Journal of Cellular Biochemistry Supplements 28/29:174–181 (1997)

Development of Human Prostate Cancer Models for Chemoprevention and Experimental Therapeutics Studies

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Abstract The progression of human prostate cancer from histomorphologic to clinical expression often requires several decades. This study emphasizes the importance of developing relevant human prostate cancer models to study the molecular events leading to prostate cancer progression. These models will provide a rational basis for chemopreventive and treatment strategies to retard the progression of human prostate cancer from its localized to its metastatic state. In our laboratory, we have established the LNCaP progression and ARCaP models and the in vitro three-dimensional growth models involving prostate cancer and bone stroma to study the progression of prostate cancer. We propose that prostate cancer may progress from an androgen-dependent to an androgen-independent state. While existing as androgen-independent tumors (defined as tumors capable of growing in castrated hosts and secreting PSA in serum), prostate cancer may assume three different phenotypes as it progresses: androgen-independent while remaining androgen-responsive; androgen-independent and unresponsive to androgen stimulation; and androgen-independent but suppressed by androgen. It is conceivable that any androgen-independent human prostate cancer may contain variable proportions of cells that exhibit these three phenotypes. This concept may have important implications in determining strategies for chemopreventive and therapeutic trials. We have established three-dimensional growth models of prostate cancer cells either in collagen gel or microgravity-simulated growth conditions to form viable and functional organoids which contain prostate cancer epithelial cells admixed with prostate or bone stromal cells. These in vitro models combined with the in vivo models described above will enhance our understanding of the regulatory mechanism of prostate cancer growth and progression, and hence could improve efficiency in screening chemopreventive and therapeutic agents which alter the biologic behaviors of human prostate cancer. J. Cell. Biochem. Suppls. 28/29:174–181.

Key words: prostate cancer progression; stromal-epithelial interaction; three-dimensional growth models; prostate cancer bone metastasis

In 1996, over 8 million American men were estimated to harbor prostate cancer cells at the microscopic level [1]. This high prevalence of prostate cancer development, and the similarity of latent prostate cancer development around the world irrespective of its mortality rate, emphasizes the importance of understanding prostate cancer *progression*, rather than initiation, as a key determinant that could make a difference in reducing the death rate in men with progressive diseases in the western countries. For this reason, it is of great value to establish models to study the biology of human prostate cancer and to design rational preventive and treatment strategies to target prostate cancer progression. A number of experimental models are available for assessing the molecular mechanisms associated with prostate cancer development and progression. These include hormonal- or carcinogen-induced rodent models of prostate cancer [2,3], spontaneously-derived rodent prostate cancer models [4], oncogene overexpression-induced prostate cancer [5], and transgenic animals harboring large T-antigen-induced prostate cancer [6]. Moreover, a number of transplantable human prostate cancers

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Received 27 January 1997; accepted 6 November 1997

[7] and established human prostate cancer cell lines [8] have been extremely valuable models to study prostatic carcinogenesis.

Our laboratory has developed three model systems to study the various stages of human prostate cancer progression. In a LNCaP human prostate cancer model, androgen-independent progression is induced in androgen-dependent LNCaP cells through in vivo cellular interaction with a non-tumorigenic human osteogenic sarcoma cell line [9,10]. To complement the LNCaP model, we have recently developed another highly metastatic androgen-repressed human prostate cancer cell line, ARCaP, to delineate underlying steps of prostate cancer progression and the possible molecular basis of androgen and estrogen receptor mechanisms that may contribute to disease progression [11]. Finally since LNCaP and ARCaP models can be studied both in vitro and in vivo, we established three-dimensional cell culture conditions to assess how hormones, growth factors, extracellular matrices, and stromal cells affect both the expression of PSA and the growth of prostatic epithelial cells in vitro. Results of our studies yield some interesting information, further defining the requirements of prostatic epithelial cell growth in vitro and tumors in vivo, which could prove to be valuable in designing future strategies for chemoprevention and therapeutic intervention of prostate cancer progression.

LNCaP MODEL: ROLE OF STROMAL CELLS

LNCaP, an androgen-dependent human prostate cancer cell line, was established from a lymph node specimen obtained from a patient with metastatic prostate cancer [12]. This cell line was shown to respond to androgen-induced growth and PSA expression both in vivo and in vitro [13]. LNCaP cells were shown to be tumorigenic initially [12,13], but the cell line we obtained from Dr. Gary Miller (University of Colorado Health Sciences Center at Denver, Colorado) proved non-tumorigenic [14]. We found that co-inoculation of LNCaP cells with either prostate or bone stromal cells in intact male (but not in intact female) mice induced both the growth and secretion of PSA by chimeric tumors in vivo [14,15]. The chimeric LNCaP tumors formed in vivo responded to testicular androgen, since castration resulted in reduced tumor growth and marked reduction of serum PSA [16]. Within several weeks, however, both growth and PSA expression by chimeric LNCaP tumors maintained in the castrated hosts rebounded [16].

From the chimeric tumors maintained in castrated hosts, we derived an array of LNCaP sublines denoted as C4 and C5, and from the C4 subline we subsequently derived additional C4-2 and C4-2 B_2, B_3, B_4, and B_5 sublines [10,17]. These LNCaP sublines share cell-lineage relationship with the parental LNCaP cell line, based upon both cytogenetic [9,10] and comparative genomic hybridization [17] data. The cell lines differ markedly in their ability to grow in either intact or castrated hosts, to metastasize to distant organs, and to produce PSA intrinsically. For example, unlike the parental LNCaP cell line, the C4-2 cell line can form tumors alone in castrated hosts, without supporting stroma. A number of the LNCaP sublines, C4, C4-2, C4-2 B_2, B_3, B_4, and B_5, also secrete high intrinsic PSA and are capable of metastasizing to the skeleton (Tony T. Wu et al. unpublished observations). Table I summarizes the behavioral and biochemical characteristics of the parental LNCaP cell line and its sublines derived from the chimeric tumors maintained in either intact or castrated hosts. In this progression model, as prostate cancer cells acquire androgen independence, they become more invasive and secrete PSA in an androgen-independent manner.

There are three important lessons in this type of approach. First, the biochemical and behavioral characteristics of LNCaP cells can be modified *irreversibly* through cellular interaction with organ-specific stroma. A non-tumorigenic and androgen-responsive LNCaP cell line can be "induced" by cellular interaction with a bone stromal cell line to acquire androgen-independent, androgen-unresponsive, and osseous metastatic potential. We believe that this interaction is a form of "induction" because the parental LNCaP cells have been cultured for numerous passages by a large number of laboratories but have never acquired the ability to grow in castrated hosts. Moreover, during the inductive phase of cellular interaction between LNCaP and bone stromal cells in vivo, we did not observe programmed cell death following castration, although dramatic reduction of PSA and slow-down of tumor growth rate were observed [9,10,14,16]. The derivative LNCaP cell sublines were cytogenetically defined and

TABLE I. Androgen Sensitivity and Tumorgenic and Metastatic Potentials of the LNCaP Cell Line and LNCaP Sublines Derived From Cellular Interaction Between the Parental LNCaP Cells and a Nontumorigenic Human Osteogenic Sarcoma Cell Line, MS*

	Androgen sensitivity[a]	PSA production[b]	Tumorigenicity		Metastatic potential		
			Male	Castrated male	Lymph node	Bone	Other organs
LNCaP	+	+	−[c]	−[d]	−	−	−
C4	+	++	+	−	−	−	−
C5	+	++	+	−	ND	ND	ND
C4-2	−	++	+	+	+	+	+

*Chimeric tumors were grown subcutaneously. All data were published by Wu et al [9] and Thalmann et al [10]. ND = not determined.

[a]Androgen sensitivity is defined by the responsiveness (growth and PSA expression) of the respective cell lines in vitro to DHT or R1881.

[b]PSA production is defined by the basal rate of synthesis and secretion of PSA by the parental LNCaP and its sublines.

[c]LNCaP when inoculated subcutaneously are non-tumorigenic [14]. However, LNCaP cells when inoculated orthotopically are tumorigenic and metastasized occasionally to the lymph nodes [15].

[d]Although C4 and C5 do not form tumors in castrated hosts, C4 and C5 do form tumors in castrated hosts when co-inoculated with MS [9,10]; parental LNCaP cells, however, failed to form tumors even when co-inoculated with MS [10,14].

closely mimicked the behavioral and biochemical characteristics of in vivo human tumors. Second, genetic changes as assessed by comparative genomic hybridization were observed in the LNCaP sublines when compared to the parental LNCaP cell line [17]. This step-wise alteration of genetic elements within the LNCaP sublines strongly suggests that genetic changes may be "induced" secondarily by cellular interaction between prostate cancer cells and stromal cells. If this interpretation is correct, it is conceivable that genetic changes are not random events and can be dictated by the epigenetic microenvironment such as stroma and extracellular matrix surrounding the tumor epithelia. Third, cellular interaction between LNCaP and its sublines with bone or host stromal cells is reciprocal. While the growth of LNCaP and its sublines in vivo is accelerated by cellular interaction with bone stromal cells and unidentified host cell infiltrates, these prostatic cancer cells, when grown in the bone, reciprocally induce the growth of osteoblasts. Marked osteoblastic reactions were observed when metastatic LNCaP sublines were grown in the skeleton [10] (also Tony T. Wu et al., unpublished observations). In a recent study, we have shown that stromal cells grown together with human prostate epithelium underwent consistent genetic amplification of chromosome 15 and deletion of chromosome Y. We suggest that prostate cancer cells are not only capable of inducing growth in a reciprocal manner with bone stromal cells, but also can induce genetic changes in their surrounding host stromal cells (confirmed by cytogenetic analysis) [18].

ARCaP MODEL: AN ANDROGEN-REPRESSED FORM OF ADVANCED HUMAN PROSTATE CANCER

ARCaP cells were obtained from the ascites fluid of a man with advanced prostate cancer. Because the patient had widely disseminated disease, ARCaP cells represent an advanced form of human prostate cancer. Unlike the androgen-dependent LNCaP cells and androgen-independent C4-2 cell lines, the growth of ARCaP cells both in vivo and in vitro was repressed by both androgen and estrogen [11]. ARCaP cells expressed androgen receptor, and the expression of PSA was suppressed by R1881, a synthetic androgen agonist. When administered orthotopically, ARCaP cells metastasized to the kidney, lung, pancreas, and bone, and induced paraplegia in the host animal. Based upon immunohistochemical analysis, we found that ARCaP cells overexpressed growth factor receptors (EGFR, c-*erb* B2/*neu*, c-*erb* B3, c-*erb* B4, and c-*met*), several neuroendocrine factors (serotonin, bombesin, neuron-specific enolase [NSE], substance P, and neurophysin), and low levels of androgen receptor and PSA (Table II). These unique characteristics of ARCaP cells in vivo and in vitro closely mimicked the clinical behaviors of some prostate cancer patients with advanced diseases.

In addition to responding negatively to androgen, ARCaP tumor growth in vivo and cell

TABLE II. Comparative Immunohistochemical Expressions of PSA, AR, EGFT Gene Family, c-*met*, HGF/SF, and Neuroendocrine Factors by LNCaP and ARCaP Cells*

Markers	LNCaP	ARCaP
PSA	++++	+
AR	++++	+
EGFR	++	++++
c-*met*	+++	++
HGF/SF	++	++
c-*erb B2*	++	++++
c-*erb B3*	++	++++
PSA	+++	+
c-*erb B4*	++	+++
Serotonin	++	+
Bombesin	+	++
NSE	++	++
Chromogranin A	++	−
Substance P	++	+
Neurophysin	+++	+
Androgen receptor	+++	+

*Relative level of expression: −, not detectable; +, low; ++, moderate; +++, high; ++++, extremely high.

growth in vitro were also repressed by estrogen [11]. This unique observation led us to examine the possibility that androgen and estrogen receptor mechanisms may be fundamentally altered in ARCaP cells. We have sequenced the entire androgen receptor cDNA obtained from ARCaP cells. We found that unlike LNCaP cells, both the DNA- and hormone-binding domains of ARCaP cells were intact [11], suggesting that other mechanisms, such as interactions of androgen receptor with cell type-specific co-factors, may account for ligand-activated repression of growth and gene expression by ARCaP cells. Likewise, we have obtained evidence to suggest that similar repression by estrogen in ARCaP cells was also mediated by repressive interaction between estrogen receptor and cell type-specific cognate factors that may account for ligand-activated repression of ARCaP cell growth and gene expression in intact female hosts (Zhang et al. unpublished observations).

THREE-DIMENSIONAL GROWTH MODELS OF PROSTATE CANCER

Recognizing the importance of stromal-epithelial interactions in prostate cancer growth, hormone responsiveness, and potential roles of stromal cells in inducing cancer progression, we explored models for studying cell-cell interaction in vitro. One of the difficulties of studying stromal-epithelial interactions in vitro is that the differential growth of stromal cells relative to that of the epithelial cells inevitably results in stromal cell dominance during subculture. Moreover, in spite of co-culture of stromal and epithelial cells in vitro in separate chambers, androgen elicited only marginal growth stimulatory responses in the epithelial cells [19], suggesting the importance of cell-cell contact as a critical determinant in androgen action on the prostate gland. Because of these potential problems facing model development in vitro, we decided to explore both a collagen gel system and a microgravity-stimulated growth model system (established by the National Aerospace Administration [NASA]) to study stromal-epithelial interactions and the mechanisms underlying androgen and estrogen inductive action.

Collagen-1 gels were used to grow both LNCaP cells and several interactive fibroblasts derived from either prostate (Pf), bone (MS, MG-63, or Saos-2), or lung (CCD16). LNCaP cells failed to survive in collagen-1 gel alone. In the presence of prostate, bone, or lung fibroblasts, however, LNCaP cells grew as an organoid and began to synthesize and secrete PSA (Fig. 1). We observed that not all bone fibroblasts support LNCaP cell growth equally (e.g., MG-63 is less effective than MS and Saos-2). Our results suggest that LNCaP cell survival in collagen gel may be ultimately dependent upon neighboring supporting stroma with some cell specificity. If this is the case, one may speculate that metastasis of prostate cancer in vivo may be the result of survival of prostate cancer cells under the influence of organ-specific stroma. Although it was demonstrated that only prostate and bone stromal cells can support the growth of LNCaP tumors in vivo [14], the collagen-1 gel model suggests that, in addition to prostate and bone stromal cells, lung fibroblasts can also support the growth of LNCaP cells in vitro (Fig. 1). This is consistent with the fact that prostate cancer not only metastasized to the bone, but occasionally to the lung also. It should be noted, however, that the collagen-1 gel data is not equivalent to in vivo tumor growth. While in this in vitro growth condition, prostate, bone, or lung stromal cells may have already undergone extensive phenotypic and genotypic changes in vitro. This is in contrast to

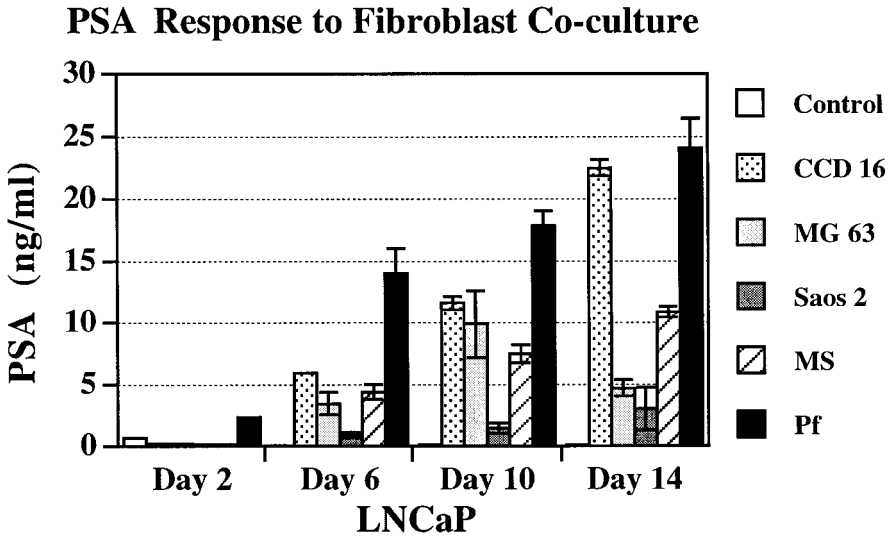

Fig. 1. PSA production by LNCaP cells in culture is affected by co-culture with organ-specific stromal cells. Note in the absence of stromal cells, LNCaP cells cultured in Collagen-1 gel produced and secreted minimal amounts of PSA.

in vivo conditions where prostate tumor growth presumably occurs with *normal* host stromal cells.

We have also adopted a microgravity-simulated growth model recently established by NASA to study stromal-epithelial interaction in three-dimensional culture. In this model, LNCaP cells quickly form large three-dimensional organoids in culture with either prostate or bone fibroblasts. Figure 2 shows histomorphologic organizations of LNCaP cells when cultured in the absence (A) or presence (B) of bone stromal cells. Note that at the histomorphologic level, LNCaP cells, when co-cultured with bone stromal cells, exhibited much more cellularity than LNCaP cells alone. These results are supported by the gross morphologic variations of organoid sizes, which varied from a few millimeters (e.g., LNCaP microgravity culture alone) to centimeters (e.g., co-culture of LNCaP with MS under microgravity-simulated conditions) in size (results not shown). These organoids apparently behaved like a prostate tumor, which synthesizes and secretes PSA efficiently under androgen regulation [20]. We observed that in the presence of prostate or bone stromal cells, such organoids seem to have excellent growth potential by expanding tumor size as well as serum PSA in vitro. It is of interest to note that when LNCaP cells interacted with selected bone stromal cells in culture, serum PSA in the medium appeared to drop precipitously as tumor size enlarged (data not shown). This down-regulation of PSA expression may be the consequence of an osteoblastic reaction induced by prostate cancer epithelium and the reciprocal interaction between bone and prostate cancer cells to induce a proliferative response of LNCaP cells at the expense of their PSA expression. In contrast, prostatic stromal cells continued to support growth as well as PSA secretion by LNCaP cells under microgravity-simulated conditions (data not shown). Overall, this microgravity-simulated condition may allow us to study the molecular mechanism underlying stromal-epithelial interaction in vitro. This will enhance our understanding of epithelial tumorigenesis, including the events associated with epigenetic and genetic mechanisms of carcinogenesis, and the acquisition of androgen independence and metastatic potential by the epithelial cells, which are under the constant influence of their surrounding stromal cells, extracellular matrices, growth factors, and hormonal milieu.

SUMMARY AND FUTURE DIRECTIONS

The progression of prostate cancer from androgen dependence to androgen independence has been further evaluated. Using LNCaP and ARCaP as model systems, we have determined that prostate cancer progresses from androgen dependence to androgen independence through the interaction of prostate cancer cells with organ-specific stroma. Both prostate and bone stromal cells have been observed to induce the ability of LNCaP cells to acquire permanent

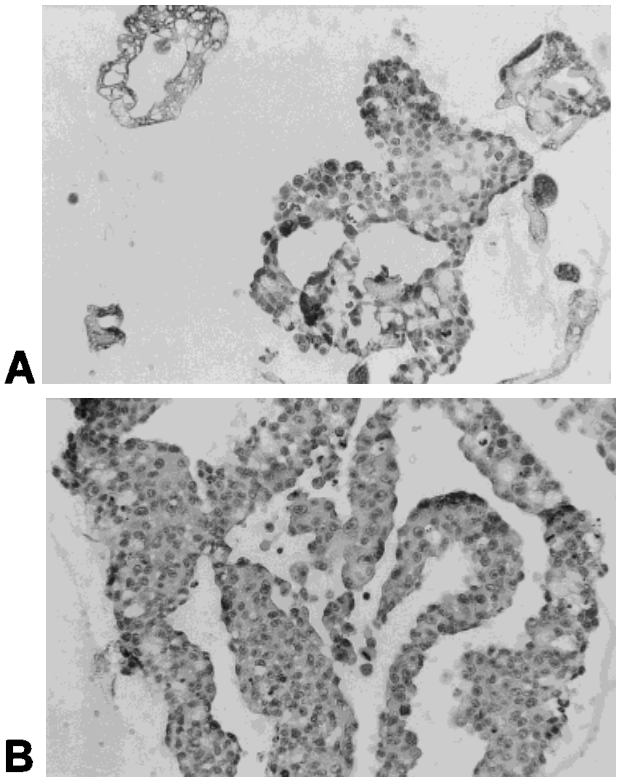

Fig. 2. Histomorphology of LNCaP cells when cultured either alone (**A**) or with bone fibroblasts (**B**) under three-dimensional microgravity-simulated conditions. Note co-culture with bone stromal cells resulted in much larger organoids with more dense cell populations detected.

and androgen independence and metastatic potential, particularly in castrated hosts. Using ARCaP as a model, we have extended these observations to suggest that prostate cancer progression from the androgen-dependent to the androgen-independent state can be further categorized into three phases: androgen-independent but androgen-responsive; androgen-independent and androgen-unresponsive; and androgen-independent but androgen-repressed. It is conceivable that all prostate cancer specimens contain a mixture of cell types, from androgen-dependent to all three androgen-independent phenotypes in various proportions. Depending upon the hormonal status of the host and the interactive stroma, prostate cancer cells can conceivably progress from androgen dependence to independence through a sequence of steps depicted in Figure 3. Initially, prostate cancer is considered to be androgen-dependent and highly responsive to androgen-stimulated growth and PSA expression. As the disease progresses, prostate cancer cells become androgen-independent and proliferate well in castrated hosts. The tumor cells are capable of synthesizing and secreting abundant PSA in the absence of circulating androgen, possibly through ligand-independent activation. As the tumor cells progress further, the growth of prostate cancer cells and their PSA expression (albeit low) are suppressed by androgen or estrogen; the androgen-independent or -repressed phenotypes are often associated with increased invasive and metastatic potential of the tumor cells.

To understand the molecular mechanism(s) of stromal-epithelial interactions, we have established three-dimensional growth models that include collagen-1 gel and microgravity-simulated conditions to study stromal-epithelial interactions in vitro. These models have yielded interesting insights into cell-cell communication, which cannot be adequately studied and defined in either single cell suspension culture or two-dimensional growth conditions on plastic dishes. Future directions in the understanding of stromal-epithelial interactions in tumor biology require the development of basic knowledge in cell biology and the integration of cell signaling systems in relevant models. Some of the following questions may be addressed:

1. How does stroma regulate the progression of epithelial carcinogenesis?

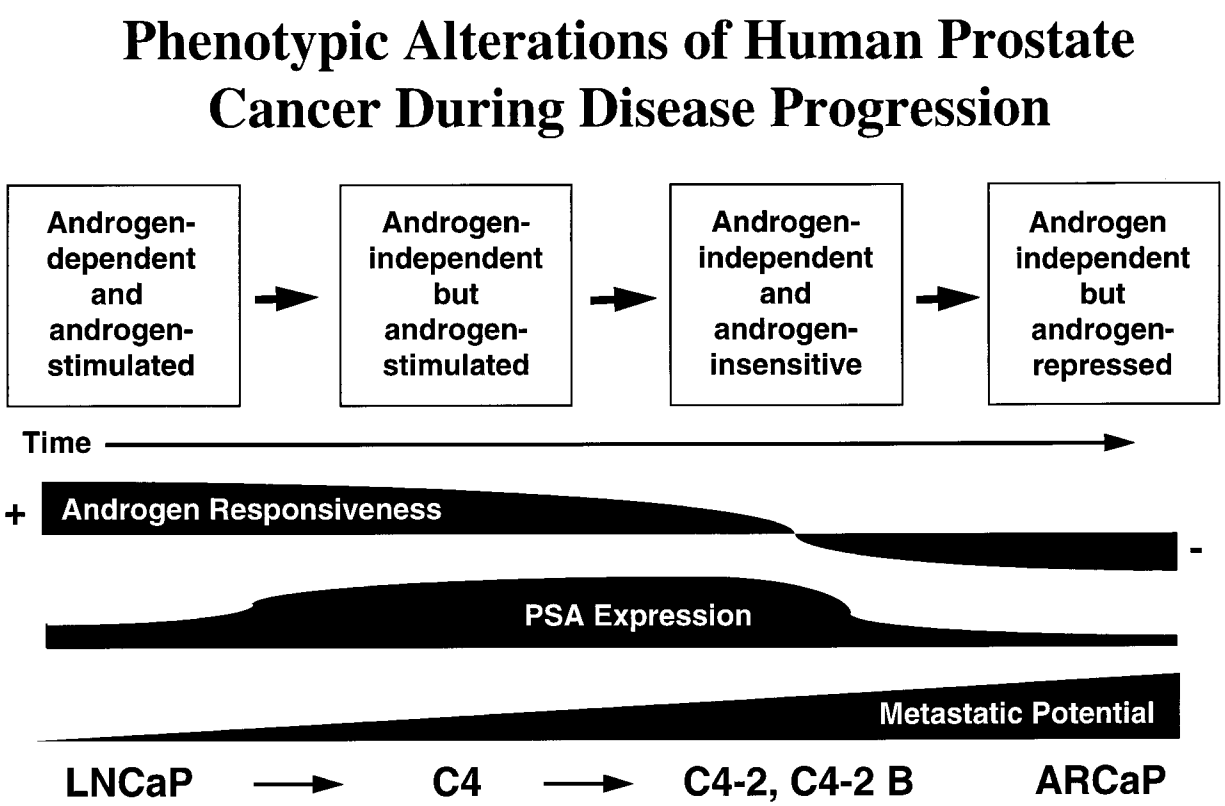

Fig. 3. A cellular model of human prostate cancer progression. We have observed that androgen-independent and metastatic LNCaP cells can be derived from its parental cells through stromal-epithelial interaction [9,10]. Androgen-repressed human prostate cancer cell line, ARCaP, expressed low levels of androgen receptor ad PSA but is highly metastatic [11]. Clinical prostate cancer specimens may contain cells of differential androgen sensitivity with wide-range of expression, from androgen-stimulated, androgen-insensitive to androgen-repressed state.

2. How are stromal-epithelial interactions affected by the presence or absence of androgen, critical growth factors, and/or extracellular matrices?
3. How may prostatic epithelial cells interact with their surrounding stroma as a three-dimensional organoid? How are the relevant molecular signals integrated among growth factors, extracellular matrices, and steroid hormone receptors? What are the most relevant targets and central pathways that could be pursued for discovering novel chemopreventive and therapeutic agents that may interfere with cell signaling and delay or retard cancer progression?
4. What are the molecular mechanisms of epigenetic and genetic interaction? How are the molecular signals altered by genetic and epigenetic mechanisms?

Future progress in developing effective chemopreventive and therapeutic agents to treat prostate cancer may depend on advancing our understanding of the interaction between cancer epithelium and host microenvironments. Effort devoted by our laboratories and others in developing preclinical models of prostate cancer could lead to further understanding of molecular mechanisms of prostate cancer growth and progression. This will ultimately help us to develop more efficient and reliable methods in discovering agents that may interrupt prostate cancer development and progression.

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