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Anabolic androgens affect the competitive interactions in cell migration and adhesion between normal mouse urothelial cells and urothelial carcinoma cells

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ABSTRACT

The urothelium is constantly rebuilt by normal urothelial cells to regenerate damaged tissues caused by stimuli in urine. However, the urothelial carcinoma cells expand the territory by aberrant growth of tumor cells, which migrate and occupy the damaged tissues to spread outside and disrupt the normal cells and organized tissues and form a tumor. Therefore, the interaction between normal urothelial cells and urothelial carcinoma cells affect the initiation and progression of urothelial tumors if normal urothelial cells fail to migrate and adhere to the damages sites to regenerate the tissues. Here, comparing normal murine urothelial cells with murine urothelial carcinoma cells (MBT-2), we found that normal cells had less migration ability than carcinoma cells. And in our co-culture system we found that carcinoma cells had propensity migrating toward normal urothelial cells and carcinoma cells had more advantages to adhere than normal cells. To reverse this condition, we used anabolic androgen, dihyrotestosterone (DHT) to treat normal cells and found that DHT treatment increased the migration ability of normal urothelial cells toward carcinoma cells and the adhesion capacity in competition with carcinoma cells. This study provides the base of a novel therapeutic approach by using anabolic hormone-enforced normal urothelial cells to regenerate the damage urothelium and defend against the occupancy of carcinoma cells to thwart cancer development and recurrence.

1. Introduction

Cancer cells have been described as "like a ruthless group of gangster running amuck the orderly functioning tissue of human body" [1]. Following this analogy, the current cancer treatment is like to eliminate gangsters from the human society. No matter how extensively the therapeutic strategy is, the cancer cells find a way to escape from attacks and evolve a new form to thrive by complex interconnecting genetic and signaling pathways [2,3].

In mammals, cell proliferation and differentiation are required for regenerate and repair the injured tissues, which are linked to cellular metabolism since the proliferation and differentiation of cells all require increased of biomass [4]. The essential hallmarks of cancer are intertwined with an altered cancer cell-intrinsic metabolism, either as a consequence or as a cause, which consequently grants cancer cells with more anabolic reactions over normal cells, resulting in the ability to thrive and metastasize in tissues [5,6]. The constitutive activation of signaling cascades in cancer cells, which stimulate cell growth has a profound impact on anabolic metabolism [7]. The metabolic programming in cancer cells has a dramatic effects to give cancer cells more efficient bioenergetic flow and anabolic reactions than normal cells, allowing cancer cells to exhibit their neoplastic phenotypes to grow and expand in normal tissues once the tissues are under stress from physical, chemical or biological agents [8]. Therefore, we need stronger cells to win over cancer cells since the higher anabolic reaction in cancer cells gives them advantages over normal cells, causing normal cells lose the battle in the past. Therefore, we need to enforce anabolic reactions in normal cells to strengthen the cells for overpowering the cancer cells. To do so, we need to change the

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cell metabolism. One of the master regulators of metabolism and growth is the anabolic androgens, which stimulate anabolic processes that convert nutrients and energy into macromolecules, including protein, lipid and nucleic acids [9]. To demonstrate anabolic androgens could increase normal cell migration and adhesion in competition with cancer cells, we selected the normal urothelial cells and urothelial carcinoma cells our experimental model.

The urothelium, composed of specialized epithelium lining the distal portion of the urinary tract which comprises the renal pelvis, ureters, urinary bladder and urethra. Urothelium has a remarkable regenerative capacity to repair tissue damage and restore urothelial integrity because urothelial cells can rapidly proliferate and differentiate under stimulus [10,11]. That is because that, in the urothelium, there are normal adult stem cells within basal cells and intermediate cells, which are responsible for renewing the tissue during normal homeostasis and regenerating the urothelium after pathological damage [10–12].

Urothelial carcinoma (UCa) arising from urothelium which lines the urethra, bladder, ureters, and renal pelvis is the most common malignancy of the urinary tract, comprising 90–95% of bladder cancer [13]. Superficial bladder UCa are treated by surgical resection and intravesical (within the bladder) immunotherapy and the 5-year survival rate approaches 90%, but for invasive bladder cancer, with radical cystectomy and systemic therapy, at least 50% of patients still die from metastases within 2 years of diagnosis and the treatment fails in 95% of patients with less than 10% 5-year survival rate for the metastatic bladder cancer [13]. The current therapy on bladder cancer has combined surgery, chemotherapy and immunotherapy, but the recurrence rate is high and once metastasis has occurred there is no effective treatment [14,15]. Therefore, to extend the patients' live and improve living quality, novel therapies are needed to prevent the high recurrence rate and reduce the need for cystectomy to minimize patient suffering from chemotherapy and economic burden due to this deadly urothelial tumor. The use of normal urothelial cell to replace carcinoma cells in tissues could be a novel approach to treat urothelial tumors.

In this study, we examined the competitive interaction between normal murine urothelial and urothelial carcinoma cells in migration and adhesion and investigate whether anabolic androgens could enhance the migration and adhesion of normal cells in competition with carcinoma cells.

2. Materials and methods

2.1. Primary culture of normal murine urothelial cells (NMU) from renal pelvis urothelium and cell culture

To harvest urothelial cells, we isolated the cells directly from the urothelium from renal pelvis. For the urothelial removal, the pelvis were immersed in collagenase IV (100 units/ml) at 37 °C on a shaker for 75 min. After the incubation in collagenase IV, the pelvis were placed in a 60-mm Petri dish containing in DMEM containing with 10% fetal calf serum (FCS), 100 units/ml penicillin and 100 μg/ml streptomycin on ice or 4 °C. The urothelial cells were gently scraped with a scalpel blade from the urothelium, collected, washed and added into 15 ml tube, and filtered them through a 70 μm filter. The cells were then centrifuged and seeded into 10 cm dish with DMEM containing with 10% fetal calf serum (FCS), 100 units/ml penicillin and 100 μg/ml streptomycin. MBT-2 cells, a murine urothelial carcinoma cell line, were cultured in DMEM supplemented with 10% fetal calf serum (FCS), 100 units/ml penicillin and 100 μg/ml streptomycin.

2.2. Cell proliferation assay

NMU cells were seeded in 96 well plates (5×10^3/well). Cellular proliferation was measured by the Cell Viability kit (XTT) (Roche Diagnostics, Indianapolis, IN, USA). The cellular proliferation was determined by the colorimetric XTT assay based on the activities of mitochondrial enzymes in viable cells follow manufacturer's instructions.

2.3. CD44 analysis

The expression profiles of CD44 in cultured cells were analyzed by flow cytometry. 5×10^5 cells were incubated with 100 μl of 5% BSA in PBS for 30 min on ice, and then labeled with APC-conjugated anti-CD44 (0.03 μg) (eBioscience, San Diego, USA) for 30 min in the dark. Labeled cells were re-suspended in PBS, and analyzed by a flow cytometer (BD LSR II).

2.4. Western blot analysis

Cell lysates were resolved by sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS–PAGE), transferred to a nitrocellulose membrane and incubated with specific primary antibodies: cytokeratin 14 antibody (Santa Cruz, Santa Cruz, CA, USA). Protein bands were visualized using horseradish peroxidase (HRP)-conjugated secondary antibodies and enhanced chemiluminescence reagent (Millipore, Bedford, MA, USA) with the Bio-Rad imaging system.

2.5. Transwell migration assay

Cells were stained with CFDA-SE (Molecular Probes, Eugene, OR, USA) before add into 8.0-μm filter upper chamber. Cells re-suspended in 250 μl of serum-free medium were plated onto each filter, and 650 μl of DMEM containing 10% FBS were added into the lower compartment of the migration chambers. After 24 h, filters were washed in 4% formaldehyde. Cells on the upper surface of the filters were removed with cotton swabs. Cells that had migrated to the lower surface of the filter were counted under the fluorescent microscope.

2.6. Luciferase reporter gene assay

NMU cells at 50–60% confluence in 24-well plates were co-transfected with 250 ng of ARE-luc reporter plasmid DNA and 2.5 ng of pRL-TK-luc plasmid DNA, using lipofectamine 2000 (Invitrogen, Carlsbad, CA, USA). After 24 h of transfection, the cells were treated with DHT for 24 h. Cells were then harvested, lysed and assayed for luciferase activity, which was determined using a Dual-Luciferase Reporter Assay kit according to the manufacturer's instructions (Promega, Madison, WI, USA) with a luminometer.

2.7. Cell adhesion assay

NMU cells were stained with 4 μM CFDA-SE (Molecular Probes, cat. no. C-1157), and then seeded with MBT-2 cells (NMU: MBT-2 = 1:1) into 6 cm plate. After 3 h co-culture, the adhered cells were collected and analyzed by a flow cytometer (BD LSR II) to assay cell proportion (CFDA-SE positive NMU cells or non-labeled MBT-2 cells).

2.8. Statistical analysis

Data are expressed as mean ± SD from at least three independent experiments. Microsoft® Office Excel 2003) were used for data processing and analyses. Results were analyzed using two-tailed