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Original Article

FZD6, targeted by miR-21, represses gastric cancer cell proliferation and migration via activating non-canonical wnt pathway

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Abstract: FZD6 plays crucial roles in human tumorigenesis. However, its mechanism in regulating cancers has not been fully elucidated. In the study, we found that FZD6 repressed gastric cancer cell proliferation and migration via activating non-canonical wnt pathway. In addition, non-canonical wnt pathway ameliorated expression of canonical wnt pathway. We also demonstrated that the FZD6 was involved in miR-21-dependent effects in the canonical and non-canonical wnt pathways in gastric cancer. These findings provide a better understanding of the development and progression of gastric cancer and may be an important implication for future therapy.

Keywords: FZD6, Wnt pathway, miR-21, gastric cancer

Introduction

Gastric cancer is the fifth most common cancer and the third most frequent cause of cancer death worldwide, and in spite of some therapeutic advances its prognosis is still unfavourable [1, 2]. Genetic background, behavioural factors (eg, diet, smoking habit and alcohol consumption), and helicobactor pylori infection have been associated with the risk of developing gastric cancer [3, 4]. However, the cascade of molecular events leading to gastric carcinogenesis are still largely unknown. An improved understanding of critical pathways involved in cancer will facilitate the development of effective targeted therapeutic strategies for gastric cancer. Many genes and pathways have been reported involved in gastric cancer formation and metastasis, such as canonical wnt/β-catenin [5], PI3K/AKT [6], p53 [7] and stat3 [8]. The canonical wnt/β-catenin pathway is involved in various differentiation events during embryonic development and associates with tumor formation when aberrantly activated. Besides canonical wnt/β-catenin pathway, the wnt pathway also includes non-canonical wnt pathway (the wnt/Ca2+ pathway, the planar cell polarity pathway), depending on the specific Wnt/Frz signal received [9]. However, the effect of non-canonical wnt pathway on cancer and the association between canonical and non-canonical wnt pathways are still controversial.

FZD6 (frizzled class receptor 6) is a member of the "frizzled" gene family, which encode 7-transmembrane domain proteins that are receptors for non-canonical Wnt signaling proteins. Mounting evidences show that FZD6 is involved in cancer development. GM Caldwell et al reported that FZD6 was overexpressed in colorectal cancer [10]. In contrast, FZD6 has also been shown reduced in leukemia cell lines [11]. Recent studies indicate that FZD6 could be regulated by microRNAs. MiR-199a-5p targeted FZD6 to regulate growth of colorectal cancer [12]. To date, the mechanism of FZD6 deregulation and its regulatory networks in gastric cancer remain elusive.

The microRNAs (miRNAs) are ~22 nucleotides noncoding RNAs and they can regulate tumorigenesis through targeting cellular function related mRNA [13]. MiR-21 has been identified as

Table 1. MiRNAs differentially expressed in gastric cancer tissues and matched noncancerous gastric tissues

MiR	P	FC (abs)	Regulation	Active_sequence	Chr
hsa-miR-21-5p	1.24E-04	3.844	Up	TCAACATCAGTCTGATAAGC	chr17
hsa-miR-196a-5p	1.42E-04	107.743	Up	CCCAACAACATGAAACTACC	chr12
hsa-miR-642a-3p	8.46E-04	2.240	Up	GGTTCCCTCTCCAAAT	chr19
hsa-miR-3198	0.002	3.121	Up	TCTCCATTCCCCAGG	chr12
hsa-miR-3651	0.004	4.035	Up	TCATGTACCAGCGACC	chr9
hsa-miR-196b-5p	0.005	32.238	Up	CCCAACAACAGGAAACTACC	chr7
hsa-miR-20a-5p	0.006	2.085	Up	CTACCTGCACTATAAGCAC	chr13
hsa-miR-106b-5p	0.009	2.068	Up	ATCTGCACTGTCAGCAC	chr7
hsa-miR-27a-3p	0.012	2.215	Up	GCGGAACTTAGCCACTG	chr19
hsa-miR-199a-3p	0.018	2.356	Up	TAACCAATGTGCAGACTACT	chr1
hsa-miR-135b-5p	0.020	16.975	Up	TCACATAGGAATGAAAAGCCATA	chr1
hsa-miR-18a-5p	0.028	11.258	Up	CTATCTGCACTAGATGCA	chr13
hsa-miR-17-5p	0.030	2.275	Up	CTACCTGCACTGTAAGC	chr13
hsa-miR-130b-3p	0.032	2.027	Up	ATGCCCTTTCATCATTGC	chr22
hsa-miR-21-3p	0.034	6.971	Up	ACAGCCCATCGACTG	chr17
hsa-miR-223-3p	0.044	2.861	Up	TGGGGTATTTGACAAACTGAC	chrX
hsa-miR-4793-5p	0.045	9.353	Up	CCTCTGCCCTGTGG	chr3
hsa-miR-3188	0.009	12.381	Down	CCCCGTATCCGCA	chr19
hsa-miR-29c-5p	0.010	15.262	Down	GAACACCAGGAGAAATCGGT	chr1
hsa-miR-29c-3p	0.011	2.067	Down	TAACCGATTTCAAATGGTGCTA	chr1
hsa-miR-551b-3p	0.012	19.344	Down	CTGAAACCAAGTATGGGTCGC	chr3
hsa-miR-378a-3p	0.017	3.195	Down	CCTTCTGACTCCAAGT	chr5
hsa-miR-451a	0.020	2.360	Down	AACTCAGTAATGGTAACGGTTT	chr17
hsa-miR-30c-1-3p	0.022	3.789	Down	GGAGTAAACAACCCTCTCC	chr1
hsa-miR-557	0.032	8.998	Down	AGACAAGGCCCACCCG	chr1
hsa-miR-129-2-3p	0.039	15.439	Down	ATGCTTTTTGGGGTAAGGG	chr11
hsa-miR-486-5p	0.047	8.5851	Down	CTCGGGGCAGCTCA	chr8

MiRNA-microarray analysis was performed using pairs of tumors and corresponding normal tissues (fold change ≥2 and p≤0.05). Up, up-regulated in tumors compared with normal tissue; down, down-regulated in tumors compared with normal tissue. Fold change of tumor to normal tissue.

the best hit in cancer related miRNAs by accumulating evidence [14]. It was shown to promote proliferation and invasion of gastric cancer, hepatocellular cancer, colorectal cancer, breast cancer, glioma and other cancers by directly targeting PTEN, PDCD4, RECK and other signal transduction pathways [15-19].

In this study, we found that FZD6 represses gastric cancer cell proliferation and migration via activating non-canonical wnt pathway. In addition, non-canonical wnt pathway ameliorates expression of canonical wnt pathway. We also demonstrated that the FZD6 was involved in miR-21-dependent effects in the canonical and non-canonical wnt pathways in gastric cancer.

Materials and methods

MicroRNA microarray analysis

Human microRNA microarray analysis was performed by Ouyi Biotechnology (Shanghai). Total RNA was extracted from gastric cancer tissues and from normal gastric tissues, according to Trizol protocol (Takara, Japan). Total RNA was used for miRNA microarray analysis (Agilent human miRNA V19.0). This chip allows the simultaneous analysis of 723 human miRNAs (miRBase release 10.1). RNA labeling and hybridization were performed in accordance to the manufacturer's indications. Agilent scanner and the Feature Extraction 10.7.1.1 software (Agilent Technologies) were used to obtain the

Table 2. Primers used for detection of the transcription of genes and miRNAs

Gene	Forward (5'-3')	Reverse (5'-3')
MiR-21	CAAAGATCACTATCCCAATCATC	GCGGTCTTTCTCAATCTAAGTC
U6	CTCGCTTCGGCAGCACA	ACGCTTCACGAATTTGCGT
FZD6	CCCAGCACAATGAAGATCAA	ACATCTGCTGGAAGGTGGAC
MMP2	TACAGGATCATTGGCTACACACC	GGTCACATCGCTCCAGACT
MMP9	TGTACCGCTATGGTTACACTCG	GGCAGGGACAGTTGCTTCT
CCND1	GCTGCGAAGTGGAAACCATC	CCTCCTTCTGCACACATTTGAA
RHOA	AGCCTGTGGAAAGACATGCTT	TCAAACACTGTGGGCACATAC
JUN	TCCAAGTGCCGAAAAAGGAAG	CGAGTTCTGAGCTTTCAAGGT
GAPDH	ATGTTCGTCATGGGTGTGAA	GGTGCTAAGCAGTTGGTGGT

microarray raw data. Microarray results were analyzed using the GeneSpring GX 12.5 software (Agilent Technologies). Differentially expressed miRNAs were identified by using a moderated t-test and Benjamini-Hochberg correction (adjusted P≤0.05 and fold change ≥2). Differentially expressed genes were measured similarity with miRNAs by the Pearson correlation and were analyzed in GO analysis, KEGG pathway analysis, and cluster analysis.

Clinical samples

Thirty-five pairs of snap-frozen gastric cancer tumor and matched adjacent non-tumor tissues were diagnosed histopathologically at the first affiliated hospital of Nanjing Medical University, Jiangsu Province, China from June 2013 to December 2013. The tissues were immediately stored in liquid nitrogen after surgery until further use. This protocol was approved by the Ethical Committee of the first affiliated hospital of Nanjing Medical University, and every patient had written informed consent.

Cell lines and cell cultures

Gastric cancer cell lines SGC-7901, AGS and immortal gastric epithelial cell line GES-1 were purchased from ATCC. Cell lines were maintained in RPMI 1640 (Gibco, USA) supplemented with 10% FBS (Gibco, USA) and 100 U/ml penicillin and 100 μg/ml streptomycin (Gibco, USA). Cells were cultured in a humidified incubator at 37°C in an atmosphere of 95% air and 5% carbon dioxide.

RNA reverse transcription and real-time quantitative PCR

Real-time PCR was performed to determine the expression levels of microRNA and mRNA in human cell lines and tissues. Total RNA was extracted with a Trizol reagent (Takara, Japan) following the instruction. Reverse Transcription was carried out using miRNA First-Strand cDNA Synthesis Kit (Gencopoeia, Guangzhou) for miRNA and reverse transcription kit (Takara, Japan) for mRNA. Real-time PCR was employed in triplicate using the SYBR Green PCR Kit (Takara, Japan) on Applied Biosystems StepOne-Plus Real-Time PCR System. All mRNAs were normalized to GAPDH, and miRNAs were normalized to U6. Relative quantification expression was calculated according to the comparative method of 2-ΔΔCT. Primer sequences were listed in **Table 2**.

Construction of 3' untranslated region luciferase plasmid and reporter assays

All 3' UTR reporter vectors were prepared by amplifying the 3' UTRs of FZD6, followed by insertion into the Pezx-MT01 vector (GeneCopoeia, Guangzhou). Site-specific mutants were generated by PCR in the Pezx-MT01 vector (GeneCopoeia, Guangzhou). SGC-7901 cells were seeded in the 24-well plates (1×10^5 cells per well) one day before transfection and then each well was transfected with a mixture of 100 ng 3'-UTR luciferase reporter vector and 50 pmol miRNA mimics, inhibitors or respective controls. Twenty four hours post transfection, the cells were lysed. Then, luciferase activity was measured using the Dual-Luciferase Reporter Assay System (Promega, USA), with a Promega Glomax 2020 Single Tube Luminometer instrument (Promega, USA). Each experiment was performed in triplicate. The ratio of Renilla luciferase to Firefly luciferase was calculated for each well.

Western blot

The total cellular protein was extracted with RIPA buffer containing a protease inhibitor. The protein concentration was detected by a BCA protein assay kit (Thermo Scientific, USA). Protein was loaded into 10% polyacrylamide SDS-PAGE gel and transferred to PVDF membranes. The membranes were blocked with 5% non-fat dry milk for 2 hour and incubated overnight at 4°C using the following antibodies: anti-FZD6 (Abcam, UK), anti-Actin (Abcam, UK). After three

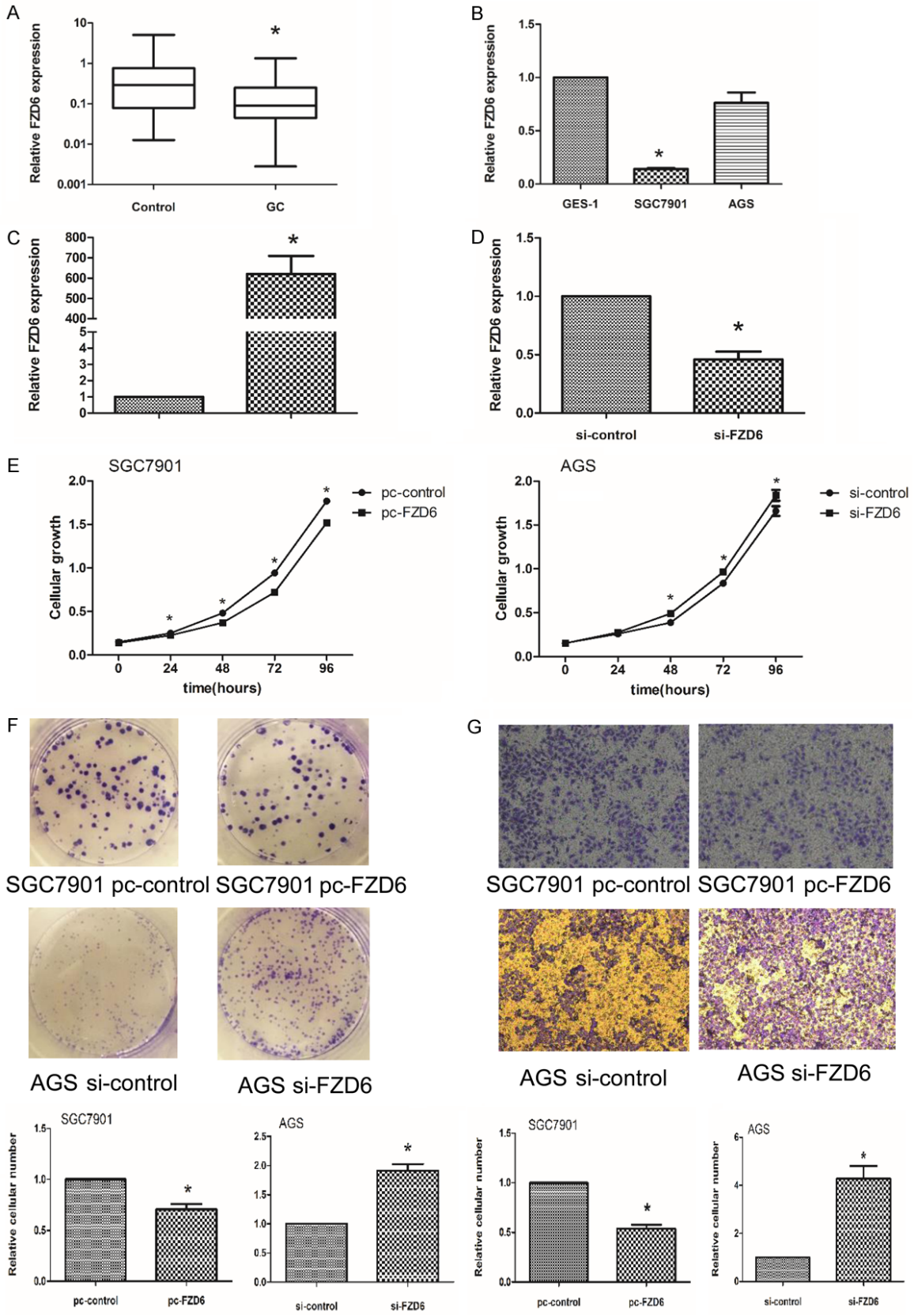

Figure 1. FZD6 suppresses tumor proliferation and migration in gastric cancer. A, B. Expression of FZD6 in gastric cancer tissues and gastric cancer cell line SGC7901 and AGS, compared with matching normal gastric tissue and normal gastric cell line GES-1. C, D. FZD6 expression was assessed by real-time PCR in SGC7901 transfected with

either pc-control or pc-FZD6, or in AGS cells transfected with si-control or si-FZD6. E-G. SGC7901 cells were transfected with either pc-control or pc-FZD6 and AGS cells were transfected with either si-control or si-FZD6. The CCK-8 analysis were performed at 0 h, 24 h, 48 h, 72 h, and 96 h. The clony formation assay was done 10 days later. Cell migration across a membrane with 8-um pores was assessed as described in the Materials and Methods section. The mean and standard error from 3 separate experiments are illustrated. *P<0.05 when compared with controls.

time washes with TBST (TBS with 0.1% tween), the membrane was incubated with secondary antibodies (HRP-conjugated goat anti-rabbit IgG, Abcam, UK) at room temperature for 1 h. After another three washes with TBST, the signal was visualized through a chemiluminescent detection system (Pierce ECL Substrate Western blot detection system, Thermo, USA) and then exposed in Molecular Imager ChemiDoc XRS System (Bio-Rad, USA).

Cck-8 assay

Cell viability was determined by CCK-8 (Dojindo, Japan). GC cells were seeded into 96-well plates at 1.0×10^4 cells per well in RPMI 1640 containing 10% FBS for 24 h and then transfected with mir-21 mimics, inhibitors, or respective controls, using Lipo2000 (Thermo, USA) according to manufacturer's instruments. After 48 hours, CCK-8 solution (10 uL/well) was added 2 h before measurement. The absorbance at 450 nm of each well was recorded by microplate reader (Bio-Rad, USA).

Colony formation assay

Colonic gastric cancer cells were transfected with miR-21 mimic or miR-21 inhibitor or their respective controls. Then, triplicate samples of 400 cells from each cell line were plated on medium. The number of colonies was counted after 10 days.

Transwell migration assay

We performed transwell migration assays in gastric cancer cell lines 48 hours after transfection with miR-21 mimic or miR-21 inhibitor or their respective controls. Assays were conducted according to manufacturer's protocol, using 20% FBS as chemoattractant. Non-migrating cells on the top side of the membrane were removed while migrating cells were fixed with methanal and stained with crystal violet 24 h post seeding. In all assays, 10 fields per insert were scored and SE was calculated.

Statistical analysis

The data were presented as the mean ± s.e.m. Statistical analyses were performed with the use of SPSS 20.0 and Graphpad Prim 5. Student's t test was used to examine the statistical difference. The correlation significance was determined by means of Spearman and Pearson correlation analyses. All used tests were two-tailed, and a *P* value of less than 0.05 was considered significant.

Results

FZD6 suppresses tumor proliferation and migration in gastric cancer

We found that FZD6 was down-regulated in gastric cancer tissues and gastric cancer cell lines SGC7901 and AGS compared with normal gastric tissues and normal gastric epithelial cell line GES-1 (**Figure 1A** and **1B**). In order to evaluate the effects of FZD6 on malignant phenotypes in gastric cancer cells, we transfected SGC7901 cells with either pc-control or pc-FZD6 and AGS cells with either si-control or si-FZD6. Transfection efficiency was confirmed through real-time PCR (both P<0.05, **Figure 1C** and **1D**). CCK-8 assays revealed that pc-FZD6 transfected cells exhibited significantly decreased growth rate than pc-control transfected cells (P<0.05, **Figure 1E**). Colony formation assays also showed that promoting FZD6 expression resulted in significant tumor growth inhibition (P<0.05, **Figure 1F**). We next examined the effect of FZD6 on migration ability of gastric cancer cells by transwell assay. Increased FZD6 expression significantly reduced migration of gastric cancer cells (P<0.05, **Figures 1G**). Inversely, si-FZD6 transfected gastric cancer cells displayed increased cell proliferation and migration compared to cells transfected with si-control. These results suggested that FZD6 could suppress gastric cancer cell proliferation and migration.

MicroRNA expression profiles in gastric cancer

We first isolated and compared the miRNA expression profile in 6 matched pairs of gastric cancer and control noncancerous gastric tissues using the Agilent human miRNA V19.0 (**Figure 2A** and **2B**). Twenty-seven miRNAs (17 up-regulated and 10 down-regulated) were expressed differentially in gastric cancer acco-

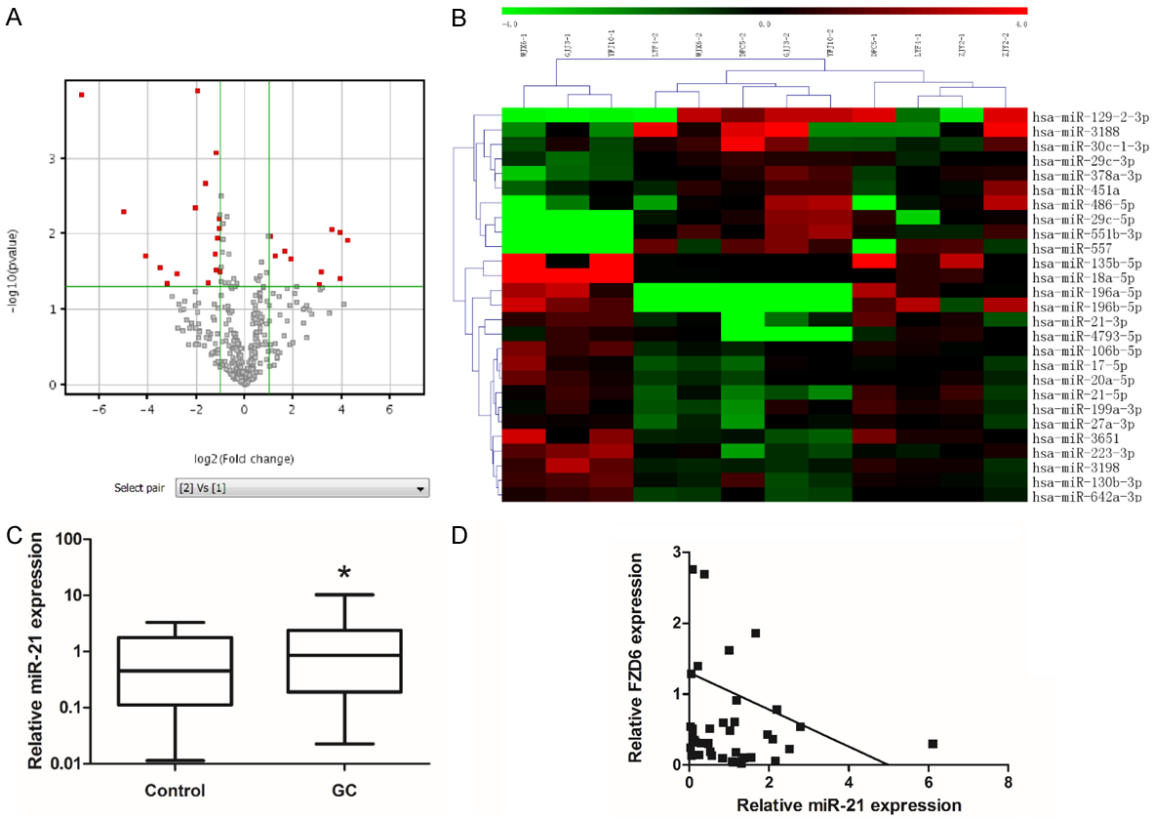

Figure 2. MicroRNA expression profiles in gastric cancer. A, B. MiRNA microarray was performed to detect differently expressed miRNA of gastric cancer and control noncancerous gastric tissues. Red dots in the volcano plot represented miR with fold change ≥2 and p≤0.05 and cluster analysis also showed the differently miR. C, D. The expression of miR-21 was measured in gastric cancer tissues and matching normal gastric tissues by RT-PCR. MiR-21 was up-regulated in gastric cancer and had the tendency of being inversely correlated with FZD6. *P<0.05 when compared with controls.

rding to class comparison analysis of fold change ≥2 and p≤0.05 (**Table 1**). The group of miRNA that were altered significantly in expression may contribute to the pathogenesis or phenotypic behavior of gastric cancer. Moreover, these miRNAs also are potential markers that may be useful for the diagnosis of gastric cancer.

FZD6 is targeted by miR-21

To establish the molecular link between FZD6 and miRNAs, we identified related miRNAs of FZD6 by computational prediction. Among hundreds of miRNAs predicted by online miRNA target prediction website (starBase, http://starbase.sysu.edu.cn/), we forcused our attention on miR-21 [20, 21]. According to the results of our microarray, miR-21 was highly significantly increased in gastric cancer tissues with the lowest *p* value of 1.24E-04. We found that miR-21 is significantly up-regulated in gastric cancer tissues (**Figure 2C**) and FZD6 was inversely correlated with miR-21 status (**Figure 2D**). What's more, Quantitative real time PCR (qRT-PCR) and western bolt revealed significantly different expression of FZD6 in cells transfected with miR-21 mimic or inhibitor relative to respective controls (**Figure 3A-E**).

To assess whether miR-21 can directly alter the expression of FZD6 in GC cells, a fragment of the 3' UTR of FZD6 mRNA, containing the putative miR-21 binding sequence, was cloned into a firefly luciferase reporter construct, which contained both luciferase and renilla expression mRNA, along with miR-21 mimic, inhibitor or respective controls. Co-transfection of miR-21 mimic and the WT FZD6 3'-UTR construct resulted in a significant inhibition of the luciferase activity compared with cells co-transfected with negative control. An increase in relative luciferase activity was noted with miR-21 inhibitor, indicating that miR-21 could modulate gene

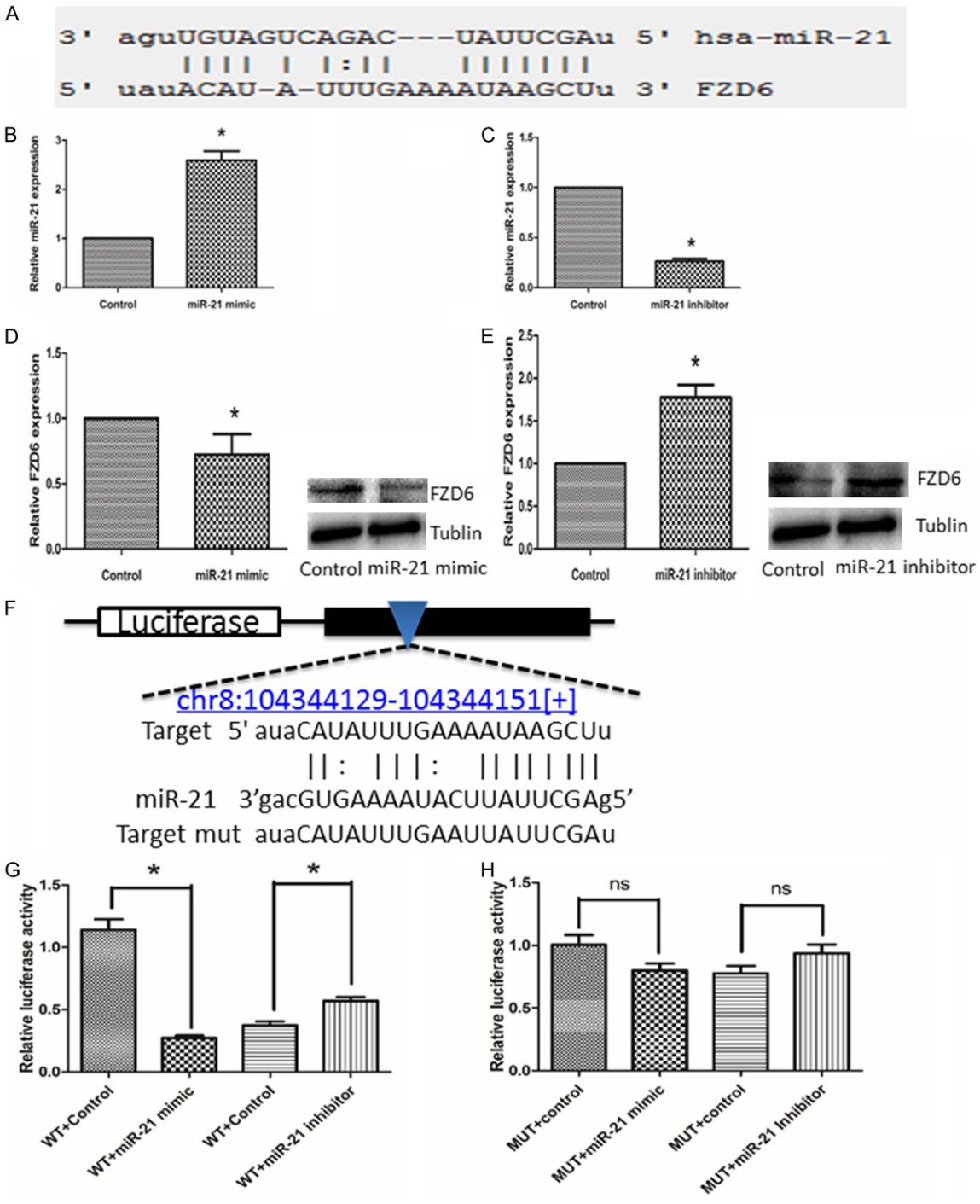

Figure 3. FZD6 is targeted by miR-21. A. Downstream targets of miR-21 by computational prediction. B-E. Gastric cancer cells were transfected with miR-21 mimic, inhibitor, or respective controls. MiR-21 was measured by QRT-PCR 48 h later. QPT-PCR and western bolt were performed for FZD6 48 and 72 h later. GAPDH and tublin were used as control and for quantitation. F-H. Gastric cancer cells were plated in 24-well plates. Cells were transfected with a firefly luciferase and renilla reporter construct, which contained 3' UTR of FZD6 mRNA, along with either miR-21 mimic, miR-21 inhibitor and controls. An decrease in relative firefly luciferase activity in the presence of miR-21 mimic and an increase in the presence of miR-21 inhibitor indicated that the 3' UTR of FZD6 contains a target that is modulated by miR-21. For the mutant firefly luciferase reporter, putative miR-21-bingding sites in 3'-UTR regions were mutated and the phenomenon disappeared. Data represent mean±standard error from 3 separate experiments. *P<0.05 when compared with controls.

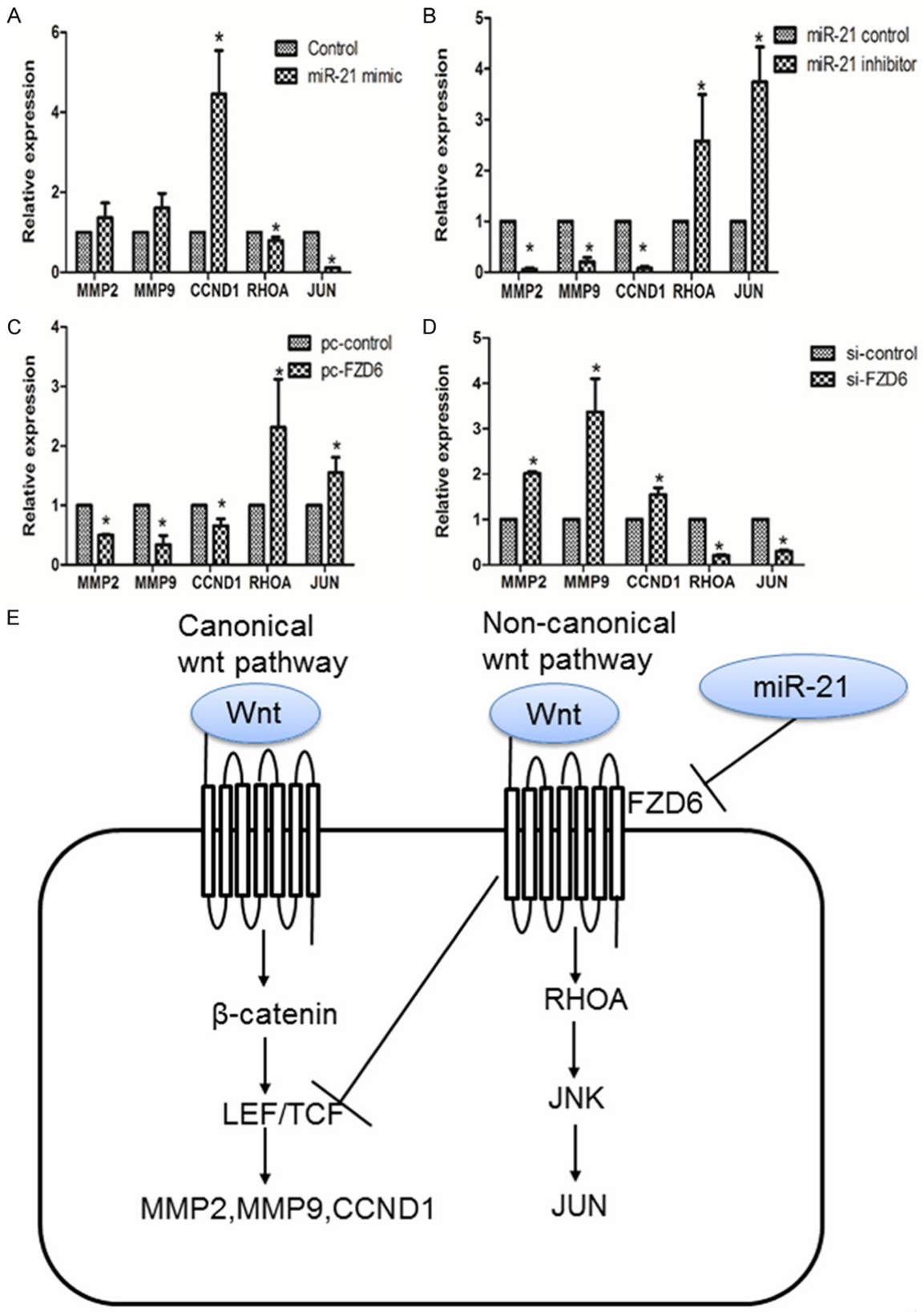

Figure 4. FZD6 is involved in miR-21- dependent effects in the canonical and non-canonical wnt pathway. A, B. Gastric cancer cells were transfected with miR-21 mimic, inhibitor, and respective controls. Quantitative real-time PCR was performed for MMP-2, MMP-9, CCND1, RHOA and JUN mRNA expression. C, D. SGC7901 cells were transfected

with either pc-control or pc-FZD6 and AGS cells were transfected with either si-control or si-FZD6. MMP-2, MMP-9, CCND1, RHOA and JUN mRNA expression was assessed by real-time PCR. E. Schematic representation of FZD6, miR-21 and wnt pathways. The experiments were performed in triplicate. *P<0.05 relative to controls.

expression directly at the FZD6 3' UTR (**Figure 3F** and **3G**). In addition, we constracted mutated luciferase reporter with mutations of putative miR-21-bingding sites in these 3'-UTR regions and found cotransfecting with miR-21 mimic and inhibitor abrogated the inhibiting and promoting effects (**Figure 3H**). Taken together, these results demonstrate that FZD6 is the direct target of miR-21-5p.

FZD6 is involved in miR-21-dependent effects in the canonical and non-canonical wnt pathway

It is known that mir-21 promotes GC via regulating wnt pathway [22]. Wnt parthway can be divided into canonical and non-canonical pathways. The representative molecular in canonical pathway includes MMP2, MMP9 and CCND1 and non-canonical pathway includes RHOA and JNK [9]. We studied the influence of mir-21 and FZD6 for wnt by real time quantitative PCR. Up regulation of MMP2, MMP9, CCND1 and down-regulation of RHOA and JNK were noticed when cells were transfected with mir-21 and si-FZD6 (P<0.05, **Figure 4A** and **4B**). In contrast, when cells were transfected with mir-21 inhibitor or pc-FZD6, the phenomenon is quite opposite (P<0.05, **Figure 4C** and **4D**). In combination, these studies defined an important role for FZD6 in canonical and non-canonical wnt pathways in human gastric cancer cells (**Figure 4E**).

Disscussion

Although many genes and pathways in cancer have been proposed, the molecular mechanisms of tumor proliferation and migration are not fully known. We show that FZD6, a gene involved in non-canonical wnt pathway, suppresses gastric cancer proliferation and migration. Moreover, it is targeted by cancer promoter miR-21 and associated with miR-21-dependent effects in the canonical and non-canonical wnt pathway. The identification of FZD6 as an important regulator of tumor cell proliferation and migration in vitro provides insight into gastric oncogenesis and molecular target treatment.

Wnt signaling pathway is closely associated with tumor development, including gastric cancer [23]. Wnts have been classified into two functional groups with separate downstream signaling pathways, canonical and non-canonical wnt pathway. FZD6 is involved in non-canonical wnt pathway and RHOA and JNK are critical downstream molecules of non-canonical wnt patheway [9]. However, the function of FZD6 in tumor is still controversial. Some report that aberrant activation of the FZD6 leads to more malignant phenotypes, such as abnormal tissue polarity, invasion, and metastasis [24]. However, Piga et al silenced FZD6 in non-tumor human type II bronchial epithelial BEAS-2B cells and found enrichment of the Gene Ontology terms "oncogenes", "cell proliferation" and "cell cycle", which reveals FZD6 may inhibit tumor formation [25] Moreover, Beatriz et al reported that WNT4 ligand regulated the cell growth of leukemia derived cells by arresting cells in the G1 cell cycle phase in an FZD6-independent manner, possibly through antagonizing the canonical WNT/β-catenin signaling pathway [11]. In addition, FZD6 was reported to activate the kinase-NEMO-like kinase pathway that blocks TCF/lymphoid enhancer, thereby inhibiting the ability of canonical wnt/β-catenin pathway [26].

miR-21 has been shown to be overexpressed in many different solid tumors, including gastric cancer. Therefore, it is highly likely that miR-21 plays a fundamental role in malignant transformation and targets tumor suppressor mRNA in tumor cells. A number of target genes have been identified [27, 28]. Dan et al reported that miR-21 regulates lung cancer via wnt pathway [22]. In our study, we found inversely expression level of mir-21 and FZD6 via real time quantitative PCR and western blot and confirmed the direct targeting of mir-21 to FZD6 via luciferase. What's more, we confirmed miR-21 can promote canonical wnt pathway and inhibit non-canonical wnt pathway via FZD6.

Although we found FZD6 and miR-21 expression levels had the tendency of being inversely correlated, the *p* value is more than 0.05. The reason may be the limited number of tissues and more clinical tissues are needed. In addition, when cells were transfected with miR-21 mimic, the changes of MMP2 and MMP9 were

not significantly. We analyzed that the phenomenon may be led by high expression of miR-21 in gastric cancer cells. This also suggest that the regulation of miR-21 and its targets is very complex and other studies explaining the mechanism are still needed.

Proliferation and metastasis are associated with a poor prognosis, and therefore targeting these mechanisms may lead to more effective treatment for gastric cancer patients. There is considerable interest in microRNA-mediated therapy. Firstly, microRNAs readily cross cell membranes [29]. Secondly, microRNAs are not immunogenic because of lacking poly-A tail [30]. Thirdly, these molecules are fairly stable both in vitro and in vivo, although they can be further modified to prolong their biological half-life [31, 32]. Therefore, we are hopeful that this study provides enticing data to support the use of microRNAs inhibitor, specifically miR-21, for the treatment of gastric cancer and other cancers.

In summary, our results demonstrate that FZD6 suppresses gastric cancer proliferation and migration and is involved in miR-21-dependent effects in the canonical and non-canonical wnt pathway. Exploring gastric cancer mechanism can provide a more effective treatment for gastric cancer patients.

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Disclosure of conflict of interest

None.

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