Available online at www.sciencedirect.com
SCIENCE DIRECT®
Journal of Steroid Biochemistry & Molecular Biology 92 (2004) 221–236
The Journal of Steroid Biochemistry & Molecular Biology
www.elsevier.com/locate/jsbmb

Hormonal, cellular, and molecular regulation of normal and neoplastic prostatic development

Gerald R. Cunha[a,b,*], Will Ricke[a], Axel Thomson[c], Paul C. Marker[d], Gail Risbridger[e], Simon W. Hayward[f], Y.Z. Wang[g], Annemarie A. Donjacour[a], Takeshi Kurita[a]

[a] *Department of Anatomy, University of California, Box 0452, 513 Parnassus Avenue, San Francisco, CA 94143-0452, USA*
[b] *Department of Urology, University of California, San Francisco, CA 94143, USA*
[c] *MRC Human Reproductive Sciences Unit, Centre for Reproductive Biology, University of Edinburgh Chancellor's Building, 49 Little France Crescent, Edinburgh, EH16 4SB, UK*
[d] *University of Minnesota, Cancer Center, MMC 806, 420 Delaware St. SE, Minneapolis, MN 55455, USA*
[e] *Monash Institute of Reproduction and Development, Monash Medical Centre, 246 Clayton Road, Clayton, Vic. 3168, Australia*
[f] *Departments of Urologic Surgery and Cancer Biology, Vanderbilt University Medical Center, A 1302 MCN, 116121st Avenue South, Nashville, TN 37232-2765, USA*
[g] *BC Cancer Agency, Department of Cancer Endocrinology, 600 West 10th Avenue, Vancouver, BC, Canada, V5Z 4E6*

Abstract

This review on normal and neoplastic growth of the prostate emphasizes the importance of epithelial–mesenchymal/stromal interactions. Accordingly, during prostatic development urogenital sinus mesenchyme (a) specifies prostatic epithelial identity, (b) induces epithelial bud formation, (c) elicits prostatic bud growth and regulates ductal branching, (d) promotes differentiation of a secretory epithelium, and (e) specifies the types of secretory proteins expressed. In reciprocal fashion, prostatic epithelium induces smooth muscle differentiation in the mesenchyme. Epithelial–mesenchymal interactions during development continue postnatally into adulthood as stromal–epithelial interactions which play a homeostatic role and in so doing reciprocally maintain epithelial and stromal differentiation and growth-quiescence. Prostatic carcinogenesis involves perturbation of these reciprocal homeostatic cell–cell interactions. The central role of mesenchyme in prostatic epithelial development has been firmly established through analysis of tissue recombinants composed of androgen-receptor-positive wild-type mesenchyme and androgen-receptor-negative epithelium. These studies revealed that at the very least ductal morphogenesis, epithelial cytodifferentiation, epithelial apoptosis and epithelial proliferation are regulated by stromal and not epithelial androgen receptors. Likewise, progression from non-tumorigenesis to tumorigenesis elicited by testosterone plus estradiol proceeds via paracrine mechanisms. Thus, stromal–epithelial interactions play critical roles in the hormonal, cellular, and molecular regulation of normal and neoplastic prostatic development.

Keywords: Epithelium; Mesenchyme; Stroma; Epithelial–stromal interactions; Epithelial–mesenchymal interactions; Hormonal carcinogenesis; Fibroblasts growth factors; Activin; Inhibin; Sonic hedgehog; Androgen receptors; Estrogen receptors

1. Introduction

This review focuses on the hormonal, cellular, and molecular regulation of normal and neoplastic prostatic development. The central underpinning of our research is that developmental mechanisms involved in organogenesis of the prostate may have relevance to the genesis and biology of prostate cancer. One of the pioneers of the relationship between normal development and carcinogenesis was the pathologist G. Barry Pierce who promulgated the concept that "Neoplasia is a caricature of differentiation" [1]. This idea is based on observations that virtually all properties of neoplasms have a counterpart in normal embryonic development. Accordingly, proliferation, differentiation, invasion and apoptosis are events especially relevant to neoplasms as well as to the developing embryo. In the prostate these events

* Corresponding author. Tel.: +1 415 476 4140; fax: +1 415 502 2270.
E-mail address: grcunha@itsa.ucsf.edu (G.R. Cunha).

0960-0760/$ – see front matter © 2004 Elsevier Ltd. All rights reserved.
doi:10.1016/j.jsbmb.2004.10.017

are cardinal features of both normal development and carcinogenesis.

2. Overview of prostatic development

In all species the prostate develops from the endodermal urogenital sinus (UGS), which is derived from the caudal terminus of the hindgut called the cloaca (Fig. 1). The urorectal septum subdivides the cloaca into the UGS ventrally and the rectum and anal canal dorsally (Fig. 1). The endodermal UGS is an ambisexual embryonic rudiment, which develops into the prostate, prostatic urethra and bulbourethral glands in males, the lower vagina and urethra in females, and into the bladder in both sexes [2]. The endodermal UGS is surrounded by embryonic connective tissue called urogenital sinus mesenchyme (UGM). Before sexual differentiation of the UGS, UGM expresses androgen receptors (AR) in both sexes and thus acquires the capacity to undergo masculine development [3,4]. In response to fetal testicular androgens, epithelial buds emerge from the wall of the UGS, grow into the surrounding

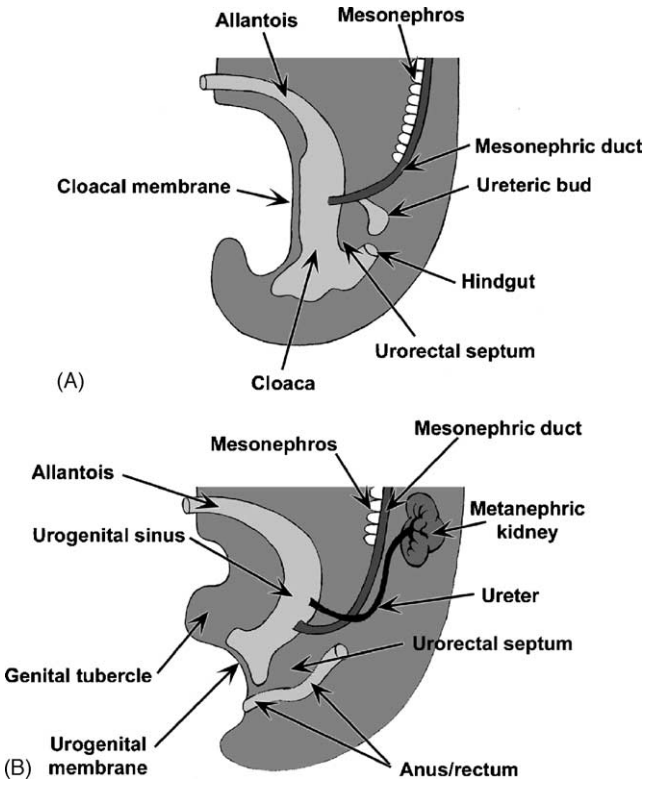

Fig. 1. Division of the cloaca into the urogenital sinus, rectum and anal canal. (A) Mid-sagittal view through the pelvis of a 4 week human fetus showing the cloaca, the blind caudal terminus of the hindgut. The cloaca has a ventral diverticulum, the allantois, extending up the anterior body wall and terminating in the umbilical cord. The urorectal septum grows caudally towards cloacal membrane to subdivide the cloaca. (B) Mid-sagittal view through the pelvis of a 7 week human fetus showing division of the cloaca into the urogenital sinus ventrally and rectum and anal canal dorsally. Note that the division of the cloaca has occurred in a manner in which the mesonephric duct and ureter empty into the urogenital sinus.

UGM in a precise spatial pattern, and thus establish in rodents the lobar subdivisions of the prostate into dorsal-lateral, ventral, and anterior prostates, each having a characteristic ductal branching pattern [5–8]. The anterior prostate grows in close association with the seminal vesicles (SV). Indeed, the epithelial rudiments of the SV and the anterior prostate develop within a common mass of mesenchyme, historically designated seminal vesicle mesenchyme (SVM) [9]. In reality "SVM" is a mesenchymal inducer of both prostate and SV with the specific tissue response being determined by germ layer derivation of the epithelium [10–12].

In the perinatal period (rats and mice), the solid prostatic buds elongate and then undergo a process of branching morphogenesis, which is completed by the end of puberty [7,8]. Initially, prostatic buds and prostatic ducts are solid. Beginning in the neonatal period the solid epithelial cords canalize. Ductal canalization begins at the urethra and progresses distally towards the ductal tips [6]. During ductal canalization luminal and basal epithelial cells differentiate. Secretory cytodifferentiation of the epithelium occurs postnatally in laboratory rodents, and prostate-specific secretory proteins are initially detected in rats and mice at 12–20 days postnatal [13]. Prostatic epithelial differentiation is accompanied by differentiation of the mesenchyme into smooth muscle cells and fibroblasts [14,15].

3. Mesenchymal–epithelial interactions in prostatic development

The prostate develops from the embryonic urogenital sinus in the presence of androgens as a result of obligatory interactions between urogenital sinus epithelium (UGE) and UGM. During prostatic development UGM (a) specifies prostatic epithelial identity, (b) induces epithelial bud formation, (c) elicits prostatic bud growth and regulates ductal branching, (d) promotes differentiation of a secretory epithelium, and (e) specifies the types of secretory proteins expressed [6,16].

4. Androgenic effects and mesenchymal–epithelial interactions

Androgenic effects on prostatic development are mediated via androgen receptors (AR) in the context of mesenchymal–epithelial interactions. An important relationship between AR and mesenchymal–epithelial interactions is revealed by the ontogeny of AR in the prostate. During prenatal development AR are initially detected solely in UGM prior to and during prostatic bud formation. AR are undetectable in developing prostatic buds suggesting that mesenchymal (and not epithelial) AR are critically involved in the early phases of prostatic development [3,4]. To elucidate the respective roles of epithelial versus mesenchymal AR in prostatic development, chimeric prostates were prepared with mesenchyme

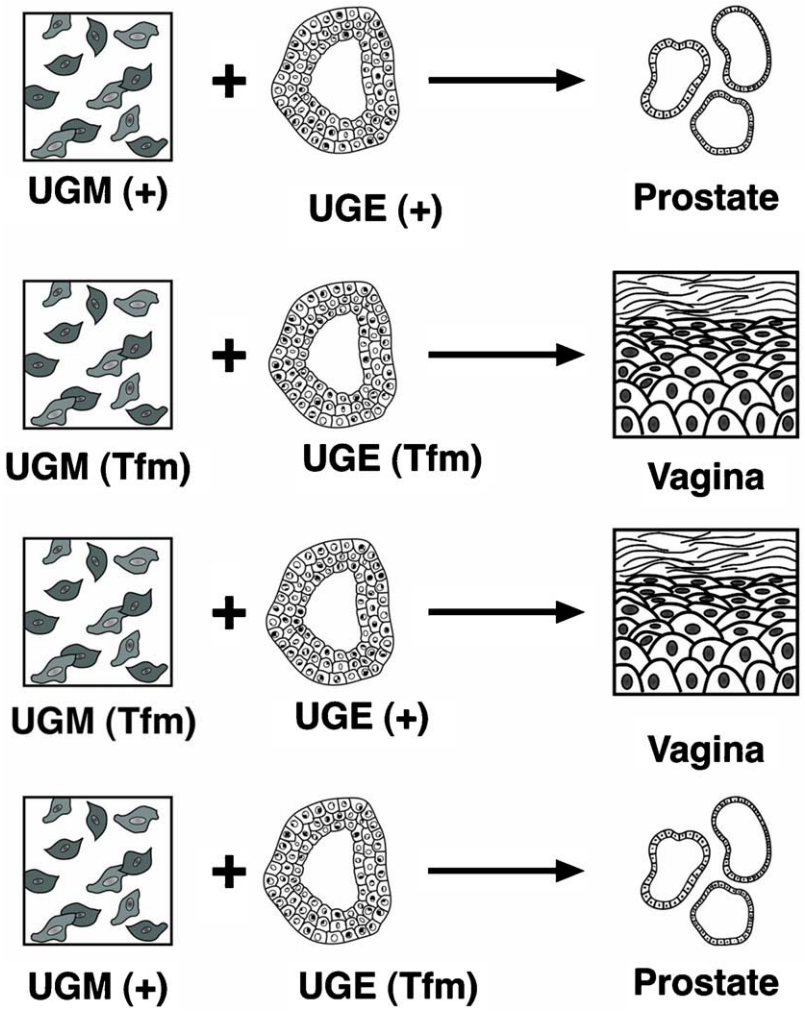

Fig. 2. Summary of tissue recombination experiments between urogenital sinus mesenchyme and epithelium from Tfm and wild-type embryos. A positive androgenic response (prostatic morphogenesis) occurs when wild-type mesenchyme is grown in association with either wild-type or Tfm epithelium. Conversely, vagina-like differentiation occurs when either wild-type or Tfm epithelium is grown in association with Tfm mesenchyme. These results demonstrate that androgens elicit many of their effects on epithelial development via mesenchymal androgen receptors (redrawn from [132]).

and epithelium from wild-type (wt) and AR-deficient testicular feminization (Tfm) mice (Fig. 2) [17]. In keeping with the feminized phenotype of Tfm mice (absence of prostate), tissue recombinants composed of Tfm-UGM + Tfm-epithelium did not form prostate even in the presence of androgens. As expected wt-UGM + wt-epithelium tissue recombinants formed prostate in response to androgens. Tfm-UGM + wt-epithelium tissue recombinants did not undergo prostatic development in the presence of androgens, which suggested a critical role of mesenchymal AR in prostatic development. This idea was confirmed in the reciprocal wt-UGM + Tfm-epithelium tissue recombinant in which AR-deficient Tfm epithelium underwent prostatic development in association with AR-positive wild-type UGM (Fig. 2). In wt-UGM + Tfm-epithelium tissue recombinants the AR-deficient Tfm epithelium underwent androgen-dependent ductal morphogenesis, epithelial proliferation and columnar cytodifferentiation thus forming glandular epithelium resembling prostate [18]. We presume that the developmental processes occurring in wt-UGM + Tfm-epithelium tissue recombinants also involved induction of prostatic epithelial identity and formation of solid prostatic buds that subsequently canalized and underwent columnar cytodifferentiation. These experiments demonstrated that many “androgenic effects” on prostatic epithelial development do not require epithelial AR. Rather, many androgenic effects expressed in epithelium are elicited by the paracrine action of AR-positive mesenchyme. Further analysis of Tfm/wild-type tissue recombinants has revealed that epithelial AR are required for expression of AR-dependent secretory proteins [19,20].

5. Specification of prostatic epithelial identity

The earliest event in prostatic development is specification of prostatic epithelial identity. As mentioned above, the primitive UGE has a very broad developmental repertoire encompassing both male and female urogenital tract struc-

tures. Under the influence of androgens, UGM determines prostatic epithelial identity so that subsequent cell–cell interactions can elicit prostatic bud outgrowth, ductal branching and prostatic differentiation. The ability of UGM to specify prostatic epithelial identity was discovered through the analysis of heterotypic tissue recombinants composed of UGM plus epithelium of embryonic or fully differentiated adult urinary bladder, in which bladder epithelium (BLE) was induced by UGM to undergo prostatic differentiation. This remarkable change in adult epithelial histodifferentiation (bladder → prostate) implies that adult BLE is not committed to a single differentiated state. Instead, adult BLE differentiation can be maintained as such or reprogrammed by paracrine cues from mesenchyme/stroma. The molecules produced by UGM that induce prostatic epithelial differentiation are unknown. Induction of prostatic epithelial identity is thought to be an early event preceding the formation of prostatic buds. In this regard, the homeobox gene, Nkx3.1, appears in UGE of male mouse fetuses about 48 h before prostatic buds emerge. Expression of this transcription factor is androgen-inducible and occurs in the male, but not female, UGE [21]. Thus, Nkx3.1 is the earliest prostatic marker whose expression occurs at a time when prostatic epithelial identity is being acquired. In UGM + BLE tissue recombinants the UGM induces Nkx3.1 in the BLE [22]. While Nkx3.1 is the earliest UGM-induced prostatic marker, studies of Nkx3.1 null mice reveal that prostatic development can occur in the absence of Nkx3.1, even though subsequent prostatic growth and differentiation are adversely affected [22].

6. Prostatic bud stage

In mice and rats prostatic buds form on days 17 and 19 of gestation, respectively [23]. Bud formation does not occur when UGE is grown by itself. Prostatic bud development is normally induced in UGE by UGM, but experimentally can be induced in a variety of endoderm-derived epithelia from the bladder, vagina and the urethra [24,25]. The mechanism of prostatic bud formation is poorly understood. When first recognizable histologically, prostatic buds are spherical protrusions about 45 μm in diameter extending from the UGE into the surrounding UGM (Donjacour unpublished). These small epithelial buds contain the progenitor cells for generation of the extensively branched ductal trees that subsequently develop. While the number of epithelial cells in a newly emerged prostatic bud is probably only a few hundred, evidence from analysis of chimeric mice reveals that at least some of the prostatic buds are polyclonal in origin [26]. Thus, each prostatic bud may contain two or more progenitor lineages. Localized proliferation in the UGE does not precede initial bud formation, and there is no local thickening or epithelial placode formation preceding bud formation. The only morphological hint of where a bud may form are small indentations of the epithelial basal lamina [27]. In early elongating buds, the distal tips have a higher ki67 labeling index than do the proximal bud segments [27]. This is similar to the pattern of proliferation seen postnatally [28].

7. Lobe and region-specific identity in the prostate

The rodent prostate is a multi-lobed gland arranged around the urethra at the base of the bladder. The lobes of the rodent prostate are named for their anatomical position: ventral prostate (VP), dorsolateral prostate (DLP, also sometimes considered as separate dorsal and lateral lobes), and anterior prostate (AP). Due to lobe-specific differences in the patterns of branching morphogenesis, the final shape of each lobe is distinct. In addition, the lobes have distinct histologic features with extensive epithelial infolding in the AP, significant but less epithelial infolding in the DLP, and minimal epithelial infolding in the VP. The prostatic lobes also express distinct groups of secretory proteins. Within each lobe, regional differences in cell morphology, rates of DNA synthesis, and secretory activity are also observed along the proximal-distal (urethra to ductal tip) axis of prostatic ducts [28,29].

In contrast to the rodent prostate, the adult human prostate is a compact gland without distinct lobes. It is roughly the size and shape of a walnut (20 g and 4 cm × 2.5 cm). The human prostate clearly exhibits distinct anatomical regions that are commonly described as three zones: central zone, transition zone, and peripheral zone, reflecting three distinct sets of ducts [30]. Comparative observations of prostatic development in rodents and humans demonstrates that prostatic morphogenesis occurs in an analogous manner in both humans and rodents with several distinct sets of epithelial buds growing out of the urethra into the UGM [5,31]. Nevertheless, compelling molecular evidence for homology between specific rodent prostatic lobes and human prostatic zones has yet to be identified, and little is known about the molecular basis for the lobe- and region-specific features observed in the prostate. Nevertheless, this aspect of prostatic biology is important because prostatic diseases occur in a highly region-specific manner since human prostatic adenocarcinoma is predominantly a disease of the peripheral zone and benign prostatic hyperplasia is predominantly a disease of the transition zone.

One gene that participates in establishing lobe-specific identity is *homeobox a*10 (*Hoxa*10). *Hoxa*10 encodes a transcription factor that is expressed in both the epithelium and mesenchyme of the developing prostate. Mice null for *Hoxa*10 have reduced branching in the AP and a partial AP to DLP transformation based on ductal morphology and branching pattern [32]. These phenotypes implicate *Hoxa*10 in the establishment of the AP-specific pattern of branching morphogenesis. A second gene, *fucosyltransferase* 1 (*Fut*1) has also been implicated in region specific differences in epithelial proliferation during prostatic development. *Fut*1 encodes a transmembrane carbohydrate-modifying enzyme present in the secretory pathway and at the cell surface. *Fut*1 is expressed in the developing epithelium of all prostatic lobes.

However, within each lobe, *Fut*1 is restricted to a subset of epithelial ducts. Inhibitory antibodies directed against the cell-surface fraction of the FUT1 protein reduce epithelial proliferation during prostatic branching morphogenesis [33]. These observations implicate *Fut*1 as part of the molecular mechanism that establishes region-specific heterogeneity within the prostate.

8. Prostatic epithelial cytodifferentiation

All of the above aspects of prostatic development (specification of prostatic epithelial identity, induction of epithelial bud formation, and prostatic bud growth and branching) are induced by UGM, which in turn promotes prostatic epithelial differentiation into secretory epithelial cells and specifies the types of secretory proteins expressed. The normal process of prostatic development involves the emergence of solid epithelial buds from the stratified epithelium of the UGS, followed subsequently by their canalization to form ducts lined by a simple columnar secretory epithelium. Concurrent with ductal elongation and branching morphogenesis, epithelial cytodifferentiation begins shortly after birth in rats and mice. Epithelial cells of the UGS and the developing solid prostatic buds express a wide spectrum of cytokeratins (cytokeratins 5, 8, 14, 18 and 19) and p63 [34]. As the solid epithelial cords elongate into the surrounding mesenchyme, ductal canalization is initiated beginning at the urethra and proceeding distally towards the ductal tips. In rats and mice, as the solid epithelial cords canalize, the epithelium reorganizes into tall columnar luminal cells and a discontinuous layer of basal cells in rats and mice. The luminal cells express cytokeratins 8, 18 and 19 and differentiate into secretory cells [35]. The basal cells express cytokeratins 5 and 14, and p63 and are localized along the basement membrane [34,35]. By this process the solid epithelial buds (co-expressing both luminal and basal cell markers) differentiate into the distinct luminal and basal cell lineages with characteristic phenotypes and functional roles, each expressing their characteristic subset of cytokeratins and other marker proteins [34,35]. The third epithelial cell type that differentiates in the prostate is the neuroendocrine cell, which makes up only a small proportion of the epithelial cells and is characterized by the expression of functional markers such as chromogranin A and synaptophysin [36,37].

Evidence suggests that epithelial differentiation described above is induced and regulated by paracrine influences from UGM. This is particularly evident in experiments in which embryonic or adult bladder epithelium (BLE) was grown in association with UGM [25]. Adult bladder epithelium has a unique histodifferentiation and is characterized as urothelium. Urothelium is stratified, but usually not keratinized. Urothelium of the bladder is composed of specialized cells that adopt a narrow dome-shape when the bladder is empty and a stratified squamous shape as the bladder fills with urine. Cell membranes of bladder urothelium express a unique spectrum of membrane proteins, the uroplakins, which are thought to be essential to accommodate the cellular shape changes during expansion and contraction of the bladder [38]. Bladder urothelium is non-glandular and does not express AR. When adult bladder epithelium (BLE) is combined with UGM, solid prostatic buds emerge from the basal aspect of the BLE [25]. These buds elongate, undergo branching morphogenesis and differentiate into an AR-positive secretory epithelium that expresses prostate-specific secretory proteins [39]. The types of secretory proteins produced by the epithelium are specified by the origin of the mesenchyme. When the ventral subdivision of the UGM is recombined with UGE or BLE, the epithelium forms prostatic ducts that express secretory proteins characteristic of ventral prostate [40,41]. Likewise, when adult ventral prostatic epithelium is partnered with mesenchyme of the anterior prostate/seminal vesicle, the induced prostatic ducts express secretory proteins characteristic of anterior and dorsolateral prostate [16]. Thus, UGM not only elicits secretory cytodifferentiation, but also specifies the types of secretory proteins produced.

Finally, it is important to note that the inductive signals from UGM that elicit transformation of adult bladder epithelium into prostatic epithelium can induce this change in epithelial cytodifferentiation across species lines [42], thus indicating that the vocabulary of this cellular dialogue is highly conserved in mammals. In this regard, we have shown that a rat prostatic mesenchymal inducer can elicit prostatic differentiation from urinary bladder epithelium derived from 60- to 70-year-old men [43]. Not only did the induced adult human epithelium exhibit prostatic ductal organization, but the induced epithelium expressed human prostate specific antigen (PSA) [43].

9. Mesenchymal differentiation

All epithelia are associated with connective tissue, which plays a critical role in epithelial development and differentiation. As discussed above in the case of the prostate, UGM (a) specifies prostatic epithelial identity, (b) induces epithelial bud formation, (c) elicits bud growth and regulates ductal branching, (d) promotes epithelial differentiation into secretory epithelial cells, and (e) specifies the types of secretory proteins expressed [6,16]. While it is well established that UGM induces epithelial development, it is likewise true that developing prostatic epithelium in turn induces UGM to undergo smooth muscle differentiation [44]. Thus, the developmental interactions between UGM and UGE are reciprocal in that UGM induces prostatic epithelial differentiation, and the developing prostatic epithelium induces and patterns smooth muscle differentiation in the UGM [45]. The role of epithelium in smooth muscle differentiation is based upon the observation that only small amounts of smooth muscle differentiate in grafts of embryonic UGM only, while tissue recombinants composed of UGM plus epithelium of either adult prostate, bladder or embryonic urogenital sinus,

developed prostatic ducts which are surrounded by α-actin-positive smooth muscle bundles [46]. Tissue recombinants composed of rat UGM plus human prostatic epithelium further demonstrated that human prostatic epithelium not only induced the rat UGM to undergo smooth muscle differentiation but also determined spatial patterning of the smooth muscle [47]. Thus, prostatic development occurs as a result of reciprocal mesenchymal–epithelial interactions in which mesenchyme induces epithelial differentiation and epithelium induces mesenchymal differentiation.

10. Role of stromal–epithelial interactions in prostatic apoptosis

Maintenance of adult prostatic epithelium is dependent upon the presence of androgens. Androgen-deprivation elicited by castration triggers apoptosis of prostatic epithelium [48,49]. Thus, failure to occupy androgen receptors (AR) is the trigger for prostatic epithelial apoptosis [50]. The mature prostate contains epithelial and stromal cells, both of which express AR [51]. This raises the question as to whether failure to occupy epithelial versus stromal AR is the trigger for prostatic epithelial apoptosis? To answer this question, chimeric prostates were constructed with epithelium (E) from wild-type (wt) or Tfm mice combined with normal rat UGM [52]. The resultant tissue recombinants were grafted into intact male nude mouse hosts. One month after grafting, both rat UGM + Tfm-E and rat UGM + wt-E tissue recombinants

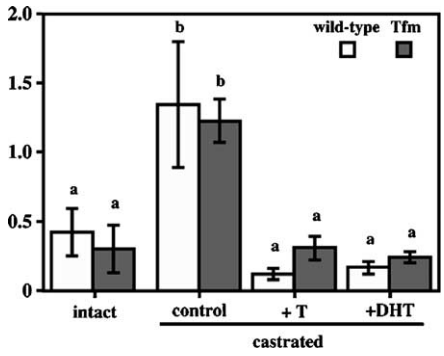

Fig. 3. Epithelial apoptotic index in prostatic recombinants. Tissue recombinants were prepared with rat urogenital sinus mesenchyme (rUGM, which is androgen receptor positive) plus either wild-type epithelium (wt-E, which is androgen receptor positive) or Tfm epithelium (Tfm-E, which is androgen receptor negative). After 4 weeks of growth as grafts to intact male hosts, the hosts were divided into four groups. One group of hosts remained intact, while the remainder were (a) castrated (control), (b) castrated and immediately treated with testosterone (+T), or (c) (b) castrated and immediately treated with dihydrotestosterone (+DHT) as described previously [52]. Each host received both rUGM + wt-E and rUGM + Tfm-E tissue recombinants. Epithelial apoptosis was assessed by TUNEL and was expressed as apoptotic index [52]. Note that castration induced epithelial apoptosis comparably in rUGM + wt-E and rUGM + Tfm-E tissue recombinants. Likewise, testosterone and dihydrotestosterone inhibited castration-induced epithelial apoptosis comparably, thus suggesting that regulation of epithelial apoptosis does not require epithelial androgen receptors but instead is mediated via stromal androgen receptors (figure from [52]).

formed prostate. The hosts were then castrated, which induced apoptosis in the prostatic epithelial cells comparably in both the rat UGM + Tfm-E and rat UGM + wt-E tissue recombinants (Fig. 3). Moreover, androgen-treatment (DHT or T) inhibited castration-induced epithelial apoptosis in both the rat UGM + Tfm-E and rat UGM + wt-E tissue recombinants. These results clearly indicate that epithelial AR are not required to regulate apoptosis of prostatic epithelium. We could not test whether stromal AR is essential to inhibit castration-induced prostatic epithelial apoptosis by androgen, because AR in UGM is essential to induce prostate. Therefore, androgen may have inhibited apoptosis of prostatic epithelium through a systemic effect on the host mouse. This issue can be settled in the future by tissue recombination experiments utilizing an inducible AR-knockout mouse, with which stromal and/or epithelial AR genes can be inactivated after full growth of the prostate.

11. Role of fibroblast growth factors in prostatic development

Studies of gene knockout mice have elucidated some of the molecules that are involved in prostatic development. Members of the fibroblast growth factor (FGF) family such as FGF7 and FGF10 clearly play important roles in prostatic development (see below). FGF10 null mice develop a urogenital sinus, but fail to develop prostate [27]. Even though the testes of FGF10 null mice produce sufficient androgens to induce prostatic development, few if any prostatic buds are observed in these animals [27]. Because FGF10 null mice die at birth, prostatic development was studied in vivo by transplantation of FGF10 null prostatic rudiments, which showed little growth but did show some signs of prostatic differentiation [27]. In organ cultures of FGF10 null urogenital sinuses, prostatic buds formed when FGF10 and testosterone were added to the medium, but FGF10 alone did not stimulate prostatic bud formation in wild-type or FGF10 null urogenital sinuses in the absence of testosterone. Thus, FGF10 is not the inducer of prostatic bud formation, but instead may be required for bud stabilization and is definitely mitogenic on developing prostatic epithelium [27].

Following the emergence of prostatic buds from the UGS, the buds elongate and undergo branching morphogenesis in a lobe-specific pattern [7]. The Tfm/wild-type tissue recombinant experiments described above (Fig. 2) demonstrated that androgenic induction of prostatic epithelial development and growth does not require epithelial AR, but instead is elicited by paracrine factors produced by the AR-positive UGM. Two members of the FGF family, FGF7 and FGF10, have been studied for their roles in the paracrine regulation of prostatic ductal growth [53]. In the developing prostate, FGF7 and FGF10 are produced by the mesenchyme. The FGF receptor (FGFR2iiib), to which these FGFs bind, is expressed exclusively in the epithelium [54,55]. Thus, the spatial pattern of expression of these molecules in the developing prostate is

consistent with paracrine action of androgens mediated by mesenchymal factors. Even though prostatic defects have not been reported in FGF7 null mice, neutralization of FGF7 with a monoclonal antibody or a soluble fragment of FGFR2iiib inhibits androgen-stimulated prostatic epithelial growth and ductal branching. Exogenous FGF7 and FGF10 largely overcome the requirement for testosterone during prostatic epithelial growth and ductal branching [27,54,56]. Even though testosterone stimulates prostatic epithelial growth in vivo or in organ culture, it appears that neither FGF7 nor FGF10 are directly regulated by androgens in vivo [54,55].

12. Role of sonic hedgehog in prostatic development

The sonic hedgehog (Shh) signaling pathway mediates epithelial–mesenchymal interactions in several tissues during development and disease, and is involved in prostatic growth and differentiation. Initial studies suggested that Shh was required for prostatic development [57] and might be regulated by androgens [57,58]. More recent studies have shown that Shh is not required for the formation of the prostate, but that Shh is involved in subsequent growth and ductal patterning [59,60]. The expression of *Shh* and its receptor Patched (*Ptc*) correlates with growth and development of the prostate, and their expression in the prostate is not directly regulated by androgens [60]. Additionally, it appears that the primary cause of prostatic agenesis in *Shh* knockout mice is due to androgen insufficiency as a result of a testicular defect [59]. Several observations suggest that *Shh*-signaling is not critical for induction of prostatic buds. For example, prostatic budding was induced in response to testosterone in *Shh* null mouse UGS explants grown in vitro [59,60], and in wild-type testosterone-treated UGS explants cultured with cyclopamine, an inhibitor of signaling of all Hedgehog ligands [58–60]. However, when *Shh* signaling was disrupted at later stages of prostatic development in vitro, there was a reduction in organ size, an increase in ductal tip number, and reduced proliferation of ductal tip epithelia, indicating that *Shh* is involved in prostatic growth. Furthermore, in prostates grown in vitro in the presence of testosterone, inhibition of *Shh*-signaling accelerated the canalization of prostatic epithelial ducts and resulted in ducts that showed morphological similarities to cribriform prostatic intra-epithelial neoplasia (PIN). The epithelia of these ducts also demonstrated precocious and aberrant differentiation, when examined by immunohistochemistry for p63 and cytokeratin 14 [60]. The observation of lesions showing morphological similarities to PIN is most intriguing and raises the question of how *Shh* might be involved in their formation. *Shh* is made by prostatic epithelium, and the *Ptc* receptor is expressed in the mesenchyme, though it is possible that very low (but undetectable) levels of *Ptc* may be present in the epithelium. If prostatic epithelium lacks the ability to respond to Shh signaling directly, then the PIN-like lesions may result from altered mesenchymal signaling (as a result of a lack of Shh activity). This correlates well with the involvement of stroma in prostate disease as discussed below [61]. Addition of recombinant *Shh* to VPs grown in vitro caused an expansion of the mesenchyme and showed that *Shh* is a mitogen for prostatic mesenchyme. Thus, it appears that *Shh*-signaling is not essential for prostatic induction, but is important for prostatic growth, branching, proliferation and differentiation.

13. The ventral mesenchymal pad and the peri-urethral smooth muscle

One crucial requirement for ductal growth and branching morphogenesis is that the emerging prostatic buds come into intimate contact with mesenchymal populations rich in epithelial mitogens and morphogens required for prostatic organogenesis. In this regard, sub-populations of UGM have been described that appear to play critical roles in prostatic ductal growth and branching. The ventral mesenchymal pad (VMP) is a peripherally located dense zone of the UGM separated from the UGE by peri-urethral mesenchyme [41] (Fig. 4). During prostatic development prostatic buds emerge from the UGE, grow through the peri-urethral mesenchyme and undergo branching morphogenesis upon entering the VMP [54]. In tissue recombination experiments, the VMP has been shown to be able to induce prostatic development from competent epithelium [41]. A key question is what is the identity of the regulatory molecules made in the VMP and how is their activity regulated? FGF10 and FGF7 are both expressed by UGM, but a striking feature of the VMP is the high level of FGF10 transcripts (Fig. 4) [54,62]. The FGF10 and FGF7 genes do not appear to be directly regulated by androgens in vivo (reviewed in Thomson [53]), yet it is clear that there is androgenic control of the inductive activity of the VMP/UGM. Recently, we have shown that a layer of smooth muscle can control the interaction of nascent prostatic buds with specialized mesenchyme such as the VMP [63]. A layer of peri-urethral smooth muscle forms in males and females between the VMP and the urethra. This smooth muscle layer is less well developed in males in which prostatic buds pass through a gap in this smooth muscle layer to enter the VMP. Androgens partially inhibit development of the peri-urethral smooth muscle layer, modulate smooth muscle differentiation, and lead to sexual dimorphism of this smooth muscle layer. When this smooth muscle layer is fully developed as in females, rare prostatic epithelial buds appearing in females after the smooth muscle layer has formed cannot enter the VMP to grow and develop in response to androgens. These data indicate that smooth muscle can act as a regulator of prostatic ductal elongation and branching morphogenesis, and that this peri-urethral smooth muscle is a barrier for prostatic ductal growth and branching morphogenesis [63]. Three dimensional reconstruction of developing prostate in males confirms that prostatic buds only elongate and undergo ductal branching in areas devoid of this peri-urethral smooth muscle sleeve [31].

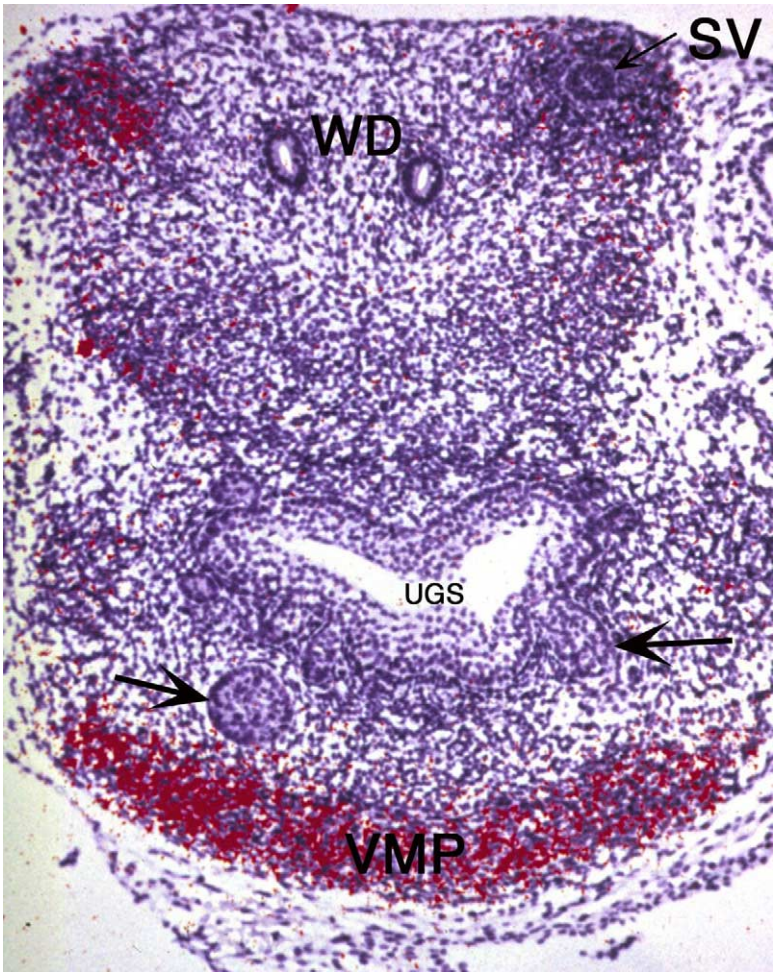

Fig. 4. Localization of FGF10 transcripts in a transverse section through a 19 day embryonic male rat UGS showing ventral prostatic buds (arrows) emerging from the urogenital sinus epithelium. The ventral mesenchymal pad (VMP), which expresses high levels of FGF10 transcripts (red), is separated from the UGE by peri-urethral mesenchyme having little or no FGF10 transcripts. At the top of the micrograph (dorsal) the seminal vesicle (SV) rudiments and Wolffian ducts (WD) are visible (figure from [54]).

14. Activins, inhibins and related proteins

Activins and certain related proteins inhibit ductal growth and branching morphogenesis of prostate as well as other branched organs. Activins are members of the TGFISβ superfamily of growth and differentiation factors, and consist of disulphide-linked homo- and heterodimers of βA and βB subunits (forming activins A, AB, and B). Inhibins consist of activin βA or βB subunits linked to an inhibin α subunit (forming inhibins A and B). Activins and inhibins were originally isolated as gonadal proteins that regulated pituitary follicle-stimulating hormone secretion [64,65], and in some systems inhibins and activins have dual but opposing actions. Follistatins are activin-binding proteins that regulate activin bioactivity, restricting ligand access to the receptors. In many systems there is evidence of an interplay between activins and follistatins.

The temporal pattern of expression of activin βA and βB subunits is consistent with a role for activin A rather than other activin or inhibin ligands in prostatic development in that activin βA subunits are expressed in the mesenchyme of developing prostate during early postnatal periods (days postnatal 2–10) when prostatic development is most intense [66]. In contrast, the inhibin α subunit is expressed in the pubertal prostate after most of the ductal architecture has developed. Follistatin, which opposes activin A action, was detected at the mRNA and protein levels in vivo from 2 days postnatal and thereafter. Activin receptors and follistatin are predominantly co-localized in the developing prostatic epithelium. Thus, paracrine control of prostatic ductal branching morphogenesis by activin A involves precise spatial and temporal expression of endogenous activin A within mesenchyme associated with ductal tips in conjunction with epithelial expression of binding proteins (activin receptors and follistatin).

Activin A, when added to developing prostatic explants in vitro, inhibited ductal elongation and branching, such that the ductal tips did not bifurcate and elongate but instead continued to expand as a solid clusters of cells. This effect of activin on the epithelium of developing prostatic explants was associated with an expansion of the mesenchymal cap surround-

ing the prostatic bud tips, suggesting a role for activin A in maintaining tip mesenchyme. The interplay between activin and follistatin in tissue homeostasis, was confirmed with the addition of exogenous follistatin to neonatal rat prostate explant cultures, which increased growth and ductal branching beyond that of controls, presumably due to neutralization of endogenous activin [66].

In other branched ductal organs such as pancreas, salivary gland and kidney, activin A is inhibitory and reduces ductal branching [66,67]. In the mammary gland, activin is essential for normal ductal elongation and alveolar morphogenesis [68]. The effects of activin B were not fully explored in the mammary gland because of limited availability of ligand. However, the effects of activin A on the mammary gland differed from that of TGFβ, which also inhibited ductal branching in that TGFβ did not inhibit elongation of the ducts or maintain tip mesenchyme.

Additional importance of activins as negative regulators of prostatic branching should be viewed in the broader context of the androgen-stimulated prostatic growth and ductal branching. Activins (and TGFβ) signal via the Smad pathway [69], and there is ample evidence that activin/TGFβ and sex steroid receptor signaling are linked due to the physical interaction between Smad-3 and the androgen receptor leading to intracellular cross-talk [70,71]. Thus, there is a new twist to the complexity of the role of activins in androgen-regulated prostatic ductal growth and branching.

15. Microenvironmental stromal aspects of carcinogenesis

Many studies have focused on the abnormal properties of emerging or established malignant epithelial cells during carcinogenesis. This approach has yielded a wealth of information, especially on the genetic alterations associated with carcinogenesis. However, the process of carcinogenesis can also be examined more broadly in the context of loss of homeostatic control over normal tissue architecture, nuclear atypia, genetic alterations, destruction of tissue boundaries, stromal changes, angiogenesis, and destruction of distant organs by metastatic cells. Indeed, from a more global view it is evident that carcinogenesis is a disease of tissue organization resulting from de-regulation of the finely orchestrated processes that determine how cells are integrated into normal tissues and tissues into organs [72]. Accordingly, our focus has been the role of the stromal microenvironment in prostatic carcinogenesis. From this approach several new models of prostatic carcinogenesis have emerged, which may be instrumental in deciphering the mechanisms of progression from normal cellular behavior to tumorigenesis and hence to metastasis.

Prostatic organogenesis culminates in the development of a mature gland composed of highly differentiated secretory epithelial cells and highly differentiated contractile smooth muscle cells. In the absence of pathological processes both the epithelium and smooth muscle are essentially growth-quiescent in the adult prostate. We have postulated that the highly differentiated growth-quiescent state of the adult prostate is maintained by reciprocal homeostatic interactions between epithelium and smooth muscle [73]. Thus, in adulthood homeostasis is maintained through reciprocal smooth muscle–epithelial interactions whose outcome is the reciprocal maintenance of functional differentiation and growth-quiescence in both the epithelium and smooth muscle. We propose that prostatic carcinogenesis occurs in part as a result of perturbation of these reciprocal homeostatic smooth muscle–epithelial interactions. It is likely that prostatic carcinogenesis is initiated by genetic damage to prostatic epithelium. We proposed that perturbation of reciprocal homeostatic smooth muscle–epithelial interactions with ensuing de-differentiation of both the emerging prostatic carcinoma cells and smooth muscle plays a key role in progression of initiated epithelial cells. Thus, following genetic insult to prostatic epithelium, the epithelium fails to signal appropriately to the adjacent smooth muscle, which in turn begins to de-differentiate towards a more fibroblastic phenotype [74]. As smooth muscle begins to de-differentiate, signaling from prostatic smooth muscle to prostatic epithelium becomes anomalous resulting in progressive loss of control over epithelial differentiation and proliferation. Accordingly, a vicious cycle is established during progression in which both prostatic epithelium and smooth muscle de-differentiate and proliferate. This hypothesis is supported by many observations. In vivo the highly differentiated state of prostatic epithelium and smooth muscle is maintained under homeostatic interactions requiring intimate association of these two cell types. If growth-quiescent prostatic epithelial and stromal cells are isolated from normal adult glands and grown separately in vitro, both the epithelial and smooth muscle cells rapidly de-differentiate and proliferate. This de-differentiation can be counteracted to some degree by growing epithelial or smooth muscle cells on various extra-cellular matrices [75,76]. Thus, the intimate cell–cell and cell–extracellular matrix interactions seen in vivo are likely to be essential for maintenance of the highly differentiated growth-quiescent state of the normal adult prostate and its constituent cells.

We have proposed a cellular mechanism that integrates the ontogeny of prostatic smooth muscle differentiation due to epithelial–mesenchymal interactions with de-differentiation of prostatic smooth muscle during carcinogenesis [77] and reduction of smooth muscle in advanced prostatic adenocarcinomas [15,45,78–80]. The hypothesis is that as prostatic epithelium undergoes neoplastic transformation it loses its ability to maintain (and induce) smooth muscle differentiation. This possibility was tested in experimental tissue recombinants in which various normal or neoplastic prostatic epithelia were grown in combination with embryonic rat UGM. We found that only normal (non-neoplastic) epithelia were fully capable of inducing differentiation of prostatic smooth muscle in UGM [45]. This observation is consistent with the idea that one aspect of the carcinogenic process is a loss of the ability of the epithelium to induce and

maintain smooth muscle differentiation. It is now evident that smooth muscle–epithelial interactions are the operative cell–cell interactions in the adult prostate, and that smooth muscle–epithelial interactions play key roles in regulating epithelial differentiation, proliferation and carcinogenesis.

The role of stroma in the initiation and promotion of carcinogenesis has been considered for many years and is based upon the observation that "tumor stroma" frequently exhibits a variety of phenotypic differences relative to normal stroma [81–83]. Thus, deregulation of epithelial–stromal interactions has been thought to contribute to both early and late stages of cancer formation. Furthermore, the continued interaction of the carcinoma cell with its stromal microenvironment plays an important role in the biology of the neoplasm. However, persuasive formal proof of the role of stroma in carcinogenesis has been lacking until recently. Thompson et al. were the first to perform experiments designed to test the idea that stromal cells may facilitate prostatic carcinogenesis. In these experiments, the urogenital sinus (prostatic anlagen) or its mesenchymal (UGM) or epithelial (UGE) components were transfected with a virus carrying the myc and ras oncogenes. In UGM + UGE tissue recombinants containing un-infected UGM plus infected UGE, epithelial hyperplasias were detected. Prostatic reconstitutions composed of infected UGM plus un-infected UGE, developed stromal desmoplasias. Carcinomas were found only in recombinants in which both UGM and UGE were infected [84]. Thus, changes were required in both the epithelium and stroma for prostatic carcinogenesis to occur.

More recently, we have demonstrated that fibroblasts associated with human prostatic carcinomas (carcinoma-associated fibroblasts, CAF) can promote carcinogenesis in initiated but non-tumorigenic human prostatic epithelial cells [61,85]. In these experiments, CAF cells were isolated from human prostate cancer surgical specimens and recombined with the non-tumorigenic SV40T-immortalized human pro-

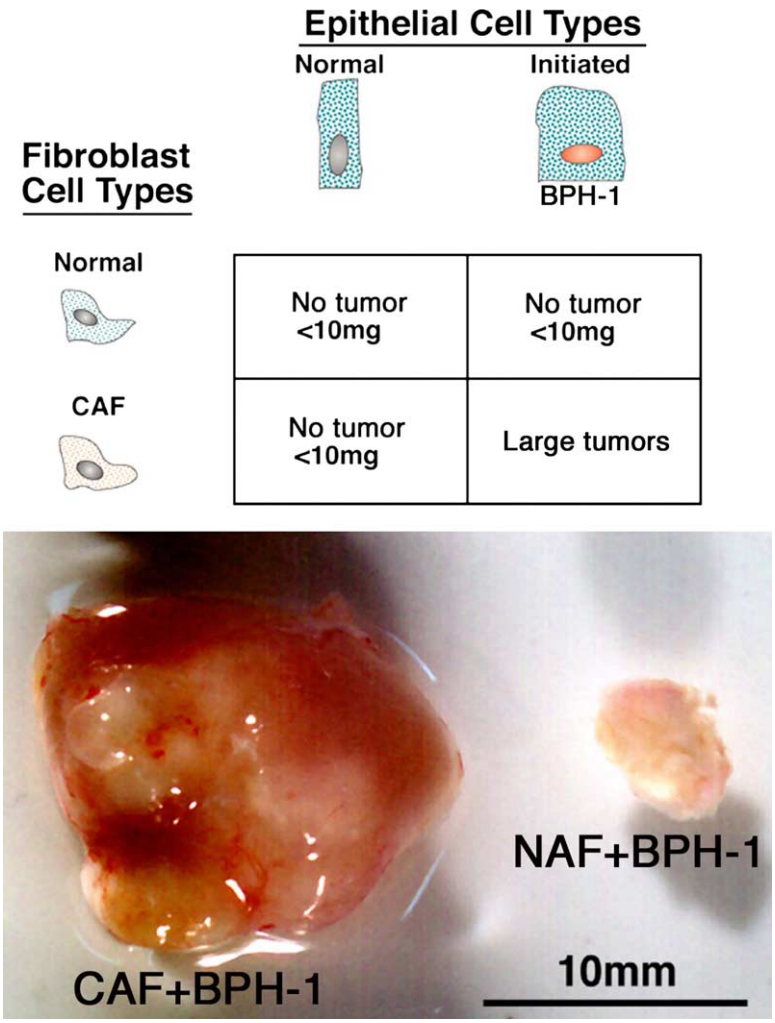

Fig. 5. Summary of tissue recombination experiments in which normal human prostatic fibroblasts (NAF) or carcinoma-associated fibroblasts (CAF) were grown in vivo in association with either normal human prostatic epithelial cells or initiated human prostatic epithelial cells (BPH-1). As indicated in the four-way grid, tumors only develop when initiated human prostatic epithelial cells (BPH-1) are grown in association with CAF. The bottom depicts the gross size differences between CAF + BPH-1 recombinants, which form tumors, versus NAF + BPH-1 recombinants, which do not. These studies demonstrate that CAF can promote initiated but non-tumorigenic prostatic epithelial cells to full tumorigenicity (modified from [61].

static epithelial cell line, BPH-1 [86]. BPH-1 cells grafted by themselves or in combination with normal prostatic stromal cells rarely formed tumors. Carcinomas developed with high efficiency when BPH-1 cells were grown in association with CAF, but not in association with normal human prostatic fibroblasts (Fig. 5). BPH-1 epithelial cells of CAF + BPH-1 tissue recombinants formed large poorly differentiated carcinomas [61]. In experiments in which fetal UGM was combined with BPH-1 cells, epithelial proliferation was also stimulated, but tumorigenesis did not occur. Thus, mere stimulation of epithelial proliferation is not the single determinant in CAF-induced promotion of tumorigenesis [87]. Rather than acting to repress epithelial proliferation (as would be expected of normal prostatic stroma), stromal cells derived from human prostate carcinoma (CAF) stimulated epithelial proliferation and promoted carcinogenesis.

The tumorigenic process promoted by CAF in the non-tumorigenic BPH-1 cells involved further alterations in gene expression and characteristic genetic alterations [88,89]. The genetic alterations induced in BPH-1 cells by association with CAFs were sufficient to allow $BPH1^{CAFTD}$ cells (tumorigenic derivative BPH-1 lines isolated from CAF + BPH-1 tumors) to subsequently grow as tumors independent of a continuing interaction with CAF. A description of the genetic changes has been published [89]. These data suggest that interactions between stroma and epithelium during tumorigenesis influence genetic changes across tissue layer boundaries.

The mechanisms by which stromal cells influence tumorigenesis are poorly understood. Differential gene expression in normal stroma versus CAF of factors modulating the local micro-environment has been proposed. In this regard, Tuxhorn et al. demonstrated that reactive stromal cells (CAF) supported establishment of tumors and increased angiogenesis in a subcutaneous grafting model [79]. Stromal cells associated with carcinomas are known to produce a variety of matrix metalloproteinases, which may affect tumor initiation, growth, migration, angiogenesis, apoptosis, invasion and metastasis [90]. Indeed, tumor stromal cells exhibit a variety of phenotypic changes that include abnormal migratory behavior in vitro [91], alterations in cell surface molecules [92,93], altered expression of growth factors [94–99], expression of prostaglandin-synthesizing enzymes [100,101] and alterations in ECM [102,103]. Future work on the cellular and molecular mechanisms of stromal–carcinoma cell interactions may provide the basis for new therapeutic strategies for regulating carcinoma growth and/or apoptosis.

16. Role of stroma in hormonal carcinogenesis

Hormones play a pivotal role in the biology of the prostate. Androgens are required for prostatic development, growth and function. The prostate is also an estrogen target organ, and estrogens can profoundly affect prostatic growth and differentiation (see Härkönen and Mäkelä, this issue). Estrogenic effects on the prostate are complex involving both direct and indirect systemic actions. In intact males estrogens suppress pituitary gonadotrophins and thus reduce production and secretion of testosterone by the testes [104]. Thus, high levels of exogenous estrogen affect the prostate in two ways: (a) androgen deprivation is an indirect effect of estrogen via the pituitary-testis axis. (b) Estrogens also can act directly on the prostate to influence epithelial growth and differentiation. The classic direct effect of exogenous estrogen on the prostate is squamous metaplasia, which is reversible following removal of the estrogenic stimulus in most cases.

Steroid hormones also play pivotal roles in prostatic carcinogenesis [105], and both androgens and estrogens have been implicated in prostatic carcinogenesis. For example, prostate cancer does not occur in eunuchs castrated early in life. Plasma testosterone levels decline with age, while plasma estradiol levels remain unchanged or increase during aging when prostate cancer develops and is diagnosed. Thus, the 17β-estradiol (E2)/testosterone (T) ratio is elevated during the development of prostate cancer [106,107]. African Americans have the highest incidence of prostatic cancer and exhibit elevations in both plasma T and E2 [108]. Testosterone in combination with estradiol (T + E) induces prostatic hyperplasia and dysplasia in mice and prostate cancer in rats [109–114]. Thus, androgens alone and in combination with estrogens play a role in prostate carcinogenesis.

Hormonal carcinogenesis of the prostate is presumably induced via signaling through androgen (AR) and estrogen (ER) receptors. The prostate expresses AR, ERα and ERβ [115–119]. In order to elucidate the respective roles of these steroid receptors, the tissue distribution of these receptors must be born in mind as the effects of androgens and estrogens on the prostate may involve both direct and indirect (paracrine) actions of these hormones. In this regard, AR are expressed in both prostatic epithelial and stromal cells. The tissue distribution of ER is somewhat controversial. ERα is found predominantly in prostatic stroma, whereas ERβ is found predominantly in prostatic epithelium [118,120,121]. However, there are reports of ERβ in prostatic stroma [122–125] and ERα in prostatic epithelium, such as in basal cells [126], squamous metaplasia [122], or prostate cancer cell lines [122]. Utilizing tissue recombinants constructed with epithelium and stroma from mice null for AR, ERα and β, we have examined the respective roles of these receptors in prostatic carcinogenesis [109].

Many androgenic responses in normal prostatic epithelial cells are mediated through stromal AR as discussed above. Based upon the analysis of the Tfm/wild-type tissue recombinants (Fig. 2), epithelial proliferation, ductal branching morphogenesis, epithelial cytodifferentiation into basal and luminal cells are mediated via androgenic effects on stromal AR [17,127]. In contrast, epithelial AR are required for expression of secretory proteins [19,20]. To elucidate the role of stromal AR in prostate carcinogenesis, tissue recombinants were prepared with embryonic rat UGM (rUGM) plus BPH-1 cells. BPH-1 cells are clonally derived, immortalized, non-tumorigenic human prostatic epithelial cells [86]. BPH-1

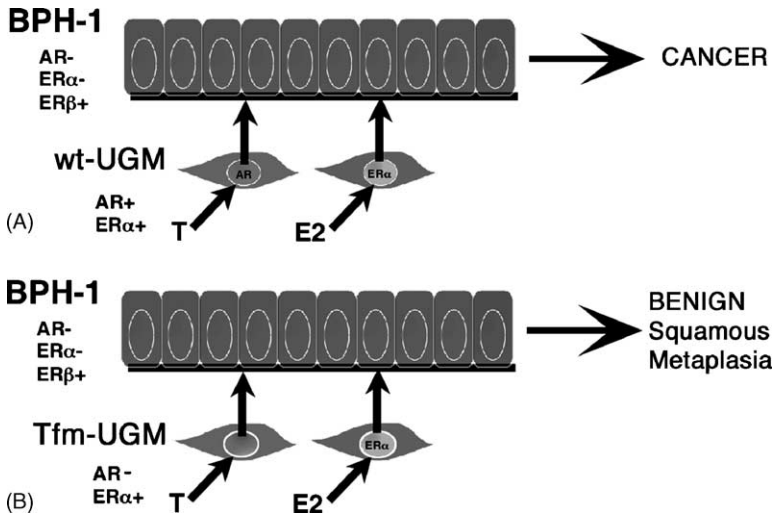

Fig. 6. Summary of tissue recombinant experiments on hormonal carcinogenesis elicited by testosterone plus estradiol (T + E) as described previously [87]. (A) BPH-1 cells, which appear to lack androgen receptor and estrogen receptor alpha, progress from non-tumorigenic to tumorigenic at high efficiency when combined with wild-type urogenital sinus mesenchyme (wt-UGM) and treated with T + E. (B) In contrast, comparable UGM + BPH-1 recombinants consistently give rise to benign squamous metaplasia when treated with T + E provided the UGM is derived from an androgen receptor null Tfm mouse.

cells grafted by themselves form small masses of undifferentiated epithelial cells and never undergo tumorigenesis when grown in intact male hosts or male hosts treated with T + E presumably due to the absence of AR and ERα in BPH-1 epithelial cells [87,128]. Rat and mouse UGM express AR and ERα [4,121,129–131]. When BPH-1 cells are combined with rUGM and grown in untreated male mice, the BPH-1 cells undergo relatively normal prostatic development and form branched solid epithelial cords that sometimes canalize into ducts. When rUGM + BPH-1 or mouse UGM + BPH-1 tissue recombinants are grown in T + E2 treated mice, the BPH-1 cells undergo carcinogenesis [87]. Because AR and ERα are only detectable in the stroma of UGM + BPH-1 recombinants [128], these findings suggest a critical role of stromal hormone receptors in this model of hormonal carcinogenesis. If hormonal carcinogenesis involves paracrine mechanisms, then tissue recombinants composed of AR-negative Tfm UGM plus BPH-1 cells should not undergo carcinogenesis in response to T + E2. Not surprisingly, wild-type AR-positive mouse UGM can substitute for rat UGM in the UGM + BPH-1 model (Ricke and Cunha, submitted). Growth was modest and benign epithelial structures developed when wt-mouse UGM + BPH-1 tissue recombinants were grown for 4–6 months in untreated hosts. In contrast, large transplantable carcinomas developed in wt-mouse UGM + BPH-1 tissue recombinants grown in T + E2-treated hosts (Ricke and Cunha, submitted). Utilizing AR-deficient Tfm mouse UGM, Tfm-UGM + BPH-1 tissue recombinants were constructed and grown in T + E2 treated hosts. Hormonal carcinogenesis in response to T + E2 did not occur in Tfm-UGM + BPH-1 tissue recombinants. Instead, Tfm-UGM + BPH-1 tissue recombinants underwent benign squamous metaplasia presumably due to un-opposed estrogen action. These experiments strongly support the concept that hormonal carcinogenesis is mediated by paracrine factors whose expression is dependent upon stromal AR, as is the case for many hormonal effects during normal prostatic development as described above. Clearly, the stromal microenvironment plays a key role in hormonal carcinogenesis (Fig. 6).

17. Conclusion

The recurring theme emphasized in this review is that reciprocal interactions between epithelium and the connective tissue stroma play key roles in both normal development and carcinogenesis of the prostate (and other organs). In the embryo these interactions are called epithelial–mesenchymal interactions, whereas in adulthood they are called epithelial–stromal interactions. Through these cell–cell interactions epithelial morphogenesis, growth, differentiation and function are elicited during normal development. In adulthood homeostatic epithelial–stromal interactions are required to maintain the highly differentiated, growth-quiescent functional state of both the epithelium and stroma. Carcinogenesis and malignant progression are regulated in part via altered stromal–epithelial interactions, which foster progression to malignancy. Epithelial–mesenchymal interactions during development and epithelial-stromal interactions in adulthood are mediated by differential regulation of growth/differentiation factors and proteases, which modulate the local micro-environment. As a result of altered cell–cell and cell–ECM interactions progression to malignancy is fostered. Accordingly, altered stromal cells associated with carcinomas profoundly affect a multitude of processes that include tumor initiation, growth, migration, angiogenesis, apoptosis, invasion and metastasis [90]. Elucidation of the cellular and molecular mechanisms of interactions between

the stromal micro-environment and carcinoma cells may provide new therapeutic strategies for regulating growth and/or apoptosis of carcinomas. The December 2002 issue of *Differentiation* provides a collection of reviews on the role of the cellular microenvironment in neoplasia.

Acknowledgements

This work was supported in part by the following grants: NIH grants CA84294, CA89520, CN-15114-MAO, CN-35115 (GRC), NCI Canada grant 014053 (YZW), NH&MRC Program Grant 143786 (GR), NIH grant CA97725 (WAR), NIH grant CA96403 and Department of Defense grant DAMD 17-02-1-0151 (SWH). The authors thank Michael McLaughlin for drawing Fig. 1.

References

[1] G. Pierce, R. Shikes, L. Fink, Cancer: A Problem of Developmental Biology, Prentice Hall, New Jersey, 1978.
[2] A. Staack, A.A. Donjacour, J. Brody, G.R. Cunha, P. Carroll, Mouse urogenital development: a practical approach, Differentiation 71 (2003) 402–413.
[3] H. Takeda, T. Nakamoto, J. Kokontis, G.W. Chodak, C. Chang, Autoregulation of androgen receptor expression in rodent prostate: immunohistochemical and in situ hybridization analysis, Prostate, in press.
[4] P.S. Cooke, P. Young, G.R. Cunha, Androgen receptor expression in developing male reproductive organs, Endocrinology 128 (1991) 2867–2873.
[5] D. Price, Comparative aspects of development and structure in the prostate, Natl. Cancer Inst. Monogr. 12 (1963) 1–27.
[6] P.C. Marker, A.A. Donjacour, R. Dahiya, G.R. Cunha, Hormonal, cellular, and molecular control of prostatic development, Dev. Biol. 253 (2003) 165–174.
[7] Y. Sugimura, G.R. Cunha, A.A. Donjacour, Morphogenesis of ductal networks in the mouse prostate, Biol. Reprod. 34 (1986) 961–971.
[8] H. Kinbara, G.R. Cunha, Ductal heterogeneity in rat dorsal-lateral prostate, Prostate 28 (1995) 58–64.
[9] G.R. Cunha, Epithelio–mesenchymal interactions in primordial gland structures which become responsive to androgenic stimulation, Anat. Rec. 172 (1972) 179–196.
[10] S.J. Higgins, P. Young, G.R. Cunha, Induction of functional cytodifferentiation in the epithelium of tissue recombinants. II. Instructive induction of Wolffian duct epithelia by neonatal seminal vesicle mesenchyme, Development 106 (1989) 235–250.
[11] A.A. Donjacour, G.R. Cunha, Induction of prostatic morphology and secretion in urothelium by seminal vesicle mesenchyme, Development 121 (1995) 2199–2207.
[12] G. Risbridger, H. Wang, P. Young, T. Kurita, Y. Wong, D. Lubahn, J.-A. Gustafsson, G. Cunha, Evidence that epithelial and mesenchymal estrogen receptor-a mediates effects of estrogen on prostatic epithelium, Dev. Biol. 229 (2001) 432–442.
[13] E.S. Lopes, B.A. Foster, A.A. Donjacour, G.R. Cunha, Initiation of secretory activity of rat prostatic epithelium in organ culture, Endocrinology 137 (1996) 4225–4234.
[14] S.W. Hayward, L.S. Baskin, P.C. Haughney, B.A. Foster, A.R. Cunha, R. Dahiya, G.S. Prins, G.R. Cunha, Stromal development in the ventral prostate, anterior prostate and seminal vesicle of the rat, Acta Anatom. 155 (1996) 94–103.
[15] S.W. Hayward, M.A. Rosen, G.R. Cunha, Stromal–epithelial interactions in normal and neoplastic prostate, Br. J. Urol. 79 (Suppl.2) (1997) 18–26.
[16] N. Hayashi, G.R. Cunha, M. Parker, Permissive and instructive induction of adult rodent prostatic epithelium by heterotypic urogenital sinus mesenchyme, Epithelial Cell Biol. 2 (1993) 66–78.
[17] G.R. Cunha, A.A. Donjacour, P.S. Cooke, S. Mee, R.M. Bigsby, S.J. Higgins, Y. Sugimura, The endocrinology and developmental biology of the prostate, Endocr. Rev. 8 (1987) 338–362.
[18] G.R. Cunha, B. Lung, The possible influences of temporal factors in androgenic responsiveness of urogenital tissue recombinants from wild-type and androgen-insensitive (Tfm) mice, J. Exp. Zool. 205 (1978) 181–194.
[19] A.A. Donjacour, G.R. Cunha, Assessment of prostatic protein secretion in tissue recombinants made of urogenital sinus mesenchyme and urothelium from normal or androgen-insensitive mice, Endocrinology 131 (1993) 2342–2350.
[20] G.R. Cunha, P. Young, Inability of Tfm (testicular feminization) epithelial cells to express androgen-dependent seminal vesicle secretory proteins in chimeric tissue recombinants, Endocrinology 128 (1991) 3293–3298.
[21] P.J. Sciavolino, E.W. Abrams, L. Yang, L.P. Austenberg, M.M. Shen, C. Abate-Shen, Tissue-specific expression of murine Nkx3.1 in the male urogenital system, Dev. Dyn. 209 (1997) 127–138.
[22] R. Bhatia-Gaur, A.A. Donjacour, P.J. Sciavolino, M. Kim, N. Desai, P. Young, C.R. Norton, T. Gridley, R.D. Cardiff, G.R. Cunha, C. Abate-Shen, M.M. Shen, Roles for Nkx3.1 in prostate development and cancer, Genes Dev. 13 (1999) 966–977.
[23] G.R. Cunha, P.S. Cooke, R. Bigsby, J.R. Brody, Ontogeny of sex steroid receptors in mammals, in: M.G. Parker (Ed.), The Structure and Function of Nuclear Hormone Receptors, Academic Press, New York, 1991, pp. 235–268.
[24] E.L. Boutin, E. Battle, G.R. Cunha, The germ layer origin of mouse vaginal epithelium restricts its responseness to mesenchymal inductors: uterine induction, Differentiation 49 (1992) 101–107.
[25] G.R. Cunha, H. Fujii, B.L. Neubauer, J.M. Shannon, L.M. Sawyer, B.A. Reese, Epithelial–mesenchymal interactions in prostatic development. I. Morphological observations of prostatic induction by urogenital sinus mesenchyme in epithelium of the adult rodent urinary bladder, J. Cell Biol. 96 (1983) 1662–1670.
[26] J.H. Lipschutz, H. Fukami, Y. M., M. Tatematsu, M. Kusakabe, G.R. Cunha, Clonality of urogenital organs as determined by analysis of chimeric mice, Acta Anat., in press.
[27] A.A. Donjacour, A.A. Thomson, G.R. Cunha, FGF-10 plays an essential role in the growth of the fetal prostate, Dev. Biol. 261 (2003) 39–54.
[28] Y. Sugimura, G.R. Cunha, A.A. Donjacour, R.M. Bigsby, J.R. Brody, Whole-mount autoradiography study of DNA synthetic activity during postnatal development and androgen-induced regeneration in the mouse prostate, Biol. Reprod. 34 (1986) 985–995.
[29] C. Lee, J.A. Sensibar, S.M. Dudek, R.A. Hiipakka, S. Liao, Prostatic ductal system in rats: regional variation in morphological and functional activities, Biol. Reprod. 43 (1990) 1079–1086.
[30] J.E. McNeal, The prostate gland: morphology and pathobiology, Monogr. Urol. 4 (1983) 3–37.
[31] B.G. Timms, T.J. Mohs, J.A. DiDio, Ductal budding and branching patterns in the developing prostate, J. Urol. 151 (1994) 1427–1432.
[32] C.A. Podlasek, R.M. Seo, J.Q. Clemens, L. Ma, R.L. Maas, W. Bushman, Hoxa-10 deficient male mice exhibit abnormal development of the accessory sex organs, Dev. Dyn. 214 (1999) 1–12.
[33] P.C. Marker, J.P. Stephan, J. Lee, L. Bald, J.P. Mather, G.R. Cunha, fucosyltransferase1 and H-type complex carbohydrates modulate epithelial cell proliferation during prostatic branching morphogenesis, Dev. Biol. 233 (2001) 95–108.
[34] Y. Wang, S. Hayward, M. Cao, K. Thayer, G. Cunha, Cell differentiation lineage in the prostate, Differentiation 68 (2001) 270–279.

[35] S.W. Hayward, L.S. Baskin, P.C. Haughney, A.R. Cunha, B.A. Foster, R. Dahiya, G.S. Prins, G.R. Cunha, Epithelial development in the rat ventral prostate, anterior prostate and seminal vesicle, Acta Anatom. 155 (1996) 81–93.
[36] Y. Xue, J. van der Laak, F. Smedts, C. Schoots, A. Verhofstad, J. de la Rosette, J. Schalken, Neuroendocrine cells during human prostate development: does neuroendocrine cell density remain constant during fetal as well as postnatal life? Prostate 42 (2000) 116–123.
[37] R.J. Cohen, G. Glezerson, L.F. Taylor, H.A. Grundle, J.H. Naude, The neuroendocrine cell population of the human prostate gland, J. Urol. 150 (1993) 365–368.
[38] T.T. Sun, F.X. Liang, X.R. Wu, Uroplakins as markers of urothelial differentiation, Adv. Exp. Med. Biol. 462 (1999) 7–18, discussion 103–114.
[39] G.R. Cunha, N. Hayashi, Y.C. Wong, Regulation of differentiation and growth of normal adult and neoplastic epithelial by inductive mesenchyme, in: J.T. Isaacs (Ed.), Prostate Cancer: Cell and Molecular Mechanisms in Diagnosis and Treatment, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, 1991, pp. 73–90.
[40] H. Takeda, N. Suematsu, T. Mizuno, Transcription of prostatic steroid binding protein (PSBP) gene is induced by epithelial–mesenchymal interaction, Development 110 (1990) 273–282.
[41] B. Timms, C. Lee, G. Aumuller, J. Seitz, Instructive induction of prostate growth and differentiation by a defined urogenital sinus mesenchyme, Microscopy Res. Technique 30 (1995) 319–332.
[42] G.R. Cunha, M. Sekkingstad, B.A. Meloy, Heterospecific induction of prostatic development in tissue recombinants prepared with mouse, rat, rabbit, and human tissues, Differentiation 24 (1983) 174–180.
[43] S. Aboseif, A. El-Sakka, P. Young, G. Cunha, Mesenchymal reprogramming of adult human epithelial differentiation, Differentiation 65 (1999) 113–118.
[44] G.R. Cunha, S.W. Hayward, R. Dahiya, B.A. Foster, Smooth muscle–epithelial interactions in normal and neoplastic prostatic development, Acta Anatom. 155 (1996) 63–72.
[45] S.W. Hayward, G.R. Cunha, R. Dahiya, Normal development and carcinogenesis of the prostate: a unifying hypothesis, Ann. N.Y. Acad. Sci. 784 (1996) 50–62.
[46] G.R. Cunha, E. Battle, P. Young, J. Brody, A. Donjacour, N. Hayashi, H. Kinbara, Role of epithelial–mesenchymal interactions in the differentiation and spatial organization of visceral smooth muscle, Epithelial Cell Biol. 1 (1992) 76–83.
[47] S.W. Hayward, P.C. Haughney, M.A. Rosen, K.M. Greulich, H.U. Weier, R. Dahiya, G.R. Cunha, Interactions between adult human prostatic epithelium and rat urogenital sinus mesenchyme in a tissue recombination model, Differentiation 63 (1998) 131–140.
[48] C. Lee, Physiology of castration-induced regression in rat prostate, in: J.P. Karr, A.A. Sandberg, G.P. Murphy (Eds.), The Prostatic Cell: Structure and Function, Part A, AR Liss, New York, 1981, pp. 145–159.
[49] N. Kyprianou, E.M. Bruckheimer, Y. Guo, Cell proliferation and apoptosis in prostate cancer: significance in disease progression and therapy, Histol. Histopathol. 15 (2000) 1211–1223.
[50] J.T. Isaacs, Antagonistic effect of androgen on prostatic cell death, Prostate 5 (1984) 545–557.
[51] G. Prins, L. Birch, G. Greene, Androgen receptor localization in different cell types of the adult rat prostate, Endocrinology 129 (1991) 3187–3199.
[52] T. Kurita, Y.-Z. Wang, A.A. Donjacour, C. Zhao, J.P. Lydon, B.P. O'Malley, J.T. Isaacs, R. Dahiya, G.R. Cunha, Paracrine regulation of apoptosis by steroid hormones in the male and female reproductive system, Cell Death Differentiation 8 (2001) 192–200.
[53] A.A. Thomson, Role of androgens and fibroblast growth factors in prostatic development, Reproduction 121 (2001) 187–195.
[54] A.A. Thomson, G.R. Cunha, Prostatic growth and development are regulated by FGF10, Development 126 (1999) 3693–3701.
[55] A.A. Thomson, B.A. Foster, G.R. Cunha, Analysis of growth factor and receptor mRNAs during development of the rat seminal vesicle and prostate, Development 124 (1997) 2431–2439.
[56] Y. Sugimura, B.A. Foster, Y.K. Hom, J.S. Rubin, P.W. Finch, S.A. Aaronson, N. Hayashi, J. Kawamura, G.R. Cunha, Keratinocyte growth factor (KGF) can replace testosterone in the ductal branching morphogenesis of the rat ventral prostate, Int. J. Dev. Biol. 40 (1996) 941–951.
[57] C.A. Podlasek, D.H. Barnett, J.Q. Clemens, P.M. Bak, W. Bushman, Prostate development requires Sonic hedgehog expressed by the urogenital sinus epithelium, Dev. Biol. 209 (1999) 28–39.
[58] M.L. Lamm, W.S. Catbagan, R.J. Laciak, D.H. Barnett, C.M. Hebner, W. Gaffield, D. Walterhouse, P. Iannaccone, W. Bushman, Sonic hedgehog activates mesenchymal Gli1 expression during prostate ductal bud formation, Dev. Biol. 249 (2002) 349–366.
[59] D.M. Berman, N. Desai, X. Wang, S.S. Karhadkar, M. Reynon, C. Abate-Shen, P.A. Beachy, M.M. Shen, Prostate defects of Sonic hedgehog mutant mice are a consequence of androgen insufficiency, Dev. Biol., in press.
[60] S.H. Freestone, P. Marker, O. Cathal-Grace, D.C. Tomlinson, G.R. Cunha, P. Harnden, T.A.A., Sonic hedgehog regulates prostatic growth and epithelial differentiation, Dev. Biol. 264 (2003) 352–362.
[61] A.F. Olumi, G.D. Grossfeld, S.W. Hayward, P.R. Carroll, T.D. Tlsty, G.R. Cunha, Carcinoma-associated fibroblasts direct tumor progression of initiated human prostatic epithelium, Cancer Res. 59 (1999) 5002–5011.
[62] P.W. Finch, G.R. Cunha, J.S. Rubin, J. Wong, D. Ron, Pattern of KGF and KGFR expression during mouse fetal development suggests a role in mediating morphogenetic mesenchymal-epithelial interactions, Dev. Dyn. 203 (1995) 223–240.
[63] A.A. Thomson, B.G. Timms, L. Barton, G.R. Cunha, O.C. Grace, The role of smooth muscle in regulating prostatic induction, Development 129 (2002) 1905–1912.
[64] H.G. Burger, M. Igarashi, D.T. Baird, W. Bardin, S. Chappel, F. de Jong, A. Demoulin, D. de Kretser, J. Findlay, R. Forage, et al., Inhibin: definition and nomenclature, including related substances, Clin. Endocrinol. (Oxf.) 28 (1988) 448–449.
[65] F. De Jong, Inhibin, Physiol. Rev. 68 (1988) 555–607.
[66] B. Cancilla, R.A. Jarred, H. Wang, S.L. Mellor, G.R. Cunha, G.P. Risbridger, Regulation of prostate branching morphogenesis by activin A and follistatin, Dev. Biol. 237 (2001) 145–158.
[67] O. Ritvos, T. Tuuri, M. Eramaa, K. Sainio, K. Hilden, L. Saxen, S.F. Gilbert, Activin disrupts epithelial branching morphogenesis in developing glandular organs of the mouse, Mech. Dev. 50 (1995) 229–245.
[68] G.W. Robinson, L. Hennighausen, Inhibins and activins regulate mammary epithelial cell differentiation through mesenchymal–epithelial interactions, Development 124 (1997) 2701–2708.
[69] R. Derynck, Y.E. Zhang, Smad-dependent, Smad-independent pathways in TGF-β family signalling, Nature 425 (2003) 577–584.
[70] J.E. Chipuk, S.C. Cornelius, N.J. Pultz, J.S. Jorgensen, M.J. Bonham, S.J. Kim, D. Danielpour, The androgen receptor represses transforming growth factor-beta signaling through interaction with Smad3, J. Biol. Chem. 277 (2002) 1240–1248.
[71] S.A. Hayes, M. Zarnegar, M. Sharma, F. Yang, D.M. Peehl, P. ten Dijke, Z. Sun, SMAD3 represses androgen receptor-mediated transcription, Cancer Res. 61 (2001) 2112–2118.
[72] D.E. Ingber, J.D. Jamieson, Tumor formation and malignant invasion: role of basal lamina, in: L. Liotta, I. Hart (Eds.), Tumor invasion and metastasis, Martinus Nijhoff, The Hague, The Netherlands, 1982, pp. 335–357.
[73] G.R. Cunha, S.W. Hayward, Y.-Z. Wang, Role of stroma in carcinogenesis of the prostate, Differentiation 60 (2002) 473–485.
[74] Y.C. Wong, N.N. Tam, Dedifferentiation of stromal smooth muscle as a factor in prostate carcinogenesis, Differentiation 70 (2002) 633–645.

[75] M.J. Bissell, H.G. Hall, Form and function in the mammary gland: The role of extracellular matrix, in: M.C. Neville, C.W. Daniel (Eds.), The Mammary Gland: Development, Regulation, and Function, Plenum Press, New York, 1987, pp. 97–146.
[76] J. Thyberg, Differentiated properties and proliferation of arterial smooth muscle cells in culture, Int. Rev. Cytol. 169 (1996) 183–265.
[77] Y.C. Wong, N.N.C. Tam, Dedifferentiation of stromal smooth muscle as a factor in prostate carcinogenesis, Differentiation 60 (2002) 633.
[78] J.T. Arnold, J.T. Isaacs, Mechanisms involved in the progression of androgen-independent prostate cancers: it is not only the cancer cell's fault, Endocr. Relat. Cancer 9 (2002) 61–73.
[79] J.A. Tuxhorn, S.J. McAlhany, T.D. Dang, G.E. Ayala, D.R. Rowley, Stromal cells promote angiogenesis and growth of human prostate tumors in a differential reactive stroma (DRS) xenograft model, Cancer Res. 62 (2002) 3298–3307.
[80] D.R. Rowley, What might a stromal response mean to prostate cancer progression? Cancer Metastasis Rev. 17 (1998) 411–419.
[81] D. Tarin, Tissue Interactions in Carcinogenesis, Academic Press, London, 1972.
[82] F.T. Bosman, A. de Bruine, C. Flohil, A. van der Wurff, J. ten Kate, W.W. Dinjens, Epithelial–stromal interactions in colon cancer, Int. J. Dev. Biol. 37 (1993) 203–211.
[83] R. Seljelid, S. Jozefowski, B. Sveinbjornsson, Tumor stroma, Anticancer Res. 19 (1999) 4809–4822.
[84] T.C. Thompson, L.D. Truong, T.L. Timme, D. Kadmon, B.K. McCune, K.C. Flanders, P.T. Scardino, S.H. Park, Transgenic models for the study of prostate cancer, Cancer 71 (1993) 1165–1171.
[85] G. Grossfeld, S. Hayward, T. Tlsty, G. Cunha, The role of stroma in prostatic carcinogenesis, Endocr. Relat. Cancer 5 (1998) 253–270.
[86] S.W. Hayward, R. Dahiya, G.R. Cunha, J. Bartek, N. Despande, P. Narayan, Establishment and characterization of an immortalized but non-tumorigenic human prostate epithelial cell Line: BPH-1, In Vitro 31A (1995) 14–24.
[87] Y. Wang, D. Sudilovsky, B. Zhang, P.C. Haughney, M.A. Rosen, D.S. Wu, T.J. Cunha, R. Dahiya, G.R. Cunha, S.W. Hayward, A human prostatic epithelial model of hormonal carcinogenesis, Cancer Res. 61 (2001) 6064–6072.
[88] S. Hayward, Y. Wang, M. Cao, Y. Hom, B. Zhang, G. Grossfeld, D. Sudilovsky, G. Cunha, Malignant transformation in a nontumorigenic human prostatic epithelial cell line, Cancer Res. 61 (2001) 8135–8142.
[89] J. Phillips, S. Hayward, Y. Wang, J. Vasselli, C. Pavlovich, H. Padilla-Nash, J. Pezullo, B. Ghadimi, G. Grossfeld, A. Rivera, W. Linehan, G. Cunha, T. Ried, The consequences of chromosomal aneuploidy on gene expression profiles in a cell line model for prostate carcinogenesis, Cancer Res. 61 (2001) 8143–8149.
[90] C.C. Lynch, L.M. Matrisian, MMPs in tumor-host cell communication, Differentiation 60 (2002) 561–573.
[91] S.L. Schor, A.M. Schor, G. Rushton, Fibroblasts from cancer patients display a mixture of both foetal and adult-like phenotypic characteristics, J. Cell Sci. 90 (1988) 401–407.
[92] K. Oishi, J.C. Romijn, F.H. Schroeder, The surface character of separated prostatic cells and cultured fibroblasts of prostatic tissue as determined by concanavalin-a hemadsorption, Prostate 2 (1981) 11–21.
[93] S. Chaudhuri, I. Koprowska, J. Rowinski, Different agglutinability of fibroblasts underlying various precursor lesions of human uterine cervical carcinoma, Cancer Res. 35 (1975) 2350–2354.
[94] D. Yee, S. Paik, G.S. Lebovic, R.R. Marcus, R.E. Favoni, K.J. Cullen, M.E. Lippman, N. Rosen, Analysis of insulin-like growth factor I gene expression in malignancy: evidence for a paracrine role in human breast cancer, Mol. Endocrinol. 3 (1989) 509–517.
[95] M.J. Ellis, C. Singer, A. Hornby, A. Rasmussen, K.J. Cullen, Insulin-like growth factor mediated stromal–epithelial interactions in human breast cancer, Breast Cancer Res. Treat. 31 (1994) 249–261.
[96] K.S. Frazier, G.R. Grotendorst, Expression of connective tissue growth factor mRNA in the fibrous stroma of mammary tumors, Int. J. Biochem. Cell Biol. 29 (1997) 153–161.
[97] T. Nakamura, K. Matsumoto, A. Kiritoshi, Y. Tano, T. Nakamura, Induction of hepatocyte growth factor in fibroblasts by tumor-derived factors affects invasive growth of tumor cells: in vitro analysis of tumor–stromal interactions, Cancer Res. 57 (1997) 3305–3313.
[98] F. Ponten, Z. Ren, M. Nister, B. Westermark, J. Ponten, Epithelial–stromal interactions in basal cell cancer: the PDGF system, J. Invest. Dermatol. 102 (1994) 304–309.
[99] G. Yan, Y. Fukabori, G. McBride, S. Nikolaropolous, W. McKeehan, Exon switching and activation of stromal and embryonic FGF/FGF receptor genes in prostate epithelial cells accompanies stromal independence and malignancy, Mol. Cell Biol. 13 (1993) 4513–4522.
[100] R.L. Shattuck-Brandt, L.W. Lamps, K.J. Heppner-Goss, R.N. DuBois, L.M. Matrisian, Differential expression of matrilysin and cyclooxygenase-2 in intestinal and colorectal neoplasms, Mol. Carcinog. 24 (1999) 177–187.
[101] R.L. Shattuck-Brandt, G.W. Varilek, A. Radhika, F. Yang, M.K. Washington, R.N. DuBois, Cyclooxygenase 2 expression is increased in the stroma of colon carcinomas from IL-10(−/−) mice, Gastroenterology 118 (2000) 337–345.
[102] S.M. Pupa, S. Menard, S. Forti, E. Tagliabue, New insights into the role of extracellular matrix during tumor onset and progression, J. Cell Physiol. 192 (2002) 259–267.
[103] Z. Werb, J. Ashkenas, A. MacAuley, J.F. Wiesen, Extracellular matrix remodeling as a regulator of stromal–epithelial interactions during mammary gland development, involution and carcinogenesis, Braz. J. Med. Biol. Res. 29 (1996) 1087–1097.
[104] P.C. Walsh, Physiologic basis for hormonal therapy in carcinoma of the prostate, Urol. Clin. North Am. 2 (1975) 125–131.
[105] J.E. Montie, K.J. Pienta, Review of the role of androgenic hormones in the epidemiology of benign prostatic hyperplasia and prostate cancer, Urology 43 (1994) 892–899.
[106] C.B. Brendler, A.L. Follansbee, J.T. Isaacs, Discrimination between normal, hyperplastic and malignant human prostatic tissues by enzymatic profiles, J. Urol. 133 (1985) 495–501.
[107] R.B. Hayes, F.H. de Jong, J. Raatgever, J. Bogdanovicz, F.H. Schroeder, P. van der Maas, K. Oishi, O. Yoshida, Physical characteristics and factors related to sexual development and behaviour and the risk for prostatic cancer, Eur. J. Cancer Prev. 1 (1992) 239–245.
[108] R. Ross, L. Bernstein, H. Judd, R. Hanisch, M. Pike, B. Henderson, Serum testosterone levels in healthy young black and white men, J. Natl. Cancer Inst. 76 (1986) 45–48.
[109] Y.Z. Wang, S.W. Hayward, M. Cao, P. Young, R. Cardiff, G. Cunha, Role of estrogen signaling in prostatic hormonal carcinogenesis, J. Urol. 165 (2001) 1320.
[110] S.M. Ho, M. Yu, Selective increase in type II estrogen-binding sites in the dysplastic dorsolateral prostates of noble rats, Cancer Res. 53 (1993) 528–532.
[111] I. Leav, S.M. Ho, P. Ofner, F.B. Merk, P.W. Kwan, D. Damassa, Biochemical alterations in sex hormone-induced hyperplasia and dysplasia of the dorsolateral prostates of Noble rats, J. Natl Cancer Inst. 80 (1988) 1045–1053.
[112] R.L. Noble, The development of prostatic adenocarcinoma in Nb rats following prolonged sex hormone administration, Cancer Res. 37 (1977) 1929–1933.
[113] M. Yu, B.A. Leav, I. Leav, F.B. Merk, H.J. Wolfe, S.M. Ho, Early alterations in ras protooncogene mRNA expression in testosterone and estradiol-17 beta induced prostatic dysplasia of noble rats, Lab Invest. 68 (1993) 33–44.

[114] Y.Z. Wang, Y.C. Wong, Sex hormone-induced prostatic carcinogenesis in the noble rat: the role of insulin-like growth factor-I (IGF-I) and vascular endothelial growth factor (VEGF) in the development of prostate cancer, Prostate 35 (1998) 165–177.
[115] K.M. Lau, M. LaSpina, J. Long, S.M. Ho, Expression of estrogen receptor (ER)-α and ER-β in normal and malignant prostatic epithelial cells: regulation by methylation and involvement in growth regulation, Cancer Res. 60 (2000) 3175–3182.
[116] W.Y. Chang, G.S. Prins, Estrogen receptor-beta: implications for the prostate gland, Prostate 40 (1999) 115–124.
[117] G.G. Kuiper, E. Enmark, M. Pelto-Huikko, S. Nilsson, J.A. Gustafsson, Cloning of a novel receptor expressed in rat prostate and ovary, Proc. Natl. Acad. Sci. U.S.A. 93 (1996) 5925–5930.
[118] S. Makela, L. Strauss, G. Kuiper, E. Valve, S. Salmi, R. Santti, J.A. Gustafsson, Differential expression of estrogen receptors α and β in adult rat accessory sex glands and lower urinary tract, Mol. Cell Endocrinol. 164 (2000) 109–116.
[119] Z. Weihua, S. Makela, L.C. Andersson, S. Salmi, S. Saji, J.I. Webster, E.V. Jensen, S. Nilsson, M. Warner, J.A. Gustafsson, A role for estrogen receptor beta in the regulation of growth of the ventral prostate, Proc. Natl. Acad. Sci. U.S.A. 98 (2001) 6330–6335.
[120] J.F. Couse, J. Lindzey, K. Grandien, J.A. Gustafsson, K.S. Korach, Tissue distribution and quantitative analysis of estrogen receptor-alpha (ER-α) and estrogen receptor-β (ER-β) messenger ribonucleic acid in the wild-type and ER-α-knockout mouse, Endocrinology 138 (1997) 4613–4621.
[121] G.S. Prins, M. Marmer, C. Woodham, W. Chang, G. Kuiper, J.A. Gustafsson, L. Birch, Estrogen receptor-β messenger ribonucleic acid ontogeny in the prostate of normal and neonatally estrogenized rats, Endocrinology 139 (1998) 874–883.
[122] J.Y. Adams, I. Leav, K.M. Lau, S.M. Ho, S.M. Pflueger, Expression of estrogen receptor beta in the fetal, neonatal, and prepubertal human prostate, Prostate 52 (2002) 69–81.
[123] I. Leav, K.M. Lau, J.Y. Adams, J.E. McNeal, M.E. Taplin, J. Wang, H. Singh, S.M. Ho, Comparative studies of the estrogen receptors beta and alpha and the androgen receptor in normal human prostate glands, dysplasia, and in primary and metastatic carcinoma, Am. J. Pathol. 159 (2001) 79–92.
[124] D. Pasquali, S. Staibano, D. Prezioso, R. Franco, D. Esposito, A. Notaro, G. De Rosa, A. Bellastella, A.A. Sinisi, Estrogen receptor beta expression in human prostate tissue, Mol. Cell Endocrinol. 178 (2001) 47–50.
[125] G. Pelletier, M. El-Alfy, Immunocytochemical localization of estrogen receptors alpha and beta in the human reproductive organs, J. Clin. Endocrinol. Metab. 85 (2000) 4835–4840.
[126] H. Bonkhoff, T. Fixemer, I. Hunsicker, K. Remberger, Estrogen receptor expression in prostate cancer and premalignant prostatic lesions, Am. J. Pathol. 155 (1999) 641–647.
[127] G.R. Cunha, A.A. Donjacour, S.W. Hayward, Reciprocal mesenchymal–epithelial interactions in development of the male urogenital tract, in: K.S. Korach (Ed.), Reproductive and Developmental Toxicology, Marcell Dekker Inc., New York, 1998, pp. 509–530.
[128] G.R. Cunha, S.W. Hayward, Y.Z. Wang, W.A. Ricke, Role of the stromal microenvironment in carcinogenesis of the prostate, Int. J. Cancer 107 (2003) 1–10.
[129] P.S. Cooke, P. Young, R.A. Hess, G.R. Cunha, Estrogen receptor expression in developing epididymis, efferent ductules and other male reproductive organs, Endocrinology 128 (1991) 2874–2879.
[130] H. Takeda, C. Chang, Immunohistochemical and in situ hybridization analysis of androgen receptor expression during the development of the mouse prostate gland, J. Endocrinol. 129 (1991) 83–89.
[131] G.S. Prins, L. Birch, The developmental pattern of androgen receptor expression in rat prostate lobes is altered after neonatal exposure to estrogen, Endocrinology 136 (1995) 1303–1314.
[132] G.R. Cunha, L.W.K. Chung, J.M. Shannon, O. Taguchi, H. Fujii, Hormone-induced morphogenesis and growth: Role of mesenchymal–epithelial interactions, Recent Prog. Horm. Res. 39 (1983) 559–598.