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Steroid Hormones, Polypeptide Growth Factors, Hormone Refractory Prostate Cancer, and the Neuroendocrine Phenotype

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Abstract The growth, development, and differentiation of the prostate gland is largely dependent on the action of androgens and peptide growth factors that act differentially at the level of the mesenchymal and epithelial compartments. It is our premise that to understand the emergence of metastatic and hormone refractory prostate cancer we need to investigate: (1) how androgen action at the level of the mesenchyme induces the production of peptide growth factors that in turn can facilitate the growth and development of the epithelial compartment; (2) how androgen action at the level of the epithelium induces and maintains cellular differentiation, function, and replicative senescence; and (3) how transformation of the prostate gland can corrupt androgen and growth factor signaling homeostasis. To this end, we focus our discussion on how deregulation of the growth factor signaling axis can cooperate with deregulation of the androgen signaling axis to facilitate transformation, metastasis, and the emergence of the hormone refractory and neuroendocrine phenotypes associated with progressive androgen-independent prostate cancer. Finally, we suggest a working hypothesis to explain why hormone ablation therapy works to control early disease but fails to control, and may even facilitate, advanced prostate cancer. J. Cell. Biochem. 91: 671–683, 2004.

Key words: prostate cancer; peptide growth factors; androgen receptor; neuroendocrine; mouse models

The purpose of this study is to present a plausible working model of prostate cancer progression that highlights the interaction between stromal and epithelial compartments and the role of peptide and steroid hormone signaling during the natural history of the disease. As well, we attempt to shed some new perspective on the paradoxical consequences of hormone ablation and the significance of the emergence of the neuroendocrine phenotype drawing heavily on our experience with genetically engineered mouse (GEM) model systems.

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PROSTATE GLAND: A COMPLEX ORGAN SYSTEM

The prostate can be divided into two major cellular compartments, the mesenchyme and the epithelial compartment (Fig. 1). The prostate mesenchyme comprises smooth muscle cells and fibroblasts, and derives from the mesenchymal component of the embryonic urogenital sinus [Cunha et al., 2003]. In contrast, the prostate epithelium is likely comprised of glandular/secretory epithelial cells, neuroendocrine cells, and basal cells [Abrahamsson, 1999a]. Recently, Bonkhoff and Remberger postulated that the epithelial compartment could itself be subdivided into (i) a stem cell/undifferentiated compartment of both androgen-independent and androgen non-responsive cells; (ii) a proliferative/undifferentiated compartment consisting of androgen-independent but hormone-responsive cells; and (iii) a differentiation compartment derived from committed

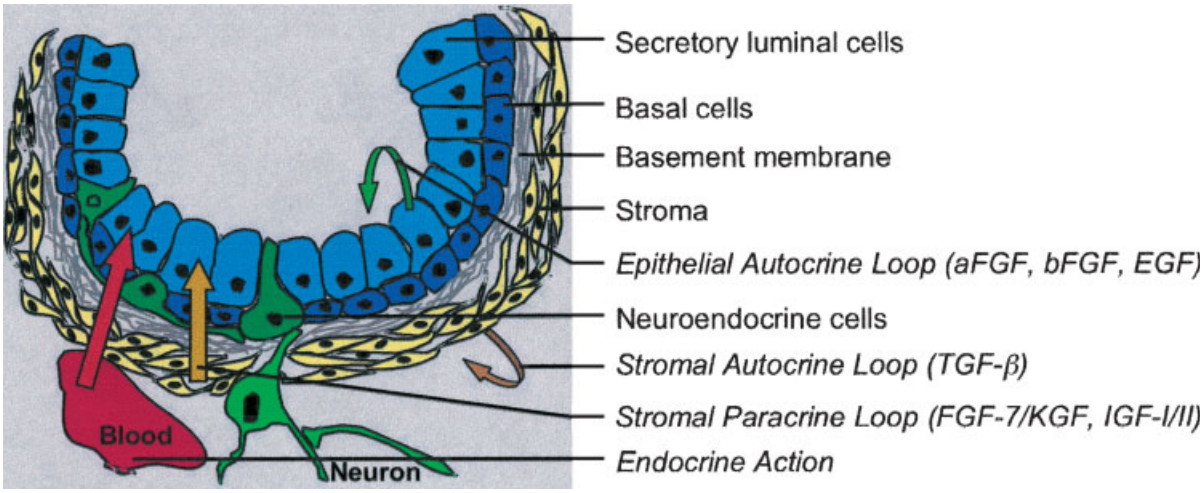

Fig. 1. The major cellular compartments of the prostate gland. Adapted from Hansson and Abrahamsson, *Annals of Oncology* 12:S145–S152, 2001.

basal cells giving rise to androgen-independent neuroendocrine cells, androgen-responsive basal cells, and androgen-dependent secretory epithelial cells [Bonkhoff and Remberger, 1996]. Even this rather simple description demonstrates that the prostate gland is a complex organ and underscores the need to identify, study, and define roles for each of the various cellular constituencies. Indeed, while considerable attention has been focused on the terminally differentiated secretory epithelial cells and their interaction with the mesenchyme, we now come to realize how relatively little we know about the neuroendocrine compartment.

The neuroendocrine cells of the prostate gland most likely represent terminally differentiated cells derived from undifferentiated neuronal precursor or basal cells [Aumuller et al., 1999a]. It is generally thought that the normal mature neuroendocrine cells are fully differentiated and postmitotic [Abrahamsson, 1996], and growth arrested in G_0 [Bonkhoff and Remberger, 1995]. The neuroendocrine cells do not express an androgen receptor (AR) and are by definition androgen independent [Abrahamsson, 1996].

At least two types of prostatic neuroendocrine cells have been observed in the prostate, the so-called "open cell" type that have long slender extensions reaching towards the lumen and the "closed cell" type that lack luminal extensions. Although "closed" neuroendocrine cells can express both neuroendocrine-specific (Chromogranin A (ChgA)) and basal cell-specific (Cytokeratin D) markers, suggesting that all three epithelial cell types in the prostate epithelium may have developed from common endodermal pluripotent stem cells, there is evidence that these cells are of neurogenic origin [Aumuller et al., 1999b].

STEROID HORMONES AND PROSTATE CANCER

The development, growth, and maintenance of the prostate gland is androgen dependent and the growth of primary prostatic tumors is at least initially dependent on androgen action. In the early 1940s, Huggins and Hodges [1941] introduced a pioneering concept that has since made androgen ablation and anti-androgen therapy the cornerstone of treatment for patients with locally advanced or metastatic prostate cancer. However, despite a positive initial response in most (80–90%) patients, those treated with androgen ablation eventually develop androgen-independent tumors, rendering further hormone therapy or complete androgen blockade ineffective [Gittes, 1991; Laufer et al., 2000]. Understanding the biology underlying the emergence of hormone-independent prostate cancer may represent the biggest challenge to the development of efficacious treatments for this disease.

During ontogeny of the prostate gland, androgens are believed to initially act at the level of the mesenchyme that expresses a functional AR, to indirectly induce ductal morphogenesis, cytodifferentiation, and the formation of a differentiated epithelial compartment [Sugimura

et al., 1996] (Fig. 2). By this paradigm, it can be said that the development of the prostate is dependent not only on the mesenchyme, but on a functional androgen signaling axis within this compartment. In fact, it has been demonstrated that expression of a wild type AR in the mesenchyme is a prerequisite for the formation of prostatic epithelial structures, and that an AR expressing mesenchyme can still direct formation of the epithelium even if the epithelial cells themselves do not express functional AR [Cunha et al., 1992, 2003]. That the maintenance of a terminally differentiated functional epithelial structure is AR dependent suggests that in the prostate the AR has distinct and compartment specific roles during development and differentiation. Based on these observations, it can be predicted that androgen insensitivity or early androgen ablation would severely impair prostate development or cause glandular regression primarily through the loss of stromal-derived growth factors. In contrast, androgen ablation following the emergence of a stroma-independent (and by definition growth factor autonomous) epithelium would have little impact on cell viability or apoptosis.

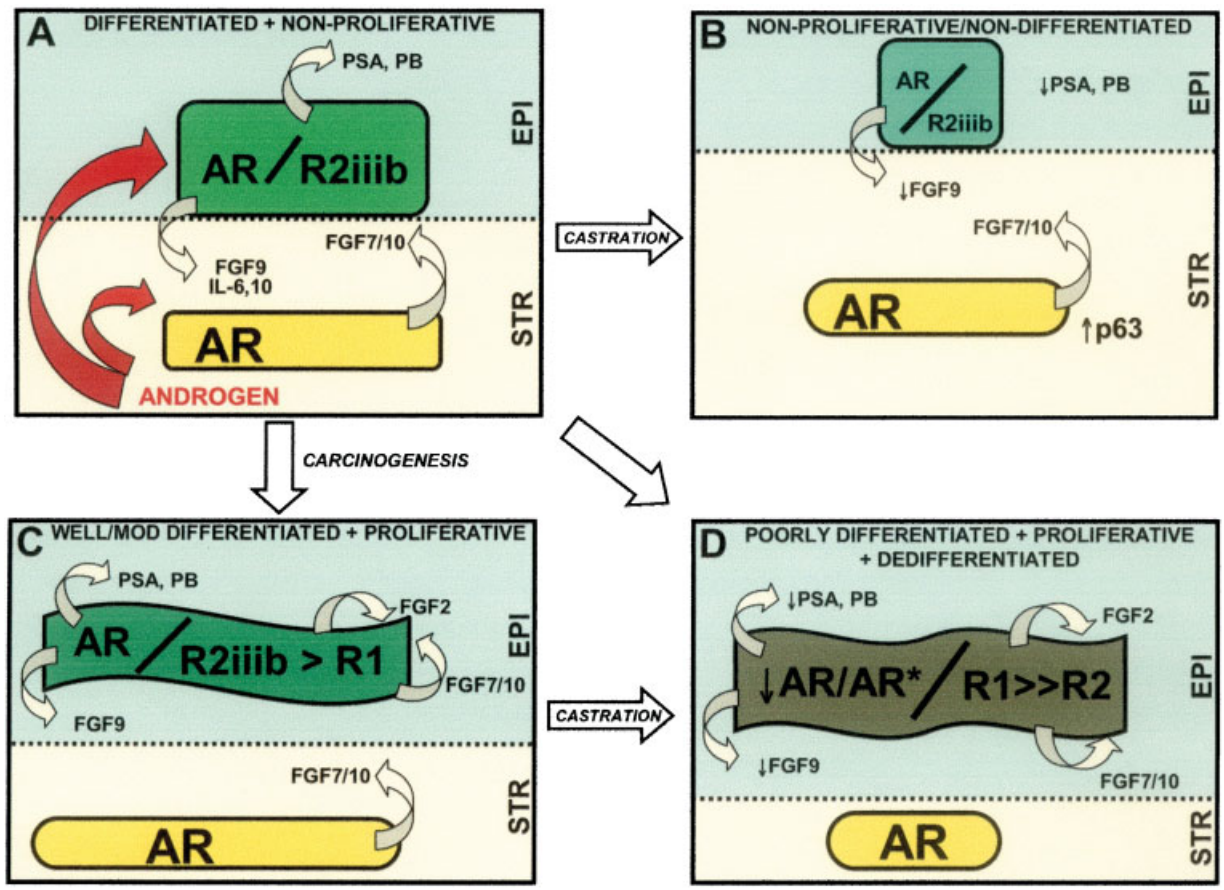

Fig. 2. Working model for prostate cancer progression. In the prostate, the FGF axis regulates the growth and differentiation of epithelial cells. FGF-7/FGF-10 secreted from the androgen-responsive stromal compartment (STR) conveys signals to the epithelial (EPI) cells via FGFR2iiib receptors to influence prostate epithelial development, growth, and differentiation. **A**: In the presence of wild type AR and FGFR2iiib, the prostate epithelial compartment is differentiated and non-proliferative. **B**: Upon castration, the stromal FGF-7/FGF-10 signal is reduced and the epithelial compartment will regress and remain quiescent. **C**: With the onset of transformation, the epithelial compartment is still FGF2Riiib responsive and well or moderately well differentiated and will continue to express androgen-regulated gene product proteins such as prostate-specific antigen (PSA). The loss of FGFR2iiib expression is concomitant with increased expression of FGFR1, epithelial-mesenchymal transformation (EMT), and proliferation. **D**: Androgen ablation therapy (or castration) after transformation may select for the growth of cells expressing FGFR1 and AR variants and the formation of poorly differentiated and highly proliferative cancers. The emergence of the neuroendocrine phenotype is consistent with trans-differentiation of the epithelial compartment and loss of E-cadherin. Although the cells may be androgen independent, they may still be androgen responsive and express PSA under control of a mutated AR (*) or other ligand-independent mechanism of regulation.

Moreover, our model predicts that androgen ablation following emergence of the stromal independent population would significantly impair epithelial differentiation and as discussed below provide selective pressure for the emergence of the neuroendocrine phenotype and development of an aggressive, poorly differentiated, and highly plastic androgen-independent epithelial population. It is interesting to note that in a recent report of the long-term clinical study on the impact of finasteride on prostate cancer development, patients who received 5 mg/day finasteride, an inhibitor of 5α-reductase, the enzyme that converts testosterone to the more potent dihydrotestosterone, exhibited a 24.8% decrease in prostate cancer incidence compared to the placebo control group. However, tumors in patients in the finasteride treated group exhibited a 66% increase in aggressive high-grade (Gleason 7–10) disease compared to tumors arising in the placebo group [Thompson et al., 2003]. In fact, the TRAMP model data predicted that inhibition of androgen signaling would provide a selective pressure favoring the growth of more aggressive androgen-independent cells. [Gingrich et al., 1996]. Hence, the roughly 6% of men who developed advanced disease following finasteride treatment likely harbored stochastic molecular lesions that conferred androgen independence and that depleted androgen signals, resulting from finasteride treatment, provided a selective pressure favoring outgrowth of these more malignant cells.

POLYPEPTIDE GROWTH FACTORS AND PROSTATE CANCER

How does the mesenchyme direct epithelial growth and differentiation? In part, the mesenchyme is known to produce a number of polypeptide growth factors, one of the best examples being members of the fibroblast growth factor (FGF) family. For instance, it has been demonstrated that the ligands FGF-7 (also known as keratinocyte growth factor or KGF) and FGF-10 are produced by the prostate mesenchyme and that they are capable of activating a specific FGF receptor, FGFR2iiib, localized on prostate epithelial cells [Cunha et al., 1992; Yan et al., 1992; Sugimura et al., 1996]. It is by activating the epithelial FGFR2iiib receptor that FGF-7 and FGF-10 are believed to influence epithelial proliferation, development, and function [Uematsu et al., 2001; Elghazi et al., 2002]. While it is widely held that the production and secretion of FGF-7/10 in the stromal cells of the prostate is, in part, a consequence of androgen action, and there is substantial evidence to implicate androgen signaling in the regulation of some FGF ligands, including FGF-7 [Fukabori et al., 1994; Fasciana et al., 1996; Planz et al., 1998] and FGF-9 [Goncharova, 1994], it remains to be proven that either FGF-7 [Thomson et al., 1997] or FGF-10 [Thomson and Cunha, 1999] represent direct AR targets in the mesenchyme.

During pathogenesis leading to adenocarcinoma of the prostate, survival of the epithelium requires independence from the stroma and often the androgen-signaling axis itself (Fig. 2C). Hence we postulate that specific changes in the FGF axis play a pivotal and functional role in the pathobiology of prostate cancer. Observations in both clinical prostate cancer and animal models of prostate cancer support our hypothesis. For example, loss of FGFR2iiib accompanied by a concomitant increase in FGFR1iiic has been demonstrated in malignant adenocarcinoma cells [Feng et al., 1997; Foster et al., 1999]. Furthermore, during tumor progression, the loss of FGFR2iiib is accompanied by activation of FGFR2iiic and FGFR1 that has a very high affinity for FGF-2 and can abrogate the FGF-7 signal [Yan et al., 1993; Wang et al., 2002; Huss et al., 2003]. Presumably the activation of FGFR1 provides tumor cells with stromal independence and a growth advantage. In fact, we and our collaborators are using GEM models to show how forced expression of the FGFR1 kinase domain in the epithelial compartment can accelerate spontaneous progression of prostate epithelial cells toward the malignant phenotype in vivo (Jin et al., in press).

An interesting prediction of our stromal-epithelial signaling model, wherein androgens and peptide hormones mediate growth and differentiation, is that the consequence of androgen ablation would be the loss of stromal mediated production of FGF ligands that would subsequently and negatively impact the viability and differentiation status of the epithelial compartment (Fig. 2). Hence, the primary consequence of androgen ablation in the normal (or minimally transformed) prostate gland may be a downregulation of stromal-derived FGFs that in turn lead to apoptosis of the stromal-

dependent epithelial compartment. Based in part on these observations, it is our impression that the primary role of androgen action at the level of the mesenchyme is to regulate growth factor production to support growth of the epithelial compartment, while the primary role of androgen action at the level of the epithelial compartment is to cooperate with FGF signals such as those downstream of FGFR2iiib to facilitate functional terminal differentiation and growth quiescence. It is also our contention that the consequence of androgen ablation would be the death or atrophy of epithelial cells that maintain a strict dependence on the stromal compartment while at the same time establishing a selective pressure and growth advantage for those cells that have achieved stromal independence by releasing them from androgen-induced terminal differentiation and growth senescence.

PROSTATE CANCER AND THE NEUROENDOCRINE PHENOTYPE

Since neuroendocrine cells are more abundant in prostate cancer tissue specimens than in non-malignant prostate tissue [Aprikian et al., 1993], neuroendocrine cells may function to provide stimulatory paracrine and autocrine growth factors in prostate cancer patients that have undergone androgen-ablation therapy leading to increased growth and progression of prostate cancer cells [Guate et al., 1997]. In fact, prostate cancer patients with advanced hormone-dependent and hormone-refractory disease often have increased levels of ChgA and neuron-specific enolase (NSE) in their sera and tissue specimens [Kadmon et al., 1991; Tarle and Rados, 1991; Angelsen et al., 1997]. It is thought that prostatic neuroendocrine cells can exert their biological effect in a combination of both endocrine and paracrine fashion.

Although the origin of neuroendocrine cells in prostate cancer is still debated, there is growing evidence in the literature that prostate cancer cells posses an intrinsic plasticity that allows them to either transdifferentiate or dedifferentiate and redifferentiate into cells with neuroendocrine-like properties. For example, human LNCaP epithelial cells can display neuronal-like morphology or differentiation when grown in steroid-reduced media or following treatment with interleukin-6 (IL-6) (50 ng/ml) or dibutyryl cAMP (0.1 mM) [Qiu et al., 1998; Cox et al., 1999; Zelivianski et al., 2001]. This supports our hypothesis that steroids (and/or other growth factors/peptides) are critical for maintaining epithelial differentiation of prostate cells. Furthermore, when prostate cancer cells are exposed to pharmacological agents that can increase their intracellular level of cyclic AMP (cAMP) in the presence of IL-6, they also trans-differentiate into neuroendocrine-like cells in culture [Bang et al., 1994]. The molecular basis of this phenomenon must have occurred early in evolution as we have now determined that mouse prostate cell lines (C1A, C2G, and C2H) will also trans-differentiate into neuroendocrine-like cells in culture when propagated in the absence of sera or under steroid-reduced conditions (Fig. 3).

We previously postulated that the peptide growth factor signaling axis could help malignant and normal epithelial differentiation and that disruption of the growth factor homeostasis could lead to loss of differentiation. Remarkably, treatment with heparin-binding epidermal growth factor-like growth factor (HB-EGF) has recently been found to induce neuroendocrine differentiation in LNCaP cells in a manner that required activation of cAMP and MAPK [Kim et al., 2002]. Treatment of these cells with cAMP-dependent protein kinase (PKA) induced the neuroendocrine differentiation [Chen et al., 1999]. Furthermore, treatment of LNCaP cells with HB-EGF also antagonized AR function and reduced AR expression [Adam et al., 2002] suggesting a potential conflict between some peptide growth factors and AR signaling in epithelial cells. This phenomenon is not restricted to HB-EGF as IL-6, which can act in a synergistic manner with HB-EGF-MAPK pathway, has also been shown to induce neuroendocrine differentiation [Deeble et al., 2001]. In addition, the IGF-binding protein-related protein 1 and the related neuroendocrine differentiation factor (NEDF) 25.1 were also found to be downstream of neuroendocrine differentiation effector proteins that can co-translocate to the nucleus and induce morphological and biochemical features in the prostate epithelial cancer cell line M12 [Wilson et al., 2001]. A similar role for FGFR2iiib mediated signals has been made in the pancreas where abrogation of the FGF-7 signal caused epithelial cells to differentiate into endocrine cells [Elghazi et al., 2002]. In fact, we have recently demonstrated the emergence of the neuroendocrine phenotype

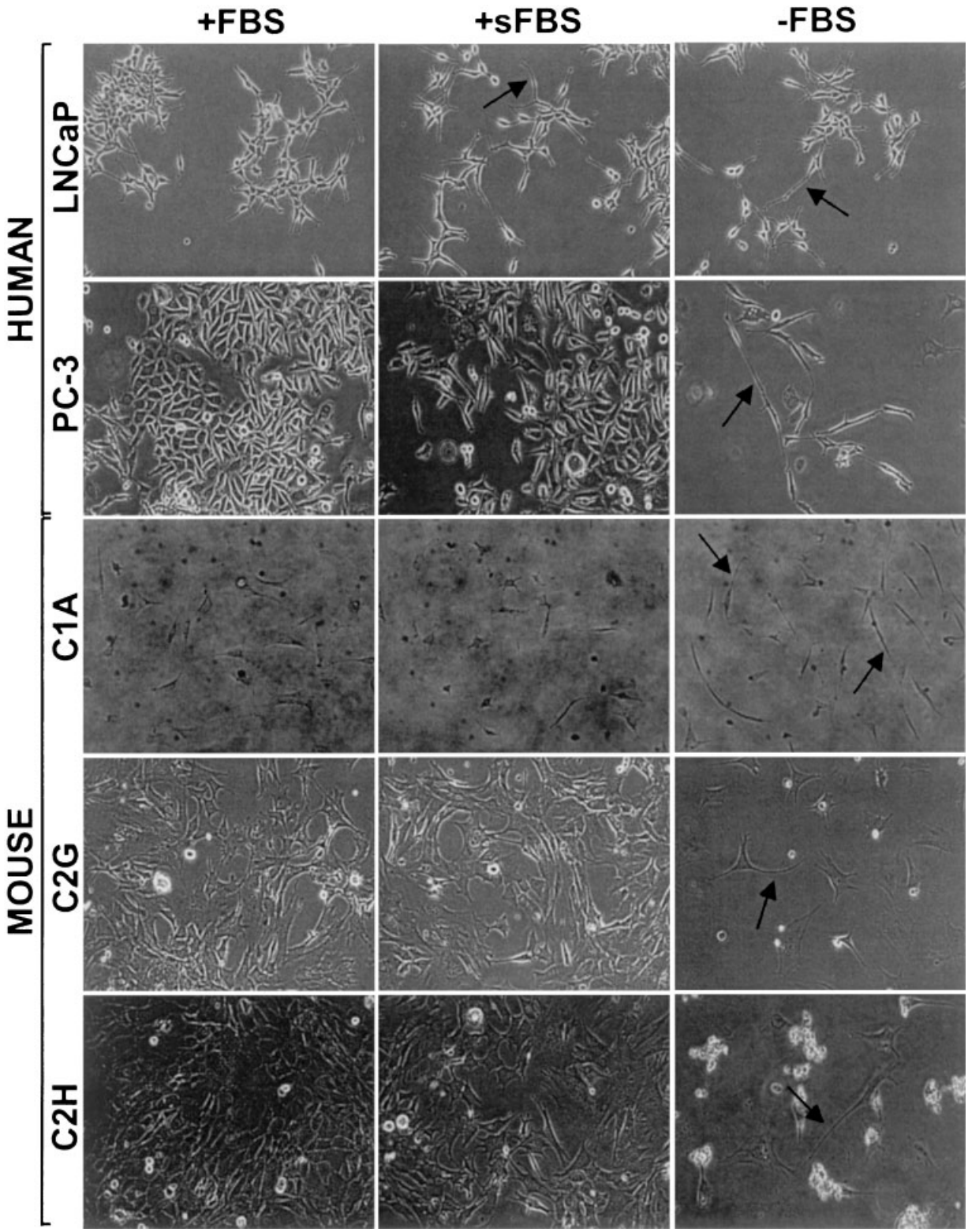

Fig. 3. Growth of human prostate cancer and mouse TRAMP cell lines in charcoal-stripped and serum-free media mediates neuronal-like morphological changes and neuroendocrine induction. Cell cultures were plated and maintained with RPMI1640 media for LNCaP and PC-3 and with DMEM for TRAMP cell lines (C1A, C2H, and C2G), and supplemented with 10% fetal bovine serum (+FBS), 10% charcoal-stripped FBS (+sFBS), or without serum (−FBS) for 72 h. Cellular morphology was inspected by phase contrast microscopy, using a Zeiss inverted microscope at 10× magnification.

as a consequence of deregulated FGF signaling in a genetically engineered transgenic mouse model [Foster et al., 2002]. In these studies, elevated numbers of synaptophysin (SynP) expressing cells were observed in the prostate glands of KDNR mice in which enforced epithelial expression of a dominant negative FGFR2iiib construct blocks the endogenous stromal mediated FGF-7 and FGF-10 signals and likely favors the growth promoting signals of FGFR2iiic or FGFR1. We have also observed the expression of SynP in primary and metastatic prostate cancer tissue specimens from TRAMP mice (Fig. 4). Clearly, the emergence of a neuroendocrine phenotype as a consequence of deregulated growth factor signaling is a hallmark of advancing prostate cancer and therefore should also be considered a favorable attribute of prostate cancer model systems.

Despite the many parallels between TRAMP and clinical prostate cancer, there has been a lot of discussion and misconceptions concerning neuroendocrine cancer and the TRAMP model [Abate-Shen and Shen, 2002; Ellwood-Yen et al., 2003]. Given that neuroendocrine carcinoma is a frequent constituent of advanced human prostate cancer [Abrahamsson, 1999a,b; Hansson and Abrahamsson, 2001], the recent study by Kaplan-Lefko et al. [2003] addressed the emergence of the neuroendocrine phenotype in the TRAMP model [Kaplan-Lefko et al., 2003]. Histologically, the most advanced and poorly differentiated tumors in the TRAMP model display neuroendocrine features that can include a very high nuclear to cytoplasmic ratio, stippled chromatin, and irregular dendrite-like processes extending underneath and between adjacent epithelial cells (Fig. 1). Interestingly, when we performed immunostaining on sections representing progressive stages of prostate cancer in TRAMP with an antibody against SynP, a marker of neuroendocrine differentiation, SynP was only detected in four small foci within a total of 162 PIN lesions (2.5%) and was not detected in any of the

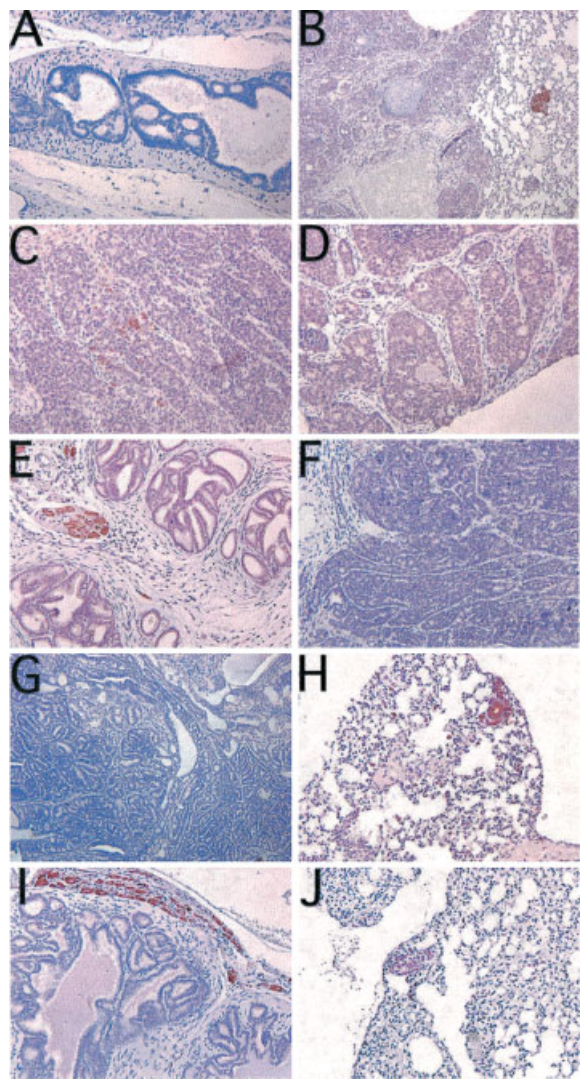

Fig. 4. Expression of synaptophysin in primary and metastatic prostate carcinomas from TRAMP. We used immunohistochemistry with an anti-synaptophysin antibody (the binding site PH510; 1/200 dilution) to analyze tissue sections procured at necropsy from TRAMP mice. All sections were visualized with ABC detection kit (Vector Labs). **Panels A–D**: Animal 1228. A: Well-differentiated primary tumor characterized by well-formed glands and desmoplastic stroma. No evidence of moderately or poorly differentiated carcinoma was identified and staining for synaptophysin (SynP) was negative. B: A large moderately differentiated lung metastasis that does not express SynP is present on the left. There is also a small, poorly differentiated metastasis that expresses SynP (right). C: Moderately to poorly differentiated liver metastasis with focal expression of SynP (center). D: Moderately differentiated liver metastasis without SynP expression. **Panels E, F**: Animal 885. E: Well-differentiated primary tumor. No evidence of moderately or poorly differentiated carcinoma was identified and stains for SynP were negative. Note the positively staining ganglia on the left that serve as an internal positive control. F: Moderately differentiated liver metastasis. No SynP expression was identified. **Panels G, H**: Animal 1113. G: Well-differentiated primary tumor. No evidence of moderately or poorly differentiated carcinoma was identified and stains for SynP were negative. H: Lung metastasis expressing SynP. A glandular structure is present in the metastasis. **Panels I, J**: Animal 1057. I: Well-differentiated primary tumor. No evidence of moderately or poorly differentiated carcinoma was identified and stains for SynP were negative. Note the positively staining ganglion along the upper portion of tissue. J: Lung metastasis and stains for SynP were negative. Original magnification: 200×.

well-differentiated (WD) or phylloides-like lesions (0/45 WD and 0/17 phylloides-like). Consistent with emergence of the neuroendocrine phenotype as a stochastic event related to progression, SynP expression was detected in 24 of 26 poorly differentiated (PD) regions (92%) and in 100% (13/13) of the PD tumors arising in castrated mice. Most interesting was our finding that only 14 of 23 (61%) lymph node metastases expressed SynP, consistent with our previous observations that metastogenesis in the TRAMP model occurs stochastically and is not necessarily dependent on primary tumor progression to poorly differentiated disease [Gingrich et al., 1996]. Clearly, emergence of the neuroendocrine phenotype also seems to be a stochastic event related to the progression of prostate cancer in TRAMP that is correlated with loss of differentiation, glandular architecture, and hormonal response, features remarkably similar to those observed in clinical disease [Abrahamsson, 1999b]. It should also be noted that cells of true neuroendocrine origin should not express AR, and 16 of 29 (55%) TRAMP tumors were found to express both SynP and AR. Furthermore, of the PD tumors in castrated mice, 9 of 13 (70%) expressed both SynP and AR while only 4 of 13 (30%) expressed SynP without AR. Given that the PD tumors were not uniformly SynP positive or AR negative, it is unlikely that these tumors could have arisen from a neuroendocrine precursor.

To further distinguish the emergence of the neuroendocrine phenotype in TRAMP from NE carcinoma, we have now used in silico analysis to compare gene expression profiles in samples representing progressive stages of prostate cancer in TRAMP and samples of neuroendocrine carcinoma in the CR2-Tag mice. As shown in Figure 5, we noted clear differences between the expression profiles of TRAMP and CR2-Tag samples. Most notably, expression of neuroendocrine markers increased as a function of disease progression in TRAMP, but these markers were uniformly and highly expressed in the primary CR2-Tag lesions, underscoring the stochastic nature of the TRAMP model and supporting the hypothesis that these adenocarcinomas display a certain intrinsic plasticity that allows them to phenocopy neuroendocrine cells and display neuroendocrine features. It is therefore our conclusion from these studies that the neuroendocrine phenotype in TRAMP emerges as a consequence of an "epithelial to neuroendocrine" transition (or switch) as a function of cancer progression.

WORKING MODEL FOR PROSTATE CANCER PROGRESSION

In an attempt to explain the paradoxical consequences of hormone ablation and the emergence of the neuroendocrine phenotype, a number of groups have begun to look at the role

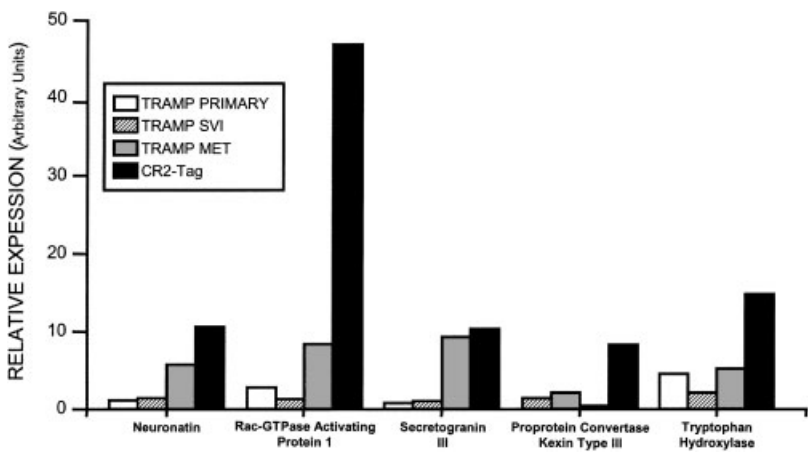

Fig. 5. Neuroendocrine progression in TRAMP and CR2-Tag mouse models. Tumor samples from TRAMP and CR2-Tag mice were harvested immediately following euthanasia and subjected to expression array profiling. Samples were processed for RNA and total RNA was isolated using RNeasy (Qiagen) mini or midi-sized column, according to manufacturer's procedures. Final RNA concentrations and quality were determined by A260:A280 absorption readings and by Agilent Lab Chip technique using a Bioanalyzer 2100. A progressive increase in neuroendocrine marker expression across stage/invasiveness of TRAMP tumors is observed, but not to degree seen in the CR2-Tag model. Elevated expression levels (greater than twofold) were detected in early TRAMP primary tumors (dorsal prostate), in seminal vesicle invasive extensions, and in lymph node metastasis. SVI, seminal vesicles invasive; MET, metastasis.

of polypeptide growth factors in ligand independent activation of AR signaling [Culig et al., 1993, 1994] in addition to AR gene amplification [Koivisto et al., 1997]; Bubendorf et al., 1999 and AR gene mutation [Tilley et al., 1996; Buchanan et al., 2001a,b; Han et al., 2001]. In particular, our lab has focused on a central hypothesis that deregulation of FGF signaling can cooperate with deregulated androgen signaling to facilitate transformation, angiogenesis, dedifferentiation, metastasis, and the emergence of hormone refractory and neuroendocrine phenotypes associated with androgen-independent prostate cancer.

Androgen-refractory prostate cancer is associated with neuroendocrine differentiation and it has been suggested that detection of neuroendocrine specific markers such as ChgA, NSE, 5-HT, and α subunit of glycoprotein hormones (α-SU), in serum detect the level of neuroendocrine differentiation. Nobels et al. [1997] have already shown that serum ChgA levels can be specifically used to detect neuroendocrine neoplasias [Nobels et al., 1997]. While Sciarra et al., 2003 suggested that serum ChgA levels be monitored to determine how to modulate treatment in patients undergoing androgen ablation therapy [Sciarra et al., 2003].

Neuroendocrine differentiation in the prostate occurs during prostate cancer progression as a result of selective pressures including but not limited to androgen ablation. Given that the role of androgen action on the normal epithelial cell seems to be more consistent with terminal differentiation and growth suppression, it is not surprising that androgen ablation facilitates neuroendocrine differentiation and creates a favorable growth condition for prostate cancer. Most recently, we have demonstrated how expression of the dominant negative FGFR2iiib specifically in prostate epithelial cells induced neuroendocrine differentiation in vivo [Foster et al., 2002]. These observations suggest that neuroendocrine differentiation in advanced prostate cancer is likely the result of a loss of homeostatic balanced communication between the epithelial and mesenchymal compartments.

Since true neuroendocrine cells do not express AR [Wright et al., 2003], it is therefore not surprising that castration has little or no effect on the progression of true neuroendocrine cancers such as occurring in the CR2-Tag model [Hu et al., 2002]. Hence, it should also be of little surprise that androgen ablation should facilitate the progression of prostate cancer given that emergence of the neuroendocrine phenotype is a direct consequence of androgen ablation [Kaplan-Lefko et al., 2003]. This provides a reasonable explanation to the failure of hormonal ablation to control prostate cancer that has advanced to the stromal independent stage.

PERSPECTIVE

Recently, Laufer et al. reported that current methods for treating advanced prostate cancer offer little evidence to support the use of hormonal ablation therapy, that is, the routine use of anti-androgens in combination with medical/surgical castration as an effective treatment for advanced prostate cancer [Laufer et al., 2000]. In this study, they discuss how endocrine control of prostate tumor growth is not always mediated by direct activation of AR, and that alternative signaling pathways exist. In fact, we now appreciate that there are several androgen-independent mechanisms that play major roles in prostate cancer progression including the activation of genes directly involved in cell proliferation, loss of apoptotic signals, stimulation of tumor angiogenesis, regulation of tumor invasion and metastasis by extracellular matrix proteins, and the loss of expression and function of AR [Feldman and Feldman, 2001]. Moreover, we have seen that androgen-independent growth signaling pathways can be active during the early stages of prostate cancer and that AR deletions, point mutations, amplifications, and polymorphisms are involved in the loss of specificity, increased sensitivity, and the complete loss of androgen-dependent activation [Bonkhoff et al., 1993; Culig et al., 1993; Tilley et al., 1996; Berthon et al., 1997; Koivisto et al., 1997; Sweat et al., 1999; Han et al., 2001; Buchanan et al., 2001a; Arnold and Isaacs, 2002].

In the normal prostate epithelium, prostate epithelial cells are dependent on androgen and AR for epithelial differentiation, G_0 growth arrest or survival, apoptosis, and prostatic secretions (e.g., PSA, FGF9, and FGF2). As discussed above, androgens also act to stimulate epithelial cell proliferation by acting on AR-positive mesenchymal cells of the prostate stromal compartment to stimulate production and secretion of growth factors (e.g., FGF7, FGF10) required

for normal growth and maintenance of the prostate epithelium. It is therefore conceivable that, following some transforming event, a primary tumor might be initiated in the prostate epithelium that would be stroma-dependent, androgen-dependent, and AR-dependent that we would define as "abnormal stage I." At this stage, the presence of testosterone would be expected to both stimulate growth as well as survival of a well-differentiated epithelium while androgen ablation therapy would result in glandular atrophy and tumor regression. Indeed, most patients that respond durably to hormone therapy likely presented with this kind of cancer, and it could be argued that these are the patients that may have never progressed to advanced disease.

With further acquisition of genetic lesions, possibly as a consequence of genomic instability and loss of Rb and p53 tumor suppressor pathways, tumor progression to "abnormal stage II" would be characterized by stromal-independent epithelium. In these tumors, the epithelial cells would be expected to elaborate their own growth factors or express alternative receptors so they would no longer require or respond to stromal-derived factors such as FGF7 or FGF10. In fact, we have observed very similar events using the TRAMP model system [Foster et al., 1998, 1999, 2002]. A major feature of abnormal stage II disease would be that the epithelial cells would still require androgen signals for differentiation. At this stage, it would be expected that a patient would demonstrate loss of epithelial differentiation after hormonal therapy (as measured by serum PSA levels for example) but would ultimately progress to develop high-grade hormone refractory disease owing to the loss of androgen-induced differentiation signals. Indeed, patients who developed high-grade (Gleason 7–10) disease following finasteride treatment probably harbored molecular lesions such that they could be classified as abnormal stage II.

Once cells have lost both stromal-dependence and the ability to respond to androgens, they would be classified as "abnormal stage III." In this case the prostate cancer might express mutated forms of the AR that could still direct expression of differentiation markers such as PSA, but in a manner independent of the cognate steroid ligand. Indeed, rise in serum PSA following complete androgen blockade is a hallmark of progressive disease and mutations in the AR that can no longer discriminate between agonist and antagonist have been identified [Feldman and Feldman, 2001]. Based on our previous discussions, it would be expected that prolonged treatment with hormone ablation therapy would select for poorly differentiated prostate cancers and emergence of the neuroendocrine-like phenotype. Our data with the TRAMP model also predict that these advanced hormone refractory tumors should be more metastatic [Gingrich et al., 1996]. Hence it should be very interesting to determine the neuroendocrine and metastatic properties of the patients that developed high-grade cancer following finasteride treatment.

Lastly, we recognize that the prostate gland is a very complex microenvironment from both a cellular and molecular perspective. Structurally the gland is composed of a number of distinct compartments that themselves comprised a diverse set of distinct cellular populations. At the cellular level, basal, neuroendocrine, and glandular/secretory cells comprise the epithelial components, while smooth muscle cells, fibroblasts, tissue macrophages, and others occupy the mesenchymal compartment. In addition, the requisite capacity of the gland to respond to exogenous and endogenous endocrine signals of both steroid and peptide hormones is the consequence of a complex set of rules that are only now being dissected at the molecular level. Clearly, the ability to study the prostate in a suitably complex model that can be manipulated at the genetic level should greatly facilitate our ability to understand the biology of the prostate gland in great detail and at greater resolution. To this end, application of genetically engineered mouse models holds great promise and has already afforded us significant insights into the natural history of spontaneous and autochthonous disease. Most importantly, these studies are beginning to shed new light on long-standing problems and the enigma surrounding hormone refractory prostate cancer. We can now, perhaps for the first time, appreciate how differentiated epithelial cells might be able to transform themselves into cells with neuroendocrine features, how the microenvironment of cancer can exert such strong influence on cell determination, differentiation and proliferation, and why androgen ablation may cure early prostate cancer but also facilitate emergence of more aggressive and metastatic disease.

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