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Suppression of Tescalcin inhibits growth and metastasis in renal cell carcinoma *via* downregulating NHE1 and NF-kB signaling

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ABSTRACT

Background: Renal cell carcinoma (RCC) is the most common form of kidney cancer. Recent studies reported that Tescalcin was overexpressed in various tumor types. However, the status of Tescalcin protein expression in RCC and its biological function is uncertain. This study was designed to investigate the expression of Tescalcin in human RCC and its biological function.

Methods: shRNA transfection was performed to abrogates the expression of Tescalcin. Quantitative real time PCR and western blotting assays were used to determine mRNA and protein expression levels, respectively. The cell viability was analyzed by MTT and colony formation. Cell flow cytometry was used to assess pHi value and cell apoptosis. Cell invasive and migratory ability was measured with modified Boyden chamber assay. Xenograft model was setup to evaluate tumor growth.

Results: Tescalcin was overexpressed in RCC tissues compared with matched normal tissues. It was also overexpressed in RCC cell lines relative that of normal cells. Suppression Tescalcin with specific shRNA resulted in the inhibition of cell proliferation, migration, invasion and apoptosis of RCC cells. Additionally, silencing of Tescalcin also caused the inhibition of the tumor growth in nude mice. Mechanistic study showed that Tescalcin regulated cell proliferation, migration and invasion *via* NHE1/pHi axis as well as AKT/NF-κB signaling pathway.

Conclusions: These findings demonstrate that atopic expression of Tescalcin facilitates the survival, migration and invasion of RCC cells *via* NHE1/pHi axis as well as AKT/ NF-κB signaling pathway, providing new perspectives for the future study of Tescalcin as a therapeutic target for RCC.

1. Introduction

Tescalcin is a 24-kDa EF-hand Ca(2+) and Mg(2+) binding protein, which is the member of calcineurin homologous protein (CHP) family (Perera et al., 2001). It is expressed in mouse testis in early development as well as hematopoietic cells, brain, stomach and heart of the adult mouse (Kolobynina et al., 2016). Characterization of Tescalcin promoter revealed that transcription factors including Sp1and Sp3 regulate its activation (Perera et al., 2010). Studies have shown that Tescalcin is able to interaction with protein kinase glycogen-synthase kinase 3 (GSK3), subunit 4 of the COP9 signalosome (CSN4) and Na+/H+ exchanger 1 (NHE1) and impact their activity (Li et al., 2003; Levay & Slepak, 2014). Previous studies have demonstrated that dysregulation of the Tescalcin is involved in many types of malignant tumors including gastric cancer, melanoma, colorectal cancer and show biological functions in these tumors (Kolobynina et al., 2016; Kang et al., 2016; Fan et al., 2015a). Our preliminary study showed upregulation of Tescalcin in real cell carcinoma (RCC). However, the biofunction of Tescalcin in RCC development and its underlying mechanism remain largely unclear.

Dysregulated pH is one of the hallmarks of cancer. Normal cells have extracellular pH (pHe) of around 7.4 and intracellular pH (pHi) of ~7.2, whereas in cancer cells pHe reduces to 6.7–7.1 and pHi is approximately 7.4. The increased pHi is in favor of tumor development and progression. The plasma membrane ion pumps, which is responsible for the H+ efflux play critical role in maintaining a higher

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Table 1
Primer Sets used for qPCR.

Gene Symbol	Primer Sets	
E-Cadherin	F: CGGGAATGCAGTTGAGGAT	R: ATTGCATAGTTAGTCACACCTC
Vimentin	F: AGGAAATGGCTCGTCACCTTC GTGAATA	R: GGAGTGTCGGTTGTTAAGAACTAG AGCT
Tescalcin	F: CCTACCATTCGCAAGGAGAA	R: TTCTCGATGTGAGGGTTTCC
COX-2	F: TTCAAATGAGATTGTGGGAAA AT	R: AGATCATCTCTGCCTGAGTATCTT
Beta-actin	F: AAG AACCGCGAGAAGAT	R: TGATGACCTGGCCGTCAGG

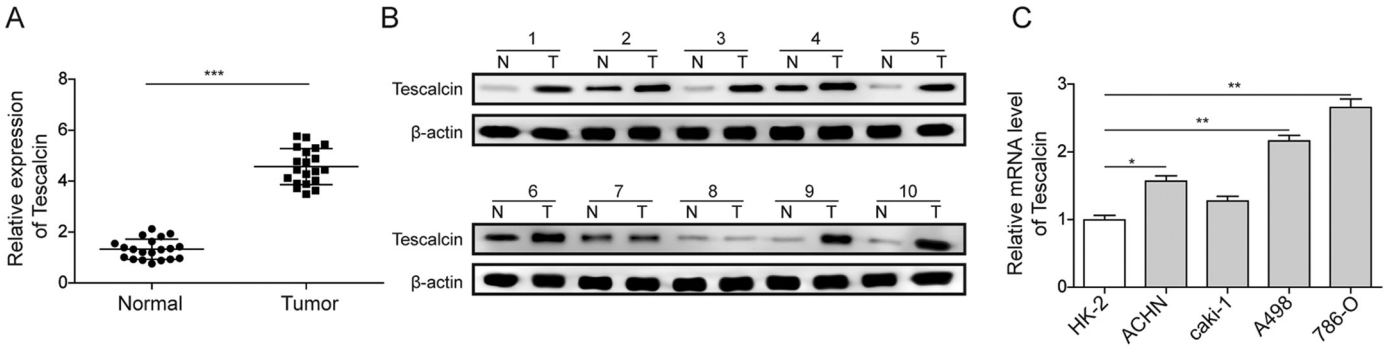

Fig. 1. Tescalcin expression in cell lines and RCC tissues. (A) The mRNA level of Tescalcin is detected in 20 pairs of RCC tissues and matched normal tissues with qRT-PCR. (B) The protein level of Tescalcin is detected in 10 pairs of RCC tissues and matched normal tissues with western blot analysis. (C) The mRNA level of Tescalcin is detected in human RCC cell lines (786-O, ACHN, Caki-1 and A498) and immortalized proximal tubule epithelial cells HK2 with qRT-PCR. "*" denotes $p < .05$, "**" denotes $p < .01$ and "***" denotes $p < .001$. Data are expressed as the mean ± SD based on three independent experiments.

pHi and lower pHe. NHE modulates the pH of the intracellular organelles and cytoplasm and prevents cells from the acidification, thereby effecting on tumor cell viability and proliferation (Harguindey et al., 2013). Upregulation of NHE1expression levelcaused the increased pHi, which further enhanced the tumorigenesis and progression. For example, NHE1 causes the decrease of pHe causing the degradation extracellular matrix and increases of invasion and metastasis in breast cancer (Amith & Fliegel, 2013). More recently, it was reported that Tescalcin was upregulated in acute myeloid leukemia (AML) cell line during continuous sorafenib treatment and was involved in sorafenib resistance mediated by NHE1(Man et al., 2014).

Nuclear factor κB (NF-κB) proteins comprise a family of transcription factors including RelA, RelB, c-Rel, p50 and p52, which form homodimeric and heterodimeric complexes that are involved in signaling through canonical and non-canonical pathways (Perkins, 2012; Zhang et al., 2011) Previous studies reported that the oncogenic mutations and inflammatory context induced constitutive NF-κB activity in various tumor types (Mak et al., 2015; Basseres et al., 2010; Nagel et al., 2015). Abnormal NF-κB signaling is emerging as a hallmark of tumor, which is involved in the regulation of tumor growth, metastasis, and resistance to treatment (Basseres et al., 2010; Pikarsky et al., 2004; Meylan et al., 2009; Basseres & Baldwin, 2006). NF-κB is a critical regulator of a wide variety of genes including chemokines, inflammatory cytokines, cell cycle-related genes, genes involved in migration and invasion (Pahl, 1999; Schmidt et al., 2007; Escarcega et al., 2007).

In the present study, we reported that Tescalcin is highly expressed in RCC tissues compared with normal tissues, and silencing of Tescalcin significant inhibited the survival and growth of RCC *in vitro* and *in vivo*, and suppressed cell migration and invasion in RCC cells. Furthermore, mechanism investigation revealed that Tescalcin is involved in the progression of RCC through NHE1/pHi axis and Akt/ NF-κB signaling pathway.

2. Materials and methods

2.1. Patients and samples

Totally 20 pairs of RCC tissues and matched normal tissues were collected from the patients, who were enrolled in the Third Xiangya Hospital of Central South University according to the protocols approved by the Ethics Review Board.

2.2. Cell culture

Human RCC cell lines (786-O, ACHN, Caki-1 and A498) and immortalized proximal tubule epithelial cells HK2 were purchased from Shanghai Cell Bank, Chinese Academy of Sciences (Shanghai, China) and cultured in DMEM (St. Louis, MO, USA) containing 10% FBS and 1% penicillin/ streptomycin at 37 °C in a humidified atmosphere of 5% CO2.

2.3. Short-hairpin RNA transfection

The shRNA targeting Tescalcin were inserted into the pGPU6/GFP/ Neo siRNA expression vector (GenePharma). When the confluency of cells was around 80%, the mixture of 4 μg of plasmid DNA and 10 μl of Lipofectamine 2000 (Invitrogen) were added to cells. A scrambled shRNA was served as a negative control. Cells were collected and subjected to subsequent analysis of Tescalcin expression with Q-PCR and western blotting at the indicated time points post transfection.

2.4. Reverse transcription-quantitative polymerase chain reaction (RT-qPCR)

RNA was extracted from RCC cell lines and normal/ tumor paired tissues using TRIzol reagent (Invitrogen, Carlsbad, CA) according to the manufacturer's protocol. A first strand cDNA kit from Sigma (Munich, Germany) was used for cDNA synthesis reaction, according to the manufacturer protocol. The SYBR Green PCR kit from Thermo was used for PCR amplification, which was run on an ABI 7300 Thermocycler

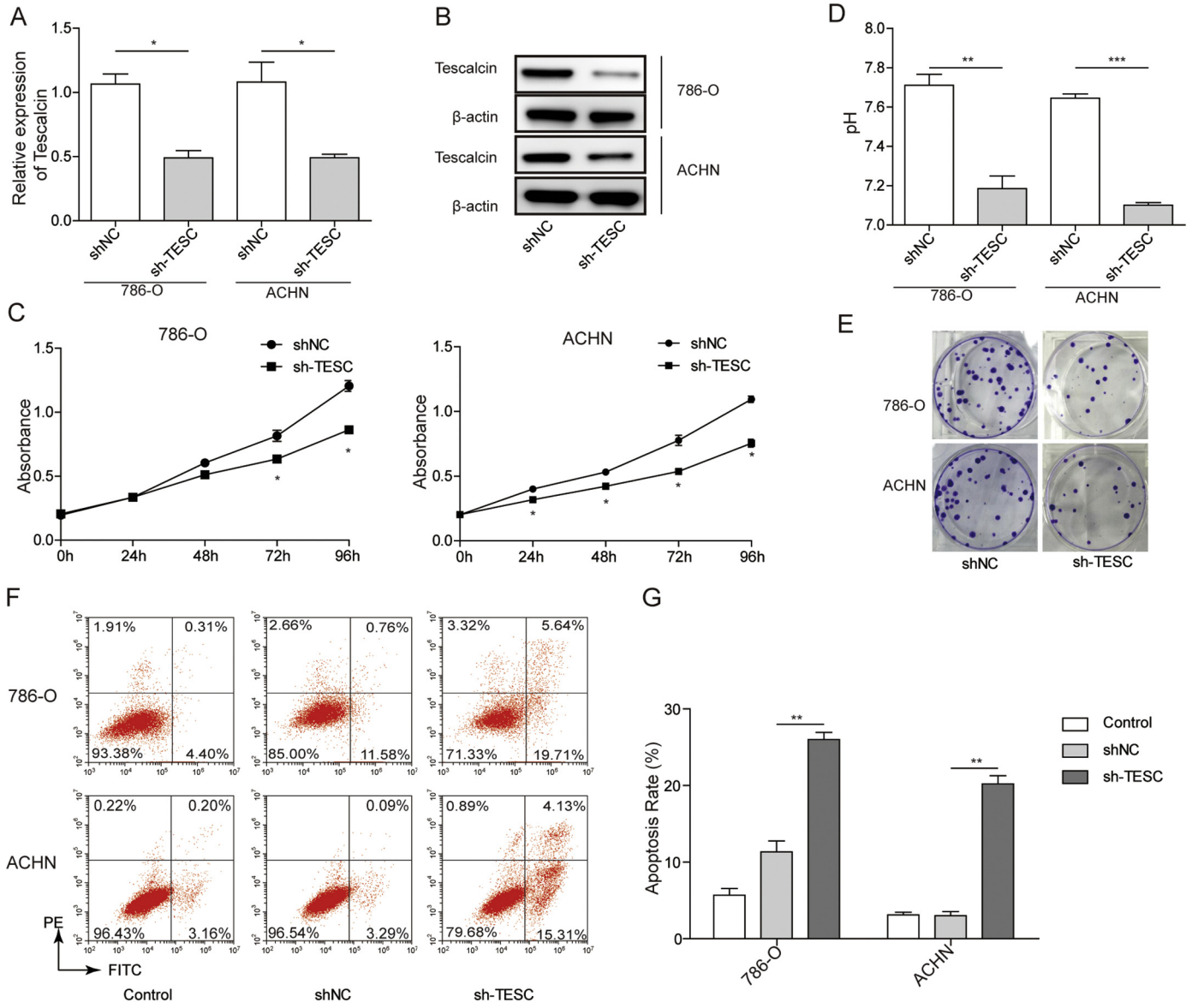

Fig. 2. Down-regulation of Tescalcin suppresses proliferation of RCC cells. (A) mRNA expression of Tescalcin was detected in ACHN and 786-O cells post transfection with the specific shRNA of Tescalcin by using qRT-PCR. (B) The protein expression of Tescalcin was detected in ACHN and 786-O cells after transfection with the specific shRNA of Tescalcin by using western blot. (C) The cell viability of ACHN and 786-O cells was analyzed after transfection with the specific shRNA for Tescalcin for 24, 48, 72 and 96 h by using MTT, respectively. (D) ACHN and 786-O cells were transfected with the specific shRNA for Tescalcin or control shRNA. After cell labeling with BCECF-AM, pHi was analyzed using flow cytometric analysis. (E) The colony formation ability of ACHN and 786-O cells was analyzed after transfection with the specific shRNA for Tescalcin, respectively. (F and G) Cell apoptosis was detected in ACHN and 786-O cells post transfection with the specific shRNA of Tescalcin by using flow cytometry analysis "*" denotes $p < .05$ and "***" denotes $p < .001$, compared with the control cell. Data are expressed as the mean ± SD based on three independent experiments.

(Applied Biosystems, Foster City, CA, USA). The specific primers used for PCR were list in Table 1. The $2^{-\Delta\Delta Cq}$ method was used to calculate differences in mRNA expression(Livak & Schmittgen, 2001).

2.5. Cell viability assay

To determine cell viability, MTT assays and colony formation assays were used. For MTT assays, the cells were cultured in 96-well plates at 3×10^3 cells per well. After removing the supernatant of each well and washing twice by PBS, 20 µl of MTT solution and 100 µl of medium were then introduced. The absorbance was measured at 490 nm. For colony formation assays, 600 cells were cultured in six-well plates at 37 °C until the cells in control plates have formed colonies with good size. The visible colonies were fixed and stained with 0.5% crystal violet and methanol.

2.6. Annexin V/propidium iodide apoptosis assay

Cell apoptosis was assessed with Annexin V-FITC Apoptosis Detection Kit purchased from Sigma according to the manufactory's instructions. 5×10^5 cells were collected by centrifugation and re-suspended in binding buffer. Add 5 µl of annexin V-FITC and 5 µl of propidium iodide (PI) and incubate at room temperature for 5 min in the dark. The annexin V-FITC binding and PI staining will be analyzed by flow cytometry.

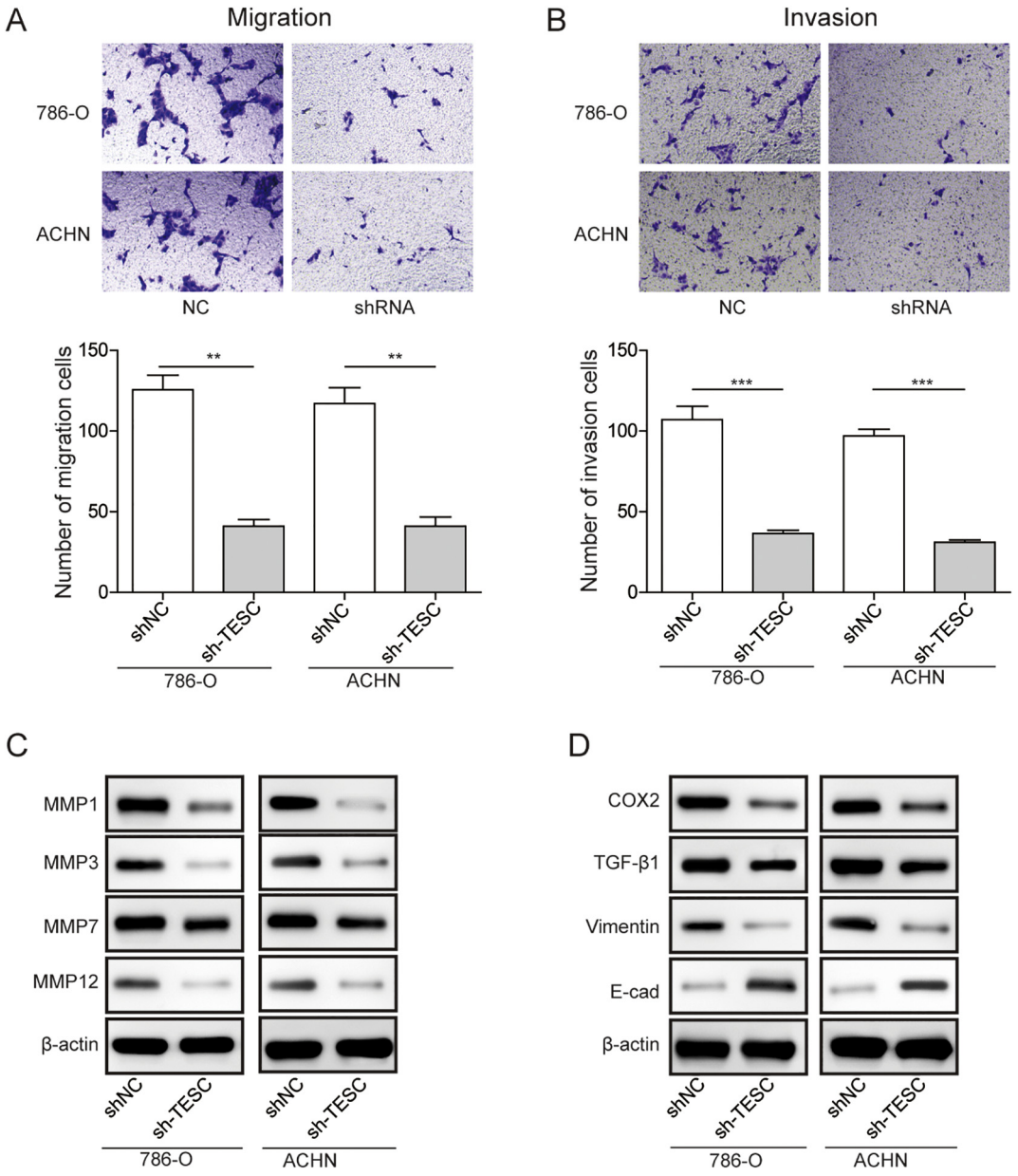

Fig. 3. Down-regulation of Tescalcin inhibits the migration and invasion of RCC cells. After transfection with specific shRNA for Tescalcin in 786-O cells for 48 h, cell migration (A) and invasion (B) were determined with modified Boyden trans-well assays. (C) After ACHN and 786-O cellswere transfected with the specific shRNA of Tescalcin for 48 h, the expressions of MMP1, MMP3, MMP7 and MMP12 were assessed by western blot.β-actin serves as internal reference. (D) After ACHN and 786-O cellswere transfected with the specific shRNA of Tescalcin for 48 h, the expressions of COX-2, TGF-β, vimentin and E-cadherin were analyzed by western blot.β-actin serves as internal reference. "**" denotes $p < .01$ and "***" denotes $p < .001$, compared with the control cells. Data are expressed as the mean ± SD based on three independent experiments.

2.7. Cell invasion and migration assay

A modified Boyden chamber assay was used to determine the invasion and migration activity of 786-O and ACHN cells. Briefly, cells (1×10^4 cells/well) transfected with shRNA or control were suspended in culture media and added into the upper trans-well chamber with or without Matrigel (BD Biosciences) on the upper surface of the membrane (Sigma). The lower chamber was soaked with DMEM medium supplemented with 10% FBS. After incubation for 24 h, the chambers were washed three times and fixed with 4% paraformaldehyde for 10 min. Added 0.5% crystal violet solution to the chambers and incubated at RT for 2 h. Remove crystal violet carefully and immerse the plates in tap water to rinse off crystal violet and the cells which passed through the membrane were counted visually under a microscope (OLYMPUS).

2.8. Protein isolation and western blot analysis

Total proteins were extracted using 1% Triton X-100 reagent (Sigma-Aldrich; Merck KGaA, Darmstadt, Germany) on ice. The protein concentration was examined with a bicinchoninic acid assay kit (EMD Millipore, Billerica, MA, USA). The proteins were subjected to SDS polyacrylamide gel electrophoresis and electro-transferring onto PVDF membranes. Anti-Tescalcin (cat. no. sc-109446; dilution, 1000), -NHE1 (cat. no. sc-136239; dilution, 1:1000), -p-P65 (cat. no. sc-166748; dilution, 1:1000), -p-IκBa (cat. no. sc-8404; dilution, 1:1000), -p-AKT (cat. no. sc-271964; dilution, 1:1000), -p-GSK-3β(cat. no. sc-81495; dilution, 1:1000) and -β-actin (cat. no. sc-4778; dilution, 1:5000)

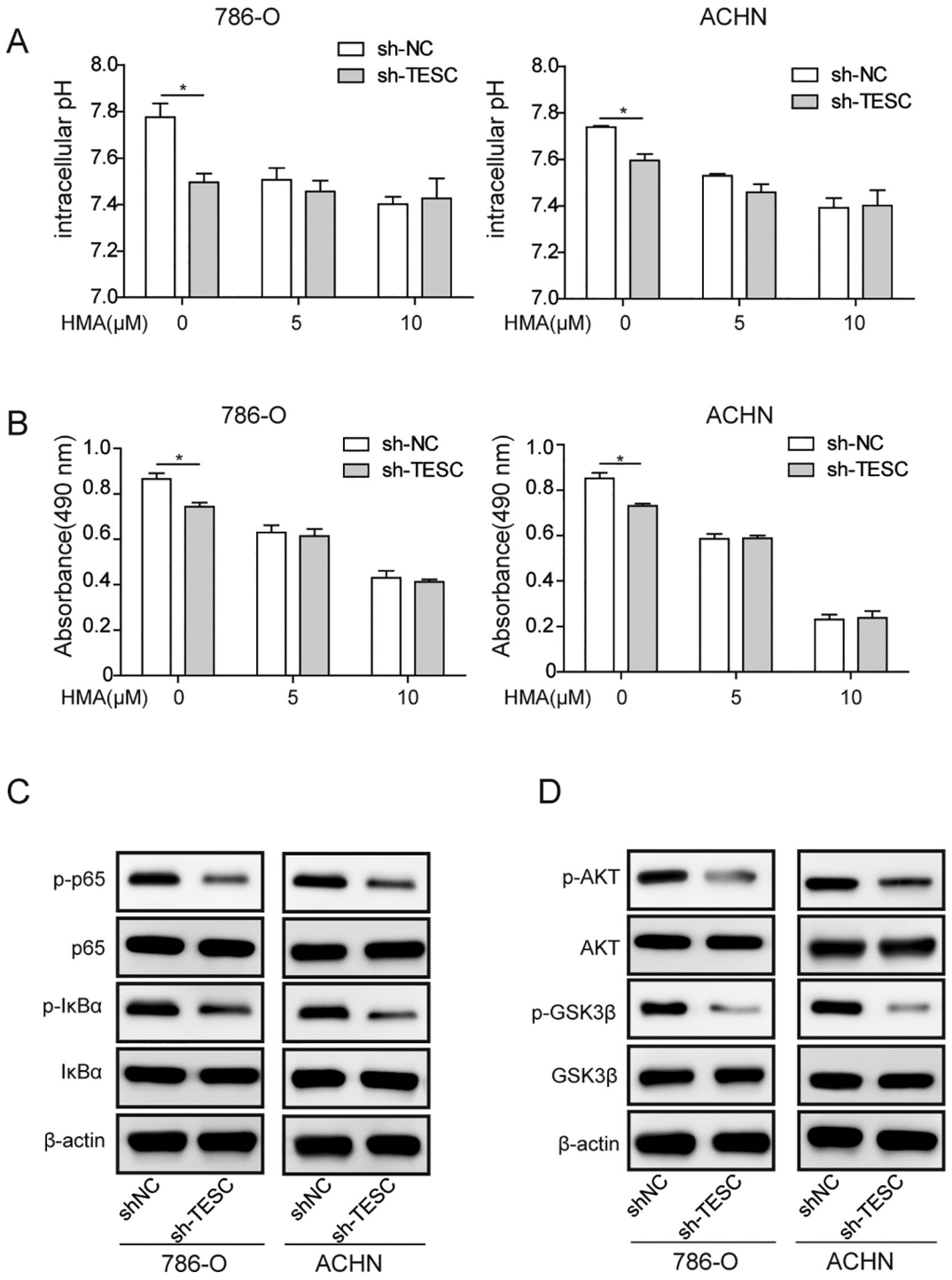

Fig. 4. Tescalcin regulate cell viability, migration and invasion *via* NHE1/pHi pathway. (A) ACHN and 786-O cells were transfected with the specific shRNA for Tescalcin or control shRNA and were co-treated with HMA at 5 μM or 10 μM for 72 h. After cell labeling with BCECF-AM, pHi was analyzed using flow cytometric analysis. (B) ACHN and 786-O cells were transfected with the specific shRNA for Tescalcin or control shRNA and were co-treated with HMA at 5 μM or 10 μM for 96 h.Cells viability was analyzed by MTT. (C and D) After ACHN and 786-O cells were transfected with the specific shRNA of Tescalcin for 48 h, the expressions of p-p65, p-IκB, p-GSK3β and p-AKT were analyzed by western blot.β-actin serves as internal reference. "*" denotes $p < .05$, compared with the control cells. Data are expressed as the mean ± SD based on three independent experiments.

antibodies (Santa Cruz Biotechnology, Inc., Dallas, TX, USA) were used to detect the expression of each protein. Secondary horseradish peroxidase-conjugated donkey anti-goat (dilution, 1:5000; sc-2020) or anti-mouse (dilution, 1:5000; sc-2314) antibodies (Santa Cruz Biotechnology, Inc.) were used and bands were developed using RapidStep™ ECL Reagent (EMD Millipore) according to the manufacturer's directions.

2.9. Evaluation of pHi

The cell flow cytometry assay was used to evaluated the pHi of cells as described previously(Gao et al., 2014). Briefly, cells in serum-free medium were labeled with pH-sensitive fluorescent probe BCECF-AM. BCECF was in the excited state at 490 and 440 nm. The generated fluorescent signal was detected at 535 nm. The Intensity ratio (490/

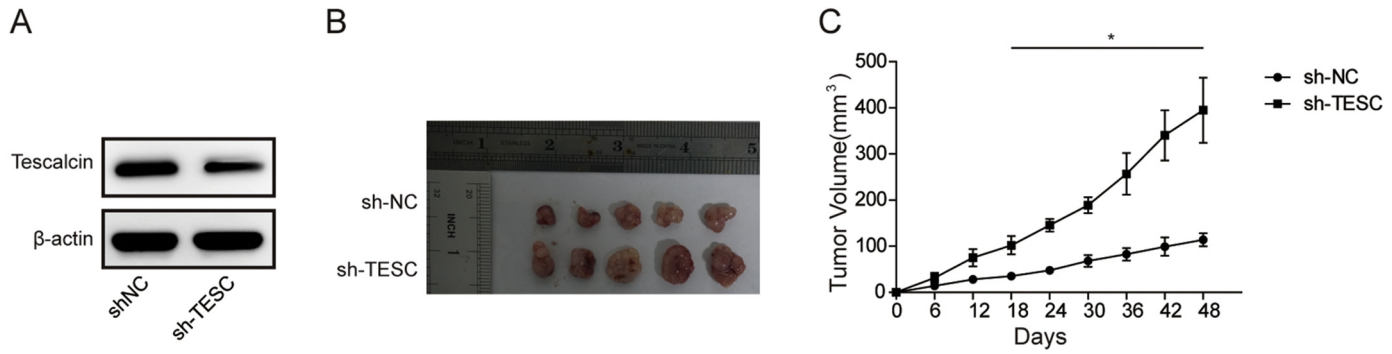

Fig. 5. Downregulation of Tescalcin suppresses tumor growth *in vivo*. ACHN cells transfected with specific shRNA for Tescalcin or negative control were subcutaneously implanted in nude mice. Tumor volumes were evaluated after injection. (A) The protein expressions of Tescalcin in tumor tissue were analyzed by western blotting. (B) Photograph of xenograft mouse model subcutaneously injected with ACHN cells of each group ($n = 5$). (C) Tumor volumes were evaluated after injection every 6 days for 48 days. "*" denotes $P < .05$, compared with the control cells. Data are expressed as the mean ± SD based on three independent experiments.

440) was plotted *vs.* pHi.

2.10. Tumor xenografts

3×10^6 ACHN cells were subcutaneously inoculated into the right flank of five six-week old BALB/c nude mice, which were purchased from Charles River Laboratories. Tumor volumes will be measured twice weekly in two dimensions using a caliper, and the volume will be expressed in mm^3 using the formula: $V = 0.5(a \times b^2)$ where a and b are the long and short diameters of the tumor, respectively.After injection for 48 days, the mice were sacrificed, and the formative tumors were photographed weighted on a digital balance. All experimental procedures involving animals were performed in accordance with animal protocols approved by Laboratory Animal Center of Third Xiangya Hospital of Central South University.

2.11. Statistical analysis

Statistical analysis was carried out with the SPSS software (version 13.0; SPSS, Inc., Chicago, IL, USA). All data were presented as the mean of at least triplicate samples ± standard deviation. The differences between groups were determined by the Student *t*-test or oneway ANOVA. $P < .05$ was considered statistically significant.

3. Results

3.1. Tescalcin is overexpressed in RCC tissues

The expression of Tescalcin is shown to be upregulated in several tumor types, but in RCC its expression is unclear. Firstly, we detected the expression of Tescalcin in RCC and matched normal tissues. The results showed that both of mRNA expression level ($P < .001$, $n = 20$) and protein level ($n = 10$) of Tescalcin was upregulated in the RCC tissues than that in the matched normal tissues (Fig. 1A and B). Moreover, the expression level of Tescalcin was also examined in RCC cell lines and proximal tubule epithelial cells HK2. The RCC cells displayed higher expression of Tescalcin relative to HK2 cells (Fig. 1C). These results suggested that Tescalcin is upregulated in RCC tissues and RCC cell lines.

3.2. Knockdown of Tescalcin suppresses the cell viability of RCC cells

To examine the biological function of Tescalcin in RCC, ACHN and 786-O was selected to perform the functional experiments. To address this issue, we used the specific shRNA to inhibit Tescalcin expression. q-RT-PCR and western blotting assays were used to detect mRNA and protein expression levels of Tescalcin ($P < .05$) (Fig. 2A and B). Our findings revealed that silencing of Tescalcin time-dependently suppressed the cell viability in ACHN and 786-O cells ($P < .05$) (Fig. 2C). We also investigated the effect of Tescalcin on pHi of RCC cells by using the fluorescent dye BCECFAM. As shown in Fig. 2D, knockdown of Tescalcin resulted in a decrease in pHi value in 786-O cells ($P < .01$) and ACHN cells ($P < .001$) (Fig. 2D). Furthermore,inhibition of Tescalcin remarkably suppressed the ability of colony formation in 786-O cells and ACHN cells (Fig. 2E). Consistently, *via* employing PI/Annexin staining assay, silencing of inhibition of Tescalcin elevated apoptosis rate (including early apoptosis and late apoptosis) in ACHN and 786-O cells (Fig. 2F and G). Taken together, Tescalcin knockdown inhibited proliferation of ACHN and 786-O cells.

3.3. Downregulation of Tescalcin represses the migration and invasion of RCC cells

Cell migration and invasion are important steps during tumor metastasis. We investigated whether Tescalcin could have influence on migration and invasion of RCC cells with a modified Boyden chamber assay. We found that specific inhibition of Tescalcin significantly repressed the migratory ability of ACHN and 786-O cells ($P < .01$) (Fig. 3A). The cell invasion of the RCC cells was further examined with a modified Boyden chamber invasion assay. Likely, knockdown of Tescalcin in ACHN and 786-O cells caused a remarkable reduction of their invasion ($P < .001$) (Fig. 3B). EMT is believed to be a crucial event in tumor metastasis. To determine whether the downregulation of migratory and invasion ability of the RCC cells caused by Tescalcin silencing were related to EMT, changes of EMT associated factors were investigated. Inhibition of Tescalcin robustly decreased the expression of matrix metallopeptidase including MMP-7, MMP-1, MMP-12 and MMP-3, compared with the control group (Fig. 3C). Additionally, suppression of Tescalcin also caused downregulation of COX2, TGF-β1 as well as vimentin whereas upregulation of E-cadherin in ACHN and 786-O cells RCC cells (Fig. 3D). qPCR also shown similar profile of EMT markers in Tescalcin knockdown cells (data not shown).

3.4. Tescalcin regulate cell viability, migration and invasion via NHE1/pHi pathway

Because NHE1/pHi pathway is well known to be a critical regulator of tumor biological process, we hypothesized that NHE1/pHi is responsible for the modulation of cell survival by Tescalcin in RCC cells. Knockdown of Tescalcin resulted in a decrease in pHi value of ACHN and 786-O cells. However, treatment of HMA, an NHE1 inhibitor, comprised the significant changes of pH level between Tescalcin silencing groups and the control groups ($P < .05$) (Fig. 4A). We further found that suppression of Tescalcin alone or treatment of HMA

significantly inhibited the cell viability, whereas no significant change was seen between the control and Tescalcin knockdown group while treated with HMA (Fig. 4B), indicating that HMA abolished the inhibitory effect of Tescalcin. We also performed western blot of p-p65, p-IκB, p-glycogen synthase kinase 3β (GSK3β) and p-AKT levels, which are reported to be involved in tumor progression including proliferation and metastasis. The data showed that inhibition of Tescalcin significantly reduced the phosphorylation of p65, IκB, GSK3β and ATK (Fig. 4C and D).

3.5. Downregulation of Tescalcin suppresses the tumorgenesis of RCC cells in vivo

Given the downregulation of Tescalcin could inhibit the proliferation of RCC cells *in vitro*, its function *in vivo* also needs to be identified. The Tescalcin knockdown ACHN cells were subcutaneously implanted into nude mice for 48 days, and in the period the sizes of formative tumors were measured at indicated times. Tescalcin silencing efficiency in xenograft tumor tissue was determined by western blot analysis (Fig. 5A). The data showed that the downregulation of Tescalcin significantly reduced the sizes of formative tumors in nude mice ($P < .05$) (Fig. 5B and C), suggesting that Tescalcin is able to promote RCC growth *in vivo*.

4. Discussion

In the current study, we demonstrated the overexpression of Tescalcin in RCC tissues compared with matched normal tissues. Our findings provided the evidence that downregulation of Tescalcin significantly inhibited the proliferation *in vitro* and *in vivo*, migration and invasion in RCC cells, which is mediated by NHE1/pHi and NF-κB signaling pathway.

So far, many studies have shown that Tescalcin is strongly expressed in various tumor types. The abnormal expression of Tescalcin gene have been shown relation to theradiation-induced papillary thyroid carcinoma (PTC) pediatric patients (Stein et al., 2010). Increased Tescalcin expression is also observed in human colorectal cancer tissues, but not in normal mucosa and premalignant lesions. The Tescalcin expression level in tumor showed clinical significance (Kang et al., 2014). In this study, we for the first time reported that the expression of Tescalcin was increased in RCC tissues compared with matched normal tissues.

Several researches reported the participation of Tescalcin in normal cell differentiation and proliferation. For example, Tescalcin is significantly increased during the differentiation and maturation of primary megakaryocytes. Tescalcin knockdown partially block megakaryocytic differentiation (Levay & Slepak, 2007). Currently, functional characterization of Tescalcin in cancer development has been investigated in colon cancer, gastric cancer, melanoma and lukemia (Kang et al., 2016; Man et al., 2014). After Tescalcin silencing, significant decline in the growth and cell migration of AGS cells and HT29 cells was observed. Fan et al., also demonstrated that Tescalcin contributes to tumor growth and metastasis and is considered as a novel oncogene in tumor development (Fan et al., 2015b).The expression of Tescalcin shRNA reduced cell migration and invasion of colon cancer cells by inhibiting MMP and the EMT pathway (Kang et al., 2016). In line with the previous findings, this study showed that silencing of Tescalcin dramatically suppressed the tumor growth of RCC both *in vitro* and *in vivo* as well as reduced the migration and invasion ability in RCC cells. These data indicate that ectopic expression of Tescalcin facilitate tumor growth and metastasis of RCC. In order to preliminarily explore the explanation for the regulation of migration and invasion by Tescalcin in RCC, we examined the changes of EMT-related genes after the silencing of Tescalcin,including matrix metallopeptidase(Radisky & Radisky, 2010; Tan et al., 2016), Cox-2 (Neil et al., 2008; Li et al., 2011), TGF-β(Willis & Borok, 2007; Ji et al., 2013), E-cadherin (Scarpa et al., 2015) and vimentin (Mendez et al., 2010; Luo et al., 2014). Our results revealed that Tescalcin regulated the expression of the factors mentioned above.

NHE1 activates the efflux of protons from inside the cell leading to the local increase in pHi, which is in favor of tumorigenesis and development. The interplay of Tescalcin with NHE1 was initially found by yeast two-hybrid screen (Mailander et al., 2001). However, the functional effects of the interaction between Tescalcin and NHE1 are controversial. Li et al. (Li et al., 2003), *in vivo* study showed that expression of Tescalcin caused suppression of the activity of the NHE1 when there was an intact C-terminus of the protein. On the other hand, previous report showed that reduction of Tescalcin expression induced a decrease of pH by ~0.15 units in acute myeloid leukemia cell lines (Man et al., 2014). There is also another report of Tescalcin induced activation of NHE1 in transfected cell models (Zaun et al., 2008). In line with this evidence, the data showed that downregulation of Tescalcin dramatically lowered the pHi in RCC cells, which is abolished with treatment of NHE1 inhibitor. We further demonstrated that NHE1 inhibitor abolished the inhibitory effect of Tescalcin on cell viability, suggesting that Tescalcin indeed participated in regulating the NHE1/pHi signaling pathway in RCC cells.

AKT/NF-κB signaling is thought to be the critical regulatory mechanism of tumor progression in RCC (Chen et al., 2017). Previous studies showed that NF-κB p65 activation, p-GSK3β and p-Akt was suppressed in Tescalcin siRNA-expressing colorectal cancer cells (Kang et al., 2016; Kang et al., 2014). In this study, we found Tescalcin shRNA decreased the phosphorylation of AKT, GSK3β and IκBa in RCC cells. Interestingly, it is shown that Akt hyperactivation could influence the stability of NHE-1 expression (James & Kumar, 2011). NHE-1 is involved in the activation of NFκB (Nemeth et al., 2002). Therefore, the working model we proposed is that Tescalcin contributes to the proliferation and metastasis of RCC through two signaling pathways including ATK/ NHE1/ pHi and AKT/ NF-κB. The two signaling pathways may have crosstalk *via* the regulation of NF-κB by NHE1.

In conclusion, our study demonstrated that the expression of Tescalcin was increased in RCC tissues compared with matched normal tissues. Downregulation of Tescalcin suppressed the progress of RCC through regulating NHE1/ pHi and AKT/ NF-κB signaling pathway, suggesting that Tescalcin may be a therapeutic target for RCC.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.yexmp.2018.12.004.

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Conflict of interest

The authors declare that they have no conflict of interest.

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