MicroRNA-27b suppresses *Helicobacter pylori*-induced gastric tumorigenesis through negatively regulating Frizzled7

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Abstract. MicroRNAs (miRNAs) are novel tools for cancer therapy. Frizzled7 (FZD7) is an important co-receptor in the WNT signaling pathway. The WNT signaling pathway is aberrantly activated in *Helicobacter pylori* (*H. pylori*)-infected gastric cancer cells. However, the role of FZD7 in *H. pylori*-induced gastric tumorigenesis remains unknown. In this study, we investigated the potential role of FZD7 in *H. pylori*-induced gastric tumorigenesis and validated the possibility that targeting of FZD7 by specific miRNA inhibits *H. pylori*-induced gastric tumorigenesis. First, we found that FZD7 was significantly induced by *H. pylori* infection in a dose- and time-dependent manner. Knockdown of FZD7 by FZD7 small interfering RNA effectively inhibited *H. pylori* infection-induced cell proliferation of gastric cancer cells. We found that microRNA-27b (miR-27b) was the predicted miRNA for FZD7 and that miR-27b negatively regulated FZD7 expression by targeting the 3'-untranslated region of FZD7. Furthermore, miR-27b overexpression significantly inhibited *H. pylori* infection-induced cell proliferation and WNT signaling pathway activation in gastric cancer cells. Restoration of FZD7 expression significantly attenuated the inhibitory effect of miR-27b overexpression on cell proliferation and WNT signaling pathway activation. Collectively, our study suggests that FZD7 triggered by *H. pylori* infection contributes to the *H. pylori* infection-induced cell proliferation that links the WNT. Thus, miR-27b may be a promising molecular target for the treatment of the disease.

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Abbreviations: miRNAs, microRNAs; *H. pylori*, *Helicobacter pylori*; 3'-UTR, 3'-untranslated region; FZD7, Frizzled7; CagA, cytotoxin-associated gene A

Key words: *Helicobacter pylori*, gastric cancer, Frizzled7, miR-27b

Introduction

Gastric cancer is the fourth most diagnosed cancer and the second leading cause of cancer-related death worldwide (1). A pivotal risk factor for gastric carcinogenesis is *Helicobacter pylori* (*H. pylori*) infection (2). *H. pylori* infection is among the most common infectious diseases. It is considered to be carcinogenic and accounts for approximately 80% of gastric carcinomas (3,4). *H. pylori* infection also influences the clinical treatment outcome of gastric cancer (5). However, the association between gastric cancer and *H. pylori* infection and its functional mechanism in gastric cancer remain largely unknown. A better understanding of this molecular mechanism may improve therapeutic prevention and treatment for gastric cancer.

H. pylori is a Gram-negative bacteria that contributes to gastric tumorigenesis mainly through its virulence factor cytotoxin-associated gene A (CagA) (6). The CagA protein has been suggested as an oncogenic protein for gastric cancer (7,8). It has been reported that *H. pylori* infection helps activate multiple oncogenic pathways, such as PI3K/Akt (9-11), WNT/β-catenin (12), and Janus kinase signal transducers, as well as activators of the transcription 3 signaling pathway (13). *H. pylori* infection also deactivates tumor suppressors, such as p53 (14,15). Therefore, focusing on oncogenic signaling pathways in *H. pylori* infection-induced gastric carcinogenesis could support novel and promising therapeutic strategies for gastric cancer.

Frizzled7 (FZD7) is a critical receptor for the WNT/β-catenin signaling pathway (16,17). Various studies have reported that FZD7 plays an important role in many types of human cancers, including renal cell carcinoma (18), cervical cancer (19), ovarian cancer (17), breast cancer (20), colon cancer (21), and hepatocellular carcinoma (22). It participates in carcinogenesis mainly through promoting the activation of the WNT/β-catenin signaling pathway. Aberrant activation of the WNT/β-catenin signaling pathway occurs frequently in gastric cancer (23). *H. pylori* infection also has been implicated

in this WNT/β-catenin signaling pathway (12). These findings suggest that FZD7 may be involved in the WNT/β-catenin signaling pathway that is induced by *H. pylori* infection. However, little is known regarding the role of FZD7 in gastric carcinogenesis induced by *H. pylori* infection.

MicroRNAs (miRNAs) are a class of small, non-coding RNAs that negatively regulate gene expression through binding the 3'-untranslated region (UTR) of target gene (24,25). Therefore, miRNAs mediate cancer cell proliferation, differentiation, and metastasis by targeting various genes and regulating numerous signaling pathways (26). Several studies have suggested miRNAs as novel candidates for cancer therapy (27). Although the role of miRNAs in *H. pylori*-induced gastric carcinogenesis has been widely investigated in recent years, their precise role and target genes remain unclear. In this study, we investigated the role of FZD7 in *H. pylori*-induced gastric carcinogenesis and demonstrated a targeting relationship between FZD7 and miR-27b, a well characterized tumor-suppressive miRNA (28,29). We found that FZD7 was highly upregulated by *H. pylori* infection and was associated with *H. pylori* infection-induced cell proliferation. miR-27b suppressed *H. pylori* infection-induced cell proliferation and the WNT signaling pathway by directly targeting and negatively regulating FZD7 expression. Our study demonstrated that miR-27b/FZD7 is implicated in *H. pylori*-induced gastric carcinogenesis by affecting the WNT signaling pathway.

Materials and methods

Cell lines. The gastric epithelial-derived cancer cell lines AGS and BGC-823 and human embryonic kidney cell line HEK-293T were obtained from the American Type Culture Collection (ATCC; Manassas, VA, USA). AGS and BGC-823 cells were grown in RPMI-1640 medium (Gibco, Rockville, MD, USA) and HEK-293T cells were cultured in Dulbecco's modified Eagle's medium (DMEM; Gibco). All cultured media were supplemented with 10% fetal bovine serum (Gibco) and 1% penicillin-streptomycin mix (Sigma, St. Louis, MO, USA) in a humidified atmosphere containing 5% CO_2 at 37˚C.

H. pylori culture. Standard strain *H. pylori* 43504, was purchased from the ATCC and cultured on rain-heart infusion plates containing 5% goat blood and incubated for 3-4 days in a humidified CO_2 incubator containing 10% CO_2, 5% O_2, and 85% N_2 at 37˚C. Thereafter, *H. pylori* was collected and re-suspended in RPMI-1640 for a concentration of $3x10^8$ colony forming units/ml. Gastric cancer cells were then infected with *H. pylori* at a multiplicity of infection (MOI) of 1:25, 1:50, and 1:100 and incubated for 6 and 12 h.

RNA extraction and real-time quantitative PCR analysis. Total RNA from cells was harvested with a miRNeasy kit (Qiagen, Dusseldorf, Germany). For analysis of FZD7 mRNA expression, RNA was reverse transcribed into cDNA using M-MLV Reverse Transcriptase (BioTeke, Beijing, China) and RT-PCR was performed using SYBR Green PCR Master Mix (Applied Biosystems, Carlsbad, CA, USA). For analysis of miR-27b expression, RNA was converted into cDNA using miScript reverse transcription kit (Qiagen), and real-time quantitative PCR (RT-qPCR) was conducted using TaqMan miRNA Reverse Transcription kit (Applied Biosystems). GAPDH or U6 small nuclear RNA was used as an internal reference for relative gene expression quantitation. The RT-qPCR reactions were performed in triplicate, and relative gene expression level was determined by using the $2^{-\Delta\Delta Ct}$ method.

Western blot analysis. An equal amount of protein from different samples was isolated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and then transferred onto a PVDF membrane (Bio-Rad, Hercules, CA, USA). The transferred protein on the membrane was confirmed by Ponceau staining solution. Then, the membrane was blocked in 3% nonfat milk for 1 h at 37˚C. Primary antibodies, including anti-FZD7 and anti-GAPDH (Santa Cruz Biotechnology, Santa Cruz, CA, USA), were added and incubated at 4˚C overnight. After three washes with Tris-buffered saline with Tween-20, horseradish peroxidase-conjugated secondary antibodies (1:5,000; Bioss Inc., Beijing, China) were added and incubated for 1 h. The membrane was then developed by use of enhanced chemiluminescence (Pierce, Rockford, IL, USA). Relative protein expression was quantitated by using Image-Pro Plus 6.0 software.

Cell transfection. FZD7 was knocked down by transfection of FZD7 siRNA (Santa Cruz Biotechnology), according to the manufacturer's instruction. Briefly, FZD7 siRNA and transfection reagent were mixed in transfection medium for 45 min. Cells were washed with transfection medium and then incubated with the FZD7 siRNA mixture for 6 h. Normal growth media were added and incubated for 24 h. The old media were then replaced with fresh normal-growth media and further incubated for 24-72 h. Negative control (NC) siRNA was used as a control. For miRNA transfection, miR-27b mimics and NC miRNA (GenePharma, Shanghai, China) were transfected into cells using Lipofectamine 2000 (Invitrogen, Carlsbad, CA, USA) at a concentration of 20 nM and incubated for 48 h. For FZD7 overexpression, pcDNA3.1/FZD7 vectors were transiently transfected into cells using Lipofectamine 2000 (Invitrogen) for 48 h. The transfection efficiency was finally detected by RT-qPCR or western blot analysis.

Cell proliferation assay. For the MTT assay, cells were seeded into 96-well plates and transfected with FZD7 siRNA, miR-27b mimics, or pcDNA3.1/FZD7 vectors for 48 h, followed by *H. pylori* infection for 12 h. MTT solution (5 mg/ml) was added at 20 μl/well. Dimethyl sulfoxide was added at 200 μl/well to dissolve the formazan products. The optical density of the solution was detected using a microplate reader (Bio-Tek Instruments, Winooski, VT, USA) at a wavelength of 490 nm. For the colony formation assay, cells transfected with FZD7 siRNA, miR-27b mimics, or pcDNA3.1/FZD7 vectors followed by *H. pylori* infection were seeded into a 6-well plate in a growth medium containing 0.3% noble agar (200 cells/well) to form natural colonies for 14 days. The cells were washed with phosphate buffer saline and fixed with 4% paraformaldehyde. The plates were stained with crystal violet (Sigma), and the number of colonies was counted and averaged.

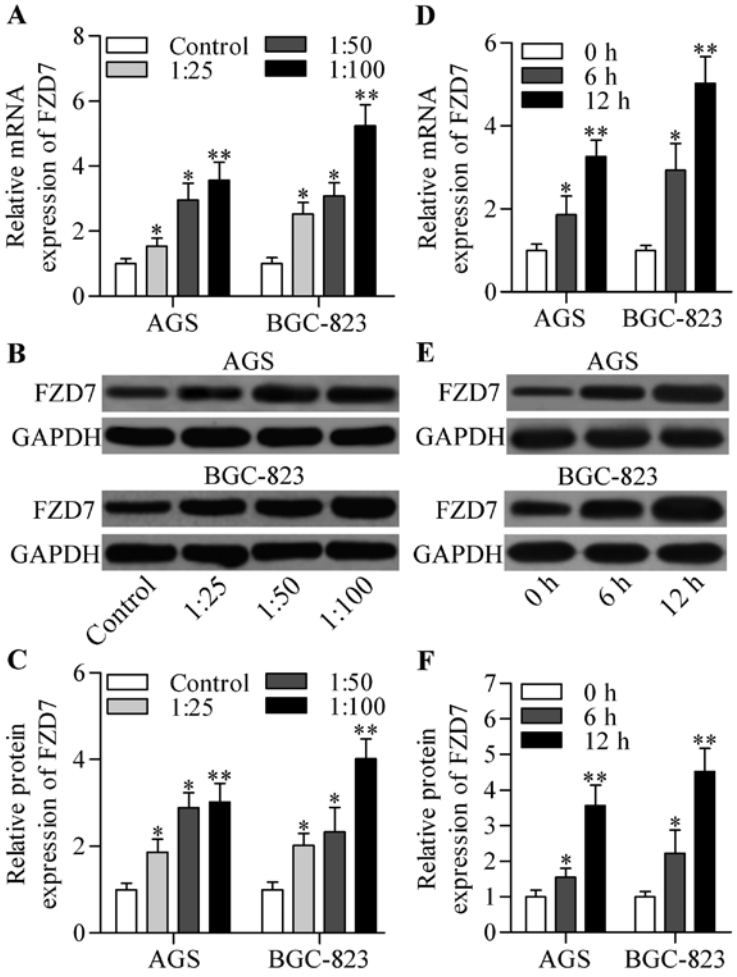

Figure 1. *H. pylori* infection promotes FZD7 expression. (A) mRNA and (B) protein expression of FZD7 in AGS and BGC-823 cells infected with *H. pylori* at MOI of 1:25, 1:50, and 1:100, as detected by RT-qPCR and western blot analysis, respectively. Non-infected cells were used as controls. Cells were harvested after infection of 12 h. (C) The relative protein expression of FZD7 in different groups, quantified using Image-Pro Plus 6.0. *P<0.05, **P<0.01 vs. control. (D) mRNA and (E) protein expression of FZD7 in AGS and BGC-823 cells infected with *H. pylori* at MOI of 1:100 for 6 or 12 h, as detected by RT-qPCR and western blot analysis, respectively. (F) Quantitative analysis of FZD7 protein expression in different groups. *P<0.05, **P<0.01 vs. 0 h.

Luciferase reporter assay. The targeted relationship between miR-27b and FZD7 3'-UTR was detected using a dual-luciferase reporter assay. Briefly, the cDNA fragments of FZD7 3'-UTR containing the miR-27b targeted site were inserted into pmirGLO vectors (Promega, Madison, WI, USA). HEK-293T cells were transfected with pmirGLO-FZD7 3'-UTR and miR-27b mimics or NC miRNA and incubated for 48 h. Cells were harvested and lysed. The activity of firefly and *Renilla* luciferase was tested with a Dual-Luciferase reporter assay kit (Promega). The WNT signaling activity was examined using Tcf luciferase reporter assays. Briefly, gastric cells were co-transfected with a TOPFlash firefly luciferase reporter vector (Addgene, Cambridge, MA, USA) and phRL-TK *Renilla* luciferase vectors (Promega) together with FZD7 siRNA, miR-27b mimics, or pcDNA3.1/FZD7 vectors, followed by *H. pylori* infection. After 48 h of transfection, cells were lysed and the activity of firefly and *Renilla* luciferase was detected, respectively.

Statistical analysis. Quantitative data are shown as mean ± standard deviation. Statistical analyses were processed with SPSS version 11.5 software (SPSS Inc., Chicago, IL, USA) with one-way analysis of variance. A p-value of <0.05 was regarded as statistically significant.

Results

H. pylori infection promotes FZD7 expression in gastric cancer cells. To explore whether FZD7 is involved in carcinogenesis related to *H. pylori* infection, we examined its expression level in two gastric cancer cell lines, AGS and BGC-823, infected with *H. pylori*. The results showed that both the mRNA (Fig. 1A) and protein (Fig. 1B and C) expression level of FZD7 were highly upregulated in AGS and BGC-823 cells by *H. pylori* infection in a dose-dependent manner. Further data showed that the mRNA (Fig. 1D) and protein (Fig. 1E and F) expression levels of FZD7 increased in a time-dependent manner. These results indicate that FZD7 was induced by *H. pylori* in gastric cancer cells.

Knockdown of FZD7 inhibits H. pylori infection-induced cell proliferation of gastric cancer cells. To investigate the potential biological role of FZD7 in *H. pylori*-induced carcinogenesis, we silenced the expression of FZD7 by using

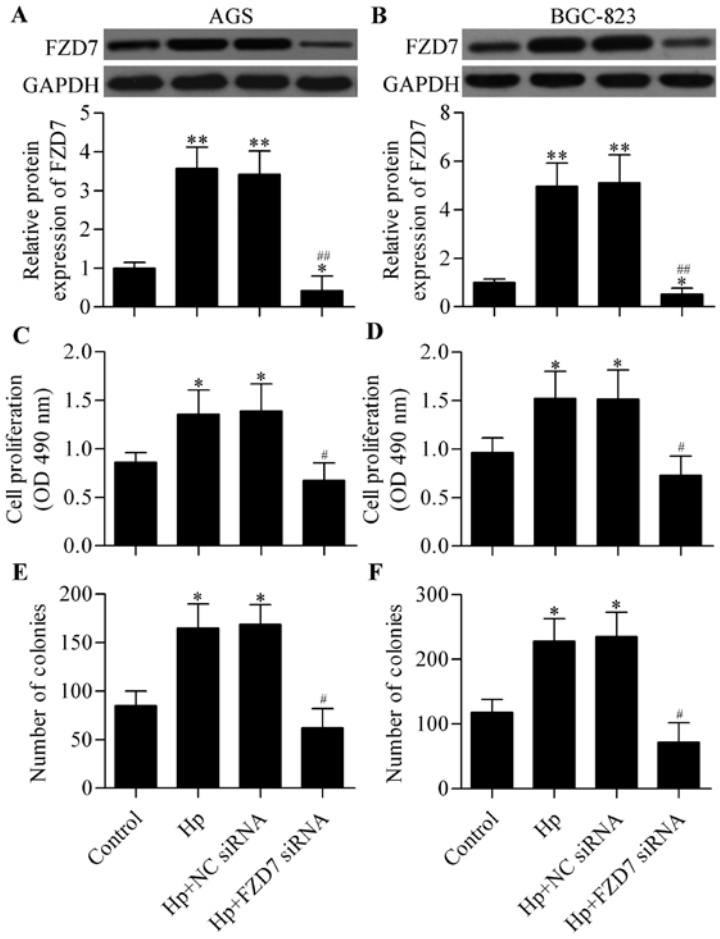

Figure 2. FZD7 is involved in gastric cancer cell proliferation induced by *H. pylori* infection. Western blot analysis of FZD7 protein expression in (A) AGS and (B) BGC-823 cells. Cells were transfected with FZD7 siRNA or NC siRNA for 48 h and infected with *H. pylori* at MOI of 1:100 for 12 h. Hp denotes *H. pylori* infection. *P<0.05, **P<0.01 vs. control; $^{\#\#}$P<0.01 vs. Hp+NC siRNA. MTT assay of (C) AGS and (D) BGC-823 cells transfected with FZD7 siRNA or NC siRNA for 48 h and infected with *H. pylori* at MOI of 1:100 for 12 h. *P<0.05 vs. control; $^{\#}$P<0.05 vs. Hp+NC siRNA. The effects of FZD7 knockdown on the colony-forming capacity of (E) AGS and (F) BGC-823 cells infected with *H. pylori*. *P<0.05 vs. control; $^{\#}$P<0.05 vs. Hp+NC siRNA.

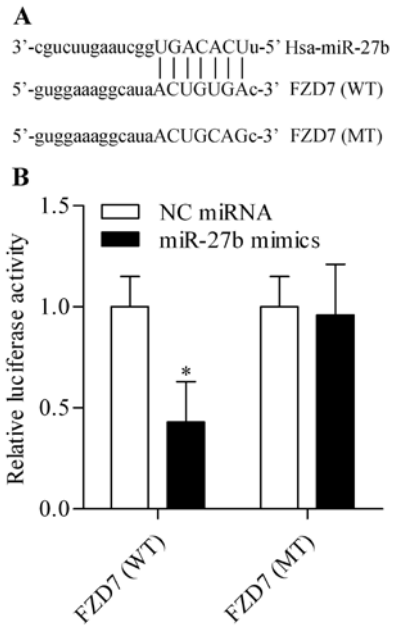

Figure 3. miR-27b directly targets the 3'-UTR of FZD7. (A) Diagram of the predicted miR-27b targeting site within the 3'-UTR of FZD7. (B) Dual-luciferase reporter assay demonstrating the effect of miR-27b on the luciferase activity of the two luciferase reporter vectors containing FZD7 3'-UTR (WT) and FZD7 3'-UTR (MT). *P<0.05 vs. NC miRNA.

FZD7 siRNA (Fig. 2A and B) and detected its effect on gastric cancer cell proliferation with *H. pylori* infection. MTT results showed that *H. pylori* infection significantly promoted gastric cancer cell proliferation and that FZD7 knockdown could reverse this promotion (Fig. 2C and D). Similarly, *H. pylori* infection markedly upregulated the colony formation of gastric cancer cells, and FZD7 knockdown reversed this upregulation (Fig. 2E and F). These results suggest that the increased expression of FZD7 induced by *H. pylori* infection might contribute to *H. pylori*-related gastric carcinogenesis.

miR-27b directly targets the 3'-UTR of FZD7 and negatively regulates FZD7 expression. miRNAs have emerged as novel tools for cancer treatment because of their regulatory function on gene expression (30). We sought to identify and characterize novel miRNA that could target and regulate FZD7 expression and were thus involved in gastric carcinogenesis related to *H. pylori*. Through bioinformatic analysis, we found that miR-27b harbored a putative binding site for FZD7 3'-UTR (Fig. 3A). This miRNA attracted our interests because of its critical role in tumorigenesis of various cancer types (29). To validate whether FZD7 is a bona fide target of miR-27b, we performed a dual-luciferase reporter assay using

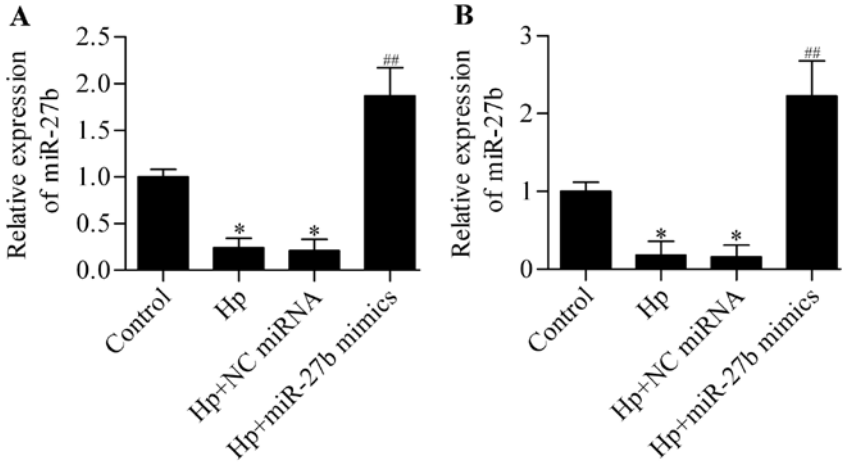

Figure 4. Detection of the miR-27b expression in different groups. RT-qPCR analysis of miR-27b expression in (A) AGS and (B) BGC-823 cells with different treatments. Cells were transfected with miR-27 mimics or NC miRNA for 48 h and infected with *H. pylori* for 12 h at MOI of 1:100. **P<0.01 vs. control; $^{\#\#}$P<0.01 vs. Hp+NC miRNA.

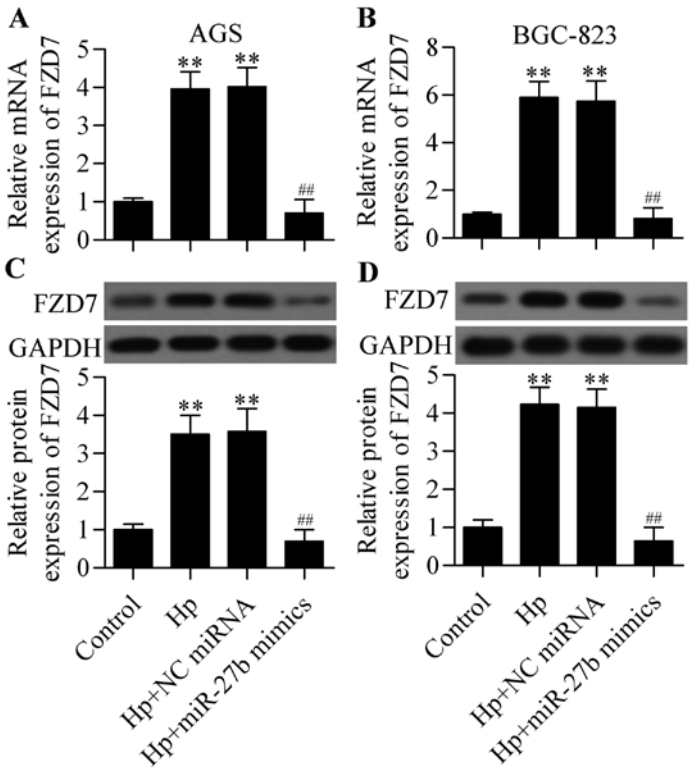

Figure 5. miR-27b regulates the expression of FZD7. RT-qPCR analysis of FZD7 mRNA expression in (A) AGS and (B) BGC-823 cells with different treatments. Western blot analysis of FZD7 mRNA expression in (C) AGS and (D) BGC-823 cells with different treatments. **P<0.01 vs. control; $^{\#\#}$P<0.01 versus Hp+NC miRNA. Cells were transfected with miR-27 mimics or NC miRNA for 48 h and infected with *H. pylori* for 12 h at MOI of 1:100.

two luciferase reporter vectors containing the putative miR-27b binding sites in the wild-type FZD7 3'-UTR (WT) and mutant 3'-UTR (MT). The results showed that co-transfection of HEK-293T cells with miR-27b mimics and luciferase reporter vectors containing the WT FZD7 3'-UTR led to a significant decrease in luciferase activity, compared to NC miRNA transfection (Fig. 3B). In contrast, the luciferase activity of luciferase reporter vectors containing the MT FZD7 3'-UTR was not affected by miR-27b mimics transfection (Fig. 3B). The data imply that miR-27b could target the 3'-UTR of FZD7 directly. Next, we detected the expression of miR-27b in *H. pylori*-infected cells and found that miR-27b expression was significantly decreased by *H. pylori* infection in AGS and BGC-823 cells, which could be upregulated by transfection of miR-27b mimics (Fig. 4). To investigate whether miR-27b regulated FZD7 expression, we overexpressed miR-27b by transfection of miR-27b mimics in AGS and BGC-823 cells. The results showed that both the mRNA (Fig. 5A and B) and protein (Fig. 5C and D) expression of FZD7 that was upregulated by *H. pylori* infection was significantly decreased by

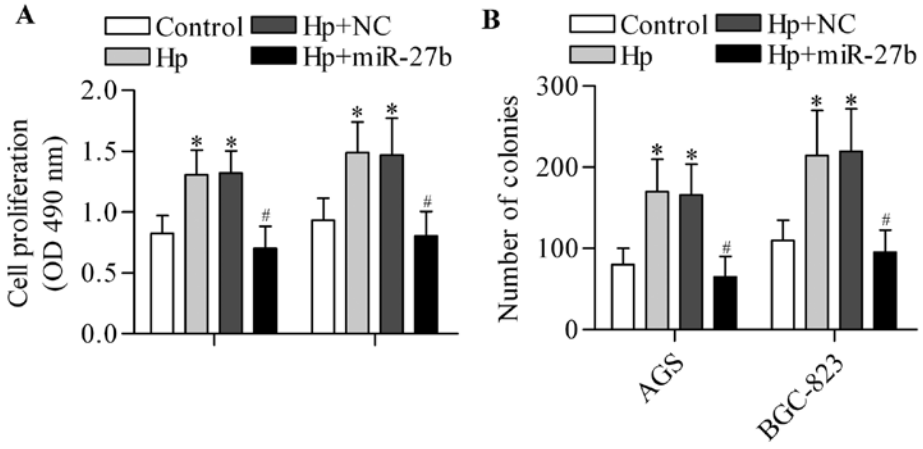

Figure 6. miR-27b is involved in gastric cancer cell proliferation induced by *H. pylori* infection. (A) MTT assay of gastric cancer cells transfected with miR-27b mimics or NC miRNA for 48 h and infected with *H. pylori* at MOI of 1:100 for 12 h. (B) Effect of miR-27b overexpression on the colony-forming capacity of gastric cancer cells infected with *H. pylori*. *P<0.05 vs. control; #P<0.05 vs. Hp+NC.

miR-27b overexpression in AGS and BGC-823 cells. Taken together, these results demonstrated that miR-27b regulated FZD7 expression.

The regulation of FZD7 by miR-27b is involved in H. pylori-induced cell proliferation. As previously described, *H. pylori* infection significantly promoted the expression of FZD7, and FZD7 was verified as a direct target gene of miR-27b. We thus speculated that miR-27b may play an important role in *H. pylori*-induced cell proliferation by regulating FZD7 expression. To test the hypothesis, we overexpressed miR-27b in *H. pylori*-infected cells and detected its effect on cell proliferation. As expected, the results showed that the cell proliferation (Fig. 6A) and colony formation (Fig. 6B) of gastric cancer cells promoted by *H. pylori* infection could be significantly reversed by miR-27b overexpression. However, restoring expression of FZD7 (Fig. 7A and B) in *H. pylori*-infected cells significantly reversed the inhibitory effect of miR-27b overexpression on *H. pylori*-induced cell proliferation (Fig. 7C and D). In summary, these results indicate that miR-27b inhibited *H. pylori*-induced cell proliferation directly through targeting miR-27b.

Suppression of FZD7 by FZD7 siRNA or miR-27b overexpression inhibits the activation of WNT signaling pathway. A previous study has reported that *H. pylori* infection is associated with activation of the WNT signaling pathway that contributes to carcinogenesis (12). We speculated that the increased expression of FZD7 induced by *H. pylori* infection might contribute to the activated WNT signaling pathway. To test this hypothesis, we suppressed the expression of FZD7 by FZD7 siRNA or miR-27b overexpression and detected their effect on the WNT signaling pathway. The results showed that *H. pylori* infection significantly increased the activity of the WNT signaling pathway, but this promotion was significantly decreased by FZD7 knockdown or miR-27b overexpression in AGS (Fig. 8A) and BGC-823 (Fig. 8B) cells. Further data showed that the inhibitory effect of miR-27b on WNT activity was significantly reversed by restoration of FZD7 expression (Fig. 8C and D). Taken together, the promoted FZD7 expression induced by *H. pylori* infection contributed to the activated WNT signaling pathway, which could be directly inhibited by miR-27b overexpression.

Discussion

In this study, we demonstrated that *H. pylori* infection significantly upregulated the expression of FZD7 in gastric cancer cells and that this promotion could be markedly reversed by miR-27b overexpression. FZD7 is the critical co-receptor for the WNT signaling pathway. Our result revealed that suppression of FZD7 could block the WNT activation induced by *H. pylori* infection. Therefore, *H. pylori* infection may induce the repression of miR-27b, resulting in overexpression of FZD7 and activation of the WNT signaling pathway, which play important roles in *H. pylori*-induced gastric tumorigenesis.

H. pylori promotes gastric tumorigenesis by facilitating proliferation, angiogenesis, and invasion of cancer cells (31). Various genes and signaling pathways are involved in this process (6). However, the precise molecular mechanism underlying this process remains poorly understood. In this study, we found that FZD7, the critical co-receptor for the WNT signaling pathway, was highly induced by *H. pylori* in AGS and BGC-823 gastric cancer cells. These results indicate that FZD7 may play an important role in *H. pylori*-induced gastric tumorigenesis. Moreover, we found that knockdown of FZD7 significantly decreased the gastric cancer cell proliferation induced by *H. pylori* infection. The results confirmed the pivotal role of FZD7 in *H. pylori*-induced gastric tumorigenesis. As an important regulator for the WNT signaling pathway, FZD7 has drawn particular interest in tumorigenesis. Studies have revealed that FZD7 is highly expressed in hepatocellular carcinoma and that it contributes to aberrant activation of the WNT signaling pathway (22,32,33). Furthermore, a pharmacological inhibitor of FZD7 showed antitumor effects linked to the inactivated WNT signaling pathway (34). FZD7 is involved in regulating the cell proliferation, invasion, and metastasis of colon cancer cells through the WNT signaling pathway (21,35). Similarly, FZD7 plays critical roles in breast cancer (36) and cervical cancer (19).

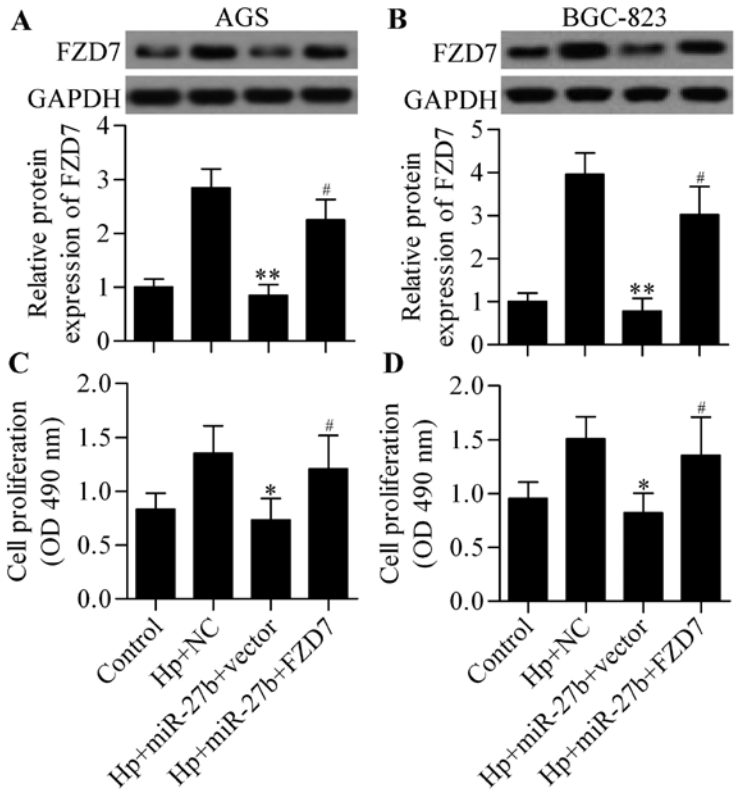

Figure 7. FZD7 overexpression rescues the inhibitory effect of miR-27b on *H. pylori*-induced cell proliferation. Western blot analysis of FZD7 expression in (A) AGS and (B) BGC-823 cells transfected with miR-27b mimics, with or without pcDNA3.1/FZD7. The transfections were incubated for 48 h and infected with *H. pylori* for 12 h at a multiplicity of infection rate of 1:100. Vector, empty vectors. **P<0.01 vs. Hp+NC; $^{\#}$P<0.05 vs. Hp+miR-27b+FZD7. MTT assay of (C) AGS and (D) BGC-823 cells transfected with miR-27b mimics and pcDNA3.1/FZD7 with *H. pylori* infection. *P<0.05 vs. Hp+NC; $^{\#}$P<0.05 vs. Hp+miR-27b+FZD7.

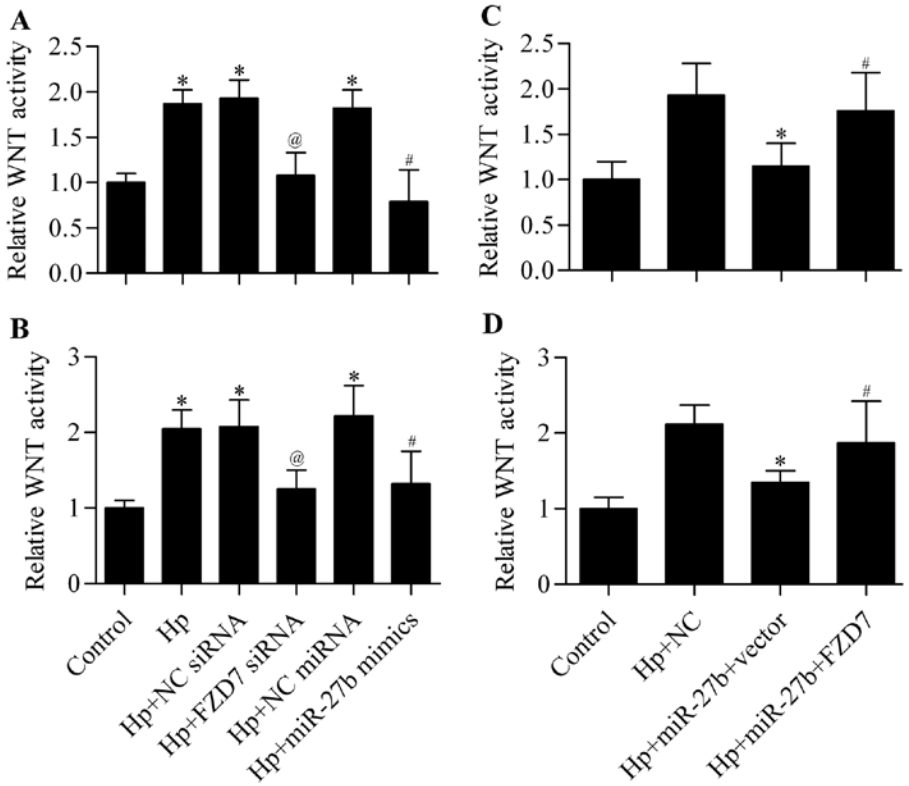

Figure 8. FZD7 regulates WNT activity. Detection of the effect of FZD7 siRNA or miR-27b overexpression on WNT activity in (A) AGS and (B) BGC-823 cells infected with *H. pylori*. *P<0.05 vs. control; $^{@}$P<0.05 vs. Hp+NC siRNA; $^{\#}$P<0.05 vs. Hp+NC miRNA. FZD7 overexpression rescues the inhibitory effect of miR-27b overexpression on *H. pylori*-induced cell WNT activation in (C) AGS and (D) BGC-823 cells. *P<0.05 vs. Hp+NC; $^{\#}$P<0.05 vs. Hp+miR-27b+vector.

In gastric cancers, it was found that FZD7 was overexpressed and was associated with activation of the WNT signaling pathway (37). Importantly, FZD7-positive gastric cancers are associated with lower survival rates (38). All these findings suggest that FZD7 is dysregulated in cancer development and is positively associated with tumorigenesis. Indeed, FZD7 is considered an emerging and promising molecular target for cancer therapy (39).

Increasing evidence has suggested that miRNAs are emerging tools for cancer therapy because of their negative regulatory effect on target genes (30). Targeting FZD7 by specific miRNA may be a promising and effective therapeutic strategy for cancer. Several studies have tested the hypothesis. For example, miR-23b was found to inhibit the tumorigenic potential of colon cancer cells by regulating pro-metastatic targets, including FZD7 (40). miR-27a suppressed the multiple drug resistance of hepatocellular carcinoma cells by targeting FZD7 and inhibiting the FZD7-mediated WNT signaling pathway (41). Other miRNAs, including miR-199a (42), miR-142-3p (43), and miR-126 (44), were found to be capable of directly targeting and regulating FZD7 expression in cancer cells, implying that these miRNAs are novel and promising approaches for cancer therapy. In this study, we identified miR-27b as a novel miRNA that targets and regulates FZD7 expression. Our results showed that overexpression of miR-27b could inhibit cell proliferation induced by *H. pylori* by suppressing FZD7 expression and by mimicking the effect of FZD7 knockdown via FZD7 siRNA. Additionally, restoring the expression of FZD7 could block the effect of miR-27b overexpression, further confirming that FZD7 was a functional downstream target gene of miR-27b. Recent studies have demonstrated that miR-27b functioned as a tumor suppressor through suppressing the expression of various oncogenic proteins. miR-27b was found to inhibit colorectal cancer progression and angiogenesis by targeting vascular endothelial growth factor C (45). miR-27b suppressed non-small cell lung cancer cell proliferation and invasion by targeting Sp1 transcription factor (29) or LIM kinase 1 (46). Cyclin A2 (47) and ectonucleotide pyrophosphatase/phosphodiesterase family member 1 (48) are also direct target genes of miR-27b in regulating tumorigenesis. Other evidence suggests that miR-27b functions as a tumor suppressor (28,49-51). Most recently, a study demonstrated that miR-27b could target and inhibit cAMP responsive element binding protein 1 which is associated with metastasis and poor outcome in gastric cancer (52). However, this study did not further investigate the function role of miR-27b on gastric cancer. By investigating the expression and functional role of miR-27b in gastric cancer, our study demonstrated that miR-27b might be a tumor suppressor for *H. pylori*-induced gastric tumorigenesis by modulating FZD7 expression. We found that miR-27b could inhibit gastric cancer cell proliferation by targeting FZD7 and inhibiting the FZD7-mediated WNT signaling pathway involved in *H. pylori*-induced gastric tumorigenesis.

In this study, we also observed that *H. pylori* infection activated the WNT signaling pathway. Other studies have confirm the activation of WNT/β-catenin by *H. pylori* (12). However, the underlying mechanism remains obscure. In normal conditions, β-catenin mainly interacts with E-cadherin at the cell membrane, whereas CagA of *H. pylori* disrupts the complex, resulting in cytoplasmic and nuclear accumulation of β-catenin for WNT signaling activation (9). Interestingly, in AGS cells lacking the expression of E-cadherin, *H. pylori* still upregulates the cytoplasmic and nuclear accumulation of β-catenin, implying that other mechanism are involved in *H. pylori*-induced WNT signaling activation (9,12). Sokolova *et al* found that *H. pylori* inhibited the activity of GSK-3β, leading to increased accumulation of β-catenin (53). It has also been reported that *H. pylori* infection induced activation of low-density lipoprotein receptor-related protein 6, another co-receptor of the WNT signaling pathway (54). In this study, we found that *H. pylori* infection induced the expression of FZD7, the co-receptor for WNT signaling pathway. Thus, we speculate that the increased expression of FZD7 may contribute to the activated WNT signaling pathway induced by *H. pylori* infection. Subsequently, we demonstrated that suppression of FZD7 by siRNA or miR-27b significantly blocked the activation of WNT induced by *H. pylori* infection. Our study thus provides novel insight into the aberrant WNT signaling pathway induced by *H. pylori* infection that involves FZD7.

Our study indicates that *H. pylori* infection triggers the high expression of FZD7 in gastric cancer cells and may contribute to the cell proliferation and WNT activation processes induced by *H. pylori*. Furthermore, we demonstrated that the expression of FZD7 and WNT activation could be inhibited by miR-27b overexpression. We conclude that the miR-27b-FZD7-WNT signaling pathway may be a promising molecular target for the treatment of gastric cancer associated with *H. pylori* infection.

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