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Down-regulation of frizzled-7 expression decreases survival, invasion and metastatic capabilities of colon cancer cells.

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The Wnt signalling pathway is composed of canonical and non-canonical signals. The canonical Wnt signalling pathway that regulates cell fate and proliferation is initiated by binding of Wnt ligands to frizzled transmembrane receptors, and low-density lipoprotein receptor-related proteins. β-Catenin associates with T-cell factor (TCF)/lymphocyte enhancer transcription factors to regulate the expression of target genes that are related to cell survival, proliferation and invasion (Moon et al, 2004; Clevers, 2006). The non-canonical Wnt signalling pathway consists of Wnt/Ca\(^{2+}\) pathway and Wnt/c-Jun N-terminal kinase (JNK) (planar cell polarity) pathway (Cohen et al, 2008). In the Wnt/Ca\(^{2+}\) pathway, Wnt activates intracellular Ca\(^{2+}\) signalling, as well as Ca\(^{2+}\)-dependent protein kinases, such as protein kinase C (PKC) and calmodulin-dependent protein kinase II. In the Wnt/JNK pathway, receptor stimulation activates Dishevelled (Dvl), which in turn activates Rho family of GTPases such as RhoA and Rac. RhoA stimulates c-Jun expression through phosphorylation of c-Jun by Rho-associated kinase (ROCK) (Marinissen et al, 2004). Accumulating evidence suggests that non-canonical Wnt signalling is important in regulating cell polarity and movement (Veeman et al, 2003).

It is well known that the canonical Wnt signalling pathway is activated in most sporadic colorectal cancers (CRCs; ~80%), which is mainly caused by mutations in the adenomatous polyposis coli (APC) gene (Segditsas and Tomlinson, 2006; Schneikert and Behrens, 2007). In a small proportion of cases (~10%), activated mutations of the β-catenin gene (CTNNB1) are found (Schneikert and Behrens, 2007). However, recent findings revealed that Wnt ligands or inhibitors could affect the growth and survival of colon cancer cells in spite of the presence of APC or CTNNB1 mutations (Bafico et al, 2004; Suzuki et al, 2004; He et al, 2005). These findings suggested that Wnt ligands and receptors that function upstream of APC might have a vital role in the development of CRCs.

We have recently reported that frizzled-7 (FZD7), 1 of 10 members of the FZD gene family, is predominantly expressed in colon cancer cells and is implicated in canonical Wnt signalling in colon cancer cells with APC or CTNNB1 mutations (Ueno et al, 2008). Moreover, the down-regulation of FZD7 with Small-interfering RNA (siRNA) in colon cancer cells resulted in decreased in \textit{vitro} invasion activity (Ueno et al, 2008), which is consistent with previous findings that inhibition of FZD7 expression with dominant-negative mutant construct or siRNA reduced the motility of hepatocellular carcinoma cells (Merle et al, 2004) or colon cancer cells (Vincan et al, 2007), respectively. These data suggest that FZD7 may be important in the invasion and metastasis of CRC. Recent studies have shown that non-canonical Wnt signalling pathways affect the motility and invasion of cancer cells.
(Weeraratna et al, 2002; Croft et al, 2004; Qiang et al, 2005), but there is little data on CRC cells. Although there is a report that conditional ROCK activation of colon cancer cells induced in vitro motility and in vivo tumour cell dissemination in nude mice (Croft et al, 2004), the relation of FZD7 with non-canonical signals in CRC cells remains unknown.

In this study, we hypothesised that FZD7 may be involved in progression of CRC probably through both canonical and non-canonical signalling pathways. To address this hypothesis, we attempted to reveal a function of FZD7 in the survival, invasion and metastatic capabilities of colon cancer cells with the use of newly prepared and selected siRNAs against FZD7. Moreover, the expression level of FZD7 mRNA was quantitatively evaluated in primary CRC tissues (n = 135) to clarify whether it could be of prognostic significance for CRC.

MATERIALS AND METHODS

Cell cultures

Human colon cancer cell lines, HCT-116 and HT-29, were purchased from the ATCC (Manassas, VA, USA). Human embryonic kidney 293T cells were purchased from RIKEN BRC (Tsukuba, Japan). HCT-116 and HT-29 cells were cultured in McCoy’s 5A medium (Gibco/Invitrogen, Carlsbad, CA, USA) supplemented with 10% heat-inactivated fetal bovine serum, 100 IU ml⁻¹ penicillin and 100 μg ml⁻¹ streptomycin (Sigma, St Louis, MO, USA). 293T cells were cultured in DMEM (Nissui Pharmaceutical, Tokyo, Japan) supplemented with 10% heat-inactivated fetal bovine serum (ATCC), 100 IU ml⁻¹ penicillin and 100 μg ml⁻¹ streptomycin (Sigma).

RNA interference

Small-interfering RNAs were constructed in the piGENE hU6 Vector (Clontech, Palo Alto, CA, USA). The nucleotide target sequence for FZD7 were as follows: siRNA1 sequence, gcaccatcagaaacagcag; siRNA2 sequence, gcagacgtcgcaagctgtc; siRNA3 sequence, acctctctagagcctgct; siRNA4 sequence, gcttcctactacttggttg; siRNA5 sequence, gcagacgtcgcaagctgtc; siRNA6 sequence, tctgtcatcagcatgct; siRNA7 sequence, ctcagacgtcgcaagctgtc; siRNA8 sequence, gcttcctactacttggttg; siRNA9 sequence, ctgtaacactttaagagggagag; siRNA10 sequence, gtaaggtgacagtacctt; siRNA11 sequence, tcctctctgagcctgct; siRNA12 sequence, gaaaggctgacagtacctt; siRNA13 sequence, gcagacgtcgcaagctgtc; EGFP siRNA sequence, gcagacgtcgcaagctgtc; scramble siRNA sequence, gcagacgtcgcaagctgtc;

Luciferase reporter assay

For Tcf luciferase assays, cells were transfected with 0.475 μg of TOPflash (Upstate, Lake Placid, NY, USA) and 100 IU ml⁻¹ Streptokinase, 100 IU ml⁻¹ Empirin (Nissui Pharmaceutical, Tokyo, Japan) supplemented with 10% heat-inactivated fetal bovine serum, 100 IU ml⁻¹ penicillin and 100 μg ml⁻¹ streptomycin (Sigma). For Tcf luciferase assays, cells were transfected with 0.475 μg of TOPflash (Upstate, Lake Placid, NY, USA) and 100 IU ml⁻¹ Streptokinase, 100 IU ml⁻¹ Empirin (Nissui Pharmaceutical, Tokyo, Japan) supplemented with 10% heat-inactivated fetal bovine serum, 100 IU ml⁻¹ penicillin and 100 μg ml⁻¹ streptomycin (Sigma). For Tcf luciferase assays, cells were transfected with 0.475 μg of TOPflash (Upstate, Lake Placid, NY, USA) and 100 IU ml⁻¹ Streptokinase, 100 IU ml⁻¹ Empirin (Nissui Pharmaceutical, Tokyo, Japan) supplemented with 10% heat-inactivated fetal bovine serum, 100 IU ml⁻¹ penicillin and 100 μg ml⁻¹ streptomycin (Sigma).

Crystal violet stain

HT-29 and HCT-116 cells were transfected with plasmid by the Nucleofector system (Amaza, Cologne, Germany). Cells were seeded in 2 ml medium in a six-well tissue culture plate. At 6 days after transfection, cells were stained with 0.5% crystal violet in 20% methanol for 10 min. Stable transfectants were seeded at 10⁴ cells per well in 2 ml medium in a six-well tissue culture plate. At 8 days after seeding, cells were stained with 0.5% crystal violet in 20% methanol for 10 min. Cell viability was determined by absorbance measurements at 595 nm using 2030 ARVO X4 (PerkinElmer, Boston, MA, USA).

Cell-cycle assay

HCT-116 cells were transfected with scramble siRNA or FZD7_sirNA8. At 48 h after transfection, cells were harvested, fixed with 75% ethanol for 2 h at 4 °C, washed with phosphate-buffered saline (PBS), treated with 100 μg ml⁻¹ RNase (Sigma) for 30 min at 37 °C and stained in 10 μg ml⁻¹ propidium iodide (Sigma) for 30 min at 4 °C. Analysis was performed on Cytomics FC500 using FC500 CXP Cytometer software (Beckman Coulter Co., Miami, FL, USA).

Western blot analysis and RhoA activation assay

At 48 h after transfection, cells were washed in ice-cold PBS and re-suspended in cold buffer containing 1% Nonidet P-40, 0.5% sodium deoxycholate, 0.1% SDS, 0.1% N-sulfonyl fluoride. Re-suspended cells were passed through the 21-gauge needle to shear the DNA and incubated for 60 min on ice followed by centrifugation at 10 000 g for 10 min at 4 °C. Total protein (10 μg) was analysed by western blotting using primary antibodies and anti-mouse and anti-rabbit IgG HRP-conjugated secondary antibodies (Dako, Glosstrup, Denmark), and were visualised with LumiGLO Reagent and Peroxide (Cell Signaling Technology, Beverly, MA, USA). Results of western blot analysis were shown as ratio of band intensity, which indicates the ratio of band intensity of tested protein to that of β-actin. Band intensity was measured by using ImageJ software (NIH, Bethesda, MD, USA). At 48 h after transfection, cells were assayed for Rho activation with a Rho Activation Assay Kit (Upstate). The GTP-bound fraction was monitored by western blot analysis. The primary antibodies used were mouse anti-V5 (Invitrogen), anti-β-actin (Abcam, Cambridge, UK), anti-p38, anti-phospho-p38, anti-ERK, anti-phospho-ERK, anti-JNK, anti-phospho-JNK, anti-c-Jun and anti-phospho-c-Jun (BD Biosciences, San Jose, CA, USA) monoclonal antibodies, and rabbit anti-RhoA monoclonal antibody (Cell Signaling Technology).

Cell invasion assay

Matrigel (1 : 5 ; BD Biosciences) was added to Transwell membrane filter inserts (8.0 μm pore size; Costar, Cambridge, MA, USA) and incubated for 5 h at 37 °C in a 5% CO₂ tissue culture incubator. HCT-116 cells were transfected with scramble siRNA or FZD7_sirNA8. At 24 h after transfection, cells were harvested and re-suspended in serum-free medium. Aliquots (10⁵ cells) of the prepared cell suspension were added into the upper chamber and the lower chamber was filled with 600 μl of culture media containing 5 μg ml⁻¹ fibronectin (Sigma), as an adhesive substrate. Cells were incubated for 48 h at 37 °C in a 5% CO₂ tissue culture incubator. Invasive cells were stained with Diff-Quick solution (Fisher Scientific, Pittsburgh, PA, USA). Cells were counted with a microscope. The average number of cells in five fields per membrane was counted in triplicate.

Quantitative PCR

Total RNA was isolated from colon cancer cell lines and primary colorectal tumour and non-tumour tissues using the RNeasy Plus Mini Kit (Qiagen, Hilden, Germany) and All prep DNA/RNA Mini kit (Qiagen), respectively. The extracted total RNA was...
reverse-transcribed into single-stranded cDNA using a High-Capacity cDNA Archive Kit (Applied Biosystems, Warrington, UK). Real-time PCR was performed using first-strand cDNA with TaqMan Universal PCR Master Mix (Applied Biosystems). The assay numbers for the endogenous control (β-actin) and target genes were as follows: 4326315E (β-actin); Hs00275833_s1 (FZD7).

Quantitative PCR was performed on an ABI Prism 7900HT Sequence Detection System (Applied Biosystems). Quantitative PCR parameters for cycling were as follows: 50 °C for 2 min hold, 95 °C for 10 min 40 cycles of PCR at 95 °C for 15 s and 60 °C for 1 min. All reactions were carried out in a 20-μl reaction volume in triplicate. For target gene assays, total RNA was isolated from colon cancer cell lines using the RNeasy Plus Mini Kit (Qiagen) 48 h after transfection. The extracted total RNA was reverse-transcribed into single-stranded cDNA using High-Capacity cDNA Archive Kit (Applied Biosystems). Real-time PCR was performed using first-strand cDNA with Power SYBR Green PCR Master Mix (Applied Biosystems). The primers used were as follows: FZD7 forward primer, 5′-ttctggaaggatcagtcctc-3′; FZD7 reverse primer, 5′-gaacaccagagagagaacagtc-3′; CD44s forward primer, 5′-tctcttcgttg-3′; CD44s reverse primer, 5′-tgtcagagtagaagttgttggatgg-3′; CD44v6 forward primer, 5′-cccaagaaaaagcttgttg-3′; CD44v6 reverse primer, 5′-agctgtccctgttgtcgaatg-3′; CD44v8-9 forward primer, 5′-caggtttggtggaagatttgg-3′; Met reverse primer, 5′-ggcagtattcgggttgtaggag-3′; Survivin forward primer, 5′-cctgggtgtctcctgg-3′; Survivin reverse primer, 5′-cctgggtgtctcctgg-3′; MT1-MMP forward primer, 5′-aagtttggctctttctctgtcc-3′; MT1-MMP reverse primer, 5′-tgccctgagctcttcttg-3′; Jun forward primer, 5′-ggaagacgcttcatgactgtg-3′; Jun reverse primer, 5′-agggtgtctctgctctgttg-3′; Hs00275833_s1 (FZD7).

Statistical analysis

The relationship of FZD7 mRNA levels with clinical stage and follow-up information after surgery was analysed using the Kruskal–Wallis and post hoc tests. Kaplan–Meier curves were compared using the log-rank test. Data were processed using GraphPad Prism 5 software (GraphPad Software, San Diego, CA, USA).

RESULTS

Preparation and selection of FZD7 siRNA

Thirteen shRNA expression vectors harbouring siRNAs against FZD7 were constructed and tested to determine which had the best knockdown effect.
of FZD7 were measured by real-time PCR. Thirteen siRNAs were designed based on the nucleotide sequence of the FZD7 gene. HCT-116 cells were transiently transfected with shRNA expression vectors harbouring siRNA against FZD7. As shown in Figure 2C, the band intensities of c-Jun, p-JNK and β-actin antibodies were decreased by FZD7_siRNA.

To examine whether the FZD7_siRNA could discriminate between FZD7 and FZD1 with the highest homology, we co-transfected FZD7-V5 or FZD1-V5 and FZD7_siRNA into 293T cells and subjected the whole proteins to immunoblotting with an anti-V5 antibody (Figure 1B). The expression of FZD7-V5 protein was abolished with FZD7_siRNA whereas that of FZD1-V5 was not. Thus the FZD7_siRNA was used to specifically inhibit FZD7.

FZD7_siRNA suppressed cell viability and invasion

HCT-116 and HT-29 cells were transfected with scramble siRNA or FZD7_siRNA, and the cells were stained with crystal violet stain 6 days after transfection. Viable cells were decreased to <10 and 40% in HT-29 and HCT-116 cultures, respectively (Figure 2A). On the basis of this result and the fact that we were unable to isolate stable HT-29 siRNA transfectants (see below), we used HCT-116 cells for the following siRNA transfection experiments.

To observe the effect of FZD7_siRNA on cell cycle, we transfected HCT-116 cells with scramble siRNA or FZD7_siRNA, and the cells were analysed by flow cytometry 48 h after transfection. The percentage of cells at G2/M phase was reduced from 40 to 30% with FZD7_siRNA transfection (Figure 2A). To test whether FZD7_siRNA indeed altered Wnt transcriptional activity, we transfected TOP-Tcf flash reporter plasmid into the transfectants, and measured the Tcf transcriptional activity (Figure 3B). We found that Tcf activity was significantly decreased in FZD7_siRNA transfectants (Figure 2A). To assess whether FZD7_siRNA affected the canonical Wnt signal-transducing activity, we transfected TOP-Tcf flash reporter plasmid into the transfectants, and measured the Tcf transcriptional activity (Figure 3B). We found that Tcf activity was significantly decreased in FZD7_siRNA transfectants (Figure 3B).

FZD7_siRNA inhibits in vivo metastasis

HCT-116 cells were transfected with pcDNA3.1-U6EGFP_siRNA or pcDNA3.1-U6 FZD7_siRNA, and the expression level of FZD7 and two target genes (MT1-MMP and Jun) in stable transfectants was measured with real-time PCR. We used two stable transfectants (FZD7_siRNA clone 1 and clone 2) that show the decreased expression of these genes compared with control cells harbouring EGFP_siRNA for the following experiments (Figure 3A). To test canonical Wnt signal-transducing activity, we transfected TOP-flash reporter plasmid into the transfectants, and measured the Tcf transcriptional activity (Figure 3B). We found that Tcf activity was decreased to ~20% in both FZD7_siRNA clones compared to control cells. Cell viability and invasion activity were also significantly decreased in FZD7_siRNA transfectants (P < 0.005 and <0.05, respectively; Figures 3C and D).
The anti-metastatic activity of FZD7 siRNA was also shown in an in vivo liver metastasis model (Figure 3E). FZD7 siRNA transfectants were transplanted into the spleen of scid mice, and after 3 weeks, the mice were killed to count liver metastasis colonies. Liver metastases in mice transplanted with FZD7 siRNA after 3 weeks, the mice were killed to count liver metastasis transfectants were transplanted into the spleen of scid mice, and analysis.

b protein (10 ng) was analysed. Expression of p38, phospho-p38, ERK, phospho-ERK, JNK, phospho-JNK, c-Jun or phospho-c-Jun was assessed by western blot analysis. β-Actin was used as a loading control. Ratio of band intensity indicates the ratio of band intensity of tested protein to that of β-actin. Band intensity was measured by using ImageJ software. (D) Effect of FZD7 siRNA on RhoA activation. HCT-116 cells were transfected with FZD7 siRNA or scramble siRNA. At 48 h after transfection, GST-RhoA expression was assessed. Total RhoA was used as a loading control. Ratio of band intensity indicates the ratio of band intensity of tested protein to that of β-actin. (E) Effect of FZD7 siRNA on cell invasion. HCT-116 cells were transiently transfected with FZD7 siRNA or scramble siRNA. At 48 h after transfection, total RNAs were reverse-transcribed and the level of mRNA expression of Wnt target genes was measured by real-time PCR.

**Figure 2** FZD7 siRNA suppressed cell viability and invasion. (A) Effect of FZD7 siRNA on cell viability. HCT-116 and HT-29 cells were transiently transfected with FZD7 siRNA or scramble siRNA control. At 6 days after transfection, HCT-116 and HT-29 cells were stained with crystal violet. Cell viability was determined by absorbance at 595 nm. (B) Effect of FZD7 siRNA on cell cycle. HCT-116 cells were transfected with FZD7 siRNA or scramble siRNA. Cells were harvested 48 h after transfection and cell-cycle analysis was performed using a Cytophysics FC500 cell sorter. (C) Effect of FZD7 siRNA on MAP kinase protein expression. HCT-116 cells were transiently transfected with FZD7 siRNA or scramble siRNA. At 48 h after transfection, cytosolic MAP kinase protein expression. HCT-116 cells were transiently transfected with FZD7 siRNA or scramble siRNA. After 48 h, p38, phospho-p38, ERK, phospho-ERK, JNK, phospho-JNK, c-Jun or phospho-c-Jun was assessed by western blot analysis. β-Actin was used as a loading control. Ratio of band intensity indicates the ratio of band intensity of tested protein to that of β-actin. Band intensity was measured by using ImageJ software. (D) Effect of FZD7 siRNA on RhoA activation. HCT-116 cells were transfected with FZD7 siRNA or scramble siRNA. At 48 h after transfection, GST-RhoA expression was assessed. Total RhoA was used as a loading control. Ratio of band intensity indicates the ratio of band intensity of tested protein to that of β-actin. (E) Effect of FZD7 siRNA on cell invasion. HCT-116 cells were transiently transfected with FZD7 siRNA or scramble siRNA. At 48 h after transfection, total RNAs were reverse-transcribed and the level of mRNA expression of Wnt target genes was measured by real-time PCR.

**DISCUSSION**

Transient transfection of FZD7 siRNA prepared for this study into colon cancer HCT-116 or HT-29 cells gave rise to a significant suppression of cell viability (Figure 2A), confirming our previous finding (Ueno et al., 2008). This also seems to be consistent with a previous finding that siRNA inhibition of FZD7 decreased the viability of mesenchymal stem cells (hMSCs; Song et al., 2006). However, in contrast to this report, no apoptotic cells were detected in our present experiments as well as in our previous studies (Ueno et al., 2008). Although the mechanism is not fully understood at present, the decrease of G2/M cells (Figure 2B) suggests the involvement of the β-catenin/Tcf target genes c-myc and cyclin-D (He et al., 1998; Tetsu and McCormick, 1999). Our
previous data demonstrated that the expression levels of c-myc and cyclin-D mRNAs increased after FZD7 transfection into HCT-116 cells and a siRNA against FZD7 suppressed c-Myc protein and cyclin-D mRNAs increased after FZD7 transfection into HCT-116 cells. These data may provide a molecular explanation for the migratory activity of colon cancer cells (Vincan et al, 2007) and hepatocellular carcinoma cells (Merle et al, 2004).

We have also demonstrated decreased invasion activity of FZD7-down-regulated HCT-116 cells (Figure 2E). In addition to migratory activity, proteolytic lysis of Matrigel is required for cells to penetrate the membrane in the invasion assay we used. The canonical Wnt pathway may be responsible for this process, because it involves several extracellular proteinases such as urokinase-type plasminogen activator (uPA) (Hiendlmeyer et al, 2004), uPA receptor (Mann et al, 1999), CD44 (Wielenga et al, 2002), matrix metalloproteinase (MMP)-7 (Brabletz et al, 1999) and MT1-MMP/MMP-14 (Takahashi et al, 2002). It is also known that the β-catenin/Tcf target gene fra-1 directly induces MMP-1 and MMP-9 promoter activity (Belguise et al, 2005). Our quantitative RT-PCR data showed that the expression levels of CD44v8-9 and MT1-MMP were decreased after FZD7 siRNA transfection into HCT-116 cells (Figure 2F).

It remains to be determined how non-canonical Wnt signalling interacts with the canonical Wnt/β-catenin/Tcf signalling in colon cancer cells. It was shown that inhibitors of the canonical
Frizzled-7 as a potential therapeutic target in CRC

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Frizzled-7 (FZD7) is a member of the Frizzled family of receptors that mediate Wnt signalling. It has been shown to be involved in the regulation of proliferation, migration, and invasion in cancer cells. However, the clinical significance of FZD7 expression in colorectal cancer (CRC) remains unclear.

**Materials and Methods**

We have detected Wnt11 induction of differentiated cell phenotypes (Yamanaka et al., 2007), osteogenic differentiation of hMSCs (Song et al., 2006), and higher expression of FZD7 in seven colon cancer cell lines (data not shown).

**Figure 4** The expression level of FZD7 mRNA in primary colorectal tumour and non-tumour tissues. (A) Comparison of tumour with non-tumour tissues. The mRNA levels of FZD7 in primary colorectal tumour and non-tumour tissues were examined by real-time PCR. Tumours were divided into four groups according to the pathological stage (see Table 1). (B) Effect of clinical course after surgery on FZD7 expression level. Patients were divided into disease free after surgery and recurrence or death after surgery groups according to the follow-up information after surgery. Disease free indicated a patient group with no recurrence after surgery. Recurrence + Death indicated a patient group with recurrence or death after surgery. (C) Kaplan–Meier analysis for overall survival of patients. High or low indicates the patients with the FZD7 mRNA levels ≥ or < the mean value (11.1) of all tumours tested.

**Results**

We have shown that stable transfectants of HCT-116 cells harbouring the FZD7 siRNA have decreased Tcf activity, viability and invasion (Figures 3B–D), and less in vivo metastatic activity using a liver metastasis model of HCT-116 cells in nude mice (Bouvet et al., 2006). The number of metastatic foci with FZD7 siRNA transfectants was significantly decreased compared to that with control cells (Figure 3E). A major molecular basis for this suppressive effect is thought to be due to the decreased expression of motility- and invasion-related genes, but it is possible that it partly reflects the reduced cell viability. As described above, it was reported that FZD7 promotes cell survival without altering cell proliferation in hMSCs (Song et al., 2006). Furthermore, canonical Wnt signalling was shown to be involved in the regulation of proliferation, as well as the migration/invasion capacity of hMSCs (Neth et al., 2006). In this context, FZD7 might be one of mesenchymal characteristics of colon cancer cells when they metastasise through epithelial–mesenchymal transition (Turley et al., 2008).

**Discussion**

An important finding of this report is the prognostic significance of FZD7 mRNA expression in primary CRC tissues. FZD7 expression was higher in the Recurrence + Death group than in the Disease-free group (Figure 4D), and patients with higher FZD7 expression levels (≥ mean of all cases) had worse overall survival (Figure 4C). However, no association was found between FZD7 expression and clínico-pathological factors except for pathological stage. This may be related to the functional diversity of FZD7 including induction of mesenchymal–epithelial transition (Vincan et al., 2007), osteogenic differentiation of hMSCs (Song et al., 2006) and Wnt11 induction of differentiated cell phenotypes (Yamanaka and Nishida, 2007; Kim et al., 2008). We have detected Wnt11 mRNA in seven colon cancer cell lines (data not shown). Nevertheless, our present clinical data support the importance of FZD7 as a therapeutic target for CRC in those patients with higher FZD7 expression.

In conclusion, we first reported that FZD7 may be important in the survival, invasion and metastatic capabilities of colon cancer cells, at least partly, through expression of c-Jun, phosphorylation of c-Jun and JNK, and activation of RhoA. Furthermore, higher expression levels of FZD7 mRNA in primary CRC tissues were shown to be associated with poor prognosis, suggesting that FZD7 may be involved in CRC progression.

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