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The Role of Interleukin-6 and Interleukin-6 Receptor as Prognostic Biomarkers in Prostate Cancer

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Abstract

Background: IL-6 is known to play important roles in the growth of prostate cancer cells, activation of the androgen receptor and prostate-specific protein expression. It is reported that IL-6 promotes the growth of prostate cancer. Prostate Specific Antigen (PSA) does not directly assess the biological behavior of individual hormone-refractory prostate cancers, whereas serum biomarkers produced by tumors may correlate with disease progression. Prostate cancer cells, especially those that are hormone-refractory, secrete IL-6. We therefore evaluated the potential of circulating IL-6 levels as a marker of disease progression. Furthermore, we investigated the correlation with the expression of IL-6 receptor (IL-6R) and the effects of hormonal therapy against prostate cancer.

Methods: We separated 21 patients with hormonal refractory prostate cancer into one group with more (higher group; n=9), and another with less (lower group; n=12) serum IL-6 than average. The correlation between prognosis and serum IL-6 level was investigated in these groups. Furthermore, in 57 specimens from patients with prostate cancer and benign prostatic hypertrophy, the expression of IL-6R was examined using immunohistochemistry. The correlation with immunoreactivity of IL-6R and Gleason grade as well as histological effects of hormonal therapy was compared using the nonparametric Mann-Whitney U-test and generalized Wilcoxon test.

Results: A comparison of disease-specific survival after PSA failure between these groups demonstrated that serum PSA and serum IL-6 levels were significantly correlated. The median survival after PSA failure was significantly shorter in the higher group than in the lower group (p=0.004). There were significant differences in IL-6R expression between medium Gleason grade and high Gleason grade (p=0.008), and BPH and high Gleason grade (p=0.004). Furthermore, the level of IL-6R expression was correlated with the histological effects of hormonal therapy against prostate cancer.

Conclusion: The serum IL-6 level may be associated with the prognosis of patients with prostate cancer, and both IL-6 and PSA levels may indicate patients with a poor prognosis. In prostate cancer patients with strong expression of IL-6R, other therapeutic options without hormonal therapy, irradiation and/or chemotherapy should be chosen first. The expression of IL-6R may be helpful in predicting the effects of hormonal therapy against prostate cancer.

Keywords: IL-6; IL-6 receptor; Prostate cancer

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Introduction

Interleukin-6 (IL-6) is a pleiotropic cytokine that regulates immune defense mechanisms and hematopoiesis, and may also be involved in malignant transformation and tumor progression. A poor prognosis for patients with multiple myeloma, renal cell carcinoma, ovarian cancer or prostate cancer is consistently associated with elevated serum IL-6 levels [1-3].

IL-6 induces prostate cancer cell proliferation *in vitro* through autocrine and paracrine mechanisms [4]. The serum IL-6 level correlates with lymph nodes and bone metastasis, or prognosis among patients with hormone-refractory prostate cancer [5]. Furthermore, cross-talk between IL-6 and the androgen pathway has been demonstrated [6]. Prostate Specific Antigen (PSA) does not directly assess the biological behavior of individual hormone-refractory prostate cancer, but serum biomarkers produced by tumors may correlate with disease progression. Prostate cancer cells, especially those that are hormone-refractory, secrete IL-6.

As IL-6 may be a mediator of morbidity in patients with metastatic disease, we evaluated the potential of circulating IL-6 levels as a marker of prostate cancer progression.

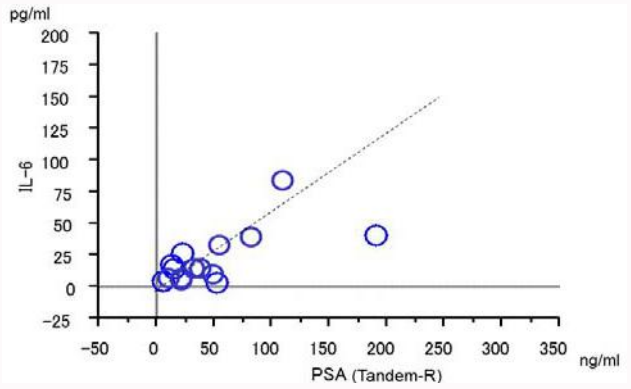

Figure 1: Serum IL-6 levels compared with PSA in patients with hormone refractory prostate cancer. Correlation between blood PSA and serum IL-6 level is significant (r^2=0.663).

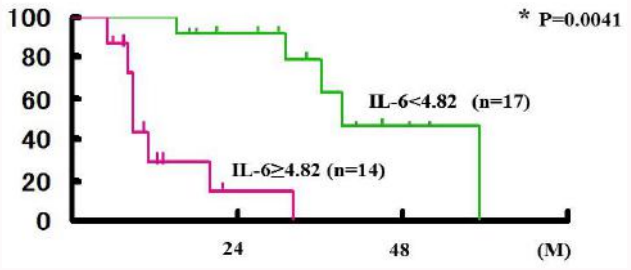

Figure 2: Cancer-specific survival curves of 21 hormone refractory prostate cancers related to serum IL-6 level. Cancer-specific survival after PSA failure was significantly longer in the higher level group than in the lower level group (p = 0.0041).

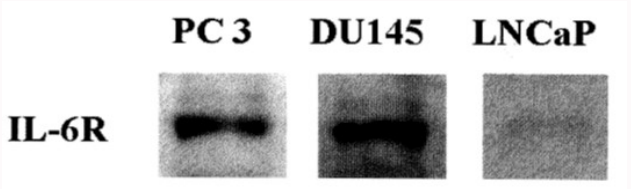

Figure 3: In three prostate cancer cell lines, positive immunoreactivity of IL-6R was detected by western blot analysis. The expression of IL-6R in androgen-independent prostate cancer cells, PC-3 and DU145, was stronger than in the androgen-responsive prostate cancer cells, LNCaP.

Furthermore, human prostate cancers and cancer cell lines almost uniformly expressed the IL-6 receptor (IL-6R) [7,8], giving rise to an IL-6 autocrine loop.

In this study, immunohistochemical analysis of IL-6R in hormone refractory prostate cancer was performed. We examined the correlation with the expression of IL-6R and the effects of hormonal therapy against prostate cancer.

Patients and Methods

Twenty-one patients with hormone-refractory prostate cancer were selected from Nagoya City University Hospital between October 2011 and September 2013. Levels of IL-6 were determined using an ELISA and correlation with prognosis and disease-specific survival after PSA failure was examined. All 21 patients were examined using X-rays, computed tomography and bone scintigraphy to detect distant metastasis.

The patients were then separated into one group with more (higher group; n=9) and another with less (lower group; n=12) than the average serum IL-6 value, and then we compared disease-specific survival after PSA failure between the two groups.

Data are presented as means ± Standard Error of the Means (SEM). Individual groups were compared using the nonparametric Mann-Whitney U-test, the generalized Wilcoxon test and Student's t-test. A probability value of $p < 0.05$ was considered significant in all analyses.

Correlation between IL-6R expression and effects of hormonal therapy in prostate cancer

Cells and Culture conditions: Cultured human prostate cancer cell lines, PC-3 and DU145 were obtained from the American Type Culture Collection (ATCC, USA). The androgen-responsive prostate cancer cell line, LNCaP was purchased from Dainihon Laboratory Products Co. (Osaka, Japan). PC-3 and DU145 were maintained in Eagle's MEM medium supplemented with 10% fetal bovine serum. LNCaP was maintained in RPMI1640 medium supplemented with 10% fetal bovine serum.

Patients and Samples: Prostatic biopsies from 52 patients who were diagnosed with prostate cancer and 5 patients diagnosed with Benign Prostatic Hypertrophy (BPH) at Nagoya City University Hospital between October 2011 and September 2013 were enrolled in this study. These patients were subdivided into stage C (8 patients), stage D (44 patients) and BPH (5 patients) groups according to the general rules for clinical and pathological studies on prostate cancer. For the detection of distant metastasis, all the patients were checked by X-ray, computed tomography and bone scintigraphy. The 52 prostate cancer specimens were subdivided into three groups by Gleason grade – low Gleason (dominant grade 1-2), medium Gleason grade (dominant grade 3) and high Gleason grade (dominant grade 4-5) (Table 1). The histological criteria of the effects of hormonal therapy are as follows, Grade 0; the viable cells occupy the entire cancer lesion in the slice, Grade 1; the non-viable cells are in less than half of the cancer lesion in the slice, Grade 2; the non-viable cells are in over half of the cancer lesion in the slice, Grade 3; only non-viable cells are in the cancer lesion in the slice.

Immunoblotting: Semiconfluent prostate cancer cells were collected and lysed in modified RIPA buffer (20 mM Tris. HCl [pH 7.4], SDS 0.1%, Triton X 100 1%, sodium deoxycholate 1%). The protein concentration was determined by BCA assay. After separation by SDS-PAGE, proteins were transferred to Immobilon-P transfer membranes (Millipore Co., Bedford, MA). The membranes were

Table 1. Patient's characteristics

		(n=31)
Age(ys.)	74.8±6.7 (63-86)	
PSA (ng/ml)	48.3±66.5(5.2-210.0)	
Gleason scores		
	< 7	9 cases
	7	11cases
	7 >	11 cases
Therapy after PSA failure (first line)	Dexamethasone	19 cases
	Estramustine	12 cases

Table 2. Serum IL-6 levels in CRPC study

Serum IL-6 (pg/ml)	4.82±8.65 (0.8-79.6)	
Mean survival after PSA failure (M)	25.6±14.5 (5-59)	
IL-6<4.82 (lower group) (n=17)	35.2±11.1 (15-59)	*
IL-6≥4.82 (higher group) (n=14)	14.1± 9.8 (5-32)	*

* P=0.0041

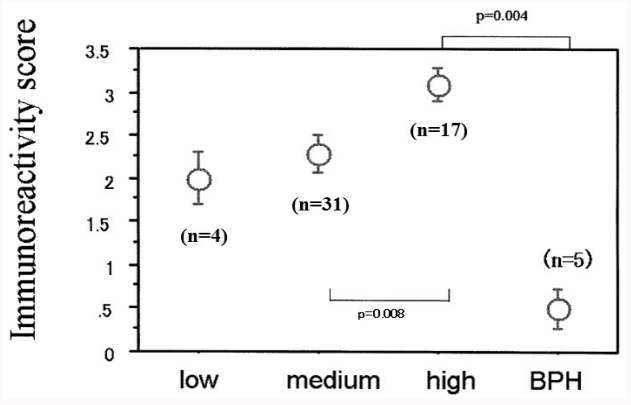

Figure 4: The positive correlation of IL-6R expression (IR) and Gleason grade is demonstrated by semi-quantitative evaluation. IL-6R expression was strongest in prostate cancer patients with a high Gleason grade. There were significant differences in IL-6R expression between medium Gleason grade and high Gleason grade (p=0.008), and BPH and high Gleason grade (p=0.004) by the Mann-Whitney U-test.

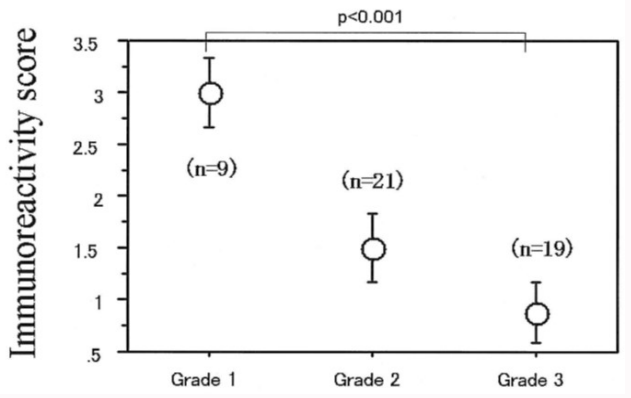

Figure 5: The level of IL-6R expression was correlated with the histological effects of hormonal therapy against prostate cancer. There was a significant difference in IR of IL-6R between the histological effects of hormonal therapy grade 1 and grade 3 (p<0.001) by the generalized Wilcoxon test.

blocked, probed with monoclonal antibody (1:1000) against IL-6R (Santa Cruz Co., CA, USA) and developed using WESTERN BLOT KIT (Kirkegaard and Perry Laboratories, Inc., Gaithersburg, MD).

Immunohistochemistry: Paraffin-embedded tissue sections obtained from 45 patients during the operation were deparafinized in a cleaning solution (Histochoice, Amresco, OH, USA), rehydrated

Table 3 Patients characteristics

Total patients		57 cases
Age		67.8±8.6 y (59-81y)
Prostate cancer		52 cases
Gleason grade		
low		4 cases
medium		31 cases
high		17 cases
Stage		
	C	8 cases
	D	44 cases
BPH		5 cases

in a graded series of ethanol (100%, 95%, 70% and 50%) and washed in distilled water. Endogenous peroxidase activity was quenched by 1.5% H_2O_2 in PBS for 15 min followed by washing twice with PBS. Non-specific protein recognition by the antibody was blocked in casein wash buffer (containing 0.3% casein and 0.5% Tween20 in PBS) for 30 min. Tissue sections were then incubated for 1h at room temperature with the primary antibody, monoclonal anti-IL-6R antibody. After being washed twice in 1:10 casein wash buffer for 5 min, the sections were incubated with 1:250 biotinylated anti-mouse IgG (Vector Laboratories, CA) for 30 min. The specific intracellular immunoreactivity was detected by incubation with avidin-biotin/horse radish peroxidase complex (Vector Laboratories, CA) for 45 min at room temperature followed by color development in 0.05% diamino-benzidine/0.01% H2O2/PBS (pH 7.6) chromogen (Sigma, MO) for 5 min. Color development was stopped by washing in distilled water, and sections were lightly counterstained in hematoxylin, dehydrated in a graded series of alcohol, cleared in xylene and finally mounted in Eukitt.

Semi-quantitative Analysis of IL-6R expression : The degree of IL-6R expression was estimated and classified into one of five grades as described previously [9]. Immunoreactivity (IR) of IL-6R was classified into a scale of 0 to 4 on the basis of tumor cell staining as follows: 0, no staining; 1, focal, weak staining; 2, strong staining of <25% of cells or moderate staining of <80%; 3, strong staining of 25 - 50% or moderate staining of >80%; and 4, strong staining of >50%. The immunostained tissue section slides were examined and scored independently by two of the authors blinded to any other pathological or clinical information: in 60% of cases the decisions were consistent, and the other 40% were reviewed until agreement was achieved.

Statistical Analysis: Data are presented as the mean ± Standard Error of the Means (SEM). Individual groups (Gleason grade and histological effects of hormonal therapy) were then compared using the nonparametric Mann-Whitney U-test, generalized Wilcoxon test and Student's t-test. For all analyses, a probability value of p<0.05 was considered significant.

Results

Table 1 presents the baseline characteristics of the 31 patients with hormone-refractory prostate cancer. The median age was 74.8 years and 89% of them had bone metastases. The secondary therapy applied after PSA failure was almost always steroids. Serum PSA and IL-6 levels significantly correlated (Figure 1).

The mean serum IL-6 value was 4.82 ng/ml (0.8 ~ 79.6 ng/ml). The median survival after PSA failure in the higher (14.1 months) group was significantly shorter than that of the lower (35.2 months) group (p=0.004) (Table 2, Figure 2).

In three prostate cancer cell lines, positive IR of IL-6R was detected by western blot analysis (Figure 3). The expression of IL-6R in androgen-independent prostate cancer cells, PC-3 and DU145, was stronger than in the androgen-responsive prostate cancer cells, LNCaP. IL-6R expression was detected by immunohistochemistry in almost all specimens from patients with prostate cancer.

Table 3 presents the baseline characteristics of the 57 patients with prostate cancer to evaluate IL-6R. In Figure 4, the positive correlation of semi-quantitative evaluation of the IL-6R expression (IR) and Gleason grade is demonstrated. IL-6R expression was strongest in prostate cancer with a high Gleason grade. There were significant differences in IL-6R expression between medium Gleason grade and high Gleason grade (p=0.008 Mann-Whitney U-test), and BPH and high Gleason grade (p=0.004). Interestingly, the level of IL-6R expression was correlated with the histological effects of hormonal therapy against prostate cancer (Figure 5). As shown in Figure 5, we found a significant difference in IR of IL-6R between the histological effects of hormonal therapy grade 1 and grade 3 (p<0.001 by generalized Wilcoxon test).

Discussion

De Vita et al. [10] demonstrated that IL-6 serum levels are significantly elevated in patients with advanced gastrointestinal cancer compared with controls. Moreover, serum IL-6 levels are significantly higher in patients with disseminated cancer than in those without apparent metastases. Univariate analysis revealed in the present study that serum IL-6 levels affected both overall survival and time to disease progression. As IL-6 signaling can activate the androgen receptor in a ligand-independent manner, it may play an important functional role in the progression of hormone-refractory prostate cancer and in patient survival. Several small studies have associated plasma and serum IL-6 levels with prostate cancer progression.

The molecular actions of IL-6 in prostate cancer cells have not been completely elucidated, but IL-6 binds to a trans-membrane receptor (IL-6Rp80) that requires the association of a second glycoprotein (gp130) [11]. Signals are then transduced via three possible routes: the ErbB3(2)-mitogen-activated protein kinase pathway, the phosphoinositide 3-kinase-Etk/Bmx pathway or the Janus-activated kinase/signal transducers and activators of transcription pathway, all of which are associated with androgen-independent prostate cancer [12]. Recent studies suggest that IL-6R activation represents a dominant pathway for accessory activation of the androgen receptor [13]. Furthermore, IL-6 alone is a relatively powerful activator of androgen receptors in the absence of androgen, and it is synergistically potent in the presence of low androgen levels [11,12]. Thus, IL-6 functions as an autocrine growth factor for the androgen-independent growth of prostate cancer cells [1,3]. However, IL-6 may also induce a more differentiated grade of cancer, resulting in decreased proliferation and apoptosis [14-22]. These studies suggest that the ultimate cellular effect of IL-6 depends on other genetic and molecular features of the cancer, including downstream pathways such as signal transducers and activators of transcription-3, as well as phosphoinositide 3-kinase. Finally, the dysregulation of IL-6 may result in paraneoplastic morbidity and early mortality. IL-6 is a potent mediator of the acute-phase response to injury and infection [11], and chronically elevated IL-6 levels in Castleman's disease are associated with a constellation of symptoms that are analogous to those that are often associated with end-stage prostate cancer [16]. Twillie et al. [22] have suggested that in patients with advanced hormone-refractory prostate cancer, serum IL-6 is a prostatic exocrine gene product, a candidate mediator of prostate cancer morbidity and a potential marker of disease activity. However, if IL-6 was the sole factor for such biology, its prognostic significance should more closely correlate with LDH, performance status and other measures of the host condition.

Serum levels of IL-6 and of IL-6R are typically elevated in patients with advanced prostate cancer, particularly bone metastases, and correlate with poor prognosis [23,24]. In the PC-3 and DU145 cell lines, increased activity of the IL-6 promotor has been traced to constitutive up-regulation of NF-κB and AP-1 transcription factors [25]. Thus, the constitutive activation of NF-κB, typical of androgen-independent prostate cancers, may promote androgen independence via up-regulated IL-6 and STAT3. Pencik et al. [26] reported that IL6/STAT3/ARF signaling promote progression and metastasis in prostate cancer. Inhibition of STAT3 signaling may become a target for advanced prostate cancer [27].

Furthermore, Wegiel B et al. [28] reported that IL6 may utilize PI3K/Akt and cyclin A1 to promote tumor cell survival in prostate cancer. Recently, it was demonstrated that autocrine IL6/STAT3 signaling mediates resistance to anti-VEGF therapy in several cancers [29].

All members of the IL-6 family share the common signal transduction subunit of their receptors, gp130 [30]. The interaction of the ligand with its receptor involves binding of the ligand to a specific receptor component, followed by heterodimerization with the gp130 molecule [31]. *In vivo* targeting of IL-6 by an anti-IL-6 antibody has already demonstrated regression of prostate cancer xenografts in nude mice [32]. In this study, we demonstrated that the expression of IL-6R in androgen-independent prostate cancer was stronger than that in androgen-dependent prostate cancer. Furthermore, the level of IL-6R expression was correlated with the histological effects of hormonal therapy against prostate cancer. If we speculate the effects of hormonal therapy against prostate cancer, the appropriate option of first therapy can be selected. In prostate cancer patients with strong expression of IL-6R, other therapeutic options, without hormonal therapy, irradiation and/or chemotherapy, should be chosen first.

We previously analyzed the effects of NF-κB inhibition using the anti-NF-κB reagent, N-acetyl-*L*-cysteine (NAC) on the viability of prostate cancer cells after chemotherapy [33]. We established that the inhibition of NF-κB activation by NAC substantially enhances the effects of chemotherapy by CDDP, dosetaxel or etoposide.

We concluded that the expression of IL-6R may be helpful in predicting the effects of hormonal therapy against prostate cancer. Furthermore, these results indicate that the serum IL-6 level is associated with the prognosis of patients with prostate cancer, and suggest that both IL-6 and PSA are factors that can identify patients with a poor prognosis who may benefit from more aggressive management such as with NAC plus CDDP, dosetaxel or Etoposide.

References

1. Giri D, Ozen M, Ittmann M. Interleukin-6 is an autocrine growth factor in human prostate cancer. Am J Pathol. 2001; 159: 2159–2165.

2. Lee SO, Lou W, Hou M, de Miguel F, Gerber L, Gao AC. Interleukin-6 promotes androgen-independent growth in LNCaP human prostate cancer cells. Clin Cancer Res. 2003; 9: 370–376.

3. Okamoto M, Lee C, Oyasu R. Autocrine effect of androgen on proliferation of an androgen responsive prostatic carcinoma cell line, LNCAP: role of interleukin-6. Endocrinology. 2003; 138: 5071–5074.

4. Borsellino N, Belldegrun A, Bonavida B. Endogenous Interleukin 6 is a resistance factor for cis-diamminedichloroplatinum and etoposide-mediated cytotoxicity of human prostate carcinoma cell lines. Cancer Res. 1995; 55: 4633-4639.

5. Howard LA, McCurdy MA, Kattan MW, Timme TL, Scardino 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.

6. Hobisch A, Eder IE, Putz T, Horninger W, Bartsch G, Klocker H, et al. Interleukin-6 regulates prostate-specific protein expression in prostate carcinoma cells by activation of the androgen receptor. Cancer Res. 1998; 58: 4640-4645.

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

8. Siegall CB, Schwab G, Nordan RP, et al. Expression of the interleukin 6 receptor and interleukin 6 in prostate carcinoma cells. Cancer Res. 1990; 50: 7786-7788.

9. Tozawa K, Akita H, Kawai N, Okamura T, Sasaki S, Hayashi Y, et al. KAI1 expression can be a predictor of stage A prostate cancer progression. Prostate Cancer and Prostatic Diseases 2001; 4: 150-153.

10. De Vita F, Romano C, Orditura M, Galizia G, Martinelli E, Lieto E, et al. Interleukin-6 serum level correlates with survival in advanced gastrointestinal cancer patients but is not an independent prognostic indicator. J Interferon Cytokine Res. 2001; 21: 45-52.

11. Heinrich PC, Behrmann I, Haan S, Hermanns HM, Müller-Newen G, Schaper F. Principles of interleukin (IL)-6-type cytokine signalling and its regulation. Biochem J. 2003; 374: 1–20.

12. 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.

13. Lin DL, Whitney MC, Yao Z, Keller ET. Interleukin-6 induces androgen responsiveness in prostate cancer cells through up-regulation of androgen receptor expression. Clin Cancer Res. 2001; 7: 1773–1781.

14. Chung TD, Yu JJ, Kong TA, et al. Interleukin-6 activates phosphatidylinositol-3 kinase, which inhibits apoptosis in human prostate cancer cell lines. Prostate. 2000; 42: 1–7.

15. Spiotto MT, Chung TD. STAT3 mediates IL-6-induced growth inhibition in the human prostate cancer cell line LNCaP. Prostate. 2000; 42: 88–98.

16. Ueda T, Mawji NR, Bruchovsky N, Sadar MD. Ligand-independent activation of the androgen receptor by interleukin-6 and the role of steroid receptor coactivator-1 in prostate cancer cells. J Biol Chem. 2002; 277: 38087–38094.

17. Chen T, Wang LH, Farrar WL. Interleukin 6 activates androgen receptor-mediated gene expression through a signal transducer and activator of transcription 3-dependent pathway in LNCaP prostate cancer cells. Cancer Res. 2000; 60: 2132–2135.

18. Hobisch A, Eder IE, Putz T, Horninger W, Bartsch G, Klocker H, et al. Interleukin-6 regulates prostate-specific protein expression in prostate carcinoma cells by activation of the androgen receptor. Cancer Res. 1998; 58: 4640–4645.

19. Matsuda T, Junicho A, Yamamoto T, Kishi H, Korkmaz K, Saatcioglu F, et al. Cross-talk between signal transducer and activator of transcription 3 and androgen receptor signaling in prostate carcinoma cells. Biochem Biophys Res Commun. 2001; 283: 179–187.

20. Yang L, Wang L, Lin HK, Kan PY, Xie S, Tsai MY, et al. Interleukin-6 differentially regulates androgen receptor transactivation via PI3K-Akt, STAT3, and MAPK, three distinct signal pathways in prostate cancer cells. Biochem Biophys Res Commun. 2003; 305: 462–469.

21. Jia L, Kim J, Shen H, Clark PE, Tilley WD, Coetzee GA. Androgen receptor activity at the prostate specific antigen locus: steroidal and non-steroidal mechanisms. Mol Cancer Res. 2003; 1: 385–392.

22. 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.

23. Shariat SF, Andrews B, Kattan MW, Kim J, Wheeler TM, Slawin KM. Plasma levels of interleukin-6 and its soluble receptor are associated with prostate cancer progression and metastasis. Urology. 2001; 58: 1008-1015.

24. Shariat SF, Kattan MW, Traxel E, Andrews B, Zhu K, Wheeler TM, et al. Association of pre- and postoperative plasma levels of transforming growth factor beta (1) and interleukin 6 and its soluble receptor with prostate cancer progression. Clin Cancer Res. 2004; 10: 1992-1999.

25. Zerbini LF, Wang Y, Cho JY, Libermann TA. Constitutive activation of nuclear factor kappa B p50/p65 and Fra-1 and Jun D is essential for deregulated interleukin 6 expression in prostate cancer. Cancer Res. 2003; 63: 2206-2215.

26. Wegiel B, Bjartell A, Culig Z, Persson JL. Interleukin-6 activates PI3K/Akt pathway and regulates cyclin A1 to promote prostate cancer cell survival. Int J Cancer 2008; 122: 1521-1529.

27. Pencik J, Wiebringhaus R, Susani M, Culig Z, Kenner L. IL6/STAT3/ARF: the guardians of senescence, cancer progression and metastasis in prostate cancer. Swiss Med Wkly. 2015; 145.

28. Han Z, Wang X, Ma L, Chen L, Xiao M, Huang L, et al. Inhibition of STAT3 signaling targets both tumor-initiating and differentiated cell populations in prostate cancer. 2014; 5: 8416-8428.

29. Eichen A, Su J, Adler AP, Zhang L, Ioffe E, Parveen AA, et al. Resistance to anti-VEGF therapy mediated by autocrine IL6/STAT3 signaling and overcome by IL6 blockade. Cancer Res. 2016; 76: 2327-2339.

30. Hibi M, Nakajima K, Hirano T. IL-6 cytokine family and signal transduction: a model of the cytokine system. J Mol Med. 1996; 74: 1-12.

31. Gearing DP, Comeau MR, Friend DJ, Gimpel SD, Thut CJ, McGourty J, et al. The IL-6 signal transducer, gp130: an oncostatin M receptor and affinity converter for the LIF receptor. Science 1992; 255: 1434-1437.

32. Smith PC, Keller ET. Anti-interleukin-6 monoclonal antibody induces regression of human prostate cancer xenografts in nude mice. Prostate 2001; 48: 47-53.

33. Tozawa K, Okamoto T, Hayashi Y, Sasaki S, Kawai N, Kohri K. N-acetyl-*L*-cysteine enhances chemotherapeutic effect to prostate cancer cells. Urol Res. 2002; 30: 53-58.