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Epithelial-Mesenchymal Interactions in Prostatic Development. II. Biochemical Observations of Prostatic Induction by Urogenital Sinus Mesenchyme in Epithelium of the Adult Rodent Urinary Bladder

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ABSTRACT Adult bladder epithelium (BLE) is induced to differentiate into glandular epithelium after association with urogenital sinus mesenchyme (UGM) and subsequent in vivo growth in syngeneic male hosts. Alteration of epithelial cytodifferentiation is associated with the expression of prostate-specific antigens, histochemical and steroid metabolic activities. These observations suggest that the inductive influence of the UGM has reprogrammed both the morphological and functional characteristics of the urothelium. In this report, differences regarding the mechanisms and effects of androgenic stimulation of prostate and bladder are exploited to determine the extent to which UGM plus BLE recombinants express a prostatelike, androgen-dependent phenotype.

Results from cytosolic and autoradiographic binding studies suggest that androgen binding is induced in UGM plus BLE recombinants and that this activity is accounted for by the induced urothelial cells. In UGM plus BLE recombinants, androgen-induced [3H]thymidine or [35S]methionine uptake analyzed by two-dimensional gel electrophoresis was qualitatively and quantitatively similar to that of prostate as opposed to bladder.

These studies indicate that expression within BLE of prostatic phenotype is associated with a loss of urothelial characteristics and that androgen sensitivity is presumably a function of the inductive activities of the stroma.
as subcapsular renal grafts in adult animals for 25-30 d before hormonal manipulation.

**Cytosolic Androgen Receptor Assays:** For the cytosolic androgen receptor assays, bladder and prostatic tissues from 28-d-old rats as well as UGM plus BLE recombinants were excised from hosts castrated 24 h before death. The tissues were homogenized at a ratio of 1:10 or 1:20 in TEDG buffer (10 mM Tris-HCl, 1.5 mM EDTA, and 1.0 mM dithiothreitol, 10% vol/vol glycerol, pH 7.4). Cytosols isolated by ultracentrifugation at 105,000 × g for 1 h were used for receptor assays. For receptor analysis, 0.25-0.84 mg of cytosol (0.25-3.84 nM protein) was incubated with TEDG buffer containing 17a-methyl-[3H]R-1881 (17a-methyl-([3H]R-1881, 87 Ci/mmol, New England Nuclear, Boston, MA) in the presence or absence of unlabelled competing R-1881 (1.0 nM). The final volume of the incubate was 0.3 ml and equilibrium was achieved after 20 h at 0°C. Free ligand was removed by adding 0.6 ml of TEDG buffer containing dextran-coated charcoal (0.003% T:70 dextran [Sigma Chemical Co., St. Louis, MO]; 0.25% charcoal, activated with 0.5% [vol/vol] DTT) incubating a brief centrifugation (8000 × g), aliquots were added to a 10.0-m1 MaxiFluor scintillation cocktail (J. T. Baker Chemical Co., Phillipburg, NJ) and counted on a Beckman LS-313P scintillation counter (Beckman Instruments, Inc., Irvine, CA). The counting efficiency for tritium was 60%. Data were analyzed by the method of Scatchard (3).

**[3H]Dihydrotestosterone (DHT) Autoradiographic Analysis of Androgen Receptors:** Hosts bearing tissue recombinants for 25-30 d were killed, and UGM plus BLE tissue recombinants were removed and minced into 1-2-mm² pieces. The specimens were incubated in a Brunswick shaker (Brunswick Instruments, New Brunswick, NJ) for 1.5 h at 37°C in serum-free Ham's F-12 medium containing 17 nM [3H]DHT (170 Ci/mmol, New England Nuclear) with or without a 600-fold molar excess of dihydrotestosterone (DHT) and [3H]R-1881. The samples were processed as described by Shannon et al. (4). Specimens of prostate, urinary bladder, and the UGM plus BLE recombinants were then evaluated by autoradiography as specified above. The rate of DNA synthesis was expressed as specific activity (counts per minute per microgram of DNA per 60 min). Under these experimental conditions, it was established that the rate of DNA synthesis is linear with regard to the weight of the tissues and the duration of incubation. Values presented are expressed as the mean ± SEM of at least six observations.

**RESULTS**

The results presented were based on the analysis of 98 UGM plus BLE recombinants grown in 18 hosts. 90 grafts of isolated UGM grown in 9 hosts served as controls for assessment of the cleanliness of separation. Contamination of the UGM by residual urogenital sinus epithelium was observed in six specimens (6%).

**Cytosolic Androgen Receptor Assays**

To provide a firm basis from which to assess the expression of androgen receptor activity, both ventral prostatic and bladder tissues from 28-d-old male CDF rats, as well as 28-d-old tissue recombinants, were subjected to receptor analysis. Scatchard plots of the data (Fig. 1) reveal that young rat ventral prostatic cytosol contained substantial (37.4 femoles/mg protein) high-affinity (the dissociation constant (Kd) is equal to 0.8 nM) binding sites, whereas cytosol from bladder was devoid of [3H]R-1881 binding. The UGM plus BLE recombinant contained high affinity (0.3 nM) binding sites in reduced, yet measurable amounts (3.9 femoles/mg protein). Total protein concentrations per sample in ventral prostatic, bladder, and UGM plus BLE assays were 2.9, 1.2, and 0.8 mg/ml, respectively. In a separate experiment (Fig. 1), intact male Copenhagen rat cytosol was diluted to protein concentrations of 0.3–4.0 mg/ml. At sample protein concentrations <1 mg/ml, there were electrophoresed the same day on SDS slab gels in the second dimension.

The two-dimensional slab gels (13 × 15 cm) were composed of a 5-5% [wt/vol] acrylamide stacking gel and a 10-5% acrylamide [wt/vol] separating gel. Proteins focused isoelectrically were transferred onto the SDS gels as described by O’Farrell (8) and run in the second dimension at 17 mA per gel in Tris-glycine buffer (0.025 M Tris-HCl, 0.192 M glycine, 0.1 M SDS, pH 8.3) for 4 h. Electrophoresis was terminated when the bromophenol blue tracking dye appeared 1 cm from the bottom of the gel.

After electrophoresis in the second dimension, the SDS slab gels were fixed by agitation for 1 h in a solution of isopropanol alcohol (10% [vol/vol]) and acetic acid (7% [vol/vol]). The gels were then transferred to a solution of ENHANCE (New England Nuclear) and agitated for 1 h. After this step, the gels were washed by agitation for 1 h in distilled H2O and dried on filter papers under an electrophoresis gel dryer (Hoefler Scientific Instruments, San Francisco, CA). A sheet of x-ray film perforated with the gel thickness was placed on the dried gels in a box at -80°C for 4-7 d. The apparent molecular weights of the proteins were estimated, using known molecular weight reference standards (bovine albumin, 68,000; glutamate dehydrogenase, 53,000; ovalbumin, 45,000; pepsin, 34,000; trypsinogen, 24,000; and ribonuclease A, 14,000). The apparent isoelectric points of the proteins were estimated from the pH of the isoelectric focusing gels.

Androgen-dependent DNA Synthesis As Measured by [3H]Thymidine Incorporation into Tissue DNA: Host animals carrying the UGM plus BLE tissue recombinants for 21-23 d were castrated on 1 wk and then implanted with a pellet containing 50 mg of TP or lactose in the treated and control animals, respectively. At days 1, 2, 3, or 4 (day 0 is equal to the date of implantation), animals were killed and the specimens were minced with fine scissors at 4°C. Aliquots of minced host prostate, bladder, and UGM plus BLE tissue recombinants (5–10 mg) were incubated in gasuated (95% O2, 5% CO2) Krebs-Ringer phosphate buffer (pH 7.4) containing 1.0 × 10–5 M unlabeled thyminde in ice-cold Krebs-Ringer phosphate buffer, centrifuged, and stored at -20°C until analysis. DNA was analyzed by the fluorometric assay of Hinegardner (9) with calf thymus DNA (Sigma Chemical Co.) as the reference standard. Sample aliquots were added to a 10-m1 MaxiFluor scintillation cocktail (J. T. Baker Chemical Co.) and counted as described earlier. The counting efficiency for tritium was 60%. The rate of DNA synthesis was expressed as specific activity (counts per minute per microgram of DNA per 60 min). Under these experimental conditions, it was established that the rate of DNA synthesis is linear with regard to the weight of the tissues and the duration of incubation.

Values presented are expressed as the mean ± SEM of at least six observations. Data were analyzed by a one-way analysis of variance for groups of unequal size. Significant differences between TP- and lactose-treated groups were determined by Dunnett's multiple comparison method (10).

**RESULTS**

The results presented were based on the analysis of 98 UGM plus BLE recombinants grown in 18 hosts. 90 grafts of isolated UGM grown in 9 hosts served as controls for assessment of the cleanliness of separation. Contamination of the UGM by residual urogenital sinus epithelium was observed in six specimens (6%).
Table 1

| Tissue             | Kd (nM) | Bmax (fmole/mg protein) |
|--------------------|---------|-------------------------|
| Ventral Prostate   | 0.8     | 37.4                    |
| UGM + BLE          | 0.3     | 3.9                     |
| Bladder            | -       | -                       |

Figure 1: Scatchard analysis of R-1881 binding to androgen receptors in cytosol obtained from rat ventral prostate, bladder, and UGM plus BLE. Note the presence of high affinity androgen binding in ventral prostate and UGM plus BLE and the absence thereof in bladder. Inset represents the relationship between protein concentration and androgen receptor levels detected in the standard assay. Bmax, maximum binding.

is an apparent decrease in cytoplasmic androgen receptor binding.

[^3H]DHT Autoradiography

Data from autoradiographic studies of[^3H]DHT binding sites in murine prostate, UGM plus BLE tissue recombinants, and bladder demonstrate that acinar cells of both host prostate (Fig. 2a) and UGM plus BLE tissue recombinants (Fig. 2b) are heavily labeled, with the majority of silver grains being localized over epithelial cell nuclei. In contrast, urothelial cells in the host bladder display low levels of silver grains without preferential localization over any cell organelles (Fig. 2c). Nuclear androgen localization in both the prostate and UGM plus BLE recombinants was abolished when the[^3H]DHT was incubated with a 600-fold excess of the radioinert steroid (data not shown).

Protein Synthesis Analyzed by Two-Dimensional PAGE

Total cellular proteins in host prostate, bladder, and 24 UGM plus BLE tissue recombinants carried in six hosts were labeled with[^35S]methionine and separated according to apparent isoelectric points as well as molecular weights (Fig. 3, a, b, and c). The overall intensity of protein labeling in the prostate (Fig. 3a) and tissue recombinant (Fig. 3c) was greater than in host bladder (Fig. 3b). Castration for 7 d decreased the intensity of labeling in the prostate and tissue recombinant but had no effect on the labeling in the bladder (data not shown). Numerous qualitative and quantitative differences were observed in the prostatic and bladder tissues. For example, individual proteins in regions P1 and P2 (Fig. 3a) are associated with the prostate, whereas the protein labeling associated with region B1 (Fig. 3b) is bladder specific. UGM plus BLE tissue recombinants exhibited protein labeling in the prostate-specific regions, while the protein spot unique to bladder was not expressed (Fig. 3c).

Figure 2: Autoradiograms of[^3H]DHT localization in murine (a) prostate (X 1,000), (b) UGM plus BLE recombinant (X 1,000), and (c) bladder tissues (X 1,600). Note the localization of silver grains in the epithelial nuclei of prostatic and UGM plus BLE specimens and the random distribution of labeling in bladder. The nuclear concentration of label observed in a and b was abolished when specimens were exposed simultaneously to[^3H]DHT and a 600-fold excess of radioinert steroid (not shown).
Figure 3 Autoradiograms of total cellular proteins separated by two-dimensional PAGE. Specimens ([a] murine prostate, [b]) murine urinary bladder, and [c] UGM plus BLE recombinants) were minced and labeled with [35S]methionine in Krebs-Ringer phosphate buffer and then subjected to two-dimensional electrophoretic separation (see Materials and Methods). (a) Murine prostate. Proteins within areas $P_1$ to $P_3$ are prostatic specific. (b) Murine urinary bladder. The protein designated $B_5$ is bladder specific. (c) UGM plus BLE recombinant. Note the similarity between protein labeling in $R_1$ to $R_5$ and that obtained from prostatic tissue ($P_1$ to $P_3$).
Androgen-dependent DNA Synthesis Measured by $[^3H]$Thymidine Incorporation into Tissue DNA

Previous findings (6, 11) that maximal rates of prostatic DNA synthesis occurred 72 h after initiation of TP administration to castrated rats were confirmed using castrated male mice treated with TP (data not shown). DNA synthesis in prostate, bladder, and 24 UGM plus BLE tissue recombinants carried out in six hosts in the presence and absence of exogenously administered androgens (Fig. 4) shows that, at the period of maximal TP-induced cellular proliferation, there was a significant ($P < 0.05$) sixfold stimulation in the prostate. A significant ($P < 0.05$) sixfold stimulation in the prostate. A significant ($P < 0.05$) sixfold stimulation in the tissue recombinants was also observed in the TP- vs. lactose-treated castrates at 72 h. The amount of TP-induced $[^3H]$thymidine incorporation into tissue DNA in the recombinants was lower than in prostates of TP-treated hosts. There were no significant alterations of $[^3H]$thymidine incorporation into the bladder DNA after TP treatment during the entire course of this study.

DISCUSSION

The influence of various mesenchymal tissues upon epithelial morphogenesis and differentiation has been analyzed in different experimental systems. Most studies have demonstrated that the mesenchyme may influence or modify the morphological organization of the epithelium, i.e. whether the epithelium is simple columnar, stratified, or organized into acinar configurations (12-16). Epithelial products characteristic of induced states are produced in experimental recombinations. Karkinen-Jaaskalainen (17) has demonstrated by immunofluorescence the expression of lens crystallins in epidermal cells of ventral trunk skin induced by the optic cup to form lentoids. Dhouflily et al. (18) showed the expression of scleroproteins possessing features of scale keratins in feather epithyses induced to form scales by association with scale dermis. However, morphogenetic inductions (changes in glandular branching patterns) may occur exclusive of biochemical reprogramming. Sakakura et al. (19) have demonstrated that while ductal arborization of mammary epithelium is modified by recombination with salivary mesenchyme to form salivarylike glands, casein continues to be produced by the tissue recombinants. The studies described herein illustrate that several biochemical activities of the UGM plus BLE recombinants exhibit features that are distinctly prostatic, the foremost being the expression of androgenic sensitivity.

The effects of androgens in target organs such as the prostate are mediated via cytosolic androphiles (20). In nontarget organs such as the urinary bladder, levels of cytosolic androphiles are exceedingly low or undetectable. Autoradiographic analysis of nuclear $[^3H]$DHT binding sites indicates a complete absence of androgen receptor activity in urothelium. Induction by UGM of prostatic differentiation in adult bladder epithelium results in the expression of nuclear androgen binding sites within the normally androgen-receptor-deficient urothelium as measured by steroid autoradiography.

Scatchard plots analyses of $[^3H]R-1881$ binding supported the autoradiographical data that the high affinity androgen receptor was present in the host prostate and the UGM plus BLE tissue recombinant but absent in the host bladder. Differences in the observed affinities and number of binding sites between ventral prostate and the recombinants may be explained by the observation that the UGM plus BLE specimens are composed of all elements of the prostatic complex (i.e., acini from different lobes, renal capsular elements, urethral epithelia, and urethral glands). The ventral prostate specimens were, in contrast, representative of a singular accessory sex organ element and contained only the active acinar parts thereof. It has been documented that the cytosolic androgen receptor in the rat ventral prostate changes as a function of age and that the receptor content in the ventral prostate is 10-fold greater than in the dorsolateral lobes (21, 22). In addition, the lower androgen receptor content detected in the tissue recombinants may be underestimated, since somewhat lower amounts of protein were used in the assay. Lower protein content per assay may result in the destruction of binding activity during incubation (23). This finding was confirmed herein (Fig. 1). It is also probable that measurement of cytosolic androgen binding activity in UGM plus BLE recombinants reflects the relative contributions of tissue components possessing differing androgenic sensitivity. The biochemical analyses of androgen receptor activity in tissue homogenates are complimented by the steroid autoradiographic data that demonstrated that the bulk of androgen binding activity is associated with the induced urothelial cells per se. In this sense, adult urothelium is similar to embryonic bladder epithelium (24), since in both cases the inductive activity of UGM can elicit the expression of nuclear androgen localization.

Expression of androgen receptor activity within epithelial cells provides the basis for the regulation of many biochemical

FIGURE 4 The effects of TP-induced DNA synthesis in murine prostate, bladder, and UGM plus BLE recombinants. Note the time- dependent TP-induced increase in DNA synthesis in prostate and an absence of this effect in bladder tissues. At the point of maximal TP-induced $[^3H]$thymidine uptake in the prostate, the UGM plus BLE tissue recombinants exhibited a comparable (six-fold) stimulation over lactose-treated control levels.
activities. Protein synthesis in male accessory sex organs is dependent upon androgenic stimulation (25-27). Total cell protein [35S]methionine labeling in the three tissues after two-dimensional gel electrophoretic separation was indicative of the induction of an androgen-sensitive, prostatic-like profile in UGM plus BLE tissue recombinant. Labeling of cellular proteins with [35S]methionine in the prostate and tissue recombinant was dependent upon the androgen status of the host (namely, castration decreased and TP administration increased the overall labeling on the gels), while the amount of labeling in the bladder was unaffected by such manipulations. In addition, a qualitative similarity in the protein synthetic profiles was observed for both the UGM plus BLE recombinant and the prostate, while the bladder exhibited a generally different profile.

Cells of prostate and UGM plus BLE recombinants also exhibit a marked stimulation of DNA synthesis after administration of TP to previously castrated hosts. By contrast, this treatment did not affect DNA synthesis in the host's bladder. In other studies, TP-induced epithelial DNA synthesis stimulation evaluated histologically in the prostate and UGM plus BLE recombinants could be blocked by the simultaneous injection of the antiandrogen, cyproterone acetate (11, 27).

Phenotype in UGM plus BLE recombinants.

The androgen receptor activity measured biochemically is due to the presence of this activity within the induced bladder epithelium. This, in turn, lends strong circumstantial evidence to the idea that androgen-induced protein and DNA synthesis was primarily a function of the induced epithelium, with little contribution from the surrounding stroma. Finally, localization of prostate-specific antigens (2) in the induced epithelium provides further evidence for the expression of additional prostatic phenotype in UGM plus BLE recombinants.

It has been proposed that developmental inductive processes reexpressed in adulthood may be involved in the pathogenesis of human benign prostatic hyperplasia (28, 30). These studies demonstrate that an inducible mesenchyme has the potential to alter the functional phenotype of a responsive adult epithelia.

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