Biochemical and Biophysical Research Communications 452 (2014) 322–327

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

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

Chi-Ping Huang [a], Teng-Fu Hsieh [a,b], Chi-Cheng Chen [a,b], Xiao-Fan Hung [a], Ai-Lin Yu [a], Chawnshang Chang [a,c], Chih-Rong Shyr [a,*]

[a] Sex Hormone Research Center, Departments of Urology and Medical Technology, Graduate Institute of Clinical Medical Science, China Medical University/Hospital, Taichung 404, Taiwan
[b] Department of Urology, Taichung Tzu Chi Hospital, Buddhist Tzu Chi Medical Foundation, Taichung, Taiwan
[c] George Whipple Lab for Cancer Research, Departments of Pathology, Urology and Radiation Oncology, University of Rochester Medical Center, Rochester, NY, United States

ARTICLE INFO

Article history:
Received 28 July 2014
Available online 23 August 2014

Keywords:
Androgen
Anabolic
Urothelial cell
Urothelial carcinoma cell
Migration
Adhesion

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

* Corresponding author. Address: Sex Hormone Research Center, China Medical University Hospital, Cancer Center Building, 6 Hsiuh-Shih Rd, Taichung 40454, Taiwan. Fax: +886 4 22057414.
E-mail address: chshyr@mail.cmu.edu.tw (C.-R. Shyr).

http://dx.doi.org/10.1016/j.bbrc.2014.08.039
0006-291X/

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

paired *t*-tests. A *P*-value < 0.05 was considered to be statistically significant.

3. Results

3.1. The culture and characterization of normal murine urothelial (NMU) cells

To determine the interaction between normal and carcinoma cells, first, we have developed a protocol to isolate and expand normal urothelial cells from male mouse urothelium with specialized culture conditions. For cancer cell part, we use murine bladder cancer cells: MBT-2 cells. MBT-2 cells are invasive urothelial carcinoma cells derived from female mouse bladder tumor induced by bladder carcinogen FANFT, which were poorly differentiated [16]. Our culture protocol has been successful to grow normal murine urothelial (NMU) cells from primary culture of urothelium (Fig. 1A). NMU cells are in proliferative status with cell doubling time around 40 h after 10 passages of cells (Fig. 1B). Therefore, we believed in our culture condition, NMU cells may contain stem cells and basal cells to acquire their proliferation ability within. To verify the stem cell-like characteristic of NMU cells, we determined the expression of urothelial stem cell marker, CD44 [11] by flow cytometry and examined the expression of Cytokeratin 14 (CK14) which was identified as a more primitive keratin marker as basal-type [11]. MBT-2 cells were also examined. We found that CD44 expression was detected in over 90% population of NMU and MBT-2 cells (Fig. 1C), suggesting the stem cell-like population is present in NMU cells and MBT-2 cells. Using Western blotting to detect the CK14 protein expression, we found that CK14 had higher expression in NMU cells compared to MBT-2 cells, indicating that NMU cells may contain more basal cell-like population than MBT-2 cells (Fig. 1D). The cell maker information about NMU cells and MBT-2 cells with similar expression pattern may explain the origin of cancer cells, which tells that cancer cells arise from stem cells in the urothelium with less differentiation properties (lower CK14 expression in MBT-2 cells).

3.2. Higher migration and adhesion capacity in carcinoma cells

Urothelial carcinoma cells exhibit migration capacity to spread out and form multifocal tumors in the urothelial tract [13]. Therefore, there are competition to the injured sites between normal urothelial cells and urothelial carcinoma cells to either repair the tissues or the cancer rises. To understand the migration capacity and the interaction between normal urothelial cells and urothelial carcinoma cells, we tested the migration ability of NMU cells and MBT-2 cells with transwell migration assay using carboxyfluorescein diacetate succinimidyl ester (CFDA-SE) fluorescent dye for cell labeling to measure the migration ability of NMU and MBT-2 cells. CFDA-SE is a lipophilic molecule that has only minimally fluorescence, but when transported inside cells, it becomes markedly fluorescent after esterase cleaves the acetyl groups of the molecule, which makes CFDA-SE an ideal cell labeling dye to monitor live cells. Using transwells, we placed labeled cells on upper wells and added serum medium on bottom well and let the cells migrate through porous membranes for 24 h and counted the cells through the membranes. The result showed that MBT-2 cells have better migration ability (Fig. 2A). And further investigation on the migration ability of NMU and MBT-2 cells toward each other in transwell assays with different cell placements with CFDA-SE labeled cells on upper wells and counted the total migrating cells into the bottom wells with flow cytometry. The result (Table 1) showed that less NMU cells moved toward to MBT-2 cells in the bottom well. More

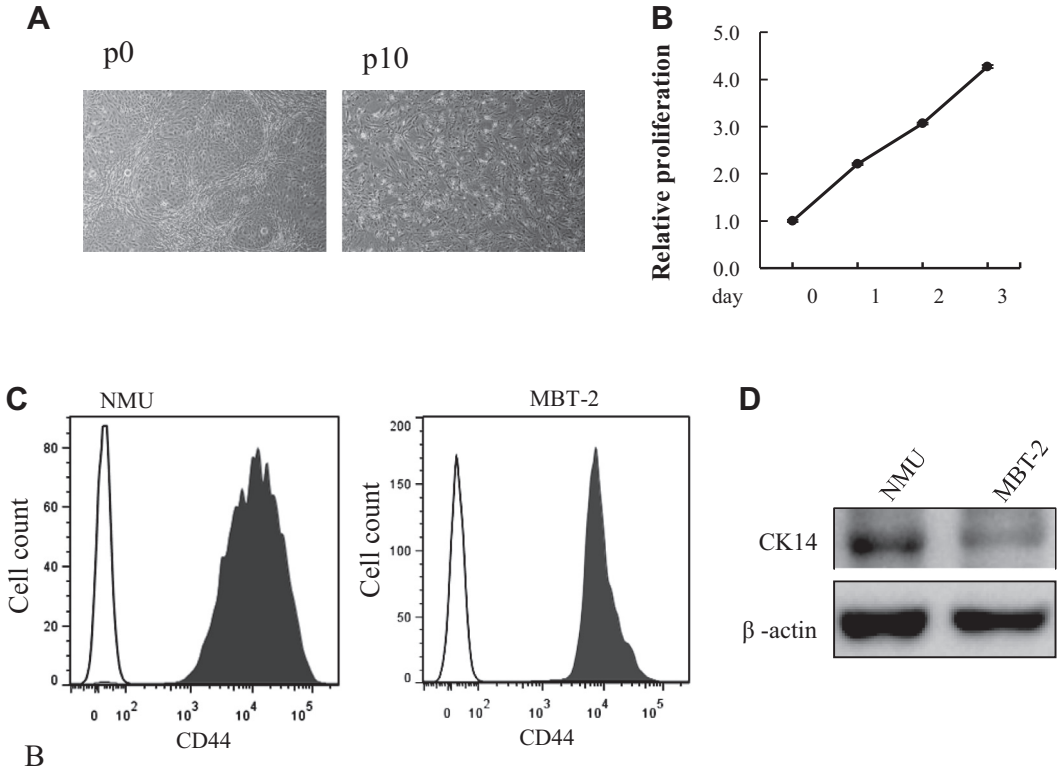

Fig. 1. The morphology, growth and cell phenotypes of normal murine urothelial (NMU) and MBT-2 cells. (A) Microscopy imaging of passage 0 (p0) and passage 10 (p10) NMU cells. At p10, most cells were short and spindle-shaped. (40× magnification). (B) The cell proliferation of NMU cells was assayed by XTT using 10 passage cells. The cell doubling time was calculated as 40 h. (C) Urothelial stem cell marker CD44 expression on NMU and MBT-2 cells (solid line: isotype control; filled peak: CD44) was analyzed by flow cytometry. (D) Urothelial basal cell maker CK14 expression in NMU and MBT-2 cells was by measured by Western blotting. β-Actin was used as a loading control.

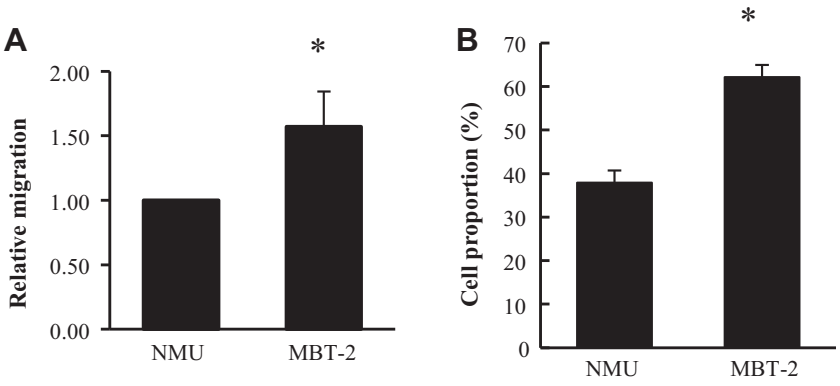

Fig. 2. Cell migration and adhesion capacity of NMU an MBT-2 cell. (A) NMU cells or MBT-2 cells were stained with CFDA-SE and the cell migration was evaluated by transwell migration assay. The cells migrated through the filter, staying on the back side of filter were photoed in a fluorescence microscope (with fluorescent labeling) and counted (middle panel). The quantitative result of cell relative migration of NMU and MBT-2 cells is calculated by the number of cells passed though the filter and set the number in NMU cells as one-fold. (B) CFDA-SE labeled NMU cells were mixed with MBT-2 cells with 1:1 ratio and placed on a 6 cm well and allowed cell to attach on the surface for 3 h. And the attached cells were analyzed by flow cytometry to determine the proportion of adhered cells. Data are expressed as Mean ± SD from three independent experiments ($n = 3$) ($^*P < 0.05$ vs control).

Table 1
The interactive migration of NMU and MBT-2 cells. The cells labeled with CFDA-SE in the upper well migrated through filter to the bottom well were analyzed by the flow cytometry on the total cells collected from suspension and attached cells.

Group		Cell	CFDA-SE positive cell (%) in bottom well	SD (%)
1	Upper well	NMU	1.5	0.6
	Bottom well	MBT-2		
2	Upper well	NMU	13.6	3.6
	Bottom well	NMU		
3	Upper well	MBT-2	20.7	4.8
	Bottom well	MBT-2		
4	Upper well	MBT-2	55.7	11.3
	Bottom well	NMU		

NMU cells moved toward NMU cells in the bottom wells. In the other hand, when placed MBT-2 cells in the upper well, more MBT-2 cells moved toward NMU cells in the bottom wells and less MBT-2 cells moved toward MBT-2 in the bottom well. These results suggest that carcinoma cells might have a better capacity to migrate than the normal urothelial cells and normal urothelial cells have the propensity not to migrate toward the site where urothelial carcinoma cells locate, but on the contrary, urothelial carcinoma cells have the propensity to move to the normal the anabolic normal urothelial cells. Cell adhesion is a complex mechanism involved in a variety of biological processes including regeneration [17]. The stronger ability to adhere to the surface than urothelial carcinoma cells will be another key step for normal urothelial cells to regenerate injury and prevent cancer cells occupying and control the injury sites for their growth. As mentioned before, with higher anabolic reactions in cancer cells, cancer cells have higher adhesive ability than normal cells. Indeed, in our result, equal amount of NMU and MBT-2 cells seeded on to a plate, 3 h after, the attached cells were collected and analyzed by flow cytometry, MBT-2 cells were shown to adhere to the surface more than NMU cells (Fig. 2B).

3.3. Anabolic androgens increased the migration and adhesion ability of NMU cells in competition with carcinoma cells

Since the migration and adhesion to the injury site is the first step for normal urothelial cells to repair the injured urothelium and prevent the urothelial carcinoma cells to take over, we then investigated whether we could reverse the advantages of urothelial carcinoma cells in migration and adhesion. The decisive step is to alter the anabolic status of NMU cells to increase their migration and adhesion capacity to move faster and toward urothelial carcinoma cells with anabolic hormones, so NMU cells could have the more opportunities accumulating in injury sites to repair the damages and block the rises of urothelial carcinoma cells. And for urothelial cells to regenerate the injured urothelium, the urothelial cells need to adhere to the injury sites. Androgens, mainly testosterone and dihydrotestosterone (DHT) are strong anabolic steroid hormone to affect cell proliferation and differentiation, which have been used in anabolic therapy to improve outcomes in patients with physical dysfunction associated with chronic illness or aging [18]. We have detected the expression of AR protein (Fig. 3A) and transactivational activity (Fig. 3B) of AR in NMU cells. Therefore, to increase migration ability of normal urothelial cells, we used the potent androgen, DHT to treat NMU cells and we found that NMU cells increased their migration capacity toward to MBT-2 cells (Fig. 3C), suggesting that anabolic steroid hormone DHT has the ability to enforce the migration ability of NMU cells. With enforced anabolic reactions in NMU cells to win over MBT-2 cells, NMU cells could migrate faster and toward MBT-2 cells in order to regenerate the urothelium and prevent urothelial carcinoma cells to take over. To enhance the adhesion ability of NMU cells, we also used anabolic androgens to boost anabolic reactions in NMU cells to increase protein synthesis, which may increase proteins essential for cell adhesion with the development of an optimal anabolic hormone regimen. The result showed that DHT increased NMU cell adhesion when in co-culture with MBT-2 cells to reverse the disadvantages of NMU cells to MBT-2 cell on the adhesion ability, further proving that by modulating the anabolic reactions of normal cells, normal cells could win over cancer cells on the attachment to the injury sites (Fig. 3D).

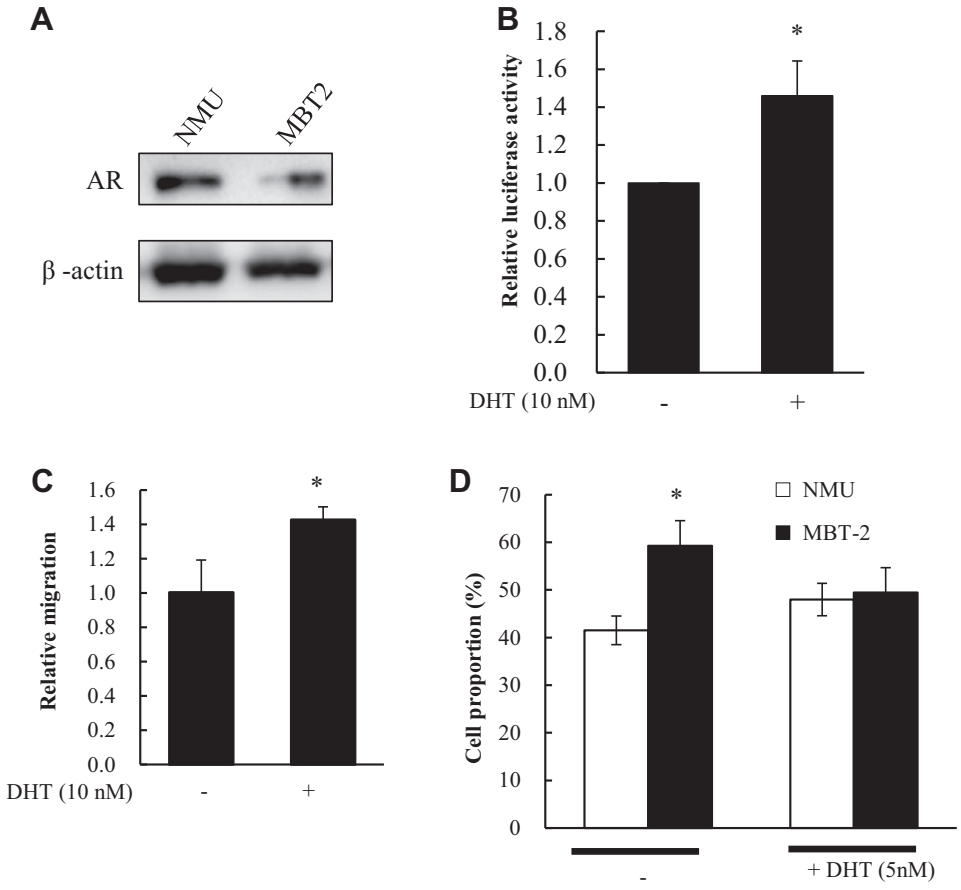

Fig. 3. The effect of anabolic androgen, DHT on NMU cell migration and adhesion in competition with MBT-2 cells. (A) The expression AR expression in NMU and MBT-2 cells was determined by Western blot. (B) The transactivation activity of AR in NMU cells was examined by luciferase reporter gene assay. NMU cells were transfected with ARE-luc plasmid and incubated for 24 h, and then treated with or without DHT for 24 h. And cell lysates were harvested for luciferase activity assay. (C) NMU cells were treated with anabolic androgen, DHT (10 nM). The cells in the upper well migrated through filter to the bottom well were analyzed by the flow cytometry on the total cells collected from suspension and attached cells in the bottom well. (D) NMU cells were treated with anabolic androgen, DHT (5 nM) for 7 days and then were mixed with MBT-2 cells with 1:1 ratio and placed on a 6 cm well and allowed cell to attach on the surface for 3 h. And the attached cells were analyzed by flow cytometry to determine the proportion of adhered cells. Data are expressed as Mean ± SD from three independent experiments ($n = 3$) (*$P < 0.05$ vs control).

4. Discussion

Cancer is a serious harm to human health. The prevailing idea is to find every way possible to kill cancer cells, therefore, surgery, immunotherapy, radiotherapy, chemotherapy, target therapy and/or the combinations of the above therapies are the principal means of treating cancer. And as the same time, we destroy the normal surrounding tissue and even remove the whole organs, bringing severe impact on the patient's quality of life with many adverse reactions, but still the cancer cells eventually come back and bring down the patient's whole system. In current therapy, we use all means to kill cancer cells and stop them from spreading with surgery, radiation, and anti-cancer drugs, but we miss out to explore the regenerative power of normal stem cells, which could replace the cancer cells in the lesion site and regenerate the tissue. Furthermore, in Virchow's hypothesis, he hypothesized that the origin of cancer was at sites of chronic inflammation because some classes of irritants, together with the tissue injury and ensuing inflammation they cause, enhance cell proliferation, which has been supported in current studies [19,20]. Based on the studies on inflammation and cancer, wound healing and tumor formation share many important properties and this similarity was phrased as "Tumors: wounds that do not heal" [21]. Indeed, many cancers arise from tissue injury sites due to infection, chronic irritation and inflammation, where cancer cells occupy and expand like gangsters take over the dysfunctional neighborhoods and develop into a gang to spread out. Therefore, the most effective way to prevent the rise of cancer should be to regenerate the injury sites with normal cells, not letting the cancer cells dominate the sites, proliferate and eventually metastasize. And then to regenerate the injury sites, normal cells need to migrate to the location where cancer cells also flock and compete with cancer cells to attach on the injury sites. These events manifest the key determining steps for normal cells to regenerate tissues not let cancer cells to takeover are more efficient and effective migration and adhesion of normal cells than those of cancer cells. In this study, we used anabolic androgens to enhance the anabolic reactions in normal cells, so that normal cells could compete with carcinoma cells to increase their migration and adhesion.

To regenerate damaged urothelium, urothelial cells divide and migrate from basal cell compartment to the surface for wound healing and restoration of function [22]. Urothelial carcinoma cells also exhibit migration capacity to spread out and form multifocal tumors in the urothelial tract [13]. Therefore, there are competition to the injured sites between normal urothelial cells and urothelial carcinoma cells to either repair the tissues or the cancer rises. Androgens, mainly testosterone and dihydrotestosterone (DHT) are strong anabolic steroid hormone to affect cell proliferation and differentiation, which have been used in anabolic therapy to improve outcomes in patients with physical dysfunction associated

with chronic illness or aging [18]. In our findings (Fig. 3A), we used the potent androgen, DHT to treat NMU cells and we found that NMU cells increased their migration capacity to MBT-2 cells suggesting that anabolic steroid hormone DHT has the ability to enforce the migration and of NMU cells. An optimal anabolic hormone regimen is worth of investigation to enforce anabolic reactions in normal cells to win over cancer cells, so that normal cells could migrate faster than carcinoma cells in order to regenerate the urothelium and prevent carcinoma cells to take over.

For urothelial cells to regenerate the injured urothelium, the urothelial cells need to adhere to the injury sites. Cell adhesion is a complex mechanism involved in a variety of biological processes including regeneration [17]. The stronger ability to adhere to the surface than urothelial carcinoma cells will be another key step for normal urothelial cells to regenerate injury and prevent cancer cells occupying and control the injury sites for their growth. As mentioned before, with higher anabolic reactions in cancer cells, cancer cells have higher adhesive ability than normal cells. Our result (Fig. 3B) showed that DHT increased normal urothelial cell adhesion when in co-culture with MBT-2 cells to reverse the disadvantages of normal cells to carcinoma cell on the adhesion ability, further proving that by modulating the anabolic reactions of normal cells, normal cells could win over cancer cells on the attachment to the injury sites to regenerate the tissues.

The upregulatory effects of anabolic androgens on the migration and adhesion capacities of normal urothelial cells could add more advantages in anabolic reactions to normal cells to compete with carcinoma cells. The findings in this study provide the in vitro evidences to prove that the movement and attachment of normal urothelial cells could be enforced by anabolic hormone to surpass urothelial carcinoma cells. It will pave the way for a new therapeutic approach to treat cancers.

Acknowledgment

This work was supported by the Grant from Ministry of Science and Technology, Taiwan (MOST 103-2314-B-303-009), China Medical University Grant (CMU102-BC-8), Buddhist Tzu Chi General Hospital Grant (TTCRD 102-10), and in part by Taiwan Ministry of Health and Welfare Clinical Trial and Research Center of Excellence (MOHW103-TDU-B-212-113002).

References

[1] R.R. Spencer, The problems of cancer biology, J. Am. Med. Assoc. 127 (1945) 509–514.
[2] B.A. Chabner, T.G. Roberts Jr., Timeline: chemotherapy and the war on cancer, Nat. Rev. Cancer 5 (2005) 65–72.
[3] A. Rehemtulla, The war on cancer rages on, Neoplasia 11 (2009) 1252–1263.
[4] R.J. DeBerardinis, J.J. Lum, G. Hatzivassiliou, C.B. Thompson, The biology of cancer: metabolic reprogramming fuels cell growth and proliferation, Cell Metab. 7 (2008) 11–20.
[5] G. Kroemer, J. Pouyssegur, Tumor cell metabolism: cancer's Achilles' heel, Cancer Cell 13 (2008) 472–482.
[6] M.R. Ponisovskiy, Cancer metabolism and the Warburg effect as anabolic process outcomes of oncogene operation, Crit. Rev. Eukaryot. Gene Expr. 20 (2010) 325–339.
[7] R.J. Deberardinis, N. Sayed, D. Ditsworth, C.B. Thompson, Brick by brick: metabolism and tumor cell growth, Curr. Opin. Genet. Dev. 18 (2008) 54–61.
[8] P.P. Hsu, D.M. Sabatini, Cancer cell metabolism: Warburg and beyond, Cell 134 (2008) 703–707.
[9] C.J. Langer, J.P. Hoffman, F.D. Ottery, Clinical significance of weight loss in cancer patients: rationale for the use of anabolic agents in the treatment of cancer-related cachexia, Nutrition 17 (2001) S1–S20.
[10] P.L. Ho, A. Kurtova, K.S. Chan, Normal and neoplastic urothelial stem cells: getting to the root of the problem, Nat. Rev Urol. 9 (2012) 583–594.
[11] J. Hatina, W.A. Schulz, Stem cells in the biology of normal urothelium and urothelial carcinoma, Neoplasma 59 (2012) 728–736.
[12] E.A. Kurzrock, D.K. Lieu, L.A. Degraffenried, C.W. Chan, R.R. Isseroff, Label-retaining cells of the bladder: candidate urothelial stem cells, Am. J. Physiol. Renal Physiol. 294 (2008) F1415–F1421.
[13] X.R. Wu, Urothelial tumorigenesis: a tale of divergent pathways, Nat. Rev. Cancer 5 (2005) 713–725.
[14] N. Ismaili, M. Amzerin, A. Flechon, Chemotherapy in advanced bladder cancer: current status and future, J. Hematol. Oncol. 4 (2011) 35.
[15] H.G. Juffs, M.J. Moore, I.F. Tannock, The role of systemic chemotherapy in the management of muscle-invasive bladder cancer, Lancet Oncol. 3 (2002) 738–747.
[16] M.S. Soloway, Intravesical and systemic chemotherapy of murine bladder cancer, Cancer Res. 37 (1977) 2918–2929.
[17] R.S. King, P.A. Newmark, The cell biology of regeneration, J. Cell Biol. 196 (2012) 553–562.
[18] S. Bhasin, O.M. Calof, T.W. Storer, M.L. Lee, N.A. Mazer, R. Jasuja, V.M. Montori, W. Gao, J.T. Dalton, Drug insight: testosterone and selective androgen receptor modulators as anabolic therapies for chronic illness and aging, Nat. Clin. Pract. Endocrinol. Metab. 2 (2006) 146–159.
[19] L.M. Coussens, Z. Werb, Inflammation and cancer, Nature 420 (2002) 860–867.
[20] F. Balkwill, A. Mantovani, Inflammation and cancer: back to Virchow?, Lancet 357 (2001) 539–545
[21] H.F. Dvorak, Tumors: wounds that do not heal. Similarities between tumor stroma generation and wound healing, N. Engl. J. Med. 315 (1986) 1650–1659.
[22] A. Staack, S.W. Hayward, L.S. Baskin, G.R. Cunha, Molecular, cellular and developmental biology of urothelium as a basis of bladder regeneration, Differentiation 73 (2005) 121–133.