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PROSTATE ANDROGEN RECEPTOR: IMMUNOHISTOLOGICAL LOCALIZATION AND mRNA CHARACTERIZATION

CHAWNSHANG CHANG,[1,2] GERALD CHODAK,[1] E. SARAC,[1] HIROYUKI TAKEDA[1] and SHUTSUNG LIAO[2,3]*

[1]Department of Surgery/Urology, [2]Ben May Institute and [3]Department of Biochemistry and Molecular Biology, University of Chicago, Chicago, IL 60637, U.S.A.

Summary—Four androgen receptor (AR) specific monoclonal antibodies were used for the immunohistochemical localization of AR in the human prostate tissue. The prostate tissue consisted of alveoli embedded in fibromuscular stroma and lined with a single layer of columnar secretory epithelial cells. The immunoreactive ARs were found predominantly in the nuclei of epithelial cell, suggesting ARs, like estrogen receptors and progesterone receptors, are mainly nuclear proteins. Northern blot hybridization showed that AR mRNA is about 9 kilobases (kb) and relative abundant in the androgen-sensitive organs, such as ventral prostate, dorsolateral prostate and seminal vesicle.

INTRODUCTION

Since the discovery of Charles Huggins[1], the primary treatment for patients with metastatic prostate cancer has been androgen suppression. Approximately 80% of patients initially can respond to this therapy but few are cured by this approach. Finding new methods for treating prostate cancer and benign prostate hyperplasia may require better understanding of etiology of these disorders. Since androgen actions in target organs are dependent on specific intracellular ARs, many attempts have been made to relate the organ levels and cellular localization of ARs. The techniques employed in these studies, however, have been based on radioactive androgen-binding by ARs and may not be suitable for analysis of cellular ARs that do not bind androgens [2].

Since we have isolated and constructed human and rat AR cDNA that can code for full-length ARs [3-5], we have employed a fusion protein technique to produce large amounts of polypeptides that represent different regions of human and rat ARs. Spleen cells from rat immunized with these proteins were used for cloning hybridoma and produce monoclonal anti-AR antibody [6]. With these monoclonal antibodies, we have found nuclear localization of ARs in the prostate cells. We also report here the result of a Northern blot analysis of AR mRNA and its tissue distribution.

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*To whom correspondence should be addressed.

EXPERIMENTAL

Materials

[α-^{32}P]dATP (3000 Ci/mmol), nylon filter and random primer kit were from Amersham Corp. All restriction enzymes were the products of Bethesda Research Laboratories. Human prostate tissue was from a 74-year-old patient with prostate cancer. The tissue was placed in OTC compound (Tissue-Tek II; Miles Laboratory Inc.) and was rapidly frozen in the liquid nitrogen. Alkaline phosphatase-anti-alkaline phosphatase (APAAP) complex and NTB(nitro blue tetrazolium)/levamisol kit were from Kirkeguard and Perry Inc. Monoclonal antibodies to androgen receptor were raised and purified as described elsewhere [6].

Immunohistological localization of androgen receptor in the human prostate cancer

Fresh frozen tissue stored at liquid nitrogen were sectioned (4 μm) in a cryostat and thaw-mounted on an uncoated glass slides. The sections were fixed immediately with methanol-acetone solution for 30 s and incubated with the first antibody (8 μg/ml of purified monoclonal antibody to AR) for 60 min. After washing the excess of first antibody in Tris-buffered saline (TBS), the sections were incubated with second antibody (goat-anti-rat IgG) containing normal human serum and then stained with APAAP complexes [7]. Briefly, the sections, after washing the excess of second antibody in TBS, were incubated with APAAP complexes (prepared from alkaline phosphatase and rat monoclonal anti-alkaline phosphatase) for 60 min. The sites of immunoprecipitate

formation was visualized (under optical microscope) with NTB as substrate. Levamisol, 1 mM was used to inhibit the endogenous enzyme activity.

Northern blot analysis of AR mRNA

Total RNA was isolated from rat ventral prostate and other tissue using guanidinium thiocynate method [8]. Total RNA was treated with formaldehyde/formamide, seperated by electrophoresis on a 1.5% agarose gel, transferred to a nylon filter and hybridized to NurI-HindIII rat AR cDNA fragment (the specific activity of this random primed probe is 7×10^8 dpm/μg). The hybridization condition was $5 \times$ SSPE ($1 \times$ SSPE is 180 mM NaCl, 1 mM EDTA, 10 mM Na_2PO_4, pH 7.4), $5 \times$ Denhard's solution (0.1% Ficoll 400, 0.1% polyvinylpyrrolidone, 0.1% bovine serum albumin), 0.3% SDS, 50 μg/ml salmon sperm DNA and 1×10^6 dpm probe/ml hybridization solution at 68°C for 16 h. The filter was washed with a solution containing $1 \times$ SSC (150 mM NaCl, 15 mM sodium citrate), 1% SDS at room temperature for 3×15 min, and $2 \times$ SSC, 1% SDS at 65°C for 2×30 min. Filter was dried and exposed for 72 h at −80°C.

RESULTS AND DISCUSSION

Monoclonal antibodies employed in this study were AR-specific and did not react with receptors for glucocorticoids, estrogens, or progestins [6]. As shown in Fig. 1, the prostate tissue showed alveoli embedded in fibromuscular stroma and lined with a single layer of columnar secretory epithelial cells. Immunoreactive ARs were found mainly in the nuclear compartments of prostate cells. Most of the immunostaining was with the epithelial cells. Stroma cells stained very weakly, suggesting that epitherial cells contain much more ARs than stroma cells. Nuclear localization of ARs in rat [9, 10] and human [11, 12] have been shown by autoradiographic methods using radioactive androgens. Using a polyclonal anti-human AR antibodies, Lubahn *et al.* also showed recently by immunocytochemical analysis that, in human prostate, AR is localized predominantly in nuclei of glandular epithelial cells [13]. ARs, therefore, are similar to receptors for estrogens [14, 15] and progestins [16, 17] that have been shown to localize mainly in cell nuclei. Glucocorticoid receptors have also been localized in the nuclei and cytoplasmic compartments of target cells [18].

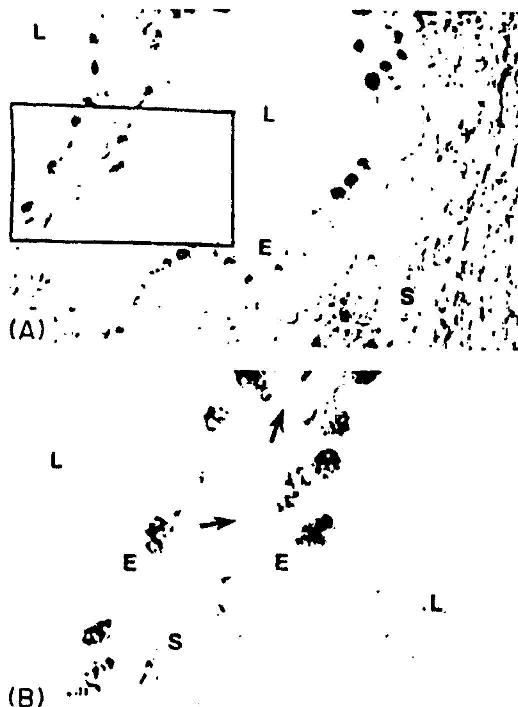

Fig. 1. Immunostaining of AR in the human prostate tissue (A) and its local enlargement (B). Monoclonal anti-AR antibody AN1-15 was used. The positive staining is observed in the nuclei of most epithelial cells while the nuclei of stromal cells are stained much less or negative (arrows). E: Epithelium; S: stroma; L: lumen.

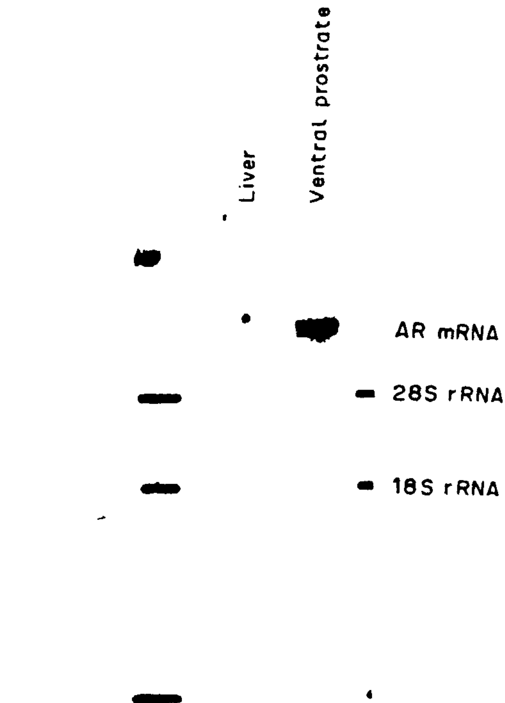

Fig. 2. Northern blot analysis of RNA from rat ventral prostate and liver using a cDNA probe to the AR. Total RNA (5 μg) was denatured with formaldehyde/formamide and applied to a 1.5% agarose gel. Following electrophoresis, RNA was transferred to a nylon filter and hybridized to NurI-HindIII rAR cDNA fragment as described in the 'Experimental'.

We have used Northern blot analysis to characterize AR-mRNA of rat ventral prostate. As shown in Fig. 2, only one band of RNA (about 9 kb) was found to hybridize with the radioactive AR-probe. This is in agreement with that reported by other investigators [13]. Using the same hybridization analysis, we

found that AR mRNA is much more abundant (per unit of RNA) in the ventral prostate than in other organs that are less sensitive to androgens. The relative AR-mRNA levels are (ventral prostate as 100%): seminal vesicle, 34%; dorsolateral prostate, 33%; kidney, 17%, liver and spleen, less than 1%. Analysis of AR-mRNA levels and immunocytochemical localization of AR in human prostate tissue may provide important information for predicting androgenic responses and selection of therapeutic methods for prostate diseases.

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