TRPM7 Regulates AKT/FOXO1-Dependent Tumor Growth and Is an Independent Prognostic Indicator in Renal Cell Carcinoma

Zhijian Zhao1, Mengping Zhang2, Xiaolu Duan1, Yiwen Chen1, Ermao Li1, Lianmin Luo1, Wenqi Wu1, Zhenwei Peng2, Huijuan Qiu3, and Guohua Zeng1

Abstract

Transient receptor potential melastatin 7 (TRPM7) is important for the tumorigenesis and progression of several cancers. However, little is known about TRPM7 expression and its clinical significance in clear cell renal cell carcinoma (ccRCC). The expression dynamics of TRPM7 was examined in a clinical cohort of RCC specimens by qPCR, immunoblotting, and IHC staining. A series of in vitro and in vivo assays were performed to elucidate the function of TRPM7 in RCC and the underlying mechanisms. For the first time, results demonstrate that TRPM7 expression is markedly higher in RCC cell lines and clinical samples and has a positive correlation with T status, tumor size, and poor patients' overall survival and progression-free survival. Preclinical studies using multiple RCC cells and a mouse model indicate that TRPM7 promotes cell proliferation and colony formation in vitro and tumor growth in vivo. Mechanistically, TRPM7 promotes AKT phosphorylation, leading to repression of the FOXO1 expression and transcriptional activity. Moreover, luciferase reporter assays demonstrate that miR-129-3p directly targets the 3'-UTR of TRPM7 and acts as a negative regulator of TRPM7. These findings reveal an important role for TRPM7 in the regulation of RCC growth and represent a novel prognostic biomarker for this disease.

Implications: TRPM7 is an independent prognostic indicator in RCC, and targeting the TRPM7 signaling pathway may be a novel therapeutic approach for the treatment of RCC. Mol Cancer Res; 1–11. © 2018 AACR.

Introduction

Clear cell renal cell carcinoma (cccRCC) is the most common adult kidney cancer, with a 5-year disease-specific survival rate of 50% to 69% (1, 2). However, a previous study has demonstrated that the median survival of patients with metastatic RCC is approximately 13 months (2). Because of the lack of early signs, diverse clinical manifestations, and the resistance of radiotherapy and chemotherapy, early diagnosis and prognosis is particularly important. cccRCC is an aggressive tumor with unpredictable outcome, which are hard to accurately predict by the currently used clinical parameters.

Numerous studies have examined the role of cancer-associated genes, mRNAs, and proteins in cancer cells, to determine their pathologic characteristics, prognostic value, and potential for use in targeted therapy, although most of them have not yet become clinically routine (3, 4). Transient receptor potential melastatin 7 (TRPM7) is a ubiquitously expressed ion channel with intrinsic kinase activity (5). It has been associated genes, mRNAs, and proteins in cancer cells, to determine their pathologic characteristics, prognostic value, and potential for use in targeted therapy, although most of them have not yet become clinically routine (3, 4). Transient receptor potential melastatin 7 (TRPM7) is a ubiquitously expressed ion channel with intrinsic kinase activity (5). It has been implicated in various human diseases including cancer, and plays a variety of functional roles in cancer cells including survival, cell-cycle progression, proliferation, growth, migration, invasion, and epithelial–mesenchymal transition (5, 6). In recent studies, TRPM7 was identified as a regulator of PI3K/Akt, MAPK, and ERK1/2 pathways, regulating several oncogenes and cell-cycle genes, and its function was potentially associated with several human cancers, such as human colon cancer, ovarian cancer, prostate cancer, breast cancer, bladder cancer, pancreatic cancer, nasopharyngeal carcinoma, and esophageal squamous cell carcinoma (7–13). Up to now, no association of TRPM7 and RCC was reported, and the significance of TRPM7 expression in cccRCC has not been elucidated.

Here, based on our cccRCC patient’s clinical data, we aimed to investigate whether TRPM7 acts as a promising prognostic indicator in cccRCC, and using a series of in vitro assays and in vivo mouse model to investigate the function of TRPM7 in RCC.

www.aacrjournals.org

Mol Cancer Res; 1–11. © 2018 AACR.

© 2018 American Association for Cancer Research.
Materials and Methods

Clinical patient cohort and tissue specimens

A total of 129 patients with ccRCC were collected from the First Affiliated Hospital of Sun Yat-Sen University (Guangzhou, China) between September 2008 and December 2011. In addition, 35 matched samples of normal adjacent noncancerous renal tissues were used for controls. Written informed consent was obtained from all patients. The study was approved by the medical ethics committee of our institute and was conducted in accordance with the ethical guideline of International Ethical Guidelines for Biomedical Research Involving Human Subjects (CIOMS). Human ccRCC tissue samples were obtained during radical nephrectomy or nephron-sparing surgery or biopsy specimens and confirmed by pathology. Before tissue sampling, these RCC patients did not receive previous treatment, without malignant disease or a second primary tumor. Data collected from each patient included age, gender, tumor size, tumor-node-metastasis (TNM) stage, tumor necrosis, and histologic grade. Tumor stage classification was carried out according to 2010 AJCC TNM classification (14). Presence of nodal and metastatic disease was confirmed according to intraoperative, pathologic, and radiographic findings. Patients were evaluated postoperatively with physical examinations, laboratory studies, chest imaging, and abdominal ultrasound or CT scans every 3 months for the first year and then every 6 months for the next 2 years, and finally annually. The endpoint of interest was the overall survival (OS). OS was defined as the time from the date of surgery to the date of death or last follow-up. Progression-free survival (PFS) was defined as the interval from the date of surgery to the date of progress or death due to RCC. Survival status of each patient was updated in July 2017. Furthermore, 35 pairs of fresh ccRCC cancer tissues and adjacent normal tissues were used to do the qPCR or Western blot analysis, and to study the expression relationship of gene or proteins involved in the current study.

IHC staining assays and selecting the optimal cut-off value

IHC analysis was performed to examine TRPM7 expression levels in ccRCC specimens. Primary antibodies against TRPM7 (1:200 dilutions, Abcam) were used in this study. Detailed procedures of IHC were as described previously (15). The intensity of staining in tumor cells was scored by two pathologists independently. First, tumor cells in five fields were randomly selected and scored on the basis of the percentage of positively stained cells (0%–100%). Then, the positive staining cells of different intensities were assessed: 0, no staining; 1, weak (light yellow); 2, moderate (yellow brown); 3, strong (brown). Finally, a semi-quantitative IHC score ranging from 0 to 3 was calculated by multiplying the percentage of positively stained cells with each category of staining intensity.

The optimal cut-off IHC score of TRPM7 expression was selected using X-tile plots (Yale University School of Medicine, New Haven, CT). The X-tile program can automatically select the optimal data cut-off point according to the highest χ² value (minimum P value) defined by Kaplan–Meier survival analysis and log-rank test (16). X-tile plots were performed with X-tile software version 3.6.1 (Yale University School of Medicine).

Cell lines and materials

RCC cell lines (786-O, A498, ACHN, OSRC-2) and one immortalized human renal epithelial cell line (HK-2) were purchased from ATCC and were grown in DMEM medium supplemented with 10% FBS. Cells were used within 10 passages of cell authenticity by genetic profiling (Geneica DNA Laboratory Inc.) and identified as mycoplasma free (by TransDetect PCR Mycoplasma Detection Kit) following ATCC’s instructions during the past 3 months. The following primary antibodies were used: anti-TRPM7 (Abcam); anti-GAPDH, anti-AKT, anti-p-AKT, anti-FOXO1, anti-p21, and anti-Ki67 (Cell Signaling Technology). miR-129-3p mimic and inhibitor were purchased from Sigma.

Plasmid preparation and lentivirus packaging

A plko.1 vector-based short hairpin RNA plasmid (TRCN0000318838 and TRCN0000039580) for silence TRPM7 gene expression was purchased from Sigma. The targeting sequences were as follows: 5'-GCCAATATGTTCTACATTGTA-3' and 5'-GCTATGAAATTGCTCACTTAT-3' for shTRPM7, 5'-GCCACCAAAACACCATTTGAA-3' and 5'-ACTACGAGTTGATGTCAAC-3' for shFOXO1. The PWPI vector was used to express ectopic TRPM7 in RCC cells. The plasmid combining the pMD2G envelope plasmid and the psAX2 packaging plasmid were then transfected into 293HEK cells using the standard calcium chloride transfection method. The lentivirus soup was collected after incubating 48 hr/and 72 hours and frozen in −80°C for later use.

Colony formation assay

Twenty-four hours after infection, 500 infected cells were placed in a fresh 6-well plate and cultured for 14 days. Colonies were fixed with methanol and stained with 0.1% crystal violet in 20% methanol for 15 minutes. Colony number was then calculated.

MTS proliferation assay

To detect relative cell proliferation abilities, 20 μl of 3-(4,5-dimethylthiazole-2-yl)-2,5-biphenyl tetrazolium bromide (MTS, 5 mg/ml) was added per day to 96-well plates till the cells were seeded for 5 days. The plates were then incubated at 37°C for 4 hours, followed by solubilization of the product in 100 μl of DMSO (Sigma-Aldrich). Absorbance was measured at 490 nm using an ELISA reader. The proliferative abilities were expressed as the relative percentage of cell numbers on the first day.

Luciferase reporter assay

A fragment (1,500 bp) of TRPM7 3’ UTR containing wide-type or mutant miRNA targeting sites was constructed into psiCheck2-vector (Promega). Cells were transfected with miR-129-3p mimic or inhibitor, plated in 24-well plates, and transfected with TRPM7 3’ UTR or its mutant plasmid using Lipofectamine 3000 (Invitrogen) according to the manufacturer’s instructions. After indicated treatments, cells were lysed and the luciferase activity was detected by the Dual-luciferase Assay (Promega). Data were presented as mean ± SD from at least three independent experiments.

Xenograft tumor model assay

Six-week-old male SCID mice were randomized into two groups. To investigate the impact of TRPM7 on proliferation in vivo, OSRC-2 shTRPM7-1/scramble cells were injected subcutaneously into the dorsal flank of each nude mouse, respectively (1 × 10⁶ cells mixing with Matrigel at 1:1 volume) on day 1 morning. Mice were followed-up every 3 days to measure tumor sizes with calipers. Tumor volume was calculated as
follows: volume = (largest diameter × smallest diameter²)/2. Twenty-seven days after injection, mice were killed and tumors were weighed and photographed. All animal studies were conducted with the approval of the Guangzhou Medical University Institutional Animal Care and Use Committee and in accordance with the Chinese regulations and standards on the use of laboratory animals.

Survival and statistical analysis
Group differences for qualitative variables were analyzed using χ², t test, Mann–Whitney U, and Pearson correlation tests as appropriate. Kaplan–Meier method and log-rank test were performed to obtain and compare the survival curves. For survival analyses based on TRPM7 expressions, TRPM7 protein expression was defined as high or low expression using the X-tile program software to generate the optimal cut-off points. The multivariate Cox proportional hazards regression analyses were conducted to evaluate independent prognostic factors associated with survival. Statistical differences between expression levels of adjacent normal kidney tissues, primary tumor, or different stage were analyzed using two-tailed paired Student t test. Bivariate correlations between study variables were calculated by the Spearman rank correlation coefficients. All data were analyzed using SPSS 13.0 software, with the level of statistical significance at P < 0.05.

Results
Elevated expression of TRPM7 expression in RCC
First, to examine the expression status of TRPM7 in ccRCC, western blotting and qPCR analysis were conducted in 4 RCC cell lines (786-O, A498, ACHN, and OSRC-2), one normal human renal epithelial cell line (HK-2), and IHC staining in 35 matched pairs of ccRCC tissues and adjacent normal tissues. All four RCC cell lines displayed increased TRPM7 mRNA and protein...
expression compared with HK-2 (Fig. 1A and B). Similarly, TRPM7 was considerably higher in RCC tumor tissue (T) specimens when compared with their paired nonneoplastic tissues (N; Fig. 1C and D). Consistently, for IHC staining, the positive TRPM7 protein immunoreactivity was mainly detected in the nucleoplasm, and some cytoplasm of normal cells was markedly increased in most of RCC samples (Fig. 1E).

Selection of cut-off score for high expression of TRPM7 in RCC

In total, 129 tumor tissues and 35 adjacent nonneoplastic renal tissues were stained by IHC. To assess the statistical significance and avoid arbitrary cut-off point selection, we applied the X-tile program to obtain cut-off scores for TRPM7 expression. Using the X-tile plots for the training cohort, we divided this cohort into low and high populations based on a cut-off score of “1.8” for TRPM7 IHC staining. Using the cut-off score, we performed Kaplan–Meier survival analyses. Patients with high TRPM7 expression had significantly poorer OS ($P = 0.008$) and PFS ($P = 0.016$) than those with low TRPM7 expression (Fig. 2A and B). According to this cut-off point, high TRPM7 expression was observed in 62 of 129 (48.1%) RCCs. The rates of high expression of TRPM7 in RCC with respect to clinicopathologic features are detailed in Table 1. The results demonstrated that high expression of TRPM7 was positively correlated with tumor size ($P < 0.001$) and T stage ($P < 0.001$). These data suggested that the expression of TRPM7 increases with RCC progression.

Elevated TRPM7 expression predicted poor prognosis

To further confirm the prognostic value of TRPM7 expression and clinicopathologic features in RCC, we applied ROC curves to test patient survival status in our study. According to the ROC curve analysis, TRPM7 was found to be a promising predictor for survival status both for OS [AUC = 0.691; 95% confidence interval (CI), 0.588–0.794; $P = 0.001$] and PFS (AUC = 0.668; 95% CI, 0.568–0.768; $P = 0.002$; Fig. 2C). Furthermore, our univariate and multivariate analysis showed that high expression of TRPM7 was an independent risk factor for adverse OS (HR = 1.55; 95% CI, 1.18–3.07; $P = 0.023$) and PFS (HR = 1.61; 95% CI, 1.00–2.84; $P = 0.051$; Supplementary Table S1).
TRPM7 promotes cell proliferation and clonogenicity of RCC cell lines 
in vitro

In Fig. 1A, we have showed higher levels of endogenous TRPM7 in four RCC than in normal renal epithelial HK-2 cells. Then, we further knock down the TRPM7 expression using two targeted shRNA in ACHN and OSRC-2, which have relatively higher TRPM7 expression among the four cell lines. A subsequent MTT analysis and colony-forming assay showed that both ACHN and OSRC-2 cells transfected with TRPM7shRNA displayed a substantial decrease in cell proliferation and clone capacity compared with control cells, while overexpression of TRPM7 in 786-O can increase the ability of cell proliferation and clone formation (Fig. 3A and B). Because TRPM7 expression appeared to be tightly linked to the RCC cell proliferation, we further investigated whether cell-cycle marker or factors, including Ki67 and p21, could be regulated by TRPM7. Our Western blot analysis showed a significant downregulation of Ki67, accompanied by upregulation of p21 protein levels in TRPM7-silenced cells compared with control cells, and the opposite trend changes by overexpression of TRPM7 (Fig. 3C).

TRPM7 suppresses FOXO1 transcript activity via activating AKT signaling pathways

As FOXO1 transcriptionally regulates p21 and cyclin D1 (17, 18), we further investigated whether TRPM7 exerted these functions by modulating the expression of FOXO1. As shown in Fig. 4A, the expression level of phosphor-FOXO1 was significantly decreased in TRPM7-silenced cells. Furthermore, immunofluorescence analysis showed the cellular localization of FOXO1 from the cytoplasm into the nucleus after knocking down TRPM7 (Fig. 4B), which also suggested that silencing TRPM7 can activate FOXO1 transcript activity. AKT kinases are known to have key roles in phosphorylating and repressing FOXO1 transcriptional activity (18, 19). As predicted, phospho-AKT (p-AKT) levels were decreased by TRPM7 silencing, suggesting that TRPM7 suppresses FOXO1 activity via activating the AKT signaling pathway (Fig. 4A). To confirm these results, we overexpressed the TRPM7 in RCC cells and treated with an AKT inhibitor (LY294002). As shown in Fig. 4C, LY294002 led a significant downregulation of p-AKT activity on the total of FOXO1, which also suggested that siRNA knockdown TRPM7 can activate FOXO1 signaling pathway (Fig. 4A). To confirm these results, we overexpressed the TRPM7 in RCC cells and treated with an AKT inhibitor (LY294002). As shown in Fig. 4C, LY294002 led a significantly reduced expression level of p-AKT and p-FOXO1 but no significant influence on the total of FOXO1 expression in overexpression of TRPM7 RCC cells. Next, we also examined the growth and clonogenicity ability of TRPM7-silenced RCC cells using LY294002. MTS and colony formation assays showed that the growth of TRPM7-overexpressed cells was significantly compromised by treatment with the AKT inhibitors compared with control cells (Fig. 4D–E). Moreover, colony formation and MTT assays showed that knocking down of FOXO1 blocked the growth rate of TRPM7-silenced RCC cells, suggesting that FOXO1 plays an important downstream role involved in the regulation of TRPM7 on proliferation in RCC cells (Fig. 4E–H). Taken together, these data indicate that TRPM7 may promote proliferation partly via activation of the AKT to suppress FOXO1 activity.

miR-129-3p is a negative regulator of TRPM7

As posttranslational regulators such as miRNA had a critical role in protein regulation, we sought to explore whether the

Table 1. Relationship between TRPM7 expression and clinicopathologic characteristics

<table>
<thead>
<tr>
<th>Variable</th>
<th>Patients TRPM7 expression</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>High (n = 62)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Low (n = 67)</td>
<td></td>
</tr>
<tr>
<td>Age, mean ± SD (years)</td>
<td>62.6 ± 11.3</td>
<td>0.160</td>
</tr>
<tr>
<td>Age &lt;60</td>
<td>58 (45)</td>
<td>64 ± 12.8</td>
</tr>
<tr>
<td>≥60</td>
<td>71 (55)</td>
<td>61.3 ± 12.1</td>
</tr>
<tr>
<td>Gender Male</td>
<td>80 (62)</td>
<td>42 (67.7)</td>
</tr>
<tr>
<td>Female</td>
<td>49 (38)</td>
<td>20 (32.3)</td>
</tr>
<tr>
<td>Tumor size, median (range)</td>
<td>5.6 (0.5–27)</td>
<td>61 (5.0–27)</td>
</tr>
<tr>
<td>Tumor size, mean (SD)</td>
<td>5.7 ± 2.0</td>
<td>6.4 ± 2.1</td>
</tr>
<tr>
<td>pT stage T1+T2</td>
<td>105 (81.4)</td>
<td>63 (94)</td>
</tr>
<tr>
<td>T3+T4</td>
<td>24 (18.1)</td>
<td>4 (6)</td>
</tr>
<tr>
<td>pN stage NO+Nx</td>
<td>125 (96.9)</td>
<td>67 (100)</td>
</tr>
<tr>
<td>N1</td>
<td>4 (3.1)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Fuhrman grade 1 or 2</td>
<td>77 (59.7)</td>
<td>32 (51.6)</td>
</tr>
<tr>
<td>3 or 4</td>
<td>52 (40.3)</td>
<td>30 (48.4)</td>
</tr>
<tr>
<td>Tumor necrosis Absent</td>
<td>111 (86)</td>
<td>61 (91)</td>
</tr>
<tr>
<td>Present</td>
<td>18 (14)</td>
<td>6 (9)</td>
</tr>
<tr>
<td>ECOG-PS 0</td>
<td>108 (83.7)</td>
<td>46 (74.2)</td>
</tr>
<tr>
<td>≥1</td>
<td>21 (16.3)</td>
<td>16 (25.8)</td>
</tr>
<tr>
<td>SSIGN score 0–3</td>
<td>98 (76)</td>
<td>37 (59.7)</td>
</tr>
<tr>
<td>&gt;3</td>
<td>31 (24)</td>
<td>25 (40.3)</td>
</tr>
<tr>
<td>UISS risk Low</td>
<td>68 (52.7)</td>
<td>26 (41.9)</td>
</tr>
<tr>
<td>Intermediate</td>
<td>49 (38)</td>
<td>24 (35.8)</td>
</tr>
<tr>
<td>High</td>
<td>12 (9.3)</td>
<td>11 (17.7)</td>
</tr>
</tbody>
</table>

Abbreviations: ECOG-PS, Eastern Cooperative Oncology Group performance status; SSIGN, stage, size, grade, and necrosis; UISS, University of California Los Angeles Integrated Staging System.
Dysregulation of miRNAs was responsible for the upregulation of TRPM7 in RCC. We first conducted bioinformatics analyses and overlapped the predicted miRNA regulators with downregulated miRNAs from miRNA expression profiles of RCC in published studies. The result showed that miR-129-3p may be a potential regulator of TRPM7 (Fig. 5A). Next, our quantitative RT-PCR analyses showed that miR-129-3p was indeed downregulated in all four RCC cell lines (Fig. 5B, left) and RCC tissues from clinical specimens (Fig. 5B, right). To verify the hypothesis that downregulation of miR-214 was responsible for the upregulation of TRPM7 in RCC, 1,500-bp fragment of 3'UTR of TRPM7 containing wild-type or mutant miR-129-3p-targeting sites was constructed into psiCheck2-vector (Fig. 5C). Results of the luciferase reporter assay showed that miR-129-3p overexpression decreased the luciferase activity of TRPM7 3'-UTR, whereas the mutant UTR failed to show an inhibitory effect on the luciferase expression (Fig. 5D). Furthermore, Western blotting assays proved that miR-129-3p mimic greatly downregulated the protein level of TRPM7, whereas miR-129-3p inhibitor increased the protein level of TRPM7 (Fig. 5E). Most importantly, we also directly illustrated the inverse correlation between miR-129-3p and TRPM7 IHC score by Pearson correlation scatter plots in adjacent noncancerous RCC specimens with \( R = -0.55, P = 0.0006 \) (Fig. 5F). These data collectively provided evidence that miR-129-3p directly suppresses TRPM7 expression, and decreased miR-129-3p contributes to TRPM7 overexpression in RCC.
Figure 4.
TRPM7 suppressed the expression and transcription capacity of FOXO1 via activation of the AKT signaling pathway. A, Western blot analysis of p-AKT, total AKT, p-FOXO1, and total FOXO1 in shTRPM7 (using two targeted sequences of sh-RNA, #1 and #2) and scramble-infected cells. B, In TRPM7 stably reduced ACHN and OSRC-2 cells, the nuclear cellular localization of FOXO1 was increased as determined by immunofluorescence staining. C, TRPM7 stable overexpression in RCC cells was treated with the AKT inhibitor LY294002 (50 μmol/L), or DMSO for 24 hours, and then harvested to examine the expression levels of the indicated proteins by Western blotting. D and E, A498 and ACHN RCC cell clonogenicity and proliferation were determined by MTS (D) and colony formation assays (E) after treatment with LY294002 or DMSO. *** P < 0.001. F, In both OSRC-2 and ACHN cells, endogenous FOXO1 was greatly upregulated in the presence of shTRPM7; the suppression was reversed by stably silencing FOXO1. G and H, The inhibition of cell viability and proliferation ability by shTRPM7 was significantly compromised by treatment with stable silencing FOXO1, as determined by MTS assays (G) and colony formation assays (H). *** P < 0.001.
Effects of TRPM7 on tumor growth in vivo

Finally, to further confirm the effect of TRPM7 required for RCC tumor growth in vivo, xenograft tumor model assays were conducted by injecting OSRC-2-shTRPM7/scramble cells into the dorsal flank of nude mice subcutaneously. The OSRC-2-shTRPM7 cells grew at a much slower rate than OSRC-2/scramble cells (P < 0.001; Fig. 6A). Furthermore, the average weight of tumor was significantly lower in the TRPM7 depletion group compared with the scramble group (P < 0.001, Fig. 6B and C). Importantly, IHC staining also showed that the proliferation marker Ki67 was significantly lower, whereas FOXO1 was higher in TRPM7 depletion group (Fig. 6D). Collectively, this gave direct evidence of TRPM7’s role in promoting RCC carcinogenesis.

Discussion

The development of RCC is a multistep process involving the loss of tumor suppressor genes and activation of oncogenes. At present, most RCC-related deaths are due to advanced disease and diagnosed when metastases have already disseminated to lymph nodes or distant organs. Therefore, it is of great clinical value to identify potential early biomarkers for diagnosis and prognosis. In this study, we take this promotion of tumor growth as a potential underlying mechanism in TRPM7-mediated RCC carcinogenesis.

The molecular status of TRPM7 and its attendant expression patterns are vastly different in various tissues and tumor types. This variation suggests that abnormal gene regulation and/or protein functions of TRPM7 in tumorigenesis are complicated and are likely to be tumor-type specific (7, 12, 13, 20–22). In most studies, TRPM7 was reported to be aberrantly overexpressed in tissue specimens and/or cell lines; however, in esophageal squamous cell carcinoma (NSCLC), suppression of TRPM7 expression increased cell proliferation, migration, and invasion (21). In this study, we report, for the first time, that the expression of TRPM7 is pervasively upregulated in RCC cell lines and tissues. Furthermore, high expression of TRPM7 in RCC is strongly associated
with an aggressive phenotype and poor survival outcomes. As a widely accepted fact, advanced stage of tumor and recurrence are major causes of cancer-related death. Notably, our analyses demonstrated that high TRPM7 expression is correlated significantly with aggressive clinical characteristics and poor survival. Our clinical data analysis indicated that TRPM7 expression might provide useful information in the evaluation prognosis for RCC patients. Consistently, our in vitro and in vivo studies showed that overexpression of TRPM7 increased cellular viability and proliferation ability, but the deletion of TRPM7 repressed these abilities.

Referring to cell proliferation, here we showed that TRPM7 can regulate the expression of p21 and Ki67. Previous studies showed that both the PI3K/AKT and the MAPK/ERK signal transduction cascades, which are required for cell-cycle progression, were frequently involved in the promotion of proliferation, and the PI3K/AKT pathway is well known to be involved in RCC (23). Moreover, activation of AKT and ERK stimulated the phosphorylation of various downstream targets, including the FOXO family of transcription factors. In particular, activated AKT and ERK could result in phosphorylation of FOXO1, which led to downregulation of FOXO1 transactivity via ubiquitin proteasome–mediated degradation and thus repression of FOXO1-mediated growth arrest (19). FOXO1, one of the most studied tumor suppressors, is involved in several malignant cellular processes, such as cell growth and proliferation. In this study, analyses showed that in silenced TRPM7 cells, the expression and transcription capacity of FOXO1 were remarkably increased along with a decreased level of phospho-AKT and phospho-FOXO1. When knocking down FOXO1 was induced into shTRPM7 cells, the inhibition of cell viability and proliferation ability exerted by decreased TRPM7 was significantly compromised. Thus, we speculate that the modulation of cell proliferation by TRPM7 is probably due to activation of the PI3K/AKT/FOXO1 pathways. Consistent to our data.

Figure 6.
Effects of TRPM7 on tumor growth in vivo. A, The xenograft tumor volumes of nude mice injected with OSRC-2/scramble cells or OSRC-2/shTRPM7 cells (n = 5). B and C, The average tumor weight in nude mice injected with OSRC-2/shTRPM7 cells were significantly decreased compared with the OSRC-2/scramble group (n = 6). D, Expression of TRPM7 is positively associated with Ki-67 and negatively associated with FOXO1 in tumor samples. One representative case from two groups is shown. ***, P < 0.001.
one published study has demonstrated that decreased FOXO1 in RCC and increasing expression of FOXO1 could prevent cell growth (24).

As the expression of TRPM7 is pervasively upregulated in RCC cell lines and tissues, one critical question was then raised: What is the mechanism by which TRPM7 is upregulated? A class of miRNAs has been proved as important regulators of gene expression; in this study with the help of bioinformatics analyses and miRNA expression profiles of RCC cells based on search from published articles, we revealed that miR-129-3p directly suppresses TRPM7 expression. This finding is based on the evidence provided as follows: First, miR-129-3p has a conserved binding site in the 3′-UTR of TRPM7; second, the luciferase activity of TRPM7 3′-UTR reporter is specifically responsive to increased miR-129-3p but the mutant UTR is nonreactive; third, the overexpression of miR-129-3p reduced the expression of TRPM7; and fourth, miR-129-3p is downregulated in RCC cells. Therefore, upregulation of miR-129-3p, such as using anti-miR-129-3p, is an effective strategy to suppress RCC, which has a high level of TRPM7.

On the basis of the different patterns of TRPM7 expression in several tumor types, the risks and benefits of sh-TRPM7 treatment for cancers should be concerning. Fortunately, nowadays nanoparticle-based gene delivery systems may be very efficient tools for the administration of therapeutic genes to solid tumors and cancer metastases, owing to the numerous advantages in terms of enhanced tissue penetrability, improved cellular uptake, and targeted gene delivery to the cells of interest compared with other gene delivery systems (25–27). Therefore, via this system, it is possible to target the therapeutic agents exactly to the target cells and protect healthy tissues outside the peritoneal cavity from side effects.

In conclusion, we describe, for the first time, the expression pattern of TRPM7 in human ccRCC tissues. Our results provide a basis for the concept that increased expression of TRPM7, a target of miR-129-3p, may be important in RCC tumorigenesis and might be a novel predictor for RCC patients. Furthermore, the deletion of TRPM7 repressed carcinogenesis of RCC cells both in vitro and in vivo through activating the expression and transcription capacity of FOXO1. In addition, the function of TRPM7 in cell proliferation is via PI3K/AKT pathways. These findings suggest that TRPM7 has a role in the development and progression of human RCC, which renders TRPM7 a potential prognostic marker and may serve as a novel therapeutic target in RCC.

Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.

Authors’ Contributions
Conception and design: H. Qiu, G. Zeng
Development of methodology: Z. Zhao, M. Zhang, X. Duan, Y. Chen, E. Li, W. Wu, Z. Peng
Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): Z. Zhao, M. Zhang, X. Duan, L. Luo, G. Zeng
Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): Z. Zhao, M. Zhang, W. Wu, Z. Peng
Writing, review, and/or revision of the manuscript: Z. Zhao, G. Zeng
Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): H. Qiu, G. Zeng
Study supervision: Y. Chen, H. Qiu, G. Zeng

Acknowledgments
This work was supported by grants from the National Natural Science Foundation of China no. 81600542 (to Z.J. Zhao) and no. 81402430 (to X.L. Duan).

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Received December 21, 2017; revised February 15, 2018; accepted March 6, 2018; published first March 15, 2018.

References


Molecular Cancer Research

TRPM7 Regulates AKT/FOXO1–Dependent Tumor Growth and Is an Independent Prognostic Indicator in Renal Cell Carcinoma

Zhijian Zhao, Mengping Zhang, Xiaolu Duan, et al.

*Mol Cancer Res* Published OnlineFirst March 15, 2018.

Updated version
Access the most recent version of this article at:
doi:10.1158/1541-7786.MCR-17-0767

Supplementary Material
Access the most recent supplemental material at:
http://mcr.aacrjournals.org/content/suppl/2018/03/15/1541-7786.MCR-17-0767.DC1

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, use this link http://mcr.aacrjournals.org/content/early/2018/04/23/1541-7786.MCR-17-0767.
Click on "Request Permissions" which will take you to the Copyright Clearance Center's (CCC) Rightslink site.