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Human Prostate Cancer Model: Roles of Growth Factors and Extracellular Matrices

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Abstract A human prostate cancer model was established by inoculating a prostate specific antigen (PSA)-producing LNCaP cell line with either prostate or bone fibroblasts. Alternatively, this human prostate cancer model can also be established by inoculating LNCaP cells with growth factor(s) (GFs) and extracellular matrix (ECM) immobilized on Gelfoam®. The resulting LNCaP tumors were used to evaluate PSA production and excretion in athymic hosts. This model was also employed to examine the biochemical nature of mesenchymal cell-derived growth-promoting protein(s) and to assess the efficacy of potential chemotherapeutic agents. Because of the propensity of human prostate cancer to metastasize to the bone, this study defined a 1.0 M NaCl-eluted fraction, MS1, from the conditioned medium of a bone stromal cell line (MS) by heparin-affinity column chromatography. The growth-promoting activity was assayed both *in vivo* (*e.g.*, tumor formation) and *in vitro* (*e.g.*, soft agar colony formation). We found that the growth-promoting activity was trypsin- and heat-sensitive, and partially degraded by acid and dithiothreitol. Immunochemical studies indicated that the polyclonal antibody raised against MS1 blocked the growth-promoting effect elicited by the bone-conditioned media. This growth-promoting factor was found to be immunochemically dissimilar to KGF, HGF, and bFGF. However, addition of bFGF, HGF and NGF, but not aFGF, TGFβ, IGF1, IGF2, PDGF, EGF, TGFα and KGF, stimulated anchorage-independent growth of prostate cells, a condition closely parallel to tumor formation *in vivo*. We found that the MS1 fraction also contained fibronectin and tenascin but not laminin or collagen IV. None of the ECM proteins induced soft agar colony formation by normal prostate epithelial cells. Therefore, it is possible that the ECM protein(s) may potentiate the tumor-inducing activity of locally produced GFs.

Key words: extracellular matrices (ECMs), bFGF, NGF, HGF and KGF, growth factors (GFs), human prostate cancer model, prostate cancer-bone interaction, stromal-epithelial interaction

Although prostate cancer has been identified as one of the major health problems facing the aging male population in the U.S. [1], fundamental understanding of this disease is lacking for the following reasons: (1) a long protracted natural history affects only a small fraction of men with either latent or early stages of the disease; (2) few well-defined animal and human prostate cancer models mimic human disease conditions; and (3) technical difficulties are encountered in culturing human prostate carcinoma cells in chemically defined media and in growing human prostate cancer explants *in vitro* or xenografts *in vivo*. Thus, to understand human prostate cancer progression, we must establish relevant models that mimic human conditions so that sensitive morphologic, biochemical, and molecular markers can be established to monitor progression of the disease. To achieve this, our laboratory evaluated potential epigenetic constraints that may limit prostate cancer growth in experimental animals. In this

Abbreviations: LNCaP: human metastatic prostate adenocarcinoma cell line; KGF: keratinocyte growth factor; HGF: hepatocyte growth factor; bFGF: basic fibroblast growth factor; NGF: nerve growth factor; aFGF: acidic fibroblast growth factor; TGFβ: transforming growth factor-β; IGF1: insulin-like growth factor 1; IGF2: insulin-like factor 2; PDGF: platelet-derived growth factor; EGF: epidermal growth factor; TGFα: transforming growth factor-α; ECGF: endothelial cell-derived growth factor.

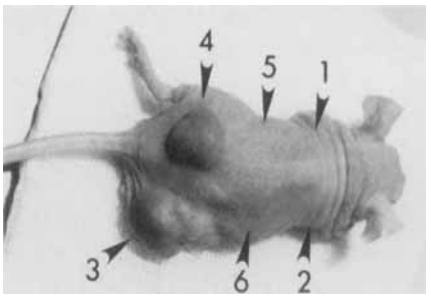
report, we summarize our laboratory findings and emphasize the important role of GFs and ECMs in modulating human prostate cancer growth and progression *in vivo*. Our conclusion was based on the observation that organ-specific fibroblasts accelerate prostate cancer growth in a nude mouse model [2–4]. We report here our recent results in the fractionation of the conditioned medium (CM) from the bone fibroblasts which appear to contain proteins which are potent growth promoters for LNCaP tumor cells *in vivo*. Using a novel growth factor delivery system *in vivo*, which parallels an *in vitro* soft agar colony-forming assay, we identified a 1.0 M NaCl-eluted heparin sepharose affinity column fraction (designated MS1) which appeared to exhibit a unique growth-stimulating effect on human prostate cancer growth *in vivo*. Detailed fractionation and characterization of the potentially active bone stromal cell-derived growth factors which contribute to prostate cancer growth *in vivo* are in progress. This report also summarizes our recent preliminary results on the use of a human prostate cancer model to assess the effect of pentosan polysulfate on human prostate cancer growth *in vivo*.

ORGAN-SPECIFIC FIBROBLASTS ACCELERATE HUMAN PROSTATE CANCER GROWTH *IN VIVO*

Our laboratory established a cell–cell recombination model where we observed that mesenchymal (stromal) cells were potent inducers capable of stimulating human prostate carcinoma growth *in vivo* [2–5]. By co-inoculating human LNCaP cells with various organ-specific fibroblasts, Gleave *et al.* [2,3] demonstrated that both prostate and bone, but not lung or kidney, stromal cells are capable of inducing LNCaP tumor growth *in vivo*. The induced human prostate LNCaP tumors secrete PSA, a human prostate epithelial-specific marker [2,3]. Levels of serum PSA were found to correlate well with tumor volume; they were regulated by androgen *in vivo* and by androgen and GFs *in vitro* [2]. The significance of organ-specific mesenchymal cells in inducing prostate tumor growth is further supported by the observation that LNCaP cells form PSA-secreting prostate tumors when injected directly into the host dorsal prostate gland of male athymic mice [6].

Table I. Induction of Human Prostate Carcinoma Growth *in Vivo* by Growth Factors

Gelfoam Treatment	Incidence of Tumor Formation
1 Collagen IV alone	0/10
2 Collagen IV + ECGF	1/10
3 Collagen IV + bFGF	6/10
4 Collagen IV + TGFα	2/5
5 Collagen IV + EGF	0/5
6 Collagen IV + PDGF	0/5



These results suggest that normal prostate stromal cells are also capable of inducing LNCaP tumor formations *in situ*. The success of inducing reproducible growth of human LNCaP prostate tumor *in vivo* prompted us to examine the mechanism of intercellular communication, *e.g.*, prostate–bone interaction, which may have a significant impact on prostate tumor growth and progression *in vivo*. The potential use of this human prostate cancer model to screen drugs that may effectively and selectively inhibit prostate tumor growth *in vivo* will also be discussed.

GROWTH FACTORS STIMULATED HUMAN PROSTATE TUMOR GROWTH *IN SITU*

Results published by our laboratory [2–6] and others [7–10] suggest that paracrine factors may mediate stromal–epithelial interaction and may determine hormonal responsiveness of many endocrine target tissues. In our laboratory, we developed an *in vivo* model of prostate tumori-co-inoculated with GFs adsorbed on well-dispersed inert matrix, Gelfoam® [3]. PSA-secret-

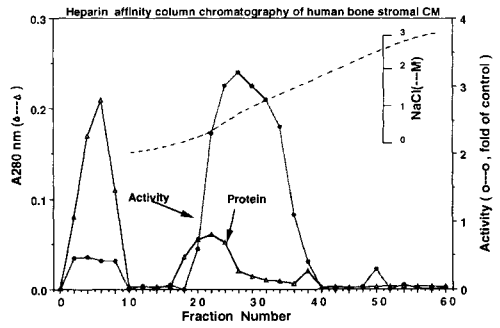


Fig. 1. Heparin sulfate-affinity column chromatographic elution of CM isolated from MS bone stromal cells. Note that the majority of LNCaP tumor-inducing activity resides in the 1.0 M NaCl-eluted fraction (MS1).

ing prostate tumors were formed with bFGF and TGFα, but not with insulin, transferrin (standard medium components), ECGF, EGF and PDGF, when delivered *in vivo* complexed with Gelfoam® (Table I). This action of bFGF can be effectively neutralized by bFGF monoclonal antibody in an *in vitro* soft agar colony formation assay; this implies that GFs act locally because tumors inoculated at adjacent sites of the same host failed to proliferate [bottom of Table I, see arrows where bFGF and TGFα induced local LNCaP tumor growth without altering the effect of EGF (marked 5) and PDGF (marked 6)]. We found that the soft agar colony-forming assay of growth factors reflected their *in vivo* prostate tumor-inducing activity, with the exception of TGFα. Although we have not detected the presence of bFGF in the CM of the prostate and bone stromal cells, our results do not exclude the possibility that a bFGF-like peptide(s) may be responsible for prostate epithelial tumor growth *in vivo*.

ISOLATION AND CHARACTERIZATION OF BONE STROMAL CELL-DERIVED FACTORS ON HUMAN PROSTATE TUMOR GROWTH *IN VIVO* AND ANCHORAGE-INDEPENDENT PROSTATE CELL GROWTH *IN VITRO*

Because certain heparin-bound GFs have been recently implicated in both benign and malignant growth of human prostate cells, we fractionated serum-free CMs collected from human bone stromal cells by heparin-affinity column chromatography. Figure 1 shows that prostate tumor-inducing activity was eluted predominantly in the 1.0 M NaCl fraction (MS1) of the bone stromal cell CM. Specificity of tumor-inducing activity was demonstrated in experiments where similar fractions eluted at 2.0 M NaCl or identical fractions eluted from 3T3 cell CM, or control serum-free TCM medium failed to exert prostate tumor-inducing activity as analyzed by both *in vivo* tumor growth and *in vitro* methods (Table II).

Table II. Effect of NaCl-eluted Protein Fractions From Heparin Sepharose Column on Prostatic Tumor Growth *in Vivo* and Prostatic Cell Colony Formation *in Vitro*

Condition	Incidence of Tumor Formation	Soft Agar Colony Formation
Gelfoam & Collagen IV	0/6 (0%)	4 ± 1.5
+ MS 1.0 M NaCl Eluate	9/12 (75%)	121 ± 7.2
+ MS 2.0 M NaCl Eluate	0/24 (0%)	—
+ 3T3 1.0 M NaCl Eluate	—	2 ± 0.7
+ TCM 1.0 M NaCl Eluate	0/6 (0%)	4 ± 0.7

We correlated *in vivo* tumor-promoting activity with soft agar colony-forming efficiency *in vitro*. The tumor-inducing activity was trypsin- and heat-sensitive (34% and 82% of the tumor growth-inducing activity was lost when the partially purified materials were heated to 70°C and 100°C for 5 minutes, respectively). Also, the tumor-inducing activity was found to be partially sensitive to acid (1 N HCl) and dithiothreitol (50 μM) treatment (40% and 50% inhibition, respectively).

The tumor-forming activity *in vitro* was further resolved by polyacrylamide gel electrophoresis. Because of the presence of high molecular weight proteins in the tumor-inducing fraction, we performed western-blot analysis with antibodies prepared specifically against fibronectin, laminin, collagen IV, and tenascin. We found that the MS1 fraction contains high molecular weight immunoreactive fibronectin and tenascin, but not laminin and collagen IV proteins (Li *et al.*, unpublished results). These results suggest that specific ECM proteins may

Table III. Inhibition of Bone Stromal CM-induced Soft Agar Colony-forming Efficiency of Prostatic Epithelial Cells by Antibodies Prepared Against MS1 or bFGF, KGF, and HGF

Condition	Soft Agar Colony Formation	% of Inhibition
EXP. 1.		
Control	9 ± 1.5	—
+ MS1	126 ± 10.1	—
+ MS1 & PoAb (rabbit)	48 ± 4.3	66
+ MS1 & PoAb (mouse)	55 ± 3.3	60
EXP. 2.		
Control	11 ± 1.4	—
+ MS1	132 ± 15.6	—
+ MS1 & bFGF Ab	109 ± 15.2	19
+ MS1 & KGF Ab	130 ± 19.5	2
+ MS1 & HGF Ab	128 ± 13.4	3

be intimately associated with GFs that confer prostate tumor-inducing activity. Both fibronectin and tenascin have been implicated in embryonic development and appear at the interphase between stroma and epithelium where morphogenesis, cell migration, and invasion occur [11]. When complexed with selected ECM, GFs may elicit morphogenetic action and may determine the tumorigenic and metastatic potentials of the adjacent tumor epithelial cells. We prepared a polyclonal antibody against MS1 fraction. Results showed that the polyclonal antibody, but not bFGF, KGF or HGF antibody, inhibited prostate epithelial soft agar colony formation (Table III).

EFFECT OF NGF, KGF AND HGF ON SOFT AGAR COLONY-FORMING EFFICIENCY OF PROSTATIC EPITHELIAL CELLS *IN VITRO*

Recent studies from several laboratories have demonstrated that NGF [12], KGF [13], or HGF [14] are candidates for paracrine mediators responsible for epithelial cell growth. Unlike bFGF, KGF and HGF contain signal peptides and thus can be secreted extracellularly, acting on cell surface receptors of short-range neighboring cells. Because KGF and HGF, but not NGF, are known to be heparin-bound GFs which can be eluted by 1 M NaCl, we focused attention on defining the possible direct growth-promoting effect of KGF and HGF which enhances the soft agar colony-forming efficiency of the prostate epithelial cells. Figure 2 shows that HGF, but not KGF, is a potent mitogen which stimulated prostate epithelial cells to form soft agar colonies in a concentration-

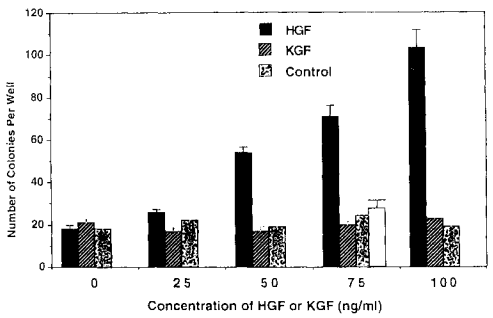


Fig. 2. HGF, but not KGF, stimulated prostatic epithelial anchorage-independent growth in soft agar dishes in a concentration-dependent manner. Note that HGF-induced increases in prostatic epithelial soft agar colonies can be effectively blocked by HGF polyclonal antibody (see open bar at 75 ng/ml HGF).

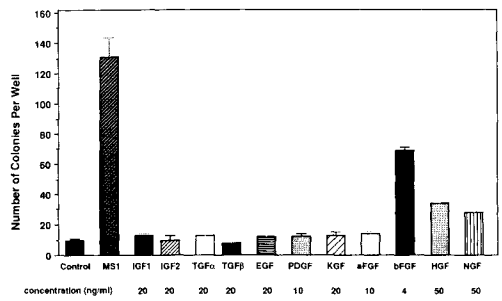


Fig. 3. Effects of growth factors on the soft agar colony-forming efficiency of the prostatic epithelial cells *in vitro*. Note that bFGF, HGF, or NGF alone, but not IGF1, IGF2, TGFα, TGFβ, EGF, PDGF, KGF or aFGF, stimulated prostatic epithelial soft agar colony formation.

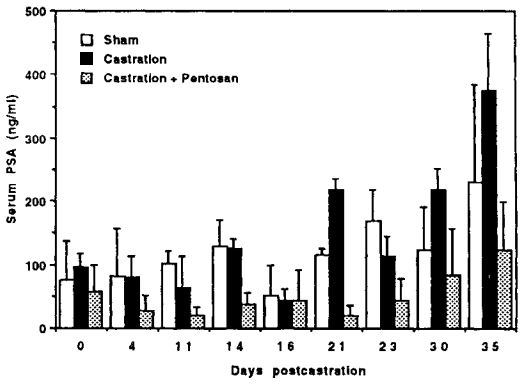


Fig. 4. Pentosan polysulfate inhibition of the serum PSA concentrations. Serum PSA was found to correlate well with prostate tumor volumes [2].

dependent manner. The soft agar colony-forming activity induced by HGF can be completely neutralized by an HGF antibody. In separate experiments, we demonstrated that the heparin-unbound NGF also exhibits a growth-promoting effect on prostate epithelial cells in soft agar plates. However, aFGF, TGFβ, IGF1, IGF2, PDGF, EGF, and TGFα were inactive in stimulating prostatic epithelial soft agar colony formation (Fig. 3). At present, the possible significance and role of HGF and NGF serving as stromal cell-derived growth-promoting proteins *in vivo* for prostate carcinoma growth is unclear.

PENTOSAN POLYSULFATE INHIBITION OF THE GROWTH OF PROSTATE TUMOR *IN VIVO*

Pentosan polysulfate, a sulfate-containing polysaccharide with a structure similar to suramin, was tested as a potential antiproliferative agent *in vivo*. This approach was chosen because certain classes of cancer chemotherapeutic drugs that may be inactive *in vitro* exert potent growth-inhibiting activity *in vivo* due to their actions on host-derived elements, such as anti-angiogenesis or interference with the host immune system. We evaluated the action of pentosan polysulfate on prostate cancer growth, both *in vitro* and *in vivo*. Unlike suramin, pentosan polysulfate is a weak inhibitor of LNCaP cell growth *in vitro* with an IC_{50} (50% inhibitory drug concentration) at least 30-fold higher than that of suramin. To determine the effect of pentosan polysulfate *in vivo*, we employed a mouse model of human prostate cancer developed in our laboratory [3]. As mentioned above, the characteristics of this model include the ability of the induced LNCaP tumors to secrete PSA *in vivo*, and the regulation of serum PSA levels by tumor volume and andro-

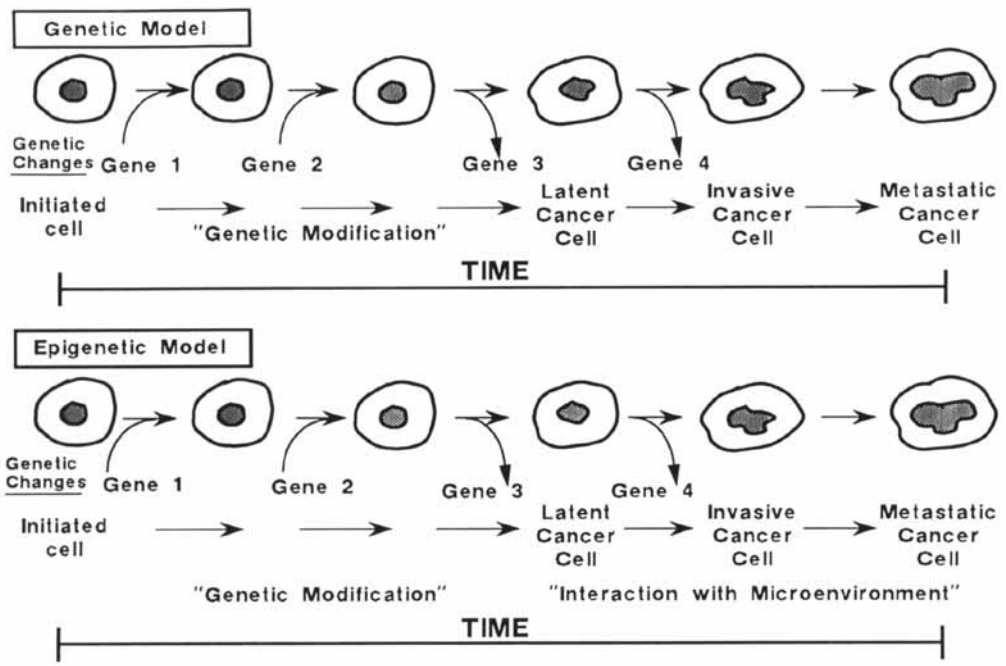


Fig. 5. Models of prostate cancer progression illustrating the genetic and epigenetic determinants which contribute to multi-step prostatic carcinogenesis. The key distinction between genetic and epigenetic models is that certain genetic changes (*e.g.*, Gene 1 to 4) may be induced by interaction of cancer cells with their microenvironment. We believe that interactions between the initiated prostatic tumor cells and their host microenvironment account for key steps leading to acquired tumor behaviors, such as altered growth rate, invasiveness and potential to metastasize.

gens. Moreover, this model mimics the human prostate cancer condition in which PSA secretion becomes autonomous and androgen-refractory when tumors were maintained in castrated hosts [2]. The basic experimental protocol was to first establish human prostate tumor growth in athymic hosts. When the tumors reached about 125 mm^3, the hosts were sham-operated or castrated, and then treated with either pentosan polysulfate or vehicle. Host serum PSA levels were followed for 35 days. Figure 4 shows that pentosan polysulfate decreased serum PSA levels, possibly by decreasing prostate tumor growth. Since the drug was not a potent inhibitor of prostate cell growth *in vitro*, we speculate that pentosan polysulfate may interfere with tumor–host interaction. For example, pentosan polysulfate may antagonize tumor angiogenesis or may inhibit GF- and ECM-mediated pathways required for *in vivo*, but not *in vitro*, growth.

SIGNIFICANCE OF LNCaP MODEL TO HUMAN PROSTATE CANCER PROGRESSION

The LNCaP tumor model above illustrates the potential importance of paracrine factors in prostate cancer growth and progression. Our model suggests that prostate tumor interaction with the host microenvironment plays a critical role in prostate cancer progression, including the possibility that unique prostate–bone cellular interaction may determine the ability of prostate cancer to disseminate to the bone. This suggestion is based upon the observation that LNCaP cells progressed to form solid tumors in hosts when they were co-inoculated with prostate- and bone-derived stromal cells. Since LNCaP tumors formed when cells were inoculated directly (without stromal cells) into the host prostate gland (but not at subcutaneous or subrenal capsular sites), this suggests that the host organ-specific microenvironment may be an important regulator of prostate tumor growth *in vivo* and may be mediated by organ-specific GFs and ECMs. These characteristics of host microenvironment may also influence the ability of inoculated tumor cells to metastasize. Fidler *et al.* [15] have shown that orthotopic (as opposed to ectopic) injection of a variety of tumor cells enhances the probability of tumor cell growth and its subsequent metastatic potential. Figure 5 schematically illustrates our current concept of prostate cancer progression whereby we propose that unlike the genetic model of multi-step carcinogenesis (top panel), interaction of transformed cells with their host microenvironment plays a crucial role in prostate cancer growth and progression. We speculate that prostate cancer progression, including the progression of prostatic intraepithelial neoplasia to the more advanced stages of prostate cancer, may be influenced by the host microenvironment. Based on our model, a therapeutic strategy can be designed to block prostate cancer progression through the influence of paracrine factor interaction with prostate cancer cells. This approach may be clinically relevant because prostate cancer is known to have a protracted natural history of progression. It is conceivable that most prostate cancer cells are in a pool of slow growth but progress from their initiated stage(s) to rapidly growing stage(s). Thus, we believe that our present model of human prostate cancer offers an opportunity to dissect the molecular mechanisms associated with the "natural history" of prostate cancer progression. If these assumptions are correct, we will be able to develop biomarkers to assess intermediate endpoints of prostate tumor progression and to design rational therapeutic approaches targeted at tumor cell interaction with the host environment in order to block or reverse prostate cancer growth and progression. Thus, the emphasis of our research focuses on interfering with prostate cancer growth at the stage of progression. Therapeutic trials using agents that interfere in GF and ECM interactions with prostate cancer cells are in progress.

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