Original Article
Androgen receptor facilitates the recruitment of macrophages in tumor microenvironment to promote upper urinary tract urothelial cell carcinoma progression

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Abstract: Interactions between infiltrating macrophages in the tumor microenvironment (TME) and tumor cells contribute to tumor progression. The potential impacts of recruited macrophages to the upper urinary tract urothelial cell carcinomas (UUTUCs) progression remain unclear. Here we found human UUTUCs might recruit more macrophages than surrounding normal urothelial cells in human clinical specimens and in in vitro co-culture experiments with UUTUC cells and macrophages. The consequences of recruiting more macrophages to UUTUCs might then enhance UUTUC cell growth, migration and invasion. Further investigation found that the androgen receptor (AR) not only enhanced UUTUC cells capacity to recruit more macrophages, it could also promote the macrophages-enhanced UUTUC cells growth, migration and invasion. Downstream AR target cytokine search found AR might function through modulating CCL5 expression to influence UTTUC progression. Interruption of CCL5 partially reversed the AR-regulated macrophage-enhanced UUTUC progression. AR in UUTUC cells also increased tumor formation in vivo. Taken together, these results suggest that macrophages recruitment may enhance UUTUC progression, modulated by AR-CCL5 signal through alterations in chromatin state to establish a tumor microenvironment with recruited macrophages and cytokines to facilitate cell growth, migration and invasion.

Keywords: Androgen receptor, upper urinary tract urothelial cell carcinoma, macrophage, tumor microenvironment

Introduction
Upper urinary tract urothelial carcinomas (UUTUCs) are relatively uncommon tumors, accounting for about 5% of all urothelial tumors and 5-10% of all renal tumors, but have severe morbidity and mortality [1, 2]. The five-year cancer-specific survival rates for patients with advanced and metastatic UUTUCs were 54, and 12 percent respectively [3]. UUTUCs with higher TAM infiltration were linked with higher expression of HIF-1α that might be associated with worse disease free survival [4]. The 5-year survival rate of UUTUC patients with positive infiltrating inflammatory cells, including neutrophils, macrophages, and lymphocytes, was 35.7%, but a much higher rate (77.1%) was found in patients negative for those inflammatory cells infiltration, suggesting the positive linkage between higher inflammatory cell infiltration to the poor prognosis [5]. The tumor microenvironment (TME) contains various inflammatory cells and cell matrix that can interact with tumor cells by exchanging enzymes and cytokines to influence the local extracellular matrix and capacity of tumor cell growth and invasion [6, 7].

UUTUCs affect more men than woman with a male-to-female ratio of 2–3 to 1 [8-10], however, with better survival rate in females [11]. The gender difference indicates the potential linkage of sex hormones, especially the male hormone androgen and the androgen receptor (AR) to the progression of UUTUCs. Early studies have demonstrated the AR might influence the UUTUC cell migration, invasion and cancer stem cell population as well as the response to chemotherapeutic agents [12-15].

Understanding the role of infiltrated macrophages and its regulation on UUTUC progres-
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The function of infiltrated macrophage cell in UUTUC was not yet clarified. To characterize and define the role of infiltrated macrophage cell in UUTUC, we set out to determine whether macrophages could supply contextual signals that serve to promote cancer progression and how they are regulated. Here we demonstrate that infiltrated macrophages can enhance the UUTUC progression, which are modulated by the AR-CCL5 signal.

Materials and methods

Cell culture

The UUTUC cell line, BFTC 909, (from a UUTUC of renal pelvis patient) was a generous gift from Dr. Tzeng CC [16] and cultured in Dulbecco’s modified Eagle’s medium (DMEM), containing 10% fetal bovine serum (FBS). 7630 cells were obtained from the tumor specimen from a UUTUC of renal pelvis patient [17] and also cultured in Dulbecco’s modified Eagle’s medium, containing 10% FBS. The establishment BFTC or 7630 hAR cells (BFTC 909 or 7630 cells infected with lentiviral vector carrying full length human AR gene and the green fluorescent protein (GFP) gene) and BFTC pWPI or 7630 pWPI cells (BFTC 909 or 7630 cells infected with parental lentiviral vector) was described previously [13, 14]. THP-1 cells were purchased from ATCC and cultured in RPMI-1640 medium, containing 10% FBS.

Transwell migration assay

THP-1 cells were first harvested from the culture dish and 1.0×10⁵ cells in 200 μL of serum-free media were transferred to the transwell inserts (the top compartment, 8-μm pore size) and BFTC 909 or 7630 cells were placed in the lower chamber in 750 μL 10% FBS DMEM medium. After incubation at 37°C for 24 h, filters were washed, fixed, and stained with Crystal violet. Cells that had moved to the lower surface of the filter were counted under the microscope. Migrated cells in each field were quantified.

Colony formation assay

Cells were harvested and counted, and a fixed number of cells were plated into 6-well tissue culture plates with conditioned media (CM) collected from co-culture of THP-1 (2.5×10⁵) and BFTC 909 or 7630 (5×10⁵) cells with different AR status. After 10-day incubation at 37°C in a humidified atmosphere containing 5% CO₂, cell monolayers were washed with saline, fixed with 100% methanol, and stained with 0.5% Crystal violet. Colonies, defined as being >50 cells, were counted under microscope.

Wound-healing migration assay

BFTC 909 or 7630 cells were seeded onto 35-mm plates until confluent. And the plates were scratched using a sterile pipette tip to generate a wound through the confluent monolayer. Cells were photographed at 0 h and 24 h after incubation of CM. The relative migration was calculated by setting the percentage of wound closure in control cells after 24 h as 1.

Transwell invasion assay

Cell invasion through a three-dimensional extracellular matrix was assessed by a Matrigel invasion assay using BD Matrigel coated-transwells with 8.0 μm filter membranes. BFTC 909 or 7630 cells resuspended in 200 μL of serum-free medium were plated onto each filter, and 750 μL of CM was added into the lower compartment of invasion chambers. After 24 h, filters were washed, fixed with 4% paraformaldehyde and stained with 1% crystal violet. Cells that had invaded to the lower surface of the filter were counted under the microscope.

Cytokine array

Conditioned media collected from co-culture of UUTUC and THP-1 cells were analyzed by Human Cytokine Array Panel A (R&D Systems, Minneapolis, MN) according to the manufacturer’s protocol.

RNA extraction and quantitative real-time PCR analysis

Total RNA was isolated using Trizol reagent (Invitrogen, Carlsbad, CA) according to the manufacturer’s instructions from THP-1 cells (in upper well) and BFTC cells (in bottom well) co-cultured in a transwell with 0.45 uM filter which blocked cell infiltration, but allowed medium exchange. One μg of total RNA was subjected to reverse transcription using Superscript III transcriptase from Invitrogen. Quantitative real-time PCR (qRT-PCR) was conducted using a
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Bio-Rad CFX96 system with SYBR green to determine the level of mRNA expression of a gene of interest. Expression levels were normalized to the expression of β-actin RNA.

Immunohistochemistry (IHC)

Immunohistochemistry was performed on paraffin embedded sections (5 μm thick). Paraffin sections were dewaxed and rehydrated.

Cell xenografts

For mouse subcutaneous xenograft model, Nude mice (BALB/cAnN.Cg-Foxn1nu/CrlNarl purchased from National Laboratory Animal Center (Taipei, Taiwan) were divided into two groups. One group was injected with BFTC pWPI cells and one was injected with BFTC hAR cells. Each animal was injected (5×10⁶ cells per injection) subcutaneously in flank sides of the mice. Animals were sacrificed and tumors were removed 8 weeks after implantation. For mouse orthotopic xenograft model, male BALB/cAnN. Cg-Foxn1nu/CrlNarl mice (5-6-week-old) were randomized into two groups for BFTC pWPI cells and BFTC hAR cells separately (n = 20). Tumor cells were harvested from 70% to 80% confluent cultures and resuspended in PBS. Mice were anesthetized by isoflurane (2.5%). A small lower dorsal incision on the left flank was made and kidney was exposed. Cells were injected into the pelvis wall muscle using sterile syringe with a 30 gauge needle. After injection, the incision was closed with suture. Mice were sacrificed 8 weeks after implantation. The kidneys were removed and the tumors were imaged with Xenogen system (Perkin Elmer, CA, USA) for their fluorescence intensity since the cells were stably transfected with GFP gene. The harvested kidneys were preserved in paraffin for later histologic diagnosis and IHC with anti-CCL5

Figure 1. UUTUC cells induce macrophage infiltration: (A) IHC staining with an anti-CD68 antibody to detect infiltrating macrophages in human UUTUC tumor of ureter and adjacent normal tissue (brown-staining, black arrows) Scale bar = 50 μm, 400× magnification. (B) Quantification of IHC-stained CD68+ macrophage cell counts in tissue at the tumor part and non-tumor parts (n = 9). (C) Schematic illustration of the THP-1 cells migration assay (upper panel). THP-1 cells (1×10⁵) were seeded on the upper chamber and BFTC 909 or 7630 cells were seeded in the bottom wells to assay the migration of THP-1 cells. After 16 hours, the bottom sides of insert wells were fixed and stained to visualize the migrated THP-1 cells. The total number of migrating THP-1 cells was calculated by manually counting four independent fields under the microscope. The results were shown as the mean ± SD (*P<0.05 vs controls).
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Figure 2. Conditioned media (CM) from BFTC 909 or 7630 co-cultured with THP-1 cell culture increases cells growth, migration and invasion: (A) Schematic illustration of experiments on the effects of BFTC 909 and THP-1 cells culture conditioned media (CM) on BFTC 909 cells colony formation growth, migration and invasion (upper panel). CM were collected from THP-1 cells only or BFTC 909-THP-1 cells or 7630-THP-1 cells co-culture. And CMs were used on BFTC 909 or 7630 cells to perform colony formation growth assay (B), wound healing migration assay (C) and transwell matrigel invasion assay with 8 µm size pore membrane coated with matrigel (D). The results were shown as the mean ± SD (*P<0.05 vs controls).

and anti-MMP9 antibodies. Protocols for animal care and experimentation were approved by the animal care and use committee of the China Medical University, Taichung, Taiwan.
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Statistical analysis

The data values were presented as the mean ± SD. P values were determined by paired Student’s t test using Microsoft excel software. P<0.05 was considered statistically significant.

Results

UUTUC cells increases macrophage infiltration

To investigate the functional consequences of the heterotypic interactions between macrophages and UUTUC cells, we applied the CM from co-culture of THP-1 cells and UUTUC cells

Figure 3. The status of AR in BFTC 909 or 7630 cells affects macrophage migration; (A) Schematic illustration of the THP-1 cell migration assay with BFTC hAR or BFTC pWPI cells cultured in the bottom well. (B) BFTC hAR and BFTC pWPI cells or 7630 hAR and 7630 pWPI cells were cultured respectively in the bottom well to attract THP-1 cells in the upper well in the transwell migration assay. (C) IHC staining of infiltrating macrophages (brown-staining, black arrows) with antibodies against F4/80, the specific cell-surface marker for murine macrophage, in BFTC pWPI or BFTC hAR cell xenograft tumors in nude mice. Scale bar = 50 μm, 400× magnification. (D) Quantification of IHC-stained F4/80+ macrophage cell counts in tissues from BFTC pWPI or BFTC hAR cell xenograft tumors in nude mice. (n = 4). The results were shown as the mean ± SD (*P<0.05 vs controls).

Infiltrated macrophages enhance UUTUC cell growth, migration and invasion

We then confirmed the human clinical data with in vitro co-culture system of UUTUC BFTC 909 or 7630 cells. The human THP-1 cell line is a monocyte-like cell line derived from a patient with acute monocytic leukemia, which resemble primary monocytes and macrophages in morphology and differentiation property [18] and was shown to turn into M2-like type as TAMs [19]. BFTC 909 or 7630 cells were derived from UUTUC of renal pelvis patient [16, 17]. BFTC 909, or 7630 cells were seeded on the lower chamber of transwell and THP-1 cells were placed on upper chamber. The total number of migrating THP-1 cells passing through the membrane filter to the underside of the inserts was calculated by manually counting four independent fields. The result showed that BFTC 909 or 7630 both cells have the capacity to recruit macrophages (Figure 1D).
AR enhances UUTUC cells capacity to recruit more macrophage THP-1 cells

Recent studies suggested that AR might play important roles to influence the UUTUC cells invasion and stem cell population [14, 15]. We were interested to see if AR also plays an important role in the UUTUC cells-enhanced macrophages recruitment and their influences on UUTUC cells. We found that BFTC hAR cells or 7630 hAR increased their capacity to recruit macrophages than BFTC pWPI or 7630 pWPI control cells (Figure 3A).

To further confirm the above in vitro cell line data in the in vivo mouse model, we examined the amount of infiltrated macrophages in BFTC cell subcutaneous xenograft tumors in BALB/c nude mice which have functional macrophages even though lacking a thymus to produce T-cells resulting in them being with immuno-compromised [20]. The IHC result staining the macrophage marker F4/80 showed that BFTC hAR cell xenograft tumors had more infiltrating macrophages than BFTC pWPI cell xenograft tumors (Figure 3B, 3C).

**AR promotes the macrophages-enhanced UUTUC cells growth, migration and invasion**

We then manipulated AR expression in the co-culture system via either addition of functional AR (in UUTUC cells) or knocking-down AR (in THP-1 cells) to study if AR could also influence the macrophages-enhanced UUTUC cell growth, migration and invasion. We incubated BFTC 909 cells with the CM collected from co-culture of BFTC 909 cells and THP-1 cells with different AR status and results revealed that higher AR expression in both UUTUC cells and macrophage THP-1 cells increased the BFTC 909 cell growth (Figure 4).
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Figure 5. The cytokine profiles in the co-culture medium of BFTC cells and THP-1 cells: (A) Cytokine array of different conditioned media (CM) of THP-1 scramble (scr) cells (plus AR) + BFTC 909 pWPIhAR cells (plus AR); THP-1 scramble (scr) cells (plus AR) + BFTC 909 pWPI cells (minus AR); and THP-1 silenced AR (shAR) cells (minus AR) + BFTC 909 pWPI cells (minus AR) were collected after 48 h incubation. Q-RT-PCR of target cytokines was performed on THP-1 cells or BFTC 909 cells with different AR status to examine the mRNA expression of CCL5/RANTES (B), Serpin E1 (C), GROα (D), and MIF (E). (F) IHC staining of CCL5 expressing (brown-staining, black arrows) cells in BFTC pWPI or BFTC hAR cell xenograft tumor in nude mice. Scale bar = 50 μm, 400x magnification. (G) Quantification of IHC-stained CCL5+ cell counts in tissues from BFTC pWPI or BFTC hAR cell xenograft tumors in nude mice. (n = 4). The results were shown as the mean ± SD (*P<0.05 vs controls).
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Figure 6. Neutralization of CCL5 by a specific antibody attenuates macrophage-induced growth, migration and invasion: Anti-CCL5 neutralizing antibody or isotype control antibody (1 µg/ml) was added into co-cultured conditioned media (CM) from THP-1 silenced AR (shAR) cells (minus AR) + BFTC 909 pWPI cells (minus AR) or THP-1 scramble (scr) cells (plus AR) + BFTC 909 pWPihAR cells (plus AR) to assay the effect of CM on BFTC 909 cells growth (A), migration (B) and invasion (C). The results were shown as the mean ± SD (*P<0.05 vs controls).

Mechanism dissection on how AR promotes the macrophages-enhanced UUTUC cells growth, migration and invasion

The aforementioned observations suggest that macrophages supply locally acting paracrine cues that induce the growth, migration and invasion of UUTUC cells and AR in UUTUC cells or macrophages could affects these interactions. To understand the mechanism behind this crosstalk better, their conditioned media from in vitro co-cultures of UUTUC cells and macrophages and were screened for the levels of various cytokines, chemokines and growth factors using western blot-based cytokine array analysis to globally search for inflammatory cytokines to further dissect the potential molecular mechanism(s). We found the presence of AR in both BFTC 909 cells and THP-1 cells led to significantly increased expression of several cytokines/chemokines including GROα, MIF, Serpin E1 and CCL5 (Figure 5A). Early studies indicated that Serpin E1/PAI-1 might play key roles to mediate the mesenchymal stem cell-colon cancer cell interactions [21], and GROα was initially isolated and characterized by its growth stimulatory activity on malignant melanoma cells [22]. MIF was reported to play positive roles to promote the prostate cancer DU-145 cell growth and invasion [23], and CCL5 might play a key role in inducing tumor cell migration and invasion [24, 25].

We then confirmed those altered cytokines/chemokines at their mRNA level in THP-1 cells or BFTC cells using qRT-PCR in transwell co-culture system that the two cell populations were separated by a permeable membrane to separate two cell populations apart, but allow media exchange. The qPCR results revealed the AR in both BFTC 909 cells and THP-1 cells significantly induced the expression of CCL5 (Figure 5B), Serpin E1 (Figure 5C), GROα (Figure 5D) and MIF (Figure 5E).

Among these cytokines/chemokines modulated by AR in the co-cultured UUTUC cells and macrophages, we found CCL5 was mainly from the THP-1 cells, and early studies indicated that CCL5 might play a key role in inducing tumor cell migration and invasion [24-26]. The results from IHC staining on CCL5 expression confirmed that BFTC-909 AR cell xenograft tumors had higher CCL5 expression than BFTC pWPI cell tumors (Figure 5F, 5G).

Importantly, when we applied the interruption approach to further verify CCL5 roles to mediate AR-promoted macrophages-enhanced UUTUC cell growth, migration and invasion, we found that addition of anti-CCL5-neutralizing antibody in CM of BFTC 909/THP-1 cells co-
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Cultures partially reversed the AR-promoted macrophage-enhanced UUTUC cell growth (Figure 6A), migration (Figure 6B) and invasion (Figure 6C).

Figure 7. BFTC hAR cells have higher tumor formation and progression in the orthotopic xenograft nude mouse model. A. Tumor incidence for BFTC hAR cells and BFTC pWPI cells. 5x10^5 cells were injected intramurally into the pelvis of left kidney. B. Representative fluorescence images of kidney of mice bearing the tumors (green color). C. The fluorescent intensity of xenograft tumors of mice implanted with BFTC hAR cells is expressed as fold increase over the control group. D. Representative haematoxylin-and-eosin-stained sections of kidney of mice bearing the tumor delineated by a dashed line. E. CCL5 expression on xenograft tumors. F. MMP9 expression on xenograft tumors. G. Western blot analysis of lysates of BFTC hAR cells and BFTC pWPI cells for modified histones. β-Actin was used as a loading control. The results were shown as the mean ± SD (*P<0.05 vs controls).
AR facilitates the growth of orthotopically xenograft UUTUC tumors in nude mice

To assess whether the effects of AR demonstrated in our in vitro cell-based experiments are present in in vivo native environment where we could evaluate authentic stromal-epithelial interactions, we then examined the roles of AR on UUTUC tumor formation in nude mice by orthotopically injecting $5 \times 10^5$ BFTC pWPI or BFTC hAR cells into the muscle walls of left renal pelvis. The orthotopic xenograft model approach we performed could let the tumor form at the site where provides abundant blood supply and a native tumor environment for tumor formation and spread to represent a muscle-invasive UUTUC tumor model. After 8 weeks, we examined the tumor formation and found that the tumor incidence (Figure 7A) was higher and tumors invaded more into renal parenchyma in BFTC hAR tumor xenograft (Figure 7B, 7C). The HE staining also showed that the tumor size of BFTC hAR cell xenografts were larger than those of BFTC pWPI cells (Figure 7C, 7D). IHC staining of CCL5 expression was higher in BFTC hAR cell xenograft tumors (Figure 7E). The protein involved in tumor cell migration and invasion such as MMP9 also was demonstrated that higher expression in BFTC hAR cell xenograft tumors (Figure 7F). Furthermore, because the epigenetic state of cancer cells could be responsible for affecting and be affected by the convergence of multiple signals that a cell gives to and receives from its nearby microenvironment [27], we then tested whether the UUTUC cell chromatin state was affected by AR, and interestingly, we found that the histone modifications in cancer cells were affected by AR, BFTC-AR cells exhibited higher levels of H3K4Me3 and H3K9Ac, “active” marks for transcriptionally expression genes but exhibited lower level of H3K9Me3, “repressive” mark for transcriptionally silenced genes without changing total H3 protein (Figure 7G).

Discussion

The potential impacts of these findings showing UUTUCs may recruit more macrophages to enhance UTTUC progression, modulated by AR-CCL5 signaling are: First, this demonstrates the important roles for the macrophages that exist in the TME surrounding the UUTUCs. Second, this shows that AR can affect the macrophage infiltration and can impact the UUTUC progression. And finally, these findings indicate that AR is the key player in the UUTUC progression and may become a potential new therapeutic target to treat UUTUC.

The progression of UUTUC, like other cancers is often accompanied by well-orchestrated events within tumors and surroundings, which involves the recruitment of a variety of stromal cells with both pro-tumorigenic activities through the release of various autocrine and paracrine growth factors and cytokines. Here we identified CCL5 as a key mediator in mediating the AR-enhanced UUTUC cell growth and invasion. An early study indicated that CCL5 secreted in TME might have the capacity to promote macrophage recruitment [28], and CCL5 might also be able to influence the tumor cell migration and invasion [24, 25]. In the study of sub-clonal heterogeneity within breast tumors, increased growth of CCL5-driven tumors was attributable to cell-autonomous expansion of CCL5-expressing cells [29]. Our results showing CCL5 may be able to mediate AR function in UUTUCs suggesting targeting CCL5 may become another therapeutic target to battle UUTUCs. Indeed, increased CCL5 in tissue or plasma has been linked to poor outcomes in breast, prostate or cervical cancer patients [30-32], and applying the CCL5 neutralization antibodies or CCR5 antagonists has suppressed the mesenchymal stem cells-induced metastasis [26]. A natural product CCR5 antagonist, anibamine was demonstrated to suppress prostate cancer cell growth, invasive and metastatic properties in mice [33].

Furthermore, recent data from tumor genome sequencing studies have not revealed substantial genetic alterations for invasive and metastatic properties of cancer cells [34, 35], and therefore, it is possibly that these properties are acquired through exposure of epithelial cancer cells to paracrine signals that they receive from stroma cells types within tumor microenvironment and the epigenetic changes in cancer cells are the major driving forces for malignant progression to either providing growth factors or cytokines to the microenvironment and receiving the cues from the microenvironment [7]. Our result (Figure 7G) showing the pattern of modified histone protein switched
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into more active chromatin state by AR, suggesting AR could play a role in affecting unstable chromatin state created by frequently mutated chromatin regulatory genes in urothelial carcinoma [35] within UUTUC cells and induce a cell state for secreting cytokines or growth factors (like GROα, MIF, and Serpin E1, which were shown to be increased in AR overexpression UUTUC cells, Figure 5C-E) to build a pro-tumor microenvironment via macrophages with increased cytokines (like CCL5, Figure 5B). This epigenetic regulatory mechanism of AR for broadly influencing the cell state could represent a novel action of AR to affect cancer progression. However the detailed mechanism and the extent of its action need to be further clarified.

In conclusion, our results identify the AR-CCL5 signaling axis is a key regulator during UUTUC progression through the modulation of infiltrating macrophages and inflammatory cytokines. Future studies using new mouse models that can spontaneously develop the UUTUCs may help us to develop the new therapeutic approach using anti-AR-CCL5 signals to better battle the UUTUCs.

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Disclosure of conflict of interest

None.

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References


[13] Hsieh TF, Chen CC, Yu AL, Ma WL, Zhang C, Shyr CR and Chang C. Androgen receptor decreases the cytotoxic effects of chemothera-
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