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Secreted Frizzled-Related Protein 4 Inhibits Proliferation and Metastatic Potential in Prostate Cancer

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BACKGROUND. Secreted frizzled-related proteins (sFRP4) inhibits Wnt signaling and thus cellular proliferation in androgen-independent prostate cancer cells in vitro. However, increased expression of membranous sFRP4 is associated with a good prognosis in human localized androgen-dependent prostate cancer, suggesting a role for sFRP4 in early stage disease. Here, we investigated the phenotype of sFRP4 overexpression in an androgen-dependent prostate cancer model.

METHODS. An sFRP4-overexpressing androgen-dependent (LNCaP) prostate cancer model was established to assess changes in cellular proliferation, the expression, and subcellular localization of adhesion molecules and cellular invasiveness, and compared with the findings in sFRP4-overexpressing androgen-independent cells (PC3).

RESULTS. sFRP4 overexpression in both cell line models resulted in a morphologic change to a more epithelioid cell type with increased localization of β-catenin and cadherins (E-cadherin in LNCaP, N-cadherin in PC3) to the cell membrane. Functionally, sFRP4 overexpression was associated with a decreased rate of proliferation ($P = 0.0005$), decreased anchorage-independent growth ($P < 0.001$), and decreased invasiveness in PC3 cells ($P < 0.0001$). Furthermore, increased membranous sFRP4 expression was associated with increased membranous β-catenin expression ($P = 0.02$) in a cohort of 224 localized human androgen-dependent prostate cancers.

The work was performed at Cancer Research Program, Garvan Institute of Medical Research, St. Vincent's Hospital, Darlinghurst, Sydney, NSW 2010, Australia.

No conflicts of interest are associated with this work.

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CONCLUSIONS. These data suggest that sFRP4 is an inhibitor of prostate cancer growth and invasion in vitro independent of androgen receptor (AR) signaling with correlative evidence in human androgen-dependent disease suggesting similar changes in the clinical setting. Consequently, potential therapeutic strategies to modulate Wnt signaling by sFRP4 will be relevant to both localized androgen-dependent prostate cancer and advanced metastatic disease. *Prostate 67: 1081–1090, 2007.*

KEY WORDS: **prostate cancer; secreted frizzled-related proteins (sFRP4); Wnt pathway; invasiveness; proliferation**

INTRODUCTION

Prostate cancer is the most commonly diagnosed cancer and a major cause of cancer death in men in western countries [1,2]. While prostate cancer is androgen dependent in its growth pattern for long periods, the majority of men with metastatic prostate cancer will eventually develop hormone-refractory disease and die of their disease [3]. Androgen-independent prostate cancer growth evolves through several mechanisms: mutation of the androgen receptor (AR) [4], increased expression of the AR [5], changes in AR co-regulatory molecules [6], ligand-independent activation of the AR [7], and activation of alternative growth pathways (e.g., the phosphatidylinositol-3-kinase pathway) [8]. Identification of pathways mediating androgen-independent growth is essential for the development of new therapeutics for patients with metastatic disease whose median survival, even using modern chemotherapy, is less than 2 years [9,10].

The Wnt signaling pathway mediates a spectrum of cellular functions including cell polarity, tissue patterning, control of cellular proliferation, and development of neoplasia. The pathway is initially activated by a Wnt ligand binding to a Frizzled receptor, which subsequently transduces a signal through one of at least three distinct intracellular signaling pathways: the classical ''Wnt/β-catenin'' canonical pathway, the ''Wnt/Ca^{2+}'' pathway, and the ''Wnt/polarity'' pathway [11,12]. When the ''Wnt/β-catenin'' pathway is inactive, glycogen synthase kinase 3β (GSK3β) forms a complex with axin and adenomatous polyposis coli which phosphorylates β-catenin marking it for degradation. Activation of the pathway leads to inhibition of GSK3β activity by phosphorylation, accumulation of β-catenin in the cytoplasm, and subsequent translocation into the nucleus where it binds and activates transcription factors such as TCF and LEF to induce expression of proliferation targets including cyclin D1 [13] and c-myc [14]. More recently, the ''Wnt/Ca^{2+}'' pathway has been associated with cellular invasion in melanoma [15]. When activated, the ''Wnt/Ca^{2+}'' pathway results in intracellular Ca^{2+} release and activation of the Ca^{2+}-sensitive enzymes Ca^{2+}-calmodulin-dependent protein kinase II and protein kinase C in a β-catenin-independent manner [11]. The Wnt ligands appear to affect a network of pathways such as HER2 [16], snail-E-cadherin [17], and phosphatidylinositol-3-kinase/Akt [18], which may explain their pleiotropic effects on normal and malignant cells.

The Wnt signaling pathway has been implicated in carcinogenesis in the colon [12,14,19] and breast [12,19]; however, more recent studies have demonstrated an association between Wnt signaling and prostate carcinogenesis [18,20–27]. Initially, β-catenin signaling was implicated in particular through activation of the phosphatidylinositol-3-kinase/Akt pathway [18]. A number of studies also demonstrated an interaction between β-catenin and the AR [20,21,28] resulting in upregulation of AR-mediated transcription [20,23,26] and conversely, inhibition of β-catenin/TCF signaling in vitro [24]. In addition, stabilization of β-catenin in the prostate of transgenic mice induced lesions similar to human prostatic intraepithelial neoplasia, suggesting a role for β-catenin and potentially the Wnt pathway in prostate cancer development [25]. More recently, Wnt3a has been demonstrated to upregulate AR transcriptional activity and promote cell growth through ''Wnt/β-catenin'' signaling [26].

A variety of soluble inhibitors of the Wnt pathway have been described, including secreted frizzled-related proteins (sFRP), Wnt-inhibitor factors (WIF), and Dickkopf proteins (Dkk). The sFRPs and WIFs bind and sequester Wnt ligands while Dkks bind low-density lipoprotein receptor-related protein (LRP), the Frizzled co-receptor [12]. sFRP1, 3, and 4 have all been implicated in prostate cancer in cell line studies. sFRP1 has been identified in the stroma of only four prostate cancers with experimental data demonstrating that stromal sFRP1 expression leads to decreased ''Wnt/β-catenin'' signaling but increased epithelial proliferation and decreased apoptosis [29]. Conversely, sFRP3 appears to suppress prostate tumor growth and cellular invasion in androgen-independent prostate cancer cells but there is no evidence for clinical relevance [30]. Similarly, evidence from our laboratory demonstrated a clear role for sFRP4 in the inhibition of proliferation in advanced androgen-independent prostate cancer [27]. However, our previous finding that

sFRP4 expression is prognostic in localized androgen-dependent prostate cancer raises the question of an additional role for sFRPs in early prostate cancer. Thus, the aim of this study is to investigate the phenotype of sFRP4 overexpression in an androgen-dependent prostate cancer model in order to gain further insight into the relationship between sFRP4 expression in human prostate cancers and clinical outcome.

MATERIALS AND METHODS

Cell Lines

LNCaP cells were obtained from the American Type Culture Collection (Manassas, VA) and grown in 10% FCS supplemented RPMI 1640 medium. LNCaP cells were transfected for 4 hr with pDEST12.2-sFRP4 [27] and lipoTAXI (Stratagene, La Jolla, CA). Control LNCaP cells were transfected with the empty pDEST12.2 vector using the same protocol. The cells were selected for 12 days in 600 μg/ml Genetacin (Invitrogen, Rockville, MA), clones isolated and seeded to 96-well plates. These were expanded in RPMI 1640/10% FCS and Genetacin 500 μg/ml. Protein was extracted using a lysis buffer containing 0.5% deoxycholate, 150 mM NaCl, 1% NP40, 50 mM Tris (pH 8.0), 0.1% SDS, 10% Glycerol, 5 mM EDTA, 20 mM NaF and protease inhibitors, 10 μg/ml aprotonin, 10 μg/ml leupeptin, 1 mM PMSF and 200 μM sodium orthovanadate. In order to assess secretion of sFRP4 into the medium, conditioned media (CM) was collected from high-density cell cultures after 48 hr of incubation. sFRP4-overexpressing PC3 cells and empty vector controls were constructed previously [27].

Immunoblotting

Samples were fractionated on a 10% polyacrylamide gel and transferred to nitrocellulose membrane. After blocking with 5% skim milk powder, tris-buffered saline, and 0.01% Tween20, the membrane was incubated with 1:12,000 sheep anti-sFRP4 polyclonal antibody [27], then horseradish peroxidase linked Protein G (Zymed, Carlton, CA) 1:10,000 followed by enhanced chemiluminescent detection (Perkin-Elmer, Boston, MA). The other primary antibodies used detected β-catenin (BD Transduction Laboratories, Lexington, KY), E-cadherin (BD Transduction Laboratories), and N-cadherin (Sigma, St Louis, MI). The secondary antibody for these was horseradish peroxidase linked anti-mouse antibody at 1:200 (Zymed).

Cell Proliferation Assays

Proliferation rate was assessed using the Cell Titer 96® kit (Promega, Madison, WI) to estimate total cell number. The parental cell line, LNCaP, LNCaP-empty vector (pooled transfectants and a clonal transfectant), and LNCaP-sFRP4 (three clonal transfectants) were plated into 96-well plates at four initial cell seeding densities, 1,500, 2,000, 3,000, and 4,000 cells per well (six replicates per plating density) to a total volume of 100 μl/well. On days 1–7, 20 μl/well of Cell Titer 96® assay solution was added to the plates which were subsequently incubated for 4 hr at 37°C in a humidified 5% CO_2 atmosphere and absorbance at 490 nm measured in a microplate reader. This experiment was repeated on three separate occasions and the results are expressed as means ± standard error of the means (SEM).

Immunofluorescence

Cells were plated onto chamber slides, fixed in 4% paraformaldehyde, permeabilized in 0.2% Triton X-100, and blocked with 2% bovine serum albumin in PBS/0.2% Tween-20. Cells were incubated with the primary antibody for 2 hr followed by a 1 hr incubation with 1:100 FITC-conjugated-anti-sheep antibody (Vector Laboratories, Burlingame, CA), 1:100 Cy3-conjugated-anti-mouse antibody (Vector Laboratories), and topro-3 staining for nuclei. Cells were visualized using confocal microscopy. The parental, empty-vector control, and two sFRP4-overexpressing clones of each LNCaP and PC3 were assessed with a minimum of 50 cells visualized for each clone. The experiment was repeated on two separate occasions. All comparative confocal images between sFRP4 overexpressing and control clones were obtained using the same confocal settings. The findings described were observed consistently across the control and the sFRP4-expressing cells.

Invasion and Soft Agar Assays

Cell invasion was assessed using modified Boyden chambers [31]. PC3 cells ± sFRP4 (3×10^5 cells) were prepared in RPMI/0.1% BSA and placed in the upper chamber. The lower chamber contained 10% FCS/RPMI/0.1% BSA as chemoattractant. After 16 hr, cells that did not migrate were removed from the top side of the inserts with a cotton swab. Cells that had migrated to the underside of the chambers were stained with Diff-Quik (Harleco, Gibbstown, NJ) and counted by assessing six random fields at 100× magnification. Soft agar assays were performed by initially coating 6-well plates with 2 ml of 1% agar diluted in RPMI 1640/10% FCS. Cell suspensions of 2×10^4 cells/ml were mixed with 0.3% agarose diluted in RPMI 1640/10% FCS and poured over the base agar (1×10^4 cells/well). Once the agar had set, 1 ml of medium was added and refreshed twice per week. At 16 days, colonies >50 μm in

diameter were counted using a dissecting microscope (40×). All assays were performed using the parental cell line, an empty vector control, and three sFRP4-overexpressing clones. Assays were repeated on three separate occasions and the results are expressed as means ± SEM.

HumanTissue Expression

Tissue microarrays containing a cohort of androgen-dependent localized prostate cancers (n = 224) were previously assessed for β-catenin [32] and sFRP4 [27] expression using immunohistochemistry. Membranous β-catenin expression was re-scored by a pathologist and the relationship between the expression patterns assessed.

Statistical Analysis

Differences in functional parameters (i.e., doubling time, anchorage-independent growth) between different cell lines/clones were analyzed using the non-parametric Mann–Whitney *U*-test. An ANOVA test and a Fisher's r to z transformation of a Pearson Correlation were used to assess the relationship between the human tissue expression patterns. A P value of <0.05 was required for significance. All reported P values are two sided. All statistical analyses were performed using Statview 4.5 software (Abacus Systems, Berkeley, CA).

RESULTS

An sFRP4-overexpressing, androgen-dependent cell line model was developed using LNCaP cells as they have minimal endogenous sFRP4 expression (Fig. 1A). Immunoblotting demonstrated that sFRP4 was expressed in the CM of sFRP4-overexpressing LNCaP cells confirming that the transfected protein was in fact secreted from the cells (Fig. 1A). This model was then used to assess the phenotype of sFRP4 overexpression in androgen-dependent prostate cancer in vitro and compare the results with the phenotype in the androgen-independent model, sFRP4-overexpressing PC3 cells.

sFRP4-Overexpression Decreases Proliferation of Androgen-Dependent Prostate Cancer Cells

Proliferation assays were performed on LNCaP, LNCaP transfected with empty vector (both a pool of transfectants and a clonal cell line) and three stable clones of LNCaP-sFRP4. The combined data from triplicate proliferation experiments on the clones demonstrated that sFRP4 overexpression in LNCaP cells decreases the growth rate of cells represented by approximately a 25% increase in doubling time ($P = 0.0005$) (Fig. 1B).

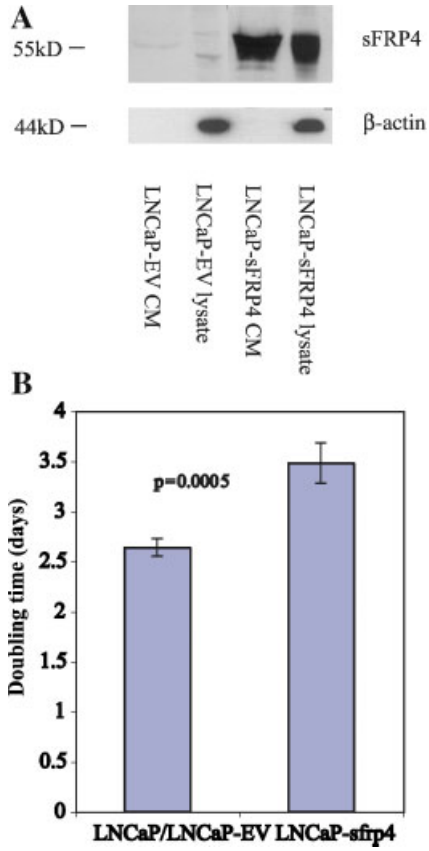

Fig. 1. Changes in cellular proliferation in LNCaP-sFRP4 cells compared to parental LNCaP cells and LNCaP-empty vector (EV) control cells. **A**: Immunoblotting demonstrating increased expression of sFRP4 in the lysate and conditioned media (CM) of LNCaP cells stably transfected with sFRP4 compared to EV controls. **B**: Histogram demonstrating the increase in mean doubling time of three LNCaP-sFRP4 clones compared with parental LNCaP and LNCaP-EV pooled clones. These data are the mean ± SEM of triplicate experiments. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

sFRP4 Overexpression Changes the Expression Patterns of Adhesion Proteins

LNCaP and PC3 cells which overexpress sFRP4 display distinct morphological changes when compared with empty vector control cells. Both cell lines appear more epithelioid in appearance with rounder cells and qualitatively less cellular projections and more adhesion between the cells (Fig. 2A–D). As these observations were common to both cell lines, immunofluorescence experiments were performed to assess the components of the catenin–cadherin adhesion complexes in both models. PC3 and LNCaP cells express different cadherins due to the fact that PC3 cells were developed from a later stage of prostate cancer than

LNCaP cells and there is a switch from E-cadherin to N-cadherin expression in PC3 cells [33]. Qualitatively, there appeared to be an increased cell–cell contact in association with increased expression of β-catenin at the plasma membrane of sFRP4-overexpressing LNCaP and PC3 cells (Fig. 2E–H). A qualitative increase in membranous expression of E-cadherin in LNCaP cells (Fig. 2I,J) and N-cadherin in PC3 cells (Fig. 2K,L) was also seen in conjunction with increased sFRP4 expression. Immunoblotting did not reveal any significant change in the overall expression of β-catenin, E-cadherin, or N-cadherin in sFRP4-overexpressing cell lines compared to controls (Fig. 2M,N) suggesting that sFRP4 expression results in a redistribution of these proteins within the cell to affect the morphologic change, rather than a change in the absolute levels.

sFRP4 Expression Attenuates Cell Invasion and Growth in Soft Agar

Although these data suggest a partial mesenchymal–epithelial transition in association with sFRP4-overexpression, it was not clear if this would result in a change in the metastatic potential of the cells as assessed by changes in motility, invasiveness, and anchorage-independent growth. Wound healing assays did not show a difference in motility between sFRP4-overexpressing PC3 and PC3-empty vector cells, while LNCaP cells are not motile and hence wound healing occurred by proliferation (data not shown). Invasion experiments were performed using a modified Boyden chamber with Matrigel™ coated membranes; invasion was determined at 16 hr. sFRP4-overexpressing PC3 cells had significantly attenuated capacity for invasion

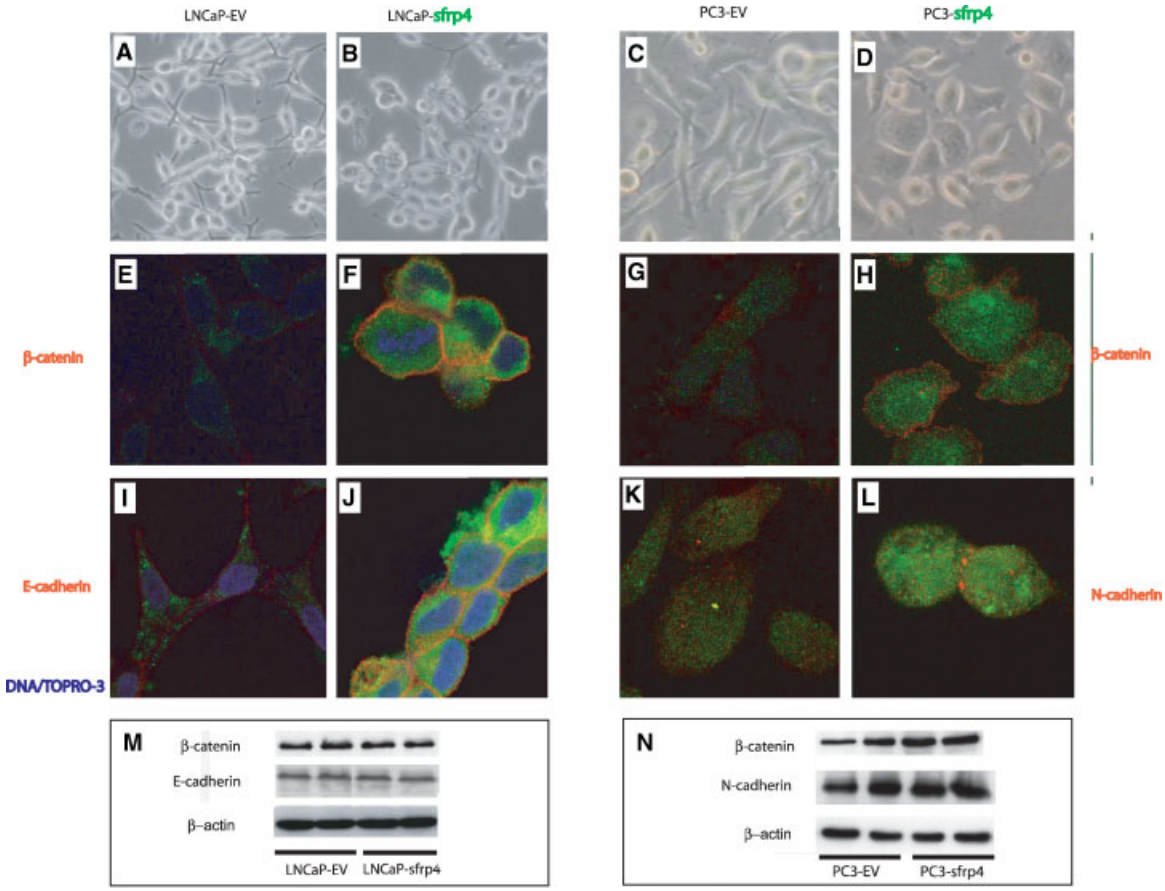

Fig. 2. The effect of sFRP4 overexpression on the expression of adhesion molecules in LNCaP and PC3 cells. Inverted phase contrast microscopy of (**A**) LNCaP-empty vector (EV), (**B**) sFRP4-overexpressing LNCaP cells, (**C**) PC3-EV, and (**D**) sFRP4-overexpressing PC3 cells (100×). Expression of sFRP4 (green), β-catenin (red), and topro (blue) in (**E**) LNCaP-empty vector, (**F**) sFRP4-overexpressing LNCaP cells, (**G**) PC3-EV, and (**H**) sFRP4-overexpressing PC3 cells. Increased expression of E-cadherin (red) in (**J**) sFRP4-overexpressing LNCaP cells compared to (**I**) LNCaP-EV cells. Increased expression of N-cadherin (red) in (**L**) sFRP4-overexpressing PC3 cells compared to (**K**) PC3-EV cells. All comparative confocal images between sFRP4 overexpressing and control clones were obtained using the same confocal settings. Changes in the expression of cell–cell adhesion proteins associated with overexpression of sFRP4 in (**M**) LNCaP and (**N**) PC3 cells by immunoblotting.

through the matrigel compared to PC3 controls (<0.0001) (Fig. 3 A–C). LNCaP cells were not used in this assay as they do not invade through matrigel, consistent with their lack of motility. To investigate the effect of sFRP4 expression on anchorage-independent growth, soft agar assays were established. These demonstrated a significant decrease in colony formation in sFRP4-overexpressing PC3 ($P = 0.0003$) and LNCaP ($P = 0.0004$) cell lines compared to controls (Fig. 4A,B). There was an obvious difference not only in the number of colonies (Fig. 4 A–D) but colonies were larger in those cell lines which express minimal sFRP4 compared to the sFRP4-overexpressing clones (Fig. 4E,F) consistent with the demonstrated changes in proliferation. Overall, sFRP4-overexpression decreased the potential metastatic phenotype of both the androgen-dependent and androgen-independent prostate cancer cell lines.

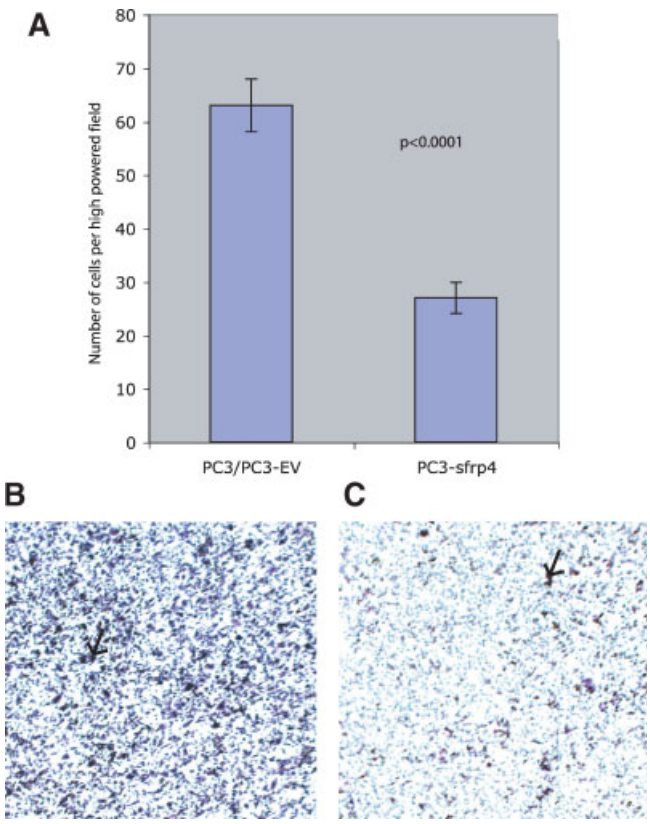

Fig. 3. Changes in the invasive potential of PC3 cells overexpressing sFRP4. **A**: Decreased invasiveness through Matrigel™ in PC3-sFRP4 cells compared to PC3 parental/PC3 EV controls demonstrated using invasion chambers. These data are the mean ± SEM of triplicate experiments of three PC3-sFRP4 clones, the parental PC3, and PC3-EV control. Density of (**B**) PC3-EV cells which invade through Matrigel™ at 16 hr compared to (**C**) PC3-sFRP4 cells (4×). The arrows indicate the dark staining prostate cancer cells which have invaded through the Matrigel™.

Human Expression Patterns of sFRP4 and β-Catenin

A cohort of men with localized androgen-dependent prostate cancer (n = 224) with known sFRP4 status [27] was now scored for membranous β-catenin expression. Analysis of the combined β-catenin and sFRP4 data demonstrated that increasing membranous sFRP4 expression in localized prostate cancer cases correlated with increasing membranous β-catenin expression ($P = 0.005$). The average number of cells expressing membranous β-catenin increased with increasing levels of membranous sFRP4 expression ($P = 0.02$) (Fig. 5A–E) paralleling the immunofluorescence data from the sFRP4-overexpressing LNCaP model.

DISCUSSION

sFRP4 overexpression in androgen-dependent and androgen-independent models of prostate cancer is associated with a consistent phenotype. In both models, sFRP4 overexpression resulted in a morphologic change to a more epithelioid cell type with increased membranous localization of β-catenin and cadherin expression (E-cadherin in LNCaP, N-cadherin in PC3). Functionally, sFRP4 overexpression was associated with decreased invasiveness in PC3 cells and a decreased rate of proliferation and decreased anchorage-independent growth in both models. These data suggest that sFRP4 is an inhibitor of prostate cancer growth and metastasis independent of AR status and thus influences the behavior of the cancer in both early and late stages of disease.

The original impetus for this study came from human data showing that higher levels of membranous sFRP4 in human prostate cancers predicted for a better disease outcome thus it was important, given the in vitro findings, to ascertain if similar changes were observed in human prostate cancer tissue. Previous studies have not shown an association between membranous sFRP4 expression and nuclear β-catenin expression despite in vitro data suggesting sFRP4 inhibits ''Wnt/β-catenin'' signaling [27,32]. On the other hand, our study demonstrated that increased membranous sFRP4 was associated with increased membranous β-catenin expression in localized androgen-dependent prostate cancer, an observation which mirrors the in vitro data.

sFRPs have diverse biological functions which may be tissue specific, or more likely related to the tissue-specific expression of Wnts and components of the signaling pathways they modulate. sFRP1 promotes apoptosis in breast cancer cell lines [34] but is pro-proliferative in prostate cancer models [29] and glioma cells [35]. sFRP2 also promotes glioma tumor growth in vivo [35] but suppresses bone formation by inhibiting osteoblast differentiation in multiple myeloma [36]. On

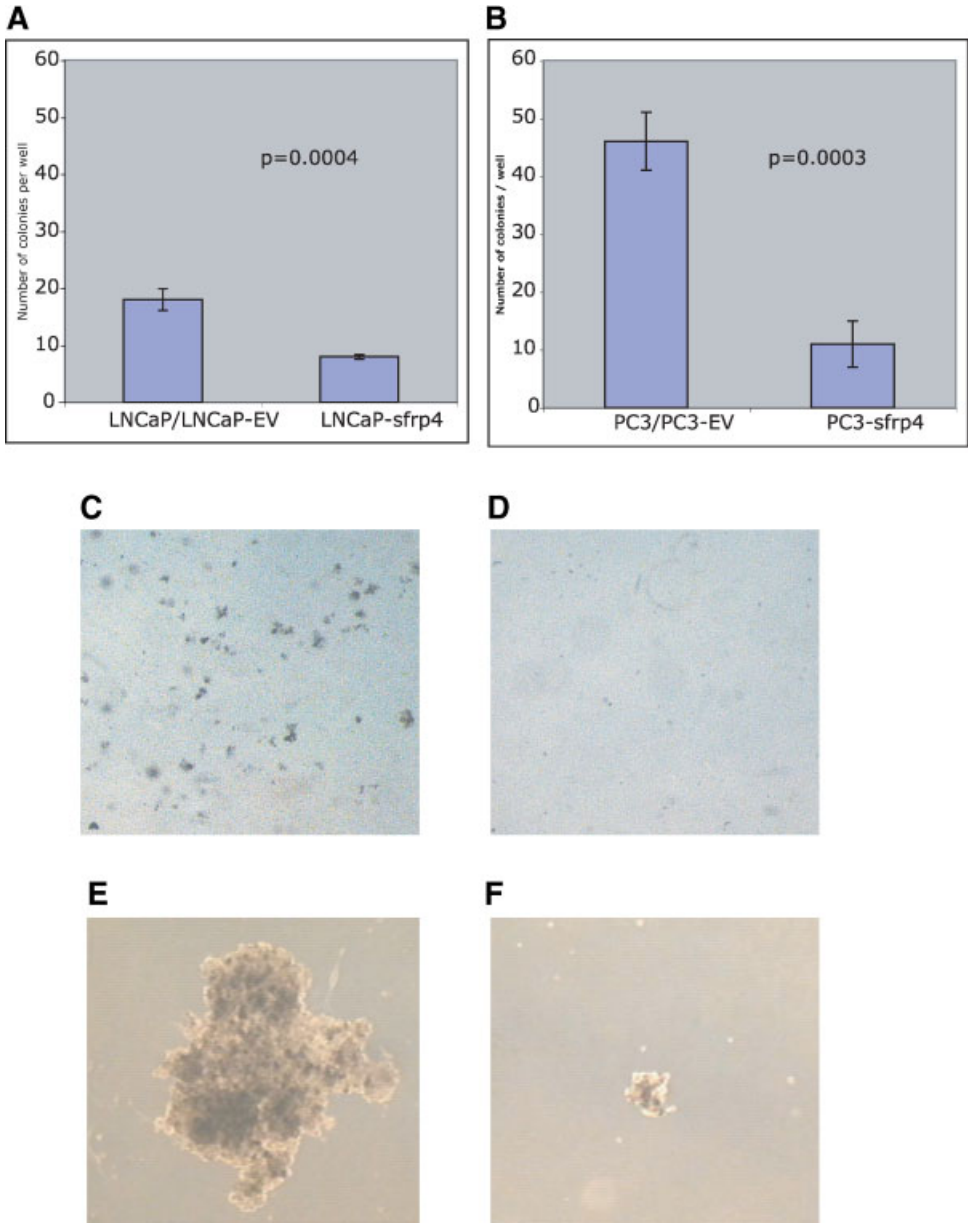

Fig. 4. Changes in anchorage-independent growth in prostate cancer cell lines overexpressing sFRP4 as demonstrated by soft agar assays. Histograms demonstrating decreased number of colonies in (**A**) LNCaP cells (±sFRP4) and (**B**) PC3 cells (±sFRP4). These data are the mean ± SEM of triplicate experiments of each of three sFRP4 overexpressing clones, the parental cell line, and EV control for each cell line. Low-power photomicrographs (2×) of soft agar assay of (**C**) PC3-EV and (**D**) PC3-sFRP4. High-power photomicrographs (40×) of (**E**) LNCaP-EV and (**F**) LNCaP-sFRP4 colonies.

the other hand, sFRP3 appears to be more consistently involved growth inhibition in prostate [30] and osteoblast models [37]. We now demonstrate that sFRP4 has a significant role in proliferation and invasiveness in prostate cancer, yet it affects apoptosis in mammary, ovarian, and endometrial tissue [38]. Transcript profiling of PC3 and LNCaP cells has demonstrated that they uniformly express a wide range of Wnts (1,3,4,6,7A,8B,10A,10B,11) and Frizzleds [3,4,6–9] though interestingly not Wnt 5A, one of the most well-characterized Wnt ligands [39,40]. No single or group of Wnts/Frizzleds has yet been found to interact with sFRP4 and it is likely that the specific Wnt ligands and/or Frizzled receptors bound by each of these inhibitors may well be the key to understanding their diverse phenotypes.

Our data show that sFRP4 overexpression results in a partial mesenchymal-to-epithelial transition, inducing significant changes in morphologic and phenotypic parameters related to metastatic potential

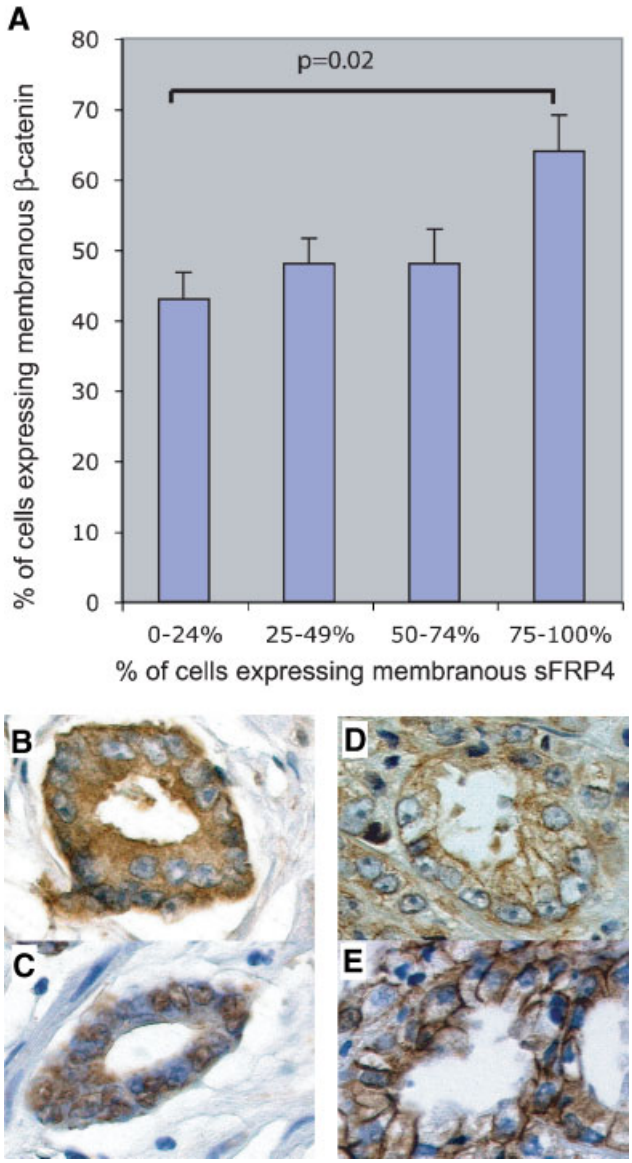

Fig. 5. Relationship between membranous sFRP4 and membranous β-catenin expression in localized prostate cancers (n = 224) as demonstrated by immunohistochemistry. **A**: Histogram demonstrating the correlation between increasing membranous sFRP4 and β-catenin. Photomicrographs of localized prostate cancer expressing (**B**) membranous sFRP4 and (**C**) membranous β-catenin in <5% of cells (400×). Photomicrographs of localized prostate cancer expressing (**D**) membranous sFRP4 and (**E**) membranous β-catenin in >95% of cells (400×).

independent of hormonal status. The changes in E-cadherin and N-cadherin cannot be explained by decreased Wnt signaling affecting the transcriptional repressors, Snail and Twist1 respectively, as there was no change in the overall level of the proteins [17,41]. On the other hand, Gottardi et al. (2004) found that there are distinct forms of β-catenin which bind cadherins rather than TCF allowing for potentially independent rather than coupled regulation of the two functions of β-catenin [42]. These latter observations suggest sFRP4-inhibition of Wnt signaling may affect the cellular architecture and phenotype independent of canonical signaling.

Despite significant evidence for the role of β-catenin signaling in prostate cancer, it was only relatively recently that upstream Wnt signaling was described in prostate cancer. Wnt3A acts as a growth factor inducing AR-mediated transcription and cell growth in prostate cancer cell lines [26]. However, expression of antisense β-catenin, TCF, and E-cadherin only partially blocks the effect of Wnt3A suggesting that other factors and/or pathways other than ''Wnt/β-catenin'' signaling alone are involved [26]. Wnt1 induces upregulation of AR mRNA through LEF-1/TCF but suppresses AR protein expression potentially through Wnt effects on Akt signaling [43]. Conversely, Wnt11 represses AR transcriptional activity and LNCaP growth but does not affect growth in androgen-independent cell lines [44]. Wnt11 expression is also repressed by R1881 while androgen depletion activates Wnt11 signaling [44]. The Wnt11 effects may be mediated by interaction with Wnts 1, 3, and 3A [44,45]. Variable patterns of Wnt expression have been identified in vitro but to date only Wnt1 has been demonstrated in human prostate tissue with increased Wnt1 expression in malignant compared to benign prostate tissue and increasing expression in the progression to more advanced disease [46]. Inhibition of Wnt activity through Dkk1 induces an osteolytic phenotype in prostate cancer bone metastasis cell lines. The levels of Dkk1 decline during the progression of prostate cancer suggesting that loss of Dkk1 may allow Wnt signaling to promote osteoblastic metastases [47]. WIF1 was downregulated in 23% of 48 human prostate cancers examined [48] while in vitro studies have demonstrated that WIF1 can increase chemosensitivity to paclitaxel by decreasing Akt activity [49]. While these studies provide compelling data implicating Wnt signaling in prostate cancer, there is very limited data linking it to human cancers. In fact, Wnt3A has not been described in human prostate cancer tissue despite the numerous in vitro studies.

CONCLUSIONS

sFRP4 mediates changes in the patterns of cellular adhesion proteins and inhibits anchorage-dependent and anchorage-independent proliferation in prostate cancer in vitro irrespective of AR status. sFRP4 also decreases invasiveness in androgen-independent prostate cancer cells. Moreover, the correlation between increased membranous sFRP4 and β-catenin expression in a large human cohort supports similar changes in the clinical setting in conjunction with strong

evidence for sFRP4 as a prognostic marker in localized androgen-dependent prostate cancer. Unlike other inhibitors of Wnt signaling, sFRP4 appears to affect androgen-dependent and androgen-independent prostate cancer and to have extensive human expression data to support the clinical significance of its role. These data suggest that sFRP4-modulated Wnt signaling is important in both early, androgen-dependent prostate cancer and advanced, androgen-independent disease and thus challenges the concept that therapeutic interventions should target only one aspect of this disease. With the advent of PEGylated biopharmaceuticals, the concept of developing a protein inhibitor such as sFRP4 into a drug therapy is a distinct possibility.

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