IL-6 and PPARγ Signalling in Human PC-3 Prostate Cancer Cells

NEA PITULIS[1], EFSTATHIA PAPAGEORGIOU[1], ROXANE TENTA[2], PETER LEMBESSIS[1] and MICHAEL KOUTSILIERIS[1]

[1]*Department of Experimental Physiology, Medical School, National and Kapodistrian University of Athens, Goudi, Athens;*
[2]*Department of Nutrition Science and Dietetics, Harokopio University, 70 El. Venizelou Ave, Kallithea 17671, Athens, Greece*

Abstract. *Background: Peroxisome proliferator-activated receptor gamma (PPARγ) ligands and interleukin (IL)-6 are key factors for controlling prostate cancer cell proliferation and survival. Materials and Methods: Herein we used the natural PPARγ ligand, 15deoxy Δ12-14 PGJ_2 ($15dPGJ_2$), and IL-6 to define their interactions on proliferation and signal transduction in PC-3 cells. Cytotoxic and trypan blue exclusion assays, Western blot analysis of mitogen-activated protein kinases (MAPK) and Janus kinase/Signal transducer and activator of transcription (JAK/Stat) and real-time polymerase chain reaction (PCR) were methods employed as investigation tools. Results: $15dPGJ_2$ reduced PC-3 cell proliferation, while IL-6 increased it. IL-6 induced PPARγ expression but did not affect the PPARγ ligand-mediated effects on the proliferation of PC-3 cells. However, $15dPGJ_2$ inhibited the IL-6-mediated increase of PC-3 cell proliferation. $15dPGJ_2$ activated Erk1/2 phosphorylation without affecting Akt phosphorylation and reduced phosphorylated and unphosphorylated Stat3 in PC-3 cells. IL-6 suppressed endogenous activation of Stat3 without affecting Erk1/2 and Akt phosphorylation and suppressed the $15dPGJ_2$-mediated activation of Erk1/2 phosphorylation in PC-3 cells. Conclusion: The interplay between PPARγ ligands and IL-6 signalling could be important in controlling the growth of androgen independent prostate cancer cells as exemplified by PC-3 cells.*

Correspondence to: Michael Koutsilieris, MD, Ph.D., Professor and Chairman, Department of Experimental Physiology, Medical School, National and Kapodistrian University of Athens, 75 Micras Asias, Goudi 11527, Greece. Tel: +30 2107462597, Fax: +30 2107462571, e-mail: mkoutsil@med.uoa.gr

Key Words: PPARγ, $15dPGJ_2$, IL-6, Stat3, PC-3 cells, prostate cancer.

Prostate cancer is an androgen-sensitive cancer, which soon progresses to an androgen-independent state, characterized by androgen ablation and chemotherapy refractoriness (1-3). Prostate cancer metastasises primarily in bones, and bone metastasis microenvironment-related growth factors, such as insulin-like growth factor 1 (IGF-1), basic fibroblast growth factor (bFGF), transforming growth factor beta 1 (TGF-β1), parathyroid hormone-related peptide (PTHrP), interleukin 6 (IL-6) and endothelin 1 (ET-1) contribute to the development of androgen ablation and chemotherapy refractoriness of prostate cancer cells (4). IL-6 is a pleiotropic cytokine involved in many physiological actions implicated in the development and progression of tumours in various organs, in particular myeloma, renal and prostate cancer, and melanoma. Notably, patients with advanced androgen ablation-refractory prostate cancer have increased levels of serum IL-6, a sign of poor prognosis (5-9). A variety of malignant tumours have been shown to express IL-6, which may use an autocrine loop as a possible mechanism for stimulating cancer cell growth (10).

Prostate cancer cell lines, such as LNCaP, DU145, and PC-3 all posses receptors for IL-6, although LNCaP do not express them normally unless stimulated by IL-1β (11, 12). Amongst these, the androgen-insensitive PC-3 cells express the most *IL-6* mRNA and have the highest IL-6 secretion in cell culture medium which is inhibited when p42/p44 MAPK signalling is chemically blocked (11, 13-15). The distinction between androgen-sensitive and androgen-insensitive prostate cancer reflects the effect of IL-6 on cell growth. Whereas it induces growth arrest of LNCaP cells, IL-6 acts as an important growth factor in PC-3 cells (7, 16). In addition, IL-6 has a survival function in PC-3 cells; adriamycin-induced apoptosis is reversed by IL-6 and blockage by specific oligonucleotide sensitizes PC-3 to cytotoxic agents (4, 17).

IL-6 signalling moves through two major pathways and involves negative feedback control mechanisms. The Janus

kinase/signal transducer and activators of transcription (JAK-STAT) pathway is one, with SOCS (suppressor of cytokine signalling) acting as JAK kinase inhibitor. The second is the mitogen-activated protein kinase (MAPK) cascade (18, 19). There is controversy about Stat3 presence and activity in PC-3 cells. Several studies did not detect phosphorylated or unphosphorylated Stat3 protein (20-25), while several others confirmed that Stat3 is active in PC-3 cells (26-28).

Another key regulator of prostate cancer is peroxisome proliferator-activated receptor gamma (PPARγ), a transcription factor with a role still to be defined in cancer biology. Indeed, several malignant tumours express PPARγ, whereas exogenous administration of PPARγ ligands produces an anticancer action, which is mediated by both genomic and non-genomic actions (29-33).

A naturally occurring PPARγ ligand, the terminal derivative of prostaglandin J_2 metabolism, 15deoxy Δ12-14 PGJ_2 ($15dPGJ_2$), does inhibit prostate cancer cell proliferation through an S-phase arrest (34). $15dPGJ_2$ affects the IL-6-induced Jak-Stat signalling pathway in several cell lines, astrocytes, microglia, endothelial cells, primary human lymphocytes, Jurkat cells and immortalized rheumatoid arthritis B cells *via* a PPARγ-independent mechanism (35-38).

In this article, we investigated the interplay between $15dPGJ_2$ and IL-6 in PC-3 androgen independent prostate cancer cells.

Materials and Methods

Cell culture. PC-3 cells are an androgen-insensitive, p53-negative, and *K-Ras*-mutated human prostate cancer cell line and were obtained from the American Type Culture Collection (ATCC, Bethesda, MD, USA). Cells were maintained in Dulbecco's modified Eagle's medium/F-12 (DMEM; Cambrex, Walkerville, MD USA) supplemented with 10% foetal bovine serum (FBS) (Biochrom, Berlin Germany), 100 U/ml penicillin/streptomycin (Cambrex) at 37˚C in a humidified atmosphere of 5% CO_2. Treatments were made in 0.5% FBS, except where stated differently. Cells were cultured in 0.5% FBS medium for twenty-four hours prior to treatment. $15dPGJ_2$ was purchased from Cayman Chemicals, Ann Arbor, MI USA and IL-6 was from R&D Systems, Minneapolis, MN USA.

Cytotoxic assay. Twenty-four hours prior to treatment, PC-3 cells were plated at cell density of 750 cells/well in 96-well plates with DMEM/F-12 containing 10% FBS. Cells were treated for 96 hours with the appropriate drug or drug combination in dose dependent manner. IL-6 concentration ranged from 1.56 to 50 ng/ml and $15dPGJ_2$ from 0.31 to 10 mM. After the treatment cells were incubated with 10% MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide] (Sigma, St. Louis, MO, USA) as described elsewhere (4) and the percentage of viable cells was measured by VectraMax plate reader.

Trypan blue exclusion assay. PC-3 cells were plated at a cell density of 3.5×10^4 cells/well in 6-well plates and treated in DMEM/F12 containing 0.5% FBS for forty-eight hours. PC-3 cells were exposed to IL-6 (1 and 25 ng/ml), $15dPGJ_2$ (2 μM) or drug combination (25 ng/ml IL-6 plus 2 μM $15dPGJ_2$) and the cell number was counted as described elsewhere (4).

Western blot analysis. PC-3 cells were plated in 6-well plates. Twenty-four hours prior to treatment, the growth medium was changed to 0.5% FBS. The cell protein content was extracted using RIPA buffer [50 mM Tris-HCl; 150 mM NaCl, 1% Igepal CA360, 0.5% NaDOC; containing protease and phosphatase inhibitors 1 mM phenylmethylsulfonyl fluoride (PMSF), 10 μg/ml aprotinin, 10 μg/ml leupeptin, 1 mM sodium orthovanadate and 1 mM NaF; all chemicals from Sigma]. Protein concentrations were determined by Bradford protein assay (BIO-RAD Laboratories) followed by electrophoresis on 10% SDS–PAGE under denaturing conditions, and transferred onto nitrocellulose membrane (BIO-RAD Laboratories, Hercules, CA USA). The blots were blocked with TBS-T (20 mmol/l Tris–HCl, pH 7.6, 137 mmol/l NaCl, and 0.1% Tween 20) containing 5% non-fat dried milk at room temperature for 1 hour. Membranes were probed overnight with primary antibodies against phosphorylated (on residue Tyr705) Stat3 and unphosphorylated Stat3, phosphorylated Erk 1/2 and phosphorylated Akt plus GAPDH as control reference for protein loading (all 1:1,000 dilution, except GAPDH 1:2,000) in TBS/T containing 5% bovine serum albumin (BSA) (all primary antibodies purchased from Cell Signalling, Beverly, MA, USA). The blots were washed and incubated with the appropriate secondary antibodies for 1 hour at room temperature (Sc2004, Sc-2005; Santa Cruz Biotechnology, Santa Cruz, CA, USA) (1:2000 dilution). The bands were visualized by exposure to x-ray film after incubation of the blots with SuperSignal ECL substrate (Pierce Biotechnology, Rockford, IL, USA). Quantification was performed using Kodak imaging software (EDAS Kodak software).

Real-time quantitative polymerase chain reaction (PCR). PC-3 were treated with IL-6 (25 ng/ml) for forty-eight hours and total RNA was extracted by homogenization of 5×10^6 cells with Trizol reagent (Invitrogen, Carlsbad, CA, USA) followed by standard chloroform extraction and isopropanol precipitation. Ten ng/μl of RNA were used for the reverse transcription reaction; the reaction was completed by the addition of RNase A (Amersham Pharmacia Biotech, Uppsala, Sweden) followed by column purification (PCR Purification Kit, Qiagen Hilden, Germany) and elution using RNAse and DNAse-free double distilled water. Reactions were carried out in a 20 μl volume with 0.5 μl primers and $MgCl_2$ concentration optimized between 2-5 mM. The assay was performed with the Light Cycler DNA Fast Start SYBR Green I kit (Roche Diagnostics, IN, USA) according to the manufacturer's instructions. Oligonucleotides used to detect *PPARγ* mRNA expression were derived from the published sequence of human *PPARγ* (Genbank Accession Number: NM_138712). Sequence alignment was performed by the GeneTool 3.0 software, while a specific set of primers was designed by the DNA Synthesizer 3900 (Perkin-Elmer Life Sciences, Boston, MA, USA).

The pair of resulting primers used was: 5'-GTC-GGA-TCC-ACA-AAA-AAA-GTA-GAA-3' (forward primer) and 5'-AGC-GGG-AAG-GAC-TTT-ATG-TAT-GA-3' (reverse primer) which gave rise to a 229-bp fragment. As a reference, transcripts of ATPase (chosen, among other housekeeping genes, as one that varies less among the treatments) for each sample were simultaneously quantified during

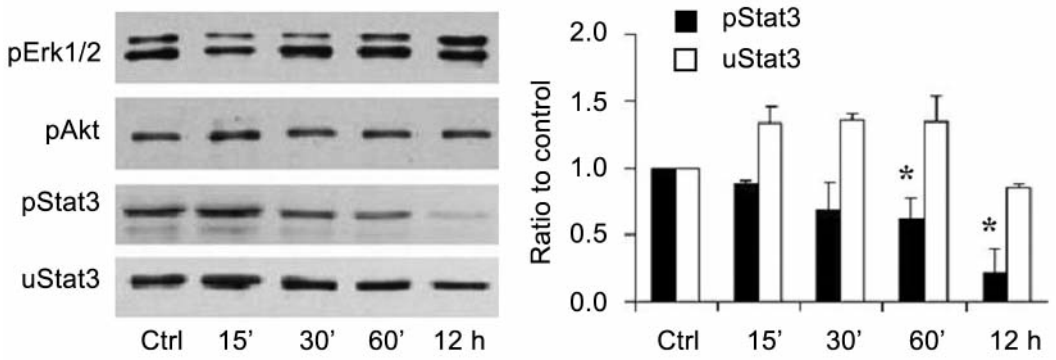

Figure 1. *An example of IL-6-mediated signal transduction in human PC-3 cells. PC-3 cells were treated with IL-6 (25 ng/ml) for 15, 30, 60 minutes and 12 hours in DMEM/F12 medium containing 0.5% FBS. Left panel: Western blot of phosphorylated (p) Erk1/2, Akt, Stat3 and unphosphorylated (u) Stat3. Right panel: The IL-6-mediated decrease of pStat3 in a time-dependent manner. Note that baseline pErk 1/2 and pAkt did not change in PC-3 cells with IL-6 treatment. *p<0.005 as compared to control.*

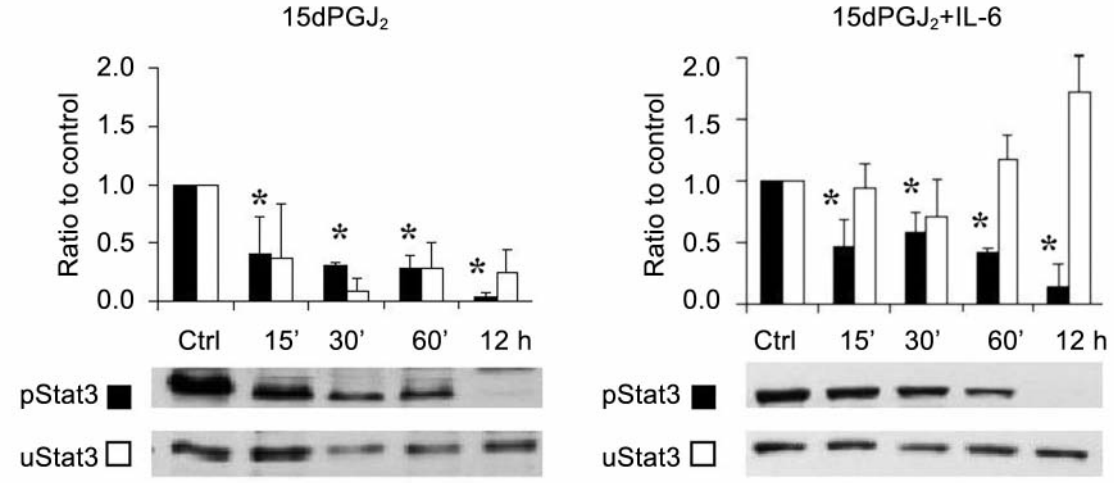

Figure 2. *Signal transduction as assessed by the analysis of pStat3 and uStat3 protein in PC-3 cells treated with 15dPGJ$_2$ (2 μM) alone (left panel) and in combination with IL-6 (25 ng/ml) (right panel). Note that 15dPGJ$_2$ reduced uStat3 and pStat3 whereas IL-6 inhibited the 15dPGJ$_2$-induced decrease of uStat3 without affecting the 15dPGJ$_2$-induced decrease of pStat3. *p<0.005 as compared to control.*

each real time-PCR run and negative controls (containing no template cDNA) were included. The cycle profile was 95˚C for 10 min and 40 cycles at 95˚C for 10 s, 58˚C for 5 s and 72˚C for 13 s. Data were collected during the extension stage of each cycle and analyzed using the Light Cycler Data Analysis Administrator.

The real time-PCR reaction 'end-point' for each sample was defined as the PCR cycle at which the fluorescence threshold was crossed. The intensity of fluorescence signals generated during PCR reactions was increased as cycles progressed The melting curve was constructed by increasing the temperature to 95˚C and by plotting the first negative derivative (–dF/dT) of the fluorescence *vs*. temperature thereby determining the melting temperature of the PCR products.

Overall, real time-PCR analysis revealed a stratification pattern between the samples which was similar to that observed in relative quantitative-PCR analysis, where different RNA extraction methods and different set of primers were used.

Statistical analysis. All experiments were performed in triplicate. Values are means±standard deviation (SD). Statistical analysis was performed by Student's *t*-test. The level of statistical significance was set at $p<0.05$.

Results

In our experiments, IL-6 did not affect Erk1/2 or Akt phosphorylation in PC-3 cells (Figure 1). On the contrary, IL-6 inhibited baseline Stat3 phosphorylation (Figure 1), whereas 15dPGJ$_2$ inhibited both phosphorylated and unphosphorylated Stat3 (Figure 2). However, 15dPGJ$_2$ activated Erk1/2 phosphorylation in PC-3 cells and this activation was inhibited by IL-6. Akt phosphorylation was not affected by 15d PGJ$_2$ with or without IL-6, (Figure 3).

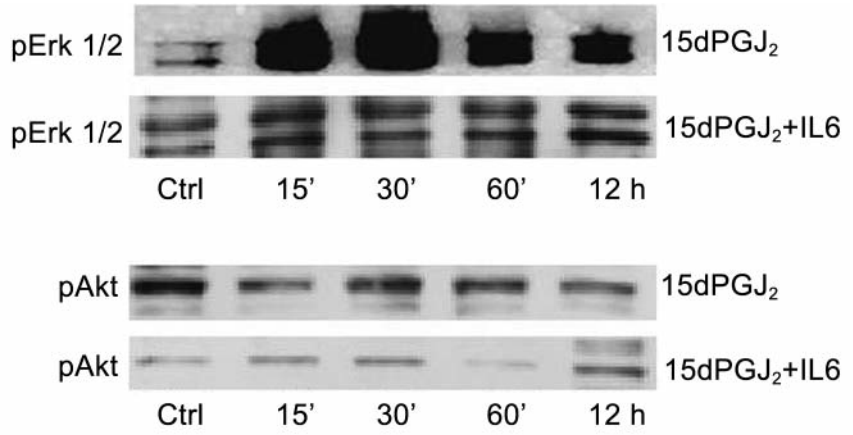

Figure 3. *An example of Erk 1/2 and Akt signal transduction in PC-3 cells. PC-3 cells were treated with 15dPGJ$_2$ (2 μM) alone and in combination with IL-6 (25 ng/ml) for 15, 30, 60 minutes and 12 hours. Note that 15dPGJ$_2$ did activate pErk in PC-3 cells, suggesting a non-genomic action on PC-3 cells. However, IL-6 abated the 15dPGJ$_2$ -induced phosphorylation of Erk 1/2 without affecting Akt phosphorylation.*

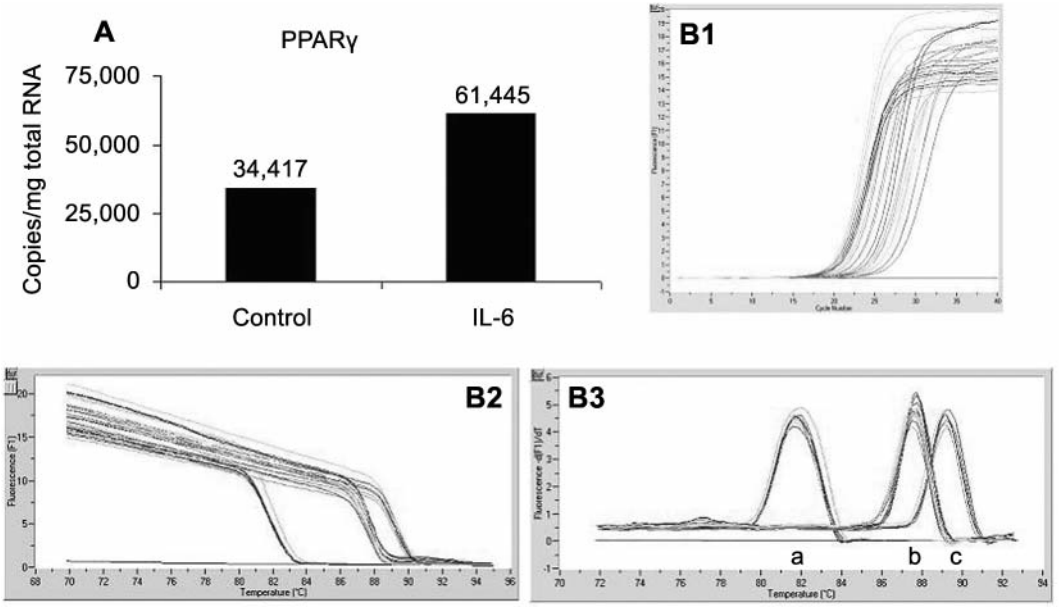

Figure 4. *An example of the absolute number of PPARγ mRNA copies/μg of total RNA determined by real-time PCR. A: PC-3 cells were exposed to 25 ng/ml of interleukin-6 (IL-6) for 48 hours, which provoked a significant increase of the PPARγ mRNA expression. B: Typical real-time PCR plot of fluorescence against cycle number for PPARγ mRNA and for the ATPase mRNA. On the x-axis, the number of PCR cycles is given. The y-axis shows the relative change in fluorescence intensity during PCR cycling. The intensity of fluorescence signals generated during PCR reactions increased as cycles progressed (B1). Real-time PCR melting curve analysis of the PPARγ amplification reaction (B2). Documentation of the gradual reduction in fluorescence as temperature increased. In addition, note the rapid fall at 87.6˚C, indicating that the specific product (PPARγ) melted at this temperature. The T_m of this product (PPARγ), as well as the T_m of two housekeeping genes ATPase and G6P (ATPase was subsequently chosen as reference gene) B3: a: ATPase, b: PPARγ, c: G6P). These plots provide confirmation for the lack of primer dimer formation and the obtainment of only one PCR product for each gene.*

IL-6 increased (by almost 2-fold) the *PPARγ* mRNA expression as determined by real-time PCR analysis in PC-3 cells treated with IL-6 for 48 hours with 25 ng/ml, (Figure 4).

15dPGJ$_2$ inhibited PC-3 cell proliferation in a dose dependent manner whereas, IL-6 (1 and 25 ng/ml) stimulated the PC-3 cell growth (Figure 5). Combinational treatments using IL-6 (25 ng/ml) with increasing doses of

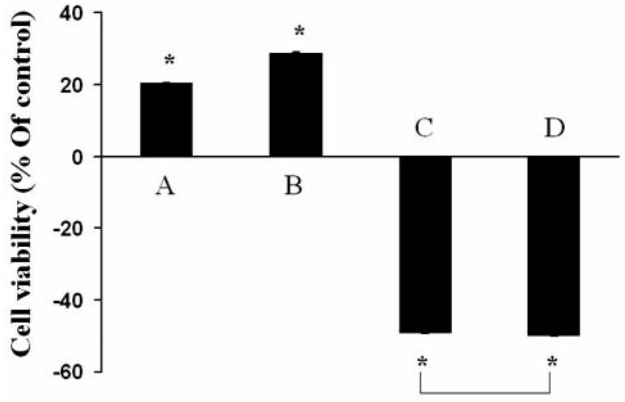

Figure 5. *Trypan blue assay for PC-3 cells treated for 48 hours (in DMEM/F12 medium containing 0.5% FBS) with A: 1 ng/ml and B: 25 ng/ml IL-6; C: 2 μM 15dPGJ$_2$; and D: combination of 25 ng/mL of IL-6 and 2 μM of 15dPGJ$_2$. Note that IL-6 increased cell proliferation in a dose-dependent manner. 15dPGJ$_2$ reduced PC-3 cell number by 50%. This effect was not modified by the addition of IL-6 (25 ng/ml). *p<0.005 as compared to control.*

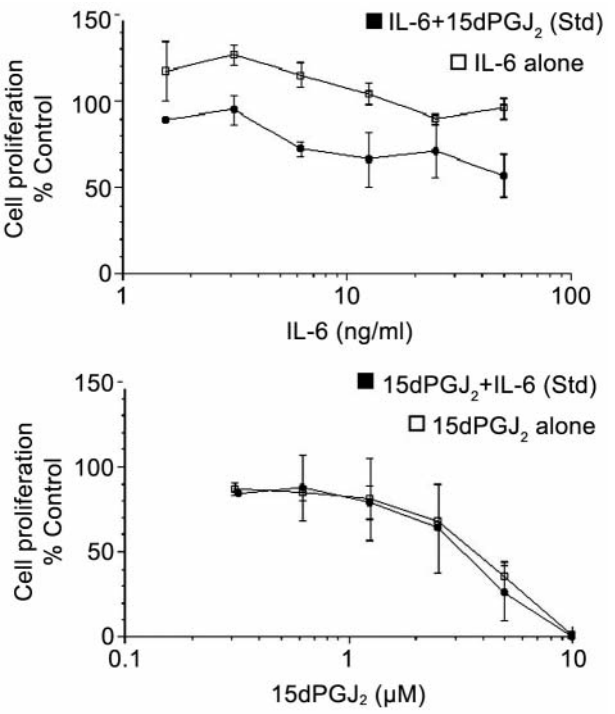

Figure 6. *MTT analysis of PC-3 cell cultured in DMEM/F12 medium containing 10% FBS for 96 hours. Upper panel: The effects of IL-6 (0-50 ng/ml) with or without 2 μM 15dPGJ$_2$. Note that IL-6 alone at low doses significantly increased PC-3 cell proliferation and that 15dPGJ$_2$ blocked the IL-6-mediated increase of PC-3 cell growth. Lower panel: Increasing doses of 15dPGJ$_2$ reduced PC-3 cell proliferation in a dose-dependent manner. IL-6 did not change the 15dPGJ$_2$-induced inhibition of PC-3 cells.*

15dPGJ$_2$ did not alter the 15dPGJ$_2$ effect on PC-3 cells. However, 15dPGJ$_2$ did block the IL-6-related increase of PC-3 cell growth. These data were documented using trypan blue exclusion and MTT assays (Figures 5 and 6).

Discussion

Previous findings have shown that troglitazone, a synthetic PPARγ ligand, has antiproliferative effect with anti-inflammatory and antidiabetic actions, both in LNCaP androgen-sensitive cells and in advanced stage prostate cancer patients (39). Furthermore, PPARγ ligands down-regulate prostate-specific antigen (PSA) levels in LNCaP cells (33, 39, 40).

Recent findings indicate that 15dPGJ$_2$, a natural PPARγ ligand, acts through a PPARγ-independent mechanism in PC-3 cells that promotes p42/p44 (Erk1/2) phosphorylation but has no effect on Akt phosphorylation. Rosiglitazone, a synthetic PPARγ ligand, blocks the IGF-1-induced Akt activation in PC-3 cells (41). This specific ligand has potent anti-inflammatory activity by inhibiting cytokine production (tumour necrosis factor (TNF)-α, IL-1β, and IL-6) (38).

Researchers that share the experimental context of using 10% FBS in growth medium did not detect Stat3 activity in PC-3 cells (20-28). As described by Pu *et al*. under these conditions, PC-3 cells constitutively secrete IL-6 and this secretion is inhibited when low serum conditions are used (17). Thus our findings for the detection of both the phosphorylated and unphosphorylated Stat3 protein in PC-3 cells grown in 0.5% FBS are here presented. In addition, we showed that IL-6 affects pStat3 protein expression by decreasing its expression to undetectable levels after 12 hours of treatment. The latter may explain why Stat3 activity is inhibited when the growth medium contains serum, which is able to stimulate IL-6 production in PC-3 and in turn down-regulates phosphorylated Stat3.

Furthermore, IL-6 does not phosphorylate Erk1/2 or Akt and, as mentioned, down-regulates pStat3 in a time-dependent manner. Apparently, PC-3 cells expressing IL-6 use uStat3 as a transcription factor. The latter has recently been described by Yang *et al*. to form a complex with unphosphorylated nuclear factor (NF) κB (42). This complex is a transcription factor that activates a subset of κB-dependent genes. Furthermore, Stat3 has the capacity of entering the nucleus in the unphosphorylated form, thus activating gene transcription without tyrosine phosphorylation (43).

An IL-6-mediated effect is the increase of PPARγ expression. However, the PPARγ ligand, 15dPGJ$_2$, induces the arrest of PC-3 cells, an effect which is not modified by IL-6, whereas IL-6 blocks both the 15dPGJ$_2$-induced phosphorylation of pErk and the down-regulation of uStat3. Finally, 15dPGJ$_2$ inhibited the IL-6-mediated increase of the

PC-3 cell growth, as assessed in MTT assays. These data provide information on the complex interaction between this PPARγ ligand, $15dPGJ_2$, and IL-6 in human PC-3 prostate cancer cells and delineate the importance for further research on this subject.

References

1 Isaacs JT: The biology of hormone refractory prostate cancer. Why does it develop? Urol Clin North Am *26*: 263-273, 1999.

2 Abate-Shen C, Shen MM: Molecular genetics of prostate cancer. Genes Dev *14*: 2410-2434, 2000.

3 Pilat MJ, Kamradt JM, Pienta KJ: Hormone resistance in prostate cancer. Cancer Metastasis Rev *17*: 373-381, 1998.

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

5 Drachenberg DE, Elgamal AA, Rowbotham R, Peterson M, Murphy GP: Circulating levels of interleukin-6 in patients with hormone refractory prostate cancer. Prostate *41*: 127-133, 1999.

6 Nakashima J, Tachibana M, Horiguchi Y, Oya M, Ohigashi T, Asakura H and Murai M: Serum interleukin-6 as a prognostic factor in patients with prostate cancer. Clin Cancer Res *6*: 2702-2706, 2000.

7 Chung TD, Yu JJ, Spiotto MT, Bartkowski M and Simons JW: Characterization of the role of IL-6 in the progression of prostate cancer. Prostate *38*: 199-207, 1999.

8 Okamoto M, Lee C and Oyasu R: Interleukin-6 as a paracrine and autocrine growth factor in human prostatic carcinoma cells *in vitro*. Cancer Res *57*: 141-146, 1997.

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

10 Paule B, Terry S, Kheuang L, Soyeux P, Vacherot F and de la Taille A: The NF-kappaB/IL-6 pathway in metastatic androgen-independent prostate cancer: new therapeutic approaches? World J Urol *25*: 477-489, 2007.

11 Lou W, Ni Z, Dyer K, Tweardy DJ and Gao AC: Interleukin-6 induces prostate cancer cell growth accompanied by activation of stat3 signaling pathway. Prostate *42*: 239-242, 2000.

12 Maliner-Stratton MS, Klein RD, Udayakumar TS, Nagle RB and Bowden GT: Interleukin-1beta-induced promatrilysin expression is mediated by NFkappaB-regulated synthesis of interleukin-6 in the prostate carcinoma cell line, LNCaP. Neoplasia *3*: 509-520, 2001.

13 Shida Y, Igawa T, Hakariya T, Sakai H and Kanetake H: p38MAPK activation is involved in androgen-independent proliferation of human prostate cancer cells by regulating IL-6 secretion. Biochem Biophys Res Commun *353*: 744-749, 2007.

14 Koul S, Huang M, Chaturvedi L, Meacham RB and Koul HK: p42/p44 Mitogen-activated protein kinase signal transduction pathway regulates interleukin-6 expression in PC3 cells, a line of hormone-refractory prostate cancer cells. Ann NY Acad Sci *1030*: 253-257, 2004.

15 Zerbini LF, Wang Y, Cho JY and Libermann TA: Constitutive activation of nuclear factor kappaB p50/p65 and Fra-1 and JunD is essential for deregulated interleukin-6 expression in prostate cancer. Cancer Res *63*: 2206-2215, 2003.

16 Mori S, Murakami-Mori K and Bonavida B: Interleukin-6 induces G_1 arrest through induction of *p27(Kip1)*, a cyclin-dependent kinase inhibitor, and neuron-like morphology in LNCaP prostate tumor cells. Biochem Biophys Res Commun *257*: 609-614, 1999.

17 Pu YS, Hour TC, Chuang SE, Cheng AL, Lai MK and Kuo ML: Interleukin-6 is responsible for drug resistance and anti-apoptotic effects in prostatic cancer cells. Prostate *60*: 120-129, 2004.

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

19 Heinrich PC, Behrmann I, Muller-Newen G, Schaper F and Graeve L: Interleukin-6-type cytokine signalling through the gp130/Jak/STAT pathway. Biochem J *334(Pt 2)*: 297-314, 1998.

20 Spiotto MT and Chung TD: STAT3 mediates IL-6-induced neuroendocrine differentiation in prostate cancer cells. Prostate *42*: 186-195, 2000.

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

22 Smith ND, Schulze-Hoepfner FT, Veliceasa D, Filleur S, Shareef S, Huang L, Huang XM and Volpert OV: Pigment epithelium-derived factor and interleukin-6 control prostate neuroendocrine differentiation *via* feed-forward mechanism. J Urol *179*: 2427-2434, 2008.

23 Bellezza I, Neuwirt H, Nemes C, Cavarretta IT, Puhr M, Steiner H, Minelli A, Bartsch G, Offner F, Hobisch A, Doppler W and Culig Z: Suppressor of cytokine signaling-3 antagonizes cAMP effects on proliferation and apoptosis and is expressed in human prostate cancer. Am J Pathol *169*: 2199-2208, 2006.

24 Yang Y, Ikezoe T, Takeuchi T, Adachi Y, Ohtsuki Y, Takeuchi S, Koeffler HP and Taguchi H: HIV-1 protease inhibitor induces growth arrest and apoptosis of human prostate cancer LNCaP cells *in vitro* and *in vivo* in conjunction with blockade of androgen receptor STAT3 and AKT signaling. Cancer Sci *96*: 425-433, 2005.

25 Abdulghani J, Gu L, Dagvadorj A, Lutz J, Leiby B, Bonuccelli G, Lisanti MP, Zellweger T, Alanen K, Mirtti T, Visakorpi T, Bubendorf L and Nevalainen MT: Stat3 promotes metastatic progression of prostate cancer. Am J Pathol *172*: 1717-1728, 2008.

26 Ni Z, Lou W, Leman ES and Gao AC: Inhibition of constitutively activated Stat3 signaling pathway suppresses growth of prostate cancer cells. Cancer Res *60*: 1225-1228, 2000.

27 Hellsten R, Johansson M, Dahlman A, Dizeyi N, Sterner O and Bjartell A: Galiellalactone is a novel therapeutic candidate against hormone-refractory prostate cancer expressing activated Stat3. Prostate *68*: 269-280, 2008.

28 Deo DD, Rao AP, Bose SS, Ouhtit A, Baliga SB, Rao SA, Trock BJ, Thouta R, Raj MH and Rao PN: Differential effects of leptin on the invasive potential of androgen-dependent and -independent prostate carcinoma cells. J Biomed Biotechnol *2008*: 163902, 2008.

29 Grommes C, Landreth GE and Heneka MT: Antineoplastic effects of peroxisome proliferator-activated receptor gamma agonists. Lancet Oncol *5*: 419-429, 2004.

30 Papageorgiou E, Pitulis N, Msaouel P, Lembessis P and Koutsilieris M: The non-genomic crosstalk between PPAR-gamma ligands and ERK1/2 in cancer cell lines. Expert Opin Ther Targets *11*: 1071-1085, 2007.

31 Lennon AM, Ramauge M, Dessouroux A and Pierre M: MAP kinase cascades are activated in astrocytes and preadipocytes by 15-deoxy-delta(12-14)-prostaglandin J(2) and the thiazolidinedione ciglitazone through peroxisome proliferator activator receptor gamma-independent mechanisms involving reactive oxygenated species. J Biol Chem *277*: 29681-29685, 2002.
32 Gardner OS, Dewar BJ, Earp HS, Samet JM and Graves LM: Dependence of peroxisome proliferator-activated receptor ligand-induced mitogen-activated protein kinase signaling on epidermal growth factor receptor transactivation. J Biol Chem *278*: 46261-46269, 2003.
33 Mueller E, Smith M, Sarraf P, Kroll T, Aiyer A, Kaufman DS, Oh W, Demetri G, Figg WD, Zhou XP, Eng C, Spiegelman BM and Kantoff PW: Effects of ligand activation of peroxisome proliferator-activated receptor gamma in human prostate cancer. Proc Natl Acad Sci USA *97*: 10990-10995, 2000.
34 Butler R, Mitchell SH, Tindall DJ and Young CY: Nonapoptotic cell death associated with S-phase arrest of prostate cancer cells *via* the peroxisome proliferator-activated receptor gamma ligand, 15-deoxy-delta12,14-prostaglandin J_2. Cell Growth Differ *11*: 49-61, 2000.
35 Jiang C, Ting AT and Seed B: PPAR-gamma agonists inhibit production of monocyte inflammatory cytokines. Nature *391*: 82-86, 1998.
36 Wung BS, Wu CC, Hsu MC and Hsieh CW: 15-Deoxy-delta(12,14)-prostaglandin J(2) suppresses IL-6-induced STAT3 phosphorylation *via* electrophilic reactivity in endothelial cells. Life Sci *78*: 3035-3042, 2006.
37 Kim HJ, Rho YH, Choi SJ, Lee YH, Cheon H, Um JW, Sohn J, Song GG and Ji JD: 15-Deoxy-delta12,14-PGJ_2 inhibits IL-6-induced Stat3 phosphorylation in lymphocytes. Exp Mol Med *37*: 179-185, 2005.
38 Park EJ, Park SY, Joe EH and Jou I: 15d-PGJ_2 and rosiglitazone suppress Janus kinase-STAT inflammatory signaling through induction of suppressor of cytokine signaling 1 (SOCS1) and SOCS3 in glia. J Biol Chem *278*: 14747-14752, 2003.
39 Hisatake JI, Ikezoe T, Carey M, Holden S, Tomoyasu S and Koeffler HP: Down-regulation of prostate-specific antigen expression by ligands for peroxisome proliferator-activated receptor gamma in human prostate cancer. Cancer Res *60*: 5494-5498, 2000.
40 Kubota T, Koshizuka K, Williamson EA, Asou H, Said JW, Holden S, Miyoshi I and Koeffler HP: Ligand for peroxisome proliferator-activated receptor gamma (troglitazone) has potent antitumor effect against human prostate cancer both *in vitro* and *in vivo*. Cancer Res *58*: 3344-3352, 1998.
41 Papageorgiou E, Pitulis N, Manoussakis M, Lembessis P and Koutsilieris M: Rosiglitazone attenuates insulin-like growth factor 1 receptor survival signaling in PC-3 cells. Mol Med *14*: 403-411, 2008.
42 Yang J, Liao X, Agarwal MK, Barnes L, Auron PE and Stark GR: Unphosphorylated STAT3 accumulates in response to IL-6 and activates transcription by binding to NFkappaB. Genes Dev 21: 1396-1408, 2007.
43 Yang J and Stark GR: Roles of unphosphorylated STATs in signaling. Cell Res *18*: 443-451, 2008.

Received September 23, 2008
Revised November 25, 2008
Accepted March 11, 2009