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Review

Growth factor involvement in progression of prostate cancer

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Understanding how the regulation of growth factor pathways alters during prostate cancer (PC) progression may enable researchers to develop targeted therapeutic strategies for advanced disease. PC progression involves the shifting of cells from androgen-dependent growth to an androgen-independent state, sometimes with the loss or mutation of the androgen receptors in PC cells. Both autocrine and paracrine pathways are up-regulated in androgen-independent tumors and may replace androgens as primary growth stimulatory factors in cancer progression. Our discussion focuses on growth factor families that maintain homeostasis between epithelial and stromal cells in the normal prostate and that undergo changes as PC progresses, often making stromal cells redundant. These growth factors include fibroblast growth factor, insulin-like growth factors, epidermal growth factor, transforming growth factor α, retinoic acid, vitamin D_3, and the transforming growth factor β families. We review their role in normal prostate development and in cancer progression, using evidence from clinical specimens and models of PC cell growth.

The Natural History of Prostate Cancer

Little is currently known about the natural history of untreated prostate cancer. The discrepancy that exists between the prevalence of prostate cancer at autopsy and the clinical incidence of prostate cancer has been recognized for many years *(1)*. The frequency of autopsy-detected cancer has been reported to be 30–40% in men over the age of 50. In contrast to these autopsy prevalence studies, the lifetime probability that a man will be diagnosed clinically with prostate cancer is ~13%, and the probability of dying of prostate cancer is only ~3% *(2)*. Despite these statistics, diagnosed prostate cancer is the most common cancer in men and the second highest cause of cancer death in men in Western society. In the US, >540 new cases are found daily, and the death rate is 104/day *(3)*. At present, separating the individuals whose cancer will not progress from those with potentially fatal disease is not possible *(4)*. Because no trial has examined the natural history of localized prostate cancers detected through screening for prostate-specific antigen (PSA),[3] this dilemma would be compounded in cancers detected through a screening program *(5)*. Such cancers may have different natural histories from those that present clinically. Moreover, prostate cancers exhibit extreme heterogeneity with respect to grade and stage within individual tumors *(6)*, making it difficult to determine their overall ability to progress. Although a number of characteristics of prostate cancer, including histological grade, tumor size, and ploidy *(7)*, are useful in predicting biological activity, more reliable prognostic markers to identify individuals with potentially fatal forms of the disease are urgently needed. A better understanding of growth factors and the changes that occur in the androgen receptor (AR) should give some information in this regard *(4)*.

Treatment for clinically detected prostate cancer includes watchful waiting, prostate surgery, or targeted irradiation. The latter two treatments can cure only cancer that is organ confined, yet up to 60% of patients present with metastases, particularly to the bone. Because determining the biological potential of localized cancers detected with any degree of certainty through screening is not possible, observation alone will result in a lost oppor-

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[3] Nonstandard abbreviations: PSA, prostate-specific antigen; AR, androgen receptor; AD, androgen dependent; AS, androgen sensitive; AI, androgen independent; KGF, keratinocyte growth factor; EGF, epidermal growth factor; IGF, insulin-like growth factor; IGF-BP, high-affinity IGF-specific binding protein; FGF, fibroblast growth factor; TGF, transforming growth factor; MPR, mouse prostate reconstitution; TbetaR, transforming growth factor-β receptor; bFGF, basic fibroblast growth factor; EGFR, epidermal growth factor receptor; FGFR, fibroblast growth factor receptor; aFGF, acidic fibroblast growth factor; and RA, retinoic acid.

tunity for cure in many patients; on the other hand, treatments such as radical prostatectomy or radiation therapy in such cases will necessarily overtreat many patients *(8)*. Advanced prostate cancer with metastases to bone and other soft tissues also presents a difficult therapeutic problem *(4)*. Management alternatives for advanced prostate cancer are palliative at best. Treatment for such cancers depends on the androgen requirement of prostate epithelial cells for growth and survival. Endocrine therapy leads to substantial periods of remission. However, it is ineffective once the tumors progress from androgen-dependent (AD), through androgen-sensitive (AS; these tumors do not require androgen for growth but proliferate more rapidly in its presence), to hormone refractory or androgen-independent (AI). This progression is almost inevitable in men after androgen ablation therapy. Total androgen ablation used in hormonally naive patients results in a median remission of about 24 months *(4)*. Therapeutic alternatives in men who have progression of the disease after androgen ablation are very limited, with a median survival between 8 and 12 months.

The need to develop more effective therapy for these patients exists. A better understanding of growth factor pathways may provide an additional target for therapy in patients with advanced prostate cancer. This review focuses on the current state of knowledge of growth factor pathways in prostate cancer. The majority of data presented were derived from cancer cell lines without functional AR and from animal models. We thus warn the reader that such data may not be directly relevant to human prostate cancer. In addition, some of the data are based on single literature reports, and they need confirmation before definitive conclusions can be drawn.

Biological Properties of the Prostate Gland

The prostate gland is composed of epithelial cells, which form two layers and stromal cells. There are three types of epithelial cells: secretory glandular cells, nonsecretory basal cells, and neuroendocrine cells *(9)*. The basal cells lack ARs, are AI, and are thought to be stem cells for secretory epithelial cells *(10)*; the neuroendocrine cells may play a role in regulating the growth and function of the secretory cells *(11)*. The stroma of the prostate is composed of smooth muscle cells, fibroblasts, lymphocytes, and neuromuscular tissue embedded in an extracellular matrix. Evidence suggests that epithelial-stromal interactions play an important role in normal prostatic morphogenesis *(12)*. In normal tissue, such interactions are often paracrine with, for example, receptors for a particular growth factor present only on epithelial cells and production of the factor only by stromal cells. In cancer, some growth factor pathways become autocrine, enabling the epithelial cells, which express a growth factor and its receptor, to grow independently of stromal cells.

Various hypotheses to explain how malignant but not benign prostatic epithelial cells become AI have been proposed [reviewed in *(13)*]. These hypotheses are based on: (*a*) clonal selection of AI cells from a heterogeneous population of AD and AI cells by androgen ablation; (*b*) an adaptive theory, which proposes the presence of AD stem cells that can adapt to self-renewal in the absence of androgen; (*c*) the potential effects of small residual amounts of androgen, which can stimulate AS cells; and (*d*) changes in the AR, such as mutation, overexpression, or loss, that occur in some but not all cases of AI prostate cancer. Currently, determining which hypothesis is correct is not possible, but given that most prostate cancers are heterogeneous, it is likely that both clonal selection of preexisting AI cells and adaptive processes may contribute to AI progression. However, the findings described below make it clear that AI progression must also be mediated by growth-regulatory factors that function independently of androgen.

Both autocrine and paracrine growth factors are up-regulated in AI prostate tumors and may replace androgen as the primary growth-stimulatory factors in cancer progression. This up-regulation may represent an adaptive response to androgen ablation in the growth regulation of AI tumors and is the basis for discussion in this review.

The AR in Prostate Cancer

Because androgen has been shown to regulate the expression of many prostatic growth factors and androgen ablation is the most common first-line treatment for prostate cancer patients [reviewed in *(14)*], no review on growth modulators of prostate cancer would be complete without some comment regarding the AR and its possible role in this disease.

The AR is a member of the steroid/thyroid hormone/retinoic acid family of receptors that bind to specific hormone-responsive elements in target genes, thus regulating transcription [reviewed in *(15)*]. In the developing prostate, only the stromal cells express ARs, suggesting that androgen regulation of prostatic development is mediated via the stromal cells [reviewed in *(16, 17)*]. In the normal adult prostate, AR expression is found mainly in the epithelium, but it is also identified in the stromal cells *(16, 17)*. In contrast, in prostate cancer specimens, AR staining in the epithelium is heterogeneous, with a marked decrease in AR-positive cells occurring in less differentiated tumors, corresponding to the reported insensitivity to androgen observed in advanced prostate cancers *(18, 19)*. The reason for this altered expression of the AR and why some cells lose their AR is still not known.

Sequence analysis of the *AR* gene in prostate cancers has revealed that many tumors contain mutations *(20–24)*. The resulting mutant proteins may be unable to bind androgen but remain constitutively activated, or they may bind androgen but be nonfunctional *(15, 25)*. *AR* mutations in prostate cancers are commonly point mutations

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but may also reflect microsatellite instability. Point mutations have been identified in many prostate cancers, including the AS cell line LNCaP *(20–22)*. In particular, a hot spot for mutation exists at codon 887 (ACT-GCT, Thr-Ala) in the hormone-binding domain of the gene. In LNCaP cells, this mutation enables the AR to bind progestogenic and estrogenic steroids, but it also causes a decreased affinity for androgen *(23)*. Such abnormal binding may induce the transcription of genes out of context, thus upsetting the delicate balance of growth factors in the prostate and promoting cancer development.

Prostate cancer development has also been linked to a change in the number of repeats in the polymorphic CAG and GGC microsatellite in exon 1 of the *AR* gene *(24–26)*. How this leads to prostate cancer development is not yet clear.

Prostate cancer cells have derived complex mechanisms that allow them to grow in the absence of androgen stimulation or in the presence of mutated or lost AR. Exposure of DU-145 AI prostate cancer cells transfected with an *AR* expression vector and a chloramphenicol acetyltransferase (*CAT*) reporter gene to keratinocyte growth factor (KGF), epidermal growth factor (EGF), or insulin-like growth factor 1 (IGF-1) could activate *CAT* gene expression in the absence of androgen *(27)*. These data suggest that growth factors may activate AR in an androgen-deprived environment.

The Role of Growth Modulators in Prostate Cancer

Although the role of androgen is important, alone it is insufficient to maintain normal prostate homeostasis. This process also requires complex interactions between peptide growth factors and growth modulators that may be regulated either by androgen or independently by other factors (Table 1) *(28–79)*. This renders the prostate gland very sensitive to any aberrations in growth factor or androgen expression. Therefore, it is not surprising that in prostate cancer the expression, regulation, and cell-related production of many of these growth modulators are altered (Table 1).

Alterations may take the form of up- or down-regulation of growth factors or their receptors or a change from paracrine to autocrine mediation of growth factor pathways. Alternatively, because many growth factor pathways send their messages to the cells via common signal transduction pathways, any mutations affecting signal transduction may affect several growth factor pathways simultaneously. Functional analysis of these potential changes is outside the scope of this review. Our focus is on growth factor families for which there is evidence to support a major role in normal and cancerous growth of the prostate. These families include the following: the fibroblast growth factor (FGF) family, the IGF family, EGF, and transforming growth factor α (TGF-α), all of which are predominantly stimulators of proliferation; retinoic acid, which causes differentiation and invasiveness; and the TGF-β family and vitamin D_3, which are predominantly inhibitors of prostatic growth. Using evidence from human prostate cancer cell lines and animal models in vivo and in vitro, we will discuss the role that these factors play in normal prostate development and in cancer progression. Evidence obtained from clinical findings is often controversial, and many different models of prostate cancer have been studied in vivo and in vitro in an attempt to define more precisely the roles particular growth factors play in prostate cancer progression.

Models for Studies of Prostate Cancer

Prostate cancer rarely arises spontaneously in animals other than humans, and an ideal model for its study does not exist. Such a model would have a reasonably slow doubling time, be AD or AS, produce PSA, metastasize to lymph nodes and bone, and progress to an AI state after castration *(80)*. The natural history of prostate cancer in humans varies widely in biological aggressiveness, androgen sensitivity, and histological appearance. Different models mimic various aspects of the clinical disease. Those most commonly used are summarized in Table 2 *(81–111)*.

RODENT MODELS

The rat prostate differs from the human gland in that it is divided into distinct individually encapsulated lobes (dorsal, ventral, and anterior), each of which has a separate set of compound ducts *(111)*. The best known model of prostate cancer is the Dunning R-3327 rat prostatic adenocarcinoma model, derived by the passage of a spontaneous prostate tumor discovered at autopsy in a Copenhagen rat *(81)*. Subcutaneously implanted tumors become palpable in ~60 days and histologically are well-differentiated adenocarcinomas with both glandular and stromal elements. Multiple sublines indicative of cancer progression have been developed [described in detail in *(82)*], including AS lines *(H)* and AI tumors (A subline), which lack 5α-reductase and ARs *(83)*, and metastatic *(84)* sublines (Table 1).

Other rodent models include the hormone- or *N*-methyl-*N*-nitrosourea-induced Noble rat prostatic adenocarcinoma model *(85, 86)*, Pollard rat tumors in Lobund-Wistar (L-W) rats derived from a spontaneous tumor *(90, 91)*, spontaneous ACI rat prostate cancers in aging August × Copenhagen hybrids *(93)*, and the Shionogi mouse mammary carcinoma model *(95, 96)*, which produces both AD and AS wild-type tumors. In Shionogi mice, the proportion of AI stem cells in recurrent AI tumors has been shown to increase 500-fold, lending support for self-renewal of stem cells in the absence of androgen *(113)*. These models have been described elsewhere in detail *(13)*.

Thompson et al. *(97)* have developed a mouse prostate

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Table 1. Role of growth factors in normal prostate and in prostate cancer progression.

Growth factor	Expression[a] and regulation in normal prostate	Expression and regulation in prostate cancer	Possible role in metastasis
TGF-β 1, 2, and 3	Rats/humans: expressed by PE and PM, a^-; TGF-βs counterbalance mitogenic effects of other growth factors; expression associated with castration-induced apoptosis (*28–32*)	Expression of members of TGF-β family correlates with responsiveness to androgens and is associated with abnormal growth; TGF-β1 and -β3 up-regulated; may be secreted with autocrine regulation; sensitivity to TGF-β inhibition is lost with tumor progression (*33–41*)	Causes osteoblast migration, angiogenesis, immunosuppression (*42–44*)
bFGF	Expressed by PE and PS but receptor only in PS, a^+; maintains homeostasis (*45, 46*)	Bone fibroblasts from PC patients stimulate LNCaP tumor formation in vivo, involving bidirectional bFGF activity; expression is higher in AI than in AS human cell lines (*47*); switch from PS autocrine to PE autocrine expression occurs in rat and human PC (*47, 48*); in Dunning rat PC, AI is accompanied by a switch in exon IIIc of the *FGF-r2* gene, changing ligand from KGF to bFGF in epithelial cells (*48*); antisense bFGF causes Dunning AT3 cells to form smaller, more slowly growing tumors in vivo; MMTV-Int-2 transgenic mice show proliferation of PE cells (*49*)	bFGF regulates protease expression, e.g., urokinase-type plasminogen activator, collagenase, which may be involved in metastatic cascade; bFGF is highly angiogenic (*50*)
aFGF	Rats: role in early prostate epithelial development (*51*)	Rats: expression changes with progression from PS in slow-growing tumors to PE in more aggressive tumors (*52*)	Not defined
KGF	Rats/humans: involved in ductal branching of fetal prostate; paracrine production by PS, receptor (BEK) on PE; a^+ (*53–55*)	Human: switch to autocrine production by PE tumor cells (*55, 56*)	Not defined
FGF-8	Androgen-regulated FGF detected by PCR in rat prostate (*57*)	Detected by PCR in LNCaP and AI DU-145 cells, suggesting that it is not androgen regulated (*57*)	Not defined
IGF-1, IGF-2, and IGF-BPs	IGFs produced by PS; IGF-type I receptors are on PE; PE secrete IGF-BP-2, -3, -4, and -6 (*58, 59*)	PE shows autocrine regulation of IGF pathways in absence of serum; dysregulation of IGF-BP production in cell lines and patients (*60, 61*)	IGFs may promote bony metastases; PSA cleaves IGF-BP-3, resulting in release of IGFs in distant tissues (*61–63*)
EGF, TGF-α/EGFR	EGF found in prostatic fluids, suggesting a regulatory role; TGF-α production by PS, but EGFR on PE; EGF and TGF-α production is a^+ but EGFR expression is a^- (*64–67*)	Both EGF and TGF-α expression are up-regulated in human cancers (*68–71*); switch from paracrine to autocrine production by PE (*72*)	EGF enhances cell invasion by induction of tumor proteases (*73*); EGF can regulate the IGF axis (*74*)
BMP	Rats/humans: BMP 2, 3, 4, and 6 present in prostate tissue (*75*)	BMP 3 predominates in rat tumors and varies in concentration in human cell lines (*75*)	May be produced at metastatic sites and stimulate bone formation (*75*)
NGF	NGF protein is localized in PS, receptors on PE (*76*)	Expression of NGF is reduced, and that of receptors is lost with cancer progression (*77*)	Not defined
IL-6	IL-6 is present in normal serum and in primary prostate epithelial cell cultures (*78*)	IL-6 is high in prostate cancer cell lines; receptors appear to be up-regulated in prostate cancer patients, with autocrine production (*79*)	

[a] PE, expression in prostate epithelial cells; PS, expression in prostate mesenchymal/stromal cells. Androgen regulation is notated as negative (a^-) or positive (a^+).
BMP, bone morphogenetic protein; NGF, nerve growth factor; IL-6, interleukin 6.

reconstitution (MPR) model that exploits the ability of fetal epithelial and stromal cells from the urogenital sinus to form a mature prostate gland when implanted under the renal capsule of adult mice. This model allows the study of paracrine interactions between cell compartments by introducing candidate genes for growth factors, oncogenes, or suppressor genes into the epithelial or fibroblast compartments derived from the fetal prostate

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Table 2. Models of prostate cancer.

Animal	Model	Androgen sensitivity	AR	5α-reductase	Comments	References
Rats						
Copenhagen	Dunning	H line, AS;	+	+	Various sublines	*81–82*
	R-3327	A line, AI;	−	−	reflect PC progression;	
		MAT-Ly-Lu, AI;	−	−	metastatic (liver/lung);	*83*
		MAT-Ly, AI	−	−	metastatic (liver);	*84*
Noble	Noble	AD and AI lines			Chronic hormones induce PCs; stroma plus epithelial cells required for tumorigenicity	*85–88* *89*
Lobund-Wistar (L-W)	Pollard	AD and AI lines			Spontaneous tumor; tumors induced by testosterone or *N*-methyl-*N*-nitrosourea administration	*90* *91, 92*
August × Copenhagen	ACI				Spontaneous PC in ventral lobe; old rats	*93, 94*
Mice						
DD/S	Shionogi mammary carcinoma	AD and AI lines	+	+	Extensively used for studying intermittent hormonal ablation therapy	*95, 96*
C57BL/6	MPR (renal capsule)	RM-6 line, AI			Overexpression of *myc* and *ras* in stromal and epithelial cells from urogenital sinus allows analysis of paracrine/autocrine pathways	*97*
Human cell lines	PC-93	AI	?	?	Established from primary AD tumors	*98*
	PC-3	AI	?	−	Established from	*99*
	DU-145	AI	?	−	prostate cancer	*100*
	LNCaP	Parent line, AS;	Mutated		metastases	*101*
		C4 line, AI;				*102*
		C4-2 line, AI;				*103*
		P104-R2 line			Stimulated by finasteride	*104*
Human xenograft lines	CWR22	AD	?			
	CWR22R	AI	?			*105*
	PC-82	AD	+			
	PC-EW	AD	+			*106*
	Honda	AD	+			*107*
	LuCaP	AS	+	+		*108*
	PR-2	?	?		Small cell carcinoma of prostate	*109* *110, 111*

gland and then combining and engrafting them under the renal capsule of syngeneic male mice.

HUMAN PROSTATE CANCER CELL LINES

Most human prostate cancer cell lines have been established from metastatic deposits, with the exception of PC-93 *(98)*, grown from an AD primary tumor. However, PC-93 and other widely used lines, including PC-3 *(99)*, DU-145 *(100)*, and TSU-PR1 *(114)*, are all AI; all lack ARs (with the possible exception of PC-93), PSA, and 5α-reductase; and all produce poorly differentiated tumors if inoculated into nude mice. The paucity of cell lines that are AD has made studies of the progression of prostate cancer using human material very difficult. However, metastatic sublines of PC-3 have been developed by injection of cells into nude mice via different routes, especially orthotopically *(115)*.

The LNCaP cell line, established from a metastatic deposit in lymph node *(100)*, is the only human prostate cancer cell line that demonstrates androgen sensitivity but not androgen dependence. After its initial characterization *(100)*, several laboratories found that this line was poorly tumorigenic in nude mice unless coinoculated with tissue-specific mesenchymal or stromal cells *(116)* or Matrigel *(117)*, suggesting that extracellular matrix and paracrine-mediated growth factors play a role in prostate cancer growth and site-specific metastasis *(118)*. LNCaP cells grown in castrated mice that had progressed to the AI state were cultured to obtain new cell lines. The C-4 LNCaP *(119)* line produces PSA and a factor that stimu-

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lates PSA production, and the C4-2 line metastasizes to lymph nodes and bone after subcutaneous or orthotopic inoculation *(102, 103)*. Another subline of LNCaP, LNCaP 104-R2, cultured in androgen-depleted medium for >100 passages, is stimulated by finasteride, causing some concern over the use of antiandrogens for the treatment of late-stage prostate cancer *(104)*.

HUMAN XENOGRAFT MODELS

The CWR22 xenograft line is highly AD in vivo and relapses to an AI line, CWR22R, after androgen withdrawal *(105)*, thus providing a useful model for studies of the progression of human prostate cancer. PC-82 is one of several xenograft lines established in Rotterdam. PC-82 and PC-EW are AD prostate cancer xenograft lines *(106, 107)* that are useful for studying AR regulation *(119)*. Honda and LuCap xenografts are also both AS *(108, 109)*. The UCRU-PR-2 xenograft line, established from a patient with prostatic adenocarcinoma, is a small cell carcinoma of the prostate that secretes pro-opiomelano-corticotropin-derived peptides *(110, 111)*. Cell lines have not been established in vitro from these lines.

TRANSGENIC MOUSE MODELS

Fusion of tissue-specific promoter elements to oncogenes has been used to target expression of the oncoprotein to a given organ, sometimes with the development of cancer in that organ. The use of a viral promoter with the oncogene *INT2* causes benign hyperplasia of the prostate in transgenic mice *(49)*, whereas TGF-α expressed under the control of a metallothionine gene promoter produced prostate epithelial hyperplasia and focal dysplasia resembling carcinoma in situ *(121)*. Regulatory elements of the rat *probasin* gene have been shown to target hormonally regulated expression of heterologous genes in the prostates of transgenic mice *(122)*. Two new transgenic models have excellent potential for studies of prostate cancer progression. These are C3(1)/SV40 large T antigen transgenic mice, which show a progression to cancer from intraepithelial neoplasia *(123)*, and the TRAMP (transgenic adenocarcinoma mouse prostate) model, in which metastases develop *(124)*.

Growth Factor Families Important in Prostate Cancer

The growth factor families involved in prostate cancer progression are shown in Table 1. Some of these families appear to play a more major role in prostate cancer progression than others. Their characteristics are described in Table 3 *(51, 125–144)*.

THE TGF-β FAMILY

Three proteins of the TGF-β superfamily, TGF-β1, TGF-β2, and TGF-β3, are expressed during prostate development and in the adult prostate in both normal and malignant tissue *(125, 126)*. More distantly related peptides, including the activins and inhibins, are not discussed here. The TGF-β family is highly conserved between species *(145)*. TGF-β1 predominates in all tissues,

Table 3. Characteristics of growth factors/receptor pathways in prostate cancer.

Factor/Receptor	Isoforms/Size, human, mature form	Biological activity	Comments	References
TGF-β	TGF-β1, 44.3 kDa 390 aa TGF-β2, 47.8 kDa 414 aa	Requires proteolytic cleavage	TGF-β1 and 2 are homodimers of 25 kDa protein; 75% homologous	*125, 126*
	TGF-β3, 47.3 kDa 410 aa		TGF-β3 is a heterodimer, 80% homology to TGF-β2	*127, 128*
TGF-β receptors	TbetaR-I, 53 kDa 505 aa TbetaR-II, 68 kDa 565aa	Transmembrane serine-threonine kinases	Trigger decreases in expression of src tyrosine-kinases	*129–131*
	TbetaR-III	Membrane proteoglycan	Does not transduce signals but binding of TGF-β activates other receptors	*132*
EGF	6 kDa, 53 aa	Secreted by normal and tumor cells	Share 35% homology; signal through same EGF receptor (170	*133, 134*
TGF-α	6 kDa, 50 aa	Secreted by tumor cells	kDa, 1210 aa)	*135*
bFGF	4 variants 18, 21, 21.5, and 22.5 kDa, all 155aa	No signal sequence	18 kDa protein found in cytosol; others nuclear	*136–138*
aFGF	17.5 kDa, 155 aa	No signal sequence	On chromosome 5; 55% homology with bFGF	*51, 139*
KGF	19 kDa	Has signal sequence	30–40% homology with other FGFs	*140, 141*
IGF	IGF-1, 70 aa IGF-2, 7.5 kDa, 60aa	Cause prostate cell proliferation		*142*
IGF receptors	Type I, 1367aa; Type II		Type I binds IGF-1 with higher affinity than IGF-2; type II prefers IGF-2	*143*
IGF-BP	Types 1–6, 24–43 kDa, all 200–300 aa	Regulate IGFs by modulating their receptor access		*144*

aa, amino acids; kDa, kilodaltons; b, basic; a, acidic.

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whereas the expression of TGF-β2 and TGF-β3 is more tissue-restricted *(126, 127, 146)*. However, all three isoforms share a multiplicity of biological effects *(126)*. TGF-βs induce angiogenesis in wound healing *(41)*; stimulate the synthesis of extracellular matrix components such as collagen, fibronectin, proteoglycans, and integrins; and also can inhibit extracellular matrix formation through down-regulation of a wide variety of proteases *(42, 145)*. TGF-βs also can induce proliferation of mesenchyme cells and can act as growth inhibitors of epithelial cells *(147)* and as important immunoregulatory molecules *(43)*.

How TGF-β1 works on prostrate cancer cells is unclear. TGF-β1 binds to the TGF-β2 receptor (TbetaR-II), which recruits the TGF-βI receptor (TbetaR-I) to initiate a signal transduction cascade. Although TbetaR-I and TbetaR-II are transmembrane serine-threonine kinases *(129)*, they can trigger decreases in the expression of members of the Src family of tyrosine kinases *(130)*, affecting protein tyrosine kinase signaling and hence growth regulation *(131)*. TGF-β3 receptor (TbetaR-III) plays a more indirect role because it delivers ligands to the signaling receptors *(132)*. TGF-β1 prevents phosphorylation of the retinoblastoma gene product *(149)* that is involved in cellular proliferation and should, therefore, inhibit proliferation. Thus, in the nondiseased prostate, TGF-β is believed to play a role in regulating cell growth through its antiproliferative effects because it can inhibit the mitogenic effects of EGF/TGF-α on epithelial cells *(28)* and of basic FGF (bFGF) on stromal cells *(29)*.

TGF-β1, TGF-β2, and TGF-β3 are important for fetal prostate development and are expressed at high concentration in 17-day murine urogenital sinus mesenchyme but not in the epithelium. In adult mice, only TGF-β1 is increased, with the highest concentrations observed during epithelial duct formation *(30)*. The expression of TGF-β1 and its receptor are negatively regulated by androgen in the prostate. In apparently healthy rats, expression of TGF-β and TbetaR-I and TbetaR-II is upregulated within 24 h after castration and has been linked to programmed cell death in the prostate *(31)*. In tumors, androgen withdrawal results in increased TGF-β1 and receptor concentrations in both rat Dunning R3327 PAP *(31)* and human PC-82 prostatic cancer cells *(32)*. Increasing expression of TGF-β1 appears to be important in prostate cancer progression, but its exact role remains uncertain *(125)*. In humans, increased mRNA and protein expression of both epithelial cell-specific and, to a lesser extent, stromal cell expression of intracellular TGF-β1 is associated with prostate cancer progression *(33, 34)*. Moreover, serum TGF-β1 concentrations in patients with lymph node and/or distant metastases were markedly higher than in patients with localized disease but did not differ substantially among localized cancers as to tumor extension *(35)*. Similarly, increased TGF-β1 expression is associated with increasing malignancy in the mouse MPR model *(36)* and, in particular, in metastatic versus primary cell lines *(37)*. Moreover, transfection of TGF-β1 into rat R3327-MATLyLu prostate cancer cells resulted in larger, less necrotic, and more metastatic cells than controls *(38)*.

Not only is expression of TGF-β1 increased with prostate cancer progression, but secretion also occurs. A factor involved in TGF-β1 secretion *(150, 151)*, the latent TGF-β1 high molecular weight complex, is associated with a latent binding protein *(150)* that is not expressed in prostate cancer *(150)*. This suggests the possibility that progression of this disease is associated with TGF-β1 switching from an autocrine/paracrine to a juxtacrine mode of action. This is reflected by secretion of TGF-β1 by the human AI cell lines DU-145 and PC-3 but not by the AS LNCaPs *(152)*. Less information is available on the other TGF-β isoforms in prostate cancer. The PC-3 cells do not respond to TGF-β2 *(153)*, indicating that an autocrine pathway is not present. Studies in the MPR model show that TGF-β3 but not TGF-β2 concentrations are increased in carcinomas *(36)*. These observations may reflect increased TGF-β1 concentrations given that TGF-β1 has been shown to up-regulate the expression of TGF-β2 and TGF-β3 in many epithelial and stromal cell lines *(154)*.

Studies of human prostate cancer cell lines suggest that changes in sensitivity to TGF-β1 may play a role in prostate cancer progression, but different results are obtained in clinical samples and in rodent cells. TGF-β1 inhibits the proliferation of AI PC-3 and DU-145 cells in a dose-dependent manner but not the proliferation of AS LNCaP cells *(39)*. TGF-β insensitivity in LNCaP cells has been attributed to a genetic change in their TGF-β receptor I gene *(39)* and can be reversed by transfection with the wild-type type I receptor gene *(39)*. However, in clinical samples of prostate cancer, an inverse correlation between the loss of expression of TbetaR-I and TbetaR-II and tumor grade is observed *(40)*. This could provide a potential mechanism for prostate cancer cells to escape the growth-inhibitory effects of TGF-β. In the rodent models, sensitivity to TGF-β growth inhibition is lost with tumor progression. In the R3227 Dunning model, functional TbetaR-I and TbetaR-II, as well as decreasing sensitivity to TGF-β1, are found in advanced prostate carcinoma cells *(41)*, whereas metastatic cell lines derived from the MPR model secrete but do not respond to TGF-β1 *(37)*. This suggests the role of TGF-β in the progression of prostate cancer cell lines from rodent and human cancers.

TGF-β can modulate extracellular matrix proteins and has effects on bone-derived cells, suggesting the possibility that it may promote the spread of prostate cancer cells and provide a suitable milieu in bone for metastatic growth. TGF-β can induce type IV collagenase matrix metalloproteinase-9 and plasminogen activator inhibitor 1 in mouse prostate-derived cell lines *(37)* and procollagen Iα-chain mRNA in human osteoblast-like cells *(131)*. TGF-β1 also stimulates adhesion of PC-3 cells to bone matrix proteins, possibly via the α2 β1 integrin receptor *(155)* and the migration of human osteoblasts *(44)*.

In summary, the role of TGF-β in the prostate is highly

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complex (Fig. 1). In the nondiseased prostate, TGF-β can counterbalance the mitogenic effects of various growth factors, thus having a role in growth regulation. Moreover, its expression and that of its receptors is associated with castration-induced prostate cell apoptosis. In cancer, TGF-β expression is increased as prostate cancer progresses. It can be secreted and may show autocrine rather than paracrine regulation, although its autocrine role is as yet unexplained. The sensitivity of prostate cancer cells from different species to respond to TGF-β varies, in one case, because of genetic changes in the receptor. TGF-β also has the capacity to modulate matrix metalloprotease production and to stimulate adhesion of prostate cancer cells to bone cells, providing a possible role in prostate cancer cell metastases. Given its role in angiogenesis and as an immunoregulatory molecule, the secretion of TGF-β by prostate cancer cells could have important effects on the cancer cell environment.

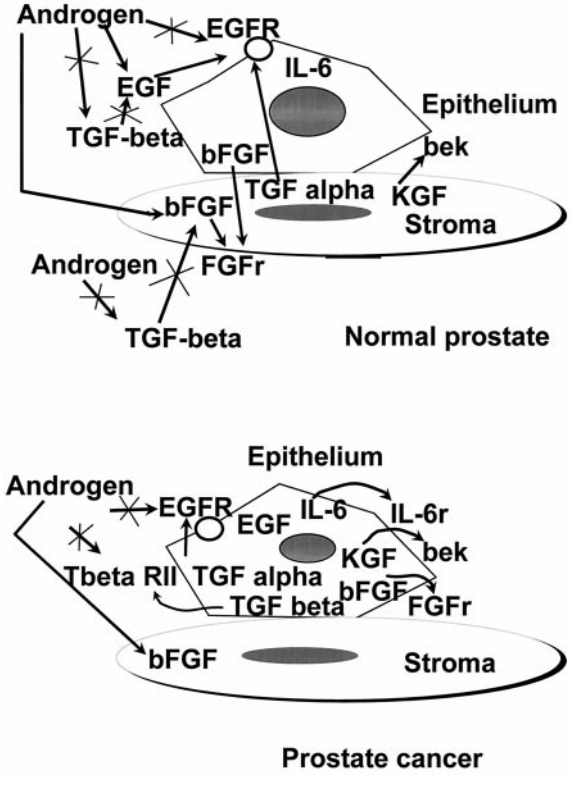

Fig. 1. Changes in growth factor pathways in prostate cancer compared with the nondiseased prostate.

In the normal prostate (*A*), most growth factor pathways are paracrine. TGF-α is produced by stromal cells but binds to the EGFR on epithelial cells; bFGF is produced by both prostate stromal and epithelial cells, but its receptors (FGFr) are only found on stromal cells; KGF is produced by stromal cells and binds to BEK receptors on epithelial cells. TGF-β down-regulates proliferation of stromal cells in response to bFGF and proliferation of epithelial cells in response to EGF or TGF-α. Androgen negatively regulates TGF-β and EGFR and positively regulates EGF. In prostate cancer (*B*), the requirement for epithelial-stromal cell interactions is reduced. Autocrine pathways for interleukin-6 (IL-6), KGF, bFGF, TGF-α, EGF, and TGF-β all exist within prostate epithelial cells. Withdrawal of androgen results in up-regulation of both EGFR and TGF-β expression. The effects of these changes on prostate cell behavior and the ability of prostate cancer to metastasize are explained more fully in the text.

EGF and TGF-α

EGF and TGF-α are two structurally and functionally related peptides (Table 3) that signal through the same 170-kDa EGF receptor, a transmembrane tyrosine kinase *(133, 134)*. Consequently, their biological activities overlap and include roles in embryogenesis, cell differentiation, and angiogenesis *(135)*.

Prostatic fluids from nondiseased human prostates contain large amounts of EGF *(64)*, which appears to be an important regulator for normal growth in both rat and human prostate *(156)*. Human prostate epithelial cells require EGF in serum-free medium for growth in primary culture *(157)*, and nondiseased human fetal prostatic fibroblasts can replicate in response to this cytokine *(65)*. Immunohistochemical studies on nondiseased and benign prostatic tissue have shown that TGF-α expression occurs predominantly in the stroma, whereas its receptor is expressed by epithelial cells, suggesting a paracrine/juxtacrine mode of regulation *(72)*.

Prostatic cell EGF expression is regulated by androgen. Castration of mice or rats results in a marked decrease in EGF expression *(66, 67)*, which can be restored by testosterone administration. There is some evidence that TGF-α may also be androgen-related. Castration of rats, followed by androgen-induced regrowth, results several days later in increased TGF-α mRNA *(67)*; because of the timing, this may reflect the induction of other regulatory factors by androgen.

Increased expression of EGF/TGF-α has been linked to prostate cancer development. A slight but important rise in EGF protein expression is observed in the epithelial cells of prostate cancer specimens *(68, 69)*, and similarly, TGF-α protein concentrations are raised in human prostate cancers in comparison with benign tissue *(70, 71)*. In many tumors, TGF-α and epithelial growth factor receptor (EGFR) are coexpressed in the epithelial cells, suggesting a switch from paracrine to autocrine regulation *(72)*.

These studies are supported by findings in vitro using human prostate cancer cell lines. LNCaP cells are stimulated in vitro *(158)* and in vivo *(159)* by EGF and TGF-α; in LNCaP cells undergoing AI progression, the EGFR is up-regulated *(160)*. Both LNCaP and DU-145 cell lines secrete EGF, with 14-fold higher expression in the DU-145 line *(161, 162)*. Interestingly, although both lines express single EGF-binding sites of similar high affinities, LNCaP cells exhibit markedly enhanced DNA synthesis in response to exogenous EGF compared with DU-145 cells, suggesting that locally produced EGF may be an important regulator of cell proliferation in the AS cells compared with AI cancer cells *(163)*. Similarly, changes in TGF-α expression are observed in human prostate cancer cell lines. The AI lines PC-3 and DU-145 express higher concentrations of TGF-α mRNA than do LNCaP cells *(164)*, but at the protein level, PC-3 cells express the least TGF-α, whereas DU-145 cells express the most *(165)*. However, PC-3 cells are more sensitive to TGF-α-induced proliferation than DU-145 cells, although they express

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lower concentrations of EGFR *(165)*. The differential sensitivity of prostate cancer cells to TGF-α suggests that the autocrine loop involving TGF-α in prostate cancer cell lines may be further regulated by other unknown factors.

The importance of these findings may relate to the ability of EGF to enhance prostate tumor cell invasion. EGF increased the invasive capacity of PC-3 cells in a Boyden chamber microinvasion assay. This was associated with increased expression of urokinase plasminogen activator, a serine protease, mRNA, and protein *(73)*.

Because both EGF and TGF-α bind to the EGFR, regulation of receptor expression is mandatory to an understanding of prostate cancer development and progression. In the nondiseased prostate, EGFR expression is largely confined to the basal cell layers *(166–168)*, whereas in human cancer cell lines, EGFR expression is increased with increasing malignancy. Thus, the AI DU-145 prostate cancer cells express 10-fold more EGFR than do AS LNCaP cells *(163)*. Results from immunohistochemistry of human prostatic tumors are inconclusive. Some studies reported no difference in EGFR expression between nondiseased and malignant cells *(168)*; others noted a decrease in EGFR expression in advanced malignancy compared with nondiseased tissue *(166, 167)*, whereas still other reports described an increased EGFR mRNA and protein expression in secretory epithelial cells that is associated with advanced cancer *(166)* or with increasing tumor grade *(169)* but not with patient survival *(170)*. Such discrepancies may relate to differences in methodology, or they may reflect heterogeneity between prostate tumors in relation to EGFR expression.

EGFR expression in nondiseased prostate tissues appears to be under negative androgen regulation. Prostate biopsies from patients with benign hyperplasia show substantially increased EGFR expression after androgen withdrawal *(171)*. Likewise in rats, castration induces increased EGFR expression with a return to normal concentrations after administration of dihydrotestosterone *(65)*, whereas exposure of LNCaP cells to the synthetic androgen, R1881, up-regulates EGFR expression from 11 500 to 28 500 sites/cell *(172)*. However, the LNCaP data may be affected by the presence of a mutated AR in these cells *(23)*. Taken together, these data suggest the possibility that the regulation of EGFR by androgen may be disrupted in prostate cancer. What impact this has on the actions of the EGFR ligands, EGF and TGF-α, remains to be elucidated.

In summary, in the nondiseased prostate, EGF appears to be an important regulator of growth *(173)*, and its expression is positively regulated by androgen, whereas that of EGFR is negatively regulated by androgen. TGF-α is predominantly expressed in a paracrine fashion by nondiseased prostate stroma. In prostate cancer, EGFR expression is up-regulated with progression as judged from prostate cancer cell lines, but evidence from clinical trials leaves its role controversial. However, up-regulation of EGF, TGF-α, and EGFR suggest their autocrine expression in advanced cancer. Increased EGF expression appears to be associated with the invasive ability of prostate cancer cells.

FGFs

The FGF family consists of nine structurally related heparin-binding peptides that share 40–55% amino acid homology *(174)*. Four genes have been identified that encode distinct high affinity (K_d = 10^{-11}mol/L) receptors for FGFs, fibroblast growth factor receptor 1 (FGFR-1), FGFR-2, FGFR-3, and FGFR-4 *(175)*. Each encodes transmembrane receptor tyrosine kinases, derived from alternative mRNA splicing, that give rise to the potential of multiple binding combinations for each FGF peptide *(176)*. These receptors also differ in their affinity for each member of the FGF family. The FGFs show different cellular locations. Some forms are secreted, and others are located in the nucleus *(176, 177)*. Three members of this family, bFGF (FGF-2), acidic FGF (aFGF or FGF-1), and KGF (FGF-7) have been implicated in prostate cancer.

bFGF

bFGF is encoded by a 36-kb single copy gene *(178, 179)* with four variants from alternative splicing (Table 3). Differing intracellular locations suggest different biological functions *(175)*. Despite no signal sequence *(178)*, bFGF has been found cell-associated or deposited into the basement membrane *(176)*, with its release possibly due to cell injury in vivo.

bFGF is synthesized by a wide variety of cell types, including epithelial cells, stromal cells *(45)*, macrophages *(180)*, and endothelial cells *(181)*. Its biological functions include a role in angiogenesis, tissue development and differentiation *(182, 183)*, and the ability to modulate neural function *(184)*.

Prostatic stromal and epithelial cells actively synthesize bFGF *(45)*. However, in the nondiseased prostate, only the stromal cells express the bFGF receptor and thus respond to this growth factor, suggesting that bFGF plays an important role in maintaining prostatic mesenchymal homeostasis *(46)*. Human fetal prostatic fibroblasts have also been shown to proliferate in response to bFGF *(185)*, which can overcome the TGF-β1 inhibition of these cells *(186)*. Some conflict concerning the response of the bFGF pathway to androgen exists. In 7-day-old rats, castration results in decreased bFGF expression, with increased bFGF mRNA 16 h after exposure of regressed prostate to androgen *(187)*. In AS LNCaP cells, androgen causes an increase in bFGF expression *(188)*, whereas other human prostatic cancer cell lines produce bFGF independently of androgen *(189)*. Similarly, AI cells from Shionogi mice produce a bFGF-like protein *(190)*. These data suggest that production of bFGF becomes AI as cancer progresses.

Further evidence of a role for the bFGF pathway in prostate cancer progression comes from studies in the Dunning tumor model *(48)*. A switch in exon IIIb (which has a high affinity for KGF) of the *FGFR* gene to exon IIIc

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(with a high affinity for bFGF and aFGF) occurs in malignant epithelial cells as they become independent of their requirement for stroma *(48)*. This suggests that prostate cancer progression may be associated with a switch from stromal autocrine bFGF regulation to epithelial autocrine regulation. It has been proposed that bFGF may enable the epithelial cell to metastasize by promoting angiogenesis in the malignant tumor. These studies are supported by findings in human prostate cancer cell lines. The AS LNCaP cell line produces very low concentrations of bFGF and FGFR *(47)* compared with the AI cell lines PC-3 and DU-145 *(47)*. However, the data are confusing; although LNCaP and DU-145 cells proliferate in response to bFGF, the more malignant PC-3 cells do not *(47)*. As with the rat model, this suggests that some form of autocrine regulation of bFGF by epithelial cells may be an important step in prostate cancer progression.

Because bFGF is angiogenic, its increased production in late stage prostate cancer may promote angiogenesis, allowing tumors to grow and metastasize *(50)*. The acquisition of metastatic ability in prostate cancer has been correlated with increasing microvessel density *(191, 192)*, implying that angiogenic ability is necessary for metastasis to occur.

In summary, bFGF appears to be produced in an autocrine fashion by nondiseased prostate stromal cells and is important for maintaining their homeostasis. As cancer occurs and progresses, the production of bFGF becomes independent of androgen and becomes regulated in an autocrine fashion by prostate cancer epithelial cells. The ability to produce bFGF may stimulate angiogenesis, allowing the cells to metastasize.

aFGF

aFGF (Table 3) is important in the development of the prostate in rats, but it has not been detected in human prostatic tissues. In 6- to 8-week-old rats, expression is confined to epithelial cells and is increased, but it declines at 14 weeks and cannot be detected by 35 weeks *(51)*. Epithelial and mesenchymal cells from nondiseased rat prostate and various grades of rat prostate tumors exhibit specific aFGF membrane receptor sites *(52)*. In the Dunning model, aFGF alone is expressed only by stromal cells of the slow-growing AD R3327PAP tumor, whereas fast-growing metastatic AT-3 cells express both aFGF and bFGF *(52)*. This suggests that, in rat models of prostate cancer, progression is associated with a switch in regulation of aFGF from paracrine to autocrine control.

KGF

Another member of the FGF family, KGF *(140)*, is important in prostate development (Table 3). In the rodent prostate, KGF is expressed and secreted by nondiseased stromal cells, but only epithelial cells express the BEK/FGFR-2 receptor gene that binds KGF, suggesting that this cytokine controls epithelial cell proliferation in a paracrine manner *(53)*. The BEK receptor has been shown to require an interaction with heparan sulfate proteoglycans to facilitate binding to its ligands *(54)*. Serum-free organ culture of neonatal rat ventral prostates, which express both KGF and BEK receptor mRNA, has been developed as a model to study prostate development *(193)*. The addition of an anti-KGF antibody could inhibit testosterone-induced branching in this model, and in testosterone-free conditions, KGF could mimic the effects of the hormone. These data suggest that, in the developing prostate, KGF may act as a paracrine mediator of androgen action. An important and similar role for KGF has also been implicated in the human prostate. KGF and its receptor are expressed in the stromal and epithelial cells, respectively, and in both fetal and adult nondiseased prostates *(55)*. This cytokine is also a potent mitogen for nondiseased human prostatic epithelial cells in vitro *(141, 194)* and promotes the growth of these cells under serum-free conditions *(195)*.

In human prostatic carcinomas, in situ hybridization studies have shown increased expression of the *KGF* gene and receptor in epithelial cells of high-grade carcinomas but not in benign hyperplasia of the prostate, suggesting a switch from a paracrine to an autocrine loop *(55, 56)*.

In summary, multiple autocrine and potentially intracrine ligand-receptor loops result from alterations within the FGF-FGFR family, which may underlie the autonomy of malignant tumor cells. bFGF and KGF appear to be produced by nondiseased prostate stromal cells in an autocrine and paracrine fashion, respectively, and are important for maintaining prostate homeostasis. As cancer occurs and progresses, the production of bFGF becomes independent of androgen and becomes regulated in an autocrine fashion by prostate cancer epithelial cells. The ability to produce bFGF may stimulate angiogenesis, allowing the cells to metastasize. Although aFGF appears to have a role in the rat prostate, its importance in the human prostate has not been ratified. The production of KGF by prostate stromal cells appears to control epithelial cell proliferation in a paracrine manner, but in human prostate cancer, autocrine production of KGF accompanies the progression to AI disease.

IGFs

IGFs are polypeptide growth factors with functional homology to insulin, but in contrast to insulin, these proteins are locally produced by a wide variety of tissues. Regulation of their production and function is extremely complex. There are two IGF peptides, IGF-1 and IGF-2, two cell surface receptors (the type 1 IGF receptor family and the type 2 IGF receptor), and at least six specific high-affinity binding proteins, IGF-BP-1 through IGF-BP-6, that regulate IGF availability and are in turn regulated by a group of IGF-BP proteases that cleave IGF-BPs to modulate IGF action (Table 3).

In the nondiseased prostate, IGFs are produced only by stromal cells, but normal epithelial cells express IGF-1 receptors and secrete predominantly IGF-BP-4. However,

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they also secrete IGF-BP-2, IGF-BP-3, and IGF-BP-6, suggesting a paracrine mode of regulation *(58, 59)*. Some controversy concerning the IGF loop in prostate cancer exists. Some authors *(74)* have shown that DU-145 cells can proliferate in response to IGF-1 but do not produce this protein, suggesting maintenance of the paracrine mode of action of IGFs in prostate cancer. However, others *(196)* describe autocrine production of IGF-1 in PC-3, DU-145, and a subline of LNCaP cells, all of which were reported to grow in serum-free medium, secrete IGF-1, and display constitutively autophosphorylated IGF-1 receptors. The LNCaP cell line also proliferates in response to IGF-1 but does not produce it; however, this only occurs in synergy with dihydrotestosterone *(197)*. IGF-1 may act directly through the AR pathway *(27)* and in turn may be regulated through an EGF autocrine growth regulatory loop *(74)*. IGF-2 protein secretion has been detected in all three human prostate cancer cell lines, and under serum-free conditions, as described above, each can produce IGF-1 *(196)*. This suggests that the capacity for autocrine production of EGFs by prostatic epithelial cells exists, and that this production may play a role in prostate cancer development.

This situation is even more complicated, however, because changes in the expression of the IGF-BPs are observed in all of these lines. For instance, PC-3 cells express large amounts of IGF-BP-2 and IGF-BP-3 and less IGF-BP-4, whereas LNCaP cells express only IGF-BP-2 *(60)*. Thus, dysregulation of the IGF-BP system may be associated with prostate cancer development (Fig. 2). Constitutively autophosphorylated IGF-1 receptors are also displayed by the PC-3, DU-145, and LNCaP cell lines *(196)*, but proliferation in response to IGF in these cell lines appears to be regulated by the autocrine secretion of IGF-BPs *(74)*. In serum-free culture, DU-145 cells produce IGF-BP-1, but the addition of an antibody that binds this protein inhibits the effects of IGF-1 *(74)*. There is also evidence that some IGF-BPs may be under androgen regulation. PC-3 cells stably transfected with an active AR construct do not produce IGF-BP-3 and proliferate in response to IGF-1 or IGF-2, in contrast to untransfected cells. PSA, a serine protease that is up-regulated by androgen, can cleave IGF-BP-3 *(61)*, and the nerve growth factor γ-subunit, which has high sequence homology with PSA, also has this capacity *(198)*. This could release IGFs locally to stimulate prostate cancer cell growth. These data have been interpreted to suggest that androgen may indirectly modulate IGF-induced proliferation of prostate cancer cells by regulating IGF-BP-3 production *(62)*.

In prostate cancer patients, serum IGF-BP-2 is increased; this is related to a rise in PSA concentrations, suggesting that the prostate is the source of IGF-BP-2 production *(61)*. Because PSA is a protease for IGF-BP-3, it likely that the rise in PSA may be related to lowered concentrations of IGF-BP-3 in prostate cancer patients *(61)*.

A potential role for the IGFs in prostate cancer progression is in the development of bone metastasis. Both IGF-1 and IGF-2 mRNA transcripts have been detected in nondiseased human osteoblast-like cells *(199)* and appear to have an important role in bone formation *(63)*. Interestingly, studies have shown that factors that decrease the activity of IGF-BP-3, such as dexamethasone, also inhibit bone formation *(63)*, suggesting an important role for IGF-BP-3 in the formation of bone metastasis in advanced prostate cancer.

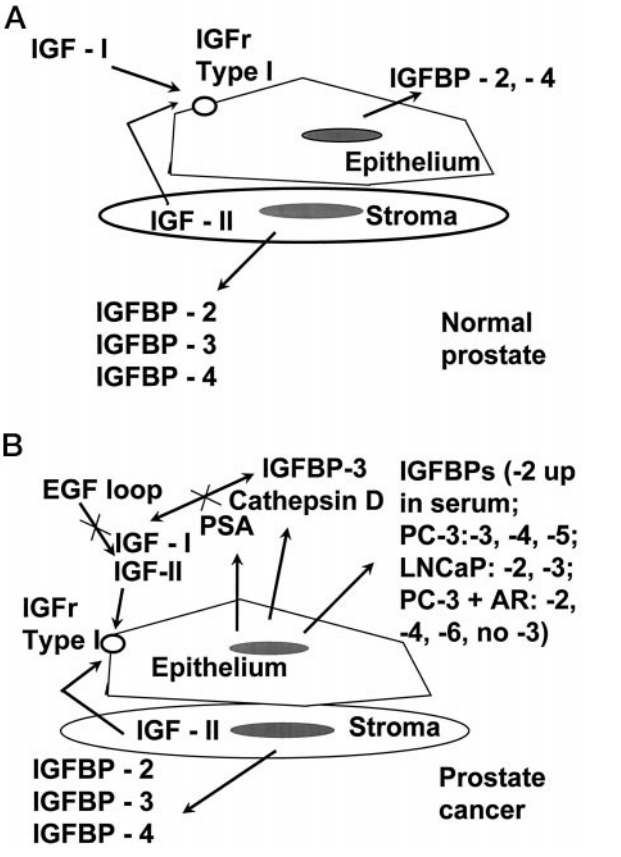

Fig. 2. Changes in the IGF axis in prostate cancer compared with nondiseased prostate cells.

In the normal prostate (*A*), IGF-2 is produced by stromal cells and, like IGF-1, binds to IGF receptor on prostate epithelial cells. The availability of IGF-2 to stimulate proliferation of prostate epithelial cells is regulated by IGF-BPs, a variety of which are produced by both epithelial and stromal cells. In prostate cancer (*B*), the balance of IGF-BPs produced is altered; this varies in different cell lines and is also reflected in changes in serum concentrations found in patients with prostate cancer (see text). Prostate epithelial cells secrete PSA and cathepsin D, enzymes that can cleave IGFBP-3 and interfere with its binding to the IGFs, thus increasing the availability of IGFs for stimulating prostate epithelial cell proliferation. The IGF axis is down-regulated by the EGF/EGFR autocrine loop, which is up-regulated after androgen withdrawal.

In summary, the IGFs appear to have an important role in the development of prostate cancer. This can be achieved by modulation of paracrine pathways, which occur in the nondiseased prostate, to autocrine pathways, seen in prostate cancer cell lines, and also by modulation of the concentrations of different IGF-BPs that are differentially expressed in the normal condition vs cancer. Androgen appears to regulate the expression of some of these IGF-BPs, and PSA, which is androgen-regulated, particularly can cleave IGF-BP-3. The local release of IGFs

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in distant tissues as a result of cleavage of IGF-BP-3 may allow prostate cancer growth as metastatic deposits.

RETINOIC ACID (RA) AND VITAMIN D_3

Strong evidence suggests that dietary factors can affect the incidence of prostate cancer *(200)*. Two such factors are RA (vitamin A) and 1,25-dihydroxyvitamin D (vitamin D_3). RA can induce the differentiation of a wide variety of cells and elicit changes in growth factor protein and receptor expression *(201–203)*. The active form of vitamin D_3, 1,25-dihydroxyvitamin D, affects calcium and phosphate homeostasis *(204, 205)* and is an important modulator of proliferation and differentiation in both nondiseased and malignant cells *(206, 207)*. Recent evidence suggests a role for these chemical hormones in regulating the proliferation and differentiation of prostate cancer cells.

RA has marked but different effects on different human prostate cancer cells in vitro. LNCaP cells are induced to differentiate in response to RA in the presence or absence of androgens *(208)*. DU-145 cells are growth-inhibited by 13-*cis*-retinoic acid *(209)*. In contrast, RA induces increased invasiveness of PC-3 cells by up-regulating urokinase plasminogen activator expression, suggesting that in more advanced tumors, RA may promote disease progression *(210)*. The different cell responses to RA may relate to their differences in AR expression. RA can inhibit the binding capacity of the LNCaP AR (which is mutated) by 30–40% *(211)*, whereas PC-3 cells lack an AR.

A deficiency in vitamin D has been proposed to increase the risk of prostate cancer *(212)*. This result is still controversial because a comparison of serum concentrations of vitamin D metabolites between prostate cancer patients and age-matched controls showed no substantial differences *(213)*. However, a striking difference between black and white men in the allelic frequencies of the gene encoding a vitamin D_3-binding protein has been reported, raising the possibility that this protein may be important in indicating risk of prostate cancer development *(214)*.

Most evidence concerning the role of vitamin D_3 in prostate cancer comes from studies of prostate cancer cell lines. The human lines DU-145, PC-3, and LNCaP all express receptors for vitamin D_3, with PC-3 showing the greatest binding capacity *(215)*. Proliferation of LNCaP and PC-3 cells is inhibited by vitamin D_3 *(215, 216)*, and proliferation of DU-145 is inhibited only by analogs of vitamin D_3 *(217)*. In LNCaP cells, exposure to vitamin D_3 can neutralize the proliferative effects of androgens, suggesting that it is a strong inhibitor of epithelial cell proliferation *(208)*. Vitamin D_3 can up-regulate expression of IGF-BP-6 mRNA in a dose-dependent manner in all three human prostate cancer cell lines *(216)*, suggesting that it may modulate growth via the IGF axis. In Lobund/Wistar rats, growth of a nontumorigenic AS epithelial cell line derived from the dorsal-lateral prostate is inhibited by vitamin D_3, whereas its AI tumorigenic counterpart is insensitive to these effects *(217)*. Taken together, these results suggest that vitamin D_3 may act as a growth modulator of prostate epithelial cell proliferation, but that its inhibitory effects may be lost in late-stage prostate cancer.

A recent study has shown that prostate cancer may be associated with vitamin D receptor gene polymorphism. Race-adjusted combined analysis showed that men who were homozygous for the *t* allele (shown to correlate with higher serum concentrations of the active form of vitamin D) have only one-third of the risk of developing prostate cancer requiring prostatectomy compared with men who were heterozygotes or homozygous for the *T* allele *(218)*. Because this is a single study, credence awaits confirmation, but this appears to suggest that vitamin D is an important determinant of prostate cancer risk and could lead to strategies for chemoprevention.

The vitamin D receptor is thought to act as a heterodimer with the retinoid X receptor, suggesting functional interactions between 1,25-dihydroxyvitamin D_3 and retinoids *(219)*. The combination of 1,25-dihydroxyvitamin D_3 and 9-*cis*-retinoic acid was shown to act synergistically in inhibiting the growth of LNCaP cells.

In summary, different activities of RA on different cancer cell lines leave its potential role in controlling prostate cancer in doubt. The ability of RA to differentiate LNCaP cells has exciting potential for control of the disease, but its ability to increase invasiveness of PC-3 cells would argue against its use. However, Phase I clinical trials on the use of liarozole, which binds to the cytochrome P450-dependent hydroxylating enzymes involved in RA catabolism *(220)*, and Phase II trials of all-*trans*-RA for control of hormone-refractory prostate cancer are in progress *(221)*. Moreover, the use of liarozole fumarate, a compound that blocks the cytochrome P450-dependent catabolism of RA and which has been successful in reducing both AD and AI tumor growth in the Dunning rat model, has been proposed *(222)*. The progression of prostate cancer to androgen independence is accompanied by a loss in sensitivity to the antiproliferative effects of vitamin D_3. Hence this vitamin could be of therapeutic benefit in patients with early disease but not once progression to late-stage disease has occurred.

INTERACTIONS OF GROWTH FACTOR PATHWAYS IN PROSTATE CANCER

The IGF axis appears to be under the control of other growth factor pathways. EGF, FGF, and TGF-β have been shown to regulate the expression of the IGFs and their binding proteins. Antibodies to EGFR inhibit the secretion of IGF-BP *(74)* and the growth-promoting effects of IGF-1. The addition of bFGF to the human osteoblast cell line MC3T-E1 inhibits IGF-1 and IFG-2 mRNA and IGF-BP-2, IGF-BP-4, IGF-BP-5, and IGF-BP-6 concentrations *(223)*, whereas TGF-β can increase the expression of IGF-1 mRNA in nondiseased human osteoblast-like cells *(224)*. Moreover, as mentioned previously, vitamin D_3 can up-

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regulate expression of IGF-BP-6 mRNA in a dose-dependent manner in LNCaP, PC-3, and DU-145 prostate cancer cells *(216)*. This suggests that the interactions of the IGFs and their binding proteins with other cytokines may in some way regulate prostate growth. Additionally, any changes to this complex IGF regulatory system may promote prostate cancer development. Moreover, several growth factor pathways including IGF-1, KGF, and EGF can aberrantly activate the AR in the absence of androgen, suggesting that the androgen-signaling chain may be activated by growth factors in an androgen-depleted environment *(27)*.

Concluding Remarks

These findings suggest that up-regulation of growth factor production and, in particular, the appearance of several autocrine pathways in prostate epithelial cells (Table 4), apparently bypassing any requirement for stromal cells, may represent an adaptive response to androgen ablation in the growth regulation of AI tumors. Future studies may lead to targeted therapy for patients with advanced prostate cancer, using an understanding of the changes that occur in growth factor pathways as prostate cancer progresses *(225)*. A recent example of a possible antigrowth factor treatment is the use of suramin *(225)*, which has antiproliferative effects against prostate cancer cells in vitro. The mode of action of suramin is unclear, but it may be mediated by its ability to disrupt the cellular energy balance of prostate cancer cells *(226, 227)*. There are conflicting reports about the ability of suramin to interfere with growth factor pathways; it can inhibit the growth-stimulating effects of exogenous testosterone and bFGF *(228)*, but it is not counteracted by 10-fold excesses of exogenous growth factors in vitro *(229)*, and the effects are reversible. Suramin is a polysulfonated naphthylurea with substantial activity in prostate cancer. When high dosages of suramin were used in 38 patients with hormone-refractory prostate cancer, declines in serum PSA of >75% were obtained in 38% of patients, and measurable disease response was observed in 35% of patients *(225, 230)*. Although preliminary studies on the effects of growth factor receptor inhibitors and neural peptide inhibitors on prostate cancer cells in vitro are very impressive, no definite conclusions regarding the efficacy or clinical applicability can yet be made. Understanding growth factors will help novel and innovative therapeutic approaches, but it will obviously require much refinement before being translated from bench to bedside.

Table 4. Expression of growth factors and their receptors in human prostate cancer cell lines.

Secreted growth factor/Receptor	Presence in human prostate cancer cell line		
	LNCaP	DU-145	PC-3
EGF/TGF-α/EGFR	+/+	+/+	?/+
TGF-β/Receptor	−/−	+/+	+/+
IGF-1/IGFr type 1	+/+	+/+	+/+
IGF-2/IGFr type 2	+/−	+/−	?/−
bFGF/bFGFr	−/+	+/+	+/+

Autocrine loops for granulocyte-macrophage-colony stimulating factor, macrophage-colony stimulating factor, and interleukin 6 also exist for each line.

IGFr, insulin-like growth factor receptor; bFGFr, basic fibroblast growth factor receptor; +, factor or receptor expressed; and −, factor or receptor not expressed.

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