**Induction of prostatic morphology and secretion in urothelium by seminal vesicle mesenchyme**

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**SUMMARY**

Mesenchymal-epithelial interactions are essential for the development of the male reproductive tract. Tissue recombination experiments have been used to define the characteristics of these interactions. When mesenchyme, embryonic connective tissue, is recombined with epithelium from another organ an instructive induction may occur in which the developmental fate of the epithelium is altered. Instructive inductions are most common when the epithelium that is removed from the mesenchyme and the epithelium that is recombined with the mesenchyme are from the same germ layer. All of the mesenchyme of the male reproductive tract is of mesodermal origin. The epithelia of these organs are derived from either the mesodermal Wolffian duct epithelium or the endodermal urogenital sinus epithelium. Urogenital sinus mesenchyme can instructively induce bladder and urethral epithelium to form prostate (Donjacour, A. A. and Cunha, G. R. (1993) *Endocrinol.* **132**, 2342-2350) and seminal vesicle mesenchyme can instructively induce epithelium from the ductus deferens and ureter (Cunha, G. R., Young, P., Higgins, S. J. and Cooke, P. S. (1991) *Development* **111**, 145-158) to form seminal vesicle. To see whether instructive interactions could occur across germ layers in this system, seminal vesicle mesenchyme, normally associated with a mesodermal epithelium, was recombined with epithelium from neonatal or adult bladder or urethra, which are of endodermal origin. The resulting tissue recombinants were analyzed histologically and by immunocytochemistry and western blotting with antibodies to prostatic and seminal vesicle secretory proteins. Full prostatic differentiation was observed in tissue recombinants made with seminal vesicle mesenchyme plus either adult or neonatal bladder or urethral epithelium. These tissue recombinants made dorsolateral but not ventral prostatic secretory proteins. None of them developed into seminal vesicle. To examine whether epithelial androgen receptors were required for this process, seminal vesicle mesenchyme was recombined with urothelium from mice with the testicular feminization mutation. These mice lack functional androgen receptors. While these tissue recombinants grew, no prostatic proteins were detected. Seminal vesicle mesenchyme acted as a potent prostatic inducer, indicating that similar mesenchymal signals can induce prostatic and seminal vesicle development. Urogenital epithelia of both endodermal and mesodermal origin appeared to be able to respond to these inductive signals produced by the seminal vesicle mesenchyme; however, their responses differed depending on their germ layer of origin: mesodermal epithelium yielding seminal vesicle (Cunha, G. R., Young, P., Higgins, S. J. and Cooke, P. S. (1991) *Development* **111**, 145-158) and endodermal epithelium yielding prostate.

Key words: prostate, mesenchymal-epithelial interactions, androgens, induction, rat, mouse

**INTRODUCTION**

Interactions between mesenchyme and epithelium are essential for the proper development of many organs, however, in no case has the mechanism of mesenchymal-epithelial interactions been established. Tissue recombination techniques have been used to characterize mesenchymal-epithelial interactions in several organ systems including the urogenital tract. In these experiments mesenchyme and epithelium from different organs or organ rudiments are separated from their native counterparts, recombined, and grown in vitro or as grafts in host animals. Such experiments have shown that mesenchyme is required for epithelial growth and differentiation (Cunha et al., 1980; Haffen et al., 1987; Saxén et al., 1976; Sengel, 1990). If the epithelium in a tissue recombinant expresses its normal prospective phenotype, despite recombination with mesenchyme from another organ, this interaction is said to be permissive. Alternatively, if the epithelium is induced to alter its phenotype the interaction is said to be instructive (Saxén et al., 1976). In instructive inductions the epithelium commonly takes on the characteristics of the epithelium normally associated with the inducing mesenchyme, for example adult epididymal epithelium recombined with seminal vesicle mesenchyme (SVM) expresses the morphology of the seminal vesicle (SV) and produces SV secretory proteins (Turner et al., 1989). The terms permissive and instructive interactions, or inductions, are operational definitions and are therefore limited by our ability to successfully construct and characterize the tissue recombi-
nant. Often mesenchymal-epithelial interactions do not fall neatly into these two categories. In some cases partial instructive inductions have been observed in which some of the characteristics of the epithelium are changed to match that of the mesenchyme’s origin while others are unchanged. For example, if mammary gland epithelium is recombined with salivary gland mesenchyme the branching pattern of the epithelium is similar to that of the salivary gland, however, the epithelium produces secretory proteins characteristic of the mammary gland (Sakakura et al., 1976). Some tissue recombinants yield uninterpretable results when the epithelium does not have the morphological or functional activity characteristics of either the epithelium or mesenchyme (Boutin et al., 1991).

The success of mesenchymal-epithelial interactions in experimental tissue recombinants depends upon many factors, including the age of the tissues and the degree to which the epithelium and mesenchyme share a developmental history. The mesenchyme from almost all organs is derived from mesoderm. However, in various organs mesenchyme supports and directs the development of epithelium from either of the three germ layers. In tissue recombinants, mesenchyme that normally interacts with epithelium from one germ layer, e.g. endoderm, appears to be more likely to undergo successful interactions with other epithelium from the same germ layer than with epithelia from another germ layer. Consequently most tissue recombination experiments have involved tissues from the same organ system (e.g. within the gastrointestinal, urogenital or integumental systems; Cunha et al., 1980; Haffen et al., 1987; Saxén et al., 1976; Sengel, 1990).

In most of the tissue recombination experiments done with tissues of the male urogenital system, the germ layer origin of the epithelium has been the same as that of the epithelium normally associated with the mesenchyme. Epithelium from the bladder, urethra, and vagina, subdivisions of the endodermal urogenital sinus, can be instructively induced by the mesenchyme from the prostatic anlagen (UGM), another region of the urogenital sinus, to form prostate if the tissue recombinant is grown in an intact male host (Cunha, 1975; Donjacour and Cunha, 1993). SVM can permissively and instructively induce SV development in epithelium from the mesodermal Wolffian duct. When recombined with ureter, Wolffian duct, epididymal or ductus deferens epithelium, SVM induces both SV morphology and protein production (Cunha et al., 1991; Higgins et al., 1989b).

In the male urogenital system mesenchymal-epithelial interactions are dependent upon androgens. Androgen action is indirect in the developing prostate and SV (Cunha et al., 1987), and probably in the other secondary sex organs as well. Androgens act upon the mesenchyme which then directs epithelial development. This is demonstrated most dramatically in tissue recombinants made with androgen-insensitive (Tfm) epithelium and normal androgen receptor-containing mesenchyme. Such tissue recombinants grow and undergo branching morphogenesis in response to androgens despite the absence of epithelial androgen receptors (Cunha and Lung, 1978; Lasnitzki and Mizuno, 1980; Shannon and Cunha, 1984).

In the present study, SVM was recombined with bladder (BLE) or urethral epithelium (URE) to examine whether the inductive mesenchymal signals that normally stimulate a mesodermally derived epithelium to form SV could affect an epithelium derived from endoderm. Epithelium from androgen insensitive (Tfm) mice was also used, to determine whether the presence of epithelial androgen receptors was required for the response of endodermal epithelium to SVM.

SVM unexpectedly induced BLE and URE to form prostate, a tissue to which neither mesenchyme nor epithelium would normally give rise. Epithelial androgen receptors were required for the development of mature prostatic morphology and secretory activity.

**MATERIALS AND METHODS**

**Animals**

Male neonatal (day 0 = day of birth) and adult Sprague-Dawley rats were purchased from Simonsen (Gilroy, CA) for use as tissue donors. Male neonatal and adult Balb/c mice were obtained from the Cancer Research Laboratory of the University of California at Berkeley. Adult male athymic 'nude' mice were purchased from Harlan (Indianapolis, IN) and used as hosts for grafting. Adult Tfm mice were obtained from a breeding colony in our laboratory. This colony was derived from mice obtained from Dr Jean Wilson. The testicular feminization mutation in mice confers complete androgen insensitivity due to a frame-shift mutation in the androgen receptor (Charest et al., 1991; Gaspar et al., 1991; He et al., 1991). All mice were housed at the UCSF animal care facility with food and water ad libitum in accordance with the NIH guidelines for animal care.

**Tissue separation, recombination and grafting**

Rat SVM (rSVM) was obtained by trypsin separation as previously described (Higgins et al., 1989a). BLE was isolated from neonatal or adult bladders by separation with 20 mM EDTA (Cunha and Donjacour, 1987) and URE was isolated after treatment of female neonatal or adult urethras with 1% trypsin (Donjacour and Cunha, 1993). Adult Tfm animals are easily identifiable by their feminized external genitalia and lack of male secondary sex organs and Müllerian duct derivatives. Tissue recombination and subrenal capsular grafting were done as previously described (Cunha and Donjacour, 1987). Nearly all of the tissue recombinations were heterospecific; one component, usually the mesenchyme, was taken from rat and the other, usually the epithelium, was taken from mouse. The SV secretions from rats and mice contain different proteins that can be identified by immunocytochemistry and western blotting (Higgins et al., 1989a). Also rat and mouse cell nuclei can be distinguished in tissue sections stained with Hoechst dye (Cunha and Vanderslice, 1984) so that any epithelial contamination of the SVM could be detected. Any contaminated tissue recombinants were excluded from further analysis.

**Histology**

Tissue recombinants were dissected from the host mouse’s kidney capsule after one month of growth, and fixed by immersion in 4% paraformaldehyde in phosphate-buffered saline (PBS). The tissues were then embedded in paraffin, sectioned (6 μm) and stained with hematoxylin and eosin. Sections were scored on the basis of their similarity to mature prostatic tissue. The scoring was done without knowledge of the composition of the tissue recombinant.

**Immunocytochemistry**

Immunocytochemical staining was done using the avidin-biotin peroxidase detection system described by Donjacour et al. (Donjacour et al., 1990). The polyclonal antiserum anti-mDLP (Donjacour et al., 1990) was used at a dilution of 1:5,000. This antiserum stains mouse DLP and anterior prostate (AP; also known as coagulating gland),
cross-reacts somewhat with rat DLP secretion, and does not stain rat or mouse SV or ventral prostate (VP; Donjacour et al., 1990). The detection of mouse VP secretion was based on staining with MP3 1:5,000 (a gift from Dr M. Parker; Mills et al., 1987) in conjunction with the absence of staining with anti-mDLP in serial sections, as the MP3 antiserum also stained mouse DLP to some extent. The antibody to a major protein of rat SV secretion (anti-S, made against protein IV, a gift from Dr S. Higgins) was used at a dilution of 1:4,000: this antibody does not stain sections of mouse SV or any lobe of the rat or mouse prostate (Fawell and Higgins, 1986). In tissue recombinations composed of r SVM and mouse urothelium, anti-S antibody was used to detect possible contamination of the SVM with rat seminal vesicle epithelium (SVE). An antiserum to whole mouse SV secretion (anti-mSVS, 1:2,000, a gift from Dr S. Higgins; Higgins et al., 1989a) was used to detect mouse SV secretion. This antiserum also stained sections of mouse DLP so the identification of mouse SV in tissue recombinants made with mouse epithelium was based on the presence of anti-mSVS staining coupled with the absence of anti-mDLP staining in serial sections. Anti-mSVS was used to detect contamination in tissue recombinants made with mouse SVM and rat urothelium.

In some cases, prior to immunocytochemical staining, sections were stained with Hoechst dye 33258 (CalBiochem, La Jolla, CA) to detect possible epithelial contamination of the mesenchyme (Cunha and Vanderslice, 1984).

Collection of secretion
Luminal material was collected from some (57 of 86) of the tissue recombinants, as well as from mouse and rat SV and DLP, as previously described (Donjacour and Cunha, 1993).

Polyacrylamide gel electrophoresis and western blotting
Samples of luminal contents from tissue recombinants and rat and mouse SV, VP and DLP were run on SDS-polyacrylamide 10-15% gradient gels (Phast System, Pharmacia, Uppsala, Sweden), transferred to nitrocellulose using the PhastTransfer semi-dry electrophoretic transfer unit (Pharmacia), and processed for antibody staining using the enhanced chemiluminescence detection system (Donjacour and Cunha, 1993). To conserve secretory proteins from tissue recombinants, blots were cut in half before staining. Pieces containing the higher molecular masses (greater than 30x10^3) were stained with anti-mouse DLP antiserum (1:5,000; Donjacour et al., 1990), which normally reacts with DLP bands at approximately 55x10^3 and 110x10^3 relative molecular mass (Mr), pieces containing the lower molecular masses were stained with MP3 (1:5,000; Mills et al., 1987), which normally reacts with a VP band at approximately 25x10^3 Mr. Rat SV protein IV and mouse SV secretory proteins were detected using the same antibodies, at the same dilutions that were used for immunocytochemistry (Higgins et al., 1989a).

Statistics
The proportion of grafts that developed as prostate in the various types of tissue recombinants were compared using χ² contingency tables (Zar, 1984); differences were considered significant at P<0.05.

RESULTS
Normal urothelium recombined with seminal vesicle mesenchyme
Tissue recombinants composed of r SVM and normal mouse urothelium, from either the bladder (BLE) or urethra (URE), developed in over 50% of cases into a prostate-like tissue after one month of growth in intact nude mouse hosts (Table 1). The tissue recombinants were qualitatively similar regardless of whether the epithelium was BLE or URE, neonatal or adult. The highest incidence of prostatic differentiation (100%, significantly greater than the other groups in pair-wise χ² comparisons) occurred in tissue recombinants composed of r SVM+neonatal URE (Table 1). Identification of the induced tissues as prostatic was based on tissue morphology and staining with antiserum to mouse prostatic secretory proteins either by immunocytochemistry or western blot analysis. Morphological criteria alone were not considered sufficient for scoring a tissue recombinant as prostate.

Histologically r SVM+BLE and r SVM+URE tissue recombinants resembled normal adult mouse prostate (Fig. 1A). The tissues consisted of branching ducts lined with a pseudostratified epithelium. In some ducts the epithelial cells were columnar with abundant apical cytoplasm, but little luminal secretion was seen. In other larger ducts the lumen was filled with secretion, and the epithelium was low columnar to cuboidal. Epithelial infolding was seen in some ducts. One to several layers of stromal cells surrounded each duct (Fig. 1C,D). The highly infolded, tall columnar epithelium with distinct apical secretory granules characteristic of the SV (Fig. 1B) was not seen in r SVM+BLE or URE tissue recombinants.

Ninety-five percent of the tissue recombinants that had prostatic morphology produced DLP secretory proteins, as revealed by immunocytochemistry with anti-mDLP antiserum. As in the adult mouse DLP, the luminal material stained consistently, and the supranuclear region of a subset of epithelial cells also stained intensely (Fig. 2). One r SVM+adult BLE tissue recombinant stained only with an antiserum raised against mouse VP secretion (MP3, Fig. 3), indicating that some differentiation into VP had occurred.

None of the r SVM+ normal urothelium tissue recombinants developed into SV. Typical SV morphology, with its tall epithelium with granular apical cytoplasm and intensely eosinophilic secretion was not observed in hematoxylin and eosin-stained sections. Immunocytochemical staining with mSV2, a polyclonal antibody that recognizes mouse SV secretory proteins was consistently negative (data not shown).

The remaining tissue recombinants (46% of r SVM+BLE and 21% of r SVM+URE; Table 1) consisted of a substantial amount of stroma plus ductal structures that varied greatly in their diameter and epithelial height (Fig. 4A). None of these tissue recombinants stained with anti-mDLP (Fig. 4B) or anti-MP3 and thus were not considered to be prostate, despite their ductal morphology.

The results of the immunocytochemistry were corroborated by western blotting of the luminal proteins from many of the tissue recombinants. Anti-mDLP stained the same set of bands, at approximately 55x10^3 and 110x10^3 Mr, that was

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Table 1. Proportion of tissue recombinants made with rat SVM + normal urothelium that showed full prostatic differentiation

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<th>Urethra</th>
<th>Bladder</th>
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<td>Adult</td>
<td>15/23(65)</td>
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<td>Neonatal</td>
<td>15/15(100)</td>
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<td>Totals</td>
<td>30/38(79)</td>
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Percentages are given in parentheses.
seen in authentic mouse DLP secretion (Fig. 5). Of the grafts from which secretory proteins were collected, 30% (10/30) of rSVM+BLE and 81% (22/27) of rSVM+URE tissue recombinants expressed mDLP proteins as judged by western blot analysis. Neither mouse VP proteins (n=49) nor mouse SV proteins (n=53) were detected by western blot analysis.

To explore further the possibility that a small proportion of the tissue recombinants might make SV, some grafts were constructed using mouse SVM+adult rat BLE. If the resulting tissues contained rat SV this should have been readily detected with immunocytochemistry using anti-S, which does not cross-react with mouse SV secretion or any secretion from the mouse or rat prostatic complex. No indication of SV differentiation was seen in any of the mSVM+rBLE (n=30) based on morphological and immunochemical analysis. It should be noted that only 27% (8/30) of the mSVM+rBLE tissue recombinants appeared to develop into prostate as determined by morphology, immunocytochemistry or western blotting. This lower frequency of prostatic induction could be due to either the inducing mesenchyme or the responding epithelium. Mouse SVM + adult mouse BLE grafts gave a similar proportion, 30% (11/33) of prostatic tissue recombinants indicating that mouse SVM is a weaker prostatic inducer than rSVM.

**Tfm adult urothelium recombined with seminal vesicle mesenchyme**

In contrast to tissue recombinants made with rSVM and urothelium from normal mice, tissue recombinants made with

| Table 2. Proportion of tissue recombinants made with rat SVM + Tfm urothelium that showed full prostatic differentiation |
|-------------------------------------------------|---------------|----------------|
| Urethra Bladder                                  | Adult         | Neonatal       |
| Urethra                                         | 0/20          | 0/13           |
| Bladder                                         | 0/13          | 0/23           |
Fig. 2. Immunocytochemical staining with anti-mDLP antiserum. All sections were counter-stained with hematoxylin. (A) Normal mouse DLP; (B) normal mouse SV; (C) rSVM + normal adult BLE; (D) rSVM + normal adult URE. Dark apical epithelial staining is seen in the DLP (A) as well as in the tissue recombinants (C,D) but not in the SV (B). Scale bar, 50 μm.

Fig. 3. Tissue recombinant made with rSVM + normal adult BLE that did not stain with anti-mDLP antiserum (A) but that did stain with MP3 antiserum (B), indicating differentiation into VP. Scale bar, 50 μm.
urothelium from neonatal or adult Tfm mice did not undergo prostatic functional differentiation when grown in intact male nude mice for one month (Table 2). These rSVM+TfmBLE or URE tissue recombinations contained relatively small, branching ducts lined with a simple epithelium that was generally cuboidal to low columnar (Fig. 6A). The lumen appeared to be empty. Abundant stroma was present between ducts (Fig. 6A). Mouse DLP proteins were not detected with immunocytochemical staining of tissue recombinants made with Tfm BLE or URE (Fig. 6B, \( n = 79 \)). In addition, secretory proteins of mouse DLP (\( n = 43 \)), VP (\( n = 32 \)) and SV (\( n = 43 \)) were not detected by western blotting in luminal secretion from rSVM+Tfm BLE or URE tissue recombinants (Fig. 5).

**Fig. 4.** Tissue recombinants made with rSVM + normal urothelium. These grafts developed ductal structures but did not differentiate into functional prostate. (A) Hematoxylin and eosin staining; (B) anti-mDLP staining. Scale bar, 100 \( \mu m \).

**Fig. 5.** Western blots stained with (A) anti-mDLP antiserum; (B) MP3 antiserum. 1, mouse DLP secretion; 2, rSVM + normal adult BLE; 3, rSVM + normal neonatal BLE; 4, rSVM + normal adult URE; 5, rSVM + normal neonatal URE; 6, rSVM + Tfm adult BLE; 7, rSVM + Tfm neonatal BLE; 8, rSVM + Tfm adult URE; 9, rSVM + Tfm neonatal URE; 10, mouse VP secretion. The two major bands of the DLP are seen in the secretion from the tissue recombinants made with normal epithelium (A, lanes 2-5) but not in the secretion of tissue recombinants made with Tfm epithelium (A, lanes 6-9). The VP-specific band stained by MP3 (B, lane 10) was absent in secretion from all of the tissue recombinants. The relative molecular mass of protein markers is indicated at left (\( \times 10^{-3} \)).

**DISCUSSION**

This study describes a unique type of mesenchymal-epithelial interaction in which a urothelial phenotype was changed to that of the prostate both functionally and morphologically by a mesenchyme, SVM, that does not induce prostate in vivo. How can SVM induce the formation of another organ from a fully differentiated epithelium?

One way to explain this interaction may be to view instructive inductions as a selective rather than a truly instructive process. A strictly instructive model of organogenetic induction would have predicted that rSVM+mouse BLE or rSVM+mouse URE tissue recombinants should develop as SV. It appears that endodermally derived BLE and URE do not have the capability of expressing the SV phenotype. Instead urogenital sinus epithelium (UGE) normally gives rise to approximately five different types of epithelia: prostatic, urothelial, urethral gland, bulbourethral gland, and vaginal. It may be surmised that in vivo different regions of the UGM, under the appropriate hormonal conditions, produces signals that select among these possible epithelial cell fates. Indeed, regional differences in inductive activity of UGM has been reported previously (Sugimura et al., 1985; Takeda et al., 1990).

Another illustration of this type of epithelial restriction occurs in the female urogenital tract. In the presence of androgens, epithelium from the sinus vagina, which is of urogenital sinus origin, can be induced by UGM to undergo prostatic differentiation (Boutin et al., 1991). However, epithelium from the sinus vagina is unable to undergo uterine differentiation in response to uterine mesenchyme (Boutin et al., 1992). The uterus is derived from the mesodermal Müllerian duct. Mesenchymal induction of uterine differentiation is not an option for an endodermally derived epithelium. A similar situation exists in the Wolffian duct; Wolffian duct epithelia can only make SV, not prostate, regardless of whether the inducer is UGM or SVM (Cunha, 1972; Cunha et al., 1991; Higgins et al., 1989b; Tsuji et al., 1994; Turner et al., 1989). Thus, in the urogenital tract it has not been possible to induce epithelial differentiation inappropriate to the developmental repertoire of the urogenital sinus, Müllerian duct, or Wolffian duct lineages. This rule appears to apply for all three germ layers as careful examination of inductive tissue interactions
does not reveal a single case in which a given epithelium has
be induced to express a differentiation unique and specific to
a different germ layer.

SVM is as strong an inducer of prostate as the UGM itself.
When recombined with BLE the proportion of rSVM+mouse
BLE tissue recombinants forming prostate (43%) is not statis-
tically different from rUGM+adult mouse BLE tissue recom-
binants (52%) which also form prostate (Donjacour and Cunha,
1993). Homotypic UGM+UGE tissue recombinants develop
into prostate with a frequency of 77 to 100% (Cunha, 1976;
Cunha and Lung, 1978). SVM can induce UGE to form
prostate as judged by morphological criteria (Cunha, 1972) and
UGM can induce SV development in SVE (Cunha, 1972).
These tissue recombinations between urogenital sinus and
Wolffian duct epithelium and mesenchyme support the idea
that UGM and SVM produce similar if not identical inductive
signals.

Prostatic inductions are dependent upon androgen both
experimentally and in vivo (Cunha et al., 1980; Price and
Williams-Ashman, 1961). During development, androgens
appear to stimulate ductal branching morphogenesis and
epithelial proliferation indirectly via the mesenchyme (Cunha
et al., 1987) However, the production of prostatic or SV
secretory proteins appears to require the direct interaction of
androgens with epithelial androgen receptors (Cunha
and Young, 1991; Donjacour and Cunha, 1993). This concept is
supported by tissue recombination experiments between
normal and Tfm epithelium and mesenchyme. Tfm mice are
androgen insensitive due to a mutation in the androgen receptor
(Charest et al., 1991; Gaspar et al., 1991; He et al., 1991).
Consistent with previous studies, prostatic ductal growth and
morphogenesis was induced by SVM in Tfm BLE or Tfm URE but
androgen-dependent SV or prostatic proteins were not
produced.

The identity of the androgen-dependent signals that mediate
mesenchymal-epithelial interactions in the prostate and SV are
unknown; however, growth factors are promising candidates.
Members of all of the major growth factor families have been
found in the adult prostate (Story, 1991). Keratinocyte growth
factor (KGF) is a member of the fibroblast growth factor family
(FGF7). It has been proposed as a likely mediator in mes-
enchymal-epithelial interactions based on its production in the
mesenchyme of developing organs (Mason et al., 1994), the
localization of its receptor in associated epithelia (Finch et al.,
1995; Orr-Urtreger et al., 1993) and the presence of a signal
sequence within KGF (Aaronson et al., 1991; Cunha et al.,
1994). Fibroblast growth factors are necessary for branching
morphogenesis in the lung as shown by the total absence of
branching, but continued growth, of the primary bronchi in
transgenic mice carrying a dominant-negative mutation for the
FGF receptor two (FGFR2) (Peters et al., 1994). In organ
cultures of neonatal mouse SV, a neutralizing monoclonal
antibody to KGF (anti-KGF) inhibited androgen-induced
branching morphogenesis. Exogenous KGF by itself did not
support SV branching morphogenesis; however, it stimulated
proliferation of the SV in the absence of testosterone (Alarid
et al., 1994). In organ cultures of rat VP, anti-KGF also blocked
testosterone-stimulated epithelial branching. Significantly,
KGF alone was able to stimulate branching morphogenesis in the
rat VP in the absence of testosterone (Foster and Cunha,
1994b; Sugimura et al., 1994). Antibodies to transforming
growth factor alpha (TGFα) blocked both androgen and KGF-
stimulated branching morphogenesis in the rat VP (Foster and
Cunha, unpublished data), suggesting that TGFα may be a
down-stream mediator of androgen and KGF. In cultured ker-
atinocytes, TGFα has been shown to mediate the effects of
KGF (Dlugosz et al., 1994).

The transforming growth factor beta (TGFβ) family may
play a counterbalancing role in SV and prostate development.
Branching morphogenesis of both organs in culture are
inhibited by TGFβ (Foster and Cunha, 1994a; Tanji et al.,
1994) TGFβ is developmentally regulated in UGM and the
mesenchyme of the neonatal prostate (Timme et al., 1994). In
many systems TGFβ decreases epithelial proliferation and
stimulates the deposition of extracellular matrix components
(Barnard et al., 1990). In the mammary gland and the lung
TGFβ is localized in ductal clefts and along ducts while it is
sparse at ductal tips where epithelial proliferation is greatest
(Serra et al., 1994; Silberstein et al., 1992).

In order to respond to common mesenchymal growth
factors, epithelia of urogenital and Wolffian duct origin would
both need to express common or related receptors for these
factors. There appears to be some redundancy among growth
factors and their receptors. For example, acidic FGF can bind
to both the IIIb and IIIc splice variants of the bek gene product
or FGFR2, even though these receptor isoforms have vastly
different affinities for KGF and basic FGF (Miki et al., 1992). It is possible that SVM is able to induce prostatic epithelial development via a growth factor different from the endogenous one.

The factors regulating the differential responsiveness of the urogenital and Wolffian duct epithelia to mesenchymal signals are unknown. Homeotic genes, which are involved in determining segment identity in invertebrates, may play a role in determining epithelial cell competence in vertebrates. Genes of the HoxD cluster are expressed in the male urogenital tract (Dollé et al., 1991). However their spatial expression pattern does not suggest involvement in epithelial determination as differential expression occurs in the mesenchyme rather than the epithelium. The spatial pattern of another homeotic gene, msx-1 is more interesting in this regard. Msx-1 is a homeotic gene outside the Hox cluster that is homologous to the drosophila msh gene (Davidson and Hill, 1991). In the developing female reproductive tract msx-1 is expressed during fetal development in the epithelia of all the Mullerian duct derivatives, and postnatally becomes restricted to the uterine epithelium (Pavlova et al., 1994). Msx-1 gene expression is associated with maintenance of developmental plasticity (Morgan and Tabin, 1993; Pavlova et al., 1994). The distribution of msx-1 in the male urogenital tract has not been determined.

The ability of SVM to elicit prostatic differentiation from urothelium supports the hypothesis that mesenchymal-epithelial interactions are selective rather than truly instructive. This interpretation shifts the search for specificity from the mesenchymal signal, which in this model acts as a trigger, to the epithelium in which sequential restrictions of developmental potential occur. The intracellular mechanisms of epithelial restriction and the molecular triggers involved in prostatic development remain to be elucidated.

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