Review Article

Prostate cancer cell survival pathways activated by bone metastasis microenvironment

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Abstract

The development of resistance to anti-cancer therapies in bones is a major hurdle preventing long-lasting clinical responses to anti-cancer therapies in hormone refractory prostate cancer. Herein, we present the major signal transduction pathways, which are activated in prostate cancer cells residing at bone metastasis microenvironment. These intracellular signal transduction pathways can inhibit anti-cancer therapy-induced apoptosis of metastatic prostate cancer cells, thereby optimizing their survival, locally. Employment of this knowledge in a clinical setting provides the conceptual framework for the development of bone-targeted therapies for advanced prostate cancer. Indeed, bone metastasis microenvironment-targeted therapies illustrate a novel paradigm in cancer treatment: anti-tumor treatment strategies may not only aim at directly inducing cancer cell apoptosis, but can also target the tumor metastasis microenvironment, and neutralize the protection it confers on metastatic cancer cells.

Keywords: Prostate Cancer, Androgen Refractoriness, Bone Metastasis, Survival Factors

Clinical aspects associated with the development of androgen ablation refractoriness in advanced prostate cancer

Prostate cancer is a best known example of an androgen-dependent neoplasia in man. Newly diagnosed prostate cancer, even at clinical stages with distal metastasis is almost always androgen ablation sensitive. Such sensitivity is defined by the presence of androgen receptor (AR) in prostate cancer cells. AR is a member of the super family of nuclear transcription factors, which participate in a variety of physiological functions, including cell growth and apoptosis[1,2]. Consequently, androgen ablation manipulation in prostate cancer inactivates AR-mediated transcription, thereby producing growth arrest and induction of apoptosis of prostate cancer cells[2]. Therefore, combination therapy consisting of orchiectomy or luteinizing hormone-releasing hormone agonists (LHRH-A) administration plus anti-androgen (combined androgen blockade; CAB or maximal androgen blockade; MAB) has become the standard therapeutic approach for the treatment of advanced prostate cancer[3-5]. However, it is now widely accepted that CAB cannot be curative for metastatic prostate cancer since metastatic prostate cancer cells, residing mainly in bone lesion microenvironment, can develop refractoriness to androgen ablation therapy[4-10]. Disease progression in stage D_2 prostate cancer patients (bone metastasis) is observed after 18-24 months of CAB[1,8]. Recent experiments showed that increased androgen receptor levels (due to increased kinase pathway signaling or altered co-activator/co-repressor ratios) in prostate cancer xenograft models cause antagonists to function as agonists resulting in resistance to anti-androgen therapy[1,2,4,11,12]. The development of androgen ablation refractoriness has been partially explained by a variety of alterations in AR, such as AR mutations[1,2,12,13] and AR overexpression (amplification)[12,13], or altered expression of AR co-regulators, which enable the AR to respond to lower concentration of androgens[14,15]. There is also evidence that AR can be activated in a ligand-independent manner by molecules such as bone metastasis microenvironment growth factors[1,5,6,8,16,17]. In this context, metastatic prostate cancer cells almost always produce blastic reaction of the host (bone) tissue (osteoblastic lesions) as opposed to the lytic reaction (osteolytic lesions) produced by other solid tumors. Therefore, under-

List of Abbreviations	
AR	androgen receptor
bFGF	basic fibroblast growth factor
BMPs	bone morphogenetic proteins
CAB	combined androgen blockade
ET-1	endothelin-1
GPCR	G-protein coupled receptor
HHM	humoral hypercalcaemia of malignancy
IGF-1	insulin-like growth factor 1
IGFBP	IGF-binding protein
IL-6	interleukin-6
IRS-1	insulin receptor substrate 1
MAPK	mitogen activated protein kinase
NFkB	nuclear factor kB
NLS	nuclear localization signal
PI3K	phosphatidylinositol kinase
PTHrP	parathyroid-hormone related peptide
TGFβ1	transforming growth factor beta 1
uPA	urokinase-type plasminogen activator

standing the cellular and molecular events occurring at bone metastasis microenvironment is a "hot" topic in prostate cancer biology of enormous clinical interest.

## Major prostate cancer cell survival pathways activated by growth substances of bone microenvironment

Metastatic prostate cancer cells arriving in bone marrow, at first, need to get access into the bone matrix. Within the bone matrix, tumor cells establish cell-to-cell interactions with bone cells ensuring their survival and growth[18]. Among host tissues affected by the process of metastasis, bone is unique in its ability to undergo frequent remodeling under the influence/control of systemic hormones and local growth factors, such as IL-6, TGFβ1, IGF-1, PTHrP, ET-1 and BMPs. High concentrations of these growth factors in the bone metastasis microenvironment provide a fertile ground for "cross talking", thereby stimulating the transcription of androgen-responsive genes via an AR ligand-independent manner and survival pathways in prostate cancer cells[1,2,8,17,19]. These, growth factor-related signal transducers and activators of transcription inhibit cytotoxic agent-induced apoptosis of prostate cancer cells and enhance survival of prostate cancer cells maintained under androgen ablation conditions[1,8,19-22] (Figure 1). Therefore, prostate cancer cells can use intracrine, paracrine or autocrine signal transduction pathways to activate AR-mediated nuclear processes in the absence or in the presence of limited concentration of androgens, such as those produced by CAB in prostate cancer patients[1,2,8,17,23,24].

1. PTHrP signal transduction pathways (Figure 2): PTHrP was originally identified as the factor responsible for the syndrome of humoral hypercalcaemia of malignancy (HHM). The physiological functions of PTHrP include the regulation of smooth muscle tone, differentiation and proliferation, transepithelial calcium transport, and tissue and organ development. However, systemic effects of PTHrP have been reported in various pathophysiological conditions[25-26]. It is interesting to note that neonatal mice with homozygous ablation of the gene encoding either PTHrP or the PTH1-R die from cardio-myocytic insufficiency at, or just before, birth and exhibit widespread skeletal abnormalities suggesting an anti-apoptotic effect of PTHrP[27,28].

PTHrP has limited homology with parathyroid hormone (PTH), however they both bind with identical affinity to the PTH/PTHrP (PTH1-R) G-protein coupled receptor (GPCR). Following its binding on PTH1-R, there is activation of the AC–PKA and PLC-PKC signaling pathway[29,30]. Considerable evidence indicates that PTHrP exerts an additional signaling as transcription factor by not being secreted extracellularly but being translocated to the nucleus by importin β, thereby influencing nuclear function directly. Mutations of the nuclear localization signal (NLS) abrogated the proliferative effects and nuclear translocation of PTHrP, indicating the involvement of an intracrine mode of action[31]. The consequences of this intracrine mode of action are not yet well characterized but they may modulate processes of vital importance to the cell such as the inhibition of apoptosis[30,31]. The intracrine growth regulatory effects of PTHrP have been studied in prostate cancer and breast cancer cell lines[32,33]. It was demonstrated that cells overexpressing wild-type PTHrP blocked cell cycle at G_2/M phase and protected transfectants from serum-starvation induced apoptosis[33]. Thereby, there exists strong evidence to support that PTHrP acts as survival factors on prostate cancer cells. In addition, transfection studies showed a higher ratio of the anti-apoptotic proteins bcl-2 and bcl-x_L to bax than those of other stable transfectants overexpressing NLS-mutated PTHrP[34]. Furthermore, PTHrP interacts with oncogenes such as the ras / src oncogenes[35]. In fact, data showed that the mechanism of PTHrP expression in cancer is ras-dependent and that other stimulators of PTHrP expression are also activators of ras[36]. In this context, it is noteworthy that the expression of a dominant negative form of ras restored androgen sensitivity in a hormone-refractory prostate cell line[37], while all three commonly studied human prostate cancer cell lines (PC-3, DU-145 and LNCaP) are overexpressing PTHrP[37,38].

Focusing on the role of PTHrP in bone microenvironment, it is important to note that bone microenvironment-related growth factors, such as IGFs and TGFβs, which are
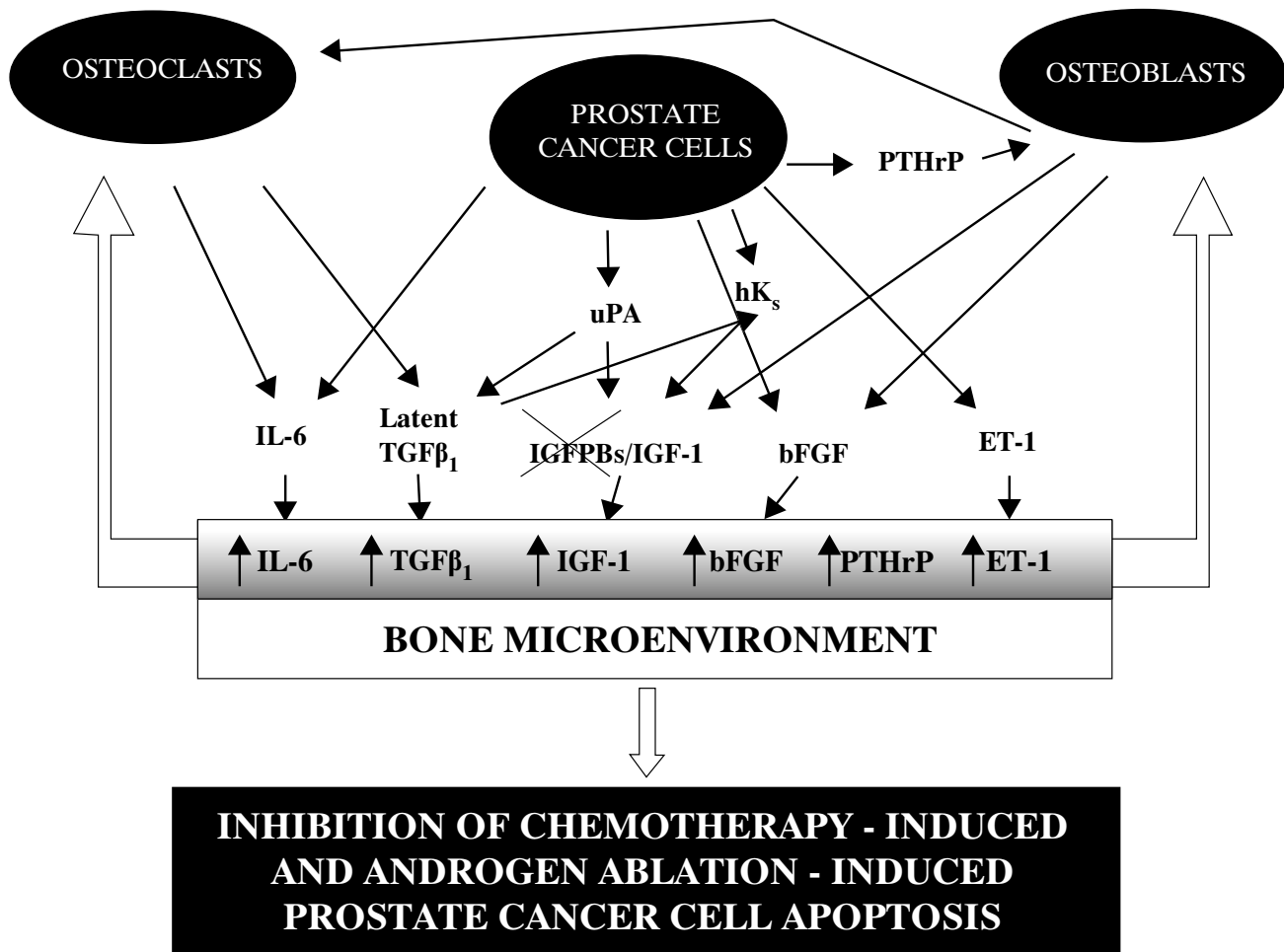
Figure 1. Molecular interactions between prostate cancer and bone microenvironment highlight the importance of paracrine acting substances in the development of resistance to anti-cancer therapies.

activated locally by prostate cancer cell-derived uPA, stimulate PTHrP expression of tumor and bone cells[39]. Therefore, PTHrP overexpression in the bone microenvironment can participate in the development of hormone refractory tumor growth in bone metastasis.

2. TGFβ1 signal transduction pathways (Figure 2): Transforming growth factor β super-family includes TGFβs, activins, BMPs and other proteins, that regulate growth, differentiation, migration, adhesion and apoptosis. TGFβ$_1$, together with IGFs, are the most abundant growth factors in bone and known to function as potent regulators of osteoblast proliferation and differentiation. TGFβ$_1$ is secreted by bone cells and stored in the bone matrix in a latent form, ready to be activated by the tumor cell-derived uPA at the sites of prostate cancer metastasis[40-43].

TGFβ$_1$ binds to two different types of serine/threonine kinase receptors known as type I and II. TGFβ$_1$ binds to the receptor type II-R and this ligand-receptor complex then recruits and phosphorylates the type I-R, which in return

activates substrates identified as Smad proteins. Smads then translocate to the nucleus, bind to DNA, co-operate with other transcription factors, interact with transcription co-repressors and co-activators like p300 and CBP and are, therefore, important transcription modulators[44,45]. Signaling downstream of TGFβ receptors cannot be considered as an isolated event, since other intracellular signaling pathways can interact with the Smad-signaling pathways in different ways. The mechanisms of cross talking between these pathways include the regulation of R-Smad (Receptor activated Smads) activity by MAPK cascades, interaction of Smads with various transcriptional co-factors and regulation of I-Smad (Inhibitory Smads) expression[46-48]. Cross talk between growth factor signal cascades can co-operate in regulating transcription of key pro-apoptotic and anti-apoptotic molecular elements, increasing the survival of prostate cancer cells. TGFβ$_1$ for instance, can activate IL-6 expression in prostate cancer cells through the synergistic collaboration of the Smad2, JNK and Ras signaling pathways[48,49].
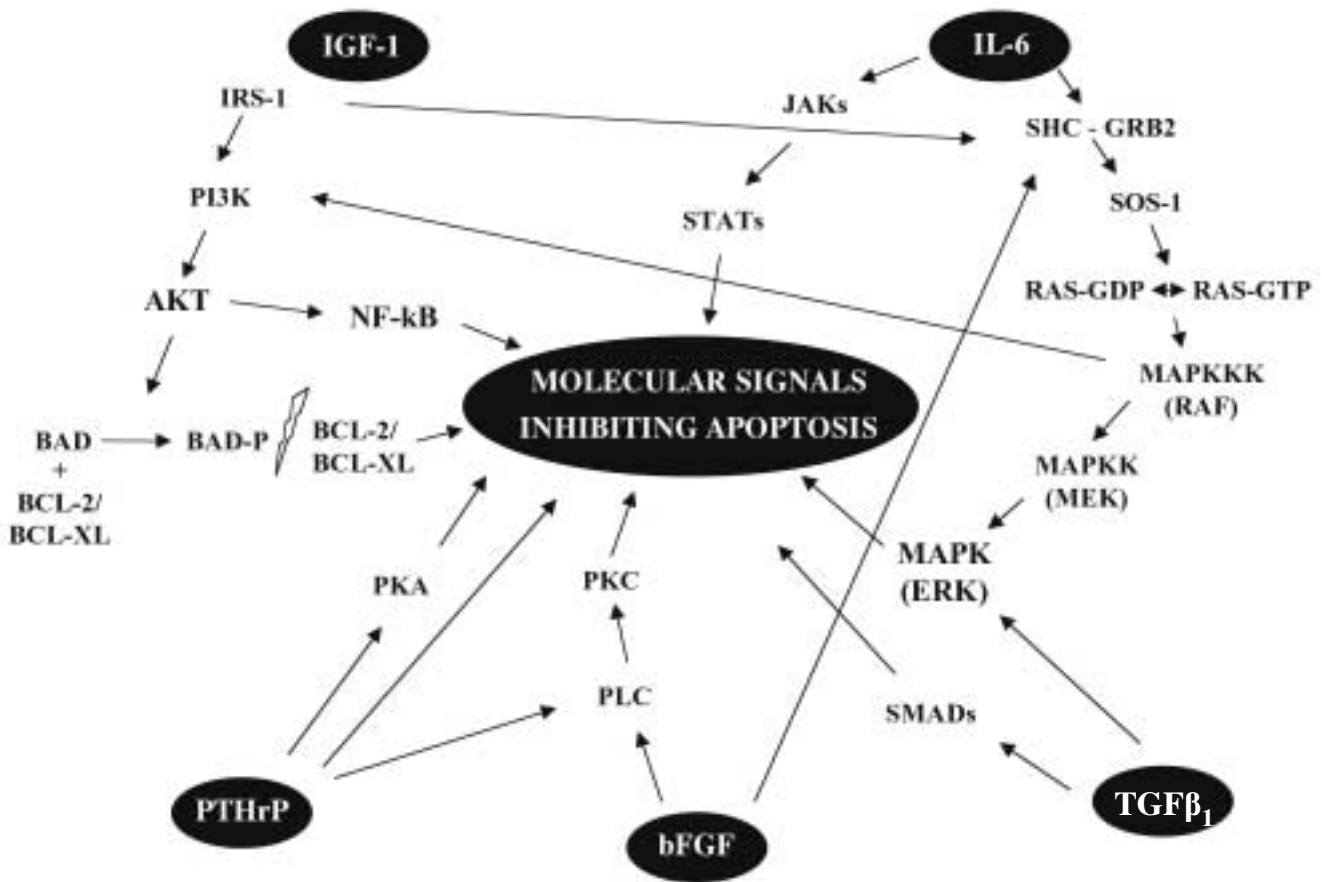
Figure 2. Complexity of IGF-1, IL-6, PTHrP, bFGF and TGFβ$_1$ signaling through unique or overlapping intracellular pathways affecting prostate cancer cell survival.

It is noteworthy that although normal prostate epithelial cells can be growth-inhibited by TGFβ$_1$, prostate cancer cells contained inactive TGFβ-receptors, however it can promote tumor growth indirectly by enhancing angiogenesis and metastasis both *in vitro* and *in vivo*[42,50]. Specifically, TGFβ$_1$ has inhibited the chemotherapy-induced apoptosis of prostate cancer cells while it was heavily implicated in the type of host tissue reaction (lytic and blastic reaction) in response to the presence of metastatic tumor cells, locally[17,18,50]. In rat prostate carcinoma lines, there was a correlation between increased TGFβ$_1$ expression by cancer cells and increased aggressiveness of the tumor, however there was loss of expression of functional TGFβ receptors (TGFβRs) in prostate cancer cells, suggesting that TGFβ$_1$ should act on host tissue cells to produce substances that may in turn influence tumor cell biology[50,51]. Finally, pre-clinical and clinical data have shown increasing TGFβ$_1$ levels in prostate cancer as compared to the TGFβ1 levels of normal subjects[51,52].

3. IGF-1 signal transduction pathways (Figure 2): The growth promoting effect of IGFs is mediated by type I IGF receptor (IGF-1R), a heterotetramer that is homologous to the insulin receptor and displays tyrosine kinase activity. The IGF-1R binds IGFs and insulin with the following specificity: IGF-1 > IGF-2 > insulin. Binding of IGF-1 to the IGF-1R results in IGF-1R auto-phosphorylation, followed by phosphorylation of intracellular substrates and activation of molecular mechanisms responsible for growth regulation and differentiation[53,54]. This includes activation of Shc, Grb2, and Sos-l to activate ras and the MAPK cascade (Raf, Mek, Erk)[55]. An alternative IGF-1 signaling pathway involves the Akt/protein kinase B activation: upon ligand binding, the Insulin Receptor Substrate 1 (IRS-l) is activated, which in return activates PI3K; PI3K then activates the Akt/protein kinase B, which phosphorylates the protein Bad. The phosphorylation of Bad doesn't allow the heterodimerization with Bcl$_{XL}$ and, consequently, Bad remains in the cytosol in its inactive form, bound to the 14.3.3 protein and incapable of inducing apoptosis. The anti-apoptotic effects of IGF-1R are attributed to this pathway[54-57].

The interaction between the IGFs and their receptors is regulated by the IGF-binding proteins (IGFBPs), which bind to the IGFs, with affinities even higher than these of IGF receptors. The cDNAs of six high-affinity members of the IGFBP family have been cloned from a variety of species,

while four additional potential members of the IGFBP family have been identified through computer analysis of GenBank sequence data. Their conserved sequence among lower species indicates the pivotal role of the IGFBPs for different organisms. Thus, IGF actions can be regulated in concert with the type of IGFBPs produced locally[58-60]. IGFBP-3 is more abundant in serum and seems to both enhance and inhibit IGF-1 action in bone. The association of IGFBP-3 overexpression with growth-arrested states in a variety of cell lines, indicates its involvement in an IGF-independent inhibition of cell proliferation[61]. In addition, neutralization of the protective effect conferred upon prostate cancer cells by the IGF-1 by reducing both the systemic growth hormone (GH)-dependent (mainly liver-derived) IGF-1 and GH-independent IGF-1 production of the bone metastasis microenvironment (uPA-mediated hydrolysis of IGFBPs) resulted in an alternative therapeutic manipulation in patients with advanced prostate cancer[7,9,10,11,20,62]. Therefore, further studies will be required to assess the role of the IGFBPs in prostate cancer.

4. bFGF signal transduction pathways (Figure 2): Basic fibroblast growth factor belongs to a family of heparin-binding polypeptides and is a potent mitogen for a number of different cell types. In prostate cancer have been described four FGF types, basic FGF (bFGF: FGF2), acidic FGF (FGF-1), FGF-7 (keratinocyte growth factor), and FGF-8. Overexpression of the FGFs and their receptors has been implicated in transformation *in vitro* and malignant progression *in vivo*[63]. bFGF (or FGF-2) induces differentiation and invasion of tumor cells and is expressed in different types of tumors, including prostate, bladder, kidney, and is also involved in the acceleration of cancer cell growth[64]. In the case of the prostate in particular, bFGF and its three receptors, FGF-R1, R2, and R3, are found in the prostatic stromal and epithelial cells in culture. Under normal conditions, bFGF is synthesized by prostatic stromal fibroblasts but can also be produced by the primary prostate cancer cells (CaP) that have metastasized, as well as by androgen-independent metastatic prostate cancer cell lines. A strong evidence for the implication of bFGF in the development of malignancy was the malignant transformation of cells, when baby hamster kidney cells were transfected with a plasmid coding for the bFGF gene, while the bFGF concentration was elevated in the serum of patients with prostate carcinoma[64-67].

Apart from mitogenic properties, bFGF has been described as an angiogenic factor[68]. Angiogenesis is a complex process, which with the exception of the feminine menstrual cycle, only takes place, in adults, only during pathological situations such as cicatrisation and oncogenesis[68-70]. During angiogenesis, bFGF regulates the activities of extracellular collagenase, proteinases, uPA, and integrins, forming a new capillary network[68]. Indeed, bFGF stimulates uPA production, which in its turns enhances $TGF\beta_1$ and IGFs activity[17,18,40,42]. $TGF\beta_1$ enhances the synthesis of PA inhibitor-l (PAI-1), which represses uPA production and which, in turn, obstructs further $TGF\beta_1$ synthesis[71-73]. This

negative feedback loop enhances tumor angiogenesis and defines a variety of bFGF-activities in bone metastasis[68].

Experiments in rat prostate cancer cell stable transfectants expressing anti-sense bFGF transcripts showed that bFGF promoted rapid activation of PKC as well as rapid phosphorylation of Shc adapter protein that mediates FGF receptor-modulated ras signaling[74]. Remarkably, the serum withdrawal-induced apoptosis in embryonic rat cells was abrogated by the addition of bFGF via the MAPK signaling pathway[73]. In other studies investigating the role of androgen receptor using stable transfectants, it was found that AR^+ PC-3 clones, cultured with bFGF, showed a significant downregulation of androgen receptor expression, thus escaping from androgen regulation[75]. The combined effects of bFGF on cell proliferation (via both the PKC and the Ras/MAPK pathways), on bcl-2 expression and on AR modulation suggest that bFGF is involved in the ability of prostate cancer cells to acquire a more aggressive phenotype and to develop androgen refractoriness[72-75].

5. IL-6 signal transduction pathways (Figure 2): Interleukin-6 (IL-6) is a cytokine that provokes a broad range of cellular and physiological responses. In the normal homeostatic state, IL-6 levels are very low, however, following appropriate stimuli, such as inflammation, many cell types produce IL-6. However, IL-6 not only plays a pivotal role in inflammation and hematopoiesis, it participates also in neuronal differentiation and bone resorption. IL-6 has, indeed, unique and important effects on bone cells and it enhances bone resorption by promoting osteoclast formation[76,77]. In addition, IL-6 enhances the osteoclast formation of PTHrP, by acting at an earlier stage of osteoclastogenesis than PTHrP. Furthermore, IL-6 has recently been shown to enhance hypercalcaemia and bone resorption mediated by PTHrP, *in vivo*[77,78]. In prostate cancer IL-6 acts through both autocrine and paracrine pathways and plays a particular role by contributing to its progression through several mechanisms. In this context, IL-6 was shown to act as an autocrine growth factor and to contribute indirectly in prostate cancer progression through its ability to stimulate osteoblast formation and bone resorption, to reduce apoptosis and to stimulate the secretion of MMP–7[77-80].

IL-6 binds to a specific membrane receptor (IL-6R) to activate cellular mechanisms such as activation of the JAK/STAT pathway and ras-mediated signaling. IL-6R activates JAK kinases, which upon their activation phosphorylate and activate STAT transcription factors, particularly STAT3, that induces transcription of genes containing STAT3 response elements and which contribute in tumor growth. The ras-mediated cellular events, on the other hand, lead to activation of the MAPK signaling pathways. Stimulation of the MAPK and STAT3 pathways by IL-6 leads to androgen-independent activation of the AR. Indeed, the androgen-insensitive PC-3 and DU-145 cells produce high amounts of IL-6, which causes an anti-apoptotic effect[81-84]. Such a phenomenon is related to the elevated levels of IL-6 measured in prostate cancer patients and is

PERIPHERAL BLOOD			BONE METASTASIS MICROENVIRONMENT
Growth Factors (range of values)	Control (mean values)	Advanced prostate cancer (mean values)	
[1*][IGF-1: 100-280 ng/ml]	140.6 ng/ml	171.1 ng/ml	
[2*][TGFβ : 1-35 ng/ml]	6.8 ng/ml	12.26 ng/ml	**?**
[3*][IL-6: 0-40 pg/ml]	1.43 pg/ml	31.6 pg/ml	*Unknown
[4*][bFGF: 0-7 pg/ml]	1.28 pg/ml	6.64 pg/ml	

1*: ref 91, 2*: ref 52, 3*: ref 52, 4*: ref 67   5*: ref 17

Table 1. Reported concentrations of bone microenvironment-related survival factors in patients with prostate cancer as compared to controls.

associated with poor prognosis[52] and successful anti-cancer therapy employed in patients with androgen ablation refractory prostate cancer were followed by reduced circulation levels of IL-6[85]. For these reasons administration of IL-6-neutralizing antibodies, anti-sense oligonucleotides or synthetic antagonists could be useful strategies for the treatment of prostate carcinoma.

6. Endothelin-1 (ET-1): The family of endothelins, includes three members ET-1, ET-2 and ET-3. ET-1 is the most active isopeptide and is produced as a preproform by vascular endothelial cells and by both normal and metastatic prostate cancer cells. Endothelin production is regulated by situations like stress and hypoxia as well as by cytokines and growth factors[86]. The main physiological role of this peptide is the blood flow maintenance in the brain, heart and kidneys, under a state of decreased perfusion. More importantly, ET-1 suppresses apoptosis, has long-term vasoconstriction effects and increases osteoblast-specific gene expression[87-89]. Two endothelin receptors have been described: ETa and ETb, with the classical seven transmembrane patterns and coupling to G proteins. Both receptors are found in human prostate and the expression of ETb is responsible for the inhibition of ET-1 secretion. Elevated ET-1 secretion is thought to increase the aggressiveness of prostate cancer[90]. Interestingly, ET-1 was also shown to potentiate the mitogenic effects of bFGF and IGF-1 which resulted in increased survival and inhibition of apoptosis, *in vitro*[87-89]. Recently, selective antagonists of the ETa receptor have been developed, capable of reversing the ET-1-induced cell proliferation and the decreased apoptosis in prostate cancer cells. Certain compounds are already tried in clinical settings for advanced prostate cancer[90].

Conclusions

Although the androgen regulatory axis has been widely studied in prostate cancer, it is now becoming apparent that many other factors are implicated in the development of androgen ablation- and chemotherapy-refractoriness in prostate cancer. These humoral factors, such as cytokines, growth factors and hormones, alter the biology of metastatic prostate cancer cells, consequently minimizing their dependence on AR function and enhancing tumor cell potential for growth and survival. "Cross talk" of growth factor signaling pathways activated by bone microenvironment contribute to the development of androgen ablation- and chemotherapy-refractoriness[17,18]. Serum concentrations of these major bone-related survival factors were found increased in cohorts of patients with prostate cancer[52,67,91-93]. The relative serum concentrations of these major survival factors suggest that the survival factor activity of IGFs (ng/ml range) should be more important than this of the other survival factors (pg/ml range), however, there exist no data for the relative concentrations of these survival factors in bone metastasis microenvironment (Table 1). Conceivably, comprehensive targeting / blockade of PTHrP, TGFβ₁, IGF-1, IL-6, bFGF and ET-1 activity in skeletal metastasis may result in significant clinical responses in metastatic cancer.

References

1. Taplin ME, Balk SP. Androgen receptor: a key molecule in the progression of prostate cancer to hormone independence. J Cell Biochem 2004; 91:483-490.
2. Culig Z, Klocker H, Bartsch G, Steiner G, Hobisch A.

Androgen receptors in prostate cancer. J Urol 2003; 170:1363-1369.

3. Labrie F. Combined androgen blockade and treatment of localized prostate cancer: a real hope when approaching the year 2000. Endocr Relat Cancer 1998; 5:341-351.

4. Mohler JL, Gregory CW, Ford OH 3III. The androgen axis in recurrent prostate cancer. Clin Cancer Res 2004; 10:440-448.

5. Koutsilieris M, Dimopoulos MA, Doillon C, Reyes-Moreno C, Choki I. The molecular concept of prostate cancer. Cancer J 1996; 9:89-94.

6. Koutsilieris M. Skeletal metastases in advanced prostate cancer: cell biology and therapy. Crit Rev Oncol Hematol 1995; 18:51-64.

7. Koutsilieris M, Mitsiades C, Bogdanos J, Dimopoulos T, Karamanolakis D, Milathianakis C, Tsintavis A. Combination of somatostatin analog, low-dose dexamethasone and standard androgen ablation therapy for stage D3 prostate cancer patients with bone metastases. Clin Cancer Res 2004;10:4398-4405.

8. Mitsiades C, Koutsilieris M. Molecular biology and cellular physiology of refractoriness to androgen ablation therapy in advanced prostate cancer. Expert Opin Investig Drugs 2001; 10:1099-1115.

9. Koutsilieris M, Mitsiades C, Dimopoulos T, Vacalicos J, Lambou T, Tsintavis A, Milathianakis C, Bogdanos J, Karamanolakis D. Combination of dexamethasone and a somatostatin analogue in the treatment of advanced prostate cancer. Expert Opin Investig Drugs 2002; 21:283-293.

10. Koutsilieris M, Mitsiades C, Dimopoulos T, Ioannidis A, Ntounis A, Lambou T. A combination therapy of dexamethasone and somatostatin analog reintroduces objective clinical response to luteinizing-hormone releasing-hormone analog in androgen ablation-refractory prostate cancer. J Clin Endocrinol Metab 2001; 86:5729-5736.

11. Koutsilieris M, Tzanela M, Dimopoulos T. Novel concept of anti-survival factor (ASF) therapy produces an objective clinical response in patients with hormone-refractory prostate cancer. Prostate 1999; 38:313-316.

12. Chen CD, Welsbie DS, Tran C, Baek SH, Chen R, Vesselle R, Rosenfeld MG, Sawyers CL. Molecular determinants of resistance to anti-androgen therapy. Nat Med 2004; 10:33-39.

13. Wallen MJ, Linja M, Kaartinen K, Schleutker J, Visakorpi T. Androgen receptor gene mutations in hormone-refractory prostate cancer. J Pathol 1999; 189:559-563.

14. Heinlein CA, Chang C. Androgen receptor (AR) coregulators: an overview. Endocr Rev 2002; 23:175-200.

15. Linja MJ, Porkka KP, Kang Z, Savinainen KJ, Janne OA, Tammela TL, Vessella R, Palvimo JJ, Visakorpi T. Expression of androgen receptor co-regulators in prostate cancer. Clin Cancer Res 2004; 10:1032-1040.

16. Evangelou AI, Winter SF, Huss WJ, Bok RA, Greenberg NM. Steroid hormones, polypeptide growth factors, hormone-refractory prostate cancer, and the neuroendocrine phenotype. J Cell Biochem 2004; 91:671-683.

17. Bogdanos J, Karamanolakis J, Tenta R, Tsintavis A, Milathianakis C, Mitsiades C, Koutsilieris M. Endocrine/ paracrine/autocrine survival factor activity of bone microenvironment participates in the development of androgen ablation and chemotherapy refractoriness of prostate cancer metastasis in skeleton. Endocr Relat Cancer 2003; 10:279-289.

18. Karamanolakis D, Bogdanos J, Sourla A, Milathianakis C, Tsintavis A, Lembessis P, Tenta R, Tiblalexi D, Koutsilieris M. Molecular evidence-based use of bone resorption-targeted therapy in prostate cancer patients at high risk for bone involvement. Mol Med 2002; 8:667-674.

19. Arnold JT, Isaacs JT. Mechanisms involved in the progression of androgen-independent prostate cancers: it is not only the cancer cell's fault. Endocr Relat Cancer 2002; 9:61-73.

20. Koutsilieris M, Mitsiades C, Sourla A. Insulin-like growth factor 1 and urokinase-type plasminogen activator bioregulation system as a survival mechanism of prostate cancer cells in osteoblastic metastases: development of anti-survival factor therapy for hormone-refractory prostate cancer. Mol Med 2000; 6:251-267.

21. Mohammad KS, Guise TA. Mechanisms of osteoblastic metastases: role of endothelin-1. Clin Orthop 2003; 415 (Suppl.):67-74.

22. Tenta R, Tiblalexi D, Sotiriou E, Lembessis P, Manoussakis M, Koutsilieris M. Bone microenvironment-related growth factors modulate differentially the anti-cancer actions of zoledronic acid and doxorubicin on PC-3 prostate cancer cells. Prostate 2004; 59:120-131.

23. Djakiew D. Deregulated expression of growth factors and their receptors in the development of prostate cancer. Prostate 2000; 42:150-160.

24. Sadar MD, Hussain M, Bruchovsky N. Prostate cancer: molecular biology of early progression to androgen independence. Endocr Relat Cancer 1999; 6:487-502.

25. Mundy GR, Guise TA. Role of parathyroid hormone-related peptide in hypercalcaemia of malignancy and osteolytic bone disease. Endocr Relat Cancer 1998; 5:15-26.

26. Suva LJ, Winslow GA, Wettenhall RE, Hammonds RG, Moseley JM, Diefenbach-Jagger H, Rodda CP, Kemp BE, Rodriguez H, Chen EY. A parathyroid hormone-related protein implicated in malignant hypercalcaemia: cloning and expression. Science 1987; 237:893-896.

27. Karaplis AC, Luz A, Glowacki J, Bronson RT, Tybulewicz VL, Kronenberg HM, Mulligan RC. Lethal skeletal dysplasia from targeted disruption of the parathyroid hormone-related peptide gene. Genes Dev 1994; 1:277-289.

28. Qian J, Colbert MC, Witte D, Kuan CY, Gruenstein E, Osinska H, Lanske B, Kronenberg HM, Clemens TL. Midgestational lethality in mice lacking the parathyroid hormone (PTH)/PTH-related peptide receptor is associated with abrupt cardiomyocyte death. Endocrinology 2003; 144:1053-1061.

29. Scluter KD. PTH and PTHP: similar structures but different functions. News Physiol Sci 1999; 14:243-248.

30. Gardella TJ, Jüppner H. Molecular properties of the PTH/PTHrP receptor. Trends Endocrinol Metab 2001; 12:210-217.

31. Henderson JE, Amizuka N, Warshawsky H, Biasotto D, Lanske BM, Goltzman D, Karaplis AC. Nucleoral localization of parathyroid hormone-related peptide enhances survival of chondrocytes under conditions that promote apoptotic cell death. Mol Cell Biol 1995; 115:4064-4075.

32. Veronica A, Sepulveda T, Falzon M. Parathyroid hormone-related protein enhances PC-3 prostate cancer cell growth via both autocrine/paracrine and intracrine pathways. Regul Peptides 2002; 105:109-120.

33. Veronica A, Sepulveda T, Shen X, Falzon M. Intracrine PTHrP protects against serum-starvation-induced apoptosis and regulates the cell cycle in MCF-7 breast cancer cells. Endocrinology 2002; 143:596-606.

34. Amling M, Neff L, Tanaka S, Inoue D, Kuida K, Weir E, Philbrick WM, Broadus AE, Baron R. Bcl-2 lies down-stream of parathyroid hormone-related peptide in a signaling pathway that regulates chondrocyte maturation during skeletal development. J Cell Biol 1997; 136:205-213.

35. Li X, Drucker DJ. Parathyroid hormone-related peptide is a downstream target for ras and src activation. J Biol Chem 1994; 269:6263-6266.

36. Aklilu F, Gladu J, Goltzman D, Rabbani SA. Role of mitogen-activated protein kinases in the induction of parathyroid hormone-related peptide. Cancer Res 2000; 60:1753-1760.

37. Bakin RE, Gioeli D, Bissonette EA, Weber MJ. Attenuation of Ras signaling restores androgen sensitivity to hormone-refractory C4-2 prostate cancer cells. Cancer Res 2003; 63:1975-1980.

38. Bryden AAG, Islam S, Freemont AJ, Shanks JH, George NJR, Clarke NW. Parathyroid hormone-related peptide: expression in prostate cancer bone metastases. Prostate Cancer P D 2002; 5:59-62.

39. Pizzi H, Gladu J, Carpio L, MiaoD, Goltzman D, Rabbani SA. Androgen regulation of parathyroid hormone-related peptide production in human prostate cancer cells. Endocrinology 2003; 144:858-867.

40. Koutsilieris M, Frenette G, Lazure C, Lehoux JG, Govindan MV, Polychronakos C. Urokinase-type plasminogen activator: a paracrine factor regulating the bioavailability of IGFs in PA-III cell-induced osteoblastic metastases. Anticancer Res 1993; 13:481-486.

41. Deftos LJ. Prostate Carcinoma (Production of bioactive factors). Cancer suppl (Skeletal Complications of malignancy) 2000; 88:3002-3008.

42. Barrack ER. TGFβ in prostate cancer: a growth inhibitor that can enhance tumorigenicity. Prostate 1997; 31:61-70.

43. Sheng Shijie. The urokinase-type plasminogen activator system in prostate cancer metastasis. Cancer Met Rev 2001; 20:287-296.

44. Miyazono K, ten Dijke P, Heldin CH. TGF-beta signaling by Smad proteins. Adv Immunol 2000; 75:115-157.

45. Derynck R. TGF-β receptor-mediated signaling. Trends Biochem Sci 1994; 19:548-553.

46. Kawabata M, Imamura T, Inoue H, Hanai J, Nishihara A, Hanyu A, Takase M, Ishidou Y, Udagawa Y, Oeda E, Goto D, Yagi K, Kato M, Miyazono K. Intracellular signaling of the TGF-β superfamily by Smad proteins. Ann NY Acad Sci 1999; 886:73-82.

47. Massague J, Hata A, Liu F. TGF-β signaling through the Smad pathway. Trends Cell Biol 1997; 7:187-192.

48. Hoi ME. Mechanism of transforming growth factor-β1 signaling: role of the mitogen-activated protein kinase. Kidney Int 2000; 58, suppl.77:53-58.

49. Park JI, Lee MG, Cho K, Park BJ, Chae KS, Byun DS, Ryu BK, Park YK, Chi SG. Transforming growth factor-β1 activates interleukin-6 expression in prostate cancer cells through the synergistic collaboration of the Smad2, p38-NF-kB, JNK, and Ras signaling pathways. Oncogene 2003; 22:4314-4332.

50. Tiecker BA. Malignant cells, directors of the malignant process: Role of transforming growth factor-beta. Cancer Met Rev 2001; 20:133-143.

51. De Caestecker MP, Piek E, Roberts AB. Role of transforming growth factor- β signaling in cancer. J Natl Cancer Inst 2000; 92:1388-1397.

52. Adler HL, McCurdy MA, Kattan MW, Timme TL, Scardno PT, Thompson TC. Elevated levels of circulating interleukin-6 and transforming growth factor - β1 in patients with metastatic prostatic carcinoma. J Urol 1999; 161:182-187.

53. Wang HS, Chard T. IGFs and IGF-binding proteins in the regulation of human ovarian and endometrial function. J Endocrinol 1999; 161:1-13.

54. Le Roith D, Werner H, Beitner-Johnson D, Roberts CT. Molecular and cellular aspects of the insulin-like growth factor I receptor. Endocr Rev 1995; 16:143-163.

55. DeBosch BJ, Deo BK, Kumagai AK. Insulin-like growth factor-1 effects on bovine retinal endothelial cell glucose transport: role of MAP kinase. J Neurochem 2002; 81:728-734.

56. Nissley P, Lopaczynski W. Insulin-like growth factor receptors. Growth Factors 1991; 5:29-49.

57. Baserga R. The IGF-I receptor in cancer research. Exp Cell Res 1999; 253:1-6.

58. Silha JV, Murphy LJ. Minireview: Insights from insulin-like growth factor binding protein transgenic mice. Endocrinology 2002; 143:3711-3714.

59. Rosenfeld RG. The blind men and the elephant-a parable for the study of insulin-like growth factor binding proteins. Endocrinology 1998; 118:1743-1758.

60. Jones JL, Clemmons DR. Insulin-like growth factors and their binding proteins: biological actions. Endocr Rev 1995; 16:3-34.

61. Dimopoulos MA, Kiamouris C, Gika D, Deliveliotis C, Giannopoulos A, Zervas A, Alamanis C, Constantinidis C, Koutsilieris M. Combination of LHRH-Analogue with somatostatin analogue and dexamethasone vs chemotherapy in hormone-refractory prostate cancer patients: a randomized phase II study. Urology 2004; 63:120-125.

62. Baxter RC. Signaling pathways involved in antiproliferative effects of IGFBP-3: a review. J Clin Pathol: Mol Pathol 2001; 54:145-148.

63. Basilico C, Moscatelli D. the FGF family of growth factors and oncogenes. Adv Cancer Res 1992; 59:115-165.

64. Dow JK, De Vere White RW. Fibroblast growth factor 2: It's structure and property, paracrine function, tumor angiogenesis and prostate-related mitogenic and oncogenic functions. Urology 2000; 55:800-806.

65. Sherwood ER, Fong CJ, Lee C, Kozlowski JM. Basic fibroblast growth factor: a potential mediator of stromal growth in the human prostate. Endocrinology 1992; 130:2955-2963.

66. Rosini P, Bonaccorsi L, Baldi E, Chiasserini C, Forti G, De Chiara G, Lucibello M, Mongiat M, Lozzo RV, Garaci E, Cozzolino F, Torcia MG. Androgen expression induces FGF2, FGF-binding protein production and FGF2 release in prostate carcinoma cells: role of FGF2 in growth, survival and androgen receptor down-modulation. Prostate 2002; 53:310-321.

67. Cronauer MV, Hittmair A, Eder IE, Hobisch A, Culig Z, Ramoner R, Zhang J, Bartsch G Reissigl A, Radmayr C, Thurnher M, Klocker H. Basic fibroblast growth factor levels in cancer cells and in sera of patients suffering from proliferative disorders of the prostate. Prostate 1997; 31:223-233.

68. Montesano R, Vassalli JD, Baird A, Guillemin R, Orci L. Basic fibroblast growth factor indices angiogenesis *in vitro*. Proc Natl Acad Sci USA 1986; 83:7297-7301.

69. Hanahan D, Weinberg RA. The hallmarks of cancer. Cell 2000; 100:57-70.

70. Van der Pluijm G. Mécanismes impliqués dans l'invasion de l'os par les cellules tumorales. Rev Rhum (Ed.Fr) 2002; 69:1002-1009.

71. Keer HN, Gaylis FD, Kozlowski JM, Kwaan HC, Bauer KD, Sinha AA, Wilson MJ. Heterogeneity in plasminogen-activator (PA) levels in human prostate-cancer cell line: increased PA activity correlates with biologically aggressive behavior. Prostate 1991; 18:201-214.

72. Sobue T, Gravely T, Hand A, Min YK, Pilbeam C, Raisz LG, Zhang X, Larocca D, Florkiewicz R, Hurley MM. Regulation of fibroblast growth factor 2 and fibroblast growth factor receptors by transforming growth factor β in human osteoblastic MG-63 cells. J Bone Min Res 2002; 17:502-512.

73. Hrzenjak M, Shain SA. Fibroblast growth factor-2 and TPA enhance prostate-cancer-cell proliferation and activate members of the Ras and PKC signal transduction pathways. Recept Signal Transduct 1997; 7:207-219.

74. Schamberger C, Cerni C. Mapk/Sapk signaling in apoptosis and bFGF-mediated survival. Sci World J 2001; 1:43.

75. Zuck B, Goepfert C, Nedlin-Chittka A, Sohrt K, Voigt KD, Knabbe C. Regulation of fibroblast growth factor-like protein(s) in the androgen-responsive human prostate carcinoma cell line LNCaP. J Steroid Biochem Mol Biol 1992; 41:659-663.

76. Swolin-Eide D, Ohlsson C. Effects of cortisol on the expression of interleukin-6 and interleukin-1β in human osteoblast-like cells. J Endocrinol 1998; 156:107-114.

77. Roodman GD. Interleukin-6: an osteotropic factor. J Bone Miner Res 1992; 7:475-478.

78. De La Mata J, Uy H, Guise TA, Story B, Boyce BF, Mundy GR, Goodman GD. IL-6 enhances hypercalcaemia and bone resorption mediated by PTHrP *in vivo*. J Clin Invest 1995; 95:2846-2852.

79. Twillie DA, Eisenberger MA, Carducci MA, Hseih WS, Kim WY, Simons JW. Interleukin-6: a candidate mediator of human prostate cancer morbidity. Urology 1995; 45:542-549.

80. Culig Z, Bartsch G, Hobisch A. Interleukin-6 regulates androgen receptor activity and prostate cancer cell growth. Mol Cell Endocrinol 2002; 197:231-238.

81. Ihle JN. Cytokine receptor signaling. Nature 1995; 377:591-594.

82. Ernst M, Oates A, Dunn AR. Gp130-mediated signal transduction in embryonic stem cells involves activation of Jak and Ras/mitogen-activated protein kinase pathways. J Biol Chem 1996; 271:30136-30143.

83. Ueda T, Bruchovsky N, Sadar MD. Activation of the androgen receptor N-terminal domain by interleukin-6 via MAPK and STAT3 signal transduction pathways. J Biol Chem 2002; 277:7076-7085.

84. Yang L, Wang L, Lin HK. Interleukin-6 differentially regulates androgen receptor transactivation ia PI3K-Akt, STAT3, and MAPK, three dinstict signal pathways in prostate cancer cells. Biochem Biophys Res Commun 2003; 305:462-469.

85. Akakura K, Suzuki H, Ueda T, Komiya A, Ichikawa T, Igarashi T, Ho H. Possible mechanism of dexamethasone therapy for prostate cancer suppression of circulating level of inerleukin-6. Prostate 2003; 56:106-109.

86. Guise TA, Mohammad KS. Endothelins in bone cancer metastases. Cancer Treat Res 2004; 118:197-212.

87. Le Roy BE, Sellers RS, Rosol TJ. Canine prostate stimulates osteoblast function using the endothelin receptors. Prostate 2004; 59:148-156.

88. Hamdy FC. Endothelin and skeletal metastases in hormone-refractory prostate cancer. Eur Urology Suppl 2003; 2:15-19.

89. Granchi S, Brocchi S, Bonaccorsi L, Baldi E, Vinci MC, Frti G, Serio M, Maggi M. Endothelin-1 production by prostate cancer cell lines is up-regulated by factors involved in cancer progression and down-regulated by androgens. Prostate 2001; 49:267-277.

90. Gohji K, Kitazawa S, Tamada H. Expression of endothelin receptor A associated with prostate cancer progression. J Urol 2001; 165:1033-1036.

91. Miyata Y, Sakai H, Hayashi T, Kanetake H. Serum insulin-like growth factor binding protein 3/prostate specific antigen ratio is a useful predictive marker in patients with advanced prostate cancer. Prostate 2003; 54:125-132.

92. Wolk A, Mantzoros CS, Andersson SO, Bergstrom R, Signorello LB, Lagiou P, Adami HO, Trichopoulos D. Insulin-like growth factor 1 and prostate cancer risk: a population-based, case-control study. J Natl Cancer Inst 1998; 90:911-915.

93. Chan JM, Stampfer MJ, Giovannucci E, Gann PH, Ma J, Wilkinson P, Hennekens CH, Pollak M. Plasma insulin-like growth factor-1 and prostate cancer risk: a prospective study. Science 1998; 279:563-565.