ANTICANCER RESEARCH *38*: 2045-2055 (2018)
doi:10.21873/anticanres.12444

Crosstalk Between Androgen-sensitive and Androgen-insensitive Prostate Cancer Cells

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Abstract. *Aim: To investigate how androgen-sensitive LNCaP cells crosstalk with androgen-insensitive DU145 or PC-3 cells. Materials and Methods: The numbers of LNCaP cells were counted when co-cultured with DU145 or PC-3 cells and vise versa. Androgen receptor (AR) activity in LNCaP cells was examined by luciferase reporter assay after transfection with a luciferase reporter driven by PSA promoter in the presence of DU145 or PC-3 cells. Concentration of androgens in the medium was measured by liquid chromatography–mass spectrometry (LC-MS/MS). The ability of migration and invasion of PC-3 and DU145 cells was investigated using a 2-layer chamber, in the presence of LNCaP cells. Results: Co-culture of LNCaP cells with DU145 cells resulted in the conversion of dehydroepiandrosterone (DHEA) to dihydrotestosterone (DHT), which stimulated cell proliferation and PSA promoter activity in LNCaP cells. The increased cell proliferation rate and AR activity, induced in LNCaP cells after DHT treatment, was further enhanced by co-culture with DU145 cells. LNCaP cells also stimulated the proliferation of DU145 and PC-3 cells, via secreting soluble factors. Finally, LNCaP cells promoted migration and invasion of PC-3 cells, in a co-culture system; however inhibited migration and invasion of DU145 cells. Conclusion: Crosstalk between androgen-sensitive PCa cells and androgen-insensitive PCa cells might develop the progression of PCa.*

Prostate cancer (PCa) is the most common malignancy and the second leading cause of cancer-related death in men in the United States (1). Advanced PCa is initially dependent on androgens, and thus, androgen-deprivation therapy (ADT) is the standard of care. However, after an initial response to ADT, PCa eventually becomes androgen-independent and progresses to castration-resistant prostate cancer (CRPC) (2).

Development of advanced PCs to CRPC during ADT is mediated *via* multiple molecular mechanisms, classified in the following two: adaptation to the low androgen concentration caused by ADT, and clonal selection (3-6). In order to adapt to the low concentration of androgen from adrenal gland after ADT, PCa cells synthesize testosterone from dehydroepiandrosterone (DHEA) in an intracrine fashion (7, 8). Especially, PCa-derived stromal cells have been reported to promote the production of androgen, dihydrotestosterone (DHT), from DHEA (9, 10). Moreover, PCa cancer often metastasizes to bones, where bone microenvironment also contributes to cancer metastasis and progression (11). Transforming growth factor-β (TGF-β1), which is released by osteoblasts and bone marrow stromal cells, has been shown to increase DHT synthesis in DHEA-treated primary PCa cells and also, to alter the expression of genes encoding androgen metabolism-related enzymes (12, 13). On the basis of this evidence, strong androgen receptor (AR) axis target drugs, abiraterone acetate and enzalutamide, have been developed and their effectiveness against CRPC has been clinically proven, suggesting that intracrine androgen biosynthesis contributed to the development of CRPC (14, 15). However, their effects are not long-lasting and new therapeutic approaches are required for the treatment of advanced CRPC (16).

Tumor stroma in PCa harbors various cell types, including fibroblasts, macrophages, and endothelial cells, which also play a crucial role in the progression of PCa. Phenotypically modulated fibroblasts, termed myofibroblasts, interact with epithelial and other cell types of the connective tissue and thus, may control tumor invasion and angiogenesis (17). Crosstalk of tumor-associated stromal cells and epithelial cells, mediated through various cytokines and chemokines,

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Key Words: Prostate cancer, androgen, androgen-sensitive, androgen-insensitive.

plays an active role in the progression, androgen-independent conversion, and distal metastasis of PCa (18). The paracrine interplay between cancer-associated fibroblasts (CAFs) and cancer cells also leads to an epithelial-mesenchymal transition (EMT)-driven gain of cancer stem cell properties associated with aggressiveness and metastatic spread (19). Cytokines and growth factors secreted by cells in the bone marrow attract and support cancer cells, thus promoting growth development and metastasis of the tumor (20, 21). More specifically, growth rate of PCa cells has been shown to accelerate when they enter the bone environment, suggesting that factors present in bone stimulate tumor cell proliferation (22).

Herein, we hypothesized that interplay between androgen-sensitive and androgen-insensitive PCa cells plays a critical role in the development of advanced PCa to CRPC, similarly to the previously described crosstalk of androgen-sensitive PCa cells with PCa-derived stromal cells (10). Therefore, androgen-sensitive and androgen-insensitive PCa cells were co-cultured, and cell proliferation and AR activity were investigated in androgen-sensitive LNCaP cells. Additionally, androgen synthesis, as well as migration and invasion of androgen-insensitive PCa cells were explored.

Materials and Methods

Cell culture. LNCaP, PC-3, and DU145 cell lines were purchased from American Type Culture Collection (ATCC, Manassas, VA, USA). LNCaP and DU145 cells were cultured in Dulbecco's Modified Eagle's Medium (DMEM; Sigma-Aldrich, St. Louis, MO, USA) including 5% fetal bovine serum (FBS; Sigma-Aldrich, St. Louis, MO, USA), PC-3 cells were cultured in RPMI medium (Sigma-Aldrich, St. Louis, MO, USA) including 5% FBS.

Co-culture and cell proliferation. LNCaP cells (5×10^4) were seeded on 12-well plates (2-layer chambers) with DMEM including 5% charcoal-stripped fetal (CCS) (HyClone Laboratories, Logan, UT, USA). After 24 h, DU145 or PC-3 (10×10^4) cells were plated on the upper chamber (1.0 μm pore size 12-well format; Becton Dickinson, Franklin Lakes, NJ, USA) with DMEM containing 5% CCS for 24 h. Afterwards, cells were treated with DHEA (0. 3, 10, 30 nM) or DHT (0, 0.1, and 1.0 nM) and cultured for 4 days. Medium was replaced once, at day 2 of treatment. LNCaP cells cultured alone for 4 days served as control. In the same way, 2×10^4 DU145 or PC-3 cells were seeded on 12-well plates with DMEM containing 5% CCS, for 24 h, and then 5×10^4 LNCaP cells were plated in cell culture inserts for additional 24 h and treated with 1 nM DHT or 10 μM enzalutamide (ENZ) for 4 days. Medium was replaced once, at day 2 of treatment. To determine cell proliferation, cell inserts were removed and cells in the bottom wells were trypsinized and counted in triplicate using a hemocytometer. The data represent the means±SD of three replicates.

Culture with conditioned medium (CM). CM was obtained from subconfluent LNCaP cells cultured in DMEM-5% CCS medium, for 24 h, in a 75 cm^2 flask. DU145 or PC-3 cells (3×10^4 or 2×10^4, respectively) were cultured with CM for 4 days, and medium was replaced at day 2. CM was used diluted at ratio of 1:4 or 2:4 with DMEM containing 5% CCS.

In vitro luciferase assay. To examine AR activity, luciferase reporter assay was performed as described previously using 0.4 μg luciferase reporter plasmid, pGL-5.8PSAp, under the control of 5.8-kb PSA promoter including androgen-response elements (23). Twelve h after transfection, 5×10^4 LNCaP cells were co-cultured with 5×10^4 DU145 or PC-3 cells on the same plates for 12 h, followed by incubation with 0, 10, and 100 nM DHEA or 0, 0.1, and 1.0 nM DHT, for 24 h, as previously described (10). Monoculture of transfected LNCaP cells, treated with DHEA or DHT for 24 h, served as control. Cells were then harvested and lysed in luciferase lysis buffer (Promega, Madison, WI, USA).

Quantitative analysis of androgens in the medium by liquid chromatography–mass spectrometry (LC–MS/MS). The concentration of androgens DHEA, T, and DHT, in medium from co-cultures was measured using LC–MS/MS, as previously described (10). Medium was collected before harvesting cells for luciferase assay, it was stored at –30˚C, and LC–MS/MS was performed by ASUKA Pharmaceutical Medical Co. Ltd (Japan). The limits of quantification of DHEA, T, and DHT in the medium were 4 pg/ml, 2 pg/ml, and 2 pg/ml, respectively according to ASUKA.

Western blot analysis. To examine levels of proteins related to EMT, 1×10^5 DU145 or PC-3 cells were seeded for 24 h. And then, they were co-cultured with 2×10^5 LNCaP cells cells in RPMI containing 10% CCS for 24 h, using 6-well plates and cell culture inserts. Afterwards, protein extraction from PC-3 and DU145 cells was performed as previously described (24). Primary antibodies against E-cadherin (mouse monoclonal, Abcam, Cambridge, UK), N-cadherin (mouse monoclonal, Abcam, Cambridge, UK), SNAIL (mouse monoclonal, Abcam, Cambridge, UK) and vimentin (rabbit monoclonal, Abcam, Cambridge, UK) were used. The secondary antibodies were goat anti-mouse HRP conjugate (1:10,000, Bio-Rad Laboratories, Hercules, CA, USA) or anti–rabbit IgG, HRP-linked antibody (1:1,000, Cell Signaling Technology, Danvers, MA, USA). Protein bands were detected using SuperSignal West Femto Maximum Sensitivity Substrate (ThermoFisher, Waltham, MA, USA), visualized by ChemiDoc XRS (Bio-Rad Laboratories, Hercules, CA, USA), and analyzed using Quantity One v4.6.5 software (Bio-Rad Laboratories, Hercules, CA, USA).

Cell migration and invasion assay. To examine the migratory ability of DU145 and PC-3 cells, 2×10^5 LNCaP cells were cultured for 24 h, on 24-well plates with RPMI containing 10% CCS, and then 5×10^3 DU145 or PC-3 cells were plated in cell culture inserts (8.0 μm pore size 24-well format; Life Sciences, One Becton Circle, Durham, USA) for 24 h. Migrated cells were fixed with paraformaldehyde and stained with Crystal Violet (Sigma, St. Louis, MO, USA) for 20-30 min, and cell number was counted. Moreover, for the cell invasion assay, LNCaP cells (2×10^5) were cultured for 12 h, on 24-well plates using RPMI-10% CCS. DU145 or PC-3 cells (1×10^5) were then plated in cell culture inserts containing Matrigel barrier (CORNING, Corning, NY, USA), for 24 h. Afterwards, matrigel was removed and invaded cells were fixed by paraformaldehyde, and then, stained with Crystal Violet for 20-30 min. Acetic acid was added for 50-60 min to solubilize Crystal Violet and the optical density (OD) was measured on a microreader at 595 nm.

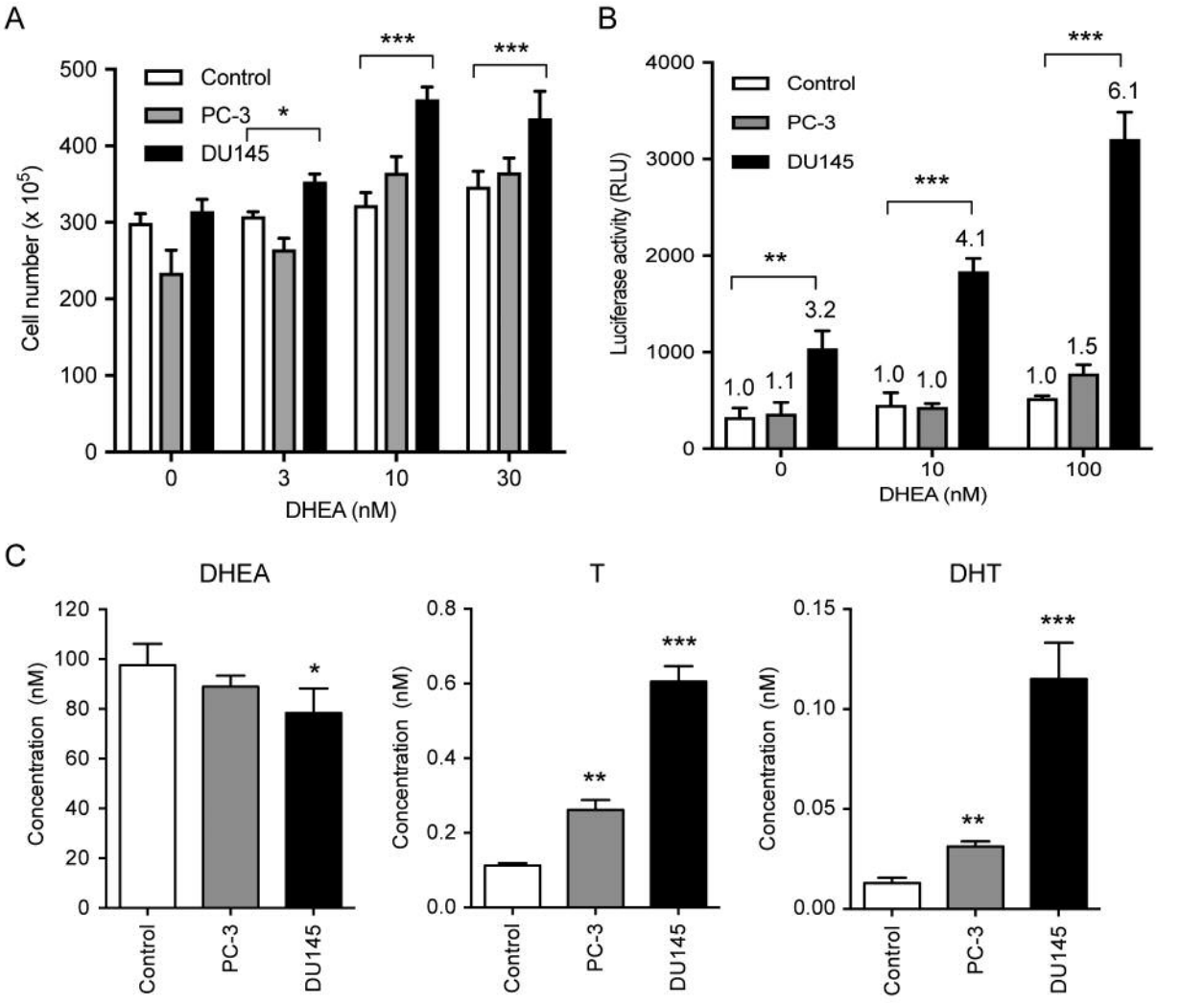

Figure 1. *Proliferation and androgen receptor (AR) activity of LNCaP cells co-cultured with PC-3 and DU145 cells in the presence of dehydroepiandrosterone (DHEA). (A) Cell proliferation assay. LNCaP cells were co-cultured with PC-3 or DU145 (no contact) for 24 h and were treated with an indicated concentration of DHEA for 4 days. (control: no co-culture) (B) Luciferase reporter assay. Twelve h after transfection of LNCaP cells with luciferase reporter pGL3PSAp-5.8, LNCaP cells were co-cultured with PC-3 or DU145 cells on the same dish for 12 h, followed by the addition of indicated concentration of DHEA for 24 h. The number above each column represents fold-change compared to the respective control. (control: no co-culture) (C) Quantitative analysis of DHEA, testosterone (T), and dihydrotestosterone (DHT) in medium from DHEA-treated co-cultures by liquid chromatography–mass spectrometry (LC–MS/MS). (control: no co-culture). Asterisks represent statistical significance (*p<0.05, **p<0.01, and ***p<0.0001 between columns linked in a line).*

Statistical analysis. Student's *t*-test was used to determine the statistical significance of comparisons between two groups, regarding proliferation, luciferase assays, androgen concentration, and migration/invasion. A p-value <0.05 was considered statistically significant. These experiments were performed at least twice in order to confirm reproducible results, and we got similar results.

Results

Androgen-independent DU145 cells enhance biosynthesis of testosterone from DHEA. It has been previously revealed that PCa-derived stromal cells and androgen-sensitive LNCaP cells could coordinately activate androgen biosynthesis of DHT from DHEA and enhance LNCaP cell proliferation in the presence of DHEA (10). However, androgen-independent PCa cells also co-exist in PCa microenvironment (3). In particular, androgen-independent DU145, but not PC-3 cells, have been confirmed to express type 5 17β-hydroxysteroid dehydrogenase (HSD17B5) that converts DHEA to the intermediate in testosterone biosynthesis, androstenediol. HSD17B5 is also expressed by PCa-derived stromal cells

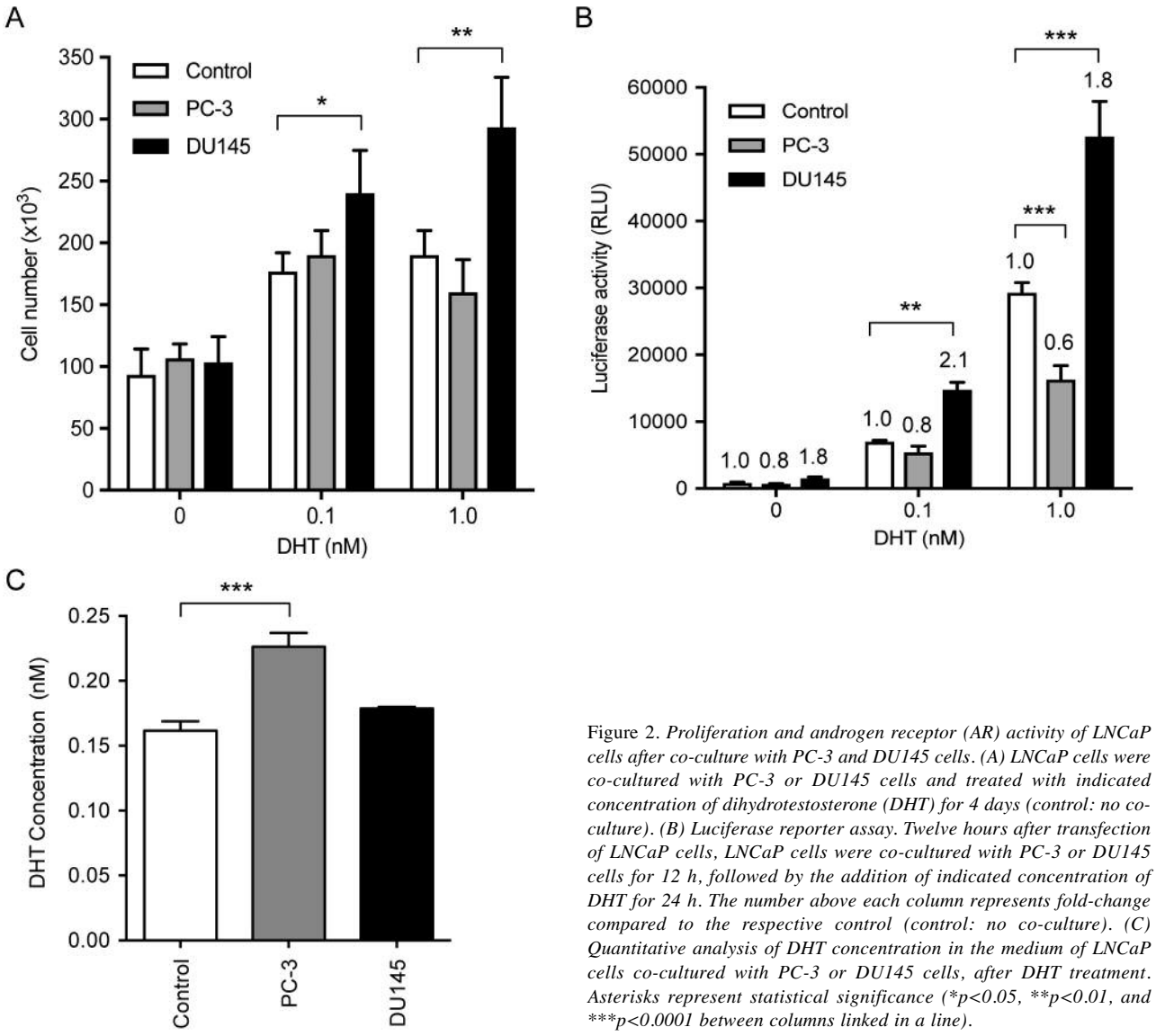

Figure 2. *Proliferation and androgen receptor (AR) activity of LNCaP cells after co-culture with PC-3 and DU145 cells. (A) LNCaP cells were co-cultured with PC-3 or DU145 cells and treated with indicated concentration of dihydrotestosterone (DHT) for 4 days (control: no co-culture). (B) Luciferase reporter assay. Twelve hours after transfection of LNCaP cells, LNCaP cells were co-cultured with PC-3 or DU145 cells for 12 h, followed by the addition of indicated concentration of DHT for 24 h. The number above each column represents fold-change compared to the respective control (control: no co-culture). (C) Quantitative analysis of DHT concentration in the medium of LNCaP cells co-cultured with PC-3 or DU145 cells, after DHT treatment. Asterisks represent statistical significance (*p<0.05, **p<0.01, and ***p<0.0001 between columns linked in a line).*

(10). Therefore, based on our previous findings on PCa-derived stromal cells, we speculated that DU145 cells might also contribute to androgen biosynthesis and enhance the proliferation of LNCaP cells in the presence of DHEA. When LNCaP cells were treated with DEHA, their proliferation was hardly stimulated, irrespective of the concentration of DHEA. In contrast, when LNCaP cells were co-cultured with DU145, but not with PC-3 cells, their proliferation was stimulated by 1.14-fold ($p<0.05$), 1.43-fold ($p<0.001$), and 1.25-fold ($p<0.001$) after 3, 10, and 30 nM DHEA treatment, respectively (Figure 1A). Furthermore, AR activity in LNCaP was investigated in the same model. LNCaP cells were transfected with a luciferase reporter (pGL3PSAp-5.8) under the control of 5.8-kb PSA promoter, which is a well-known androgen-responsive promoter (23). DHEA (100 nM) treatment hardly induced AR activity in LNCaP monoculture. In contrast, co-culture of LNCaP cells with DU145 cells induced AR activity by 3.2-fold ($p<0.001$) compared to LNCaP monoculture in the absence of DHEA. AR activity by 100 nM DHEA was further increased by 6.1-fold ($p<0.0001$) in co-culture of LNCaP cells with DU145 cells (Figure 1B). However, DHEA-induced AR activity was not altered in LNCaP cells co-cultured with PC-3 cells.

We explored whether the activation of AR activity in LNCaP cells co-cultured with DU145 cells in the presence of DHEA might be due to induction of DHT synthesis from DHEA. Then, LC-MS/MS was used to estimate the concentration of DHEA, testosterone (T), and DHT in the

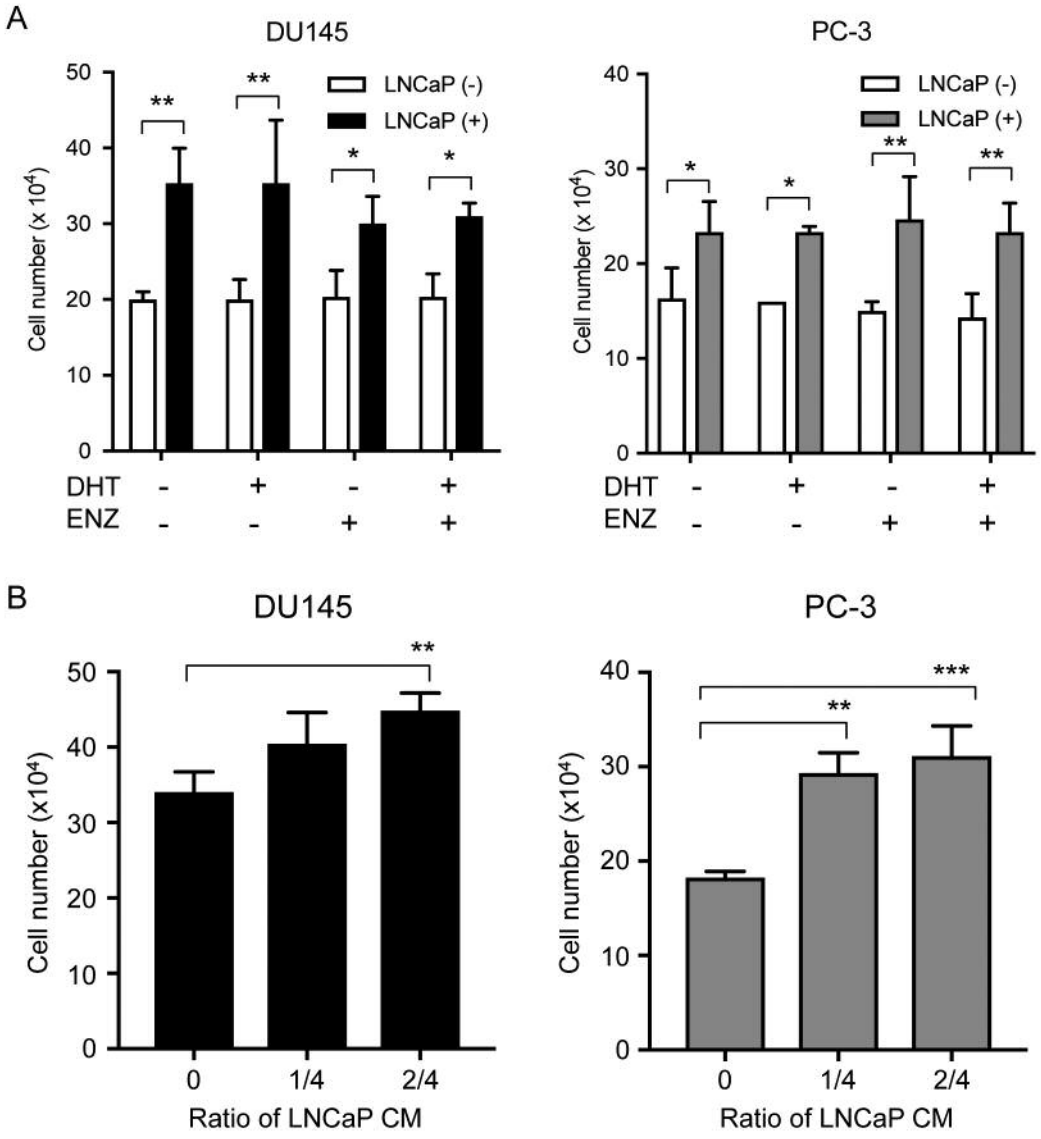

Figure 3. *Effect of LNCaP cells on the proliferation of DU145 and PC-3 cells. (A) DU145 or PC-3 cells were co-cultured with LNCaP cells on an two-layer insert (no contact) for 24 h and treated with 1 nM dihydrotestosterone (DHT) or 10 μM enzalutamide (ENZ) for 4 days. (B) DU145 or PC-3 cells were co-cultured with or without CM from LNCaP cells and counted after 4 days. Asterisks represent statistical significance (*p<0.05, **p<0.01, and ***p<0.0001 0.01 compared to white column).*

medium from co-cultures of LNCaP cells with PC-3 or DU145 cells, after a 24-h treatment with 100 nM DHEA (Figure 1C). Co-culture with DU145 cells elevated the concentration of T and DHT (5- and 8-fold of control, respectively, both $p<0.0001$), while co-culture with PC-3 cells led to a slighter increase of T and DHT concentration (both 2-fold, $p<0.01$). These results indicated that the increased DHT synthesis by DU145 cells might activate AR and also elevate the proliferation of LNCaP cells in the presence of DHEA.

DU145 cells further enhance AR activity induced by DHT in LNCaP cells. It was further examined whether androgen-independent PCa cells could enhance the proliferation of LNCaP cells in the presence of DHT, in a 2-layer co-culture system (Figure 2A). When LNCaP cells were treated with DHT, the proliferation was stimulated by 0.1 and 1.0 nM DHT. The proliferation of LNCaP cells in the presence of 0.1 and 1.0 nM DHT was further enhanced to 1.36-fold ($p<0.05$) and 1.54-fold, ($p<0.001$), respectively by co-culture with DU145 cells compared to LNCaP monoculture. However,

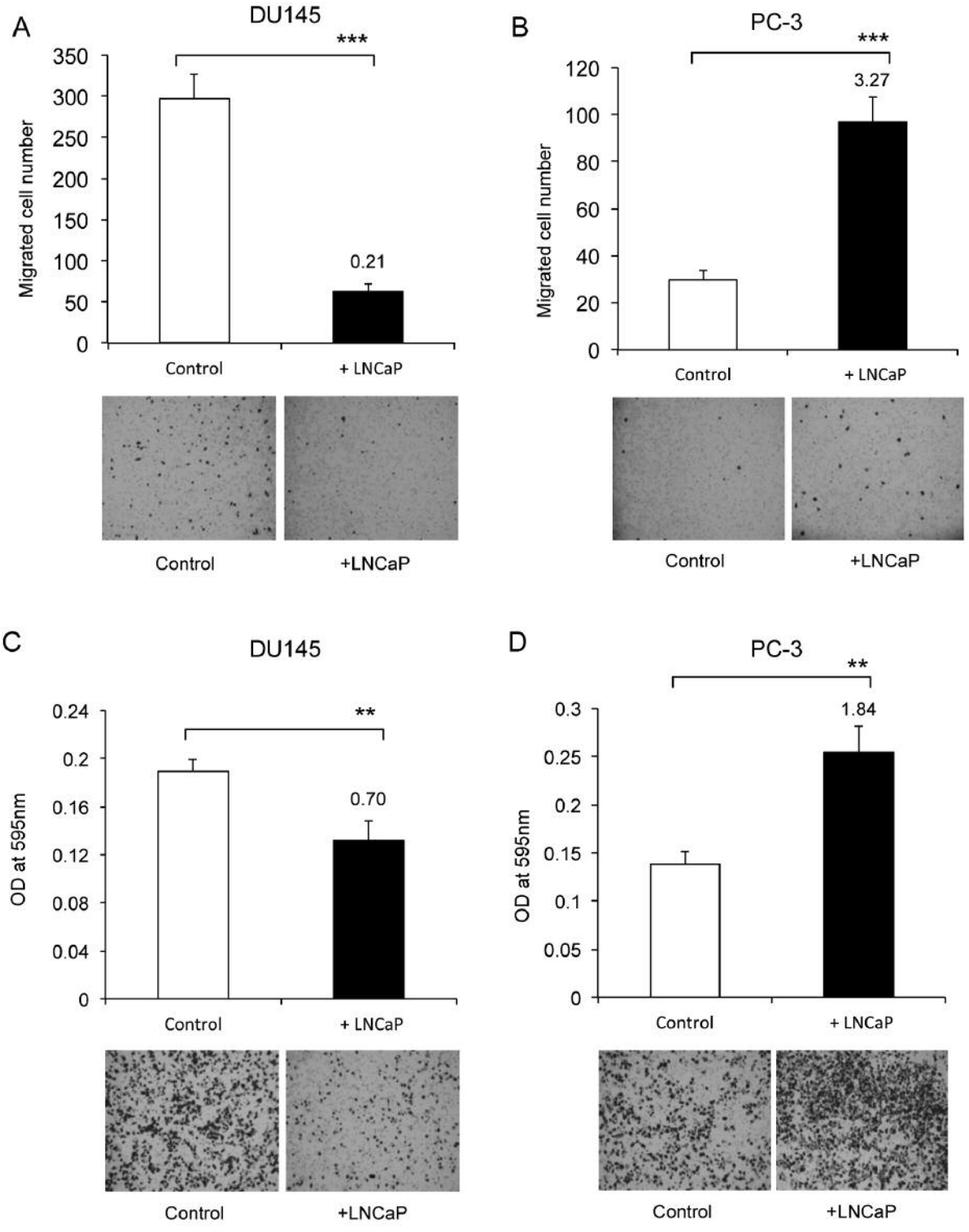

Figure 4. *Cell migration and invasion assay after co-culture of LNCaP with DU145 or PC-3 cells. (A, B) Migration of DU145 and PC-3 cells. LNCaP cells were co-cultured with DU145 or PC-3 cells (no-contact) for 24 h. Migrated cells were fixed and counted after staining with Crystal Violet. The numbers above the black columns represent fold change compared to the control (no co-culture). (C, D) Invasion of DU145 and PC-3 cells. LNCaP cells were co-cultured with DU145 or PC-3 cells (no-contact) for 24 h, with the addition of matrigel barrier in cell culture inserts. Invaded cells were fixed and stained with Crystal Violet. The numbers above the black columns represent fold change compared to the control. Asterisks represent statistical significance (**p<0.01 and ***p<0.0001 compared to white column).*

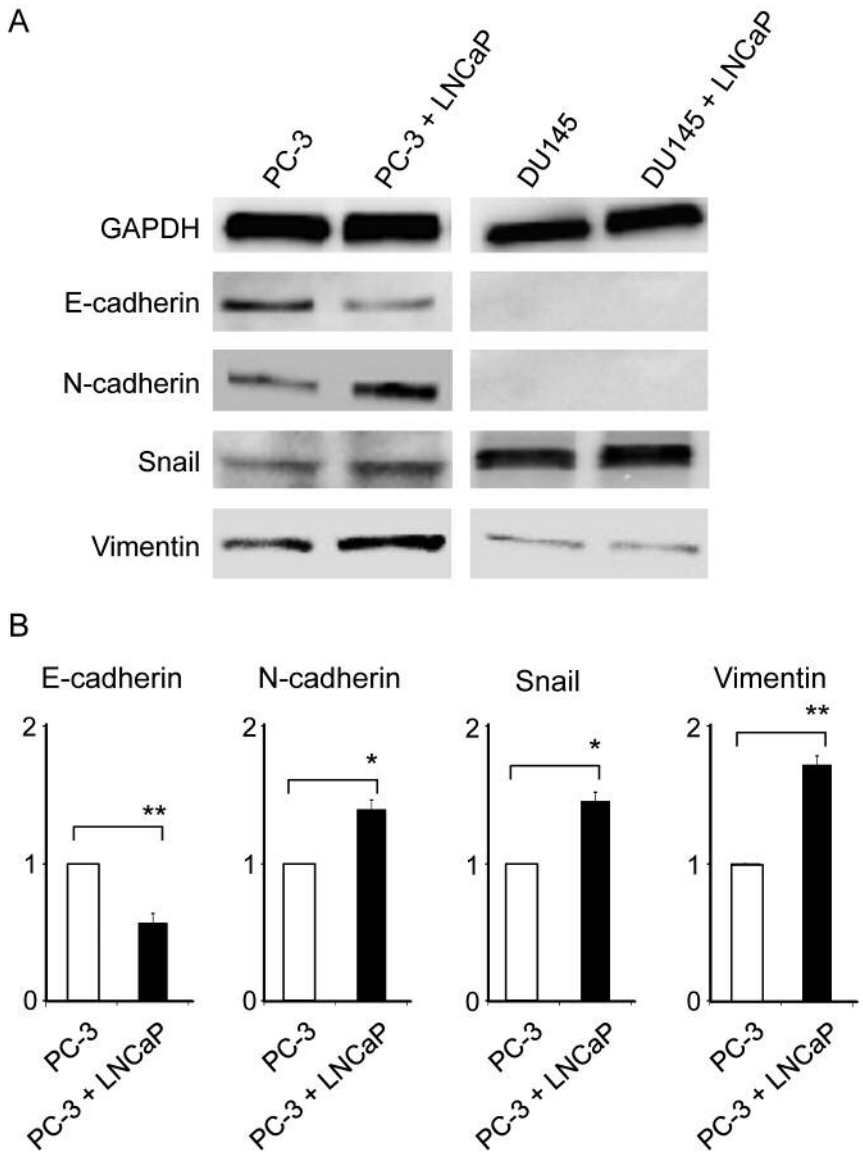

Figure 5. *Western blot analysis of proteins related to epithelial–mesenchymal transition (EMT). (A) DU145 and PC-3 cells were co-cultured with LNCaP cells were plated (no contact) for 24 h. Subsequently, protein extract from DU145 and PC-3 was analyzed for the expression of EMT-related proteins. (B) The relative intensity of each protein band for PC-3 cell monoculture and PC-3 cells co-cultured with LNCaP cells. The intensity of asterisks represents statistical significance (*p<0.05, **p<0.01, compared to the control-monoculture).*

when LNCaP cells were co-cultured with PC-3 cells, LNCaP cell proliferation was not altered, irrespective of DHT presence. It was also examined whether androgen-independent cells affect AR activity in LNCaP cells in the same model (Figure 2B). The PSA promoter activity induced by 0.1 or 1.0 nM DHT was further elevated (2.1-fold, $p<0.001$ and 1.8-fold $p<0.0001$, respectively) in co-culture with DU145 cells, compared to LNCaP monoculture. However, the PSA promoter activity, induced by 1.0 nM DHT, was rather repressed by co-culture with PC-3 cells compared to LNCaP monoculture (0.6-fold, $p<0.001$).

In order to examine the effect of androgen-independent cells on the DHT metabolism in LNCaP cells, DHT concentration was measured in the medium of LNCaP cells and androgen-independent cell co-culture, 24 h after DHT treatment (Figure 2C). In LNCaP monoculture (24 h) 1 nM DHT was degraded to 0.16 nM, while co-culture with PC-3 cells increased DHT concentration to 0.23 nM ($p<0.0001$,

compared to monoculture control). In contrast, DHT concentration in the medium of LNCaP cells was not changed in co-culture of LNCaP with DU145 cells.

LNCaP cells enhance the proliferation of DU145 and PC-3 cells, in co-culture. We further investigated whether LNCaP cells stimulated the proliferation of DU145 and PC-3 cells. When DU145 and PC-3 cells were co-cultured with LNCaP cells, the proliferation of DU145 and PC-3 cells was stimulated by LNCaP cells (1.77-fold, $p<0.01$ and 1.43-fold, $p<0.05$, respectively), irrespective of DHT presence (Figure 3). This stimulation was not inhibited by antiandrogen enzalutamide (10 μM). Therefore, it was assumed that some soluble factors from LNCaP cells might induce the proliferation of androgen-independent cells. To test this, CM was collected from LNCaP cell culture and it was shown to stimulate the proliferation of PC-3 (at CM dilution1:4, 1.61-fold, $p<0.01$; at CM dilution 1:2, 1.7-fold, $p<0.001$) and DU145 cells (at CM dilution 1:2, 1.32-fold, $p<0.01$) (Figure 3B). These results indicated that soluble factors secreted by LNCaP cells cultured *in vitro*, could affect the proliferation of androgen-insensitive cells.

Each cell lines have an influence on each migration and invasion ability. In order to investigate how LNCaP cells affect migration and invasion of PC-3 or DU145 cells, migration of DU145 and PC-3 cells was evaluated using 2-layer chamber. When DU145 cells were co-cultured with LNCaP cells for 24 h, migration of DU145 cells was suppressed (0.21-fold, $p<0.001$), compared with LNCaP monoculture control (Figure 4A). In contrast, when PC-3 cells were co-cultured with LNCaP cells, migration of PC-3 cells was rather promoted (3.27-fold, $p<0.001$) in the presence of LNCaP cells (Figure 4B). Although migration of LNCaP cells was examined by 2-layer chamber method and wound healing assay in the presence of PC-3 or DU145, no significant difference was observed (data not shown).

Moreover, DU145 and PC-3 cell invasion was examined using 2-layer chambers with Matrigel barrier. Co-culture with LNCaP cells was shown to suppress invasion of DU145 cells (0.7-fold, $p>0.01$) (Figure 4C), while contrarily, promoted invasion of PC-3 cells (1.84-fold, $p>0.01$) (Figure 4D).

EMT was related to PC-3 cell migration and invasion. In order to explore whether migration and invasion of PC-3 cells induced by LNCaP cells is associated with EMT, the expression of EMT-related proteins were examined. After co-culture of PC-3 cells with LNCaP cells for 24 h, the expression level of E-cadherin was significantly decreased (0.56-fold, $p<0.01$), and the expression level of N-cadherin (1.40-fold, $p<0.05$), SNAIL (1.45-fold, $p<0.05$), and vimentin proteins (1.71-fold, $p<0.05$) was increased, but not in DU145 cells (Figure 5). These results indicated that factors secreted by LNCaP cells might promote migration and invasion of PC-3 cells *via* EMT.

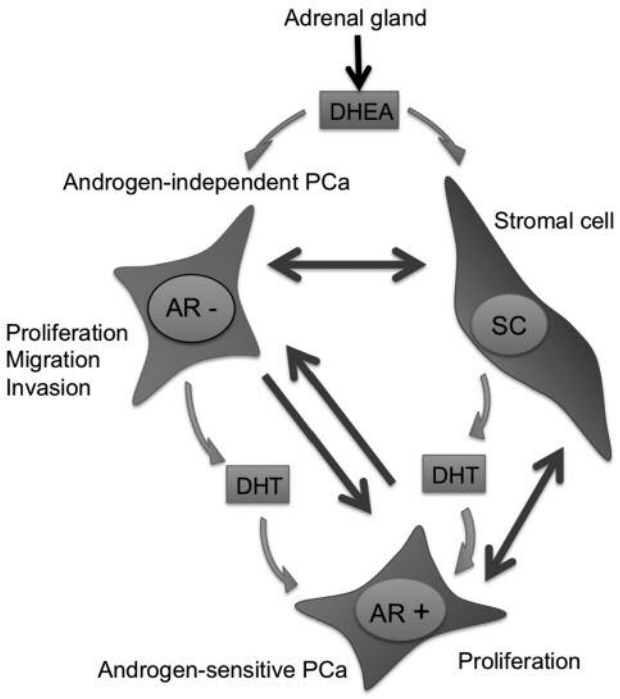

Figure 6. *Crosstalk among androgen-sensitive prostate cancer (PCa) cells, androgen-independent PCa cells, and PCa-derived stromal cells (SC). Dihydrotestosterone (DHT), metabolized from dehydroepiandrosterone (DHEA) in androgen-independent PCa cells (AR negative cells, AR-) as well as in stromal cells, activates androgen receptor (AR) in androgen-sensitive PCa cells (AR positive cells, AR+). Crosstalk among these cells may promote the migration and invasion potential of androgen-independent PCa cells via epithelial-mesenchymal transition.*

Discussion

Tumor microenvironment consists of a variety of cell types that play a pivotal role in cancer cell proliferation, and tumor progression. Especially, CAFs and bone marrow-derived myeloid cells such as tumor-associated macrophages (TAMs), tumor-associated neutrophils (TANs), and myeloid-derived suppressor cells (MDSCs), affect cancer progression, EMT, angiogenesis, and resistance to anticancer therapies (25-28). Recently, immortalized CAFs have been reported to promote proliferation and invasion of PCa cells (29). CAFs are also involved in intratumoral synthesis of androgens during the progress of PCa to CRPC (10). Since PCa tissue consists of heterogeneous cell populations, clonal selection of androgen-insensitive PCa cells is thought as one of the mechanisms by which PCa progresses to CRPC during hormonal therapy (5). However, the interaction between androgen-sensitive PCa cells and androgen-insensitive PCa cells has hardly been described.

Our hypothesis was that, within the tumor micro-environment, androgen-sensitive PCa cells interact with androgen-insensitive PCa, contributing to the progress of PCa to CRPC (Figure 6). In the present study, a crosstalk between androgen-sensitive LNCaP cells and androgen-independent PC-3 or DU145 cells was demonstrated. First, it was confirmed that DU145 cells, though not PC-3 cells, further enhance the proliferation rate and DHEA-induced AR activity in LNCaP cells. Furthermore, the concentration of androgens, testosterone and DHT, was shown to increase when LNCaP cells were co-cultured with DU145 cells, compared to the monoculture. We have previously reported that HSD17B5, which converts DHEA to androstenediol, was well-expressed in DU145 cells and PCa-derived stromal cells, compared to PC-3 and LNCaP cells. However, the expression level of 3β-hydroxysteroid dehydrogenase (HSD3B) that converts androstenediol to testosterone was shown to be lower in DU145 cells and PCa-derived stromal cells, compared to LNCaP cells (10). This evidence combined with the results presented here, suggest that DU145 cells as well as PCa-derived stromal cells might coordinately contribute to androgen biosynthesis from DHEA to DHT and activate AR in LNCaP cells. In other words, DU145 cells and PCa-derived stromal cells exhibited a similar behavior regarding androgen biosynthesis.

Moreover, DU145 cells further enhanced DHT-induced LNCaP cell proliferation and AR activity. AR has been reported to be activated in PCa cells by growth factor and cytokines, such as insulin-like growth factor-I (IGF-1), keratinocyte growth factor (KGF), epidermal growth factor (EGF), and interleukin 6 (IL-6) (30,31). In addition, DU145 cells have been shown to secrete interleukin-6 (IL-6) (32), indicating that, apart from the enhanced expression of androgen biosynthesis-related enzymes, DU145 cells might induce AR activation *via* secreting cytokines. Taken together, androgen-insensitive PCa cells are shown to contribute to the adaption of androgen-sensitive PCa cells to the low androgen concentration, and acceleration of cell proliferation during ADT, in a similar manner as PCa-derived stromal cells do.

Interestingly, AR activity in LNCaP cells was reduced after co-culture with PC-3 cells, although LNCaP and PC-3 cells increased the concentration of DHT in the medium (Figure 2B and 2C). A possible mechanism implicated in this might be the regulation of DHT efflux by P-glycoprotein, which has been reported to reduce androgen accumulation in LNCaP cells, thus reducing androgen responsiveness (33). The reduction of AR activity in LNCaP cells by co-culture with PC-3 cells should be further investigated.

Concerning the effect of LNCaP cells on androgen-independent cells, they promoted proliferation of PC-3 and DU145 cells. LNCaP cells also increased the migration and invasion of PC-3 cells, whereas they had the opposite effect on DU145 cells. It has been previously reported that monocyte chemotactic protein-1 (MCP-1) is secreted form various cells including LNCaP cells (34). MCP-1 has been shown to stimulate proliferation and increase invasion of PC-3 and DU145 cells (35). These reports suggest that secreted proteins from LNCaP cells might regulate proliferation and invasion of androgen-independent PCa cells. On the other hand, migration and invasion of DU145 cells were rather suppressed by LNCaP cells. Hence, further investigation is necessary to reveal the mechanism of this interaction.

The interactions between androgen-sensitive PCa cells and androgen-independent PCa cells are critical for the selection of the appropriate treatment method. ADT is the most frequently used first-line therapy for patients with progressive PCa. However, according to the literature, ADT results in the expansion of androgen-insensitive PCa cells, which coexist with androgen-sensitive PCa cell populations in advanced PCa, thereby promoting progress to CRPC (5). After progression to CRPC, new hormonal therapy (abiraterone acetate or enzalutamide) is often employed as an initial treatment, and docetaxel is employed as a next line treatment (14, 15). Interestingly, recent clinical studies revealed that chemohormonal therapy was beneficial in metastatic PCa as an initial therapy, compared with standard hormonal therapy (36, 37). Our results along with the evidence presented above, suggests that treatments targeted to both androgen-sensitive and -insensitive PCa cells, preventing cross-talk between the cells, should be considered before progression to CRPC.

Taken together, data from the present and our previous studies support that interplay among androgen-sensitive PCa cells, androgen-insensitive PCa cells, and PCa-derived stromal cells induces androgen synthesis in the PCa microenvironment (10). This interaction might contribute to the development and progression of CRPC. Therefore, it is important to disrupt the interaction among androgen-sensitive PCa cells, androgen-insensitive PCa cells, and PCa-derived stromal cells, aiming to improve treatment efficacy.

Conclusion

Crosstalk between androgen-sensitive and androgen-insensitive PCa cells was shown to promote androgen synthesis, AR, and EMT, *in vitro*, and might contribute to CRPC development. Therefore, our results indicate that treatments targeted against androgen-independent PCa cells should be considered for advanced PCa therapy, in order to prevent/delay the progression of the disease.

Conflicts of Interest

The Authors declare no conflict of interest.

Acknowledgements

This work was supported by Grants from JSPS KAKENHI (Grant Number 17H04325). We thank S. Fujinuma for skilled technical assistance (Kanazawa University).

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Received November 13, 2017
Revised February 14, 2018
Accepted February 26, 2018