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Down-regulation of RNF146 promotes gastric cancer cell apoptosis and inhibits cell migration via Wnt/β-catenin signaling pathway

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ABSTRACT: Gastric cancer (GC) is one of the high mortality rate cancers in the world, and seeking more effective treatment methods has always been one of the urgent clinical problems to be solved. Therefore, a thorough understanding of GC pathogenesis is necessary. The E3 ubiquitin ligase ring finger protein 146 (RNF146) has been implicated as the important factor in tumor evolution. However, its role in GC has not been well investigated. In the present study, our results first showed that RNF146 was up-regulated in both GC tissues and GC cell lines. In addition, RNF146 knockdown induced cell apoptosis and inhibited cell migration and invasion in HGC27 cells. However, these phenomena could be reversed by RNF146 over-expression. Furthermore, our results demonstrated that RNF146 possessed oncogenic role through down-regulating the expression of Axin1 to activate β-catenin signaling pathway. Collectively, these results indicated that RNF146 might be regarded as a carcinogen in human GC and representing as a promising and valuable therapeutic target for the GC treatment.

KEYWORDS: gastric cancer, RNF146, Wnt, β-catenin, apoptosis

INTRODUCTION

Gastric cancer (GC) is considered as one of the most lethal cancers worldwide, and contains varieties of clinical behaviors and pathological entities [1]. Currently, the molecular mechanism under GC remains unclear. For patients with early-stage GC, surgery is still the initial treatment with complete resection, but recurrence occurs in a significant proportion of patients [2]. It is urgently needed to reveal the underline mechanisms that are involved in GC development, which will contribute to provide novel prognostic biomarkers and valuable targets for the treatment of GC.

Numerous studies have shown that the Wnt/β-catenin signaling pathway plays critical role in human cancer development, progression, and prognosis [3, 4]. For instance, the pathway is involved in the specification and differentiation in mesenchymal stem cells [5], In addition, Wnt/β-catenin signaling pathway regulates cell autophagy and suppresses the adipogenic program [6]. Researchers have found that the pathway was activated and related to cell growth and migration in GC cells [7–9]. Moreover, targeting Wnt/β-catenin signaling has been indicated to be helpful to inhibit cell proliferation of GC cells [10].

It is well known that E3 ubiquitin ligases are involved in the management of cell growth, cell cycle arrest, and cell apoptosis [11]. RNF146, a RING finger protein belonging to E3 ubiquitin ligase family [12], interacts with poly(ADP-ribose) via a PAR-binding motif in the domain of Trp-Trp-Glu [13]. RNF146 has neuroprotective effect through inhibiting parthanatos via binding with PAR [13]. Studies showed that RNF146 controled the Wnt signaling pathway by regulating the degradation of Axin1 [13]. In addition, overexpression of RNF146 in Non-Small Cell Lung Cancer induced cell proliferation and migration via the Wnt/β-catenin signaling pathway [12]. Moreover, it has been suggested that RNF146 exerted oncogenic role in human colorectal cancer by activating the Wnt/β-catenin signaling pathway [14]. However, the function of RNF146 in the development of GC and its crosstalk with the Wnt/β-catenin signaling pathway in GC remain unclear. In the present research, we investigated the role of RNF146 in GC cells and revealed that RNF146 silencing possessed anticancer effects by promoting cell apoptosis and inhibiting cell proliferation via Wnt/β-catenin signaling pathway in GC cells.

MATERIALS AND METHODS

Gastric cancer tissues

In this study, we recruited 40 GC patients in Urumqi Friendship Hospital. Patients did not receive any chemotherapy or radiation before operation. Tissue samples were collected and snap frozen in liquid nitrogen, and then were stored at −80 °C until further uses. All experiments were performed in accordance with the ethic committee of Urumqi Friendship Hospital. The informed Consent Form was supplied following the Declaration of Helsinki [15].

Cell lines and cell culture

GES-1 (human gastric epithelial cell line) and MKN45, HGC27, AGS, MKN74 (Gastric cancer cell lines) cells

were obtained from Nanjing Cobioer Biotechnology Co., Ltd (Nanjing, Jiangsu, China). Cells were cultured in RPMI1640 with 10% fetal bovine serum (Invitrogen, Carlsbad, CA, USA), 1% penicillin, and 1% streptomycin at 37 °C with 5% CO_2.

Cell transfection

HGC27 cells were plated onto 24-well plates at approximately 50% confluence. RNF146 was cloned into the pcDNA3.1 to increase RNF146's expression. Then, cells were transfected with SiRNA control (si-NC) or si-RNF146 (si-RNF146; Santa Cruz Biotechnology, Santa Cruz, CA, USA) using Lipofectamine RNAiMAX Reagent (Invitrogen) for 24 h. Transfection efficiency was confirmed by western blotting, and GAPDH was used as reference to evaluate the protein expression level.

Wound-healing assay

Approximately 1×10^6 HGC27 cells were seeded into 6-well plate and incubated for 24 h at 80–90% confluence. A 10 μl plastic pipette tip was used to make scratched wounds, and then the cells were cultured. Cells were washed three times with PBS, serum-free medium was added to the cells, and the wound margins were photographed at 0 and 24 h under macroscope (Olympus; IX51). The distance between wounds' margins in the microscopic field (200X) was measured to quantify cell motility.

Transwell assay

200 μl cell suspensions containing 2.5×10^3 cells/ml were added to the upper chamber with the lower chamber containing 600 μl medium of 10% fetal bovine serum. The Transwell membrane was coated with Matrigel (diluted 1:4, BD Biosciences, Franklin Lakes, NJ, USA) to build a matrix barrier. Cells were incubated at 37 °C with 5% CO_2 for 24 h. After that, cells were washed with PBS, fixed with 95% ethyl alcohol at room temperature for 15 min and, then, stained with 1% crystal violet for 10 min. Finally, photographs (200X) were taken, and cells were counted under a microscope (Olympus; IX51).

Cell proliferation and apoptosis assay

pcDNA3.1-RNF146 and siRNF146 transfected cells or pcDNA3.1 and si-NC transfected cells were plated on 96-well plates. The cell proliferation was ascertained by Cell Counting Kit-8 (CCK-8; Meilunbio, China) following the producer's guidelines. Annexin V/7-AAD Apoptosis Detection Kit (Keygentec, Nanjing, China) was used to evaluate cell apoptosis. The information was obtained by flow cytometry (BD FACSCalibur, USA) and analyzed using FlowJo software (FlowJo, USA).

RT- PCR and western blot experiments

Total RNA was extracted using TRIzol (Invitrogen) following the manufacturer's direction. GAPDH's level served as the inner control. Primers used were [12] RNF146, sense: 5′-GGACGTCGCAGGAAGATTAAG-3′ and anti-sense: 5′-CAATGGAGGTGTCTGGTGCT-3′; and GAPDH, sense: 5′-GAAGGTCGGAGTCAACGGAT-3′ and anti-sense: 5′- CCTGGAAGATGGTGATGGG-3′. Data were analyzed by the comparative $2^{-\Delta\Delta Ct}$ method.

After harvesting, cells were treated with RIPA (P0013; Beyotime Biotechnology, Shanghai, China) lysis buffer to obtain the total protein, and then the proteins were separated by SDS-PAGE. Thereafter, the proteins were transferred onto polyvinylidene fluoride membranes and blocked in 5% non-fat milk. Next, the membranes were incubated overnight at 4 °C with antibodies against RNF146 (1:1000; Abcam, Cambridge, England), Caspase-3 (1:1000; CST, Boston, USA), c-Caspase-3 (1:1000; CST), Bax (1:1000; CST), β-catenin (1:1000; Abcam), Axin1 (1:1000; CST), and GAPDH (1:1000; CST). Finally, the membranes were incubated with HRP-conjugated secondary antibody (1:2500, CST) at room temperature for 1 h and quantitated using BioImaging. The protein levels were valued using GAPDH as control.

Statistical analysis

The data was statistically analyzed using SPSS version 17.0 (SPSS Inc., Chicago, IL, USA) and expressed as Mean ± SD. The differences between two groups were performed by two-tailed Student's *t*-test. And one-way ANOVA followed with post hoc Dunnett's analysis were used for comparison of differences among multiple groups. $p < 0.05$ was considered to have statistically significance.

RESULTS

RNF146 was up-regulated in GC tissues and cells

To explore the role of RNF146 in the development of GC, we measured the mRNA and protein expression of the RNF146 in GC tissues and cell lines. The expression levels of RNF146 were significantly increased in GC tissues compared with the adjacent normal tissues as displayed in Fig. 1a,b. Consistently, the expression of RNF146 was also markedly up-regulated in GC cells compared with GES-1 cells (Fig. 1c,d). All these findings demonstrated that the RNF146 was up-regulated in gastric carcinoma samples and cells.

Silencing RNF146 promoted cell apoptosis in GC cells

We then knocked down and overexpressed RNF146 in HGC27 cells using RNF146-targeting small interfering RNA (siRNA) and pcDNA3.1-RNA146 overexpression plasmid, respectively. As shown in Fig. 2a, after transfection for 24 h, the expression of RNF146

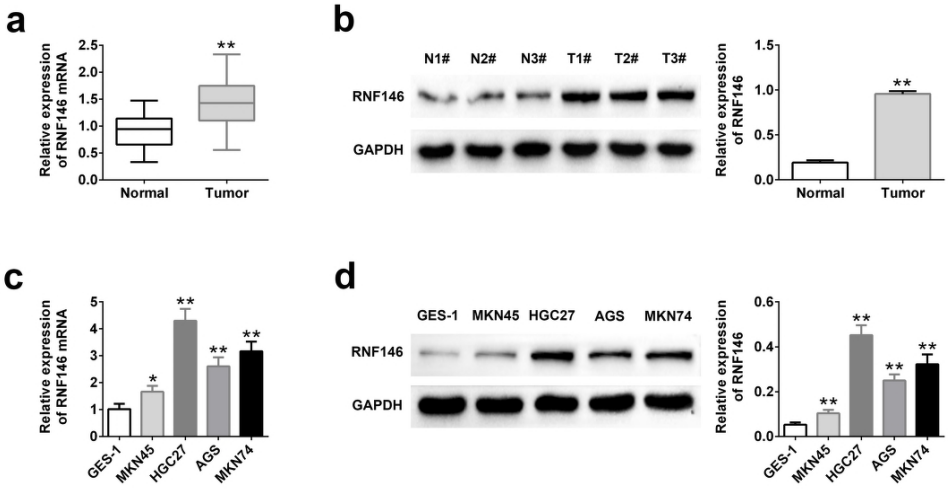

Fig. 1 Expression of RNF146 in GC tissues and cell lines. (a) RT-PCR and (b) Western-blot analysis of RNF146 levels in gastric normal and cancer tissues, $n = 40$. ** $p < 0.01$, compared with gastric normal tissues; (c) the mRNA and (d) protein levels of RNF146 in human gastric epithelial cell line (GES-1) and gastric cancer cells (MKN45, HGC27, AGS, MKN74). * $p < 0.05$, compared with GES-1 cells; ** $p < 0.01$, compared with GES-1 cells.

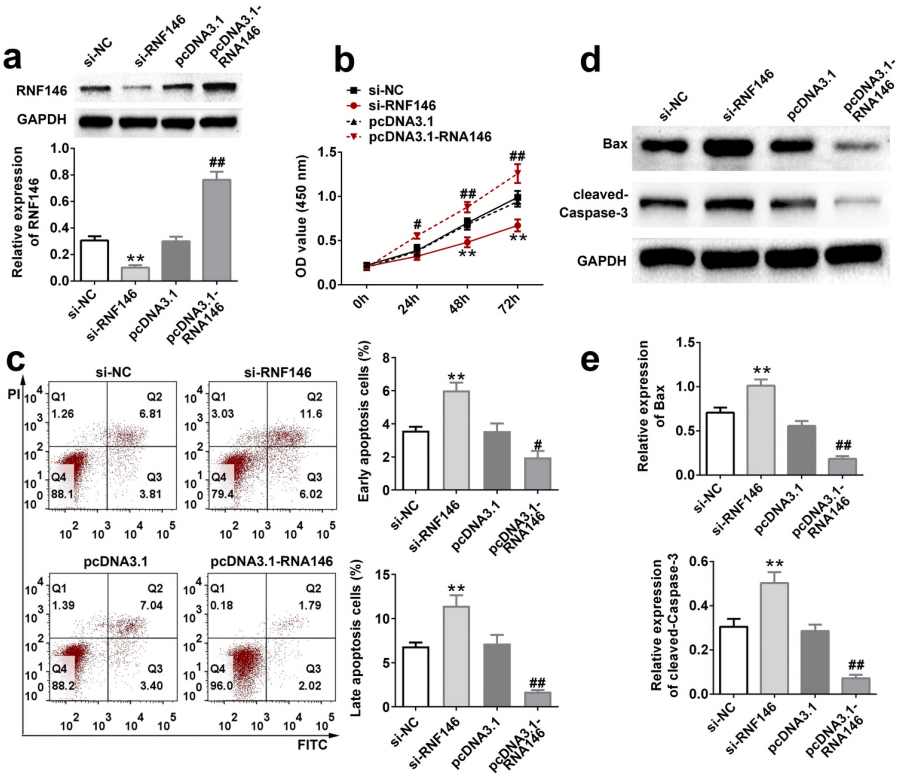

Fig. 2 RNF146 knockdown promoted cell apoptosis in GC cells. (a) Western blot analysis of RNF146 expression in HGC27 cells treated with si-NC, si-RNF146, pcDNA3.1, and pcDNA3.1-RNA146 for 24 h; (b) HGC27 cells transfected with si-NC, si-RNF146, pcDNA3.1, and pcDNA3.1-RNA146 for 24 h; (c) cell apoptosis assessed using flow cytometry with the Annexin V/7-AAD staining; (d) the levels of Bax and cleaved forms of caspase-3 examined by western blotting. * $p < 0.05$ & ** $p < 0.01$, compared with control siRNA group. # $p < 0.05$ & ## $p < 0.01$, compared with control pcDNA3.1 group.

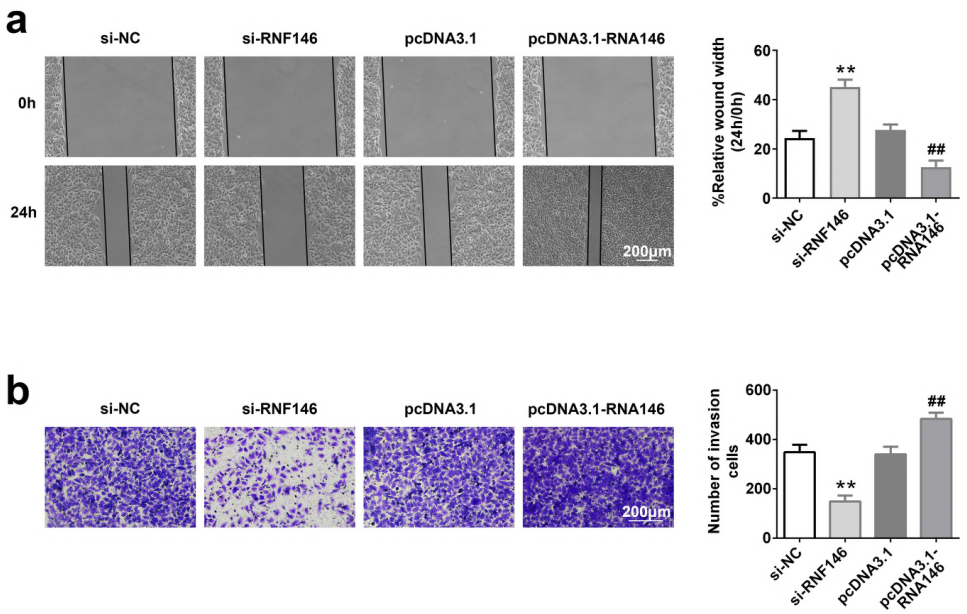

Fig. 3 RNF146 influenced cell migration and invasion in HGC27 cells. (a) Wound healing assay in HGC27 cells with RNF146 knockdown or over-expressed and the ratio of the distance between 24 h and 0 h of wounds' margins indicates the cell motility; (b) cell invasion was evaluated using Transwell method. $** p < 0.01$, compared with control siRNA group. All photomicrographs were presented as 100X. $\#\# p < 0.01$, compared with control pcDNA3.1 group.

was obviously changed. RNF146 silencing significantly repressed cell proliferation, while up-regulation of RNF146 significantly induced cell growth as determined by using CCK-8 in HGC27 cells (Fig. 2b). In addition, down-regulation of RNF146 induced cell apoptosis, but this phenomenon was reversed in RNF146 over-expression cells (Fig. 2c). In keeping with RNF146's anti-apoptotic function, the protein levels of Bax and cleaved forms of caspase-3 were substantially elevated in RNF146 knockdown HGC27 cells; whereas these results were dramatically reversed in RNF146 over-expression HGC27 cells (Fig. 2d). Thus, these results suggested RNF146 knockdown induced cell apoptosis in GC cells.

Silencing RNF146 inhibited cell migration and invasion in GC cells

The cell migration and invasion abilities were affected by the knockdown and overexpression of RNF146 in GC cells. Thus, we conducted the Wound-healing and the Transwell experiments to evaluate cell migration and invasion abilities, respectively. The results of Wound healing assay showed that the width of the wounds' margins was obviously increased after down-regulation of RNF146, and these results were significantly reversed by up-regulation of RNF146. Moreover, the cell invasion was increased in the RNF146 overexpressed group and decreased in the RNF146 silencing group (Fig. 3a,b). Taken together, the overall results indicated that RNF146 played an important role on cell migration and invasion in GC cells.

RNF146 regulated Wnt /β-catenin signaling pathway in GC cells

We proposed that RNF146 may regulate the growth of cancer cells through activating Wnt/β-catenin signaling in GC. To test this speculation, we used HGC27 cells transfected with si-NC, si-RNF146, pcDNA3.1, and pcDNA3.1-RNA146 and found that RNF146 silencing significantly repressed the expression of β-catenin; whereas the Axin1 expression was markedly induced in the si-RNF146 group. The expressions of c-Myc and MMP-7, the two target genes of β-catenin, were also decreased in si-RNF146 knockdown cells. However, overexpression of RNF146 obviously reversed these phenomena (Fig. 4a). Further investigation on the correlation of RNF146 expression and the Wnt/β-catenin signaling in GC cells was done, and PNU-74654 (a pharmacological inhibitor of Wnt /β-catenin) was used. The results showed that the regulation of Wnt/β-catenin signaling pathway by RNF146 was reversed by PNU-74654 (Fig. 4b). The effect of RNF146 on promoting cell proliferation was also blocked (Fig. 4c). Thus, these data collectively showed that RNF146 performed an important role in promoting cell migration and invasion via Wnt/β-catenin signaling pathway.

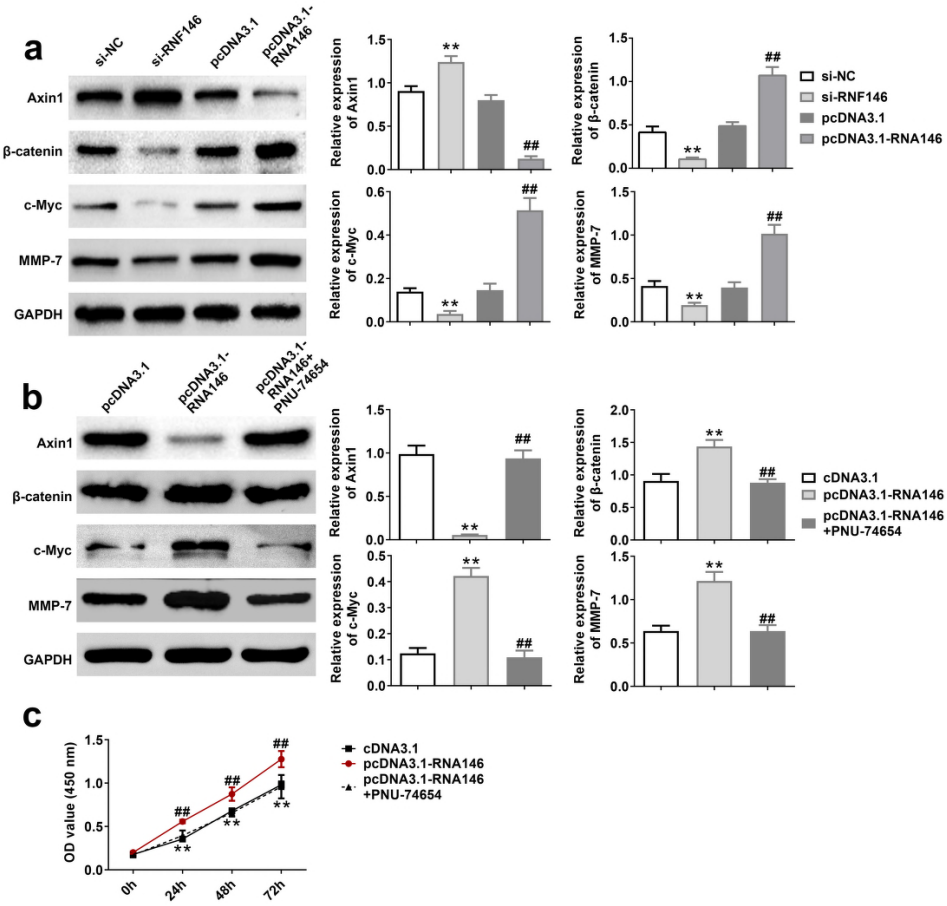

Fig. 4 RNF146-mediated regulation of Wnt/β-catenin signaling pathway. (a) The protein levels of Axin1, β-catenin, c-Myc, and MMP-7 in HGC27 cells treated with si-NC, si-RNF146, pcDNA3.1, and pcDNA3.1-RNA146 for 24 h; HGC27 cells treated with pcDNA3.1, pcDNA3.1-RNA146 and PNU-74654 (200 μM) for 24 h. (b) The levels of Axin1, β-catenin, c-Myc and MMP-7 were detected by western-blot. (c) Cell activity was detected by CCK-8 assay. ** $p < 0.01$, compared with control siRNA group; ## $p < 0.01$, compared with control pcDNA3.1 group.

DISCUSSION

Despite the great progress made by modern treatments of cancers, GC is still one of the leading causes of high mortality in cancers worldwide [16, 17]. Although efforts have been made to search for effective treatments to improve the outcomes of GC patients, the survival rate after GC surgery is still extremely low with an overall 5-year survival rate of less than 10% in the patients [18]. Therefore, it is required and clinically important to identify effective diagnostic and prognostic targets for GC treatment.

RNF146, a RING-type E3 ubiquitin-protein ligase, is a major regulator in Wnt signaling [13]. Studies indicate that RNF146 promotes DNA repair and protects cell from cell death caused by DNA impairment [19]. In addition, RNF146 presents as a promising tumor oncogene target in several cancers [12, 20, 21]. For example, Li et al [22] found that RNF146 induced the ubiquitination and degradation of PTEN, thus promoting tumor cell growth. Up-regulated expression of RNF146 was also identified in non-small cell lung cancer that related to cell proliferation and invasion [12]. Nevertheless, the clinical importance and biological role of RNF146 in GC have not even been reported. In the present study, we discovered that RNF146 was up-regulated in both GC tissues and cell lines. In addition, RNF146 silencing induced cell apoptosis, while high RNF146 expression induced cell proliferation and invasion in GC cells. Our data illustrated that RNF146 could be a possible remedial target for the treatment of GC.

It is well known that epithelial-to-mesenchymal transformation (EMT) is a key mechanism driving invasion and metastasis in most cancers [23]. Wnt/β-catenin promotes epithelial-to-mesenchymal transition (EMT) by binding to members of the TCF/LEF fam-

ily [24–26]. Besides, β-catenin forms a complex with epidermal growth factor receptor (EGFR), which also promotes the metastasis and invasion of tumor cells [27]. Moreover, Wnt/β-catenin signaling pathway is involved in tumor drug resistance through a cascade with the PI3K/AKT/GSK-3 pathway [28]. Furthermore, Wnt/β-catenin signaling also orchestrates with other cell signaling factors (such as nuclear factor kappa-B (NF-κB), Hippo/YAP, and Notch) which play pivotal role in the pathogenesis of cancers [29, 30]. Thus, Wnt/β-catenin signaling is a promising target for cancer therapy.

In the Wnt/β-catenin signaling pathway, the status of β-catenin depends on Wnt. On the one hand, in the absence of Wnt, β-catenin can bind to the β-catenin destruction complex, including the tumor suppressor adenomatous polyposis coli (APC), scaffolding protein Axin, glycogen synthase kinase 3 (GSK3), and casein kinase 1α (CK1α). On the other hand, once the Wnt ligand binds to the Frizzled receptor and the LRP5/6 co-receptor promotes the LRP5/6's phosphorylation, the activity of β-catenin destruction complex and stabilization of β-catenin was reduced [31]. As the main scaffolding protein of β-catenin [32], Axin directly interacts with β-catenin, GSK3, and APC, and plays critical role in the β-catenin destruction complex's formation [33]. Since the Axin's level associates with the β-catenin destruction complex's concentration, Axin is considered as an important factor in Wnt/β-catenin signaling. Moreover, activation of Wnt/β-catenin has been reported to be associated with tumor cell development in GC. According to previous literature, RNF14 regulates the Wnt/β-catenin signaling pathway by acting on Axin1 and subsequently affecting the expression of downstream target genes of β-catenin, such as c-Myc and MMP-7 [12–14]. Herein, our results showed that RNF146 negatively regulated Axin's expression and positively induced the expression of β-catenin (Fig. 4a). The protein levels of c-Myc and MMP-7 were reduced in the RNF14 knockdown HGC27 cells but increased in the RNF14 up-regulated HGC27 cells (Fig. 4a). However, the regulation effects of RNF146 on Wnt/β-catenin signaling pathway were blocked by PNU-74654 treatment (Fig. 4b). Thus, we propose that Wnt/β-catenin signaling may play a crucial role in regulating RNF146 induced GC cell growth and metastasis.

In conclusion, our study herein provided the first evidence of RNF146's oncogenic role in GC. Down-regulation of RNF146 decreased cell proliferation and induced cell apoptosis through repressing the Wnt/β-catenin signaling in GC cells. However, further *in vivo* experiments are needed to verify the interaction between RNF146 and Wnt/β-catenin in GC. Collectively, RNF146 may act as potential prognostic and therapeutic targets in GC patients, which will be beneficial to the anti-GC therapies.

REFERENCES

1. Carmona-Bayonas A, Jiménez-Fonseca P, Lorenzo MLS, Ramchandani A, Martínez EA, Custodio A, Garrido M, Echavarría I, et al (2016) On the effect of triplet or doublet chemotherapy in advanced gastric cancer: results from a National Cancer Registry. *J Natl Compr Canc Netw* **14**, 1379–1388.
2. Song Z, Wu Y, Yang J, Yang D, Fang X (2017) Progress in the treatment of advanced gastric cancer. *Tumor Biol* **39**, 1010428317714626.
3. Pai SG, Carneiro BA, Mota JM, Costa R, Leite CA, Barroso-Sousa R, Kaplan JB, Chae YK, et al (2017) Wnt/beta-catenin pathway: modulating anticancer immune response. *J Hematol Oncol* **10**, 101.
4. Khatoon E, Deka N, Deka M, Saikia K, Baruah M, Ahmed G (2020) Clinical significance of β-catenin, hTERT, p53, and Wnt7A as biomarkers for ovarian cancer. *Eur J Gynaecol Oncol* **41**, 181–187.
5. Ling L, Nurcombe V, Cool SM (2009) Wnt signaling controls the fate of mesenchymal stem cells. *Gene* **433**, 1–7.
6. Ross SE, Hemati N, Longo KA, Bennett CN, Lucas PC, Erickson RL, MacDougald OA (2000) Inhibition of adipogenesis by Wnt signaling. *Science* **289**, 950–953.
7. Zheng R, Deng Q, Liu Y, Zhao P (2017) Curcumin inhibits gastric carcinoma cell growth and induces apoptosis by suppressing the Wnt/β-catenin signaling pathway. *Med Sci Monit* **23**, 163–171.
8. Yang X-Z, Cheng T-T, He Q-J, Lei Z-Y, Chi J, Tang Z, Liao Q-X, Zhang H, et al (2018) LINC01133 as ceRNA inhibits gastric cancer progression by sponging miR-106a-3p to regulate APC expression and the Wnt/β-catenin pathway. *Mol Cancer* **17**, 126.
9. Wang H, Wu M, Lu Y, He K, Cai X, Yu X, Lu J, Teng L (2019) LncRNA MIR4435-2HG targets desmoplakin and promotes growth and metastasis of gastric cancer by activating Wnt/β-catenin signaling. *Aging* **11**, 6657–6673.
10. Gao J, Zhao C, Liu Q, Hou X, Li S, Xing X, Yang C, Luo Y (2018) Cyclin G2 suppresses Wnt/β-catenin signaling and inhibits gastric cancer cell growth and migration through Dapper1. *J Exp Clin Canc Res* **37**, 317.
11. Nguyen KM, Busino L (2020) Targeting the E3 ubiquitin ligases DCAF15 and cereblon for cancer therapy. *Semin Cancer Biol* **67**, 53–60.
12. Gao Y, Song C, Hui L, Li C-y, Wang J, Tian Y, Han X, Chen Y, et al (2014) Overexpression of RNF146 in non-small cell lung cancer enhances proliferation and invasion of tumors through the Wnt/β-catenin signaling pathway. *Plos One* **9**, e85377.
13. Zhang Y, Liu S, Mickanin C, Feng Y, Charlat O, Michaud GA, Schirle M, Shi X, et al (2011) RNF146 is a poly (ADP-ribose)-directed E3 ligase that regulates axin degradation and Wnt signalling. *Nat Cell Biol* **13**, 623–629.
14. Shen J, Yu Z, Li N (2018) The E3 ubiquitin ligase RNF146 promotes colorectal cancer by activating the Wnt/β-catenin pathway via ubiquitination of Axin1. *Biochem Biophys Res Commun* **503**, 991–997.
15. World Medical Association (2013) World Medical Association Declaration of Helsinki: ethical principles for medical research involving human subjects. *JAMA* **310**, 2191–2194.
16. Hea M, Fanb J, Jiangc R, Tangd W-X, Wanga Z-W (2013)

Expression and inhibition of MBD2 in gastric cancer. *ScienceAsia* **39**, 486–491.
17. Lin X, Haiyang Z, Bo Y, Hai H, Ke Z, Zou K, Wu X-Y (2020) Plasma cell-free DNA for screening patients with benefit-assisted neoadjuvant chemotherapy for advanced gastric cancer. *ScienceAsia* **46**, 462–471.
18. Ang TL, Fock KM (2014) Clinical epidemiology of gastric cancer. *Sing Med J* **55**, 621–628.
19. Kang HC, Lee Y-I, Shin J-H, Andrabi SA, Chi Z, Gagné J-P, Lee Y, Ko HS, et al (2011) Iduna is a poly (ADP-ribose)(PAR)-dependent E3 ubiquitin ligase that regulates DNA damage. *Proc Natl Acad Sci USA* **108**, 14103–14108.
20. Gold B, Kirchhoff T, Stefanov S, Lautenberger J, Viale A, Garber J, Friedman E, Narod S, et al (2008) Genome-wide association study provides evidence for a breast cancer risk locus at 6q22.33. *Proc Natl Acad Sci USA* **105**, 4340–4345.
21. Menachem TD, Laitman Y, Kaufman B, Friedman E (2009) The *RNF146* and *ECHDC1* genes as candidates for inherited breast and ovarian cancer in Jewish Ashkenazi women. *Fam Cancer* **8**, 399–402.
22. Li N, Zhang Y, Han X, Liang K, Wang J, Feng L, Wang W, Songyang Z, et al (2015) Poly-ADP ribosylation of PTEN by tankyrases promotes PTEN degradation and tumor growth. *Genes Dev* **29**, 157–170.
23. Hu J, Wang W, Lan X, Zeng Z, Liang Y, Yan Y, Song F, Wang F, et al (2019) CAFs secreted exosomes promote metastasis and chemotherapy resistance by enhancing cell stemness and epithelial-mesenchymal transition in colorectal cancer. *Mol Cancer* **18**, 91.
24. Liang Z, Lu L, Mao J, Li X, Qian H, Xu W (2017) Curcumin reversed chronic tobacco smoke exposure induced urocystic EMT and acquisition of cancer stem cells properties via Wnt/β-catenin. *Cell Death Dis* **8**, e3066.
25. Gonzalez DM, Medici D (2014) Signaling mechanisms of the epithelial-mesenchymal transition. *Sci Signal* **7**, re8.
26. Li Q, Lai Q, He C, Fang Y, Yan Q, Zhang Y, Wang X, Gu C, et al (2019) RUNX1 promotes tumour metastasis by activating the Wnt/β-catenin signalling pathway and EMT in colorectal cancer. *J Exp Clin Canc Res* **38**, 161.
27. Suzuki M, Shigematsu H, Nakajima T, Kubo R, Motohashi S, Sekine Y, Shibuya K, Iizasa T, et al (2007) Synchronous alterations of Wnt and epidermal growth factor receptor signaling pathways through aberrant methylation and mutation in non-small cell lung cancer. *Clin Cancer Res* **13**, 6087–6092.
28. Tomar VS, Patil V, Somasundaram K (2020) Temozolomide induces activation of Wnt/β-catenin signaling in glioma cells via PI3K/Akt pathway: implications in glioma therapy. *Cell Biol Toxicol* **36**, 273–278.
29. Jang G-B, Kim J-Y, Cho S-D, Park K-S, Jung J-Y, Lee H-Y, Hong I-S, Nam J-S (2015) Blockade of Wnt/β-catenin signaling suppresses breast cancer metastasis by inhibiting CSC-like phenotype. *Sci Rep* **5**, 12465.
30. Zhang Y, Wang X (2020) Targeting the Wnt/β-catenin signaling pathway in cancer. *J Hematol Oncol* **13**, 165.
31. Cheng X, Xu X, Chen D, Zhao F, Wang W (2019) Therapeutic potential of targeting the Wnt/β-catenin signaling pathway in colorectal cancer. *Biomed Pharmacother* **110**, 473–481.
32. Huang S-MA, Mishina YM, Liu S, Cheung A, Stegmeier F, Michaud GA, Charlat O, Wiellette E, et al (2009) Tankyrase inhibition stabilizes axin and antagonizes Wnt signalling. *Nature* **461**, 614–620.
33. Ji L, Lu B, Zamponi R, Charlat O, Aversa R, Yang Z, Sigoillot F, Zhu X, et al (2019) USP7 inhibits Wnt/β-catenin signaling through promoting stabilization of Axin. *Nat Commun* **10**, 4184.