#### ORIGINAL RESEARCH

# Upregulated RACK1 attenuates gastric cancer cell growth and epithelial–mesenchymal transition via suppressing Wnt/ $\beta$ -catenin signaling

This article was published in the following Dove Press journal: OncoTargets and Therapy



**Patients and methods:** Normal gastricepithelial cereanders, GC cell lines were used to detect the mRNA expression of RAG 1. Overexpressing NACK1 was transfected in HGC27 and MGC803 cells. The effects of overexpression RACK1 on cell viability, migration, and invasion were determined to cell counting kit-to wound scratch, and Transwell assay, respectively. The expressions of epithelial–mesenchymal transition (EMT) and Wnt/β-catenin signaling related gives were detended using quantitative real-time PCR or Western blot. Wnt pathway agonist Lie was added into RACK1 overexpressing GC cells, and then cell viability, migration were also detected.

**Results:** RACK1 was do a contacted in GC cell lines. Under the circumstance that overexpression PACK1 was successfully transfected in the two lowest RACK1-expressing GC cells signifient inhibition of cell viability, migration, and invasion, promotion to the mRNA expression of E-cadherin, as well as a decrease in the N-cadherin and Snail expressions could be observed. Overexpressing RACK1 also enhanced the protein level of phospherelation- $\beta$ -catenin/ $\beta$ -catenin and attenuated c-Jun protein expression. Additionally, LiCl could or trially reverse the inhibitory effects of cell viability, migration and invasion by preexpressing RACK.

**Collusion:** We found RACK1 possibly inhibited epithelial–mesenchymal transition of GC cells through limitation of the Wnt/β-catenin pathway, thereby suppressing cell migration and invasion; RACK1 could also suppress cell growth.

**Keywords:** RACK1, gastric cancer, LiCl, Wnt/β-catenin signaling, epithelial-mesenchymal transition

#### Introduction

Gastric cancer (GC) is a heterogeneous, multifactorial disease.<sup>1,2</sup> It poses a serious threat to the physical and mental health of human beings and brings heavy social and economic burden in both developed and developing countries.<sup>3</sup> According to the GLOBOCAN series of the International Agency for Research on Cancer report, in 2012 there were about 951,000 new cases of GC and 723,000 deaths in the world, making it the fifth in morbidity and third in mortality of malignant tumor worldwide.<sup>4</sup> Among them, more than 70% of GC cases occurred in developing countries, half of the world total cases occurred in East Asia, and the mortality rate of GC in East Asia was also the highest in the world.<sup>5</sup> China was the main high incidence and high

OncoTargets and Therapy 2019:12 4795-4805

4795

Lihui Zhu\* Wen Chen\* Guoqing Li Honghui Chen Wenqiu Liao Li Zhang Xiaoli Xiao Department of Gastroenterology, The Second Hospital Affiliated to

The Second Hospital Affiliated to the University of South China, Hengyang, Hunan Province, People's Republic of China

\*These authors contributed equally to this work



Correspondence: Honghui Chen Department of Gastroenterology, The Second Hospital Affiliated to the University of South China, No. 35 Jiefang Avenue, Hengyang, Hunan Province 421001, People's Republic of China Tel +86 734 889 9680 Email hhuic\_chhhui@163.com



© 19 Jule et al. This work is published and licensed by Dove Medical Press Limited. The full terms of this license are available at https://www.dovepress.com/terms.php you hereby accept the Terms. Non-commercial uses of the work are permitted without any further permission foro Dove Medical Press Limited, provided the work is properly attributed. For permission for commercial use of this work, please see paragraphs 4.2 and 5 of our Terms (https://www.dovepress.com/terms.php). mortality area of GC.<sup>4</sup> According to statistics of cancer in China in 2015, the number of new cases of GC was about 679,100, and the death toll was about 489,000.<sup>6</sup> Studies have shown that environmental and genetic factors play a key role in the development of GC, with highly relevant environmental factors such as smoking, Helicobacter pyloriinfection, high salt and smoked food intake, and non-steroidal anti-inflammatory drug abuse.<sup>7</sup> However, the incomplete early screening and the limitation of current cancer treatment are the main reasons for the high mortality rate of GC.<sup>8,9</sup> At present, the main treatment of GC is radical surgery combined with radiotherapy and chemotherapy.<sup>10</sup> More than half of patients with GC undergoing radical resection had a local recurrence or distant metastasis or were diagnosed when the tumor spread, and the median survival time was rarely more than 12 months.<sup>11</sup> In the case of metastatic GC, the 5-year survival rate was lower than 10%, and in GC without metastasis, the 5-year survival rate was only about 20%.<sup>11</sup>

Recent studies have found that early diagnosis combined with advanced surgical treatment can effectively improve the prognosis and survival rate of patients with GC, and early diagnosis is the most critical factor in treatment.<sup>12-15</sup> Receptor for activated C kinase 1 (RACK1), as a scaffold protein interacting with protein kinase C (PKC), was first discovered as a receptor of PKCB II.<sup>16,17</sup> RACK1 can bind a variety of kinness and receptors through different WD40 sites and articip e in many signal transduction pathways, including PKC ing, cAMP/PKA signaling, MAPK signaling Src signaling, and play an important rolling immune sponse. cell growth, migration, and dimerents on.<sup>18-23</sup> In addition, studies have shown at RACK1 bys a role in regulating cell prolifere on, apoptosis, and migration in many cancers.<sup>24–27</sup>

To the best of our know dge, the dain mechanisms of RACK1 in GC are in plved a proclear factor- $\kappa$ B pathway and Wnt/ $\beta$  pathenin at duray <sup>28–3</sup>. In the present study, we aimed to express effects of RACK1 on the biological function of GC was through the Wnt/ $\beta$ -catenin pathway using Wnt pathway agonist LiCI.

#### Materials and methods Cell culture

Normal gastric epithelial cells (GES-1 cells) and GC cell lines (SGC7901, BGC823, MKN45, AGS, HGC27, MGC803 cells) were purchased from Cell Bank of the Chinese Academy of Sciences (Shanghai, China). GES-1 and MKN45 cells were cultured in DMEM (Beijing, Solarbio, China), and others were cultured in RPMI1640 medium (Solarbio, Beijing, China). All of them were incubated with 10% FBS (Thermo Fisher Scientific, Waltham, MA, USA), 100 U/mL penicillin and 50  $\mu$ g/mL streptomycin (Gibco, Thermo Fisher Scientific) at 37°C with 5% CO<sub>2</sub>.

HGC27 and MGC803 cells were selected to establish a LiCl-disposed cell model. Twenty mM LiCl was added into the medium after cell adherence end then cells were continuously cultured. The medium was changed every 1 d with 20 mM LiCl.

#### Cell transfection

re seed in 6-well plates -HGC27 and MGC2 3 cc  $(1.0 \times 10^5)$  for 2 h before constraint. Overexpressing RACK1 and regards control (N ) plasmids were synthesized by Invitrog (Thermo Fisher Scientific). Lipof stamine 2000 (Inv. rogen) was performed to detertransient transfection according to the manufacturer's mir ol. A total of overexpressing RACK1 or NC and prot lipofec nine 2 00 were respectively added to Opti-MEM ium. Lipofectamine/overexpressing RNA mixtures re cultured at 20°C for 10 min and then added into Opti-MEM RPMI1640 medium. After 6 h culturing, the uid was changed back to RPMI1640 medium containing 10% FBS.

#### Cell counting kit-8 (CCK-8)

Cell viability of GC cells after treatment or transfection was performed by CCK-8 assay. Cells were plated into 96well plates at a seeding density of  $1 \times 10^4$  cells per well for 24 h. After the cells had been transfected for 12, 24, 48 h, 10 µL/well CCK-8 solution was added to each well and incubated for another 3 h at 37°C. OD was recorded at 450 nm by a microplate reader (Thermo Fisher Scientific).

#### Cell wound scratch assay

Cells were seeded in 12-well plates at 37°C incubation for 24 h. A wound was drawn using the sterile 10 ul pipette tip in the center of the plate, PBS was used to gently wash the cells 3 times, and then the serum-free medium was then added. Cell migration was observed by inverted microscope at 0 and 24 h. Scratch area was measured using Image J software.

After transfection treatment, cells were resuspended in serum-free medium and  $3 \times 10^4$  cells/well were added into the upper chamber coated with matrigel.<sup>32</sup> RPMI1640 medium containing 10% FBS was added in the lower 24-well chamber, and the cells were incubated for 24 h at 37° C with 5% CO<sub>2</sub>. The cells were fixed with 4% formalde-hyde for 20 min at 25°C and stained with 1% crystal violet for another 15 min. Invasion cells were counted at 200× magnification.

### Quantitative real-time polymerase chain reaction (qRT-PCR)

RNA was isolated from cultured cells by use of Trizol reagent (Invitrogen) according to the manufacturer's protocol. qRT-PCR was performed to detect RACK1, E-cadherin, N-cadherin, and Snail in different groups. Reverse transcription was performed by OrimeScript TM RT reagent kit (Takara, Otsu, Japan) following the manufacturer's instructions at 42°C for 60 min and 70°C for 15 min. qRT-PCR was performed by SYBR Fast qPCR Mix (Invitrogen) and primer sequences are listed in Table 1. RACK1 ran at 94°C for 7 min, 94°C min, 55°C for 45 s followed by 28 cycles of 72°C or 2 min and 72°C for 10 min; other samples ran with following cycling parameters: 95°C for min 25°C 15 s, 60°C for 1 min followed by 42 ycles of 12°C fo 30 s and 72°C for 10 min. The above Amers were synthesized by Sangon Bir ch (Shah, ai, China). Amplified products were rection horesed though 2% agarose gels. The amount of RNA s calculated using the  $2^{-\Delta\Delta CT}$  method.

Table I Print Led in T-PCR		
Gene	Prin r	Sequince
RACKI	rward verse	5-GGGGTCACTCCCACTTTGTT-3' 5'-AATCTGCCGGTTGTCAGAGG-3'
E-cadherin	Forward Reverse	5'-GCTGGACCGAGAGAGTTTCC-3' 5'-TCAAAATCCAAGCCCGTGGT-3'
N-cadherin	Forward Reverse	5'- ATGGGAAATGGAAACTTGATGGC -3' 5'-TGGAAAGCTTCTCACGGCAT -3'
Snail	Forward Reverse	5'- TCTAGGCCCTGGCTGCTACAA -3' 5'-ACATCTGAGTGGGTCTGGAGGTG-3'
GAPDH	Forward Reverse	5'- AATCCCATCACCATCTTCCAG -3' 5'- CCTTCTCCATGGTGGTGAAGAC -3'

#### Western blotting analysis

Proteins were collected from cultured cells in Radio Immuno Precipitation Assay (RIPA) lysis buffer (Beyotime, Shanghai, China). The concentrations of proteins were detected via BCA protein kit (Beyotime). Aliquots of protein were separated by 12% SDS-PAGE and resolved proteins were transferred to polyvinylidene fluoride (PVDF) membranes (EMD Millipore, Billerica, MA, USA). Membranes were blocked with 5% milk in PBS with 0.1% Triton X-100 and incubated with different primary antibodies: anti-RACK 1 antibody (ab62735, 1:1,000, 30 bcam, Cambridge, MA, USA), anti-E-cadherin ar body (ab), 48, 1:500, 135 kDa, Abcam, USA), anti-N-therin antil dy (ab18203, 1:1,000, 125 kDa, Abern), anti-ail anti-dy (ab82846, 1:500, 68 kDa, Aberra), anti-hosph  $don (p)-\beta$ -catenin antibody (ab27798, 1:5), 86 kDa, Abcam), anti-βcatenin antiboy (ab32, 2, 1:5,07, 92 kDa, Abcam), anti-500, 43 kDa, Abcam) and c-Jun anti o (ab32137, anti-GAPDH (at \$1602, 1:10,000, 36 kDa, Abcam, USA) t at 4°C. The membranes were then incubated with e appropriate horseradish peroxidase (HRP)-conjugated condary abody (Proteintech, Rosemont, IL, USA). tein band were detected with enhanced chemiluminescence, LeL, Thermo Fisher Scientific) and visualized by tity one (Bio-Rad Laboratories Inc., Hercules, CA, USA).

#### Statistical analysis

Statistical analysis was performed by Prism Graphpad version 6.0 software. All data were presented as mean  $\pm$  SD. Differences were performed using one-way ANOVA following Turkey's multiple comparison. A *P* <0.05 was considered as significant.

#### Results

### Expression of RACK1 in gastric normal cells and cancer cell lines

In order to explore the expression of RACK1 in GC, we detected mRNA level of RACK1 in gastric normal cells (GES-1 cells) and six cancer cell lines (SGC7901, BGC823, MKN45, AGS, HGC27, and MGC803 cell lines). As Figure 1 shows, the mRNA expression of RACK1 was significantly downregulated in the GC cell lines (BGC823 and MKN45, P<0.05; others, P<0.01) compared with GES-1 cells. HGC27 and MGC803 cell lines were selected to conduct the following experiments as RACK1 was expressed at a lower level in the two cell lines.



**Figure 1** Downregulation of RACK1 in GC cell lines. qRT-PCR was performed to detect the mRNA expression of RACK1 in gastric normal cells (GES-1 cells) and cancer cell lines (SGC7901, BGC823, MKN45, AGS, HGC27, and MGC803 cells). Data were shown as mean ± SD in three independent experiments.

Note: Compared with gastric normal cells, \*P<0.05, \*\*P<0.01.

 $\label{eq:abbreviations: GC, gastric cancer; qRT-PCR, quantitative real-time polymerase chain reaction.$ 

#### Upregulated expression of RACKI inhibits cell viability, migration, and invasion in HGC27 and MGC803 cells

When overexpressing RACK1 was transfected into HGC27 (Figure 2A and B) and MGC803 cells (Figure 2D and E), mRNA and protein levels of RACK1 were determined to

detect transfection efficiency of overexpressing RACK1. The results showed that both in HGC27 and MGC803 cells, the mRNA and protein levels of RACK1 were noticeably high expressed in RACK1 group in comparison to control or NC (P<0.01, Figure 2A, B and D, E). Compared to control or NC, overexpressing RACK1 could decrease the cell viability at 12, 24 and 48 h in the two cell lines. However, in HGC27 cells, the significantly decreased cell viability was mainly presented at 12 (P<0.05) and 48 h (P<0.01) (Figure 2C). In MGC803 cells, the remarkably decreased cell viability was mainly presented at 24 h (P<0.05) and 48 h (P<0.01) (Figure 2F). In regard to cell migration and vasion, und scratch (Figure 3A and E) and Transwell say (Figure C and G) were performed. In HGC27 ells, overpressi RACK1 significantly decreased cell migration and on compared with control or NC (mightion, -0.01, Figure 3B; invasion, P < 0.05, Figure 3D In MO 03 cells similar tendency of an inhibitory g of overexp. w RACK1 on migration erved as in HGC27 cells (P<0.01, and invasion was Figure d H).



Figure 2 The inhibitory effects of overexpressing RACK1 on cell viability in HGC27 and MGC803 cells. qRT-PCR (**A**) and Western blot (**B**) were used to detect the transfection efficiency of overexpressing RACK1 in HGC27 cells. (**C**) The effect of overexpressing RACK1 on cell viability at 12, 24, and 48 h was detected by CCK-8 assay in HGC27 cells. qRT-PCR (**D**) and Western blot (**E**) were used to detect the transfection efficiency of overexpressing RACK1 in MGC803 cells. (**F**) The effect of overexpressing RACK1 on cell viability at 12, 24, and 48 h was detected by CCK-8 assay in HGC27 cells. qRT-PCR (**D**) and Western blot (**E**) were used to detect the transfection efficiency of overexpressing RACK1 in MGC803 cells. (**F**) The effect of overexpressing RACK1 on cell viability at 12, 24, and 48 h was detected by CCK-8 assay in MGC803 cells. Expression of each protein in cells was following normalization with a loading control GAPDH. Data are shown as mean  $\pm$  SD in three independent experiments.

Notes: Compared with control, \*P<0.05, \*\*P<0.01; compared with NC ^P<0.05, ^^P<0.01.

Abbreviations: qRT-PCR, quantitative real-time polymerase chain reaction; CCK-8, cell counting kit-8; NC, negative control.



Figure 3 The inhibitory effects of overexpressing RACK1 on cell migration and invasion in HGC27 and MGC803 cells. (A) Wound scratch assay was performed to detect the effect of overexpressing RACK1 on HGC27 cell migration at 0, 24 h. (B) Wound closure is shown as bar diagrams in HGC27 cells. (C) Transwell assay was used to detect the effect of overexpressing RACK1 on HGC27 cell invasion. (D) Invasion capability is shown as bar diagrams in HGC27 cells. (E) Wound scratch assay was performed to detect the effect of overexpressing RACK1 on MGC803 cell migration at 0, 24 h. (F) Wound closure is shown as bar diagrams in MGC803 cells. (E) Wound scratch assay was performed to detect the effect of overexpressing RACK1 on MGC803 cell migration at 0, 24 h. (F) Wound closure is shown as bar diagrams in MGC803 cells. (G) Transwell assay was used to detect the effect of overexpressing RACK1 on MGC803 cell invasion. (H) Invasion capability is shown as bar diagrams in MGC803 cells. Data are shown as mean ± SD from three independent experiments.

**Notes:** Compared with control, P<0.05, P<0.01; compared with NC, P<0.05, P<0.01. **Abbreviation:** NC, negative control.

#### Upregulated expression of RACKI regulates the expressions of epithelialmesenchymal transition (EMT) related genes in HGC27 and MGC803 cells

The above experiments confirmed that upregulation of RACK1 could inhibit cell migration and invasion, so we further detected the expressions of EMT related genes. The results showed that there were no obvious differences in the expressions of EMT related genes in control and NC groups. Overexpressing RACK1 could noticeably increase the mRNA and protein levels of E-cadherin in HGC27 cells (P<0.01, Figure 4A, D and E). Meanwhile, both mRNA and protein expressions of N-cadherin and Snail showed a rapid reduction (P<0.01, Figure 4B–E) in RACK1 overexpressing HGC27 cells. In MGC803 cells, overexpressing RACK1 significantly increased the expression of E-cadherin and Snail in both mRNA and protein levels (P<0.01, Figure 4F–J).

## Upregulated expression of RACKI regulates the related proteins expression of Wnt/ $\beta$ -catenin signaling

In order to explore the possible mechanism of the inhibitory effects of overexpressing RACK1 on Al via ility, migration and invasion, the related process of m+/R catenin signaling in HGC27 (Figure A) a **MGC803** cells (Figure 5D) were detected Western ot. The results showed that in both the cell in s, overexpressing RACK1 could increase p-catenin/ $\beta$ -c. nin (P<0.01, Figure 5B and E) expression and dramatically decrease c-Jun expression (P<0. Figure 5C and F) at the protein level. To further rify where the inhibitory effects of overexpressing RACL on the vicell lines were through Wnt/ $\beta$ -cate n signs for we applied the Wnt signaling nown in Figure 6, no obvious difference agonist, LiCl. in cell viability in VGC27 (Figure 6A) and MGC803 cells (Figure 6E) was observed in LiCI group and in the control or NC group; however, in the LiCI group, cell viability in HGC27 (Figure 6A) and MGC803 cells (Figure 6E) were increased, compared to group overexpressing RACK1 (P<0.01). Overexpressing RACK1 could reverse the increase of cell viability induced by LiCl (P<0.01). Wound scratch and Transwell assay were performed to detect cell migration and invasion, respectively (Figure 6D and H). In HGC27 cells, the results showed

that LiCl could significantly induce cell migration and invasion in comparison to RACK1 (P<0.01, Figure 6B and C). In the case of RACK1 combined with LiCl, a significant inhibitory tendency was observed in cell migration compared to that under LiCl treatment alone (P < 0.01), while no noticeable reduction of invasion was observed compared to that under LiCl treatment alone (P>0.05). In MGC803 cells, compared to the RACK1 group, LiCl could also remarkably increase cell migration and invasion (P<0.01, Figure 6F and G). Overexpressing RACK1 could significantly decrease cell migration induced by LiCl (P<0.01). In the ase of CK1 combined with LiCl, a slight reduction of cell in sion was observed in comparison to that under iCl treatment alone (P>0.05). All the above results indicate that Wnt/ $\beta$ catenin signaling was possible underlying mechanism of the inhibitory dects of erexpression RACK1 on cell sis in GC growth and m

#### Discrion

using research is carried out on RACK1, especially in Incr relation to cance.<sup>3</sup> Studies have shown that RACK1 can prome tumor wasion and metastasis and multiple cel-Jular funct oy PKC.<sup>34</sup> At the same time, RACK1 binds and performs corresponding translation and regto ation of ribosomes, thereby activating factors related to piological functions such as tumor invasion and etastasis.<sup>34</sup> The abnormal expression of RACK1 is closely associated with the occurrence and development of tumor, and increased expression of RACK1 occurred in most tumors such as breast cancer, non-small cell lung cancer, liver cancer, and melanoma, indicating that it acts as an oncogene.  $^{35-38}$  In the present study, the finding showed that RACK1 was downregulated in several GC cell lines compared with normal gastric epithelial cells. The result was in accordance with Deng's report that RACK1 was a tumor suppressor gene of GC.<sup>39</sup> Furthermore, it was expressed at a low level in GC and associated with tumor invasion was and low differentiation.<sup>39</sup> Although Liu reported that RACK1 proteins were expressed at a low level in GC patients,<sup>40</sup> and Chen reported that low expression of RACK1 predicted poor prognosis for overall five-year survival in all GC cases,<sup>29</sup> our study lacked the information about RACK1 prognosis from TCGA database. We are planning to launch this analysis in a future study.

In addition, overexpressing RACK1 was successfully transfected into HGC27 and MGC803 cells, and showed



Figure 4 The effects of overexpressing RACK1 on EMT-related genes. qRT-PCR was used to detect the overexpressing RACK1 on mRNA expressions of E-cadherin (A), N-cadherin (B) and Snail (C) in HGC27 cells. (D) Western blot was used to assess the overexpressing RACK1 on protein expressions of E-cadherin, N-cadherin, and Snail in HGC27 cells. (E) The three proteins were quantitative as bar diagrams in HGC27 cells. qRT-PCR was used to detect the overexpressing RACK1 on mRNA expressions of E-cadherin (F), N-cadherin (G) and Snail (H) in MGC803 cells. (I) Western blot was used to assess the overexpressing RACK1 on protein expressions of E-cadherin, N-cadherin, and Snail in MGC803 cells. (J) The three proteins were quantitative as bar diagrams in MGC803 cells. Expression of each protein in cells was following normalization with a loading control GAPDH. Data are shown as mean ± SD in three independent experiments.

Notes: Compared with control, \*P<0.05, \*\*P<0.01; ^compared with NC, ^P<0.05, ^^P<0.01.

Abbreviations: EMT, epithelial-mesenchymal transition; qRT-PCR, quantitative real-time polymerase chain reaction; NC, negative control.



Figure 5 The effects of overexpressing RACK1 on the Wnt/ $\beta$ -catenin signaling-related proteins in HGC27 and MGCs cocells. (A) p- $\beta$ -catenin,  $\beta$ -catenin, and c-Jun were detected by Western blot to obverse the effects of overexpressing RACK1 on protein expression and c-27 cells. (B) p- $\beta$ -catenin/ $\beta$ -catenin, protein level is shown as bar diagrams in HGC27 cells. (C) c-Jun were detected by Western blot to obverse the effects of overexpressing RACK1 on protein expression in HGC27 cells. (D) - $\beta$ -catenin,  $\beta$ -catenin,  $\beta$ -catenin protein level is shown as bar diagrams in HGC27 cells. (D) - $\beta$ -catenin,  $\beta$ -catenin,  $\beta$ -catenin,  $\beta$ -catenin,  $\beta$ -catenin protein level is shown as bar diagrams in HGC207 cells. (D) - $\beta$ -catenin,  $\beta$ -catenin,  $\beta$ -catenin,  $\beta$ -catenin protein expressing RACK1 on protein expressions in MGC803 cells. (E) p- $\beta$ -catenin protein level is shown as bar diagrams in MGC803 cells. (F) c-Jun protein level is shown as bar diagrams in MGC803 cells. Expression of each protein in cells we following normal protein with a loading control GAPDH. Data are shown as mean  $\pm$  SD in three independent experiments.

**Notes:** Compared with control, \*P<0.05, \*\*P<0.01; compared with NC,  $^{P}<0.05$ ,  $^{P}<0.01$ . **Abbreviations:** p- $\beta$ -catenin, phosphorylation- $\beta$ -catenin; NC, negative control.

significant inhibitory effects on cell viabili , mig tion, and invasion. We also detected the effect of over sing RACK on EMT genes in GC π of our knowledge, EMT is a critical are necessary cess in the early stage of tumor met stasis. In this process, tumor cells lose epithelial ell character ics, including E-cadherin expression, *increally* ar adhesion, and epithelial cell polarity; and a uire senchymal cell characteristics, including results and invarian.<sup>42-45</sup> Our results ing R. V. regulated markers of expre found that ov it inhibited the expression of EMT. Specifically N-cadherin promoted E-cadherin expression. pressing RACK1 suppressed Snail Meanwhile, over expression, which could induce the occurrence of EMT and downregulate E-cadherin expression in the progression of cancer.<sup>46,47</sup>

In regard to the underlying mechanism of RACK1 on the above biological function, we explored the Wnt signaling. There is a study reported that RACK1 contributed to the occurrence of GC through activation of the Wnt pathway by increasing dissociation of the  $\beta$ -catenin complex.<sup>39</sup> In our work, overexpression RACK1 could significantly ncrease the protein expression of p-β-catenin/β-catenin and decrease the protein expression of c-Jun. β-catenin is a key step in the Wnt pathway.<sup>48</sup> The abnormal activation of Wnt/β-catenin signaling pathway leads to a significant increase in the accumulation of β-catenin in the nucleus, triggering excessive proliferation, and malignant transformation of cells.<sup>49,50</sup> C-Jun is a member of the Jun gene family and is an oncogene.<sup>51</sup> It encodes the components of transcription factor AP-1 in dimer form.<sup>52</sup> Takeda reported that in the activated Wnt pathway, β-catenin/TCF can bind to the c-Jun promoter and induce c-Jun transcription, which plays an important role in the development and progression of colorectal cancer.<sup>53</sup> Therefore, we inferred the effect of overexpressing RACK1 on GC cells through inhibition of Wnt/β-catenin signaling.

Furthermore, to verify the correlation between this pathway and the anti-cancer effect of RACK1, we added Wnt pathway agonist LiCl to RACK1 overexpressing GC cells. The results presented that LiCl could noticeably increase cell viability, induce cell migration and invasion in comparison to control or NC. Meanwhile, LiCl could also partially reverse the inhibitory effects of



Figure 6 The verification experiments of Wnt/ $\beta$ -catenin in GC cells. (A) The suppression effects of overexpressing RACK1 on increasing cell viability induced by LiCl in HGC27 cells. (B) The suppression effects of overexpressing RACK1 on increasing cell migration induced by LiCl in HGC27 cells. (C) The suppression effects of overexpressing RACK1 on increasing cell migration and invasion in HGC27 cells were respectively performed by wound scratch and Transwell assay. (E) The suppression effects of overexpressing RACK1 on increasing cell viability induced by LiCl in MGC803 cells. (F) The suppression effects of overexpressing RACK1 on increasing cell viability induced by LiCl in MGC803 cells. (F) The suppression effects of overexpressing RACK1 on increasing cell migration induced by LiCl in MGC803 cells. (G) The suppression effects of overexpressing RACK1 on increasing cell migration induced by LiCl in MGC803 cells. (G) The suppression effects of overexpressing RACK1 on increasing cell migration induced by LiCl in MGC803 cells. (H) Cell migration and invasion in MGC803 cells. (F) The suppression induced by LiCl in MGC803 cells. (H) Cell migration and invasion in MGC803 cells. (H) Cell migration and invasion in MGC803 cells. (H) Cell migration and invasion in MGC803 cells were respectively performed by wound scratch and Transwell assay. Data are shown as mean  $\pm$  SD in three independent experiments. Notes: Compared with control, \*P<0.01; compared with NC, ^P<0.05, ^AP<0.01; compared with RACK1, \*P<0.05, \*\*P<0.01; compared with LiCl, #P<0.05, \*\*P<0.01; Abbreviations: GC, gastric cancer; NC, negative control.

overexpressing RACK1 on cell viability, migration, and invasion. RACK1 may inhibit the proliferation, migration, and invasion of GC cells by blocking the Wnt/ $\beta$ -catenin signaling pathway. There may be other pathways at the same time, and more in-depth research is needed.

In this study, there are still many limitations, for instance, a lack of research on cell mortality, experiments regarding the relation between cell migration/invasion and cell viability, as well as JNK activator, to validate the involved mechanism of Wnt/ $\beta$ -catenin signaling. With regard to this, we are planning to launch a comprehensive, confirmative and in-depth study to make up the limitations.

#### Conclusion

In this study, we found that RACK1 possibly inhibited EMT of GC cells through limitation of the Wnt/ $\beta$ -catenin pathway, thereby suppressing cell migration and invasion; and also inhibited cell growth. This study provides a theoretical basis for determining the anti-cancer effects of RACK1 and its application in the clinical study and targeted therapy of GC.

#### Acknowledgments

This work was supported by the National Natural Scient Foundation of China [81372378].

#### Disclosure

The authors report no conflicts of interest

#### References

 Gigek CO, Calcagno DQ, Raspessen LT, et al cienetic variants in gastric cancer: risks and clusteral implications. In *Mol Pathol.* 2017;103(1):101–111. doi: 1016/j.ymp.2017.07.004

this

- Ratti M, Lampis A, Hahi, C, Passa Acqua R, Valeri N. Microsatellite instability in gastric cancer: A bundar bases a mical perspectives, and new treatment approaches. *Cell Col Life 7 :* 2018;75(22):4151–4162. doi:10.1007/s00.18-018-906-9
- Ferlay J, S in HR, F y F, Forma D, Mathers C, Parkin DM. Estimates on porld ac on port f cancer in 2008: GLOBOCAN 2008. Int J Can. 2010;127(12):2893–2917. doi:10.1002/ijc.25516
- Ferlay J, Soerjonn, cam I, Dikshit R, et al. Cancer incidence and mortality worldwide, cources, methods and major patterns in GLOBOCAN 2012. Int J Cancer. 2015;136(5):E359–E386. doi:10.1002/ijc.29210
- Rahman R, Asombang AW, Ibdah JA. Characteristics of gastric cancer in Asia. World J Gastroenterol. 2014;20(16):4483–4490. doi:10.3748/ wjg.v20.i16.4483
- Chen W, Zheng R, Baade PD, et al. Cancer statistics in China, 2015. CA Cancer J Clin. 2016;66(2):115–132. doi:10.3322/caac.21338
- Karimi P, Islami F, Anandasabapathy S, Freedman ND, Kamangar F. Gastric cancer: descriptive epidemiology, risk factors, screening, and prevention. *Cancer Epidemiol Biomarkers Prev.* 2014;23(5):700–713. doi:10.1158/1055-9965.EPI-13-1057

- Takahashi T, Saikawa Y, Kitagawa Y. Gastric cancer: current status of diagnosis and treatment. *Cancers*. 2013;5(1):48–63. doi:10.3390/ cancers5010048
- Chen XZ, Zhang WH, Hu JK. A difficulty in improving population survival outcome of gastric cancer in mainland China: low proportion of early diseases. *Med Oncol.* 2014;31(12):315. doi:10.1007/s12032-014-0374-0
- Orditura M, Galizia G, Sforza V, et al. Treatment of gastric cancer. World J Gastroenterol. 2014;20(7):1635–1649. doi:10.3748/wjg.v20.i7.1635
- Torre LA, Bray F, Siegel RL, Ferlay J, Lortet-Tieulent J, Jemal A. Global cancer statistics, 2012. *CA Cancer J Clin.* 2015;65(2):87–108. doi:10.3322/caac.21262
- Gill RS, Al-Adra DP, Nagendran J, et al. Treatment of gastric cancer with peritoneal carcinomatosis by cytoreductive surgery and HIPEC: a systematic review of survival, mortality of morbidity. *J Surg Oncol.* 2011;104(6):692–698. doi:10.1000\_s0.2201.
- Bairamov RB, Abdullaeva RT. [The supect of early distric cancer diagnosis on indices of survival in superior after ray al surgical intervention]. *Klinichna Khirutta*. 2015, p18–21.
  Smyth EC, Verheij M, Alfrad W, Cunnington Di Cervantes A,
- 14. Smyth EC, Verheij M, Alban W, Cunning am De Cervantes A, Arnold D. Gastric cancer ESMO unical proceeding guidelines for diagnosis, treatment and allower Ann Oncol. 2016;27(suppl 5): v38–v49. doi:10.100 /anno.000/w350
- Tartaglia D, Bernaucci A, Garrioto C, et al. Incidental appendectomy? Microst py cells another successful retrospective cohort study in patients py senting cutte right lower quadrant abdominal pain. *Int J Surg.* 2016;28:143–52. doi:10.1016/j.ijsu.2016.02.085
- Wey Z., Edang B, Jiang L, tal. RACK1, an excellent predictor for per clinical outcome in oral squamous carcinoma, similar to Ki67. *r J Cancer.* 2(1):45(3):490–496. doi:10.1016/j.ejca.2008.11.012
- 17. A multifaceted scaffolding products structure and function. *Cell Commun Signal*. 2011;9:22. doi:10.1016/j.8-811X-9-22
- perns H, Humar R, Hengerer B, Kiefer FN, Battegay EJ. RACK1 is producted in angiogenesis and human carcinomas. *Faseb J*. 2000;14(15):2549–2558. doi:10.1096/fj.99-1038com
- 19. Mamidipudi V, Cartwright CA. A novel pro-apoptotic function of RACK1: suppression of Src activity in the intrinsic and Akt pathways. *Oncogene*. 2009;28(50):4421–4433. doi:10.1038/onc.2009.293
- Yan Y, Jiang Y. RACK1 affects glioma cell growth and differentiation through the CNTN2-mediated RTK/Ras/MAPK pathway. *Int J Mol Med.* 2016;37(1):251–257. doi:10.3892/ijmm.2015.2421
- 21. Liu Q, Wang X, Liu Y, et al. RACK1 inhibits morphine re-exposure via inhibition of src. *Neurol Res.* 2011;33(1):56–62. doi:10.1179/ 016164110X12714125204236
- 22. Li J, Guo Y, Feng X, et al. Receptor for activated C kinase 1 (RACK1): a regulator for migration and invasion in oral squamous cell carcinoma cells. *J Cancer Res Clin Oncol*. 2012;138(4):563–571. doi:10.1007/s00432-011-1097-7
- Ruan Y, Sun L, Hao Y, et al. Ribosomal RACK1 promotes chemoresistance and growth in human hepatocellular carcinoma. *J Clin Invest.* 2012;122(7):2554–2566. doi:10.1172/JCI58488
- 24. Hu F, Tao Z, Wang M, et al. RACK1 promoted the growth and migration of the cancer cells in the progression of esophageal squamous cell carcinoma. *Tumour Biol.* 2013;34(6):3893–3899. doi:10.1007/s13277-013-0977-7
- 25. Li JJ, Xie D. RACK1, a versatile hub in cancer. Oncogene. 2015;34 (15):1890–1898. doi:10.1038/onc.2014.127
- 26. Shen F, Yan C, Liu M, Feng Y, Chen Y. RACK1 promotes prostate cancer cell proliferation, invasion and metastasis. *Mol Med Rep.* 2013;8(4):999–1004. doi:10.3892/mmr.2013.1612
- 27. Wu J, Meng J, Du Y, et al. RACK1 promotes the proliferation, migration and invasion capacity of mouse hepatocellular carcinoma cell line in vitro probably by PI3K/Rac1 signaling pathway. *Biomed Pharmacother = Biomedecine & Pharmacotherapie*. 2013;67 (4):313–319. doi:10.1016/j.biopha.2013.01.011

- Yong-Zheng X, Wan-Li M, Ji-Ming M, Xue-Qun R. Receptor for activated protein kinase C 1 suppresses gastric tumor progression through nuclear factor-kB pathway. *Indian J Cancer*. 2015;52(Suppl 3):E172–E175. doi:10.4103/0019-509X.186572
- 29. Chen L, Min L, Wang X, et al. Loss of RACK1 promotes metastasis of gastric cancer by Inducing a miR-302c/IL8 signaling loop. *Cancer Res.* 2015;75(18):3832–3841. doi:10.1158/0008-5472. CAN-14-3690
- Logan CY, Nusse R. The Wnt signaling pathway in development and disease. Annu Rev Cell Dev Biol. 2004;20:781–810. doi:10.1146/ annurev.cellbio.20.010403.113126
- 31. Sareddy GR, Challa S, Panigrahi M, Babu PP. Wnt/beta-catenin/Tcf signaling pathway activation in malignant progression of rat gliomas induced by transplacental N-ethyl-N-nitrosourea exposure. *Neurochem Res.* 2009;34(7):1278–1288. doi:10.1007/s11064-008-9906-3
- Wang L, Li B, Zhang L. et al. miR-664a-3p functions as an oncogene by targeting hippo pathway in the development of gastric cancer. *Cell Prolif*;2019. e12567. doi:10.1111/cpr.12567
- Brina D, Miluzio A, Ricciardi S, Biffo S. eIF6 anti-association activity is required for ribosome biogenesis, translational control and tumor progression. *Biochim Biophys Acta*. 2015;1849 (7):830–835. doi:10.1016/j.bbagrm.2014.09.010
- Lopez-Bergami P, Huang C, Goydos JS, et al. Rewired ERK-JNK signaling pathways in melanoma. *Cancer Cell*. 2007;11(5):447–460. doi:10.1016/j.ccr.2007.03.009
- 35. Cao XX, Xu JD, Xu JW, et al. RACK1 promotes breast carcinoma migration/metastasis via activation of the RhoA/Rho kinase pathway. *Breast Cancer Res Treat*. 2011;126(3):555–563. doi:10.1007/s10549-010-0955-3
- 36. Nagashio R, Sato Y, Matsumoto T, et al. Expression of RACK1 is a novel biomarker in pulmonary adenocarcinomas. *Lung Cancer*. 2010;69(1):54–59. doi:10.1016/j.lungcan.2009.09.015
- 37. Egidy G, Jule S, Bosse P, et al. Transcription analysis in the beLive swine model identifies RACK1 as a potential marker of malig incy for human melanocytic proliferation. *Mol Convers* 2008; 1 doi:10.1186/1476-4598-7-34
- Park SM, Gaur AB, Lengyel E, Peter MF The miR 200 family determines the epithelial phenotype of can cells be a true the E-cadherin repressors ZEB1 and 7 B2. *Dev.* 2008;22 (7):894–907. doi:10.1101/gad.16406.2
- 39. Deng YZ, Yao F, Li JJ, et al. References gangic tumorigenesis by stabilizing the deta-callene destruction complex. *Gastroenterology*. 2017142(4):812–82, 9815. doi:10.1053/j. gastro.2011.12.046

# $\mathcal{A}^{\vee}$

- 40. Liu C, Ren L, Wang Y, Liu Y, Xiao J. The interaction between RACK1 and WEE1 regulates the growth of gastric cancer cell line HGC27. *Oncol Lett.* 2017;14(4):4784–4792. doi:10.3892/ol.2017.6741
- Kraljevic Pavelic S, Sedic M, Bosnjak H, Spaventi S, Pavelic K. Metastasis: new perspectives on an old problem. *Mol Cancer*. 2011;10:22. doi:10.1186/1476-4598-10-93
- Thiery JP, Acloque H, Huang RY, Nieto MA. Epithelialmesenchymal transitions in development and disease. *Cell*. 2009;139(5):871–890. doi:10.1016/j.cell.2009.11.007
- 43. Thiery JP, Sleeman JP. Complex networks orchestrate epithelial-mesenchymal transitions. *Nat Rev Mol Cell Biol*. 2006;7 (2):131–142. doi:10.1038/nrm1835
- 44. Savagner P, Boyer B, Valles AM, Jouanneau J, Thiery JP. Modulations of the epithelial phenotype during embryogenesis and cancer progression. *Cancer Treat Res*, 100 (21):229–249.
- Tam WL, Weinberg RA. The epigrandes of the helial-mesenchymal plasticity in cancer. *Nat Med.* 27, 3;19(11):1438, 449. doi:10.1038/ nm.3336
- 46. Wang X, Wang B, Xie J, Iou D, Yang H, Hung H. Melatonin inhibits epithelialtomest chymal transfer in genetic cancer cells via attenuation of IL1ber AFkappar MMP2/2019 signaling. *Int J Mol Med.* 2018;42(4):22. 2228 poi:10.3892/ijinm.2018.3788
- Cano A, Perez Forence for Rodrigo Jost al. The transcription factor snail controls epithelia, essencional transitions by repressing E-cadhe transmission. *Nat evidentia*. 2000;2(2):76–83. doi:10.1038/ 35006.25
- 48. Li VS, Ng SS, Bernema PJ, et al. Wnt signaling through inhibition a outa-catenin degregation in an intact Axin1 complex. *Cell*. 2012;149(6):1245–1256. doi:10.1016/j.cell.2012.05.002
- Ueno K, Hinga H, Hinoda Y, Dahiya R. Frizzled homolog proteins, microRNAs and Wnt signaling in cancer. *Int J Cancer*. 2013;132 (8):1731–17 J. doi:10.1002/ijc.27746
- 50. N. Joseph Parker Struckhoff A, Trillo-Tinoco J, et al. Activation of c-Myc and cyclin D1 by JCV T-antigen and beta-catenin in colon per. *PLoS One.* 2014;9(9):e106257. doi:10.1371/journal. pone.0106257
- 51. Tanner B, Grimme S, Schiffer I, et al. Nuclear expression of apurinic/ apyrimidinic endonuclease increases with progression of ovarian carcinomas. *Gynecol Oncol.* 2004;92(2):568–577. doi:10.1016/j. ygyno.2003.10.037
- Turpaev KT. [Role of transcription factor AP-1 in integration of cellular signalling systems]. *Mol Biol (Mosk)*. 2006;40(6):945–961.
- Takeda K, Kinoshita I, Shimizu Y, et al. Clinicopathological significance of expression of p-c-Jun, TCF4 and beta-Catenin in colorectal tumors. *BMC Cancer*. 2008;8:328. doi:10.1186/1471-2407-8-172

#### **OncoTargets and Therapy**

#### Publish your work in this journal

OncoTargets and Therapy is an international, peer-reviewed, open access journal focusing on the pathological basis of all cancers, potential targets for therapy and treatment protocols employed to improve the management of cancer patients. The journal also focuses on the impact of management programs and new therapeutic agents and protocols on patient perspectives such as quality of life, adherence and satisfaction. The manuscript management system is completely online and includes a very quick and fair peer-review system, which is all easy to use. Visit http://www.dovepress.com/ testimonials.php to read real quotes from published authors.

Submit your manuscript here: https://www.dovepress.com/oncotargets-and-therapy-journal

#### **Dove**press