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Heterogenous Induction of Carcinoma-Associated Fibroblast-Like Differentiation in Normal Human Prostatic Fibroblasts by Co-Culturing With Prostate Cancer Cells

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ABSTRACT

In the tumor microenvironment, carcinoma-associated fibroblasts (CAFs) are considered to play a critical role in the promotion of tumorigenesis. However, the mechanisms that generate CAFs are not well elucidated. To understand how CAFs are generated during primary cancer progression, we investigated the biochemical characteristics of normal human prostate stromal cells (PrSC) co-cultured with human prostate cancer (PCa) cells in vitro. In primary cultures of human PCa-derived stromal cells (PCaSC-8 and PCaSC-9), expression of *TNC*, *ACTA2*, *EGF*, *FGF7*, and *IGF1* mRNA was generally higher than PrSC but gene expression patterns were not uniform between PCaSC-8 and PCaSC-9 cells. Transforming growth factor β (TGFβ) and vascular endothelial growth factor (VEGF) protein levels in both PCaSC-8 and PCaSC-9 cells were generally higher than PrSC but levels of both secreted proteins were not same. When PrSCs were co-cultured with androgen-sensitive LNCaP cells or its sublines, androgen-low-sensitive E9 cells and androgen-insensitive AIDL cells, mRNA expression of *IGF1* was significantly increased in all combinations. In contrast, expression of *COL1A1*, *TNC*, and *ACTA2* mRNA was significantly increased only in LNCaP + PrSC and E9 + PrSC co-cultures. Protein production of VEGF was significantly increased only in LNCaP + PrSC and E9 + PrSC co-cultures. Increase of TGFβ protein was observed only in E9 + PrSC co-cultures. These biochemical characteristics of PrSC were partially recapitulated in TGFβ-treated PrSC. We have demonstrated that normal fibroblasts co-cultured with cancer cells become activated and exhibit biochemical characteristics of CAFs in a heterogenous manner. Our results suggest that heterogenous induction of CAF-like differentiation might be strongly dependent on biochemical characteristics of adjacent cancer cells. J. Cell. Biochem. 112: 3604–3611, 2011. © 2011 Wiley Periodicals, Inc.

KEY WORDS: TUMOR MICROENVIRONMENT; TUMOR–STROMAL INTERACTIONS; CARCINOMA-ASSOCIATED FIBROBLASTS (CAFs); PROSTATE CANCER; ANDROGEN-SENSITIVITY; TRANSFORMING GROWTH FACTOR β (TGFβ)

In the tumor microenvironment, activation of tumor–stromal interactions is considered to play a critical role in the promotion of tumorigenesis [Olumi et al., 1999; Tuxhorn et al., 2002b; Orimo et al., 2005]. Tumor stroma surrounding cancer cells is enriched in activated fibroblasts/myofibroblasts called "carcinoma-associated fibroblasts" (CAFs) [Micke and Ostman, 2004]. CAFs surrounding cancer cells secrete extracellular matrix (ECM) proteins and a number of growth factors to support the survival and proliferation of cancer cells in a paracrine fashion [Bhowmick et al., 2004a]. However, the mechanisms that generate CAFs in the tumor microenvironment are not well elucidated.

Prostate cancer (PCa) is interesting because of the multi-focal and heterologous progression in primary tumors [Qian et al., 1997]. Recent in vivo studies have demonstrated that the heterogenous

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stromal compartment of the prostate has multiple populations of fibroblasts that are associated with tumorigenesis [Franco et al., 2011; Kiskowski et al., 2011]. Clinically, reactive stromal grading in radical prostatectomies or biopsies is a predictor of recurrence. In addition, high reactive stromal grading is associated with lower biochemical recurrence-free survival rates than low reactive stromal grading [Ayala et al., 2003; Yanagisawa et al., 2007]. Recently, we have reported that periostin expression in CAFs was increased in the early stages of PCa tissues (Gleason score 6–7) and was significantly correlated with the degree of malignancy [Tsunoda et al., 2009]. Up-regulation of collagen I, tenascin-C (TN-C), and transforming growth factor β (TGFβ) have been observed in PCa specimens [Tuxhorn et al., 2002a]. In addition, the phenotype of cultured prostate CAFs expressing high levels of CD90, a marker of mesenchymal stem cells (MSCs), has been reported to show more tumor-promoting than that of cells expressing low CD90 [Zhao and Peehl, 2009]. $CD90^{hi}$ cells expressed higher levels of many genes associated with tumor promotion, including TGFβ, angiogenic factors vascular endothelial growth factor (VEGF) and fibroblast growth factor (FGF)-2, and cytokines interleukin (IL)-6 and chemokine (C-X-C motif) ligand (CXCL) 12. Several studies have shown that isolated CAFs from PCa specimens are not uniform and have specific biochemical characteristics [San Francisco et al., 2004; Mizokami et al., 2009; Sun et al., 2010]. The variety of CAFs may reflect different cell lineages or may be the result of site-specific induction [Kalluri and Zeisberg, 2006]. In PCa specimens, different malignant cell types in a tissue are heterogenously observed. This pathological feature led us to hypothesize that the generation of CAFs in PCa may be dependent on the biochemical characteristics of adjacent cancer cells. We still know very little about what tumor-promoting CAFs are, and what distinguishes them from other fibroblasts found in the same tissue. CAFs express vimentin and the smooth muscle marker alpha-smooth muscle actin (αSMA). Thus, CAFs resemble myofibroblasts but the origin of these cells is still unclear. CAFs could be derived from fibroblasts, fibroblast precursors, myofibroblasts, or different cell types such as bone marrow-derived MSCs, preadipocytes, or smooth muscle cells [Shimoda et al., 2010].

Decrease or loss of androgen-sensitivity in PCa is a clinical concern. To compare the biochemical characteristics of androgen-insensitive cancer cells with those of androgen-sensitive PCa cells, we generated two sublines from androgen-sensitive LNCaP cells: E9 cells (low androgen-sensitivity) [Iguchi et al., 2007] and androgen-insensitive AIDL cells [Onishi et al., 2001]. The parental LNCaP cells and the E9 and AIDL derivative cells express androgen receptor (AR) protein in equal amounts, but androgen-dependent prostate-specific antigen (PSA) secretion is detected only in LNCaP cells [Ishii et al., 2009]. We have shown that recombination of E9 cells or AIDL cells with embryonic rat urogenital mesenchyme (UGM) promoted tumor progression in vivo even under androgen ablation [Ishii et al., 2009].

We hypothesized that different biological characteristics of cancer cells might generate various CAFs in a tumor tissue. In this study, to investigate the differential ability of cancer cells to induce CAF-like differentiation, we performed in vitro co-culture experiments using a commercialized normal human prostate stromal cell (PrSC) and LNCaP, E9, and AIDL cells.

MATERIALS AND METHODS

REAGENTS

Recombinant human TGFβ1 and mouse sonic hedgehog (SHH; C25II) N-terminus were purchased from R&D Systems, Inc. (Minneapolis, MN). Phenylephrine (PE), dihydrotestosterone (DHT), and estradiol (E_2) were purchased from Sigma–Aldrich, Inc. (St. Louis, MO).

CELL CULTURE

The androgen-sensitive, AR-positive, human PCa cell line LNCaP was obtained from American Type Culture Collection (Rockville, MD). Androgen-low-sensitive E9 cells were obtained from the parental LNCaP cell population by a limiting dilution method in regular culture conditions [Iguchi et al., 2007]. In contrast, the androgen-insensitive AIDL cells were established from parental LNCaP cells by continuous passaging in hormone-depleted conditions [Onishi et al., 2001]. LNCaP and E9 cells were cultured in phenol red (+) RPMI-1640 supplemented with 10% fetal bovine serum (FBS) and 1% antibiotic/antimycotic solution. AIDL cells were cultured in phenol red (−) RPMI-1640 supplemented with 10% charcoal-stripped (CS)-FBS and 1% antibiotic/antimycotic solution. The androgen-sensitivity of parental LNCaP, E9, and AIDL cells was confirmed by the change of PSA mRNA expression in cell cultures treated with synthetic androgen R1881 [Ishii et al., 2009].

Normal human PrSC were purchased from Lonza Group Ltd. (Basel, Switzerland). PCaSC-8 and PCaSC-9 cells were primary cultured from different human PCa specimens as previously described [Mizokami et al., 2009]. PCaSC-8 cells were isolated from a patient whose clinical characteristics were serum PSA on diagnosis: 246 ng/ml; Gleason score: 4 + 5; stage on diagnosis: T4, N0, M1. In addition, PCaSC-9 cells were isolated from another patient whose clinical characteristics were serum PSA on diagnosis: 180 ng/ml; Gleason score: 5 + 4; stage on diagnosis: T3b, N0, M0. PrSC, PCaSC-8, and PCaSC-9 were maintained using the SCGM Bullet Kit (Lonza Group Ltd.).

CO-CULTURE OF PrSC WITH PCa CELLS

PrSC and PCa cells, parental LNCaP, E9, or AIDL, were cultured in six-well plates using cell culture inserts (BD Falcon, Franklin Lakes, NJ), which physically separates the two cell types but allows diffusion between the two cell compartments (pore size, 1.0 μm). On day 1, PrSC (8×10^3 cells/well) were seeded in SCBM media into the cell culture inserts, while parental LNCaP, E9 or AIDL cells (4×10^4 cells/well) were seeded into six-well plates in phenol red (+) RPMI-1640 supplemented with 10% FBS or in phenol red (−) RPMI-1640 supplemented with 10% CS-FBS, respectively. On day 2, the culture media for PrSC and PCa cells were replaced with keratinocyte-SFM (K-SFM; Gibco BRL, Grand Island, NY) supplemented with 2% FBS and the inserts with PrSC were then placed into six-well plates containing PCa cells for a further 3 days.

TREATMENT OF PrSC WITH GROWTH FACTORS, NEUROTRANSMITTER, AND SEX STEROID HORMONES

On day 1, PrSC (8×10^3 cells/well) were cultured in SCBM media in a six-well plate. On day 2, the culture media was replaced with 2%

FBS-K-SFM media containing 1 ng/ml TGFβ, 100 ng/ml SHH, 10 μM PE, 1 nM DHT, and 1 nM E_2 for 3 days.

ELISA

For the quantitative determination of TGFβ1 and VEGF proteins, aliquots of conditioned medium were collected and subjected to ELISA using the Quantikine® human TGF-β1 or the Quantikine® human VEGF immunoassay kit (R&D Systems).

RNA EXTRACTION AND cDNA PREPARATION

Total RNA was extracted using the RNeasy Mini Kit (Qiagen Inc., Valencia, CA) in accordance with the manufacturer's instructions. The RNA concentration was then determined spectrophotometrically. From 50 ng of total RNA, cDNA was reverse transcribed using the High Capacity RNA-to-cDNA Kit (Applied Biosystems, Foster City, CA).

REAL-TIME POLYMERASE CHAIN REACTION (PCR) ANALYSES

TaqMan quantitative PCR was performed with an Applied Biosystems StepOne™ Real-Time PCR system (Applied Biosystems). TaqMan® Gene Expression Assays for *COL1A1* (Hs01076777_m1), *TNC* (Hs01115665_m1), *ACTA2* (Hs00426835_g1), *EGF* (Hs01099999_m1), *FGF2* (Hs00960934_m1), *FGF7* (Hs00940253_m1), *HGF* (Hs00300159_m1), and *IGF1* (Hs01547657_m1) were used with EagleTaq Master Mix containing ROX (Roche Diagnostics, Mannheim, Germany). All data were analyzed with StepOne™ Software v2.1 (Applied Biosystems) and normalized to *GAPDH* mRNA levels.

STATISTICAL ANALYSIS

The results were expressed as the mean ± SD. Differences between the two groups were determined using a Student's *t*-test. Values of $P < 0.05$ were considered statistically significant.

RESULTS

BIOCHEMICAL CHARACTERISTICS OF HUMAN PCa-DERIVED STROMAL CELLS

Our previous study reported the fibroblastic characteristics of the commercialized normal human PrSC [Hori et al., 2011]. As illustrated in Figure 1A, PCaSC-8 and PCaSC-9 cells showed an expanded and flattened cell shape as compared with PrSC. In PCaSC-8 cells, expression of mRNA for *TNC*, *ACTA2*, *EGF*, *FGF7*, *HGF*, and *IGF1* was significantly higher than PrSC (Fig. 1B). In PCaSC-9 cells, expression of mRNA for *COL1A1*, *TNC*, *ACTA2*, *EGF*, *FGF2*, *FGF7*, and *IGF1* was significantly higher than PrSC (Fig. 1B). Although elevated mRNA expression of *TNC*, *ACTA2*, *EGF*, *FGF7*, and *IGF1* was observed in both PCaSC-8 and PCaSC-9 cells, the biochemical characteristics of each were strongly heterogenous. Both PCaSC-8 and PCaSC-9 cells secreted abundant TGFβ1 protein as compared with PrSC (Fig. 1C). However, the production of TGFβ protein in PCaSC-9 cells was higher than PCaSC-8 cells. Expression of VEGF protein followed this same pattern (Fig. 1D). Thus, PCaSC-8 and PCaSC-9 cells had both common and specific biochemical characteristics.

INDUCTION OF CAFS-LIKE DIFFERENTIATION IN PrSC CO-CULTURED WITH PCa CELLS

To understand the physiological changes occurring in the hormone-refractory state, we used the E9 and AIDL cell sublines derived from androgen-sensitive LNCaP cells. When PrSC were co-cultured with each of the three PCa cell lines in vitro, expression of *COL1A1*, *TNC*, *ACTA2*, and *IGF1* mRNA was significantly increased only in LNCaP + PrSC and E9 + PrSC co-cultures (Fig. 2A). Induction of *IGF1* but not *EGF*, *FGF2*, *FGF7*, or *HGF* mRNA was observed in all PCa + PrSC co-cultures. Increased expression of TGFβ1 protein was observed only in E9 + PrSC co-cultures (Fig. 2B). VEGF protein levels were increased in both LNCaP + PrSC and E9 + PrSC co-cultures but not in AIDL + PrSC co-cultures (Fig. 2C).

EFFECTS OF GROWTH FACTORS, NEUROTRANSMITTER, AND SEX STEROID HORMONES ON BIOCHEMICAL CHARACTERISTICS OF PrSC

During prostatic development, growth factors, neurotransmitters, and sex steroid hormones are involved in regulating growth and differentiation of prostatic stromal cells [Smith et al., 2000; Cunha et al., 2004]. To investigate the specific factors that induced the CAF phenotype in PrSC co-cultured with PCa cells, we tested the effects of growth factors (TGFβ1 and SHH), the neurotransmitter (PE), and the sex steroid hormones (DHT and E_2) on the biochemical characteristics of PrSC.

TGFβ1-treated PrSC were exhibited a spindle-shape as compared with untreated PrSC (Fig. 3B). No significant difference in cell morphology was observed in SHH-, PE-, DHT-, or E_2-treated PrSC. In TGFβ1-treated PrSC, expression of *COL1A1*, *TNC*, *ACTA2*, and *IGF1* mRNA was significantly increased, while *FGF7* and *HGF* were significantly decreased (Fig. 4A). In SHH-treated PrSC, only *COL1A1* mRNA expression was significantly induced (Fig. 4B). No significant difference in mRNA expression was observed in PE-treated PrSC (Fig. 4C). In DHT-treated PrSC, expression of *COL1A1* and *ACTA2* were significantly decreased (Fig. 4D). In E_2-treated PrSC, mRNA expression of *FGF7* was significantly decreased (Fig. 4E).

Production of VEGF protein was significantly increased only in TGFβ1-treated PrSC, while it was significantly decreased in PE- and DHT-treated PrSC (Fig. 4F). Thus, TGFβ1-treated PrSC recapitulated biochemical characteristics similar, but not identical, to those seen in PrSC + PCa co-cultured cells.

To confirm whether TGFβ1 derived from PCa cells could induce a CAF-like phenotype in PrSC in vitro, we measured the secretion level of TGFβ1 protein from PrSC and human PCa cells (LNCaP cells, E9 cells, and AIDL cells). TGFβ1 secretion (fg/cell) in PrSC, LNCaP cells, E9 cells, and AIDL cells were 8.320 ± 0.651, 0.468 ± 0.011 (LNCaP/PrSC: 1/17.8), 0.626 ± 0.011 (E9/PrSC: 1/13.3), and 1.348 ± 0.187 (AIDL/PrSC: 1/6.2), respectively (Fig. 5). This result indicated that TGFβ1 secretion from LNCaP cells and its sublines was quite low as compared with that from PrSC.

DISCUSSION

It is widely accepted that most human cancer results from the accumulation of somatic mutations in epithelial cells. The behavior

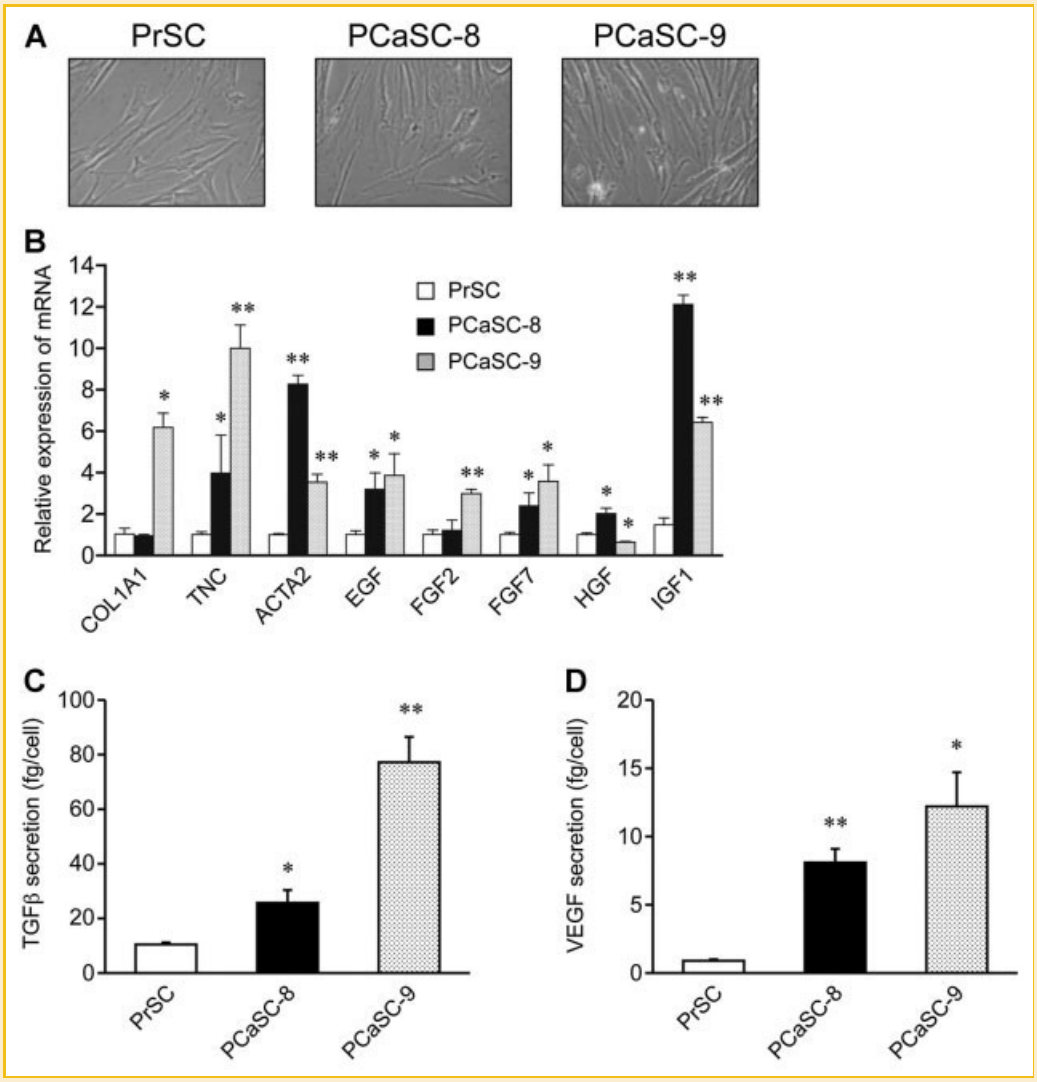

Fig. 1. Characteristics of human PCa specimen-derived stromal cells. A: Cell morphology of PrSC, PCaSC-8, and PCaSC-9 cells in vitro. Magnification ×200. B: Total RNA was isolated from the cells and then subjected to TaqMan quantitative RT-PCR analysis. The relative expression of mRNA for stromal markers (*COL1A1*, *TNC*, and *ACTA2*) and growth factors (*EGF*, *FGF2*, *FGF7*, *HGF*, and *IGF1*) were determined in PrSC, PCaSC-8, and PCaSC-9 cells. All of the qRT-PCR data were normalized to *GAPDH* mRNA levels. C,D: Secretion of TGFβ1 and VEGF from human PCa specimen-derived stromal cells. For the quantitative determination of TGFβ1 (C) and VEGF (D), aliquots of conditioned medium were subjected to ELISA. $^{*}P < 0.05$, $^{**}P < 0.01$ versus PrSC.

of cancer cells is influenced by the tumor microenvironment including CAFs, ECM proteins, blood vasculature, and inflammatory cells. At present it is not clear how cancer cells act to influence the generation of this reactive stroma and to what extent this is a result of phenotypic changes in resident cells versus recruitment of cells from other sites.

During primary cancer progression, CAFs communicate with cancer cells through the secretion of growth factors, chemokines, and cytokines [Bhowmick et al., 2004b]. For instance, CAF-derived TGFβ, EGF, FGFs, VEGF, MMPs, and a number of other factors have been implicated in epithelial cancer progression [Bhowmick et al., 2004b; Kalluri and Zeisberg, 2006; Ao et al., 2007]. Recently, Ishii et al. [2010] reported that CAFs derived from human lung cancer specimens retain their enhanced migratory activity for a while after separation from the cancer cells. Thus, CAFs can maintain their ability to stimulate cancer progression suggesting that inhibition of generation of CAFs in tumor tissues could be a new target for control of primary cancer progression.

Appearance of CAFs and ECM deposition in tumor tissues have been reported in many types of human cancer [Kalluri and Zeisberg, 2006]. In particular, CAFs provide potentially oncogenic signals: (1) CAF-derived TN-C and TGFβ participate in acceleration of cancer cell invasion; (2) CAF-derived growth factors and angiogenic factor VEGF can stimulate cancer progression including angiogenesis. In this study, primary cultured PCaSC-8 and/or PCaSC-9 cells derived from human PCa specimens displayed significantly higher mRNA expression of *COL1A1*, *TNC*, *EGF*, *FGF2*, *FGF7*, *HGF*, and *IGF1*. The role of ECM proteins in tumorigenesis includes effects on epithelial

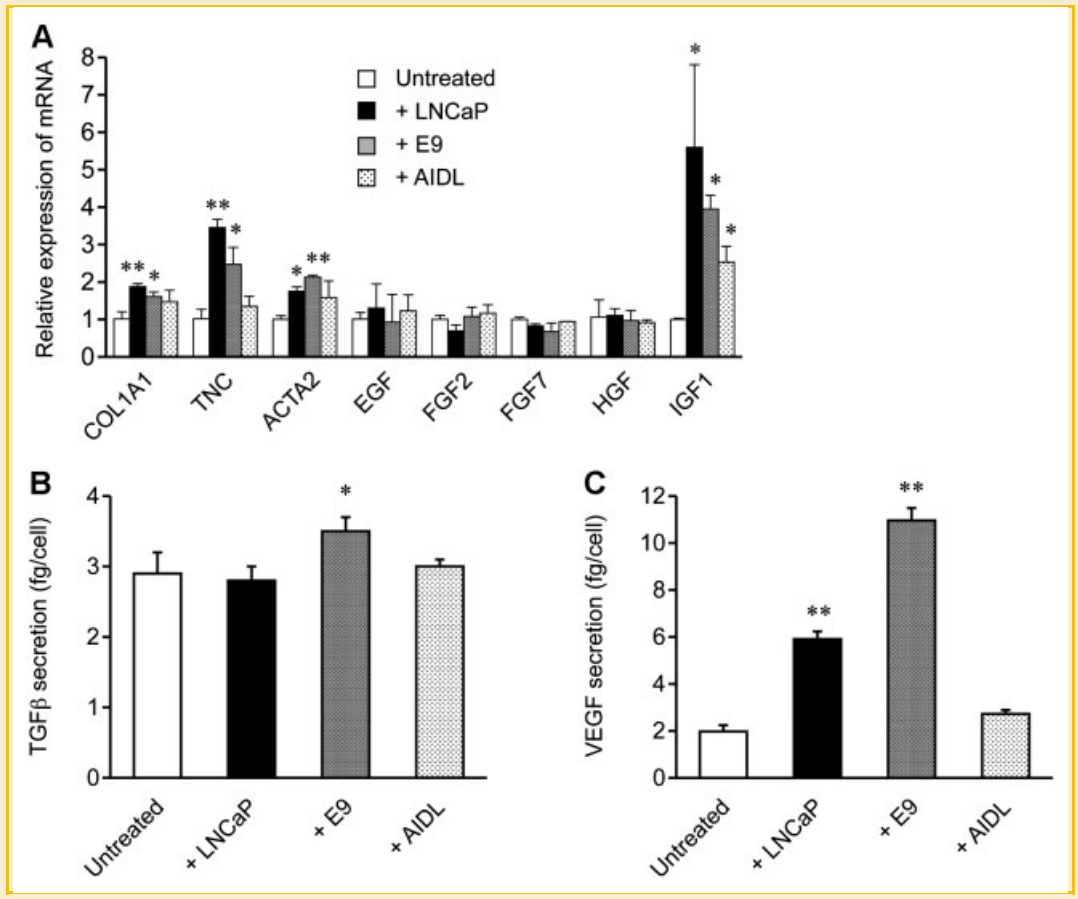

Fig. 2. Induction of CAF differentiation in PrSC co-cultured with PCa cells. PrSC were co-cultured with PCa cells, either parental LNCaP or its sublines E9 and AIDL, using cell culture inserts for 3 days. Total RNA was isolated from each PrSC co-culture and then subjected to TaqMan quantitative RT-PCR analysis. A: The relative mRNA expression of stromal markers (*COL1A1*, *TNC*, and *ACTA2*) and growth factors (*EGF*, *FGF2*, *FGF7*, *HGF*, and *IGF1*) was determined in untreated PrSC, LNCaP + PrSC, E9 + PrSC, and AIDL + PrSC. All of the qRT-PCR data were normalized to *GAPDH* mRNA levels. B,C: Secretion of TGFβ1 and VEGF from PrSC co-cultured with PCa cells. For the quantitative determination of TGFβ1 (B) and VEGF (C), aliquots of conditioned medium were subjected to ELISA. $^{*}P < 0.05$, $^{**}P < 0.01$ versus untreated PrSC.

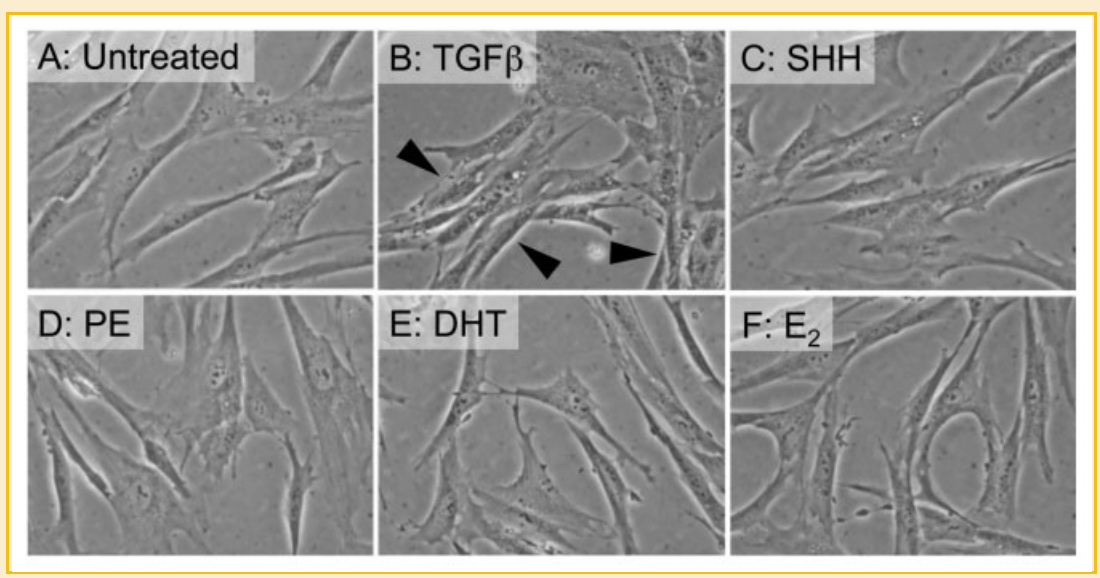

Fig. 3. Effects of growth factors, neurotransmitter, and sex steroid hormones on cell morphology of PrSC. PrSC was treated with 1 ng/ml TGFβ1 (B), 100 ng/ml SHH (C), 10 μM PE (D), 1 nM DHT (E), and 1 nM E_2 (F) for 3 days. Arrowheads indicate the spindle shape of PrSC as compared with untreated PrSC (A). Magnification ×200.

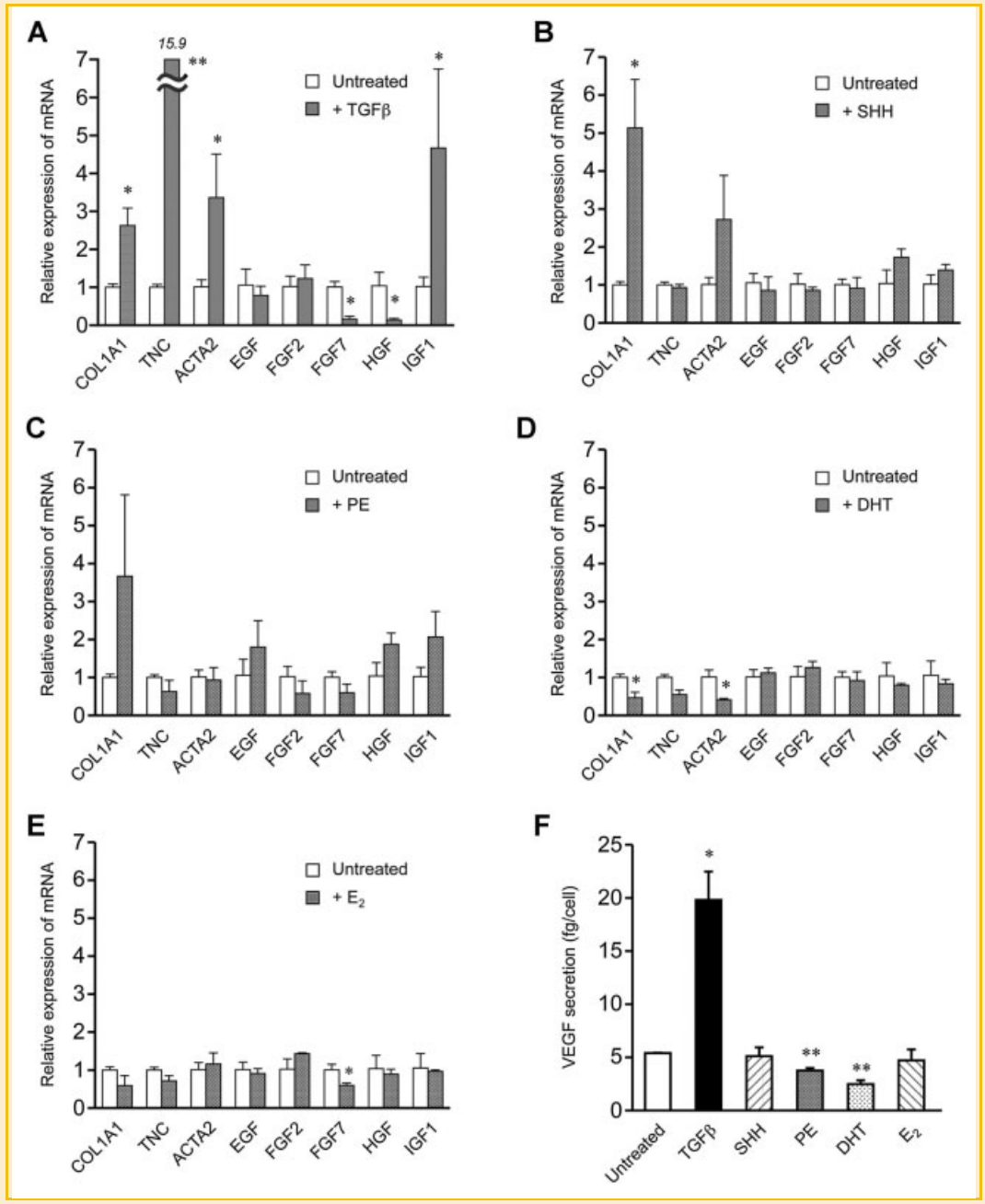

Fig. 4. Effects of growth factors, neurotransmitter, and sex steroid hormones on gene expression profiles and VEGF secretion in PrSC. PrSC was treated with 1 ng/ml TGFβ1 (A), 100 ng/ml SHH (B), 10 μM PE (C), 1 nM DHT (D), and 1 nM E_2 (E) for 3 days. Total RNA was isolated from each co-culture of PrSC and subjected to TaqMan quantitative RT-PCR analysis. The relative mRNA expression of stromal markers (*COL1A1*, *TNC*, and *ACTA2*) and growth factors (*EGF*, *FGF2*, *FGF7*, *HGF*, and *IGF1*) were determined. All of the qRT-PCR data were normalized to *GAPDH* mRNA levels. F: For the quantitative determination of VEGF, aliquots of conditioned medium were subjected to ELISA. $^{*}P < 0.05$, $^{**}P < 0.01$ versus untreated PrSC.

polarity and angiogenesis [Park et al., 2000]. In contrast, CAF-derived growth factors are predominantly stimulators of cancer cell proliferation and can play a part in promoting the carcinogenic process [Bhowmick and Moses, 2005]. Importantly, expression profiles of CAF-related genes were not uniform between PCaSC-8 and PCaSC-9 cells suggesting that the biochemical characteristics of human PCa specimen-derived CAFs must be quite heterogenous. Previous studies focused on the different biochemical characteristics of human PCa specimen-derived CAFs and adjacent normal fibroblasts showing biochemical differences between these cells [San Francisco et al., 2004; Sun et al., 2010].

In this study, we used three cancer cell lines for in vitro co-culture experiments: androgen-sensitive LNCaP cells and its derivative sublines, androgen-low-sensitive E9 cells and androgen-insensitive

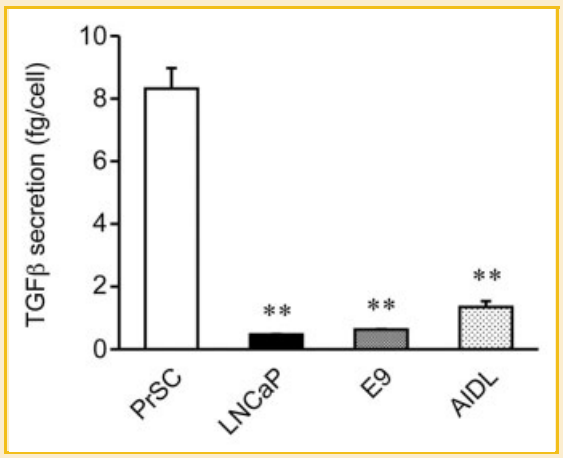

Fig. 5. Secretion of TGFβ1 from PrSC and human PCa cell lines. For the quantitative determination of TGFβ1, aliquots of conditioned medium were subjected to ELISA. $^{**}P < 0.01$ versus PrSC.

AIDL cells. Co-cultures of PrSC with PCa cells in vitro changed cytogenetic and biochemical profiles in a cancer cell line-specific manner. Our previous studies demonstrated that E9 cells and AIDL cells acquired a more aggressive phenotype in vitro and in vivo as compared with parental LNCaP cells [Iguchi et al., 2004, 2007; Ishii et al., 2009]. However, we have not investigated in detail the differences in biochemical characteristics among these three cell lines. Here, we have shown that the biochemical characteristics of PrSC co-cultured with E9 cells but not LNCaP cells and AIDL cells resembled the phenotypes of PCaSC-8 and PCaSC-9 cells. This supports the idea that the heterogenous induction of a CAF-like phenotype might be strongly dependent on the specific characteristics of adjacent cancer cells.

The CAFs in tumor tissues must be generated in response to specific signals from adjacent cancer cells. Although the origins of CAFs have not been well defined, abundant normal fibroblasts are present in prostate tissues. Verona et al. [2007] reported that TGFβ-stimulated PrSC express a number of genes related to myofibroblastic differentiation including *COL11A1*, *TNC*, and *ACTA2* and promoted CAFs formation and carcinoma growth in vivo. Our results confirmed their findings (Fig. 4) suggesting that TGFβ derived from human PCa cells could be a main factor to generate CAFs in adjacent normal fibroblasts. TGFβ participates in cellular proliferation and differentiation not only during normal processes such as embryonic development and wound healing but also during abnormal processes such as cancer progression and angiogenesis [Bhowmick et al., 2004a]. There is often increased expression of TGFβ in many tumor tissues. The sources of TGFβ include cancer cells and inflammatory cells as well as the CAFs themselves in many cases [Gold, 1999]. Loss of TGFβRII in a proportion of stromal cells is a common observation adjacent to PCa foci [Li et al., 2008]. Such changes can potentially result in interactions between subpopulations of cells within the stroma facilitating increased TGFβ expression by the stromal cells which have lost the receptor [Franco et al., 2011; Kiskowski et al., 2011]. In this study, we found that TGFβ secretion from LNCaP cells and its sublines was quite low as compared with that of PrSC (Fig. 5). Recently, Jotzu et al. [2011] demonstrated that human adipose tissue-derived stem cells (hASCs) can differentiate into a CAF-like phenotype when treated with human breast cancer cell line-derived TGFβ. They confirmed that secretion of TGFβ protein from hASCs was not detected, while a significant amount of TGFβ protein was detected in the conditioned medium from the human breast cancer cell lines. The experiments presented here suggest that the situation is more complex than a single factor resulting in a normal to CAF phenotypic switch. The limited amount of PCa cell-derived TGFβ as compared to the larger contribution from PrSC suggests that TGFβ alone may not be responsible for induction of CAF-like differentiation in PrSC + PCa co-cultures.

In conclusion, we have presented evidence that normal fibroblasts co-cultured with cancer cells become activated and exhibit the biochemical characteristics consistent with CAF in a heterogenous manner. Our results suggest that heterogenous inductions of CAF-like differentiation might be strongly dependent on the phenotypic and biochemical characteristics of adjacent cancer cells. Identifying mechanisms underlying the heterogenous induction of CAF-like differentiation in normal fibroblasts is an initial step toward designing CAF-targeted therapy for treatment of PCa. Future studies will attempt to identify the specific profile of factors responsible for the CAF phenotype.

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