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Interleukin-6 Regulation of Prostate Cancer Cell Growth

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Abstract Interleukin-6 (IL-6) is involved in regulation of immune reaction and cell growth and differentiation. It causes multifunctional responses ranging from inhibition of proliferation to promotion of cell survival. IL-6 effects may depend on experimental conditions such as passage numbers and serum composition. IL-6 signals in target tissues through the receptor that is composed of the ligand-binding and signal-transducing subunits. IL-6 is expressed in benign and malignant prostate tissue and the levels of the cytokine and its receptor increase during prostate carcinogenesis. IL-6 is considered a positive growth factor for most prostate cells. The only exemption seems to be the LNCaP cell line, in which IL-6 causes growth arrest and induces differentiation function. In contrast, IL-6 acts as an autocrine growth factor in the subline LNCaP-IL-6+ established after chronic treatment with IL-6. IL-6 is a candidate for targeted therapy in prostate cancer because of its association with morbidity. Activation of signaling pathways of Janus kinase/signal transducers and activators of transcription factors, mitogen-activated protein kinase (MAPK), and phosphatidylinositol 3-kinase has been reported in various prostate cancer cell lines. IL-6 and the related cytokine oncostatin M induce activation of the androgen receptor (AR) in the absence of androgen. IL-6 is also involved in regulation of vascular endothelial growth factor expression as well as neuroendocrine differentiation in prostate. Anti-IL-6 antibodies showed an inhibitory effect on the PC-3 xenograft. However, the development of this therapy in prostate cancer is in early stages. J. Cell. Biochem. 95: 497–505, 2005.

Key words: interleukin-6; prostate cancer; JAK/STAT; MAPK; androgen receptor

Interleukin-6 (IL-6), a cytokine of molecular weight between 21 and 28 kDa, is involved in regulation of a multitude of cellular functions including proliferation, apoptosis, angiogenesis, and differentiation. It is implicated in development and progression of tumors of various organs, in particular myeloma, renal and prostate cancer, and melanoma. In addition, it has a role in modulation of immune responses through differentiation of immune cells. IL-6 signals through the membrane receptor that is composed of the ligand-binding subunit gp80 which forms a low affinity complex with the cytokine and the signal transduction subunit gp130. The gp130 subunit's action is redundant since it is activated in response to IL-6-related cytokines, such as leukemia inhibitory factor (LIF) and IL-11. IL-6 receptors are expressed in a variety of benign and malignant cells. Following homodimerization of gp130, there is a formation of a hexameric complex consisting of two molecules each of IL-6, gp80, and gp130. This complex forms a high-affinity binding site for IL-6. IL-6 signaling is enhanced by the soluble IL-6 receptor, which can form a fully hexameric complex.

One reason for induction of different responses is the ability of IL-6 to activate signaling through distinct pathways. In various cell types, IL-6 binding to its receptor leads to phosphorylation of Janus kinases (JAK) that in turn phosphorylate the gp130 receptor subunits. The next step in IL-6 signaling is translocation and phosphorylation of signal transducers and transcription (STAT) factors by JAK. Among these transcription factors, STAT3 has a predominanant role in IL-6 signal transduction. The role of STAT3 in malignant transformation of several cell lines has been well documented [Horiguchi et al., 2002; Wang et al., 2004a]. There are three classes of negative regulatory molecules for the JAK/STAT

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pathway: suppressors of cytokine signaling (SOCS), protein inhibitors of activated STAT (PIAS), and protein tyrosine phosphatases. SOCS act as typical negative feedback regulators. Their expression is induced by IL-6 and related cytokines [Fujimoto and Naka, 2003]. This step is followed by blockade of the recruitment of STAT to the IL-6 receptor or inhibition of JAK activity. PIAS proteins bind to activated STAT dimers thus preventing them from interacting with DNA [Kile et al., 2001]. IL-6 signaling through the STAT3 pathway may cause contrasting proliferative responses. Treatment of cells with IL-6 may lead to phosphorylation of p42/p44 mitogen-activated protein kinase (MAPK) or activation of the signaling pathway of phosphoinositol 3-kinase (PI3-K). In most cases, MAPK activation is associated with a proliferative response, whereas the PI3-K pathway prevents cell death. Intermediary molecules in IL-6 signal transduction, such as the tyrosine phosphatase SHP-2, may play an important role in controling activation of a particular signaling pathway. Activated JAK can phosphorylate tyrosine residues on cytokine receptors that serve as docking sites for adapter proteins, such as SHP-2 or Shc. They can then transmit the signal through the GRB2 adapter to the MAPK pathway [Hideshima et al., 2001; Cunnick et al., 2002]. Increased activation of the PI3-K pathway by IL-6 was observed in association with phosphorylation of SHP-2.

IL-6 EXPRESSION IN HUMAN PROSTATE CANCER

Prostate cancer remains the most frequently diagnosed malignant tumor in male in the Western world. There are an increasing number of tumors diagnosed in early stages, in which the disease is curable by radical surgery or radiation therapy. Therapy for late stages prostate cancer is palliative and is based on observations made by Huggins and Hodges [1941] that androgen ablation inhibits tumor growth. There are increasing efforts to establish new therapies on the basis of identification of appropriate targets. Prostate cancer is a heterogenous disease and metastatic lesions from the same patient show a great variability in expression of protein markers.

There is increasing interest in IL-6 expression and function in carcinoma of the prostate. Initial studies were based on measurements of IL-6 levels in sera from patients with therapy-resistant metastatic disease [Twillie et al., 1995]. They revealed the cytokine's upregulation in the absence of a clinically manifest infection. Those data, although did not identify the source of IL-6, suggested a link between prostate malignancy and production of IL-6. Patients with metastatic prostate cancer who present with elevated IL-6 also have increased serum levels of transforming growth factor-β (TGF-β) [Adler et al., 1999]. TGF-β is a multifunctional cytokine, which causes an inhibition of proliferation of prostate epithelial cells in vitro. Prostate xenografts engineered to overproduce TGF-β are in contrast characterized by rapid tumor growth, most probably because of suppression of immune response and induction of angiogenesis [Steiner and Barrack, 1992]. The possibility that the two cytokines act in a cooperative manner to accelerate tumor progression in vivo requires further investigation. IL-6 serum levels higher than 7 pg/ml are associated with bad prognosis [Nakashima et al., 2000]. These findings, taken together with the results by Giri et al. [2001] and associates who showed that the levels of IL-6 and its receptor increase in organ-confined tumors, suggest that IL-6 has a growth-promoting role at earlier stages of prostate carcinogenesis.

Increase in IL-6 expression in prostate cancer might be a result of concerted action of various signaling pathways. One of the key regulators of IL-6 expression is nuclear factor (NF) kappa B, which is composed of the p50 and p65 subunits. In most prostate cancers, its expression is upregulated [Lessard et al., 2003; Ismail et al., 2004]. Importantly, NF kappa B levels are higher in androgen-independent xenografts than in androgen-dependent ones [Chen and Sawyers, 2002]. Inhibition of NF kappa B expression in prostate cancer by ingredients present in fruit and vegetables, such as indole-3-carbinol, silibinin, apigenin, or genistein is associated with a decreased tumorigenicity [Dhanalakshmi et al., 2002; Li and Sarkar, 2002; Sarkar and Li, 2004; Shukla and Gupta, 2004]. On the other side, NF kappa B also regulates expression of other genes that have a role in angiogenesis, invasion, and metastasis and novel strategies aimed to antagonize its activation in prostate cancer may be justified. IL-6 is also upregulated by TGF-β through the intermediary molecule Smad2 [Park et al.,

2003]. In fact, TGF-β casused nuclear translocation of NF kappa B, which was blocked by inhibitors of the p38 MAPK signaling pathway. Consequently, treatment of androgen receptor (AR)-negative prostate cancer cells with the superrepressor Ikappa B diminished IL-6 expression. Activated c-jun and Ras-Raf-MAPK pathway are involved in IL-6 upregulation by TGF-β. The activating protein-1 (AP-1) compounds JunD and Fra-1 heterodimers and lysophosphatidic acid are also important regulators of IL-6 expression in carcinomatous tissue [Zerbini et al., 2003; Sivashanmugam et al., 2004]. IL-6 expression in prostate cancer correlates with that of the *GBX2* homeobox gene [Gao et al., 2000]. In addition, the presence of the tumor suppressor retinoblastoma inhibits the IL-6 promoter [Santhanam et al., 1991]. Loss of retinoblastoma expression in prostate cancer might thus contribute to the upregulation of IL-6. Taken together, these observations from experimental studies suggest that increased IL-6 level in prostate cancer may occur because of alterations in one or more signaling cascades.

IL-6 and IL-6 receptor expression were investigated in benign and malignant prostate tissue by immunohistochemistry and ELISA [Hobisch et al., 2000]. Stromal cells secrete high IL-6 levels into culture supernatants whereas basal cells are the main epithelial source of IL-6 in the benign prostate (Fig. 1). On immunohistochemistry, tumor cells were shown to express IL-6 and the IL-6 receptor. Loss of the IL-6 receptor was not observed in prostate cancer specimens. Androgen-independent prostate cancer cell lines PC-3 and DU-145 secrete high IL-6 levels in the supernatants [Chung et al., 1999]. This is not surprising since they are derived from metastatic lesions and have disregulated NF kappa B and AP-1 pathways. Treatment of these cells with an anti-IL-6 antibody resulted in a diminished growth thus confirming the presence of the autocrine loop. In contrast, AR-positive LNCaP cells were found to be IL-6-negative. One possible explanation for the absence of IL-6 is the fact that steroids downregulate the expression of IL-6 in bone cell cultures [Bellido et al., 1995]. Dihydrotestosterone has an inhibitory effect on NF kappa B [Keller et al., 1996]. IL-6 signaling is potentiated by its soluble receptor IL-6sR, which induces homodimerization of the gp130 subunit. Preoperative levels of IL-6sR expression are associated with tumor volume, Gleason score, and lymph node metastastes [Shariat et al., 2004].

IL-6 REGULATION OF CELL-CYCLE AND CELL DEATH

Most data on regulation of cell-cycle by IL-6 are available from studies with LNCaP cells and their derivatives. It should be noted, however, that primary prostate epithelial cells and a cell line derived from a precancerous lesion, high-grade prostate intraepithelial neoplasia, respond to IL-6 by growth stimulation [Giri et al., 2001; Liu et al., 2002]. Divergent results with LNCaP cells could be explained by use of

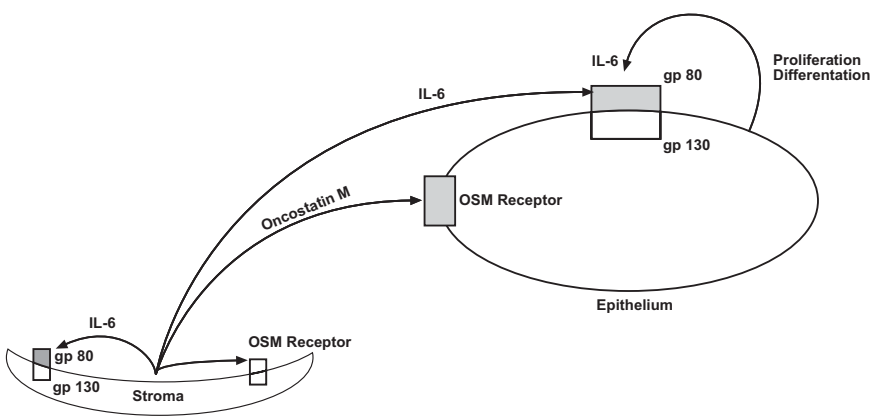

Fig. 1. IL-6 and OSM expression in prostate epithelium. IL-6 is present in both epithelium and stroma whereas OSM expression is stromal.Their receptors are expressed both in epithelial and stromal cells in the prostate. It is presumed that cytokine autocrine and paracrine loops regulate prostate growth in an autocrine and paracrine manner.

different LNCaP passages and culture conditions. Growth response and activation of intracellular signaling pathways in LNCaP cells might in general differ between low and high passages. In an early study in which LNCaP growth regulation by IL-6 was examined, it was identified as a factor in conditioned medium from stromal cells that causes an inhibition of proliferation [Degeorges et al., 1996]. Bonavida's group [1999] analyzed the mechanism underlying the growth-inhibitory effect of IL-6 in LNCaP cells. They found that the G_1/S cell-cycle checkpoint is an IL-6 target in LNCaP cells. This is reflected in inhibition of expression and activity of cyclin-dependent kinases 2 and 4. Changes in regulation of cell-cycle were studied in the new subline LNCaP-IL-6+. LNCaP-IL-6+ cells represent a clinically relevant model that was generated in the authors' laboratory with aim to study signaling alterations during continuous treatment with IL-6 [Hobisch et al., 2001]. Cells selected in the presence of IL-6 have a higher basal proliferation rate and do not show a growth inhibition in response to exogenous IL-6. Similar changes in responsiveness were reported in other tumors, such as melanoma, in which IL-6 is associated with pathogenesis [Böhm et al., 2001]. LNCaP-IL-6+ cells show the presence of endogenous IL-6, in contrast to their counterparts serially passaged in the absence of the cytokine [Hobisch et al., 2001]. Thus, chronic treatment with exogenous IL-6 leads to a positive feedback in prostate cancer cells. In LNCaP-IL-6+ cells, there is a higher percentage of cells in the S phase of the cell-cycle, a finding which could be explained by an increased expression of cyclin-dependent kinases and loss of the tumor suppressors p27 and pRb [Steiner et al., 2003]. Absent expression of tumour suppressors is common in human prostate cancer. These data show that induction of the G_1/S growth arrest by IL-6 may be a dominant regulatory mechanism in some prostate cancer cells. In concordance with these findings, LNCaP-IL-6+ xenografts volumes were significantly larger than those generated with the control subline [Steiner et al., 2003]. In summary, LNCaP-IL-6+ cells could be used for studying IL-6 transition from a paracrine growth inhibitor to an autocrine growth stimulator. Data from other researchers showed that IL-6 might act as a positive growth factor for LNCaP cells [Okamoto et al., 1997; Qiu et al., 1998; Giri et al., 2001]. One possibility to explain this finding is that, under certain experimental conditions, the IL-6 receptor associates with ErbB2 thus leading to activation of the signaling pathway of MAPK [Qiu et al., 1998].

Regulation of cell death by IL-6 is a subject of great importance. A typical feature of prostate cancer is a decreased rate of cell death. Recent study demonstrated that, in prostate cancer cells, there is redundant signaling of both pathways of MAPK and PI3-K in regulation of cell survival [Uzgare and Isaacs, 2004]. The PI3-K pathway is activated in response to IL-6 in PC-3 cells. This was demonstrated in experiments in which the tyrosine phosphorylation of p85, the regulatory subunit of the PI3-K was studied [Chung et al., 2000]. p85 coprecipitated with the signal-transducing subunit of the IL-6 receptor. IL-6 thus contributes to an increase in phosphorylation of Akt, which is a typical feature of aggressive prostate cancer. Akt phosphorylation in prostate cancer Gleason scores 6 or 7 have a prognostic significance [Ayala et al., 2004].

IL-6 AND SIGNALING PATHWAYS OF STAT AND MAPK

Activation of the signaling pathway of JAK/STAT3 by IL-6 has been analyzed in various malignancies. The tyrosine phosphorylation of STAT3 was observed in association with malignant transformation but also in cells that undergoes differentiation after IL-6 treatment. These contrasting responses were also reported in LNCaP cells by groups who observed inhibitory or proliferative response to IL-6, respectively [Spiotto and Chung, 2000a; Giri et al., 2001]. The authors of this review were able to demonstrate STAT3 phosphorylation in LNCaP-IL-6−, but not in more malignant LNCaP-IL-6+ cells, which showed activation of the MAPK pathway [Steiner et al., 2003]. Treatment with the inhibitor of the MAPK kinase PD98059 caused a partial retardation of growth of the LNCaP subline selected with IL-6. These data, however, do not allow the generalization that STAT3 is a tumor suppressor in prostate cancer. STAT3 phosphorylation, assessed by Western blot or immunohistochemistry, is increased in patients with prostate cancer [Dhir et al., 2002; Barton et al., 2004]. This was observed in patients who received palliative surgical treatment but also in indivi-

duals who underwent radical prostatectomy. Therefore, increased STAT3 activity cannot be attributed to endocrine treatment. At present, it is difficult to reconcile these clinical findings with those obtained with LNCaP or LNCaP-IL-6− cells.

Gao's group [2003] used a different approach to investigate the mechanisms underlying regulation of cell growth by IL-6; they generated a novel LNCaP subline by stable transfection of IL-6 cDNA. Similarly as with LNCaP-IL-6+ cells, forced expression of IL-6 resulted in acquisition of growth advantage. It was associated with induction of both STAT and MAPK pathways. Importantly, LNCaP cells that were generated by stable IL-6 transfection grow in an androgen-independent manner in vivo. This is an important observation showing that overproduction of IL-6 in prostate cancer may be sufficient to abrogate androgen-dependency of prostate cancer cells.

Spiotto and Chung [2000a,b] demonstrated that the induction of STAT3 phosphorylation in the LNCaP cell line parallels growth inhibition and neuroendocrine (NE) differentiation. Transdifferentiation of LNCaP cells was observed by several researchers after androgen ablation, treatment with analogues of cAMP, and by typical elongations and processes [Bang et al., 1994; Deeble et al., 2001]. Increase in expression of NE markers chromogranin A and neuron-specific enolase in LNCaP cells was reported in those studies. Induction of NE phenotype was more prominent in lower passages LNCaP cells, which retain androgenic responsiveness [Zelivianski et al., 2001]. NE cells do not proliferate but are known to secrete a variety of peptides that are mitogenic for adjacent cells. Similar results were obtained in vivo; in a recently published study, IL-6 caused an inhibition of growth of LNCaP xenografts in which NE marker expression became evident [Wang et al., 2004b]. More information about the significance of increased STAT3 phosphorylation in prostate cancer could be obtained if specific subpopulations are dissected and probed with an anti-phospho STAT3 antibody. IL-6-induced neuroendocrine differentiation in LNCaP cells is influenced by Etk/Bmx, a tyrosine kinase that acts downstream to PI3-K [Lee et al., 2001]. Induction of NE phenotype was abrogated in cells in which a dominant-negative Etk was overexpressed. Lipid rafts appear to be involved in regulation of NE differentiation [Kim et al., 2004]. The IL-6 effect was disrupted in the presence of the cholesterol-binding drug filipin. The role of STAT3 in DU-145 cells that proliferate in response to endogenous IL-6 was also investigated. Inhibition of proliferation by STAT3 antisense oligonucleotides was reported [Mora et al., 2002].

One of the oncogenic functions of STAT3 is stimulation of expression of vascular endothelial growth factor (VEGF), which is a potent angiogenetic regulator. This regulation was also observed during a short-term incubation of the LNCaP-IL-6− subline with IL-6 [Steiner et al., 2004]. The cells selected after long-term treatment with IL-6 present with constitutively upregulated VEGF and its receptor-2. That finding suggests the role of IL-6 in generation of VEGF autocrine loop in human prostate cancer. It could be concluded that IL-6 is one of the cytokines that regulate angiogenesis and proliferation in prostate cancer through regulation of VEGF. The possibility that IL-6 production is associated with prostate cancer metastasis deserves further investigation. IL-6 increases the expression of the matrix metalloproteinase promatrilysin [Stratton et al., 2001]. High production of IL-6 in prostate cancer cell lines may be one of the reasons why these cells poorly respond to various chemotherapy agents [Borsellino et al., 1995]. PC-3 and DU-145 cells are resistant to treatment with *cis*-diamminodichloroplatinum, etoposide, or adriamycin. Use of an anti-IL-6 antibody, alone or in combination with etoposide, inhibited PC-3 xenograft growth in vivo and stimulated apoptosis [Smith and Keller, 2001]. Development of reasonable anti-IL-6 therapy in prostate cancer is, however, in early stages. Anti-IL-6 antibody did not show an effect on tumor volume of the PC-3M xenograft; however, it prevented the development of cachexia [Zaki et al., 2004]. The reasons why PC-3 and PC-3M tumors differentially respond to the antibody are not known. In experimental models of myeloma, an IL-6 superantagonist caused a growth retardation thus suggesting that, in some malignant tumors such as myeloma, melanoma, and prostate cancer, a therapy strategy aimed to interfere with IL-6 signaling could be justified [Petrucci et al., 1999]. IL-6 expression in prostate cancer cell lines PC-3 and DU-145 was reduced after treatment with dexamethasone due to signaling disruption at the NF kappa B level [Nishimura et al., 2001].

IL-6 AND AR ACTIVITY

The concept of ligand-independent and synergistic activation of the AR is relevant to understanding cellular events in an environment with a reduced androgen level. Activation of the AR by HER-2/neu could be directly linked to prostate cancer progression, as evidenced in the LAPC-4 model [Craft et al., 1999]. It became evident from several research studies that there is a broad spectrum of nonsteroidal compounds that interact with the AR pathway [Culig et al., 2002]. Their effects may be enhanced after prolonged withdrawal of steroids. Due to elevated expression of IL-6 and its receptor in carcinoma of the prostate, a possible interaction between their signaling pathways is of interest. First evidence that IL-6 activates AR-mediated transcription was presented by Hobisch et al. [1998]. In that study, AR activity was induced by IL-6 in a ligand-independent and synergistic manner in DU-145 cells that transiently expressed AR cDNA and an androgen-inducible reporter and in LNCaP cells, in which the AR-target gene prostate-specific antigen was induced by IL-6. From that initial study, it became clear that the stimulation of AR activity by IL-6 is important for maintenance of prostate differentiation function. The effects of IL-6 were blocked by the non-steroidal anti-androgen bicalutamide. Whether IL-6 causes an effect on expression of the AR, remains to be clarified. AR functional activity was also induced by the IL-6-related cytokine oncostatin M (OSM); however, this induction was associated with an acquisition of agonistic properties of hydroxyflutamide [Godoy-Tundidor et al., 2002]. The reasons why anti-androgens behave in different ways in the presence of IL-6 or OSM have not been clarified yet. However, it should be emphasized that, in some nonprostatic cell lines, OSM generates a signal that is more persistent than that of IL-6. In prostate cancer, OSM may act as a positive growth factor, in contrast to studies with breast cancer models [Mori et al., 1999]. It regulates cell growth either through the OSM or LIF receptor [Godoy-Tundidor et al., 2002]. The OSM receptor is expressed in DU-145 cells. Increased expression of the OSM receptor was detected in high Gleason score prostate cancers, whereas the LIF receptor was predominantly found in less aggressive tumors [Royuela et al., 2004]. OSM and LIF were detected in tumor cells in samples obtained from prostate cancer patients.

Interference with IL-6 signaling by administration of inhibitors of signaling pathways of JAK/STAT, MAPK, or PKA substantially decreased the cytokine-induced reporter gene activity [Hobisch et al., 1998]. N-terminal region of the AR appears to be essential for IL-6 induced transcriptional activity [Ueda et al., 2002a]. Interaction between STAT3 and the amino acids 234–558 of the N-terminal region AR was induced by IL-6. Interestingly, STAT3-induced genes may be upregulated by androgen [Matsuda et al., 2001]. More recent studies revealed that intracellular kinases Pim1 and Etk and AR coactivators p300 and SRC-1 that is phosphorylated by MAPK are important for activation of the AR by IL-6 [Debes et al., 2002; Ueda et al., 2002b; Kim et al., 2004]. Interestingly, the expression of these coactivators correlates with malignant potential of prostate cancer cells [Gregory et al., 2001; Debes et al., 2003]. Divergent results on activation of the AR by IL-6 were reported by Coetzee's group [2003]. In their experimental condition, there was an inhibitory effect of IL-6 on androgen-induced prostate-specific antigen expression. They proposed that the underlying mechanism for inhibition of AR-mediated transcription is prevention of recruitment of the coactivator p300 to the transcription initiation complex and partial inhibition of histone H3 acetylation [Jia et al., 2003].

CONCLUSIONS AND FUTURE DIRECTIONS

IL-6 and related cytokines are clearly relevant to development and progression of prostate cancer. Most studies on IL-6 signaling were carried out after establishment of clinical significance of IL-6 and IL-6 receptor expression. In most prostate cancer models, IL-6 acts as a positive regulator of growth. However, several investigators described a prodifferentiation effect of IL-6 in the LNCaP cell line. This is not surprising since it is known that in various target tissues IL-6 causes multifunctional responses. It is, however, of importance to better understand intracellular signaling mechanisms leading to either growth stimulation or inhibition by IL-6. These studies should facilitate development of rational anti-IL-6 therapies in prostate cancer. At this stage, it could be speculated that a therapeutic intervention that

interferes with IL-6 signaling may be justified both at early and late stages of prostate carcinogenesis. Chronic inflammatory lesions seem to contribute to prostate carcinogenesis and IL-6 could be implicated in this process. On the other hand, the importance of IL-6 for androgen-independent growth of tumor cells makes it a good candidate for targeted therapy in human carcinoma of the prostate.

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