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Regulation of Prostate Cell Growth by the Insulin-Like Growth Factor Binding Proteins and Their Proteases

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The Human Prostate Contains all the Elements of a Functional IGF System

Prostate epithelial (PC-E) and stromal cells in primary culture as well as prostatic cell lines express protein and mRNA for IGFs, the IGF-1R and IGFBP-2 through -7. Prostate cancer (CaP) cell lines and PC-E respond to IGFs with growth enhancement which is inhibited by IGFBPs. IGFBP-3 induces apoptosis in the prostate cell lines PC-3 and LNCaP and acts via both IGF-dependent and IGF-independent mechanisms involving binding to several putative IGFBP-3 receptors. IGFBP-3 is also involved in apoptosis induction by other agents including TGFß and p53. A growing number of prostatic proteolytic enzymes have been shown to cleave IGFBPs. These IGFBP proteases (BPPs) have been detected in body fluids such as serum and seminal plasma. Serum BPPs have been detected in a variety of clinical conditions, including CaP. Different BPPs are secreted by both nonmalignant and CaP cell lines and several have now been biochemically characterized. While some BPPs may prove to be unique gene products, all of those identified so far have been previously cloned molecules, many of which are known to act on ECM components. The growing family of prostatic BPP subtypes now includes three categories: kallikreins (such as PSA, hK-2, urokinase), cathepsins (such as cathepsin D & B), and MMPs (MMP-1, -2, and -9).

Experiments utilizing the various different BPPs have demonstrated that the cleavage of the IGFBPs results in IGFBP fragments with much lower affinity to IGFs — the cleavage products typically have one to two orders of magnitude lower affinity relative to the intact peptide. Proteolysis of the IGFBPs results in a local release of previously equestered IGFs as free peptides and in a consequential increase of the IGF-dependent mitogenic effect through interaction with the IGF-R. Thus, it has been speculated that BPPs are co-mitogens with the IGFs. These BPPs have different, and selective, effects on the six known IGFBPs. Some IGFBPs appear to be completely resistant to the effects of BPP which potently cleaves other IGFBPs. The secretion of BPPs and IGFBPs is regulated coordinately by TGFß and other cytokines. It appears that the growth modulation achieved by these cytokines may be related to the induction or suppression of IGFBPs and BPPs. PSA and other proteases can prevent the inhibition of IGF-mediated growth by IGFBPs and thus, can act as co-mitogens with IGFs. In fact, IGFBP-3 proteolysis by PSA prevents both the growth inhibitory and apoptosis-inducing effects of IGFBP-3.

The activity of BPPs in the prostate is modulated by specific natural protease inhibitors which participate in the growth regulatory system here described. α1-antichmotrypsin inhibits PSA and MMPs are regulated by their naturally occurring inhibitors, the TIMPs (tissue inhibitor of metaloproteinases). The ability to specifically inhibit proteases which target the IGFBPs, especially those seen in high concentrations in CaP, might permit renormalization of intact IGFBP levels and reduce abnormal cell growth, invasion, and metastasis. Matrix components as well as IGFBPs constitute the substrates for many of the IGFBP proteases studied. We, therefore, propose that these proteases serve a dual role in the regulation of malignant proliferation and invasion. By cleaving IGFBPs, these proteases provide a mitogenic signal in the form of free IGF. Additionally, through the degradation of ECM, these enzymes coordinate tissue remodeling to allow proliferating cells to penetrate the surrounding matrix. It is further likely that reduced local IGFBP levels may result in the abnormal decrease in apoptosis, observed in CaP. The understanding of these proteases, and the development of specific inhibitors which can alter their postulated procarcinogenic effects, holds promise for the treatment of prostate cancer and other diseases. In summary, benign and malignant prostate tissues are highly responsive to the

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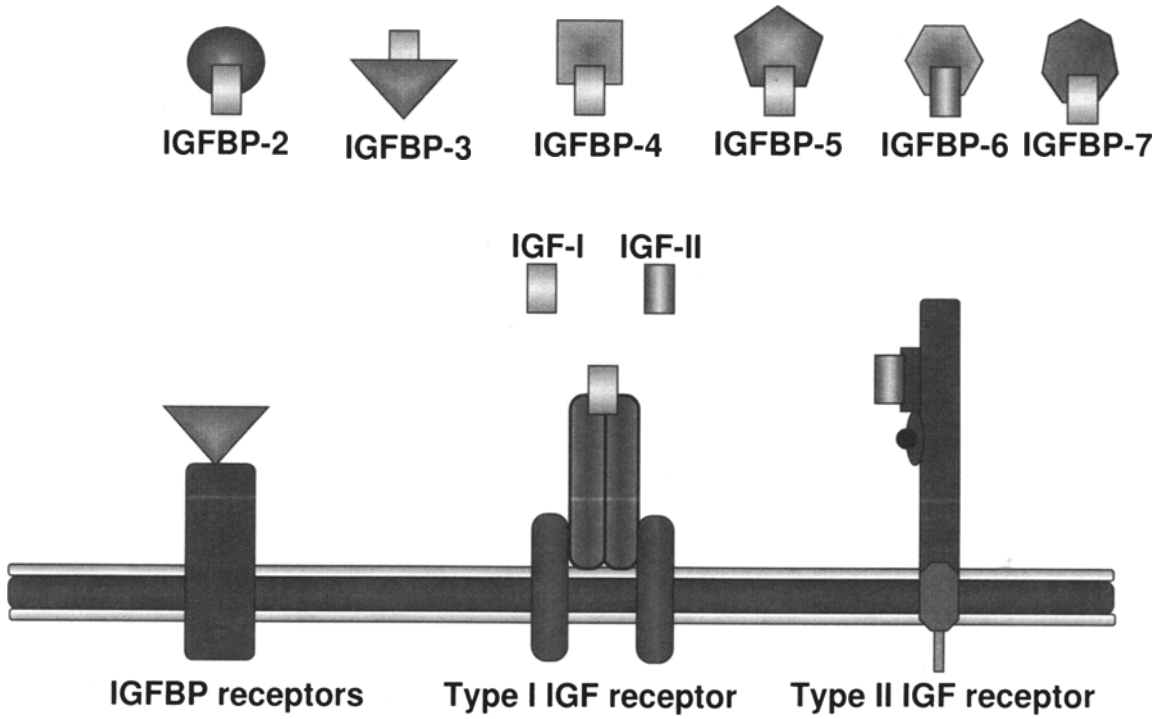

Fig. 1. The prostatic IGF axis. Prostate cells produce IGFs, IGF receptors and IGFBPs, see text for details.

effects of IGFs, IGFBPs and their proteases. These molecules may be important in the patho-physiology, diagnosis and treatment of CaP.

The normal growth and differentiation of tissues results, in part, from a delicate balance of growth factors and their comitogens, and involves coordinated cellular proliferation and extracellular matrix remodeling. Tumor growth and metastasis are processes in which this delicate balance is disrupted and the subsequent proliferation and remodeling occur in an uncontrolled and detrimental way *(1–3)*. It has been well-established that insulin-like growth factors (IGFs) are critical elements of this balance. IGFs are mitogenic agents for multiple cell types, including prostatic epithelial and stromal cells. The actions of IGFs are modulated by a family of at least six IGF binding proteins (IGFBPs) that commonly inhibit, but sometimes enhance, the interactions of the IGFs with the mitogenic IGF receptors (IGF-R) (Fig. 1) *(4)*. The IGFs bind both their receptors and binding proteins with high affinity. The IGFBPs are thought to modulate the action of IGFs in several ways, including an inhibitory model in which IGFBPs sequester IGFs from their receptors, an enhancing model in which IGFBPs transport IGFs to their site of action, or by a receptor-independent model that may involve direct interaction of IGFBPs with IGFBP receptors *(4)*. The IGFBPs also interact with cells to mediate IGF-independent, growth inhibitory effects *(5)*. IGFBP-3, in addition to its growth-inhibitory effects, has been shown to induce apoptosis in prostate cancer cells and to mediate the effects of TGFβ on apoptosis *(6)*. Recently, a growing number of proteolytic enzymes have been shown to cleave IGFBPs. These IGFBP proteases (BPPs) have been detected in numerous body fluids such as serum, urine, and seminal plasma *(7,8)*. Serum BPPs have been detected in a variety of clinical conditions, including pregnancy and prostate cancer (9). Different BPPs are secreted by both nonmalignant and transformed cell lines and several have now been biochemically characterized. Whereas some BPPs may prove to be unique gene products, all of those identified so far have been previously cloned molecules, many of which are also known to act on ECM components. The growing family of

Table 1
Prostatic IGFBP Proteases and Their Characteristics

Protease	Class	IGFBPs cleaved	Other (ECM) substrates	Known inhibitor(s)
PSA (hK-3)	Kallikrein	3,5	Fibronectin,	α1-ACT laminin
hK-2	Kallikrein	1–5	?	PCI, kallistatin
Urokinase	Serine	3	Fibronectin	
Plasmin	Serine	All	?	α-plasmin
Cathepsin D	Aspartyl	1–5	T-kininogen,	Pepstatin fibronectin
MMP-2	Metallo	3	Collagen	TIMPs

PSA, prostate specific antigen; hK-2, human kallikrein 2; MMP, matrix metalloproteinase; TIMP, tissue inhibitor of metalloproteinase; PCI, protease C inhibitor.

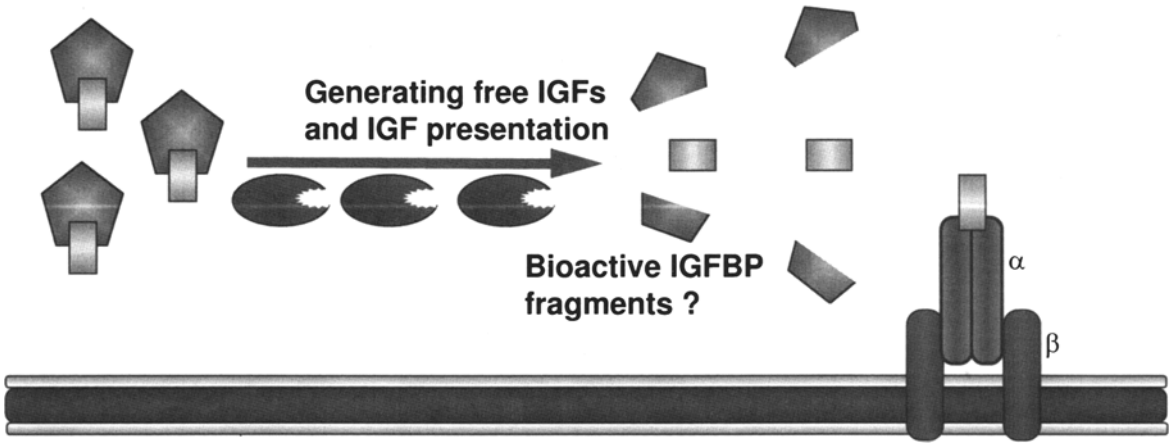

Fig. 2. Prostatic IGFBP proteases. Prostate cells produce several IGFBPs proteases which act as comitogens, see text for details.

BPP subtypes now includes three categories. These include the kallikrein enzymes, such as prostate specific antigen (PSA), which is found at abnormally high concentrations in prostatic cancer, and gamma nerve growth factor (γNGF), a neural cell stimulator whose gamma subunit shares 65% homology to PSA, the matrix metalloproteinases (MMPs), including MMP-1, -2, and -3, shown to be involved in IGFBP proteolysis in pregnancy serum and cellular models, and the cathepsins—lysosomal enzymes which have been implicated as promoters of tumor growth and metastasis and usually are only active at acid pH. These BPPs have different, and selective, effects on the six known IGFBPs. Some IGFBPs appear to be completely resistant to the effects of BPP which potently cleaves other IGFBPs.

Recent experiments utilizing the various different BPPs have demonstrated that the cleavage of the IGFBPs results in IGFBP fragments with much lower affinity to IGFs—the cleavage products typically have one to two orders of magnitude lower affinity relative to the intact peptide. Compatible with the more typical growth-inhibitory role of IGFBPs, proteolysis of the IGFBPs results in a local release of previously sequestered IGFs as free peptides, and in a consequential increase of the IGF-dependent mitogenic effect through interaction with the IGF-R. Thus, it has been speculated that BPPs are comitogens with the IGFs. BPPs may also regulate other actions of IGFs and IGFBPs such as apoptosis and differentiation. Malignancy occurs when cells lose this ability and, thus, grow uncontrollably. BPPs may, by reducing the levels of apoptosis-inducing IGFBPs, contribute to tumor growth and metastasis. The maintenance of normal IGFBP levels is critical to normal rates of cell growth and cell death. When this balance is upset by increased IGFBP proteolysis, the result is increased cellular proliferation and decreased apoptosis—a typical malignant scenario.

The authors and others have characterized several prostatic proteases as BPPs. These proteases include the kallikreins, PSA, and hK-2, which are potent IGFBP-3 and IGFBP-5 protease found in seminal plasma and serum, cathepsin D, which is responsible for the acid activated IGFBP proteolysis in seminal plasma and in prostate epithelial cell conditioned media. MMPs, which are secreted by different prostatic cell types, and urokinase (which has been demonstrated to regulate IGFBP levels and actions in prostate cell lines) *(10–15)*. The secretion of BPPs and IGFBPs is regulated coordinately by TGFβ and other cytokines. It appears that the growth modulation achieved by these cytokines may be related to the induction or suppression of IGFBPs and BPPs (Table 1).

The activity of BPPs in the prostate is modulated by those factors that regulate their production, and by specific natural protease inhibitors which participate in the growth regulatory system described here (Fig. 2). Synthetic inhibitors that provide pharmaceutical mode of intervention are also studied. The comitogenic response of IGFs and BPPs can be blocked by specific inhibitors. Cathepsin D is inhibited by the naturally occurring trypsin-chymotrypsin inhibitor (or BBI, found in leguminous seeds) and also by the synthetic cathepsin inhibitor, Pepstatin. α1 antitrypsin inhibits PSA. MMPs are regulated by their naturally occurring inhibitors, the TIMPs (tissue inhibitor of metalloproteinases) and can be inhibited by the synthetic chelating agent, EDTA. The ability to specifically inhibit proteases that target the IGFBPs, especially those seen in high concentrations in pathologic states, might permit normalization of intact IGFBP levels and reduce abnormal cell growth and differentiation, as in tumor invasion and metastasis.

Matrix components, as well as IGFBPs, constitute the substrates for many of the IGFBP proteases studied. The authors, therefore, propose that these proteases serve a dual role in the regulation of malignant proliferation and invasion. By cleaving IGFBPs, these proteases provide a mitogenic signal in the form of free IGF. Additionally, through the degradation of ECM, these enzymes coordi-

nate tissue remodeling to allow proliferating cells to penetrate the surrounding matrix. It is also likely that reduced local IGFBP levels may result in the abnormal decrease in apoptosis, observed in malignant states. The understanding of these proteases, and the development of specific inhibitors that can alter their postulated procarcinogenic effects, holds promise for the treatment of prostate cancer and other diseases.

References

1. Cohen, P., Peehl, D. M., Bhala, A., Dong, G., Hintz, R. L., and Rosenfeld, R.G. (1994). In: *The IGFs and their regulatory proteins.* Baxter, R. C., Gluckman, P. D., Rosenfeld, R. G., (eds.). Elsevier Science B.V.: Amsterdam.
2. Bhala, A., Peehl, D. M., and Cohen, P. (1994). Proceedings of the Endocrine Society Meetings.
3. LeRoith, D., Baserga, R., Helman, L., and Roberts, C. T., Jr. (1995). *Ann. of Int. Med.* **122,** 54–59.
4. Cohen, P., Peehl, D. M., and Rosenfeld, R. G. (1994). *Horm. Metab. Res.* **26,** 81–84.
5. Oh, Y., Muller, H. L., Lamson, G., and Rosenfeld, R. G. (1993). *J. Biol. Chem.* **268,** 14,964–14,971.
6. Rajah, R. Valentinis, B., and Cohen, P. (1997). *J. Bio. Chem.* In press.
7. Cohen, P., Graves, H. C. B., Peehl, D. M., Kamarei, M., Giudice, L. C., Rosenfeld, R. G. (1992). *J. Clin. Endocrinol. Metab.* **75,** 1046–1053.
8. Lee, K. O., Oh, Y., Cohen, P., Peehl, D. M., and Rosenfeld, R. G. (1994). *J. Clin. Endocrinol. Metab.* **79,** 1367–1372.
9. Fowlkes, J. L., Suzuki, K., Nagesh, H., and Thrailkill, K. M. (1994b). *Endocrinology* **135,** 2810–2813.
10. Rajah, R., Katz, L., Nunn, S., Solberg, P., Beers, T., and Cohen, P. (1996). *Prog. Growth Factors Res.* **6,** 273–284.
11. Cohen, P., Peehl, D. M., Graves, H. C. B., and Rosenfeld, R. G. (1994). *J. Endocrinol.* **142,** 407–415.
12. Angelloz-Nicoud, P., and Binoux, M. (1995). *Endocrinology* **136,** 5485–5492.
13. Conover, C. A., Perry, J. E., and Tindall, D. J. (1995). *J. Clin. Endocrinol. Metabol.* **80,** 987–993.
14. Fowlkes, J. L., Enghild, J. J., Suzuki, K., and Nagesh, H. (1994a). *J. Biol. Chem.* **269,** 25,742–25,746.
15. Montcourrier, P., Mangeat, P.H., Salazar, G., Morisset, M., Sahuquet, A., and Rochefort, H. (1990). *Cancer Res.* **50,** 6045–6054.