Expression of Frzb/Secreted Frizzled-Related Protein 3, a Secreted Wnt Antagonist, in Human Androgen-Independent Prostate Cancer PC-3 Cells Suppresses Tumor Growth and Cellular Invasiveness

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Abstract

The ability of Frzb/secreted Frizzled-related protein 3 (sFRP3) to inhibit Wnt signaling and the localization of Frzb/sFRP3 on chromosome 2q to a region frequently deleted in cancers has led some investigators to hypothesize that Frzb/sFRP3 is a tumor suppressor gene. Here, we examined the biological effects of Frzb/sFRP3 on an androgen-independent prostate cancer cell model. We showed that expression of Frzb/sFRP3 in PC-3 cells resulted in decreased colony formation in soft agar and a dramatic inhibition of tumor growth in a xenograft mouse model. When cellular morphology was examined, PC-3 cells expressing Frzb/sFRP3 exhibited an increase in cell-cell contact formation accompanied by a pronounced induction of epithelial markers E-cadherin and keratin-8 and down-regulation of mesenchymal markers N-cadherin, fibronectin, and vimentin. This phenomenon suggested a reversal of epithelial-to-mesenchymal transition and a less invasive phenotype. Indeed, further in vitro studies with a Matrigel assay showed that Frzb/sFRP3 decreased the invasive capacity of PC-3 cells. These changes in the biology of PC-3 cells are associated with a decrease in the expression and activities of both matrix metalloproteinase (MMP)-2 and MMP-9 as well as decreases in AKT activation, expression and phosphorylation of β-catenin, and transcription activity, and expression of Slug and Twist. In addition, transfection of PC-3 with a dominant-negative low-density lipoprotein receptor–related protein 5 (DN-LRP5) coreceptor showed similar biological effects as Frzb/sFRP3 transfection. Together, these data suggest that Frzb/sFRP3 and DN-LRP5 exhibit antitumor activity through the reversal of epithelial-to-mesenchymal transition and inhibition of MMP activities in a subset of prostate cancer.

Introduction

Understanding the biological basis of cancer is one of the most effective methods for developing safe and effective prevention and treatment strategies. Given that each prostate cancer diagnostic type represents a heterogeneous group of lesions (1), multiple growth and survival characteristics likely contribute to the carcinogenesis and neoplastic progression of this disease. Activation of the Wnt/β-catenin pathway has been observed in a portion of prostate cancer patients (2, 3), and this pathway has been shown to modulate androgen action in the prostate (4, 5), implying that alterations in Wnt signaling may influence prostate tumor biology. Wnts, a family of secreted cysteine-rich glycoproteins, act as ligands to activate Frizzled receptor–mediated signaling pathways (6). During normal development, cells respond to Wnts in a context-dependent fashion by undergoing changes in cell proliferation, patterning, fate determination, and movement (6). In adults, aberrant activation of Wnt signaling has been reported to be involved in tumorogenesis (6). Wnt ligands seem to activate one or more intracellular signaling pathways depending on the type of ligands, Frizzled receptors, and cells (6). The best-studied Wnt signaling pathway is the Wnt/β-catenin pathway, in which Wnt ligands form a complex with Frizzled receptor and coreceptor low-density lipoprotein receptor–related protein 5 (LRP5) or LRP6 (6, 7). Receptor activation inhibits the adenomatous polyposis coli-Axin "destruction complex," where β-catenin is phosphorylated by both casein kinase 1 and glycogen synthase kinase-3β (GSK-3β) and targeted for ubiquitination by βTrCP or Siah leading to degradation in the 26S proteasome (7). This inhibition results in cytoplasmic β-catenin stabilization and facilitates its translocation into the nucleus (6, 7). In the nucleus, β-catenin relieves inhibition of transcription factors T-cell factor-dependent Wnt-dependent pathways leading to changes in cell movement and polarity (8).

Secreted Wnt antagonists, classified as secreted Frizzled-related protein (sFRP) family, Dickkopf family, and Wnt inhibitory factor-1, are potential negative modulators of Wnt signaling (9). The sFRPs contain a cysteine-rich domain (CRD), highly homologous to the extracellular, ligand-binding domain of Frizzled receptors (9). sFRPs have been shown to inhibit Wnt signaling either by sequestering Wnt ligands or by forming nonfunctional complexes with Frizzled receptors (9). Recently, down-regulation of sFRPs by gene deletion or promoter hypermethylation has been shown in many human cancers (10–15).

Frzb/sFRP3, the first member of the sFRP family, was isolated as a chondrogenic factor in developing cartilage (16). Frzb/sFRP3 binds to both Wnt-8 and Wnt-1 and acts as a functional inhibitor of Wnt-8 activity in Xenopus embryos (17, 18). Human Frzb/sFRP3 has recently mapped to human chromosome 2q31-33 (19). Deletions of chromosome 2q occur in prostate carcinoma (20, 21).
One study reported loss of heterozygosity at 2q32-q36 in 42% (6 of 14) of prostate carcinomas (21). Allelic loss at chromosome 2q has been observed at high frequency in advanced disease in contrast to much lower rates in early-stage tumor specimens from patients with gastric cancer (22), papillary bladder cancer (23), and non–small cell lung carcinoma (24). Moreover, mortality in patients with head and neck cancer was reported to strongly correlate with loss of heterozygosity on chromosome 2q (25). These data suggest that inactivation of one or more tumor suppressors on chromosome 2q may result in aggressive behavior of various human malignancies. Given the oncogenic properties of certain Wnts and the frequent loss of chromosome 2q in human cancers, Frzb/sFRP3 has been hypothesized by some investigators to act as a tumor suppressor (17–19). Here, we examined the biological effects of Frzb/sFRP3 on prostate cancer using an androgen-independent cellular model. Ectopic expression of Frzb/sFRP3 in PC-3 cells resulted in inhibition of colony formation and loss of tumorigenicity in nude mice as well as a decrease in cellular invasiveness. These effects of Frzb/sFRP3 are associated with a dramatic induction of E-cadherin and keratin-8, down-regulation of N-cadherin, fibronectin, and vimentin, and inhibition of the activities and expression of both MMP-2 and MMP-9.

Materials and Methods

Cell culture, plasmid, and stable transfection. PC-3, 22RV1, LNCaP and DU145 cells were obtained from American Type Culture Collection (Manassas, VA) and maintained in RPMI supplemented with 10% fetal bovine serum (FBS) and antibiotics. Primary prostate epithelial cells came from Cambrex Bioscience (Walkersville, MD) and were grown in prostate epithelial basal medium. The PCDNA3.1, containing a full-length 2.4-kb BamHI-Xhol fragment of bovine Frzb/sFRP3, was a generous gift of Frank P. Luyten (Universitaire Ziekenhuizen, Leuven, Belgium). Dominant-negative (DN) LRPS was a generous gift of Dr. Matthew Warman (Case Western Reserve University, Cleveland, OH). DN-TCF4 was from Dr. T.C. He (University of Chicago Medical Center, Chicago, IL). DN-LRPS is a secreted form of LRPS that lacks the transmembrane and cytoplasmic domains (GM ref. 26). Frzb/sFRP3 and DN-TCF4 were described previously in detail (16, 27). The LEF1 expression construct FL9B, the TCF4 luciferase reporter (TOPFLASH), and a mutated control reporter (TOPFLASH) were from Dr. Marian Waterman (University of California, Irvine, CA). DN-LEF1 was constructed as follows: A neomycin/kanamycin resistance expression cassette was PCR amplified from pCMV-Script using primers 5’-CACCC-GGTGCACTTAAATGCGCCG-3’ and 5’-AGTTCGCTAGGTCTGTCG-3’. The product was restriction digested with ApaI and cloned into an ApaI-digested FL9B to generate the FL9B-Neo construct. Oligonucleotides 5’-GATCCACGGACACGAG-3’ and 5’-GTTGTCAGCTGTCG-3’ were annealed and ligated into DraIII/BamHI-digested FL9B-Neo to generate the DN-LEF1 construct used in these studies. For stable transfection, PC-3 cells were plated at 1 × 10^5 per 100-mm dish. At 60% confluency, cultures were transfected with PCDNA3.1, Frzb/sFRP3, DN-LRPS, DN-LEF1, or DN-TCF4 using FuGENE 6 (Roche, Indianapolis, IN). Transfected cells were selected with G418 (800 μg/mL) starting at 48 hours after transfection, and all of the stable transfectants were allowed to migrate through a porous, uncoated membrane (BD Biosciences) or a Matrigel (BD Biosciences) coated membrane and probed with antibodies against β-catenin (Upstate Biotechnology, Charlottesville, VA), E-cadherin, P-cadherin, and N-cadherin (BD Biosciences, San Diego, CA), keratin-8, keratin-18, fibronectin, and vimentin (Lab Vision, Fremont, CA), GSK-3β and phospho-GSK-3β (Cell Signaling, Beverly, MA), LRPS (Oribigen, San Diego, CA), e-myc (Calbiochem, San Diego, CA), cyclin D1, Slug, Twist, FRP3 (sFRP3/Frzb), and β-actin (loading control; Santa Cruz Biotechnology, Santa Cruz, CA). Proteins were revealed using secondary antibodies and visualized by an enhanced chemiluminescence detection system (Amersham Bioscience, Piscataway, NJ).

Immunocytofluorescence assay. Cells were cultured in chamber slides (Lab-Tek). After methanol fixation and permeabilization with Triton X-100, cells were incubated with anti-E-cadherin antibody (BD Biosciences), an anti-FLAG antibody (Invitrogen), or an anti-MYC antibody (Cell Signaling) and then with an Alexa 488–conjugated secondary antibody (Molecular Probes, Inc., Eugene, OR). Localizations of E-cadherin, FLAG-tagged Frzb/sFRP3, MYC-tagged DN-LRPS, and DN-TCF4 were analyzed under confocal microscopy (Zeiss, Thornwood, NY) using the 488 nm excitation wavelengths of the laser.

Real-time PCR. RNA isolation and real-time PCR methods were described in Supplementary Fig. S1A legend. Real-time PCR for quantitation of the mRNA levels of E-cadherin, Twist, and Slug was done using a Bio-Rad MyIQ thermocycler (Bio-Rad, Hercules, CA) and primer sets listed in Supplementary Table S1. Data were analyzed by using the comparative Ct method (29), where Ct is the cycle number at which fluorescence first exceeds the threshold. The C\text{t} \text{ values from each sample were obtained by subtracting the values for β-actin} C\text{t} \text{ from the E-cadherin} C\text{t}, \text{ Twist} C\text{t}, \text{ or Slug} C\text{t} \text{ value. The variation of β-actin} C\text{t} \text{ values was <0.5 among different samples. One difference of } C\text{t} \text{ value represents a 2-fold difference in the level of mRNA. Specificity of resulting PCR products was confirmed by melting curves.}

Matrigel invasion assay. To assay cell motility, 2.5 × 10^4 cells per well in serum-free RPMI were placed in the upper chamber. RPMI plus 10% FBS was placed in the lower chamber as a source of chemotacticant. Cells were allowed to migrate through a porous, uncoated membrane (BD Biosciences) for 24 hours at 37°C. Nonmigratory cells in the upper chamber then were removed with a cotton-tip applicator. Migrated cells on the lower surface were fixed with methanol and stained with hematoxylin. The number of cells migrated through an uncoated membrane was calculated as follows:

No. of cells migrated through a Matrigel – coated membrane No. of cells migrated through an uncoated (control) membrane × 100

All of the cell lines were assayed in triplicate for each experiment, and each experiment was repeated thrice.
Gelatin zymography. Samples were applied to nondenaturing 10% polyacrylamide gels containing 1 mg/mL gelatin. After electrophoresis, the gels were washed with 2.5% Triton X-100, incubated overnight at 37°C in zymography buffer, and stained with Coomassie brilliant blue. Gelatinolytic activity was visualized as clear areas of lysis in the gel.

Soft agar colony formation. A soft agar colony formation assay was done using six-well plates. Each well contained 2 mL of 0.5% agar in complete medium as the bottom layer, 1 mL of 0.38% agar in complete medium and 3,000 cells as the feeder layer, and 1 mL complete medium as the top layer. Cultures were maintained under standard culture conditions. The number of colonies was determined with an inverted phase-contrast microscope at ×100 magnification; a group of >10 cells was counted as a colony. The data are means ± SE of four independent wells at optimum time of 14 days after the start of cell seeding.

In vivo tumor model. NCR-scid/scid (nude) mice were obtained from Taconic (Germantown, NY). Cells from each stable line were concentrated to 2 × 10^6 per 200 μL and injected s.c. into the left flank of each mouse. Once xenografts became established, their sizes were measured every 4 days. The tumor volume was calculated by the formula: 0.5236L1L2^3/3, where L1 is the long axis and L2 is the short axis of the tumor. All of the animal studies were approved by the Institutional Animal Care and Use Committee at University of California (Irvine, CA).

Statistics. Comparisons of cell density, number of colonies, invasion index, relative levels of mRNA expression, and relative levels of protein expression between the different transfections were conducted using Student’s t test. For tumor growth experiments, repeated-measures ANOVA was used to examine the differences in tumor sizes among different transfections, time points, and transfection-time interactions. Additional post-test was done to examine the differences in tumor sizes between vector control and other transfections at each time point by using conservative Bonferroni method. All statistical tests were two sided. P < 0.05 was considered statistically significant.

Results

Frzb/secreted Frizzled-related protein 3 specifically decreases anchorage-independent growth and increases anchorage-dependent growth of PC-3 cells. Compared with other prostate epithelial cell lines, the expression of the LRP5 coreceptor (required for canonical Wnt signaling) was significantly up-regulated in PC-3 cells (Fig. 1A, top). In addition, Frzb/sFRP3 protein expression was detected as a doublet only in the normal prostate epithelial cell line (PrEC) but absent in prostate cancer cell lines (22RV1, LNCaP, DU145, and PC-3). This doublet band of Frzb/sFRP3 may be a result of glycosylation (16, 17). As shown by real-time PCR, PC-3 cells also lack expression of several other secreted Wnt antagonists (Supplementary Fig. S1A). In a microarray data set generated by Dhnasekaran et al. (30), down-regulation of Frzb mRNA (cDNA clone IMAGE:1400711, Frizzled-related protein) was found in bone metastatic prostate cancer specimens compared with commercial prostate reference pool, benign prostatic hyperplasia, and prostate-titis. Together, these data lead us to select PC-3 cells (derived from bone metastatic prostate cancer) as a model to examine the biological effects of Frzb/sFRP3 or disruption of LRP5-mediated signaling on prostate cancer.

We stably transfected PC-3 cells with either Frzb/sFRP3 or DN-LRP5 or PCDNA3.1 control vectors. The expression of Frzb/sFRP3 or DN-LRP5 in stable clones was confirmed by Western blot analysis of FLAG and MYC tag protein in both conditioned medium and cell lysates (Fig. 1B). The concentration of Frzb/sFRP3 in the conditioned medium was estimated to be ~15 ng/mL (by comparison with known concentration of recombinant sFRP3). Immunocytofluorescence staining of Frzb/sFRP3 transfected cells showed that Frzb/sFRP3 was localized in the membrane and cytoplasm (Supplementary Fig. S1B). By immunocytofluorescence, we estimated the expression of Frzb, DN-LRP5, and DN-TCF4 transgenes to be ~70% to 80% positive (data not shown). We were unable to estimate the percentage expression of DN-LEF1 transgene due to lack of a tag protein in this construct.

Figure 1 C and D showed that expression of Frzb/sFRP3 inhibited the anchorage-independent growth of PC-3 cells. For control transfectants, 63 ± 3.5 (mean ± SE of four wells) colonies...
per field were counted compared with seven colonies per field counted in Frzb/sFRP3 transfectants (Fig. 1D). Compared with control cells, transfection of Frzb/sFRP3 and DN-LRP5 results in 89% and 95% inhibition of colony formation, respectively (Fig. 1D; Student’s t test, P < 0.01). For anchorage-dependent growth of each transfected cell line measured by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay, Supplementary Fig. S1C showed that PC-3 cells transfected with Frzb/sFRP3 and DN-LRP5 exhibited a 1.77- and 3.57-fold higher rate of growth than PC-3 cells transfected with PCDNA3.1, respectively, after 5 days of cell seeding (growth was expressed relative to control cells where day 1 equals 100%; Student’s t test, P < 0.05). There was no significant difference in cell growth rate between PCDNA3.1 transfectants and parental PC-3 cells (data not shown). We were also unable to detect any poly(ADP-ribose) polymerase cleavage (a hallmark for apoptosis) in any transfected cell lines, suggesting that transfections did not cause apoptosis in PC-3 cells (data not shown).

**Frzb/secreted Frizzled-related protein 3 inhibits tumor growth in a xenograft mouse model.** We next examined the in vivo effect of Frzb/sFRP3 and DN-LRP5 by inoculation of $1 \times 10^5$ PC-3 cells (transfected with Frzb/sFRP3, DN-LRP5, or PCDNA3.1) into the left flank of nude mice. Frzb/sFRP3 and DN-LRP5 transfectants initially formed small tumors, which disappeared over time. In contrast, parental PC-3 cells and PCDNA3.1 transfectants exhibited rapid tumor growth over time (each group contains 10 mice; ANOVA test, P < 0.01; Fig. 2). The regressed tumors from groups of Frzb/sFRP3 and DN-LRP5 transfectants did not recur during a 30-day observation period after sacrifice of the control mice.

**Frzb/secreted Frizzled-related protein 3 induces expression of epithelial markers and down-regulates mesenchymal markers in PC-3 cells,** suggesting a reversal of epithelial-to-mesenchymal transition. When cell morphology was examined, PC-3 cells expressing Frzb/sFRP3 were more compact and adherent to adjacent cells than PC-3 cells expressing PCDNA3.1, as seen in Fig. 3A. This change to a more “adhesive” cellular morphology resembles a trans-sition from a fibroblastic to an epithelial appearance. Figure 3A also shows that DN-LRP5 results in a similar morphology to Frzb/sFRP3 transfection.

Cells undergoing epithelial-to-mesenchymal transition are characterized by a loss of epithelial cell adhesion and cytoskeleton components and acquisition of mesenchymal components (31). We next examined the effect of Frzb/sFRP3 and DN-LRP5 transfections on the expression of epithelial marker (e.g., E-cadherin, keratin-8, and keratin-18) and mesenchymal markers (e.g., N-cadherin, vimentin, and fibronectin). Consistent with the changes in morphology, transfection of Frzb/sFRP3 and DN-LRP5 in PC-3 cells caused a dramatic induction of E-cadherin and keratin-8 and down-regulation of N-cadherin, vimentin, and fibronectin (Fig. 3B). P-cadherin expression was not detectable by Western blot in either control or Wnt antagonist-transfected cells (data not shown). Together, these results suggest a reversal of epithelial-to-mesenchymal transition in PC-3 cells by Frzb/sFRP3 and DN-LRP5.

The loss of E-cadherin is a hallmark of epithelial-to-mesenchymal transition (31). Transcriptional repressors, such as Slug, Snail, and Twist, have been shown to repress E-cadherin expression via binding to E-box motifs in the E-cadherin promoter (31). Among them, Slug has been recognized as a Wnt target gene (32), whereas Twist responded to Wnt-1 stimulation (33) and could be an activator of N-cadherin (34). Using real-time PCR, we showed an opposing pattern of mRNA expression for E-cadherin and Slug or Twist (Fig. 3C). Compared with PCDNA3.1 (Fig. 3C), Frzb/sFRP3 and DN-LRP5 transfectants increased E-cadherin expression by 23- and 12-fold but decreased Slug expression by 99.9% and 96.9%, respectively (Student’s t test, P < 0.01). In addition, Frzb/sFRP3 and DN-LRP5 transfectants decreased Twist expression by 65% and 42% in relation to PCDNA3.1 transfectants, respectively (Student’s t test, P < 0.05). The protein levels of Slug and Twist were also decreased in Frzb/sFRP3 and DN-LRP5 transfectants (Fig. 3B).

Figure 3D shows that intense staining of E-cadherin was observed along the entire cell-cell contact region among neighboring cells, whereas staining in the contact-free borders was weaker. These data suggest that the increased cell-cell contact seen in Frzb/sFRP3 and DN-LRP5 transfectants may be mediated by up-regulation of E-cadherin expression.

**Frzb/secreted Frizzled-related protein 3 results in a decrease in invasive capacity of PC-3 cells.** Based on the effects of Frzb/sFRP3 on expression of E-cadherin and N-cadherin and morphologic changes, we next examined the in vitro invasiveness of PC-3 cells expressing Frzb/sFRP3, DN-LRP5, DN-LEF1, DN-TCF4, or vector control in a Matrigel invasion assay. The capacity of these cells to invade through a Matrigel-coated membrane was expressed as average number of migrated cells on the lower surfaces of triplicate membranes and adjusted by...
cell motility. Cell motility was measured by average number of cells migrating through a control, uncoated insert. Number of cells on each membrane was averaged from 10 fields (×100). Frzb/sFRP3-, DN-LRP5-, DN-LEF1-, and DN-TCF4-transfected cells exhibited a significant decrease in invasive capacity (by 96%, 95%, 32%, and 60%, respectively) compared with control cells (Student’s $t$ test, $P < 0.05$ to $P < 0.01$, respectively; Fig. 4).

Frzb/secreted Frizzled-related protein 3 on matrix metalloproteinase-2 and matrix metalloproteinase-9 activities and expression in PC-3 cells. Given an important role of MMP-2 and MMP-9 in prostate cancer progression and its involvement of cell-matrix interaction and tumor invasion (35–38), we examined the effect of Frzb/sFRP3 on MMP-2 and MMP-9 activities and expression. In addition, DN-LRP5, DN-TCF4, and DN-LEF1 were used to explore possible Wnt-related signaling mechanisms in regulation of MMP-2 and MMP-9. Zymography showed that ectopic expression of Frzb/sFRP3 and DN-LRP5 in PC-3 cells resulted in decreased activities of both MMP-2 (lower band) and MMP-9 (upper band) in conditioned medium (Fig. 5A). This decrease in MMP-2 and-9 activities correlated with lower MMP-2 and MMP-9 protein levels (Fig. 5C and D, respectively). MMP-2 and MMP-9 activities were also quantified by densitometric analysis and adjusted by total number of cells in each culture. Compared with vector control transfectants, Frzb/sFRP3, DN-LRP5, DN-LEF1, and DN-TCF4 transfectants showed a decrease in MMP-2 activity by 58%, 48%, 58%, and 30% and a decrease in MMP-9 activity by 80%, 67%, 58%, and 70% (Fig. 5B; Student’s $t$ test, $P < 0.05$ or $P < 0.01$).

Figure 3. Ectopic expression of Frzb and DN-LRP5 is associated with changes in the profile of epithelial and mesenchymal markers and cellular morphology. A, representative photograph of transfected cells at 60% confluence taken under an inverted phase-contrast light microscope at ×200 magnification. B, Western blot analysis of expression of E-cadherin, keratin-8, keratin-18, fibronectin, N-cadherin, vimentin, Slug, Twist, and β-actin shown by a representative blot from four independent experiments. C, quantitative real-time PCR analysis of E-cadherin, Slug, and Twist mRNA in the transfectants. Gene expression is presented as fold increase in $ΔC_T$ compared with PCDNA3.1 vector control transfectants. Columns, mean of four independent quantitative real-time PCR experiments; bars, SE. D, the transfectants were stained with anti-E-cadherin and Alex 488-conjugated secondary antibodies. Representative photograph taken under a confocal microscope (Zeiss) at ×400 magnification.
Frzb/sFRP3 and Prostate Cancer

In this study, we have characterized the tumor suppressor activity of Frzb/sFRP3 in an androgen-independent prostate cancer cellular model by showing the remarkable inhibitory effect of Frzb/sFRP3 on Matrigel invasion, colony formation, and in vivo tumorigenesis of PC-3 cells. We have subsequently repeated the animal experiment with a type of mesenchymal cancer. The human fibrosarcoma HT-1080 cells expressing Frzb/sFRP3 or DN-LRP5 implanted into nude mice also exhibited marked inhibition of

Discussion

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require further investigation. Wnts, and thus inhibit their activities in prostate cancer cells, these overexpressed Wnts in prostate cancer play a role in prostatic tumor growth compared with control cells. This result provides further evidence for the antitumor activities of Frzb/sFRP3.

Differences in the biological effects of the various sFRPs have been reported (10–15, 42–44). In contrast to FrzA/sFRP1, FrzA/sFRP1 and sFRP2 have been shown to increase in vivo tumor growth of glioma cells (42). Both FrzA/sFRP1 and sFRP2 increased clonogenicity and enhanced resistance to serum starvation in glioma cell lines (42), whereas sFRP4 decreased the colony formation of glioma cells in soft agar (11). At present, it is still unclear whether differences in biological function of sFRPs reflect their specificity for various Wnt ligands and/or Frizzled receptors. The aberrantly activated Wnt signaling pathway exists in a portion of prostate cancer tissues has been reported (45–47). Whether unclear whether differences in biological function of sFRPs reflect their specificity for various Wnt ligands and/or Frizzled receptors. The aberrantly activated Wnt signaling pathway exists in a portion of prostate cancer tissues has been reported (45–47). 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epithelial-to-mesenchymal transition process of prostate cancer cells.

The inhibition of β-catenin-dependent Wnt signaling by expression of Frzb/sFRP3 in our study was evidenced by activation of GSK-3β and a decrease in the cytosolic β-catenin levels and TCF transcriptional activity. In addition, we observed that DN-TCF4 or DN-LRP5 shared inhibitory effects on MMP-2 and MMP-9 and on cellular invasiveness similar to Frzb/sFRP3, suggesting that these effects of Frzb/sFRP3 may be involved, at least in part, in β-catenin-dependent Wnt signaling. However, the inhibitory effect of the secreted antagonists Frzb/sFRP3 and DN-LRP5 on cellular invasiveness was more pronounced than that of DN-TCF4 and DN-LEF1 in PC-3 cells, suggesting that TCF/LEF-independent pathways are also involved in invasion. Indeed, we showed that expression of Frzb/sFRP3 in PC-3 cells significantly inhibited AKT activation. There is constitutively activated AKT in PC-3 cells due to lack of functional phosphatase and tensin homologue. Grille et al. (41) reported that overexpression of AKT in squamous cell lines resulted in epithelial-to-mesenchymal transition and increased the invasive capacity and tumorigenicity of these cell lines. Because AKT activation plays a central role in prostate cancer and AKT activation in carcinomas resulted in an invasive phenotype, further studies are needed to examine the contribution of AKT inhibition on Frzb/sFRP3-mediated effects in PC-3 cells.

In summary, our data show the tumor-suppressing activities of Frzb/sFRP3 with the marked inhibition of tumorigenicity and invasiveness of androgen-independent prostate cancer PC-3 cells. The mechanisms of the action of Frzb/sFRP3 are associated with the reversal of epithelial-to-mesenchymal transition and decreased MMP activity. The clinical relevance of our results remains to be determined in a population study. Our findings should encourage the development of Frzb/sFRP3 as a novel agent for the prevention or delay of prostate cancer progression.

Acknowledgments

References


Expression of Frzb/Secreted Frizzled-Related Protein 3, a Secreted Wnt Antagonist, in Human Androgen-Independent Prostate Cancer PC-3 Cells Suppresses Tumor Growth and Cellular Invasiveness

Xiaolin Zi, Yi Guo, Anne R. Simoneau, et al.


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