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# Interleukin-6 and prostate cancer: Current developments and unsolved questions

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ABSTRACT

Interleukin (IL)-6 is a pro-inflammatory cytokine that is expressed in prostate tumors and in the stromal tumor micro-enviroment. It is known to regulate proliferation, apoptosis, angiogenesis, and differentiation. The signaling pathway of Janus kinase and signal transducer and activator of transcription (STAT)3, which is activated by IL-6, is in the focus of scientific investigations for improved treatment approaches. Different effects of IL-6 and/or STAT3 on tumor cell growth have been observed in human and murine prostate cancer (PCa) models. Experimental therapies have been proposed in order to block the IL-6/STAT3 signaling pathway. In this context, the anti-IL-6 antibody siltuximab (CNTO 328) has been demonstrated to inhibit growth of prostate tumors *in vitro* and *in vivo* and delays progression towards castration resistance. However, clinically, the anti-IL-6 antibody was not successful as a monotherapy in phase II studies in patients with metastatic PCa. IL-6 is implicated in regulation of cellular stemness by increasing phosphorylation of STAT3. The cytokine has also a role in development of resistance to the non-steroidal anti-androgen enzalutamide. Endogenous inhibitors of IL-6 are suppressors of cytokine signaling and protein inhibitors of activated STAT. Although they inhibit signal transduction through STAT3, they may also exhibit anti-apoptotic effects. On the basis of complexity of IL-6 action in PCa, an individualized approach is needed to identify patients who will benefit from anti-IL-6 therapy in combination with standard treatments.



## 1. Introduction

Recent investigations have resulted in identification of a large number of potential targets for prostate cancer (PCa) therapy. Although established therapies target the androgen receptor (AR) or elements of the AR signaling pathway, it is clear that several molecules regulated by cytokines contribute to growth at different stages of prostate carcinogenesis. The oncogenic role of inflammatory cytokines has been studied in PCa in different human and animal models and experimental settings. In most studies, signal transduction and effects on proliferation and apoptosis by interleukin (IL)-6, −8, and −4 have been investigated. The role of IL-6 in PCa is particularly interesting because of its association with multiple signaling pathways, as well as different regulation of proliferative and apoptotic responses. Thus, there is great potential for therapeutic intervention. Although results of preclinical research have provided encouraging data related to potential anti-IL-6 strategies, translation of these findings into clinical practice is still associated with considerable difficulties. Recent studies have therefore focused on the role of endogenous inhibitors of cytokine signaling in PCa. In this review, these topics will be primarily addressed and discussed. Basic knowledge of IL-6 signal transduction and regulation of cellular events in PCa have been summarized previously (Culig and Puhr, 2012). A central role in regulation of the inflammatory responses in PCa could be attributed to signal transducer and activator of transcription (STAT) factor-3. It is regulated not only in response to IL-6, but also by other cytokines such as IL-11 and epidermal growth factor (EGF) in the tumor and in the tumor micro-environment. In this updated review, we analyze recent developments in the field and discuss problems as well as perspectives of IL-6 targeted PCa therapies.

## 2. IL-6: a therapeutic target or tumor suppressor in PCa

Prostate carcinogenesis and progression are determined by the presence and activation status of the AR. Its activation in normal and pathological conditions can be potentiated by numerous co-

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activators that interact with one or more domains of the receptor. AR expression is increased in castration resistant PCa (CRPC) due to gene amplification or protein stabilization. Mutant receptors may be activated by various steroids and anti-hormones, thus contributing to accelerated tumor growth. Appearance of constitutively active, truncated AR in tissues of patients undergoing PCa therapy resulted in application of new experimental treatments, such as the antihelmintic drug niclosamide (Liu et al., 2016). In this context, it is particularly important to inhibit expression and/or function of the AR variant 7, which is highly expressed in CRPC (Maughan and Antonarakis, 2015). Regulation of PCa cell growth by IL-6 was studied in AR-positive and −negative cells. Although it is clear that androgen-insensitive cells are stimulated by IL-6, contrasting results were obtained only with LNCaP cells in which either growth stimulation or inhibition was observed (Degeorges et al., 1996; Giri et al., 2001). In that particular cell line, it has been demonstrated that differences may occur depending on the presence of stably transfected cells with IL-6 cDNA or treatment with exogenous IL-6 (Degeorges et al., 1996; Lee et al., 2003). In the first case, cells may show a growth stimulatory effect, especially in androgen-deprived conditions. Thus, IL-6 is one of the factors that may contribute to increased growth in the absence of androgens. In summary, the majority of published results support the view that IL-6 is an oncogenic factor in PCa. Recently, Albino et al. (2016) provided evidence that benign prostate cells undergo transformation upon treatment with exogenous IL-6. Growth-stimulatory effects of IL-6 may be potentiated by insulin-like growth factor-I (IGF1), a peptide that is known as an inhibitor of apoptosis in prostate and other cancer entities (Rojas et al., 2011). Stimulation by IL-6 and IGF1 could be particularly important in the tumor micro-environment in which both cytokines are expressed. The effect of IL-6 is mediated by up-regulation of IGF1. Some investigators, however, observed LNCaP paracrine growth inhibition and cell cycle arrest, associated with increased expression of cell cycle inhibitors (Mori et al., 1999). Regulation of cellular growth by IL-6 could also depend on the duration of stimulation with the cytokine. Paracrine growth inhibition was not detected in cells generated after prolonged treatment with the cytokine (Steiner et al., 2003; Lee et al., 2007). It was apparent that autocrine stimulation of growth by IL-6 was not associated with STAT3 phosphorylation. Another important factor which has to be considered for interpretation of studies on growth regulation by IL-6 and STAT3 signaling is selection of a relevant tumor model. In mouse prostate tumors in which the tumor suppressor PTEN is deleted, it was demonstrated that IL-6 and STAT3 are implicated in regulation of cellular senescence (Pencik et al., 2015). Co-deletion of STAT3 and PTEN resulted in enhanced metastatic potential of murine PCa cells. STAT3 is therefore considered to induce cellular senescence through coordinating action of ARF, Mdm2, and p53. ARF inhibits Mdm2 and, in consequence, promotes activation of p53 thus causing cell cycle arrest and apoptosis. Interplay between STAT3 and members of the tumor suppressor machinery may be therefore further investigated in other models relevant to human PCa to provide explanation for these findings. However, Toso et al. (2014) reported that, in PTEN-null senescent tumors, activated STAT3 pathway is associated with immunosuppression, progressive tumor growth, and chemo-resistance. Future research may provide a possible explanation for differences between those two experimental studies. Interestingly, a recent study revealed an increase of STAT3 and ZEB1 which contributes to cancer invasiveness and metastasis, during early stages of prostate carcinogenesis, thus demonstrating a potential importance of STAT3 in pre-neoplastic lesions (Cha et al., 2016). It is however not clear whether overexpression of STAT3 has a direct effect on transformation of prostate cells. Animal models relevant to early prostate carcinogenesis are not frequently used. Due to that fact, our knowledge on STAT3 function in PCa is mostly obtained from models representing an advanced disease. Therefore, several conclusions related to the role of the STAT3 signaling pathway in early carcinogenesis are only indirect. Recently, a mechanism related to control of function of STAT3 in PCa has been revealed (Dallavalle et al., 2016). In normal conditions, STAT3 is degraded by the E3 ubiquitin ligase COP1, which is inhibited by miR-424. In conditions in which this miRNA is overexpressed, COP1 cannot act as negative regulator of STAT3 thus leading to enhanced prostate tumorigenesis. The transcription factor ERG is frequently overexpressed in PCa patients. Co-expression of IL-6 and ERG was correlated in tissue samples thus further supporting oncogenic role of IL-6 (Merz et al., 2016).

Furthermore, it was demonstrated that neuroendocrine peptides are elevated in growth-arrested cells and regulate growth of adjacent tumor cells in a paracrine manner. The presence of neuroendocrine compounds in PCa is an indicator of bad prognosis. On the other hand, studies have linked “altered” STAT3 expression with neuroendocrine differentiation (Spiotto and Chung, 2000). Besides IL-6, it is known that androgen withdrawal or treatment with cAMP cause morphological changes and increased expression of neuroendocrine peptides (Debbie et al., 2001). STAT3 activation was also reported as a consequence of deregulation of the AR/CCL4 axis (Fang et al., 2013). CCL4 up-regulation is associated with high expression of Snail, a transcription factor that is relevant to epithelial to mesenchymal transition (EMT). The results of Fang and colleagues suggest that interaction between either epithelial or stromal AR may have different implications for PCa. STAT3 activation was also observed during macrophage recruitment by the chemokine CCL2 and its receptor CCR2 (Izumi et al., 2013). CCL2 expression was additionally found to be associated with poor prognosis of PCa patients.

STAT3 expression was detected in the vast majority of PCa lymph node and bone metastases (Don-Doncow et al., 2017). Promotion of a migratory phenotype by STAT3 could therefore contribute to metastatic spread (Gu et al., 2010). Another member of STAT family, STAT5, which is induced by prolactin and growth hormone is also involved in regulation of tumor cell proliferation. Several studies thus indicate that both STAT factors, which are phosphorylated in response to different cytokines, may be targeted in prostate carcinogenesis. In contrast to LNCaP cells, STAT3 is not detectable in PC3 cells as a consequence of genomic deletion. STAT3 is involved in maintaining constitutive nuclear factor kappa B activity in many malignancies, thus contributing to a positive feedback loop. Nuclear factor kappa B is a master regulator of several IL's which are critical for inhibition of apoptosis and enhancement of angiogenesis.

There are several possibilities to achieve the blockade of activated STAT3 in pathological conditions. Blockade of STAT3 will likely inhibit the expression of downstream targets like MCL1 and survivin. MCL1 is required for the anti-apoptotic effect of IL-6 in PCa (Cavarretta et al., 2007) and Survivin is also expressed in cells representing therapy-reistant PCa (Zhang et al., 2005). Furthermore, STAT3 inhibition likely affects cyclin D1, c-Myc, and Bcl-2. For example, overexpression of a dominant negative STAT3 has been reported to inhibit PCa growth *in vitro* and *in vivo* (Ni et al., 2000). Similar results could be achieved with the Janus kinase (JAK) 2 inhibitor tyrphostin AG490. Activation of STAT3 in PCa is not limited to IL-6, since the same regulation could be observed with epidermal growth factor (EGF) or IL-11. STAT3 inhibition may be achieved also by indirubin, which is an active component of a traditional Chinese herbal medicine (Nam et al., 2005). Resveratrol, which is contained in fruits and red wine, has a negative effect on STAT3 expression in prostate cells (Kotha et al., 2006). Treatment of PCa cells with the flavanone silibinin may have a similar effect, thus