conducted a subcutaneous tumor formation experiment in nude mice to determine whether *in vivo* experiments yielded similar results. Subcutaneous injection of ESCC cells in nude mice revealed that ESCC cells treated with an shRNA-ECT2 lentivirus had significantly inhibited tumor growth (Figure 4A-C) compared with the control groups. We further performed HE staining (Figure 4D). The level of Ki-67 and ECT2 signal determined by immunohistochemistry (Figure 4E and F). It also confirmed that tumor growth was significantly reduced following the injection of ESCC cells treated with shRNA-ECT2 lentivirus compared with the injection of control groups of cells. Similarly, *in vitro*, immunohistochemistry results confirmed that the expression of two important proteins downstream of the signaling axis, VEGF and MMP9 (Figure 4G and H), was significantly reduced following knockdown of ECT2 expression in ESCC; these results partly indicated that ECT2 action was mediated through this signaling pathway to promote the proliferation of ESCC.

Discussion

To promote the clinical detection of tumors, surgical resection, neoadjuvant chemotherapy, and treatment of tumors have been improved. However, for esophageal squamous cell carcinoma, because of its distant metastasis and lymph

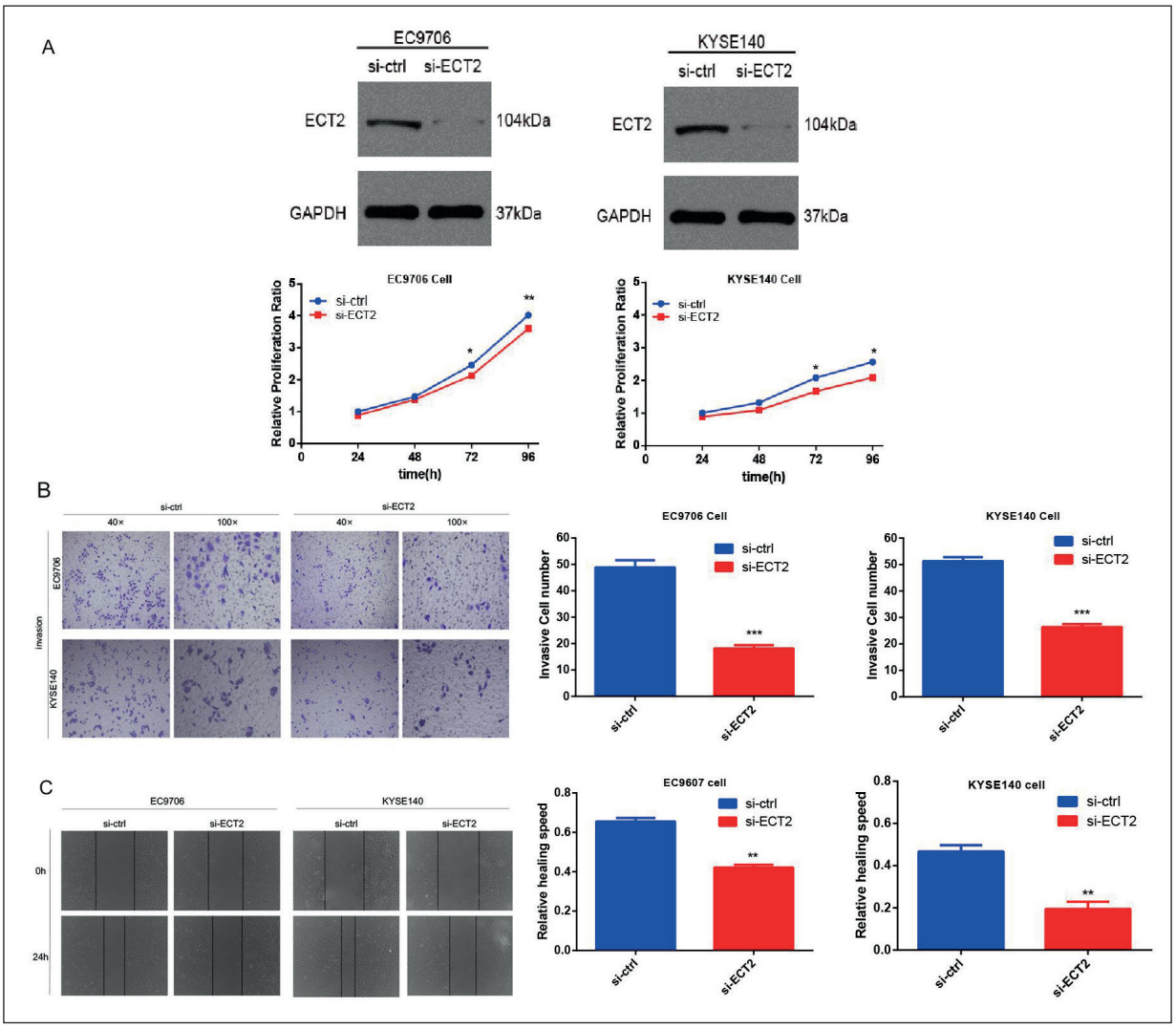

Figure 2. ECT2 can promote proliferation, invasion and migration of ESCC *in vitro*. **A**, CCK8 assays were performed in EC9706 and KYSE140 cells after 4 days. **B**, Transwell assays were performed in EC9706 and KYSE140 cells after 48 h (×40 and ×100). **C**, Wound-healing assays were performed in EC9706 and KYSE140 cells after 24 h (×100). $p<0.05$*, $p<0.01$**, $p<0.001$***.

node metastasis, its overall survival rate is still lower than that of other tumors. The development of esophageal cancer is also caused by the abnormal expression of oncogenes. ECT2 is a 104 kDa protein molecule. The N-terminal region of ECT2 is a triple BRCT domain that regulates the cell cycle and DNA repair[17,18]. The C-terminal contains DH and PH domains. The DH domain is a functional domain, and the PH domain is a catalytic domain[19,20]. In this study, we found that ECT2 was overexpressed in ESCC relative adjacent tissues. The expression is correlated with TNM stages, which can promote the malignant outcome of ESCC. We, then, demonstrated that ECT2 plays a critical regulatory role in the proliferation, tumorigenesis and metastasis of esophageal cancer cell. The reason for up-regulation of ECT2 expression in ESCC remains unclear. ECT2 interacts with several members of the Rho GTPase family and is one of the most important factors catalyzing guanine nucleotide exchange (GEF) of the small Rho GTPase[5-7]. We have also found in some studies[21,22] that ECT2 can promote phosphorylation of ERK and promote disease progression. In this study, we further discovered that ECT2 may potentially affect multiple cellular processes that drive esophageal cancer progression and metastasis through regulation of ERK activity. We hypothesized that when the regulatory factors upstream of the Rho/ERK pathway are activated,

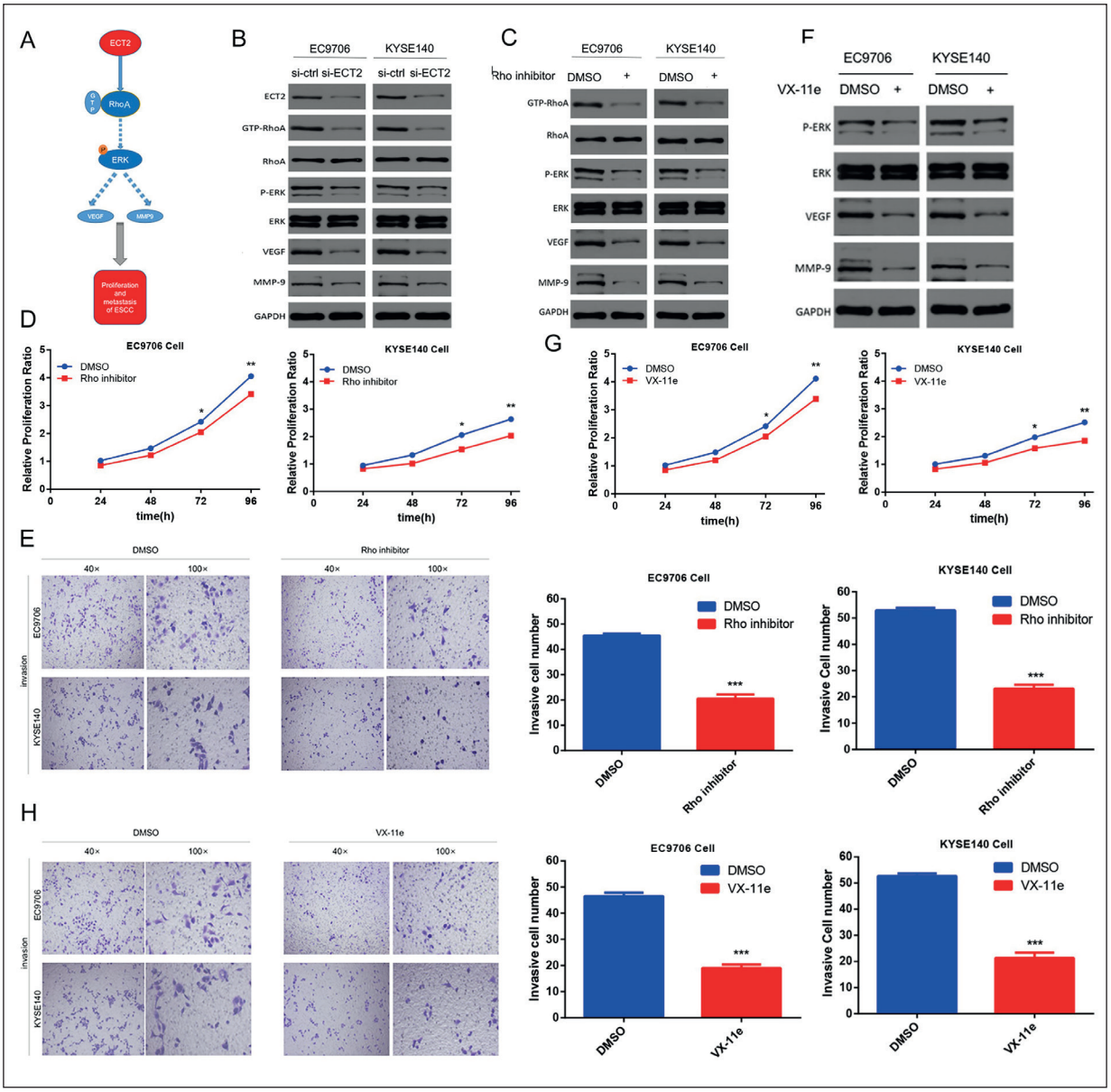

Figure 3. ECT2 can promote the carcinogenic ability of ESCC through the ERK-RhoA-VEGF/MMP9 signaling pathway. **A**, Diagram depicts the downstream signaling axis that may be activated by overexpressed ECT2 in ESCC. Solid arrows indicate direct activation, and dotted arrows indicate possible direct or indirect activation. **B**, Expression levels of proteins in the signaling axis were detected by western blot in ECT2 siRNA or scramble-treated EC9706 and KYSE140 cells. Western blot assays were used to determine the GTPase activity of Rho after different treatments. **C**, The levels of downstream signaling proteins were detected by Western blot assays after treatment with a Rho inhibitor or DMSO. **D, G,** CCK-8 assays were performed in two different treated cell lines. **E, H,** Transwell assays were performed in two different treated ESCC cell lines (×40 and ×100). **F**, The levels of downstream signaling proteins were detected by Western blot after treatment with VX-11e or DMSO. $p<0.05$*, $p<0.01$**, $p<0.001$***.

the number of subpopulations in the tissue that are affected by the Rho/ERK signaling pathway is increased, making this tissue more likely to develop into a malignant tumor. It is likely that ECT2 is a tissue-dependent and multifunctional oncogenic driver in human cancers and participates in multiple pathways to promote malignant transformation in different tissues. Previous studies confirm that ECT2 regulates the RhoA/ERK signaling axis to promote early recurrence in human hepatocellular carcinoma. This pathway is mainly composed of signaling proteins ECT2, GTP-RhoA, RhoA, ERK

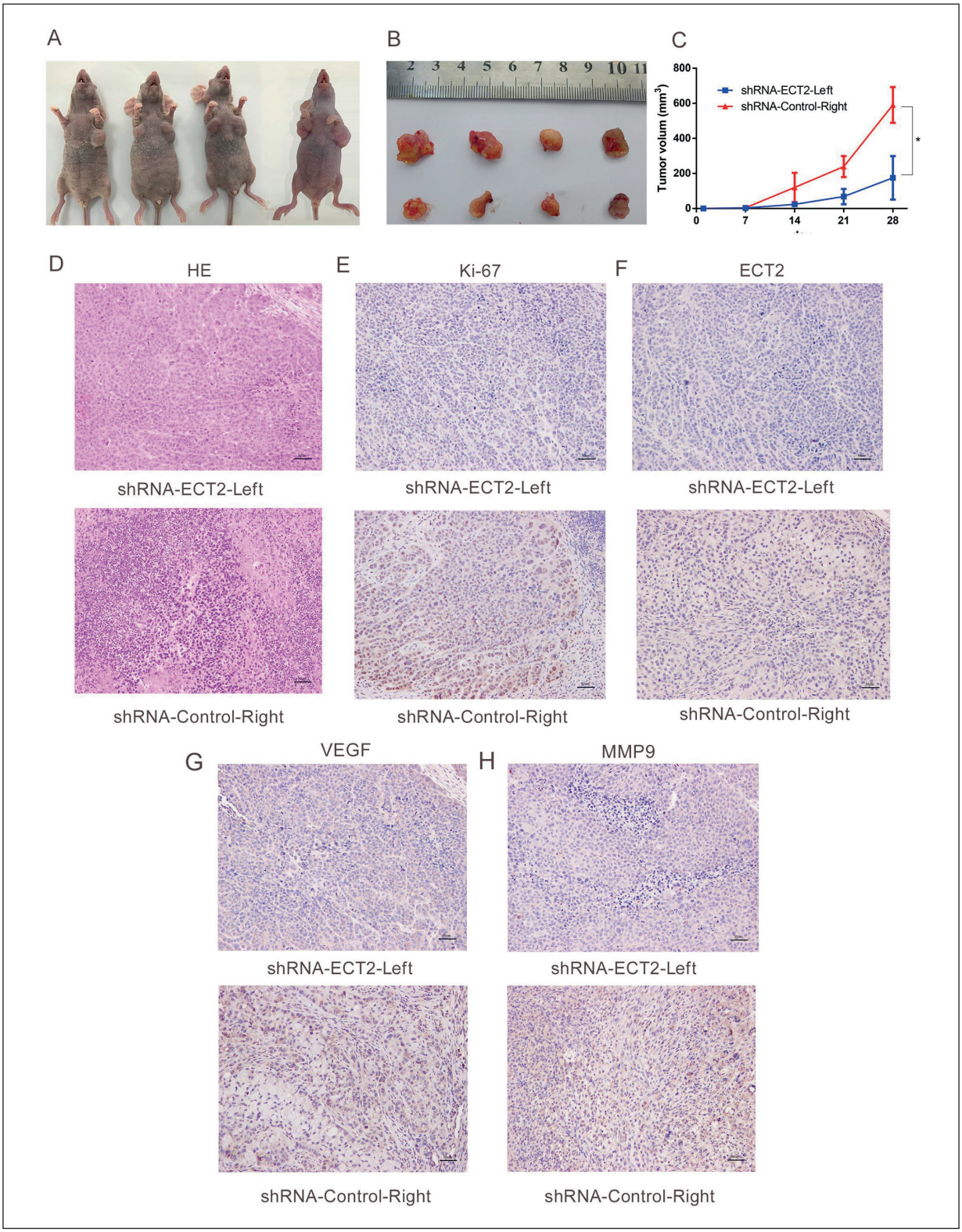

Figure 4. ECT2 can also promote the tumorigenic ability of ESCC through the RhoA-ERK-VEGF/MMP9 signaling pathway *in vivo*. **A, B**, Nude mice were injected with shRNA (short hairpin RNA)-ECT2 EC9706 cells on the left side and shRNA-NC EC9706 NC cells on the right side. The tumor size was compared after 4 weeks. **C**, Measurements of tumor volume are shown for both groups in 4 weeks. **D, E, F, G, H**, ESCC xenografts were stained with H&E. Immunohistochemistry was used to detect Ki-67, ECT2, VEGF and MMP9 (×50). $p<0.05$*, $p<0.01$**, $p<0.001$***.

and p-ERK. In this study, overexpression of ECT2 can activate RhoA to generate GTP-RhoA protein, and then it can activate ERK to generate p-ERK, thus promoting the progression of cancer[14]. In addition, Xu et al[23] showed that propofol inhibits the proliferation of ESCC cells by downregulating the ERK-VEGF/MMP-9 signaling axis. In NSCLC, ECT2 has been linked in PKC-iota Par6-alpha/Rac1 pathway activation and transformation[24]. In ovarian cancer, ECT2 interacts with PKC-iota to activate a MEK/ERK signaling pathway that drives tumorigenesis[25]. In glioma, the ECT2/PSMD14/PTTG1 axis promotes the proliferation of glioma by stabilizing E2F1[26]. Mutant p53 and RB genes have been reported to upregulate the expression of ECT2[27,28]. In addition, by inhibiting the expression of ECT2[29] or by disrupting a domain of ECT2, the activation of related pathways can be inhibited, and the progression of cancer can be repressed.

In vitro, knocking down ECT2 in ESCC cells or treating them with a Rho inhibitor is equivalent to changes in downstream effector proteins after treatment with ERK inhibitors. Therefore, ECT2, RhoA and ERK are considered linear components in this signaling axis. The results of studies using these inhibitors can be used to explain the inhibition of ERK and RhoA. However, kinase activation or inactivation is regulation in the context of a network, and the results obtained with these inhibitors do not fully reflect the effects of ERK. However, combining signaling axis inhibitors may increase their anticancer effects. ERK is a mitogen-activated protein kinase that activates ERK to phosphorylate and activate several important transcriptional regulators. This experiment demonstrates that the ECT2-RhoA-ERK pathway plays an important role in the regulation of VEGF and MMP9, which control cell proliferation, survival, and migration.

Conclusions

Our results reveal the discovery of the ECT2-RhoA-ERK signaling pathway and provide a theoretical basis for clinical staging treatment of ESCC. These conclusions provide a theoretical basis for target of esophageal cancer.

Conflict of Interest

The Authors declare that they have no conflict of interests.

Acknowledgements

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Authors' Contribution

BS and QW designed this study. BS, QW and CL performed the experiments. LZ and GL analyzed the data. TL, SY and ML gave guidance to the experiment. ML drafted the manuscript. All authors approved the final version of the manuscript.

Ethics Approval and Consent to Participate

All patients were informed of the experimental protocol and written informed consent was obtained from every participant. The present study was approved by the Ethics Committee of the Affiliated Hospital of Southwest Medical University. The entire animal experiment followed the National Institutes of Health (NIH) Animal Use Guidelines and passed the Animal Ethics Committee of Southwestern Medical University. .

References

1) Chen WQ, Zheng RS, Baade PD, Zhang SW, Zeng HM, Bray F, Jemal A, Yu XQ, He J. Cancer statistics in China, 2015. CA Cancer J Clin 2016; 66: 115-132.
2) Short MW, Burgers KG, Fry VT. Esophageal cancer. Am Fam Physician 2017; 95: 22-28.
3) Yue C, Li M, Da C, Meng H, Lv S, Zhao X. Association between genetic variants and esophageal cancer risk. Oncotarget 2017; 8: 47167-47174.
4) Zhang L, Ma JJ, Han Y, Liu JQ, Zhou W, Hong L, Fan DM. Targeted therapy in esophageal cancer. Expert Rev Gastroenterol Hepatol 2016;10: 595-604.
5) Tatsumoto T, Xie X, Blumenthal R, Okamoto I, Miki T. Human ECT2 is an exchange factor for Rho GTPases, phosphorylated in G2/M phases, and involved in cytokinesis. J Cell Biol 1999; 147: 921-928.
6) Miki T, Smith CL, Long JE, Eva A, Fleming TP. Oncogene ect2 is related to regulators of small GTP-binding proteins. Nature 1993; 362: 462-465.

7) Cook DR, Rossman KL, Der CJ. Rho guanine nucleotide exchange factors: regulators of Rho GTPase activity in development and disease. Oncogene 2013; 31: 4021-4035.

8) Saito S, Liu XF, Kamijo K, Raziuddin R, Tatsumoto T, Okamoto I, Chen X, Lee CC, Lorenzi MV, Ohara N, Miki T. Deregulation and mislocalization of the cytokinesis regulator ECT2 activate the Rho signaling pathways leading to malignant transformation. J Biol Chem 2004; 279: 7169-7179.

9) Zhou S, Wang P, Su X, Chen J, Chen H, Yang H, Fang A, Xie L, Yao Y, Yang J. High ECT2 expression is an independent prognostic factor for poor overall survival and recurrence-free survival in non-small cell lung adenocarcinoma. PLoS One 2017; 12: e0187356.

10) Jin Y, Yu Y, Shao Q, Ma Y, Zhang R, Yao H, Xu Y. Up-regulation of ECT2 is associated with poor prognosis in gastric cancer patients. Int J Clin Exp Pathol 2014; 7: 8724-8731.

11) Justilien V, Fields AP. Ect2 links the PKCiota-Par6alpha complex to Rac1 activation and cellular transformation. Oncogene 2009; 28: 3597-3607.

12) Wang HK, Liang JF, Zheng HX, Xiao H. Expression and prognostic significance of ECT2 in invasive breast cancer. J Clin Pathol 2018; 71: 442-445.

13) Chen Z, Liu J, Zhang Y. Role of epithelial cell transforming sequence 2 (ECT2) in predicting prognosis of osteosarcoma. Med Sci Monit 2017; 23: 3861-3868.

14) Chen J, Xia H, Zhang X, Karthik S, Pratap SV, Ooi LL, Hong W, Hui KM. ECT2 regulates the Rho/ERK signalling axis to promote early recurrence in human hepatocellular carcinoma. J Hepatol 2015; 62: 1287-1295.

15) Li Y, Cai X, Chen B, Gu H, Liu C. Overexpression of epithelial cell transforming 2 protein in colorectal carcinoma predicts a poor prognosis. Exp Ther Med 2017; 14: 4862-4868.

16) Pfaffl MW. A new mathematical model for relative quantification in real-time RT-PCR. Nucleic Acids Res 2001; 29: e45.

17) Solski PA, Wilder RS, Rossman KL, Sondek J, Cox AD, Campbell SL, Der CJ. Requirement for C-terminal sequences in regulation of ECT2 guanine necleotide exchange specificity and transformation. J Biol Chem 2004; 279: 25226-25233.

18) Vanni C, Parodi A, Mancini P, Visco V, Ottaviano C, Torrisi MR, Eva A. Phosphorylation-independent membrane relocalization of ezrin following association with Dbl in vivo. Oncogene 2004; 23: 4098-4106.

19) Li M, Bian C, Yu X. Poly (ADP-ribosyl)ation is recognized by ECT2 during mitosis. Cell Cycle 2014; 13: 2944-2951.

20) Tian L, Li W, Yang L, Chang N, Fan X, Ji X, Xie J, Yang L, Li L. Cannabinoid receptor 1 participates in liver inflammation by promoting m1 macrophage polarization RhoA/NF-κB p65 and ERK1/2 pathways, respectively, in mouse liver fibrogenesis. Front Immunol 2017; 8: 1214.

21) Chen Y, Tian P, Liu Y. P53 and protein phosphorylation regulate the oncogenic role of epithelial cell transforming 2 (ECT2). Med Sci Monit 2017; 23: 3154-3160.

22) Chen X, Zhang S, Wang Z, Wang F, Cao X, Wu Q, Zhao C, Ma H, Ye F, Wang H, Fang Z. Supervillin promotes epithelial-mesenchymal transition and metastasis of hepatocellular carcinoma in hypoxia via activation of the RhoA/ROCK-ERK/p38 pathway. J Exp Clin Cancer Res 2018; 37: 128.

23) Xu YB, Du QH, Zhang MY, Yun P, He CY. Propofol suppresses proliferation, invasion and angiogenesis by down-regulating ERK-VEGF/MMP-9 signaling in Eca-109 esophageal squamous cell carcinoma cells. Eur Rev Med Pharmacol Sci 2013; 17: 2486-2494.

24) Justilien V, Fields AP. Ect2 links the PKCiota-Par6alpha complex to Rac1 activation and cellular transformation. Oncogene 2009; 28: 3597-3607.

25) Wang Y, Hill KS, Fields AP. PKCiota maintains a tumor-initiating cell phenotype that is required for ovarian tumorigenesis. Mol Cancer Res 2013; 11: 1624-1635.

26) Zhi T, Jiang K, Xu X, Yu T, Zhou F, Wang Y, Liu N, Zhang J. ECT2/PSMD14/PTTG1 axis promotes the proliferation of glioma through stabilizing E2F1. Neuro Oncol 2019; 21: 462-473.

27) Scoumanne A, Chen X. The epithelial cell transforming sequence 2, a guanine nucleotide exchange factor for Rho GTPases, is repressed by p53 via protein methyltransferases and is required for G1-S transition. Cancer Res 2006; 66: 6271-6279.

28) Eguchi T, Takaki T, Itadani H, Kotani H. RB silencing compromises the DNA damage-induced G2/M checkpoint and causes deregulated expression of the ECT2 oncogene. Oncogene 2007; 26: 509-520.

29) Yu Y, Cai O, Wu P, Tan S. MiR-490-5p inhibits the stemness of hepatocellular carcinoma cells by targeting ECT2. J Cell Biochem 2019; 120: 967-976.