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REVIEW

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Role of stroma in carcinogenesis of the prostate

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Abstract Prostatic development is induced by androgens acting via mesenchymal–epithelial interactions. Androgens elicit their morphogenetic effects by acting through androgen receptors (ARs) in urogenital sinus mesenchyme (UGM), which induces prostatic epithelial development. In adulthood reciprocal homeostatic stromal–epithelial interactions maintain functional differentiation and growth-quiescence. Testosterone plus estradiol (T + E2) have been shown to induce prostatic carcinogenesis in animal models. Thus, tissue recombinant studies were undertaken to explore the mechanisms of prostatic carcinogenesis in BPH-1 cells in which ARs and estrogen receptors (ERs) are undetectable. For this purpose, BPH-1 cells were combined with UGM, and the UGM + BPH-1 recombinants were grafted to adult male hosts. Solid branched epithelial cords and ductal structures formed in untreated UGM + BPH-1 recombinants. Growth was modest, and tumors did not develop. UGM + BPH-1 recombinants treated with T + E2 formed invasive carcinomas. BPH-1 cells lack ARs and ERs, whereas rat UGM expresses both of these receptors. These data show that immortalized nontumorigenic human prostatic epithelial cells can undergo hormonal carcinogenesis in response to T + E2 stimulation via paracrine mechanisms and demonstrate that the stromal environment plays an important role in mediating hormonal carcinogenesis. During prostatic carcinogenesis the stroma undergoes progressive loss of smooth muscle with the appearance of carcinoma-associated fibroblasts (CAF). This altered stroma was tested for its ability to promote carcinogenesis of nontumorigenic but immortalized human prostatic epithelial cells (BPH-1). CAF + BPH-1 tissue recombinants formed large carcinomas. In contrast, recombinants composed of normal prostatic stroma + BPH-1 cells exhibited minimal growth. This stroma-induced malignant transformation was associated with additional genetic alterations and changes in gene expression. Thus, alteration in the stromal microenvironment was sufficient to promote malignant transformation of human prostatic epithelial cells.

Key words mesenchymal · epithelial interactions · prostate · prostatic carcinogenesis · urogenital sinus mesenchyme · human prostatic epithelial cells

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Introduction

The prostate develops as a result of interactions between epithelium and mesenchyme. This basic developmental mechanism is in fact employed for all organs and organ systems composed of an epithelial parenchyma, e.g. male and female urogenital tracts, the gastrointestinal system and the integument. For many organs, epithelial–mesenchymal interactions are not influenced by the hormonal milieu. However, development of the prostate is dependent upon fetal testicular androgens, whose effects are mediated via androgen receptors expressed in fetal prostatic mesen-

chyme. Thus, androgens elicit prostatic development via epithelial–mesenchymal interactions.

Mesenchymal–epithelial interaction in prostatic development

The prostate develops from the embryonic urogenital sinus as a result of interactions between the endodermal epithelium of the urogenital sinus (UGE) and the mesoderm-derived urogenital sinus mesenchyme (UGM). Interactions between UGM and UGE are obligatory for prostatic development. When isolated UGE and UGM are grown by themselves, prostatic development does not occur. However, if UGM and UGE are reassociated, prostatic development proceeds provided androgens are present. During development of the prostate, androgen-dependent inductive signals from UGM direct the overall developmental process (Cunha et al., 1987). Prostate-inducing signals emanate from spatially discrete areas within the UGM (Sugimura et al., 1985; Timms et al., 1995). Regional differences in inductive activity of UGM are responsible for development of the well-defined lobar subdivisions of the prostate in the rodent (Sugimura et al., 1985; Takeda et al., 1990; Timms et al., 1995). UGM specifies prostatic identity in the undifferentiated UGE, induces prostatic bud formation, promotes prostatic bud growth and branching morphogenesis, elicits prostatic epithelial differentiation into secretory epithelial cells, and specifies the types of secretory proteins expressed. These conclusions have been drawn from mesenchymal–epithelial recombination experiments in which UGM has been isolated and grown in association with a variety of epithelia. Perhaps the most informative experiment is the tissue recombinant composed of embryonic UGM plus epithelium of the urinary bladder (BLE). When UGM + BLE tissue recombinants were grafted under the renal capsule of male hosts (and thus exposed to endogenous androgens), the BLE differentiated into prostatic epithelium. Surprisingly, UGM induced prostatic development from either embryonic BLE or fully differentiated adult BLE (Cunha et al., 1983a, 1983b). Adult BLE is a highly specialized unique androgen receptor-negative epithelium that lines the bladder and is nonglandular. In UGM + BLE tissue recombinants, inductive influences emanating from the UGM respecify epithelial identity from bladder to prostate. Three to four days after transplantation of the UGM + adult BLE tissue recombinants, prostatic buds emerge from the basal aspect of the BLE (Cunha et al., 1983a). These solid epithelial buds are induced by UGM to elongate and undergo branching morphogenesis in a manner similar to that of developing prostate. During ductal branching morphogenesis, the solid epithelial cords undergo canalization, and the epithelium differentiates into tall columnar luminal cells interspersed by basal cells located on the basement membrane. These two cell types express the correct spectrum of differentiation markers characteristic of mature prostatic epithelium. The induced BLE resembles prostatic epithelium at both light and electron microscopic levels. Androgen receptors (ARs) appear in the originally AR-negative bladder epithelium of UGM + BLE tissue recombinants, and prostate-specific secretory proteins are expressed (Cunha, 1984; Cunha et al., 1980, 1983a; Cunha and Young, 1991). Thus, embryonic UGM induces prostatic identity, formation and elongation of prostatic buds, ductal branching morphogenesis and functional histodifferentiation of prostatic epithelium. The fact that an adult BLE can be induced to undergo an apparently complete change in histodifferentiation implies a continued importance of these epithelial–connective tissue interactions into adulthood, presumably for the maintenance of adult epithelial differentiation via stromal–epithelial interactions. A likely interpretation of these data is that adult epithelia contain a pluripotent population, which is capable of responding to inductive mesenchyma. Such pluripotent epithelial cells, which might represent a stem cell population, have the capacity to give rise to entirely new organotypic phenotypes.

While UGM induces prostatic epithelial differentiation, these epithelial–mesenchymal interactions are reciprocal in that prostatic epithelium induces UGM to undergo smooth muscle differentiation. Evidence in support of a role of prostatic epithelium as the inducer of smooth muscle is based upon studies in which undifferentiated embryonic mouse or rat UGM was grafted and grown beneath the renal capsule of male hosts for 1 month. Such UGM grafts produced only small amounts of smooth muscle. In contrast, when rodent UGM was grafted with epithelium of either adult prostate, bladder or embryonic urogenital sinus derived from rat or mouse, prostatic ducts developed and became surrounded by α-actin-positive smooth muscle cells organized into thin sheathes as is appropriate for rodent prostate (Cunha et al., 1992b). Interestingly, rat UGM formed thick sheets of smooth muscle in tissue recombinants composed of rat UGM plus human prostatic epithelium (Hayward et al., 1996, 1998b), which is the smooth muscle pattern characteristic of human prostate. This demonstrates that the human prostatic epithelium not only induced the rat UGM to undergo smooth muscle differentiation but also dictated the spatial pattern of the smooth muscle. Thus, the cell–cell interactions involved in prostatic development are reciprocal with UGM inducing prostatic epithelial differentiation, and developing prostatic epithelium inducing smooth muscle differentiation in the UGM. The final result of these reciprocal epithelial–mesenchymal interactions is the development of mature prostatic tissue in which the epithelium expresses a highly differentiated secretory phenotype and specific secretory proteins. Likewise,

these reciprocal epithelial–mesenchymal interactions result in the development of a mature prostatic stroma predominantly composed of smooth muscle cells.

Paracrine influences of androgens on prostatic development

Mesenchymal–epithelial interactions play critical roles in development of the prostate, and prostatic development is absolutely dependent upon androgens, which act via androgen receptors. During prostatic development androgens acting via mesenchymal–epithelial interactions induce prostatic buds, ductal elongation and branching and epithelial growth. In this sense, the field of prostatic development lies at the interface of developmental biology and endocrinology. To reconcile and relate these two fields of study to prostatic development, the analysis of the ontogeny of ARs has been investigated. Several investigators have demonstrated that prior to and during prostatic development, the UGM expresses high levels of ARs, while ARs are initially undetectable in the epithelium (Cooke et al., 1991a; Takeda and Chang, 1991). Indeed, when prostatic buds emerge from the UGE, androgen receptors are undetectable in the induced epithelial buds. This observation suggests that mesenchymal ARs (and not epithelial ARs) are involved in the early phases of prostatic development. Androgen receptors are initially detected in developing prostatic epithelium shortly after birth in the mouse and rat as the ducts begin to canalize (Moeller et al., 1987; Prins and Birch, 1995; Prins et al., 1996). Thus, the appearance of epithelial ARs coincides with later aspects of prostatic development. To clarify the respective roles of epithelial versus mesenchymal ARs in prostatic development, tissue recombinants were analyzed that were prepared with epithelium and mesenchyme from wild-type (wt) and AR-negative testicular feminization (Tfm) mice (He et al., 1991) (Fig. 1). Tissue recombinants composed of wt-UGM + wt-epithelium and wt-UGM + Tfm-epithelium both undergo prostatic development. The common feature of these two tissue recombinants is the expression of ARs in the mesenchyme. The other two possible tissue recombinants (Tfm-UGM + Tfm-epithelium and Tfm-UGM + wt-epithelium) do not undergo prostatic epithelium in the presence of androgens (Fig. 1). The absence of prostatic development in these two tissue recombinants prepared with Tfm-UGM suggested a critical role for mesenchymal ARs in prostatic development. This idea was confirmed through analysis of tissue recombinants composed of wt-UGM + Tfm-epithelium, which demonstrated that when associated with wild-type UGM, AR-deficient Tfm epithelium can undergo androgen-dependent ductal morphogenesis, epithelial proliferation and columnar cytodifferentiation, thus forming glandular epithelium resembling prostate (Cunha et al., 1992a). This demonstrates that certain "androgenic effects" on epithelium are independent of epithelial ARs. Instead, many androgenic effects on epithelium are elicited by paracrine factors produced by AR-positive mesenchyme.

The function of epithelial androgen receptors has been revealed through comparison of tissue recombinants composed of AR-positive wt-UGM + AR-positive wt-epithelium versus AR-positive wt-UGM + AR-negative Tfm epithelium. Such experiments demonstrated that while the Tfm epithelium was induced to undergo prostatic differentiation, it failed to express prostatic secretory proteins (Cunha and Young, 1991; Donjacour and Cunha, 1993). Thus, epithelial ARs are required for expression of AR-dependent prostatic secretory proteins.

In a broader context, it is known that estrogen and progesterone also regulate epithelial proliferation in their respective target organs of the male and female genital tracts. Comparable wild-type/steroid receptor null tissue recombinants have been prepared with wild-type, estrogen receptor-α-null, or progesterone receptor-null mice. Such experiments demonstrated that for all three classes of steroids epithelial proliferation *in vivo* is regulated via the appropriate hormone receptors in the stromal cells (Cooke et al., 1997; Kurita et al., 1998; Sugimura et al., 1986). Thus, regulation of proliferation of normal epithelia by sex steroids occurs via paracrine mechanisms. Conversely, hormonal regulation of epithelial differentiation and function requires direct hormone action mediated by epithelial hormone receptors (Buchanan et al., 1998a, 1998b). It should be emphasized that these conclusions are applicable to normal epithelia. Androgenic regulation of prostatic epithelial cells during malignant transformation of prostatic epithelial cells appears to involve conversion from a paracrine to an autocrine mechanism of androgen-stimulated growth (Gao et al., 2001).

Estrogen action in the prostate

While the prostate is primarily considered to be an androgen target organ, it is also sensitive to estrogens. One well-recognized effect of estrogen on the prostate is squamous metaplasia (Risbridger et al., 2001a). Estrogenic effects are mediated via estrogen receptors (ERs), which are expressed in the developing and adult prostate (Cooke et al., 1991b; Jung-Testas et al., 1981). The paracrine action of androgen on the prostate mediated via stromal ARs raises the possibility that estrogenic effects on the prostate may be similarly elicited via stromal ERs. Estrogenic effects on the prostate are complex and may involve both indirect and direct actions. In intact males, pharmacologic doses of estrogen elicit androgen deprivation by suppressing pituitary gonadotrophin, thus reducing the production of testosterone by the testes (Walsh, 1975). This indirect effect of estrogen has

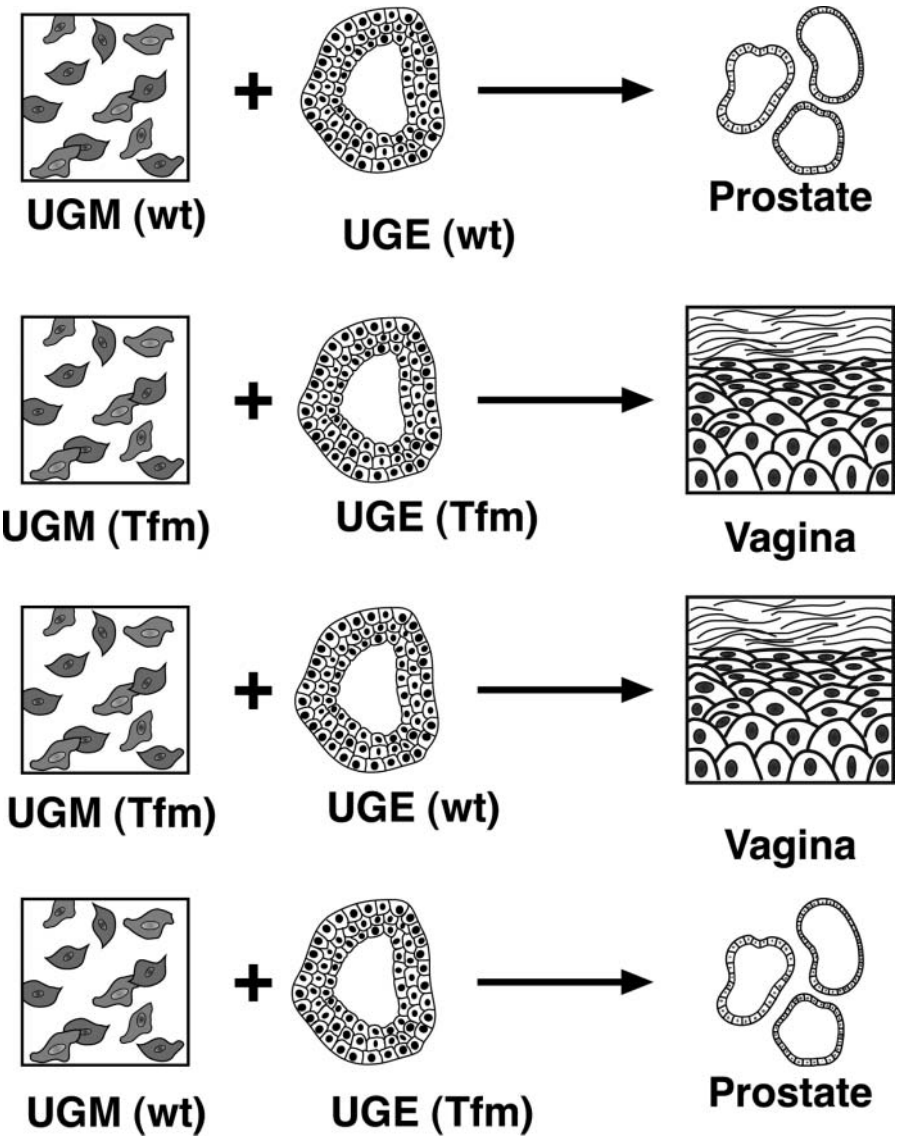

Fig. 1 A summary of tissue recombination experiments between urogenital sinus mesenchyme and epithelium from Tfm and wild-type embryos. A positive androgenic response (prostatic morphogenesis) occurs when wild-type mesenchyme is grown in association with either wild-type or Tfm epithelium. Conversely, vagina-like differentiation occurs when either wild-type or Tfm epithelium is grown in association with Tfm mesenchyme.

profound implications on the prostate because in response to high levels of exogenous estrogen two events occur: (i) the prostate is first deprived of androgens as a result of indirect effects on the pituitary; (ii) estrogens then act directly on the prostate unopposed by androgens to elicit squamous metaplasia. Experiments using ERα and ERβ null mice demonstrated that ERα, but not ERβ, is essential in estrogen induction of prostatic squamous metaplasia (Risbridger et al., 2001a, 2001b). To determine the respective roles of epithelial versus stromal ERα in the induction of prostatic squamous metaplasia, the following tissue recombinants were constructed with prostatic epithelia (PRE) and stroma (S) from wt and αERKO mice: wt-S + wt-PRE, αERKO-S + αERKO-PRE, wt-S + αERKO-PRE and αERKO-S + wt-PRE. Diethylstilbestrol (DES) elicited squamous metaplasia only in wt-S + wt-PRE tissue recombinants. Tissue recombinants containing αERKO-PRE and/or αERKO-S (αERKO-S + αERKO-PRE, wt-S + αERKO-PRE and αERKO-S + wt-PRE) failed to be induced by DES to become metaplastic. Therefore, estrogen induction of prostatic squamous metaplasia requires the action of estrogen via stromal ERα (paracrine mechanism) as well as direct action of estrogen via epithelial ERα. The importance of paracrine action of estrogen on the prostate has also been suggested in studies on neonatal imprinting of the prostate (Chang et al., 1999; Prins et al., 2001). Immunocytochemical and steroid autoradiographic studies have demonstrated ERα and ERβ in both prostatic stromal and epithelial cells during various stages of development even though ERs can be difficult to detect at certain stages (Cooke et al., 1991b; Prins

and Birch, 1997; Prins et al., 1998, 2001; Weihua et al., 2001). Nonetheless, the tissue recombinant studies formally point to the importance of both stromal and epithelial ER in estrogenic response in the prostate.

Role of stroma in hormonal carcinogenesis of the prostate

A number of epidemiological studies suggest a vital role of steroid hormones in prostate carcinogenesis (Montie and Pienta, 1994). As discussed above, the prostate is a target for both androgens and estrogens, and during prostatic carcinogenesis both of these hormones have been implicated. The importance of androgens and estrogens in prostatic carcinogenesis is suggested by several observations: (a) Prostate cancer does not occur in eunuchs. (b) In men and dogs (both susceptible to prostatic carcinogenesis) testosterone secretion as well as plasma testosterone levels decline with age, while plasma free estrogen levels remain unchanged or increase during senescence. Thus, the period of development and diagnosis of prostate cancer coincides with an elevation in the ratio of estrogen to testosterone (T) (Brendler et al., 1983; Hayes et al., 1992). (c) American Blacks, who have the highest incidence of prostatic cancer in the world, exhibit elevations in both plasma free T and estrogen levels (Ross et al., 1986). (d) T in combination with 17β-estradiol (E2) induces prostatic hyperplasia and dysplasia in mice, and induces prostate cancer in rats and in rUGM + Rb-/-PRE tissue recombinants (Ho and Yu, 1993; Leav et al., 1988; Noble, 1977; Wang et al., 2000, 2001b; Wang and Wong, 1998; Yu et al., 1993).

Hormonal induction of prostate cancer is thought to be the result of signaling through androgen receptors and estrogen receptors that are expressed in the prostate. The respective roles of AR, ERα and ERβ in prostatic carcinogenesis is currently under investigation through (i) use of mice null for genes encoding ERα (αERKO mice) or ERβ (βERKO mice), (ii) use of BPH-1 cells null for AR, and (iii) use of tissue recombination systems that allow dissection of ARs, ERα and ERβ signaling pathways.

1. Hormonal carcinogenesis in the prostate is independent of epithelial AR

There are distinct androgenic responses in the prostate mediated through stromal versus epithelial androgen receptors. Epithelial proliferation, ductal branching morphogenesis, ductal canalization and cytodifferentiation into basal and luminal cells is mediated by the mesenchymal ARs during normal development (Cunha and Chung, 1981; Shannon and Cunha, 1984; Sugimura et al., 1986) and do not require epithelial ARs. Likewise, in adulthood androgens act via stromal ARs (predominantly localized in the prostatic smooth muscle [Cunha, 1994; Hayward et al., 1997; Prins et al., 1991; Prins and Birch, 1995]) to maintain a fully differentiated growth-quiescent gland. Epithelial AR are required for secretory function in the mature prostate (Cunha and Young, 1991; Donjacour and Cunha, 1993).

To elucidate the role of ARs in prostate carcinogenesis, tissue recombinant studies have been performed by combining embryonic rat UGM (rUGM) plus BPH-1 cells (a clonally derived immortalized human prostatic epithelial cell line) (Wang et al., 2001a). ARs are undetectable in BPH-1 epithelial cells by immunocytochemical and RT-PCR techniques (Hayward et al., 1995). ERα and ERβ are also not detectable in BPH-1 cells by immunocytochemical techniques. When BPH-1 cells are grafted by themselves under the renal capsule of untreated or T + E2-treated male hosts, the BPH-1 cells survive, but do not undergo tumorigenesis. This lack of a tumorigenic response in grafts of BPH-1 cells is in keeping with an apparent lack of ARs and ERs. When BPH-1 cells are combined with rUGM (rUGM + BPH-1 recombinants) and grown in intact untreated male mice, relatively normal human prostatic development occurs with the morphogenesis of branched solid and canalized ductal structures resembling prostatic ducts. However, when rUGM + BPH-1 tissue recombinants are grown in T + E2 treated mice, BPH-1 cells are induced to undergo malignant transformation. As described above, ARs and ERs are undetectable in BPH-1 cells (Hayward et al., 1995; Wang et al., 2001a), while ARs and ERα are expressed in the rat stromal cells of the rUGM + BPH-1 tissue recombinants (Fig. 2). Thus, the only AR and ERα expressed in rUGM + BPH-1 re-

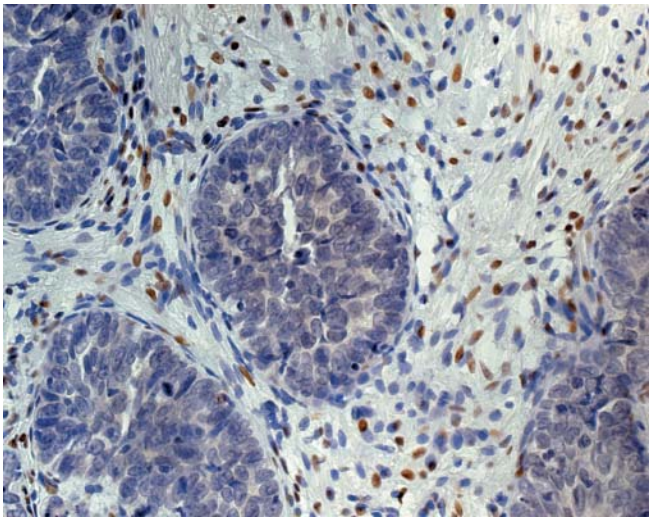

Fig. 2 A rat UGM + BPH-1 tissue recombinant stained for androgen receptor. Note AR-positive cells (brown nuclei) in the stroma and the absence of AR staining in the epithelial structures formed by the BPH-1 cells.

combinants resides in the stroma, which emphasizes the critical role of stroma and stromal hormone receptors in mediating hormonal carcinogenesis of the prostate. Based upon these observations, the effects of androgens (and probably also estrogens) as hormonal carcinogens appear to involve paracrine mechanisms, as is the case for hormonal effects during normal prostatic growth and development. It is important to recognize that the carcinogenic effect of T + E2 in this tissue recombinant model involves the promotion of pre-existing genetic lesions as the parental BPH-1 epithelial cells, while non-tumorigenic, are immortal and aneuploid (Hayward et al., 1995; Phillips et al., 2001). Thus, this model differs from the induction of tumors in genetically normal animals and may well mimic in some way the promotion of spontaneously occurring human prostate tumors.

2. Hormonal carcinogenesis in the prostate is mediated by estrogen receptor-alpha, but not estrogen receptor-beta

Estrogen plays an important role in the pathobiology of the prostate. Estrogens act through two specific receptors, ERα and ERβ (Couse and Korach, 1999; Kuiper et al., 1996). While immunocytochemical detection of ERs can be difficult in the prostate, ERα has been consistently detected in prostatic stroma (Lau et al., 1998; Prins and Birch, 1997) and is also expressed in prostatic squamous metaplasia (Adams et al., 2002; Wang and Cunha, unpublished data). ERβ, which was originally cloned from prostatic tissue, is expressed at high levels in both prostatic epithelium and stroma in the developing prostate. In adulthood, ERβ is restricted primarily to prostatic epithelium (Adams et al., 2002). Thus, ERα and ERβ are present in both stromal and epithelial tissues of the prostate during various developmental and adult stages. However, it is notable that steroid autoradiographic studies in various species and different developmental stages have consistently demonstrated the binding of ^{3}H-estradiol in the prostatic stroma but not in the epithelium (Cooke et al., 1991b; Hatier et al., 1990; Weaker and Sheridan, 1982). As described above, the induction of squamous metaplasia in prostatic epithelium is dependent upon ERα (and not ERβ), and ERα is required simultaneously in both the epithelium and the stroma (Risbridger et al., 2001b). The role of epithelial versus stromal ERα and ERβ in prostatic carcinogenesis remains to be elucidated and is currently a subject of investigation.

Estradiol in combination with testosterone is an effective method of inducing prostatic cancer in adult Noble rats (Ho and Yu, 1993; Leav et al., 1988; Noble, 1977; Wang and Wong, 1998; Yu et al., 1993). Likewise, mice treated with T + E2 developed prostatic hyperplasia and dysplasia (Wang et al., 2001b). Extending this model of hormonal carcinogenesis to mice means that the power of mouse genetics can be used to elucidate the underlying molecular mechanisms of hormonal carcinogenesis. In this regard, αERKO mice treated with T + E2 did not develop prostatic hyperplasia or dysplasia (Wang et al., 2001b). In contrast, ERβ knockout mice treated with T + E2 developed prostatic hyperplasia and dysplasia comparable to that seen in wild type mice (Wang and Cunha, unpublished data). This emphasizes the importance of signaling through ERα in the induction of prostatic carcinogenesis and squamous metaplasia. To determine the respective roles of epithelial versus stromal ERα in prostatic pathogenesis, we have underway tissue recombinant experiments in which prostatic epithelium (PRE) and stroma (S) is derived from wt and αERKO mice as follows: wt-S + wt-PRE, αERKO-S + αERKO-PRE, wt-S + αERKO-PRE and αERKO-S + wt-PRE. Grafts of these tissue recombinants under the renal capsule of male nude mice will be treated with T + E2 to assess the effects of estrogen in hormonal carcinogenesis.

Reciprocal homeostatic interactions between prostatic stroma and epithelium in adulthood

The normal adult prostate is composed of two compartments: a glandular epithelial compartment and a fibromuscular stroma. The stroma of the adult prostate contains predominantly smooth muscle cells and fibroblasts, which are derived from embryonic UGM. In the normal adult prostate, cells of both the epithelial and the stromal compartments are highly differentiated. Under steady-state androgenic conditions of the intact adult male, the prostate contains fully differentiated smooth muscle cells in intimate association with a highly differentiated and functional prostatic epithelium. Both cell types are essentially growth-quiescent with levels of proliferation and cell death low and in balance. It should be emphasized that this adult growth-quiescent, homeostatic state exists in the presence of high levels of systemic androgens, which at earlier stages (fetal, neonatal and pubertal) were profoundly growth stimulatory. This homeostatic growth-quiescent state of the adult normal prostate appears to be dependent upon maintenance of the normal architectural associations between adult prostatic epithelium and adult prostatic stroma, because proliferation occurs when epithelial and stromal cultures are established from growth-quiescent adult prostatic tissue. Proliferative activity in cultures of adult prostatic epithelial and stromal cells is associated with marked changes in the differentiation profile of both cell types. Thus, perturbation or elimination of normal stromal–epithelial interactions in adulthood causes dedifferentiation and elicits proliferation of both adult prostatic stromal and epithelial cells.

Another method of altering the cell–cell interactions in the adult prostate is to associate adult growth-quiescent epithelium (PRE) with embryonic or neonatal pros-

tatic inductive mesenchyme. This is illustrated in experiments in which tissue recombinants were prepared with embryonic rat UGM plus a ~300-μm segment of an adult mouse prostatic duct (rUGM + mPrE). Such 300-μm fragments of adult prostatic ducts contain only about 5000 epithelial cells. When grown under the renal capsule of a male host, rUGM + mPrE recombinants form about 30–50 mg wet weight of prostatic tissue in 1 month, which contains 20 to 30×10^6 mouse prostatic epithelial cells (Hayashi et al., 1993; Norman et al., 1986). Another important observation is that if the adult prostatic duct is derived from the ventral prostate, UGM or seminal vesicle mesenchyme (SVM) elicits the development of prostatic epithelium expressing secretory proteins specific to the dorsal-lateral and anterior prostates (Hayashi et al., 1993; Norman et al., 1986). This observation on mesenchyme-induced re-specification of lobar identity in adult prostatic epithelium in conjunction with the ability of UGM and SVM to induce prostatic differentiation in adult bladder epithelium (Donjacour and Cunha, 1988) provides yet another example of the responsiveness of adult epithelia to the stromal microenvironment. Thus, the adult stromal microenvironment is thought to play a central role in regulating adult epithelial proliferation and differentiation. The corollary to this idea is that alterations in the normal homeostatic stromal–epithelial interactions may play a role in the pathogenesis of the prostate and other glands.

The role of stroma in carcinogenesis: a historical perspective

The idea that stroma may play a role in the initiation and promotion of carcinogenesis has been considered for many years and is based upon the pathological literature demonstrating that "tumor stroma" is frequently different than normal stroma (Bosman et al., 1993; Seljelid et al., 1999). In the 1960s, it was recognized that "peritumoral dermis" stimulated the proliferation of embryonic epidermis (Redler and Lustig, 1968). Studies on the malignant transformation of embryonic mouse submandibular gland epithelium by polyoma virus was shown to occur when salivary gland epithelium was grown in association with its mesenchyme, but not when the epithelium was grown by itself (Dawe, 1972). A key landmark in the field of microenvironmental influences in cancer was the publishing of the book "Tissue Interactions in Carcinogenesis" in 1972 (Tarin, 1972). This monograph emphasized that deregulation of epithelial–stromal interactions may contribute to both early and late stages of cancer formation and that the continued interaction of the carcinoma cell with its stromal microenvironment played an important role in the biology of the neoplasm.

Once a carcinoma emerges, the biology of the neoplasm may be responsive to stromal influences that may have therapeutic significance. In this regard, De Cosse et al. (1973, 1975) reported that mammary carcinoma cells exhibit a more orderly histodifferentiation and a lower proliferative rate when grown in association with mammary mesenchyme. Cooper and Pinkus (1977) demonstrated that when basal cell carcinomas are grown in association with normal stroma, the malignant epidermal cells differentiated with an apparent loss of their former malignant properties. Transitional cell carcinomas of the urinary bladder when grown in association with UGM were induced to differentiate into adenocarcinomatous structures resembling prostatic neoplasms (Fujii et al., 1982). Similarly, human colon carcinoma cells differentiated in response to embryonic rat intestinal mesenchyme (Fukamachi et al., 1986, 1987). All of these examples serve to emphasize the continued importance of stromal–epithelial interactions in carcinomas. Continued responsiveness of carcinoma cells to their stromal microenvironment can provide the biological means of regulating both differentiation and proliferation of the carcinoma, perhaps to therapeutic benefit.

Modification of the cellular and extracellular matrix (ECM) of the stroma immediately adjacent to carcinoma cells has been documented in several tumors (Basset et al., 1993; Chiquet-Ehrismann et al., 1986; Singer et al., 1995; Wright et al., 1994; Yee et al., 1989). For example, the histology and growth characteristics of mammary carcinoma-associated fibroblasts are different from that of fibroblasts associated with normal breast epithelial cells; abnormal myofibroblasts are associated with invasive breast carcinoma cells (Ronnov-Jessen et al., 1996). Other phenotypic changes ascribed to carcinoma-associated fibroblasts include abnormal migratory behavior *in vitro* (Schor et al., 1988), alterations in cell surface molecules (Chaudhuri et al., 1975; Oishi et al., 1981), altered expression of growth factors (platelet-derived growth factor, insulin-like growth factors-I and -II, transforming growth factor-β1, hepatocyte growth factor/epithelial scatter factor and keratinocyte-growth factor (Ellis et al., 1994; Frazier and Grotendorst, 1997; Nakamura et al., 1997; Ponten et al., 1994; Yan et al., 1993; Yee et al., 1989), expression of prostaglandin-synthesizing enzymes (Shattuck-Brandt et al., 1999, 2000), and alterations in ECM (Pupa et al., 2002; Werb et al., 1996). While these phenotypic changes have been documented in carcinoma-associated fibroblasts from a variety of sources, their significance or contribution to tumor growth and development are poorly understood. Recently, we have tested the hypothesis that carcinoma-associated fibroblasts may affect tumor progression.

Role of carcinoma-associated fibroblasts (CAF) in progression of prostate cancer

Adult human prostatic stroma is primarily composed of smooth muscle and ECM surrounding the prostatic

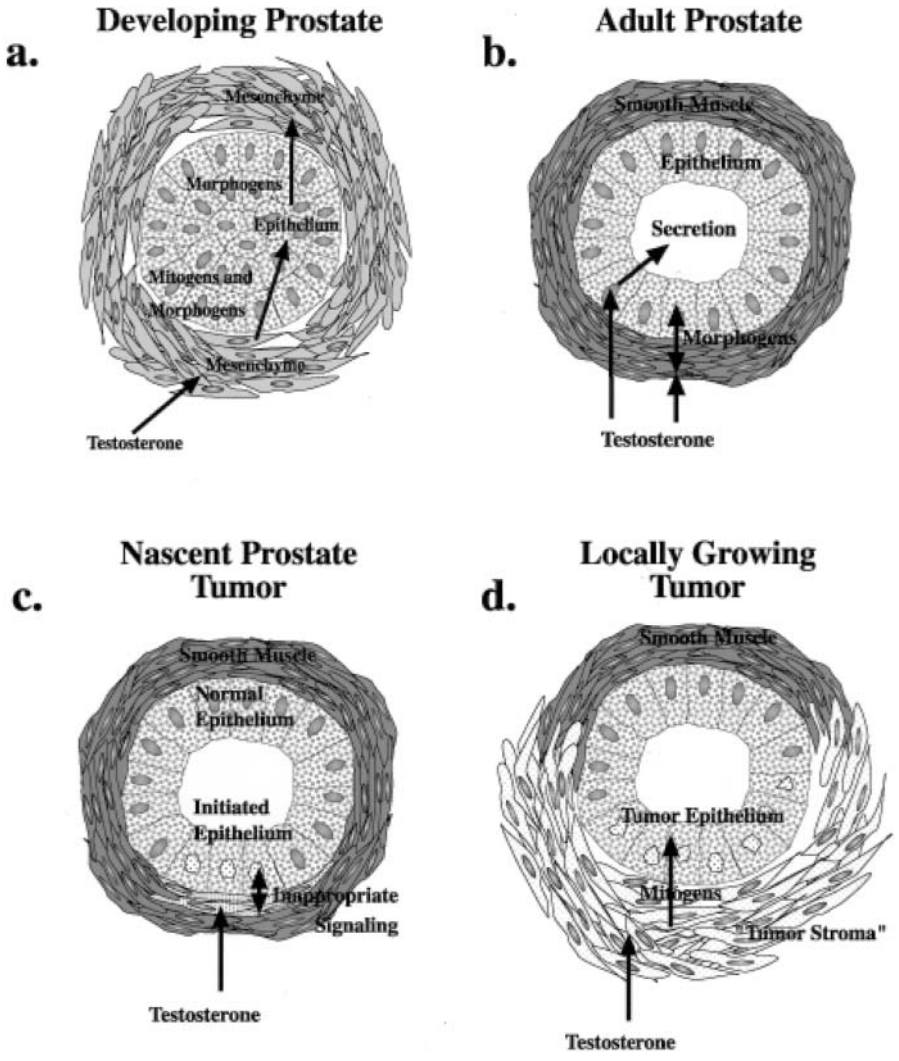

Fig. 3 Interactions between the epithelial and the stromal/mesenchymal components of the prostate during development and adulthood. During prostatic development (**A**) low levels of androgens, acting through the mesenchymal androgen receptor (AR), stimulate proliferation and differentiation of prostatic epithelium. Concurrently, the epithelium induces differentiation of the mesenchyme into smooth muscle. In the normal adult (**B**) high levels of circulating androgens, acting through AR located in the smooth muscle, maintain the morphology of growth-quiescent adult epithelium. Secretory function is elicited by direct androgenic stimulation of AR in the differentiated columnar epithelium. Epithelial and smooth muscle differentiation is maintained by reciprocal paracrine-acting homeostatic factors. In early tumors we have hypothesized that following genetic insult to the epithelium (**C**), signaling between the epithelium and the adjacent smooth muscle begins to become abnormal, leading (**D**) to the formation of a fibroblastic "tumor stroma", which responds to androgenic stimulation by producing paracrine-acting mitogens, fueling a cycle of tumor proliferation and stromal dedifferentiation.

ducts. These stromal cells express androgen receptors and respond to androgens by restraining prostatic epithelial proliferation via homeostatic stromal–epithelial interactions (Hayward and Cunha, 2000). In contrast, stromal cells surrounding a prostate carcinoma are more typically fibroblastic or myofibroblastic (Arnold and Isaacs, 2002; Hayward et al., 1996, 1997; Rowley, 1998; Tuxhorn et al., 2001). In the prostate we have described the stromal cell type within and surrounding a tumor as the carcinoma-associated fibroblast (CAF) (Olumi et al., 1999). Others, notably Rowley and coauthors, have described the same phenotype as "reactive stroma" (Rowley, 1998; Tuxhorn et al., 2001). As described by Rowley's group, these peritumoral stromal cells have many features characteristic of wound repair, including the expression of a myofibroblastic phenotype and the deposition of ECM components.

As described above, stromal–epithelial interactions play a critical role in normal development and adult function of the prostate (Cunha et al., 1992a). We hypothesized (as summarized in Fig. 3) that prostatic carcinogenesis is associated with changes in the local interactions between the stromal microenvironment and genetically initiated incipient tumor cells (Hayward et al., 1996, 1997, 1998a). These changes in stromal–epithelial interactions are suggested to promote malignant progression in genetically initiated prostatic epithelial cells resulting in tumorigenesis (Grossfeld et al., 1998; Hayward et al., 1996, 1997, 1998a).

One of the first experiments to test the idea that stromal cells may facilitate prostatic carcinogenesis was performed in a tissue recombination system by Thompson and colleagues. In these experiments either the urogenital sinus (prostatic anlagen) or its individual mesenchymal (UGM) or epithelial (UGE) components were transfected using a virus carrying the *myc* and *ras* oncogenes. In tissue recombinants containing uninfected UGM + infected UGE, epithelial hyperplasias were detected. Similarly, in prostatic reconstitutions composed of infected UGM + uninfected UGE, stromal desmoplasias were observed. Carcinomas were found only in recombinants in which both UGM and UGE were in-

fected (Thompson et al., 1993). These findings demonstrated that changes were required in both the epithelium and the stroma for prostatic carcinogenesis to occur. These experiments are also important because they illustrated the use of viral vectors to introduce genes into either epithelial or stromal cells in tissue recombinants.

We have demonstrated that human prostatic carcinoma-associated fibroblasts (CAF) can promote carcinogenesis in initiated but nontumorigenic human prostatic epithelial cells (Olumi et al., 1999). By isolating CAF cells from human prostate tumors and recombining these with the SV40T immortalized human prostatic epithelial cell line BPH-1 (Hayward et al., 1995), the CAF, but not normal prostatic fibroblasts, promoted carcinogenesis (Table 1). CAF + BPH-1 tissue recombinants gave rise to large poorly differentiated tumors (Olumi et al., 1999). It appears that, unlike the normal adult prostatic stroma, CAF cells do not respond to androgens by restricting epithelial proliferation, but rather stimulated epithelial proliferation and carcinogenesis. In experimental models where fetal UGM was combined with BPH-1 cells, epithelial growth was also stimulated but tumorigenesis does not occur, suggesting that mere stimulation of epithelial proliferation is not the single determinant in CAF-induced promotion of tumorigenesis (Wang et al., 2001a). Thus, rather than acting to repress epithelial proliferation (as would be expected of normal prostatic stroma), the stromal cells surrounding a human prostate carcinoma (CAF) stimulated proliferation and promoted carcinogenesis.

The tumorigenic process promoted by CAF in the parental nontumorigenic BPH-1 cells involves alteration in gene expression and further genetic alteration. Thus, CAF cells appear to actively participate in the process of tumor progression. Specifically, fibroblasts derived from areas of human prostatic carcinoma (CAF) induced tumors and elicited a characteristic pattern of genetic change associated with malignant progression (Hayward et al., 2001; Phillips et al., 2001). A comparison of BPH-1 epithelial cells, which had been induced to become tumorigenic by exposure to CAF cells (BPH-1^{CAFTD}), with BPH-1 cells, which were tumorigenic as a result of hormonal carcinogenesis (BPH-1^{TETD}), revealed many common changes as well as changes that were unique to the method of cancer induction. Thus, BPH-1^{CAFTD} and the BPH-1^{TETD} cells share amplification of chromosomes 11q and 20q and loss of chromosomes 3q, 8p and 10p. However, the cells that were exposed to CAF contain multiple nonreciprocal translocations and a series of complex harlequin chromosomes based on chromosome 7. In contrast, the cells exposed to hormonal carcinogens are much more likely to contain reciprocal translocations and show harlequin chromosomes based on chromosome 5. For a more complete description, see Hayward et al., 2001 and Phillips et al., 2001. These data support the contention that interactions between the stromal and epithelial compartments of a tumor might in some way influence genetic changes across tissue layer boundaries.

Table 1 Summary of the results of recombining human prostatic fibroblasts from normal human prostate (NPF) or from prostate cancer (CAF) with either normal human prostatic epithelium or with the SV40T immortalized (and thus genetically initiated) human prostatic epithelial cell line BPH-1. Recombinants were grafted to athymic mouse hosts and allowed to grow for 41 days. Tumors only developed when the initiated epithelium (BPH-1) was associated with CAF cells

Cell recombination	Result (epithelial differentiation and graft wet weight)
NPF + normal epithelium	Normal differentiation, wet weight >10 mg
CAF + initiated epithelium	Squamous differentiation, wet weight > 10 mg
NPF + normal epithelium	Solid epithelial cords, no tumor, wet weight > 10 mg
CAF + initiated epithelium	Tumor, median wet weight 132 mg

Two independent investigations have determined that the CAF populations used in these experiments are genetically normal as determined by comparative genomic hybridization (Olumi et al., 1999) and karyotypic analysis by spectral karyotyping (Phillips et al., 2001). In contrast, Macintosh et al. (1998) showed that similar CAF cells contained genetic alterations. It could certainly be suggested that "CAF" cells containing these genetic modifications resulted from an epithelial to mesenchymal transition (EMT) of previously genetically abnormal epithelium. The difference in results between our studies and those of Macintosh et al. could well reflect differences in the disease stage and grade of the patient samples examined. The phenomenon of EMTs in cancer is important because it raises questions as to the source of genetic alteration seen in stromal cells surrounding tumors. Carcinomas are, by definition, epithelial tumors. However, as noted above there have been reports of genetic changes occurring in stromal cells. As suggested, these "changes" could, in some cases, be a result of EMTs. In other cases, genetic or phenotypic changes in stroma may be induced by adjacent carcinoma cells. For example, injection of the human prostate cancer cell line, C4–2, into athymic male nude mice induced sarcomas of murine origin (Pathak et al., 1987). The mechanism by which this occurs is unknown, but epigenetic mechanisms may have elicited changes in gene expression leading to chromosomal rearrangements and malignant transformation. Thus, the findings of genetic changes in stromal cells resulting from association with human prostatic carcinoma cells (Pathak et al., 1997) and our own observations of genetic changes in human prostatic epithelial cells as a result of association with CAF may in fact reflect common mechanisms.

The mechanisms by which stromal cells influence the process of tumorigenesis are not well defined. Differen-

tial regulation of factors, which modulate the local microenvironment, is an obvious candidate. For example, Tuxhorn et al. (2002) recently demonstrated that reactive stromal cells supported establishment of tumors and increased angiogenesis in a subcutaneous grafting model. It is also possible, indeed likely, that tumor stromal cells respond to androgens to produce growth factors that induce epithelial proliferation (Haughney et al., 1998; Hayward and Cunha, 2000; Hayward et al., 1998b; Wong and Wang, 2000). Stromal cells associated with carcinomas are known to produce a variety of matrix metalloproteinases that profoundly affect the stromal–epithelial signaling and may affect tumor initiation, growth, migration, angiogenesis, apoptosis, invasion and metastasis (see review by Lynch and Matrisian in this issue). Future work on the cellular and molecular mechanisms of stromal–carcinoma cell interactions may provide new therapeutic strategies for regulating carcinoma growth and/or apoptosis to the benefit of patients suffering from cancer.

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