

Development of Male Urogenital Epithelia Elicited by Soluble Mesenchymal Factors

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ABSTRACT: Androgen-dependent development of male secondary sexual glands is mediated by paracrine mesenchymal–epithelial interactions that regulate a complex array of biological events such as epithelial morphogenesis, growth, and cytodifferentiation. It is not known whether the action of mesenchyme on epithelial development in the male genital tract requires cell–cell contact or whether soluble, diffusible mediators are involved. To examine paracrine effects of urogenital sinus mesenchyme (UGM) on epithelial development, conditioned media (CM) from embryonic mouse UGM of normal (wild-type) and androgen-insensitive Tfm (testicular feminization) mice were tested for growth and morphogenetic effects on heterotypic tissue recombinants composed of rat or mouse bladder mesenchyme plus neonatal mouse seminal vesicle epithelium (BLM+SVE) or rat or mouse bladder mesenchyme plus neonatal mouse bulbourethral gland epithelium (BLM+BUG-E). Addition of a concentrate of CM from wild-type UGM grown in the presence of dihydrotestosterone (DHT) induced epithelial growth and complex epithelial morphogenesis in

BLM+SVE recombinants, whereas CM from DHT-treated Tfm UGM or a saline control were without effect. CM from wild-type UGM elicited similar trophic effects in BLM+BUG-E recombinants, but in addition induced precocious mucous epithelial differentiation in BLM+BUG-E recombinants. These results suggest that the normally androgen-dependent epithelial growth and branching morphogenesis in developing male urogenital organs is elicited by soluble mesenchymal factors. Two-dimensional gel electrophoresis of proteins synthesized and secreted by wild-type UGM revealed several androgen-dependent proteins with molecular weights of approximately 30 kDa that are absent in CM of Tfm UGM either in the presence or absence of DHT. These androgen-dependent proteins may be involved as paracrine mediators of the growth and morphogenetic effects of UGM on epithelial development.

Key words: Seminal vesicle, bulbourethral gland, urogenital sinus mesenchyme, mesenchymal–epithelial interactions.

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Effects of androgens on development of androgen target organs are mediated by paracrine mesenchymal–epithelial interactions. The indirect paracrine action of androgens is supported by three observations: 1) During urogenital development epithelial response to androgens occurs before epithelial androgen receptors (AR) are detectable. Developmental effects of androgens are apparently mediated via mesenchymal AR, which are detectable from the ambisexual stage onwards into adulthood (Shannon and Cunha, 1983; Cooke et al, 1991; Takeda and Chang, 1991). 2) Epithelial cells from embryonic or adult androgen target organs do not proliferate in response to androgens when cultured by themselves or when grown in association with mesenchyme from nontarget organs that lack AR (Cunha, 1976; McKeehan et al, 1984). 3) Epithelial cells from the androgen-insensitive, AR-defective Tfm (testicular feminization) mice express a variety

of androgenic effects when grown in association with AR-positive urogenital mesenchymal inductors from wild-type animals (Cunha et al, 1992). Thus, the mesenchyme of developing androgen target organs is the actual target and mediator of androgenic effects on the epithelium.

The key role of mesenchyme as a paracrine mediator of androgenic effects correlates with the fact that urogenital mesenchyme induces epithelial ductal branching morphogenesis, epithelial proliferation, expression of epithelial AR, and the expression of tissue-specific secretory proteins in the developing male genital tract. Induction of prostatic differentiation by urogenital sinus mesenchyme has been shown without exception to occur solely in endodermal epithelia of urogenital sinus derivation (Boutin et al, 1991). Analogous findings have been made for the seminal vesicle (SV) in that the induction of the SV epithelial differentiation only occurs in epithelia derived from the mesodermal Wolffian duct (Higgins et al, 1989a,b). Thus, whereas mesenchyme induces epithelial differentiation in the male genital tract, the developmental history of the epithelium can determine the possible phenotypes that can be induced by the mesenchyme.

A variety of growth factors have been postulated to be the paracrine mediators of mesenchymal–epithelial interactions (Han et al, 1987; Finch et al, 1989; Nilsen-Hamilton, 1989; Lyons and Hogan, 1990; Aaronson et

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al, 1991). For the male genital tract, growth factors from as many as six growth factor families have been described, some of which are potential mediators of mesenchymal-epithelial interactions (Story, 1991). Identification of the paracrine-acting mesenchymal factors that regulate the complex morphogenetic androgen-dependent effects on epithelial development will require a bioassay system appropriate for the expression of simple growth effects as well as the more complex morphogenetic effects of mesenchyme upon the epithelium. In this report we describe a novel test system (nonpermissive tissue recombinants) to detect the morphogenetic activity of conditioned medium from inductive urogenital sinus mesenchyme (UGM). In this system serum-free conditioned medium from UGM of normal, but not Tfm mice, promoted morphogenesis and differentiation of urogenital epithelia in nonpermissive tissue recombinants composed of bladder mesenchyme plus seminal vesicle epithelium (BLM+SVE) or bladder mesenchyme plus bulbourethral gland epithelium (BLM+BUG-E). Possible candidates for the mesenchymal inductors were detected by two-dimensional gel electrophoresis as several androgen-dependent proteins in conditioned medium from cultures of UGM from normal but not from Tfm UGM.

Materials and Methods

Animals and Tissue Recombination

The rudiments of seminal vesicle (SV), bulbourethral gland (BUG), and bladder (BL) were excised from newborn (0-day-old) BALB/c male mice or newborn male rats obtained from Simonsen laboratories (Hollister, California) and/or the Cancer Research Laboratory at the University of California (Berkeley, California). BL were excised at the bladder neck with fine forceps and cut into 1.5- to 2-mm squares. From these organ rudiments the following tissues were isolated following tryptic digestion as described earlier (Tsuji et al, 1994): seminal vesicle epithelium (SVE), bulbourethral gland epithelium (BUG-E), and bladder mesenchyme (BLM). A single SVE or BUG-E was recombined with BLM, as described previously (Tsuji et al, 1994). It should be noted that the SVE and BUG-E were trimmed to uniform sizes. The resultant tissue recombinants were cultured at 37°C in a humidified atmosphere of 5% CO₂ and 95% air in serum-free medium (1:1 mixture of Ham's F12 and Dulbecco's modified Eagle's medium [DMEM] H16 [1 g/L glucose] with L-glutamine [2 mM/L], insulin [10 µg/ml], and transferrin [10 µg/ml]) in the presence of 10⁻⁸ M dihydrotestosterone (DHT). To this basic medium was added a concentrate of the conditioned medium (CM) from cultures of urogenital sinus mesenchyme from normal or androgen-insensitive Tfm mice, as described below. This strategy takes advantage of the fact that BLM is incapable of supporting development of either SVE or BUG-E. This makes possible a test of serum-free CM from normal versus Tfm urogenital sinus mesenchyme (UGM) because normal UGM is a potent glandular inductor, whereas Tfm UGM is not (Cunha et

al, 1980). Following culture, tissue recombinants were fixed in Bouin's fixative and stained with hematoxylin and eosin or with the periodic acid-Schiff (PAS) method (Humason, 1979). This study is based on the analysis of results of three separate experiments of four to five tissue recombinants per experiment.

Preparation of CM

UGMs of normal and Tfm mice (obtained from Dr. Jean Wilson, University of Texas, Dallas, Texas) at 16 days of gestation were isolated following tryptic digestion as described earlier (Cunha and Lung, 1978). Normal and Tfm UGM (five each in 35-mm petri dishes) were cultured as intact mesenchymal explants on Millipore filters supported with stainless steel grids at the surface of serum-free medium (see above) in the presence of 10⁻⁸ M DHT. The CM was collected every 2 days for 2 weeks of culture, concentrated 100-fold (10,000 molecular weight cutoff), and stored at -80°C. CM (final concentration = 2 × original concentration) was added to organ cultures of the following tissue recombinants: rat BLM + mouse SVE, mouse BLM + mouse SVE, or rat BLM + mouse BUG-E.

Electrophoretic Analysis of Proteins in the CM

To identify androgen-dependent proteins that may be the paracrine mediators of prostatic induction, two-dimensional polyacrylamide gel electrophoresis (2D-PAGE) (O'Farrell et al, 1977) was performed on proteins synthesized by 16-day embryonic normal and Tfm UGM in the presence of 100 µCi of [³⁵S]methionine (1125 Ci/mmol; Amersham). UGM from normal or Tfm mice were incubated in methionine-free medium (1:1 mixture of Ham's F12 and DMEM H16 [1 g/L glucose] with L-glutamine [2 mM/L], insulin [10 µg/ml], bovine serum albumin [BSA; 100 µg/ml], and transferrin [10 µg/ml]) containing [³⁵S]methionine for 23 hours. For UGM from normal mice the medium was either unsupplemented or supplemented with 10⁻⁸ M DHT. For UGM from Tfm mice the medium was supplemented with 10⁻⁸ M DHT. Proteins secreted into the CM were precipitated with zinc sulfate, dialyzed against lysis buffer (O'Farrell et al, 1977), and radioactivity was determined. Because the dialysate contained substantial amounts of BSA, 30,000 to 100,000 counts per minute (cpm) of protein were loaded per gel. For any given single experiment an equal amount of cpm were loaded per gel for each type of CM. Proteins were resolved by 2D-PAGE according to O'Farrell et al (1977) and detected by autoradiography on X-ray film. Nineteen separate experiments (two to three gels per experiment) were performed.

Results

Epithelium of the newborn mouse SV is a simple cane-like structure composed of tall columnar cells (Fig. 1A,B) that, under the influence of endogenous androgens, undergo a complex branching morphogenesis *in vivo* (Fig. 1C). CM from normal UGM induced epithelial branching morphogenesis in rat BLM + mouse SVE (Fig. 1D) or mouse BLM + mouse SVE tissue recombinants. The

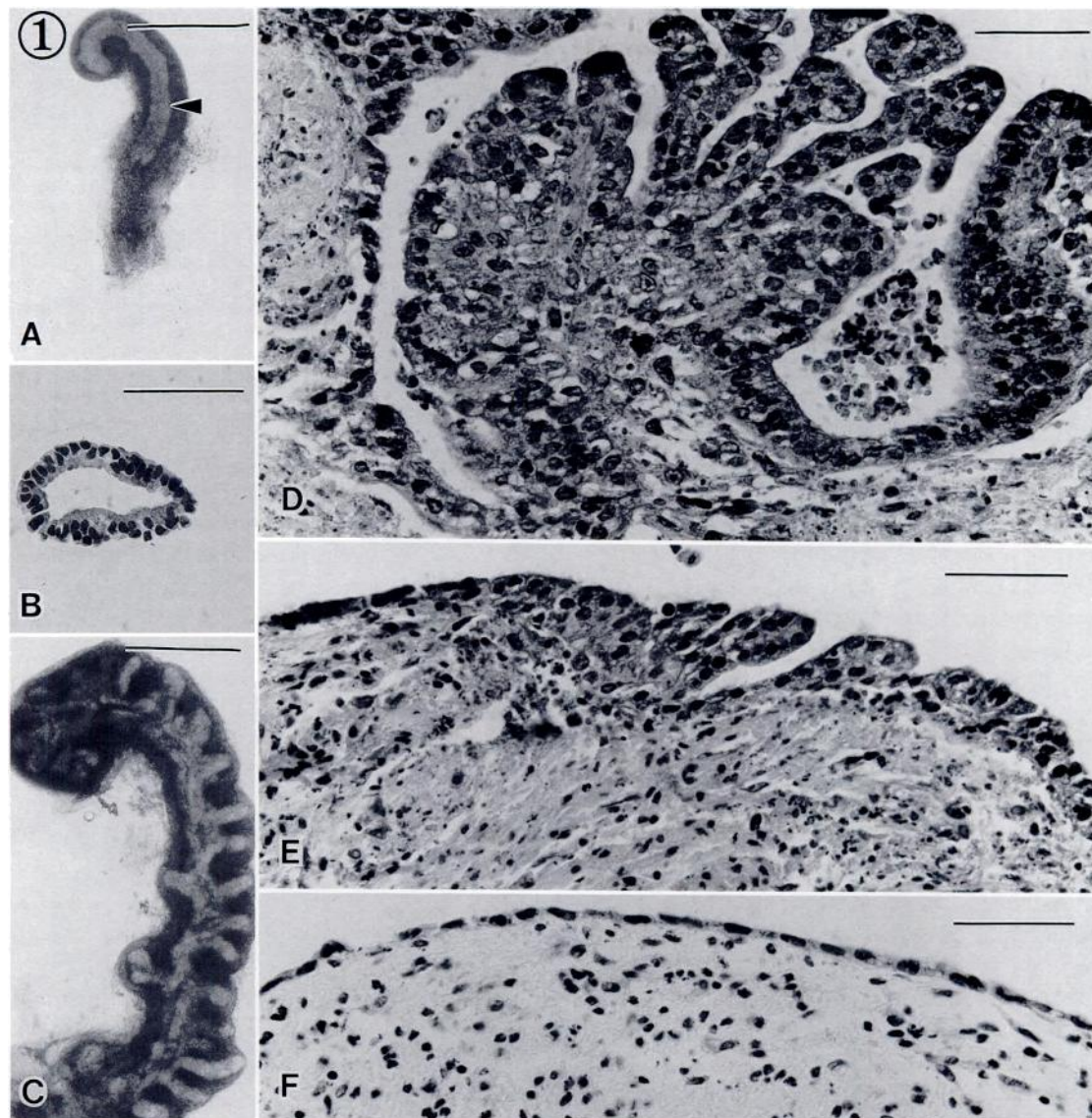


FIG. 1. (A) Wholemount of the newborn mouse SV whose epithelium (arrowhead) is a simple cane-like structure. (B) Transverse section of newborn mouse SV epithelium, isolated free from the mesenchyme following trypsinization, demonstrating the tall columnar cytodifferentiation and the absence of any contaminating mesenchymal cells. (C) Wholemount of the SV of a 6-day-old mouse, demonstrating complex epithelial branching morphogenesis unique to the developing SV. (D–F) Transverse sections of organ cultures of tissue recombinants composed of newborn rat BLM + newborn mouse SVE grown in the presence of 10^{-8} M DHT in serum-free medium supplemented with CM derived from normal UGM (D), Tfm UGM (E), or PBS (F). Epithelial branching morphogenesis of the SVE occurred when the CM was derived from normal murine UGM (D), but not when CM from Tfm murine UGM (E) was used, or in the PBS control (F). In B and D, taken at the same magnification, note the obvious increase in epithelial mass. Bar = 0.5 mm in A and C, and 50 μ m B and D–F.

complex, highly convoluted morphogenetic pattern was similar to that characteristic of the SV and was associated with maintenance of tall columnar epithelial cytodifferentiation. Considerable epithelial proliferation also occurred in that epithelial size increased dramatically from the original minute size of the SVE at the start of the experiment (compare Fig. 1B and D). Of the 15 rat BLM + mouse SVE tissue recombinants analyzed, five were unsuccessful in that the SVE was not present at the end of the culture period and apparently did not adhere to the

BLM (Table 1). All of the successful rat BLM + mouse SVE tissue recombinants exhibited epithelial branching morphogenesis and tall columnar epithelial cytodifferentiation when cultured in medium containing CM from normal UGM. When CM derived from androgen-insensitive Tfm UGM was used, epithelial branching morphogenesis failed to occur altogether or was minimal (Fig. 1E) in rat BLM + mouse SVE or mouse BLM + mouse SVE tissue recombinants, even though columnar epithelial cytodifferentiation was maintained. Instead, in the presence

Table 1. Effects of conditioned medium from normal or Tfm urogenital sinus mesenchyme on tissue recombinants*

Tissue recombinants	Branching morphogenesis (treatment)			Tall columnar epithelial cytodifferentiation (treatment)			Mucous differentiation (treatment)		
	Normal CM	Tfm CM	PBS	Normal CM	Tfm CM	PBS	Normal CM	Tfm CM	PBS
Rat BLM + mouse SVE	†10/10†	12/12	12/12	†10/10†	12/12	12/12	N/A	N/A	N/A
Mouse BLM + mouse SVE	12/12	12/12	12/12	12/12	12/12	12/12	N/A	N/A	N/A
Mouse BLM + mouse BUG-E	12/12	12/12	12/12	12/12	12/12	12/12	12/12	0/12	0/12

* Of four to five tissue recombinants prepared per group per experiment, three experiments total.

† Of the 15 rat BLM + mouse SVE tissue recombinants analyzed, 5 were unsuccessful in that the SVE was not present and apparently did not adhere to the BLM.

of Tfm UGM CM, the original three-dimensional tubular epithelial morphology disappeared as the epithelium spread as a monolayer over the BLM. Preliminary image analysis (data not given) of epithelial morphology using the "Shape Factor" module of the Prism View Morphometrics program (Dapple, Incorporated, Sunnyvale, California) verified a dramatic increase in morphological complexity, as indicated by the histological differences (Fig. 1D and E) in morphology between tissue recombinants treated with CM from normal versus Tfm UGM. Substitution of phosphate-buffered saline (PBS) for CM merely maintained the SV epithelium as a monolayer of undifferentiated squamous epithelial cells; branching morphogenesis was nil (Fig. 1F).

The epithelial rudiment of the mouse BUG is club shaped, unbranched at birth and is composed of a solid mass of undifferentiated cells (Fig. 2A,B). By day 5 *in situ*, considerable branching morphogenesis has occurred (Fig. 2C), and lumen formation has been initiated, but the cells lining the lumen have not undergone mucinous differentiation (Fig. 2C). Treatment of mouse BLM + mouse BUG-E recombinants with CM from normal (but not with Tfm) UGM elicited complex epithelial branching morphogenesis (Fig. 2D) and precocious epithelial cytodifferentiation, i.e., the formation of tall columnar mucinous PAS-positive epithelial cells (Fig. 2D, inset). Addition of CM from Tfm urogenital sinuses induced precocious lumen formation, minimal epithelial branching morphogenesis, and differentiation of a nonmucified columnar epithelium (Fig. 2E). A multilayered urothelium was never observed in any of the tissue recombinants, indicating that the bladder mesenchyme was devoid of contaminating bladder epithelium.

The proteins synthesized and secreted into the serum-free medium by normal and Tfm UGM from 16-day fetuses were analyzed by two-dimensional gel electrophoresis following incorporation of [³⁵S]methionine. For all specimens, most newly synthesized proteins of ≥40 kDa were faint and variable from run to run and therefore will not be described in detail. At approximately 30 kDa, three major proteins were consistently found in normal UGM

grown in the presence or absence of DHT as well as in Tfm UGM (Fig. 3, proteins 1–3). In the vicinity of this constellation four unique protein spots at different isoelectrofocusing points (approximately 30–35 kDa) (proteins 4–7) were released into the medium by normal UGM grown in the presence of DHT (Fig. 3A), but they were absent when DHT was deleted from the medium (Fig. 3B). These proteins were never detected in the medium of Tfm UGM, even in the presence of DHT (Fig. 3C). Other minor spots on two-dimensional gels were variable and not androgen dependent in either normal or Tfm UGM. One major protein (protein 8), at approximately 75 kDa, pI = 7, was present in Tfm specimens only. Proteins >200 kDa were not detected.

Discussion

Development of male accessory sexual glands is a complex process involving the formation of organ-specific patterns of epithelial ductal branching, proliferation, secretory cytodifferentiation, and production of tissue-specific secretory proteins, all of which are induced by mesenchyme under the influence of androgens (Cunha et al, 1987). The rationale for this study is based upon observations suggesting that androgens elicit prostatic development from the urogenital sinus via AR present in embryonic UGM. In the developing prostate AR are initially undetectable in epithelium, but they are readily detected in the mesenchyme (Shannon and Cunha, 1983; Cooke et al, 1991; Takeda and Chang, 1991). During periods when epithelial AR are undetectable in the fetal urogenital tract, a wide spectrum of androgen-dependent developmental processes are expressed in these apparently AR-negative epithelia, including: 1) prevention of programmed cell death in the embryonic Wolffian duct during the ambisexual stage of sex differentiation, 2) appearance and early morphogenesis of the seminal vesicle anlagen, 3) and appearance and initial branching morphogenesis of ducts of the prostate and BUG. Additional support for a paracrine mechanism of androgen action is derived from the analysis of

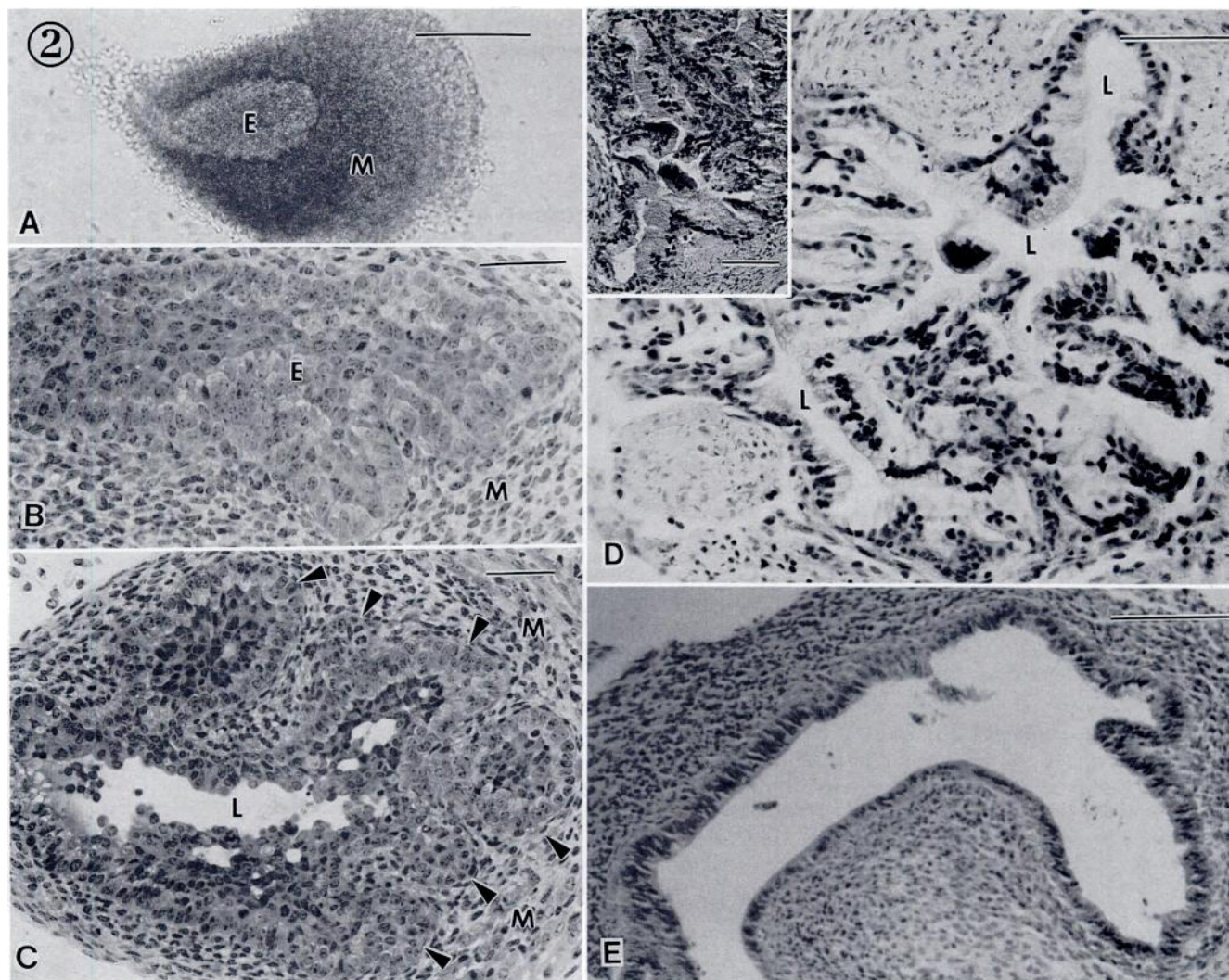


FIG. 2. (A) Wholemout of the newborn mouse BUG whose epithelium (E) is a simple bulbous club-like structure surrounded by mesenchyme (M) (bar = 500 μ m). (B) Longitudinal section of a BUG from a newborn mouse demonstrating the undifferentiated nature of the epithelium (E) (bar = 50 μ m). (C) Longitudinal section of the BUG of a 5-day-old mouse illustrating complex epithelial branching morphogenesis (arrowheads), lumen (L), and the undifferentiated nature of the epithelium (absence of tall mucinous cells) (bar = 50 μ m). (D–E) Histologic sections of organ cultures of tissue recombinants composed of newborn mouse BLM + newborn mouse BUG-E (12 recombinants total from three separate experiments) grown for 7 days in the presence of 10^{-8} M DHT in serum-free medium supplemented with CM derived from normal UGM (D), or Tfm UGM (E). When CM from normal UGM was used (D) note epithelial ductal branching morphogenesis resulting in complex lumen formation (L) and mucinous differentiation (PAS-positive epithelial cells [inset]). In (E) when CM from Tfm UGM was used, the epithelial branching morphogenesis is minimal, and lumen formation had occurred, but the epithelium did not undergo mucinous differentiation (D, E, and inset: bar = 100 μ m).

mesenchymal–epithelial interactions between normal and androgen-insensitive Tfm tissues, which lack functional AR (He et al, 1991). Tissue recombinants composed of normal mesenchyme + Tfm epithelium undergo androgen-induced prostatic epithelial development when grafted *in vivo* (Cunha and Lung, 1978; Lasnitzki and Mizuno, 1980; Cunha and Chung, 1981; Shannon and Cunha, 1984; Sugimura et al, 1986; Cunha and Young, 1991; Donjacour and Cunha, 1993). Prostatic development in AR-negative Tfm epithelium means that the Tfm epithelium underwent “androgen-induced” ductal morphogenesis, epithelial growth, and secretory cytodifferentiation even though

the Tfm epithelium lacked functional AR (Shannon and Cunha, 1984; Sugimura et al, 1986). These observations suggest that the mesenchyme is the actual target and mediator of androgenic effects upon the epithelium and provides the rationale for testing CM *in vitro* from normal and Tfm UGM for their ability to regulate epithelial development. To assay the complex inductive effects of mesenchyme on epithelial development, a novel *in vitro* assay system was devised based upon nonpermissive tissue recombinants. The rationale was to establish tissue recombinants that fail to promote normal epithelial development so that the developmental effects of UGM-CM could

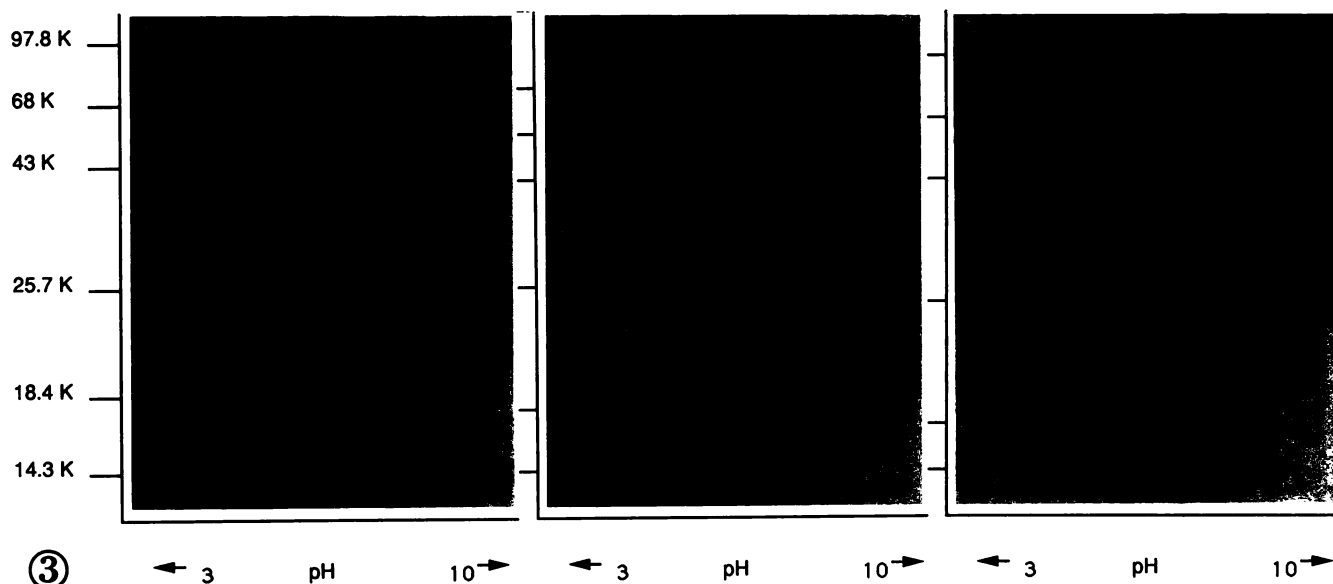


FIG. 3. 2D-PAGE of proteins synthesized and secreted by normal and Tfm UGM as described in the Materials and Methods. In (A) and (B), the exponential sodium dodecyl sulfate gradient gel was 5–20% acrylamide, whereas while in C the gradient was 7.5–15%. For (A) and (B) 40,000 cpm were loaded per gel; in (C), 60,000 cpm were loaded. The upper left corner is the acidic end (pH 3), and the upper right corner the basic end (pH 10) in each autoradiogram. Four protein spots (4–7) with molecular weights of 29–35 kDa were synthesized and secreted by normal UGM into the medium in the presence (A) but not the absence (B) of DHT. These four proteins were also not detected in CM from Tfm UGM treated with DHT (C). Three major proteins (1–3) were detected in CM of normal UGM cultured in the presence (A) and absence (B) of DHT, as well in CM of Tfm UGM. One protein (8) was detected in CM of Tfm UGM only. For (A), CM from wild-type UGM treated with DHT, 40,000 cpm of protein were loaded and the film was exposed for 21 days. For (B), CM from wild-type UGM cultured without DHT, 40,000 cpm of protein were loaded and the film was exposed for 22 days. For (C), CM from Tfm UGM cultured with DHT, 60,000 cpm of protein were loaded and the film was exposed for 45 days.

be examined. This more complex assay system allows for examination of other types of biological response (branching morphogenesis and epithelial cytodifferentiation) that are generally beyond the scope of most unidimensional growth assays employing epithelial target cells growing on plastic dishes. In keeping with the developmental properties of BLM (a nonglandular inducer), epithelial branching morphogenesis did not occur when SVE or BUG-E were grown in association with BLM *in vitro* or *in vivo* (Tsuji et al, 1994). The complex branching morphogenesis and tall columnar epithelial cells in BLM+SVE tissue recombinants treated with UGM-CM from normal mice indicate that the paracrine effects of UGM in epithelial development are mediated by soluble factors. Although epithelial proliferation was not examined directly, morphological evidence indicated that the minute neonatal SV epithelial rudiments had increased dramatically in size, suggesting that UGM-CM from normal mice has growth-promoting as well as morphogenetic activity. Adult prostatic stroma, which is derived from UGM, has been shown to produce at least four types of growth factors: basic fibroblast growth factor (b-FGF) (Story 1991; Sherwood et al, 1992), keratinocyte growth factor (KGF) (Rubin, unpublished data), hepatocyte growth factor (Rubin et al, 1991), and a nerve growth-like factor (Djakiew et al, 1991). More recently, KGF has been shown to be a mesenchyme-produced paracrine factor that regulates growth and morphogenesis of the neonatal mouse seminal

vesicle and prostate (Sugimura and Cunha, unpublished data; Alarid et al, 1994).

Because the AR-negative BLM is not normally an inducer of epithelial ductal morphogenesis (Tsuji et al, 1994), the participation of BLM in epithelial branching morphogenesis is particularly noteworthy. In several developing systems (salivary gland, mammary gland, prostate, seminal vesicle, lung), mesenchyme is known to induce and specify patterns of epithelial ductal branching (Alescio and Cassini, 1962; Sakakura et al, 1976; Cunha, et al, 1987; Higgins et al, 1989b). The active role of mesenchyme in epithelial branching morphogenesis is further manifested by the production, deposition, and degradation of interstitial collagen and other extracellular matrix substances that stabilize early ductal branchpoints and ductal morphology (Spooner and Faubion, 1980; Bernfield et al, 1984; Nakanishi et al, 1986, 1988; Reponen et al, 1992). Mesenchyme is known to synthesize a neutral hyaluronidase and type IV collagenase, which is thought to be involved in degradation of the epithelial basement membrane and in epithelial branching morphogenesis (Bernfield et al, 1984; Reponen et al, 1992). Mesenchyme is also a source of other trophic factors required for tubulogenesis (Montesano et al, 1991a,b; Ram et al, 1991; Kanazawa and Hosick, 1992). Epimorphin, a recently described mesenchymal factor, has been postulated to also be involved in ductal morphogenesis and lumen formation (Hirai et al, 1992). The participation of BLM in ep-

ithelial branching morphogenesis following treatment with CM from normal UGM implies that the BLM may also be a target for trophic substances in the CM, although it is unclear whether BLM is playing an active or a passive role in epithelial branching morphogenesis. Clearly, factors in UGM-CM have a general trophic effect because this CM promotes branching morphogenesis in both the SVE and BUG-E.

Addition of CM from both normal and Tfm UGM maintained the original tall columnar cell shape of the SVE, whereas addition of PBS to the tissue recombinant bioassay system led to the formation of a simple squamous epithelium. Cell shape (the columnar versus the squamous configuration) has been shown to be involved in epithelial responsiveness to lactogenic hormones in mammary epithelial cells (Bissell and Ram, 1989) and responsiveness of corneal epithelial cells to growth factors (Gospodarowicz et al, 1978). In this regard, CM from normal UGM also precociously stimulated epithelial functional cytodifferentiation in BUG-E, because tall columnar, PAS-positive mucinous epithelial cells were detected in BLM+BUG-E recombinants treated for 6 days *in vitro* with CM from normal UGM, even though such highly specialized cells usually do not differentiate in the BUG until puberty (Hart and Greenstein, 1968).

The positive effects of UGM-CM reported therein and the known biological effects of UGM (Cunha et al, 1987) are in stark contrast to the growth-inhibitory activity described in CM obtained from long-term cultures of UGM (Rowley and Tindall, 1987; Rowley, 1990). Such inhibitory activity has been detected through the use of classical CM growth assays in which various tumor cell lines have been used as target cells. Rowley's (1990) UGM-derived growth-inhibitory factor is clearly very different from the activities detected in CM from short-term cultures of normal UGM using our more complex nonpermissive tissue recombinant test system. Rowley's factor has been suggested to influence tumor cell differentiation, although this conclusion is only supported by a modest increase in protein synthesis in the target epithelial cells (Rowley and Tindall, 1987). Recently, we have shown in tissue recombinants grafted *in vivo* that UGM induces the undifferentiated epithelial cells of the Dunning prostatic adenocarcinoma (DT) to differentiate into tall columnar secretory epithelial cells (Hayashi et al, 1990). This change in epithelial histodifferentiation is associated with a reduction in epithelial proliferation and an apparent loss of tumorigenesis (Hayashi and Cunha, 1991).

Protein candidates responsible for the inductive activities of UGM-CM on epithelial development have been detected by two-dimensional electrophoretic analysis and have a molecular weight of approximately 30 kDa. The candidates exhibit the appropriate hormonal dependency, being androgen-inducible in cultures of normal UGM and

completely absent in cultures of Tfm UGM under any conditions. Proteins smaller than 10 kDa may not be required for induction of epithelial branching morphogenesis by UGM-CM because they were eliminated during concentration. Residual androgens or androgen metabolites are unlikely to be responsible for the effects of UGM-CM because androgen receptors are undetectable in BLM, SVE, and BUG-E of neonatal mice (Cooke et al, 1991). It is unlikely that proteins with molecular weights >200 kDa are candidates for the mesenchyme-derived inducers of the epithelial branching morphogenesis because proteins with molecular weights >200 kDa were not detected in two-dimensional gel electrophoretic analysis of UGM-CM. It is also possible that CM from Tfm urogenital sinuses may produce proteins inhibitory for growth and morphogenetic processes. At least one major protein (protein 7) was consistently observed in Tfm and not in normal UGM. It is not yet possible to establish the identity of the protein differences between normal and Tfm UGM. Secreted proteins produced by normal UGM could be growth factors, proteases capable of activating latent growth factors or tissue degrading proteinases, morphogens, differentiation factors, or cell surface receptors for the above. Further research will be required to elucidate these possibilities.

The putative inductive factors have been characterized in induction of mesodermal differentiation in *Xenopus*. Three soluble factors have been identified as inducers of mesodermal differentiation in the developing amphibian: b-FGF (Kimelman and Kirschner, 1987; Slack et al, 1987; Kimelman et al, 1988); transforming growth factor- β_2 (TGF- β_2) (Rosa et al, 1988); and activin (Smith et al, 1990). Keratinocyte growth factor (KGF) is a particularly attractive candidate as a mesenchymal paracrine factor for the following reasons: 1) KGF is produced solely by mesenchymal cells (Aaronson et al, 1991); 2) Epithelial cells have KGF receptors (Orr-Urtreger et al, 1993); and 3) KGF stimulates epithelial proliferation and differentiation (Aaronson et al, 1991). Indeed, recent studies in our laboratory have implicated KGF as an important mediator of androgen action in the developing mouse seminal vesicle and prostate (Sugimura and Cunha, unpublished data; Alarid et al, 1994). Hepatocyte growth factor, also produced by fibroblastic cells, has been shown to have tubulogenic activity of epithelial cells (Montesano et al, 1991a,b), and it has been shown to be produced in the prostate (Rubin, unpublished data). From the standpoint of molecular weight, the putative UGM-derived inducers reported herein are probably not FGF or TGF β , although alternately spliced forms of these growth factors are potential candidates. Significantly, the diffusible nature of UGM-derived inductive activity raises the possibility of purifying these regulators of epithelial development from CM.

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