Role of Secreted Frizzled-Related Protein 3 in Human Renal Cell Carcinoma

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Abstract

The secreted frizzled-related protein (sFRP) family plays an important role in the inhibition of the Wnt signaling pathway in various cancers. The functional significance of Wnt antagonist sFRP3 has not been investigated in renal cancer. We performed tissue microarray and found that the level of sFRP3 protein was high in normal kidney, low in primary renal cancer tissues, and high in metastatic renal cancer tissues. Therefore, we hypothesized that sFRP3 may play an important role in metastatic renal cancer. To test this hypothesis, we performed a series of experiments to determine the role of sFRP3 using primary and metastatic renal cancer cell lines. Functional analysis showed increased numbers of viable and invaded cells and tube formation and decreased numbers of apoptotic cells in the sFRP3-transfected renal cancer cell line A498. Promotion of tumor growth was also observed in nude mice injected with sFRP3-transfected A498 cells. In contrast, the number of viable cells and invasive cells was decreased in sFRP3 mRNA knockdown metastatic cells (ACHN and Hs891.T). To investigate the mechanism of sFRP3 function, we performed microarray analysis to see which genes were upregulated or downregulated by sFRP3 expression. Among these genes, MMP-3 and ANGPT1 were significantly upregulated in sFRP3-transfected cells. In conclusion, this is the first report to show that sFRP3 expression promotes cell growth, invasion, and inhibition of apoptosis in renal cancer cells. Cancer Res; 70(5); 1896–905. ©2010 AACR.

Introduction

Renal cell carcinoma (RCC) is the third leading cause of death among urologic tumors, accounting for 2% of adult malignancies (1). Although the rate of detection of incidental RCC has increased with improved diagnostic techniques, metastatic lesions are still found at diagnosis in ∼30% of RCC patients (2). Wnt/β-catenin signaling is involved in renal cancer. Canonical Wnt ligands bind to frizzled family receptors and the LRP5/LRP6 coreceptor, which stabilize β-catenin. Subsequently, β-catenin interacts with members of the lymphoid enhancer factor 1/T-cell factor family, resulting in generation of a functional transcription factor complex and the expression of downstream target genes (3, 4). Noncanonical Wnt ligands also bind to frizzled family receptors and ROR2 and RYK coreceptors (4–7). This signaling is mainly involved in cytoskeletal reorganization during cancer cell invasion and metastasis (6, 7). Among Wnt antagonist families, secreted frizzled-related protein (sFRP3) is generally thought to be an inhibitor of Wnt signaling in several cancers (8–10). However, sFRP2, which is also a member of the sFRP family, has been reported previously to be involved in oncogenic function in human glioma cells (11). Because there have been no reports about sFRP3 in renal cancer, we focused on the role of sFRP3 in RCC. Initially, we performed immunohistochemical analysis to clarify whether sFRP3 is expressed in human normal kidney, primary renal cancer, and metastatic renal cancer tissues. We found that the expression level of sFRP3 was decreased in primary renal cancer tissues compared with normal kidney tissues but was restored in metastatic renal cancer tissues. Therefore, we next performed reverse transcription-PCR (RT-PCR) to confirm the mRNA expression of sFRP3 in primary and metastatic renal cancer cells and observed results similar to those of immunohistochemistry. These results suggested that there may be a change in sFRP3 function from that of a tumor suppressor to an oncogene in renal cancer progression and metastasis. Based on these results, we hypothesized that (a) the function of sFRP3 may be changeable during renal cancer progression, (b) overexpression of sFRP3 in primary renal cancer cells may promote cell growth and invasive ability, and (c) underexpression of sFRP3 in metastatic renal cancer cells may inhibit cell growth and invasive ability. To test this hypothesis, we initially evaluated the effect of sFRP3 overexpression on cell viability, colony formation, in vitro invasion, apoptosis, and the cell cycle using sFRP3-transfected primary renal cancer cells (A498). Then, we evaluated the effect of sFRP3 underexpression in metastatic cell lines on cell viability, in vitro invasion, apoptosis, and cell cycle using...
si-sFRP3–transfected metastatic renal cancer cells. In contrast to the results from overexpressing sFRP3 in primary cancer cells, the number of viable and invasive cells decreased in si-sFRP3–transfected metastatic renal cancer cells. The molecular mechanisms involved in sFRP3 function were also examined by microarray analysis to determine which genes were upregulated or downregulated by enhanced sFRP3 expression.

Materials and Methods

**Tissue microarray analysis.** We purchased a kidney tissue microarray from US Biomax. This tissue microarray included 5 normal human kidney tissues, 26 primary human renal cancer tissues, and 5 metastatic renal cancer tissues. Immunostaining of sFRP3 was done in formalin-fixed, paraffin-embedded specimens using rabbit polyclonal antibody against human sFRP3 (sc-13941; Santa Cruz Biotechnology). The staining procedure was according to a commercial kit (Lab Vision). The sections were counterstained with Harris’ hematoxylin, and immunohistochemical staining was evaluated by assessing staining intensity (0-2) using a microscope at ×200. All specimens were scored blindly by two observers. The criteria of intensity were as follows: 0, negative expression; 1+, weakly positive expression; and 2+, strongly positive expression.

**Cell culture.** Primary renal cancer cell lines (A498 and 769-P), metastatic renal cancer cell lines (ACHN and Hs891.T), and a normal kidney cell line (HK2) were purchased from the American Type Culture Collection. The A498, 769-P, ACHN, and Hs891.T cell lines were cultured in RPMI 1640 or DMEM supplemented with 10% fetal bovine serum, 1 mmol/L sodium pyruvate, and 10 mmol/L HEPES. The HK2 cell line was grown in keratinocyte serum-free medium supplemented with 5 ng/mL human recombinant epidermal growth factor and 0.05 mg/mL bovine pituitary extract. Human umbilical vein endothelial cells (HUVEC) were purchased from Genlantis and cultured in endothelial cell growth medium.

**RNA and protein extraction.** RNA was extracted from cell lines using a QIAamp RNA kit (Qiagen). Cells were lysed with a RIPA buffer (Pierce) containing protease inhibitors (Sigma). Protein quantification was done using a BCA protein assay kit (Pierce).

**Plasmid construction.** Plasmids containing the human full-length cDNA fragment of sFRP3 (GenBank accession no. NM_001463) was purchased from Origene. This clone (pCMV6-FRZB) expresses the complete sFRP3 open reading frame with a Tag (MYC/DDK) at the COOH terminal.

**Stable clone establishment.** To prepare stable cell lines overexpressing sFRP3, we transfected A498 cells with the pCMV6-sFRP3 expression vector encoding sFRP3 cDNA using FuGENE HD (Roche Diagnostics) according to the manufacturer’s instructions. Transfected cells were selected by culturing with G418 (150 μg/mL) for 2 months. Single colonies of stably transfected cells were isolated and expanded for further analysis based on the level of sFRP3 expression. Experiments were done with several independent clones to avoid clonal effects.

**Cell viability assay.** SFRP3 stably transfected A498 cells were maintained in medium supplemented with 150 μg/mL G418. Cell viability was measured with MTS (CellTititer 96 Aqueous One Solution Cell Proliferation Assay; Promega). Data are mean ± SD of six independent experiments.

**Soft-agar colony formation assay.** Soft-agar colony formation was assayed with A498 mock cells and A498 sFRP3 stably transfected cells using a Cell Biolabs Cell Transformation Assay kit. Cells were incubated 7 days in a semisolid agar medium before being solubilized and detected by using the provided MTT solution in a microplate reader (A570 nm). The absorbance was compared between mock cells and sFRP3-transfected cells. Data are mean ± SD of 10 independent experiments.

**Cell invasion assay.** Cell invasion assay was done with the CytoSelect 24-well cell invasion assay kit as described previously (Cell Biolabs). Cells were resuspended to the top chamber in triplicate. Cells migrating through the membrane were stained and counted with a microscope. Five random fields were chosen for each membrane, and the results were expressed as migrated cells per field or quantified at A560 nm after extraction.

**Apoptosis and cell cycle analysis.** Cells were trypsinized, washed once in complete medium, centrifuged at 2,000 rpm for 5 min at 4°C, and resuspended in ice-cold 1× binding buffer. Annexin V-FITC solution (10 μL) and 7-amino-actinomycin D viability dye (20 μL) were added to 100 μL of the cell suspensions. After incubation for 15 min in the dark, 400 μL ice-cold 1× binding buffer was added. The apoptotic distribution of the cells in each sample was then determined using fluorescence-activated cell sorting (Cell Lab Quanta SC, Beckman Coulter). The various cell phases were determined using a DNA stain (4′,6-diamidino-2-phenylindole). Cell populations (G0-G1, S, and G2-M) were measured using fluorescence and contrasted against cell volume. Data are mean ± SD of four independent experiments.

**Angiogenesis assay.** Angiogenesis involves the very important process of generating new blood vessels, and the principal cells involved in angiogenesis are endothelial cells. The relationship between sFRP3 and angiogenesis is unknown. Therefore, in vitro angiogenesis was assessed with sFRP3 or empty vector–transfected A498 cells and endothelial cells using a Cell Biolabs endothelial tube formation assay kit (Cell Biolabs). The ability of endothelial cells to form three-dimensional capillary-like tubular structures was assessed and compared between stable sFRP3-transfected and empty vector–transfected A498 cells (12). At first, 96-well cell culture plates were coated with extracellular matrix gels, and sFRP3-transfected empty vector A498 cells were added (2 × 10^4 per well). We also prepared wells containing A498 regular medium and extracellular matrix gels to see the effect of regular medium. The extracellular matrix gels with A498 cells or regular medium were mixed simultaneously incubated for 1 h at 37°C. After solidifying, HUVECs were added (2 × 10^4 per well) on top of the extracellular matrix gels. Then, endothelial cells and A498 cells (or regular medium only) were incubated for 18 h, and endothelial tube formation was assessed using a light microscope at high magnification.
**In vivo** study. Groups of six female nude mice (strain BALB/c nude; Charles River Laboratories), 4 to 5 weeks old, received s.c. injections of $1 \times 10^7$ mock or sFRP3-transfected A498 cells in the right flank area in a volume of 200 μL. Tumor size was determined using calipers once per week for 6 weeks, and tumor volume was calculated based on width ($x$) and length ($y$): $x^2y/2$, where $x < y$. After the mice were killed, tumors were resected and weighted. Total RNA was extracted from tumor tissues for RT-PCR analysis. In addition, tumor tissues were fixed in 10% formalin, embedded in paraffin, and stained with H&E for histological examination. Animal experiments were approved by the Animal Studies Subcommittee of the Veterans Affairs Medical Center (protocol no. 08-003-01).

**Knockdown of sFRP3 mRNA in metastatic renal cancer cells.** Metastatic renal cancer cells (ACHN and Hs891.T) were transfected with sFRP3 small interfering RNA (siRNA; si-sFRP3; Invitrogen) or negative control siRNA (si-NC; Invitrogen) according to the manufacturer’s instructions. Briefly, cells were grown in six-well plates and transfected with the siRNAs [HSS103831 (No. 1), HSS103832 (No. 2), and HSS177637 (No. 3)] at a concentration of 200 pmol/well. Transfection was done with X-tremeGene siRNA Transfection Reagent (Roche Diagnostics).

**cDNA microarray and quantitative real-time RT-PCR in cell lines.** We profiled cell cycle, apoptosis, and invasion-related genes with the Human RT² Profiler PCR Array. The RT² first-strand kit, which includes a proprietary buffer to eliminate any residual genomic DNA contamination in cell line RNA samples, was used to convert mRNA into cDNA. The manufacturer’s instructions were strictly followed. To analyze the PCR array data, we downloaded a MS Excel sheet from the manufacturer’s Web site. The PCR Array Data Analysis Web Portal automatically performed several calculations as follows: scatter plots, three-dimensional profiles, and a volcano plot. Based on the scatter plot data (relative expression comparison between mock and sFRP3-transfected cells), we selected candidate genes whose expression levels were increased or decreased in sFRP3-transfected cells compared with mock cells (fold cutoff was 3.5). Data were normalized based on correcting all Ct (threshold cycle) values for the average Ct values of several constantly expressed housekeeping genes present on the array. Quantitative real-time RT-PCR was done to confirm the results of microarray analysis in triplicate with an Applied Biosystems Prism 7500 Fast Sequence Detection System using

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*Figure 1. Tissue microarray showing sFRP3 expression in normal kidney, primary renal cancer, and metastatic renal cancer tissues. Representative immunostaining of sFRP3 in human normal kidney (A), primary RCC (clear cell carcinoma) samples (B), and metastatic renal cancer tissues (C). Percentage of tissue microarray samples expressing sFRP3 (D).*
TaqMan universal PCR master mix according to the manufacturer’s protocol (Applied Biosystems). TaqMan probes and primers were purchased from Applied Biosystems. Human glyceraldehyde-3-phosphate dehydrogenase was used as an endogenous control. Levels of mRNA expression were determined using the 7500 Fast System SDS software version 1.3.1 (Applied Biosystems).

Western blotting. Total protein (20 μg) was used for Western blotting. Samples were resolved in 4% to 20% Precise Protein Gels (Pierce) and transferred to polyvinylidene difluoride membranes (Amersham Biosciences). The membranes were immersed in 0.3% skim milk in TBS containing 0.1% Tween 20 for 1 h and probed with primary polyclonal and monoclonal antibodies against glyceraldehyde-3-phosphate dehydrogenase (Cell Signaling), matrix metalloproteinase (MMP)-3 (Abcam), and angiopoetin (ANGPT)-1 (Abcam) overnight at 4°C. To confirm sFRP3 stably transfected cells, we used anti-sFRP3 antibody (Sigma-Aldrich). Blots were washed in TBS containing 0.1% Tween 20 and labeled with horseradish peroxidase–conjugated secondary anti-mouse or anti-rabbit antibody (Cell Signaling). Proteins were enhanced by chemiluminescence (Amersham ECL Plus Western Blotting Detection System) for visualization. Protein expression levels were expressed relative to glyceraldehyde-3-phosphate dehydrogenase levels.

Statistical analysis. All statistical analyses were done using StatView version 5 (SAS Institute). P < 0.05 was regarded as statistically significant.

Results

Immunohistochemical analysis using tissue microarray including normal kidney, primary renal cancer, and metastatic renal cancer tissues. We compared sFRP3 protein expression levels between normal kidney, primary renal cancer, and metastatic renal cancer tissues using tissue microarray. The percentage of samples expressing sFRP3 was lower in primary cancer tissues (23% of 23 samples) compared with normal kidney tissues (100% of 5 samples). However, the percentage of samples expressing sFRP3 was significantly higher in metastatic renal cancer tissues (100% of 5 samples) compared with primary renal cancer tissues (Fig. 1). The detailed immunohistochemistry data are shown in Supplementary Table S1.

sFRP3 mRNA and protein expression levels in renal cancer cell lines and normal kidney cells. We compared sFRP3 mRNA and protein expression levels in a normal kidney cell line (HK2), primary renal cancer cells (A498 and 769-P), and metastatic renal cancer cell lines (ACHN and Hs891.T). Expression of sFRP3 was lowest in primary renal cancer cell

![Graph A](image1.png)

*P < 0.01 (HK2 vs primary renal cancer cell lines)

**P < 0.01 (HK2 vs metastatic renal cancer cell lines)

![Graph B](image2.png)

Figure 2. sFRP3 mRNA and protein expression in normal kidney (HK2), primary renal cancer (A498 and 719-P), and metastatic renal cancer (ACHN and Hs891.T) cell lines. mRNA (A) and protein (B) expression of sFRP3 was lower in primary renal cancer cell lines (A498 and 769-P) but was significantly higher in metastatic renal cancer cell lines (ACHN and Hs891.T) compared with primary renal cancer cells and normal kidney cells.
lines (A498 and 769-P) and highest in metastatic cell lines (ACHN and Hs891.T) compared with primary renal cancer cells and normal kidney cells (Fig. 2).

**sFRP3 expression level in sFRP3-transfected A498 cells and mock cells.** Four independent sFRP3 stably transfected A498 clones were established, and the levels of sFRP3 mRNA and protein expression were confirmed (Fig. 3A).

**Effect of sFRP3 on cell viability, colony formation, and cell invasion.** We performed cell viability analysis (MTS assay), colony formation assay, and cell invasion assay using stably transfected A498 cells that overexpressed sFRP3. At day 2, the number of viable cells (Fig. 3B), the number of colonies (Fig. 3C), and the *in vitro* invasion ability was significantly increased in sFRP3 stably transfected cells (Fig. 3D) compared with mock-transfected controls.

**Apoptosis and cell cycle analyses.** Apoptosis and cell cycle analysis was carried out to investigate whether sFRP3 transfection affected apoptosis and the cell cycle in A498 renal cancer cells. Compared with empty vector cells, the population of apoptotic cells was significantly lower (Fig. 4A) and the cell population in the G0-G1 phase was significantly decreased in sFRP3-transfected cells. In contrast, the population of S- and G2-phase cells was significantly increased in sFRP3-transfected cells (Fig. 4B).

**Angiogenesis assay.** To monitor whether sFRP3 stimulates angiogenesis, we performed an *in vitro* angiogenesis assay and observed tube formation in sFRP3-transfected A498 cells and HUVECs but not in those transfected with empty vector and or when regular medium and HUVECs were used (Fig. 4C).

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**Figure 3.** Characterization of sFRP3 stable transfectants and comparison of cell viability and invasion between sFRP3- and mock-transfected A498 cells. A, four independent sFRP3 stably transfected A498 clones were established, and levels of sFRP3 mRNA and protein expression were confirmed. B, cell viability assay in mock and sFRP3 transfectants. C, colony-forming assay in mock and sFRP3 transfectants. D, invasion assay in mock- and sFRP3-transfected A498 cells.
In vivo study. A498 cells stably transfected with empty vector or the sFRP3 gene were injected s.c. into the right flank of nude mice. Tumor growth was significantly increased in mice with sFRP3-transfected cells compared with those with empty vector (Fig. 4D).

siRNA transfection, MTS, invasion, and fluorescence-activated cell sorting in metastatic renal cancer cells. SFRP3 mRNA expression was high in metastatic renal cancer cell lines (ACHN and HS891.T) compared with normal and primary renal cancer cells. To assess the function of SFRP3 in metastatic renal cancer cells, we knocked down SFRP3 mRNA expression in ACHN and HS891.T cells using siRNA (Fig. 5A). We used three different si-sFRP3 RNAs (Nos. 1-3), and si-sFRP3 RNA (No. 3) was most effective in decreasing the expression of SFRP3 in ACHN cells. Therefore, we used si-sFRP3 (No. 3) for further experiments with ACHN and HS891.T cells (MTS, invasion, and fluorescence-activated cell sorting; Fig. 5B and C). We found inhibition of cell growth in si-sFRP3–transfected cells by MTS assay, and si-sFRP3 also inhibited the in vitro invasion ability of ACHN and HS891.T cells. However, there were no significant differences in apoptosis and the cell cycle between si-NC and si-sFRP3–transfected cells (data not shown).

cDNA microarray, quantitative real-time RT-PCR in mock and sFRP3-transfected cells, and Western blotting. Promotion of cell growth, decreased apoptosis, and increased invasive ability were observed with sFRP3-transfected cells, suggesting that SFRP3 affects these functions in renal cancer cells. Therefore, we focused our study on apoptosis, cell cycle, and invasion-related genes and profiled them with the Human RT2 Profiler PCR Array. Three cDNA microarrays were used in this study (87 genes per microarray for a total of 261 genes; Human apoptosis, Human cancer PathwayFinder, and Tumor metastasis arrays). Clustergrams are shown in Supplementary Fig. S1. Those genes found to be differentially expressed were clustered using hierarchical cluster analysis to visualize the pattern of gene expression for each group. In the heat map, red represents highly expressed genes, whereas green represents underexpressed genes. The black color on the heat map represents equal gene expression relative to

Figure 4. Comparison of apoptosis, cell cycle, tube formation, and in vivo growth between sFRP3-transfected and empty vector–transfected A498 cells. A, flow cytometry analysis of apoptosis in mock and sFRP3 stably transfected A498 cells. Annexin V-FITC and 7-amino-actinomycin D were measured by flow cytometry. Representative results. B, cell cycle analysis in mock and sFRP3 stably transfected A498 cells. Detection of the various cell phases was determined using a DNA stain (4′,6-diamidino-2-phenylindole). G0–G1, S, and G2–M populations were measured by fluorescence and contrasted against cell volume. Mean ± SD of four independent experiments. C, tube formation using HUVECs. D, assessment of tumor growth in nude mice with empty vector–transfected and sFRP3-transfected cells. Representative pictures of nude mice with sFRP3 and empty vector–transfected cells are shown.
the control. Based on these microarray data, 34 genes were identified as being either upregulated or downregulated in sFRP3-transfected cells compared with mock cells in the three microarrays (Supplementary Fig. S1). Among the identified candidate genes (threshold of 3.5), mRNA and protein levels of MMP-3 and ANGPT1 were significantly upregulated in sFRP3-transfected A498 cells compared with mock cells (Fig. 6).

Discussion

The sFRP family plays an important role in inhibition of the Wnt signaling pathway, and most sFRP expression is silenced epigenetically in various cancers (13–19). However, sFRP3 has no CpG islands in its DNA promoter region, and there have been no reports about the relationship between sFRP3 expression and epigenetic silencing. sFRP3 has been shown to be involved in the inhibition of the Wnt signaling pathway in prostate cancer and fibrosarcoma (9, 10), but there have been no reports about its role in renal cancer. According to previous reports, sFRP2, a sFRP family member, strongly promoted the growth of intracranial glioma xenografts in nude mice (11). Also, sFRP2 has been reported to be upregulated in the vasculature of human breast cancer and stimulates angiogenesis (20, 21). Recently, our laboratory found that sFRP1 depletion decreased the invasive potential of the metastatic renal cancer cell lines and showed that overexpression of sFRP1 may be related to invasiveness and metastatic behavior in renal cancer (22). Therefore, we initially investigated sFRP3 protein levels in human normal kidney, primary renal cancer, and metastatic renal cancer tissues. We observed that the expression level of sFRP3 protein was decreased in primary renal cancer tissues compared with normal kidney tissues. However, the expression of sFRP3 was restored in metastatic renal cancer tissues. We also found that sFRP3 mRNA expression was low in primary renal cancer cell lines and high in metastatic renal cancer cell lines. These results suggested that sFRP3 may change function from a tumor suppressor gene to an oncogene in renal cancer progression and metastasis. To test this hypothesis, we used dual sFRP3 mRNA expression experiments to determine the role of sFRP3 in primary renal cancer cell lines and metastatic renal cancer cell lines. Initially, we established sFRP3-overexpressing stable cell lines using a primary renal cancer cell line (A498) because the expression of sFRP3 was low in primary renal cancer cell lines compared with the normal kidney cell line (HK2). We then performed functional analysis including cell viability, colony formation, cell invasion, apoptosis, and cell cycle assays. In sFRP3 stably transfected
A498 cells, the number of viable cells or colonies after culture was significantly increased compared with mock-transfected cells. In addition, the number of cells in the apoptosis and G0-G1 phase was significantly decreased after sFRP3 transfection. We also assessed the effect of sFRP3 expression on tumor growth in nude mice using sFRP3 and mock-transfected A498 cells. Tumor growth in mice was dramatically increased in sFRP3-transfected cells compared with mock cells. Invaded cells also significantly increased in sFRP3-transfected cells.

Next, we knocked down sFRP3 expression in metastatic renal cancer cell lines (ACHN and Hs891.T) because the expression level of metastatic renal cancer cell lines was significantly higher compared with normal kidney and primary renal cancer cell lines. Then, we performed functional assays similar to those described above. In si-sFRP3–transfected metastatic renal cancer cell lines, the number of viable cells was significantly decreased compared with si-NC cells. In addition, invaded cells were decreased in si-sFRP3–transfected metastatic renal cancer cells. These results suggest that there may be a change in sFRP3 function from a tumor suppressor gene to oncogene during renal cancer progression so that high sFRP3 expression may induce tumor invasion and metastasis.

To investigate the mechanism of sFRP3 function in sFRP3-overexpressing cells, we used microarray to screen for cell cycle, apoptosis, and invasion-related genes and found altered expression of several genes compared with mock control cells. MMPs are usually induced in cancer tissues, and increased MMP expression is predictive of tumor aggressiveness and invasive ability in several cancers (23). mRNA and protein levels of MMP-3 were also increased in sFRP3-transfected cells. MMP-3 is known to be expressed in renal cancer and has been identified as playing a role in renal cancer progression (24). Therefore, the present results may suggest that sFRP3 is involved in renal cancer invasion and progression via MMP-3.

In cancer progression and invasion, angiogenesis is an important process consisting of extracellular matrix remodeling, endothelial cell proliferation, and new blood vessel formation. In the angiogenesis pathway, vascular endothelial growth factor is well known as a predictor of invasion in renal cancer (25). However, we did not observe a significant change in vascular endothelial growth factor expression in sFRP3-transfected cells (data not shown). The Tie2/angiopoietin system also plays an important role in angiogenesis in cancer cells (26). Angiopoietin consists of ligands of Tie2.
and the family contains ANGPT1, ANGPT2, and ANGPT4 (26). Based on the microarray data, we observed that expression of ANGPT1 was significantly increased in sFRP3-transfected cells but not that of ANGPT2 or ANGPT4. These data were confirmed with real-time RT-PCR and Western blotting. We also found that tube formation was increased with HUVECs plus sFRP3-transfected A498 cells. It was recently revealed that expression of DKK4, a Wnt antagonist, increased migration, invasion, and the tube-forming properties of colon cancers (27). Moreover, Baldeuwijns and colleagues reported that high-grade renal clear cell carcinoma had high angiogenic activity, and Yamakawa and colleagues reported that the angiopoietin/Tie-2 system may participate in tumor angiogenesis in renal carcinoma (28, 29). We also found that ANGPT1 mRNA expression was significantly decreased in si-sFRP3-transfected metastatic renal cancer cell lines (ACHN and Hs891.T; data not shown). However, we did not observe ANGPT2 and ANGPT4 mRNA expression in renal cancer metastatic cells (data not shown). Also, there was no change in Tie-2 mRNA in si-NC and si-sFRP3-transfected ACHN cells (data not shown).

Taken together, these data suggest that sFRP3 may be associated with angiogenesis in renal cancer via ANGPT1. However, additional studies using an in vivo metastatic model will be required to reveal the exact molecular mechanism of how sFRP3 regulates ANGPT1 expression. Therefore, these findings may be very important for future studies on the mechanism of renal cancer invasion and metastasis.

In conclusion, this is the first report to show that sFRP3 expression promotes cell growth, invasion, and inhibition of apoptosis in renal cancer cells. Furthermore, our results suggest that sFRP3 may play a role in invasion and angiogenesis by enhancing MMP-3 and ANGPT1 expression. The changes in sFRP3 expression levels in normal, primary cancer, and metastatic renal cancer tissues suggest that sFRP3 may play dual roles as a tumor suppressor gene and an oncogene during renal cancer progression.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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