The Expression and Evaluation of Androgen Receptor in Human Renal Cell Carcinoma

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OBJECTIVE To investigate the expression of androgen receptor (AR) with clinical and pathologic features in patients with renal cell carcinoma (RCC) and to explore the function of AR using human RCC cells.

MATERIALS AND METHODS The expression of AR was detected by immunohistochemistry in 44 adjacent normal kidney tissues of 120 RCC patients and also in 16 metastatic RCC patients with their respective primary and metastatic tissue samples. The expression of AR was examined by western blot in commonly used human RCC cell lines and normal kidney epithelial cells, and the luciferase assay was performed in those AR-positive RCC cells.

RESULTS The expression rate of AR was higher in adjacent normal kidney (90.9%) than in RCC tissues (30.0%, $P < .001$), and it was negatively associated with pT stage and Fuhrman's grade. Specifically, there were 40.7% AR-positive cases in pT1 compared with 8.0% in pT3 ($P = .013$), and 50.0% of grade I cases were found to be AR positive compared with 12.9% in grade III ($P = .008$). AR expression was slightly higher in primary RCC tissues (12.5%) than their respective metastases (0%, $P = .484$). AR strongly expressed in CAKI-2 and OSRC-2 cells with little transactivation, which might indicate that AR in those 2 RCC cells has little function.

CONCLUSION Our results suggest that any attempt to investigate the roles of AR in RCC progression might need to combine the detection of AR expression in tissue samples with examining its function to make a correct correlation between AR and RCC progression. UROLOGY 83: 510.e19–510.e24, 2014. © 2014 Elsevier Inc.

Renal cell carcinoma (RCC) accounts for 3% of all adult malignancies.[1] Surgery remains the only effective treatment for RCC because metastatic disease is usually resistant to radiotherapy and chemotherapy, and immunotherapy shows limited response rates of 15%-20%.[2] Although the development of targeted therapy opens a new door for advanced RCC patients, its effect is still limited for patients with selective pathologic type.[3]

The epidemiology study indicates that RCC has a gender difference in tumor incidence (male-to-female ratio 1.6:1),[4] and early studies suggested that many types of diseases with incidence of gender difference might be linked to steroid hormone receptor expression and function, such as hematologic malignancies with the higher incidence in men[5] and autoimmunity diseases more commonly found in women.[6] Importantly, the gender difference in cancer susceptibility is one of the most consistent findings in cancer epidemiology.[7] However, previous studies on the linkage between AR expression and RCC progression remain unclear and controversial.[8-12]

In this study, we systematically examined the expression of androgen receptor (AR) in RCC tissue samples by immunohistochemistry (IHC) and its potential linkage to various clinical stages and pathologic grades, focusing on the type of clear cell carcinoma, which is the most commonly seen histologic subtype of RCC. In addition, we explored the expression of AR and its function in commonly used human RCC cell lines.

MATERIALS AND METHODS

Tissue Samples

One hundred twenty paraffin embedded RCC specimens from 73 male and 47 female patients, 44 adjacent normal kidney

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tissue specimens from 24 male and 20 female patients, and 16 primary RCC tissue specimens with their respective metastatic tissue specimens from 12 male and 4 female patients who received radical or partial nephrectomy and cytoreductive nephron-sparing surgery, respectively, between January 2000 and March 2012 were retrieved from the files of the Department of Urology, the First Affiliated Hospital of Medical College of Xi'an Jiaotong University for analysis. All patients provided written informed consent of using their tissue specimens in this study. The mean patient age at operation was 63 years (range, 35-82). All specimens were re-evaluated with respect to pT stage, Fuhrman's grade, and histologic subtypes by 2 pathologists. pT stages were adjusted according to the 2009 edition of the TNM system, and nuclear grading was performed according to the World Health Organization guidelines. Stage pT1, pT2, and pT3 disease was present in 54 (45.0%), 41 (34.2%), and 25 (20.8%) tumors, respectively. Tumor grade was G-I, G-II, and G-III in 28 (23.3%), 61 (50.9%), and 31 (25.8%) cases, respectively. Histologic subtypes of the main component were clear cell in 96 (80.0%), papillary in 12 (10.0%), chromophobe in 6 (5.0%), collecting duct carcinoma in 4 (3.3%), and sarcomatoid cell in 2 (1.7%) cases.

Immunohistochemistry

IHC was conducted using the Dako EnVision System (Dako Corporation, Carpinteria, CA). Rabbit polyclonal antibody against AR (N-20, SC-816, 1:500 diluted) was from Santa Cruz Biotechnology (Santa Cruz, Dallas, TX). Tissues were deparaffinized, rehydrated, and subjected to 5 minutes pressure-cooking antigen retrieval in citrate buffer (10 mM, pH 6.0), 10 minutes Double Endogenous Enzyme Block, 90 minutes primary antibody incubation, and 30 minutes Dako Cytomation EnVision + HRP reagent incubation. Signals were detected by adding the substrate hydrogen peroxide using diaminobenzidine as a chromogen followed by 1 minute hematoxylin counterstaining. Specimens were dehydrated, mounted, and analyzed using an E800 microscope (Nikon, Melville, NY) and a SPOT camera (Diagnostic Instruments, Arnold, MD). All reagents except those specified were obtained from Dako Corporation (Carpinteria, CA). The specificity of IHC staining for all studied protein expressions was confirmed using negative and positive controls. For negative controls, the adjacent sections of each specimen were stained with normal rabbit serum at appropriate dilutions. For positive controls, one slide of human prostate cancer tissue specimen was used. The expression of AR was investigated in semiquantitative fashion independently by 2 pathologists blinded to the clinicopathologic data. Tissue samples were considered AR positive even when only a few cells showed distinct nuclear and or cytoplasm staining. Immunoreactivity was analyzed as previously described,[13] which was semiquantified in categories as 0—no reactivity, 1—<10%, 2—10% to 25% positive, 3—25% to 50% positive, 4—50% to 75% positive, 5—75% to 90% positive, and 6—>90% of normal kidney epithelial cells or RCC cells positive for AR staining.

Cell Lines and Cell Culture

Human RCC cell lines, including CAKI-2, OSRC-2, ACHN, 786-O, and 769-P were purchased from ATCC, the normal human kidney proximal tubular cell line HKC-8 were kindly provided by Dr. Syed Khundmiri from the University of Louisville (Louisville, KY), and all cells were maintained in Dulbecco's modified Eagle's medium (DMEM, Gibco, San Diego, CA) supplemented with 10% fetal bovine serum (FBS) at 37°C with 5% CO_2 in a humidified incubator.

Protein Extraction and Western Blot

Total cellular protein lysates from human RCC cell lines, including CAKI-2, OSRC-2, ACHN, 786-O and 769-P, normal human kidney proximal tubular cell line HKC-8, and LNCaP and PC-3 prostate cancer cells were prepared with radioimmunoprecipitation assay (RIPA) buffer (50 mM Tris/pH 8.0, 150 mM NaCl, 0.1% SDS, 1% NP40, and 0.5% sodium deoxycholate) containing proteinase inhibitors, 1% cocktail and 1 mmol/L PMSF (Sigma, St Louis, MO). A total of 20 μg (LNCaP cell lysates) or 80 μg (RCC, HKC-8 cell and PC-3 lysates) of protein was separated by 10% SDS-PAGE and transferred to nitrocellulose membranes. After blocking with 5% skim milk in Tris-buffered saline with 0.1% Tween 20/pH 7.6 (TBST), the membranes were incubated with a rabbit polyclonal primary antibody against AR (SC-816, 1:1000 diluted) from Santa Cruz Biotechnology (Santa Cruz, Dallas, TX) at room temperature for 1.5 hours. After being washed with TBST, membranes were incubated with goat antirabbit secondary antibody coupled to horseradish peroxidase (65-6120, 1:5000 diluted) from Invitrogen Life Technology (Invitrogen, Grand Island, NY) at room temperature for 1 hour and visualized with an ECL chemiluminescent detection system (Thermo Scientific, Rockford, IL). Loading differences were normalized using a monoclonal mouse against human β-Tubulin antibody (SC-5274, 1:3000 diluted) from Santa Cruz Biotechnology (Santa Cruz, Dallas, TX). The cell lysate from LNCaP and PC-3 prostate cancer cells was used as the positive and negative controls, respectively.

Plasmids

The plasmids used were pSG5-AR, full-length complementary deoxyribonucleic acid of wild-type human AR, MMTV-Luc (MMTV) a luciferase reporter plasmid, and pRL-TK-Luc as internal control, all of which were constructed as described previously.[14-17]

Luciferase Assay

Luciferase activity, transfection, and reporter assays were performed using Lipofectamin 2000 (Invitrogen, Grand Island, NY) according to the manufacturer's protocol. Human RCC cell lines, OSRC-2 and CAKI-2, which have endogenous AR expression demonstrated by western blot, were transfected with MMTV-Luc. Cells transfected with pSG5-AR were used as positive controls, and all cells were cotransfected with pRL-TK-Luc as the internal control. The assay was performed as described previously.[18] Briefly, OSRC-2 and CAKI-2 cells at 5×10^4 were plated respectively into 24-well dishes with 10% charcoal stripped—FBS DMEM medium for 24 hours, medium was refreshed, cells in testing group were transfected with MMTV-Luc and pRL-TK-Luc, whereas cells in positive group were transfected with MMTV-Luc, pRL-TK-Luc, and pSG5-AR for 24 hours, and all wells repeated in triplicate. After transfection, the medium of all the aforementioned cells was refreshed to 10% charcoal stripped—FBS medium in the presence of dihydrotestosterone (DHT) at different concentration (1 nM and 10 nM, ethanol was used as control) for 24 hours. These cells were then harvested and assayed for luciferase activity using the Dual-Luciferase Reporter Assay System (Promega Corporation, Madison, WI). Data were expressed as relative luciferase activity normalized to the internal Renilla luciferase control.

Statistic Analysis

Subgroups with respect to tissue types (normal kidney or RCC tissue specimens), gender, pT stages, and grades were compared