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Featured Review Article

Proinflammatory cytokine interleukin-6 in prostate carcinogenesis

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Abstract: Interleukin-6 (IL-6) is a multifunctional pro-inflammatory cytokine which is expressed in clinical specimens obtained from patients with prostate cancer and in multiple cell lines. IL-6 expression is regulated in prostate cancer by several oncogenes and tumor suppressors. IL-6 activates in prostate cancer pathways of Janus kinases/signal transducers and activators of transcription (STAT), mitogen-activated protein kinases, and phosphatidylinositol 3-kinase. In several tumor models, proliferative and anti-apoptotic effects were described, although androgen-sensitive prostate cancer cells LNCaP are inhibited by IL-6. IL-6 is also involved in regulation of neuroendocrine differentiation and angiogenesis in prostate cancer. IL-6 activation of the androgen receptor is important for tumor growth and differentiation. IL-6 activation of STAT3 is crucial for maintenance of the tumor progenitor cells phenotype. Suppressors of cytokine signaling inhibit permanent activation of STAT3, however they may have also IL-6-independent effects. Experimental therapies with aim to inhibit IL-6 signaling in prostate cancer were developed with the monoclonal antibody CNTO328. Although progression towards castration resistance was delayed by CNTO328 in a xenograft model, clinical monotherapies in patients with castration therapy-resistant disease with the antibody did not yield a satisfactory response.

Keywords: Prostate cancer, interleukin, apoptosis, tumor progenitor cells, antibodies

Chropnic inflammation and prostate carcinogenesis

Prostate cancer is a heterogenous neoplasm which is primarily regulated by androgenic hormones and is influenced by dietary habits. There are several factors which could contribute to the development of chronic inflammation, prostatitis, some of which may be contained in red meat [1]. The issue of development of prostate cancer from chronic prostatitis is a subject of discussion among experts in pathohistology and will not be analyzed in detail in this review. However, it should be mentioned that prostate intrapithelial atrophy and high grade prostate intraepithelial neoplasia are lesions which are considered pre-malignant. Appropriate models to study the development of prostate cancer from chronic inflammation are largely missing and it is therefore difficult to analyze contribution of inflammatory cytokines to early prostate carcinogenesis in vivo. Thus, many studies on prostate pro- and anti-inflammatory cytokines are performed with models representing advanced prostate cancer. For this reason, the role of interleukin (IL)-6 in prostate cancer progression is better understood and will be discussed in the most part of this review. However, a report on prostaglandin E(2) stimulation through the IL-6 signaling pathway in a prostatic intraepithelial neoplasia cell line suggested that the cytokine may have a stimulatory role at early stages of prostate carcinogenesis [2]. In accordance with those observations, treatment of prostate intraepithelial neoplasia cells with an inhibitor of cyclooxygenase-2 or an IL-6 neutralizing antibody decreased cellular proliferation.

Regulation of interleukin-6 expression in prostate cancer

IL-6 is a cytokine whose expression and function are altered in several human cancers. Binding of IL-6 to the membrane receptor subunit gp80 is followed by initiation of signal transduction through the gp130 subunit. Subsequently, multiple signaling pathways could be

activated in target cells. Janus kinase (JAK) and signal transducer and activator of transcription (STAT) factor 3 are specifically activated by IL-6. Enhanced activation of the JAK/STAT pathway is observed in many tumors and the development of novel therapies that target STAT3 is one of the priorities in oncology. In addition, signaling pathways of mitogen-activated protein kinase and phosphatidylinositol 3-kinase could be activated upon IL-6 treatment. In addition to regulation through the membrane receptor, IL-6 also acts through trans-signaling in regulation of proliferation, migration, and invasion [3]. Trans-signaling in prostate cancer is dependent on the presence of the soluble IL-6 receptor.

Most studies published in prostate cancer link IL-6 and tumor aggressiveness. IL-6 is expressed in most prostate cancer cell lines and the expression is at higher levels in those which do not express the androgen receptor (AR) and show an enhanced malignant potential [4]. Quantification of IL-6 expression in tissue specimens is possible and those studies revealed that there is increased expression of the cytokine in samples obtained from patients undergoing radical prostatectomy [5]. Consistent with those results, expression of IL-6 and its receptor was consistently demonstrated in tissues from prostate cancer specimens on immunohistochemistry [6]. IL-6 is considered a downstream target of the GBX2 homeobox gene, however also other factors play a role in regulation of its expression. For instance, the tumor suppressor retinoblastoma (RB) is important for inhibition of IL-6 expression. Since RB loss is frequently observed in prostate cancer, this mechanism may be relevant to IL-6 up-regulation in late stages of the disease. IL-6 is also typically regulated by nuclear factor kappa B, similarly to several other cytokines [7]. The elements of the activator protein-1 complex Fra-1 and JunD are also upstream to IL-6 in prostate cancer. In addition, intracellular IL-6 may also be elevated due to up-regulation of transforming growth factor-beta, a growth factor that is inhibitory in vitro [8]. In vivo, growth-promoting effects of transforming growth factor-beta could be explained by immunosuppression, stimulation of angiogenesis, and promotion of epithelial to mesenchymal transition. Vasoactive intestinal peptide was also reported to stimulate IL-6 expression in prostate cancer cells [9]. Loss of annexin A1 expression may also lead to increased IL-6 production [10]. Importantly, chemotherapy with docetaxel leads to an increase in nuclear factor kappa B activity and expression levels of IL-6. Serum levels of IL-6 in patients with prostate cancer are elevated during metastatic progression of the disease [4]. Serum IL-6 is a prognostic factor for patients with prostate cancer. The levels higher than those of 7 pg/ml are associated with a lower survival rate [11]. In summary, up-regulated expression of IL-6 in prostate cancer is a result of multiple alterations in expression of oncogenes and/or tumor suppressors.

Interleukin-6 and regulation of signaling pathways and cellular events in prostate cancer

Earlier studies on IL-6 in prostate cancer revealed that the cytokine mediates resistance to chemotherapy [12]. Cellular treatment with an anti-IL-6 antibody potentiated the effects of cisplatinum and etoposide in prostate cancer. There are several studies related to IL-6 and proliferation in LNCaP cells [13-15]. It is evident that growth-stimulation and -inhibition were reported in different laboratories, most probably because of differences in number of seeded cells or different composition of sera used in various laboratories. LNCaP cells respond to IL-6 also in terms of up-regulation of prostate-specific antigen expression [15]. In experiments in which a negative effect of IL-6 was observed an induction of the cell cycle inhibitors p27 and p21 was reported [16]. On the other hand, proliferative response induced by IL-6 could be explained by recruitment of the oncogene Erb-B2 for the cytokine signaling [17]. Those experiments provide an explanation how IL-6 could act through the signaling pathway of mitogen-activated protein kinases p44/p42. Other researchers observed inhibition of prostate cancer cell growth after treatment with the Janus kinase inhibitor tyrphostin, AG490 [18]. It should be mentioned that STAT3 in prostate cancer may be elevated also in response to IL-11 and growth factors, such as epidermal growth factor [19]. Higher levels of activated STAT3 were found in tissue specimens obtained from patients with a higher Gleason score [20]. The question whether activated STAT3 may have different functions in subgroups of prostate cancers remains to be answered in the future.

IL-6 is known for its ability to induce neuroendocrine differentiation in human prostate cancer [21]. Neuroendocrine prostate cancer is associ-

ated with bad prognosis because of a paracrine effect of neuropeptides which may regulate proliferation and angiogenesis. Neuroendocrine cells themselves are growth-arrested. Tyrosine kinase Etk/Bmx is involved in IL-6-phosphatidyl inositol 3-kinase-regulated neuroendocrine differentiation in prostate cancer. IL-6 regulation of phosphatidyl inositol 3-kinase also leads to inhibition of programmed cell death in prostate cancer cells [22]. The JAK-STAT3 pathway is also implicated in regulation of neuroendocrine differentiation in prostate cancer cells [23]. Regulation of neuroendocrine cells by IL-6 is a reversible process [24].

Anti-apoptotic effect of IL-6 in prostate cancer is mediated by the Mcl-1 oncogene [25]. The expression of Mcl-1 is also elevated in human prostate cancer tissues. The presence of Mcl-1 is important for the effectiveness of an anti-IL-6 antibody. In the absence of Mcl-1 in prostate cancer cells, no therapeutic effect of an antibody could be observed. In addition, the anti-apoptotic effect of IL-6 in prostate cancer through the phosphatidylinositol 3-kinase pathway is accompanied by regulation of cyclin A which is another molecule in the pro-survival network [26]. There are several other possibilities by which IL-6 contributes to prostate cancer progression. One of them is stimulation of intracellular androgen synthesis [27]. In particular, genes encoding HSD3B2 and AKR1C3 involved in biosynthesis of androgens are up-regulated by IL-6. These findings are particularly important because of the use of abiraterone acetate in prostate cancer therapy. The cytokine may also activate insulin-like growth factor receptor thus contributing to prostate carcinogenesis [28]. This interaction between the two signaling pathways is particularly important for regulation of invasive processes. The results of that study indicate that growth factors and cytokines interactions may be particularly important in tumor microenvironment. IL-6 is also known as a regulator of epithelial to mesenchymal transition which is in prostate cancer initiated by the chaperone heat shock protein 27 [29]. Importantly, IL-6 has a crucial role in regulation of stemness of prostate cancer cells [30]. Prostate cancer stem/progenitor cells are of substantial interest in research because of their insensitivity to endocrine and chemotherapy in prostate cancer. Inhibition of activated STAT3 by the anti-IL-6 antibody siltuximab yielded suppression of clonogenicity of prostate cancer stem cells. The results published by Kroon and associates indicate that STAT3 is a target for therapy in the most primitive prostate cancer cells.

Interleukin-6 and androgen receptor signaling

There is an important issue of IL-6 regulation of the androgen receptor (AR). The AR is a ligand-induced transcription factor which is expressed in primary and metastatic prostate cancer. The development of therapy resistance may be associated with increased expression of the AR because of gene amplification or stabilization of the protein, point mutations which lead to increased activation through other steroids and anti-androgens, emergence of truncated AR which are constitutively active, enhanced coactivation, or ligand-independent activation by protein kinases. AR activation by IL-6 is of significance because of the presence of higher concentrations of cytokine during tumor progression. AR activation by IL-6 leads to enhanced growth of MDA PCa 2b tumors in vitro and in vivo [31]. Those effects could be antagonized by the non-steroidal anti-androgen bicalutamide. Mechanistically, both signaling pathways of JAK-STAT and mitogen-activated protein kinase are important for AR activation by IL-6 [32, 33]. Mitogen-activated protein kinases phosphorylate the N-terminal of the AR. STAT3 was shown to associate with the AR in an androgen-independent manner. In addition, IL-6 may also up-regulate the expression of AR in prostate cancer cells [34]. Nonsteroidal anti-androgens do not act as AR agonists in the presence of IL-6. However, oncostatin M, which is an IL-6 type cytokine, caused activation of the AR that is associated with increased agonistic properties of the anti-androgens hydroxyflutamide and bicalutamide [35]. For these reasons, use of the two anti-androgens may be compromised in prostate cancer. Similar acquisition of agonistic properties of steroid receptor antagonists has been discussed in breast cancer. There is experimental evidence suggesting the role of STAT3 in the development of resistance to the anti-androgen enzalutamide in prostate cancer [36]. Although administration of enzalutamide resulted in improved survival of prostate cancer patients, resistance to this therapy also develops. It is expected that future studies will address the issue of STAT3 inhibi-

Table 1. Preclinical anti-IL-6 therapy effects in prostate cancer models

Cellular/xenograft model	Therapy effect	Reference Nr.
PC-3	Induction of apoptosis, inhibition of in vivo growth	48
PC-3M	Inhibition of cachexia	49
LNCaP-IL-6+	Insignificant inhibition of in vivo growth, decrease of Ki67-positive cells	51
LuCaP-35	Delayed tumor progression, blocked orchiectomy, induced p300/CBP expression	50

tion and resistance to novel anti-androgens in detail.

Ligand-independent activation of steroid receptors is in part regulated by coactivators, proteins with histone acetyltransferase activity. One of the coactivators which is important in regulation of cellular events in prostate cancer is SRC-1, a protein whose expression is high in tumors with a higher Gleason score. It was found that SRC-1 enhances AR activation by IL-6 [37]. SRC-1 is phosphorylated by mitogen-activated protein kinases in the presence of IL-6. Importantly, AR-SRC-1 interaction is enhanced by the cytokine. Another coactivator that is important for AR activation by IL-6 is p300 [38]. P300 is a transcriptional activator that is implicated in many cellular processes. It is up-regulated by androgen ablation [39]. A mutant p300 which lacks histone acetyltransferase activity cannot efficiently co-activate the AR [38]. Increased levels of p300 in human prostate cancer correlate with cellular proliferation and extension of the disease [40]. p300 is structurally similar to the coactivator CBP whose expression is down-regulated by IL-6 [41]. Thus, novel experimental therapies targeting p300 may lead to inhibition of AR activation by IL-6. Cells treated with IL-6 were shown to develop resistance to the anti-androgen bicalutamide [42]. This could be explained by increased expression of the coactivator TIF2. Following down-regulation of TIF2, the cells increased their sensitivity to bicalutamide. Cytoplasmic kinases Pim1 and Etk are also required for AR activation by IL-6 [43]. The presence of neither kinase alone is sufficient for ligand-independent AR activation.

There is also an issue of possible alterations in IL-6 signaling in cells that may occur during a prolonged time period. Chronic treatment of cells with IL-6 is relevant because of increased levels of IL-6 in circulation. In the authors' laboratory, parental LNCaP were inhibited by IL-6. After a chronic treatment with the cytokine, a subline with a growth advantage was generated [15]. In vitro results were confirmed in vivo and explained by increased expression of elements of the mitogen-activated signaling pathway in those cells [44]. In addition, it was demonstrated that the expression of cyclin-dependent kinases increases, whereas those of the cell cycle inhibitors decreases in cells generated after prolonged treatment with IL-6. The expression of RB was also lost in the subline generated after prolonged treatment with IL-6. Changes in IL-6 effects from a growth inhibitor to stimulator in prostate cancer cells was confirmed in the Gao's laboratory [45]. The role of IL-6 in promotion of androgen-independent growth in prostate cancer is also evident in LNCaP cells transfected with IL-6 cDNA [46]. In that experimental work, it could be demonstrated that cells which overexpress IL-6 could grow faster under androgen-deprived conditions. IL-6 is also important for regulation of angiogenesis in human prostate cancer [47]. Cells generated after prolonged treatment with IL-6 show the presence of autocrine loop of vascular endothelial growth factor.

Therapeutic and endogenous inhibition of interleukin-6 in prostate cancer

On the basis of several publications mentioned above, it was concluded that experimental therapy against IL-6 should be developed. In initial studies, an anti-IL-6 monoclonal antibody induced apoptosis in PC-3 cells and inhibited tumor growth in vivo [48]. CNTO328, an IL-6 monoclonal antibody prevented induction of cachexia in another prostate cancer xenograft [49]. CNTO-328 was demonstrated to delay progression of the prostate cancer xenograft LuCaP 35 [50]. IL-6 inhibition in vivo was associated with increased apoptosis and reduced cellular proliferation. A less pronounced inhibition of cancer cell growth by CNTO328 was observed in the LNCaP-IL-6+ xenograft [51]. A summary of preclinical studies with CNTO328 is presented in **Table 1**. A phase I clinical study with CNTO328

accompanied with an array analysis provided a basis for further patients' treatment [52]. It was revealed that the expression of several genes immediately downstream of the IL-6 signaling pathway and androgen synthesis enzymes decreased after CNTO328 treatment. However, clinical studies with CNTO328 in advanced prostate cancer did not show an improvement in the disease status [53, 54]. There may be several reasons for this lack of efficacy of anti-cytokine therapy. Importantly, any monotherapy may not be effective in castration-resistant prostate cancer because of tumor heterogeneity. IL-6 production was also reduced by administration of vitamin D and subsequent inhibition of the p38 mitogen-activated protein kinase pathway [55].

Endogenous inhibitors of IL-6 signaling are suppressors of cytokine signaling (SOCS). Under physiological conditions, SOCS prevent continuous up-regulation of IL-6 signal transduction and, probably, chronic inflammation. Most frequently studied SOCS proteins in prostate cancer and SOCS-1 and -3. It was demonstrated that a SOCS-1 peptide mimetic inhibited constitutive and IL-6-induced STAT3 activation in cancer cells [56]. This peptide mimetic blocked progression to the S phase of cell cycle. In this area of research, Neuwirt and colleagues documented SOCS-1 expression in prostate cancer cell lines and clinical specimens as well as its role in down-regulation of cyclins and cylin-dependent kinases [57]. In contrast, SOCS-3 expression was detected predominantly in AR-negative cell lines [58]. In these cells, SOCS-3 has an anti-apoptotic effect which is regulated by the Bcl-2 oncogene. Inhibition of expression of SOCS-3 in prostate cancer cells yielded increased phosphorylation of STAT3 [59].

Conclusions

Because of its increased expression in prostate cancer and involvement in regulation of proliferative responses and cell death, IL-6 is a valid therapy target in prostate cancer. There is an evidence suggesting that anti-IL-6 antibodies target appropriate models and cause inhibition of tumor volume or delayed progression towards castration therapy-resistance. Although anti-IL-6 monotherapies did not yield a success in clinical trials in patients with therapy resistant-prostate cancer, there is an opportunity to design studies in which anti-IL-6 therapies are combined with other agents at earlier stages of the disease. Additional studies should be performed in order to evaluate the potential of modulation of SOCS in prostate cancer in order to interfere with IL-6 signaling and prevent disease progression.

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