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Overexpression of CXCL10 in human prostate LNCaP cells activates its receptor (CXCR3) expression and inhibits cell proliferation

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Abstract

Chronic or recurrent inflammation plays a role in the development of many types of cancer including prostate cancer. CXCL10 (interferon-γ inducible protein-10, IP-10) is a small secretory protein of 8.7 kDa. Recently, it has been shown that normal prostate epithelial (PZ-HPV-7) cells produce lower amounts of angiogenic CXC chemokines (GRO-α, IL-8) and higher amounts of angiostatic chemokines (CXCL10, CXCL11) as compared to prostate cancer cells (CA-HPV-10 and PC-3). Accordingly, we studied the effects of overexpression of CXCL10 in human prostate cancer LNCaP cells. LNCaP cells were transiently transfected with CXCL10 cDNA in pIRES2-EGFP vector. CXCL10, CXCR3, PSA and G3PDH mRNA levels were determined by semi-quantitative conventional and quantitative real-time RT-PCR and fluorescence-activated cell sorting (FACS). The expression of CXCL10 was markedly enhanced in the transfected cells at mRNA and protein levels in the cells. Overexpression of CXCL10 inhibited cell proliferation of the transfected cells by 30%–40% in serum-limited medium (1% FCS in RPMI1640 medium) and decreased PSA production. CXCR3 expression was significantly induced by the overexpression of CXCL10 as determined by RT-PCR and FACS. These results indicated that CXCL10 inhibited LNCaP cell proliferation and decreased PSA production by up-regulation of CXCR3 receptor. CXCL10 may be potentially useful in the treatment of prostate cancer.

Keywords: CXCL10; Prostate cancer; CXCR3; Cell proliferation; PSA

1. Introduction

Prostate cancer is the most commonly diagnosed cancer among men in the Western countries. Genes, dietary factors and lifestyle-related factors contribute to the development of prostate cancer. The initial growth of prostate cancer is usually androgen-dependent, but subsequently becomes androgen-independent over time when treated with androgen-deprivation therapy [1]. It has been recently suggested that the changes in the androgen receptor non-genomic signaling pathway may play an important role in the progression of human prostate cancer to androgen-independence [2,3].

Chronic or recurrent inflammation is associated with development of many human cancers, including cancer of the liver, stomach, intestine, bladder and prostate [4]. In the tumor bed, cytokines are secreted by both tumor-infiltrating immune cells and by the tumor cells themselves, and these cytokines can have pleiotropic effects. The intratumoral injection of CXCL10 improved long-term survival of severe combined immune deficiency (SCID) mice with non-small cell lung cancer [5]. Intratumoral/intraperitonial administration of only 3×10^7 replication units of MV/Mp/CXCL10 per animal strongly inhibited the progression of established polyoma virus middle T antigen (HSV)-vascular tumors [6]. Moreover, high affinity binding sites for CXCL10 have been reported on in vitro cultured normal lung epithelial and lung cancer cells [7]. In the

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testis, CXCL10 is expressed predominantly in macrophages and Leydig cells [8,9]. Its expression is inhibited by human chorionic gonadotropin (hCG), but induced by murine interferon-γ (mIFN-γ), murine interleukin-1α (mIL-1α) and murine tumor necrosis factor-α (mTNF-α). It was further demonstrated that CXCL10 has a strong inhibitory effect on steroidogenesis [8]. Recently, it has been shown that normal prostate epithelial (PZ-HPV-7) cells produce lower amounts of angiogenic CXC chemokines (GRO-α, IL-8) and higher amounts of angiostatic chemokines (CXCL10, CXCL11) as compared to prostate cancer cells (CA-HPV-10 and PC-3) [10]. Accordingly, we studied the effects of overexpression of CXCL10 in human prostate cancer LNCaP cells.

2. Materials and methods

2.1. Materials

Human CXCL10 immunoassay kit, phycoerythrin-CXCR3 Ab and goat antihuman CXCL10 Ab were purchased from www.RnDSystems.com. RPMI1640, fetal calf serum (FCS), Trizol reagent, oligo-dT primer, SuperscriptIII and enzymes were procured from www.invitrogen.com. Charcoal stripped FCS (CS-FCS) was obtained from www.BioMeda.com. EndoFree Plasmid Maxi Kit was obtained from www.qiagen.com. FUGENE 6 reagent was purchased from www.roche-applied-science.com. Mibolerone (Mib) a non-metabolizable synthetic androgen, was purchased from http://las.perkinelmer.com. RT-PCR reagents and MTS assay kit were purchased from www.Promega.com, and Real-Time PCR reagents including SYBR Green reagent were purchased from www.discover.bio-rad.com. Anti-goat secondary Alexa594 conjugate and rhodamine phalloidin were obtained from Molecular Probes (Eugene, OR). Draq5 (nuclear stain) was obtained from www.alexis-e.biz. and pIRES2-EGFP vector from www.bdbiosciences.com.

2.2. Cell culture

LNCaP cells were obtained from American Type Culture Collection (www.atcc.org). LNCaP cells were cultured in RPMI1640 supplemented with 5% FCS and 1% antibiotic penicillin and streptomycin. The cells were maintained under standard cell culture conditions at 37 °C and 5% CO_2 in a humid environment until reaching ~50–70% confluence.

2.3. Treatment of cells

The medium was changed to serum-free RPMI 1640 to deplete undesired steroids for 4 h prior to experiments. Cells were then treated with RPMI 1640 containing FCS (1%–5%) or CS-FCS (1%) with/without mibolerone (Mib) (1–100 nM). Mib was dissolved in dimethyl sulfoxide (DMSO). The final concentration of DMSO was 0.1% for each treatment. Equivalent amounts of solvent were added to control cells.

2.4. Cell proliferation assay

LNCaP cells were seeded at 2×10^3 cells/well in 96-well plates. After 72 h, they were treated with serum-free RPMI 1640 for 4 h and incubated with varying amounts of FCS, CS-FCS with/without 1 nM Mib. An MTS assay was performed using a Microplate Reader (www.discover.bio-rad.com). Four wells per treatment were used for the assays and each experiment was repeated thrice.

2.5. Cloning the human CXCL10 gene in a mammalian high-expression vector

Total RNA was isolated from peripheral blood mononuclear cells (PBMC) (from a frozen stock) using Trizol reagent. RNA was treated with ribonuclease-free DNaseI to remove any contaminating DNA. The published sequence for human CXCL10 (GenBank accession number 15012098) was used to derive PCR primers (sense, 5′-GAA GCT AGC- ATG AAT CAA ACT GCC ATT CTG ATT-3′; antisense, 5′-GAC GAA TTC- TTA AGG AGA TCT TTT AGA CCT TTC C-3′), incorporating *Nhe*I and *Eco*RI restriction sites.

2.6. Transfection of cells with pIRES2–EGFP–CXCL10 (pI2E–CXCL10)

The pI2E–CXCL10 plasmid DNA was amplified and purified with EndoFree Plasmid Maxi Kit. Cells were transfected with 10 μg plasmid DNA in FUGENE 6 reagent for 48 h. The cells were further treated with different types of media, with or without mib, for various time periods and harvested, and the medium and harvested cells were stored at −80 °C for further analysis.

2.7. hCXCL10 assay in spent media

Cells were grown for 3 days to 6 days, media were collected, centrifuged and stored as spent media at − 80 °C until the assays were conducted. The hCXCL10 assays were carried out by the sandwiched enzyme-linked immunosorbent assay (ELISA) according to the manufacturer's protocol (RNDSystems.com). Briefly, standards, controls and samples were pipetted into the wells of a microplate pre-coated with a polyclonal antibody specific for hCXCL10. Any hCXCL10 present was bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked polyclonal Ab specific for hCXCL10 was added to the wells. Following a wash, a substrate solution was added. The enzyme reaction yielded a blue product that turned yellow when the Stop Solution was added. The intensity of the color measured (at 450 nm and correction carried out at 540 nm) is in proportion to the amount of hCXCL10 bound in the initial step. The recovery tests were carried out by measuring hCXCL10 in spent media with or without adding known amounts of rCXCL10. Standard curve was generated with each set of data.

2.8. Comparative RT-PCR for mRNA expression

cDNA was generated using 5 μg total RNA, 2.5 μg oligo-dT primer and SuperscriptIII at 42 °C for 1 h. Total cDNA (1/20 of the reaction) was used as the template for PCR. The amplification reaction buffer also contained 0.2 mM of each dNTP, 10 pM of each primer, cDNA template and 1.5 U pfu DNA polymerase. The amplification were carried out on PTC-200 (MJ Research, Inc., Waltham, MA). Cycling programs were 94 °C for 2 min, followed by 26–40 cycles of 94 °C for 30 s, 48 °C for 30 s, 72 °C for 1.5 min, with a final extension of 72 °C for 4 min.

The primers used in this study were synthesized by the Oligonucleotide Synthesis Core Facility, University of South Carolina, and were as follows:

hG3PDH (gi: 32891804), 5′-GGA AAC TGT GGC GTG CTG G-3′ and 5′-ACT CCC CAG CAG TGA GGG TC-3′, PCR product size 601 bp;
hCXCL10 (gi 15012098), 5′-CTG CCA TTC GAT TTG CTG C-3′ and 5′-ACC TTT CCT TGC TAA CTG CT-3′, PCR product size 270 bp;
EGFP (using pIRES2-EGFP sequence), 5′-GAA GGA AGC AGT TCC TCT GG-3′ and 5′-TCA TCG TGT TTT TCA AAG GA-3′, PCR product size 366 bp;
hCXCR3 (gi 31455575), 5′-CTC TGC TGG ACC CCC TAT CA-3′ and 5′-GTC TCA GAC CAG GAT GAA TC-3′, PCR product size 283 bp;
hPSA (KLK3 gene) (gi 14422306), 5′-CCT CCT GAA GAA TCG ATT CCT-3′ and 5′-GAG GTC CAC ACA CTG AAG TT-3′, PCR product size 360 bp.

2.9. Quantitative Real-time PCR for mRNA expression

Real-time quantitative PCR amplification reactions were carried out using MyiQ system (www.discover.bio-rad.com), which incorporates 96-channel optical unit. Primers used for PSA amplification were: forward, 5′-ACT CAC AGC AAG GAT GGA GCT GAA-3′; reverse, 5′-TGA GGG TTG TCT GGA GGA CTT CAA-3′; PCR product size 197 bp [11] and for hG3PDH were: 5′-ATC ACT GCC ACC CAG AAG AC-3′ and 5′-GCC ATG CCA GTG AGC TTC CC-3′; PCR product size 153 bp [12].The reaction mixture consisted of 1 ×

PCR buffer containing SYBR-Green; 3 mM $MgCl_2$, 100 nM of each primer, 0.2 mM each dNTP, iTaq DNA polymerase (hot-start enzyme) 25 units/ml, 10 nM SYBR Green, and stabilizers. Fifty nanograms of cDNA template was added to each reaction. The final volume was adjusted to 20 μl with H_2O. The PCR conditions were: 95 °C, 8:30 min; 48 cycles of 95 °C, 0:30 min; 60 °C, 0:30 min; 72 °C, 1:00 min. To distinguish specific amplicons from non-specific amplifications, a melt curve was generated. Standard curves were generated for G3PDH and PSA.

2.10. Detection of protein expression by immunocytochemical analysis

The cells were grown on chamber slides, fixed for 10 min in 4% formalin in PBS at r.t. The cells were permeabilized in 0.2% Triton-X100 for 3 min at r.t. The slides were incubated with goat anti-human CXCL10 primary Ab (1:250 dilution). Slides were rinsed twice with PBS and incubated with Alexa594-conjugated donkey anti-goat Ab (1:1000 dilution). Confocal microscopy was performed using 40× oil immersion objective using BioRad MRC-1024 Laser Confocal Microscope. The fluorescence of EGFP, Draq5 and Rhodamine staining were detected by the confocal microscopy using specific light filters.

2.11. Fluorescence activated cell sorter (FACS) analysis for CXCR3 protein expression

The cells were harvested by mild trypsinization, treated with the phycoerythrin-conjugated CXCR3 Ab, washed in PBS containing 2% FCS and resuspended in PBS. The cells were passed through cell strainer (35 μM nylon mesh) (www.fishersci.com). The single cell suspensions were then subjected to FACS using a Coulter Epics XL-MCL flow cytometer.

2.12. Statistical analyses

Results are the means±SEM of 4–6 separate experiments. One-way ANOVA followed by Newman–Keuls multiple comparison tests were used for statistical analyses (GraphPad Prism, version 3.0; GraphPad Software Inc., San Diego). $P \leq 0.05$ was considered statistically significant.

3. Results

3.1. Overexpression of CXCL10 in LNCaP cells

A complete human CXCL10 cDNA was generated from the total RNA isolated from peripheral blood mononuclear cells (PBMC). The PCR product was digested with *Nhe*I and *Eco*RI, gel purified and cloned into the gel purified *Nhe*I and *Eco*RI digested pIRES2–EGGP vector. The direction of cloning and correctness of the sequence were further checked by restriction mapping and sequencing.

3.2. Localization of CXCL10 in LNCaP cells

Cells were transiently transfected with 10 μg of pI2E–CXCL10 using Fugene6 reagent. CXCL10 expression was visualized by goat-antihuman CXCL10 Ab and Alexa594-conjugated donkey anti-goat second Ab by Laser Confocal Microscopy. The nuclear staining (Blue with Draq5) and actin (red with rhodamine phalloidin were detected in control (untransfected) (Fig. 1A) and pII2E–CXCL10 transfected cells (Fig. 1B). Green fluorescence was detected only in the pI2E–CXCL10 transfected cells (Fig. 1B). The red fluorescence shows the localization of CXCL10 in pI2E–CXCL10 transfected cells (Fig. 1C). CXCL10 is mainly localized in the cytoplasm and membrane (Fig. 1C).

3.3. Secretion of CXCL10 by the transfected LNCaP cells

Cells were grown for 3–8 days, media were collected, centrifuged and stored at −80 °C. The CXCL10 secreted by transfected cells increased significantly over the growth period (Fig. 2B). Transfected cells secreted 3–6 ng/ml of CXCL10 after 6–8 days of cell culture, (80%–90% confluent) ($n=4$), whereas the control or vector transfected cells secreted no detectable amounts of CXCL10. The detectable amounts of CXCL10 by ELISA assay were 0.04–0.06 ng/ml.

3.4. Effect of overexpression of CXCL10 on cell proliferation

There was no significant difference in cell proliferation in control and pI2E–CXCL10 transfected cells grown in serum-rich medium (5% FCS) (Fig. 3). However, overexpression of CXCL10 inhibited cell proliferation of the transfected cells by

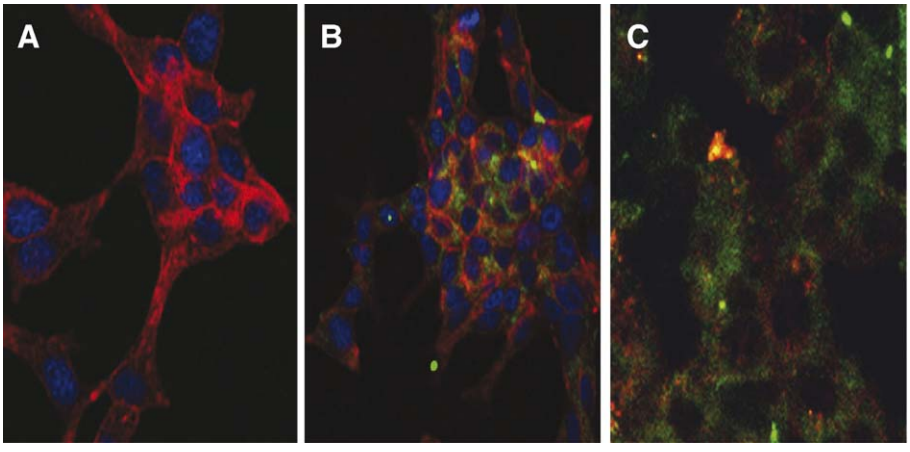

Fig. 1. Localization of EGFP and CXCL10 in human prostate LNCaP transfected cells. (A) Untransfected cells stained with Draq5 (Blue; Nuclear stain) and rhodamine phalloidin (Red; actin stain). (B) Cells transfected with the pI2E–CXCL10 plasmid, and stained with Draq5 (Blue; Nuclear stain) and rhodamine phalloidin (Red; actin stain). EGFP fluorescence (Green) is visible in these cells indicating successful transfection. (C) Cells transfected with the pI2E–CXCL10 plasmid and stained for CXCL10 (goat anti human CXCL10 and Donkey anti goat Alexa 594). The red fluorescence shows the localization of CXCL10. CXCL10 is mainly localized in the cytoplasm and membrane, but not in the nucleus. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

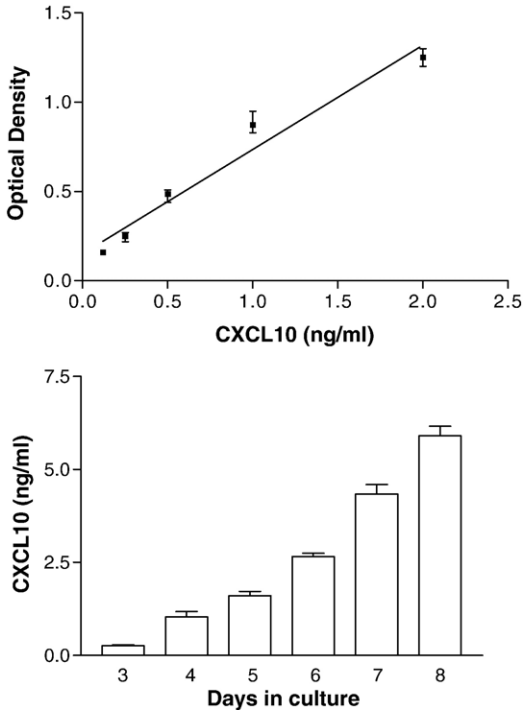

Fig. 2. Secretion of CXCL10 by the transfected LNCaP cells. (A) Standard curve generated for the assay of CXCL10 using ELISA. (B) Transfected cells (1×10^5 cells in 2 ml medium/well) were seeded in 12-well plates. Media were removed after various days of cell growth, centrifuged and assayed for CXCL10. Error bars represent the SD of 3 independent experiments.

30%–40% in serum-limited medium (1% FCS in RPMI 1640) (Figs. 3 and 4). Mibolerone (1 ng/ml) in 1% CS-FCS did not induce cell proliferation of LNCaP cells in control and transfected cells (Fig. 3).

3.5. Effect of overexpression of CXCL10 on PSA expression

The expression of CXCL10 was markedly enhanced in the transfected cells as revealed by RT-PCR (Fig. 5). PSA mRNA

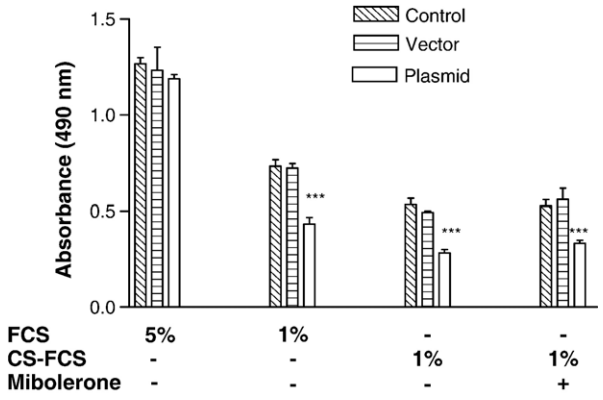

Fig. 3. Overexpression of CXCL10 inhibits cell growth of human LNCaP prostate cancer. cells in serum limited medium (1% FCS). At the indicated time points post-seeding, cells were analyzed by the MTS assay. The data represent the mean±SEM of quadruplicate wells from 3 independent experiments. ***$P<0.0001$ vs. respective controls (ANOVA and Dunnett's test).

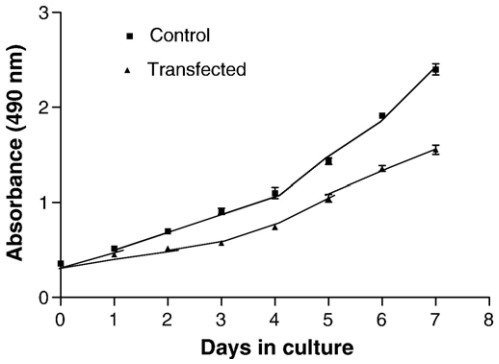

Fig. 4. Overexpression of CXCL10 inhibits cell proliferation. Control and transfected cells were incubated in 96-well plates in triplicates. Cell proliferation was measured by Cell Titer96 MTS assay. Graphs illustrate Mean±SEM values averaged for 3 experiments.

levels increased by 1 nM mibolerone in 1% CS-FCS-medium, and decreased significantly in the transfected cells as compared with the controls. PSA expression was quantitated by real-time RT-PCR (Fig. 6). The PSA mRNA expression in transfected cells was 30% of that of the control. Mibolerone (1 nM/ml) induced PSA mRNA levels of the transfected cells were approximately 60%–70% of that of control cells (Fig. 6D).

3.6. Effect of overexpression of CXCL10 on CXCR3 expression

The enhanced expression of CXCL10 in transfected cells increased CXCR3 mRNA levels as revealed by RT-PCR (Fig. 7). The quantification of the gel bands by densitometric measurements estimated the increase by 3–4-fold. Furthermore, flow cytometry showed the evidence of an increase in CXCR3 expression over the control cells (Fig. 8).

4. Discussion

In this study, we have shown that overexpression of CXCL10 strongly inhibits LNCaP cell growth, up-regulates

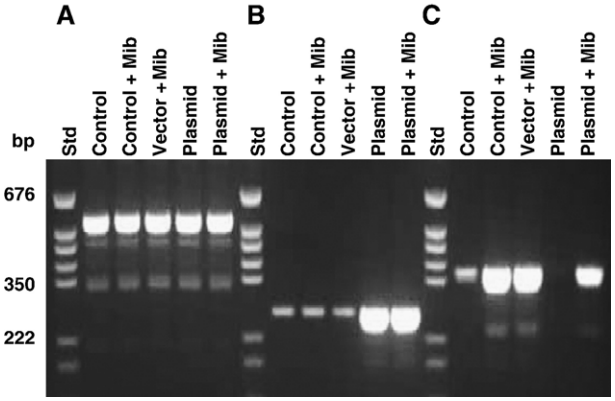

Fig. 5. Expression of G3PDH, CXCL10 and PSA in control, vector (pIRES2–EGFP)- and plasmid (pI2E–CXCL10)-transfected LNCaP cells. Lane Std indicates standard markers (pGEM, Promega) and the numbers on the left indicate the sizes of the bands in base pairs. (A) G3PDH; 601 bp. (B) CXCL10; 270 bp. (C) PSA; 360 bp.

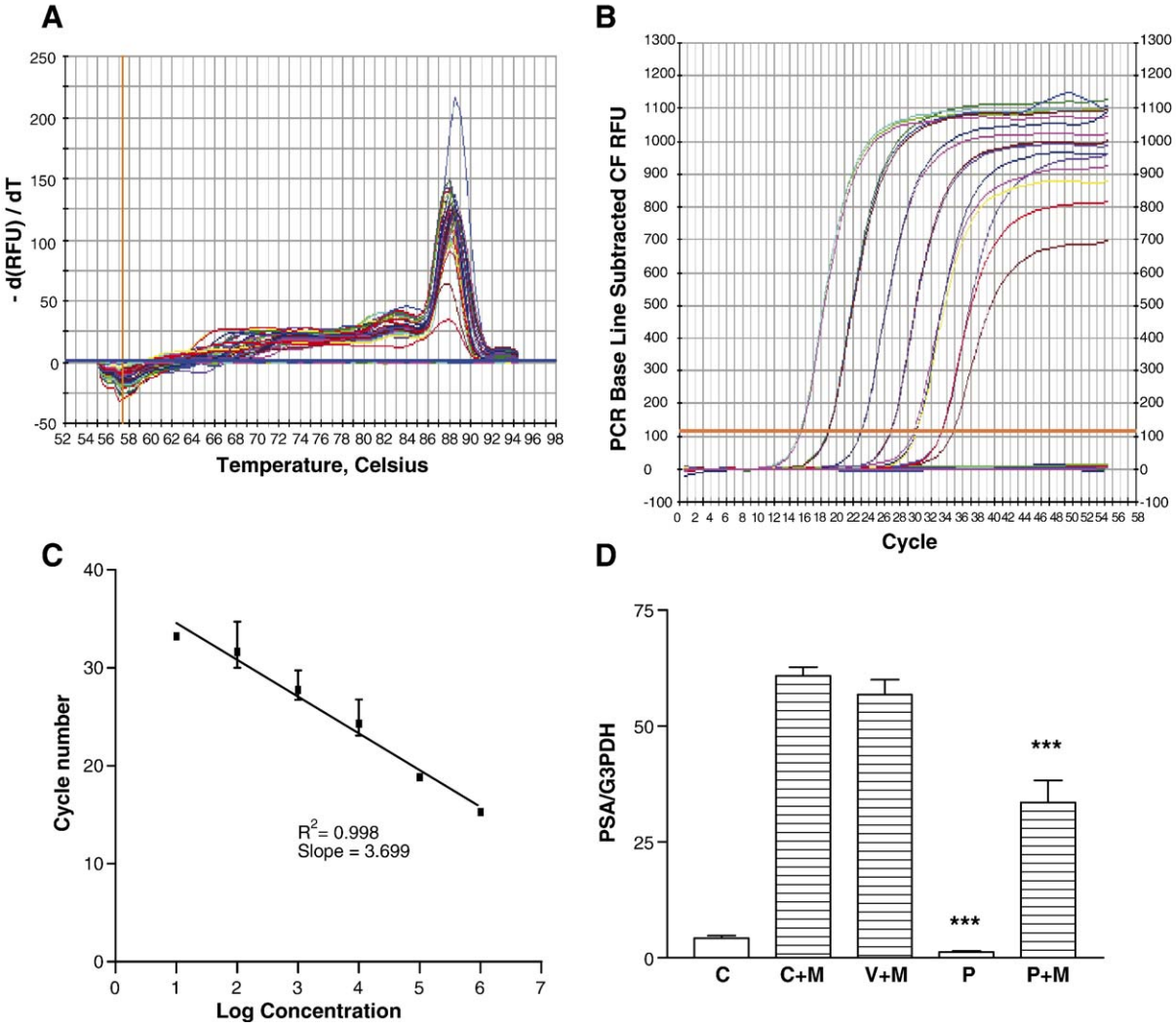

Fig. 6. Effect of overexpression of CXCL10 on PSA mRNA expression. (A) Melt-curve data collected after PSA amplification. All wells except the zero template control produced a single amplification product. (B) The standard curve was prepared with cDNA from LNCaP cells using a set of primers for PSA. The cDNA was used in decreasing concentrations with 1:10 dilutions in triplicates. (C) A plot of the threshold cycle number as a function of the input target quantity shows a linear relationship. (D) PSA/G3PDH mRNA levels in treated and untreated cells. After medium change, LNCaP cells (control, vector-transfected, plasmid-transfected) were treated with mibolerone (1 ng/ml) and cultured for 24 h. The values represent the mean±SEM of the combined samples from 3 experiments each with 3 replicates. ***$P<0.0001$ vs. respective controls.

CXCR3 expression and decreases PSA production. The anti-proliferation properties of CXCL10 in prostate cancer cells have not yet been described. We have utilized a vector, pIRES2–EGFP developed by Clontech, Inc, to overexpress human CXCL10 in LNCaP prostate cancer cells. This vector contains the internal ribosome entry site (IRES) of the encephalomyocarditis virus (ECMV) and the enhanced green fluorescent protein (EGFP). This permits both the gene of interest and the EGFP to be translated from a single bicistronic mRNA, but the resultant gene products are separate proteins, and not a fusion protein. We demonstrated that overexpression of CXCL10 inhibited cell growth and PSA production in LNCaP cells. The efficiency of this system has been reported previously by several investigators [13,14]. Transient over-expression of IRES–GFP constructs of PDGF resulted in the proliferation of senescent cardiac fibroblasts via AKT kinase [13]. Overexpression of E2F2 in pIRES2–EGFP resulted in

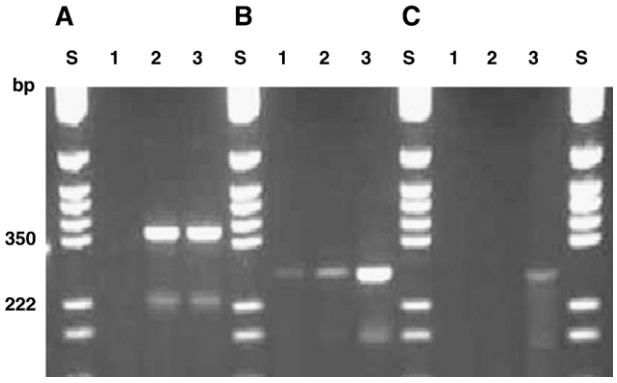

Fig. 7. Activation of CXCR3 expression by overexpression of CXCL10. Total RNA was extracted from control (lane 1), vector (IRES2–EGFP)-transfected (lane 2) and CXCL10 transfected (pI2E–CXCL10) (lane 3) LNCaP cells, and subjected to RT-PCR. (A) EGFP, 366 bp; (B) CXCL10, 270 bp and (C) CXCR3, 280 bp.

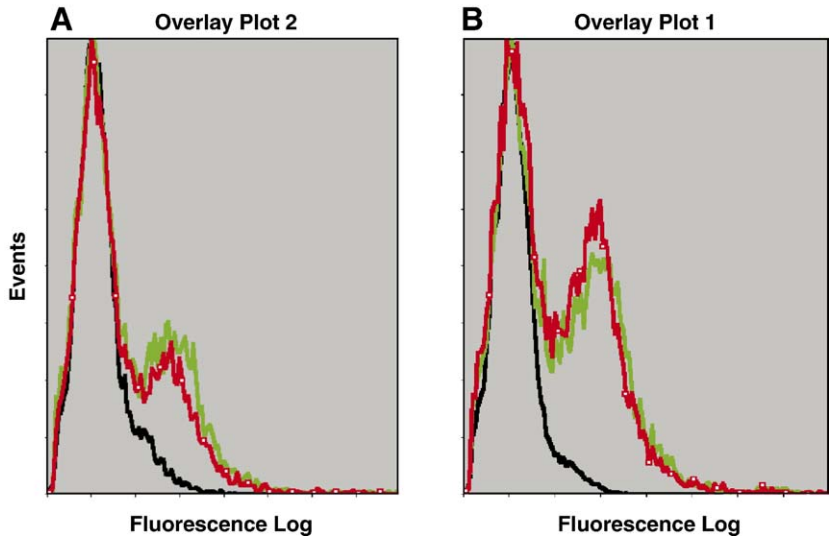

Fig. 8. The CXCR3 expression was analyzed by FACS. Cells were stained with both antibodies, primary and PE-conjugated secondary. Black line, unstained; Green line, EGFP +ve; and Red line, CXCR3 +ve cells. Panel A, Control LNCaP cells; Panel B, pI2E–CXCL10 transfected LNCaP cells. This is a representative of 4 similar experiments. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

higher levels of reaction product for Ki67 (a 5.1-fold increase) and cyclin B1 (a 2–3-fold increase) in rabbit corneal endothelial cells than in the control cells [14]. We have reported previously that overexpression of CXCL10 in MA-10 mouse Leydig tumor cells inhibited cell proliferation [15].

It is now becoming clear that chemokines are not just leukocyte chemoattractants, but play pleiotropic roles in cellular transformation, tumor growth, invasion and homing, metastasis and immune modulation. CXCL10 is a member of the chemokine family. This family is divided into 4 subfamilies; CXC, CC, C and CX3C [16]. The CXC in CXCL10 protein is CysAsnCys. The human CXCL10 gene consists of 4 exons. It yields a single band of 1.5 kb mRNA by Northern blotting [9,17]. Human CXCL10 cDNA encodes 99 amino acid residue precursor protein with a 22 amino acid residue signal peptide that is cleaved to form 77 amino acid residue secreted protein with a molecular weight of 8.7 kDa [17]. The mechanism of action of CXCL10 is believed to be mediated by its binding to cell surface receptors, such as CXCR3, heparan sulfate glycosaminoglycan (GAG) and unknown receptors [7,18,19]. The human CXCR3 cDNA has an open reading frame of 1104 bp encoding a protein of 368 amino acids with a molecular mass of 40,659. The sequences include seven putative transmembrane segments characteristic of G-protein coupled receptors (GPCR). CXCR3 receptors are coupled to Gαi proteins, making cellular responses to chemokines inhibitable by pertussis toxin. Receptor activation inhibits cAMP production, but other signal transduction pathways (PI3 kinase and MAPK) are clearly involved as well [20]. Two variants of CXCR3, CXCR3-A and CXCR3-B were identified by using rapid amplification of cDNA ends (RACE) and Northern blot analysis [21]. By transfection studies in human microvascular endothelial cell line-1 (HMEC-1), it was further observed that CXCR3-A mediated cell proliferation effects, whereas CXCR3-B mediated antiproliferative effects. Finally, monoclonal antibodies raised to selectively recognize CXCR3-B reacted with endothelial cells from neoplastic tissues, providing evidence that CXCR3-B is also expressed in vivo and may account for the angiostatic effects of CXC chemokines [21]. A variant of CXCR3, termed as CXCR3-alt, has been cloned from human PBMC cells, whose expression is considerably lower (~15-fold) than the full-size receptor and its function is not known yet [22]. Recently, it has been shown that the expression of ELR-CXC chemokines (CXCL10 and CXCL11), which are angiostatic, is significantly lower in prostate cancer cells (CA-HPV-10 and PC-3) as compared to normal epithelial cells (PZ-HPV-7) [10]. CXCL10 is a potent chemoattractant for stimulated T cells and NK cells. Anti-tumor effects of CXCL10 may be mediated by the recruitment of $CD8^+$ and $CD4^+$ cells to tumors [23–25]. Furthermore, CXCL10 inhibits angiogenic processes and antagonizes the effects of basic fibroblast growth factor (bFGF) and CXCL8 [26,27].

Our results showed that CXCL10 protein secreted in the medium by transfected cells was 3–6 ng/ml after 6–8 days of cell culture, whereas the levels of CXCL10 in control cells were undetectable as estimated by ELISA. Interestingly, the CXCL10 overexpression caused significant increase in its receptor CXCR3 expression. Previously, we observed that overexpression of CXCL10 in MA-10 mouse Leydig tumor cells significantly increased CXCR3 mRNA and protein expression [15]. Goldberg-Bittman et al. [28] reported that unlike many other chemokine receptors, CXCR3 expression was not down-regulated by exposure to high concentrations (500 ng/ml) of its ligand, but was rather promoted, which corroborates our results. CXCL10 induced upregulation of CXCR3 expression in the three human breast adenocarcinoma cell lines, which was inhibited by cycloheximide, indicating that de novo protein synthesis was required for this process.

The concomitant expression of CXCR3 and CXCL10 in breast tumor cells suggests that a CXCR3–CXCL10 axis may function in these cells, and paves the way for an in depth analysis [28]. It has been shown that chemokine receptors are rapidly desensitized and internalized following ligand binding, a process that attenuates receptor-mediated responses [29,30]. Known inhibitors of chemokine-induced chemotaxis, such as pertussis toxin or wortmannin, did not reduce ligand-induced internalization, suggesting that a distinct signal transduction pathway mediates internalization [30]. CXCR3–CXCL10 axis may have a direct role in membrane integrity, as higher concentrations of CXCL10 are antiproliferative (Our laboratory is currently exploring these effects).

In this study, we showed that CXCL10 down-regulated mobilerone-induced PSA expression. In LNCaP cells, miboleronone at very low levels (1 nM) increase AR-mediated transcription of PSA by 50–60-fold [31]. In this regard, the molecular mechanism(s) by which CXCL10 regulates the androgen signaling in prostate cells needs to be explored further. Recent studies have shown that besides AR mutations, the modulation of the AR protein by phosphorylation, acetylation, and sumoylation also regulates AR activity [32–34]. Modulation of the activities of AR cofactors is another factor that can change PSA transcription [35,36].

Our data showed that overexpression of CXCL10 inhibited cell proliferation. Our results are in agreement with the previous reports that CXCL10 inhibits cell proliferation [5,18,37]. CXCL10 is highly effective in the inhibition of primary human microvascular endothelial cell proliferation [38]. The growth of CXCL10-transduced melanoma cells was markedly diminished compared to the parental or null-transduced cells. They suggested that CXCL10 gene therapy might be an effective therapy in patients with cancer [39]. Previously, it has been shown that CXCL10 and MIG mimic the antitumor effects of IL-12 [40–42]. Neutralizing antibodies to CXCL10 and MIG partially abrogated IL-12-mediated regression in the RENCA model [40].

In conclusion, in the present study, we demonstrated that the expression of CXCL10 by transfection with pIRES2–CXCL10–EGFP of LNCaP cells was markedly enhanced at mRNA and protein levels in the cells. Significant levels of secreted CXCL10 were detected in the spent medium of transfected cells as measured by enzyme-linked immunosorbent assay (ELISA). Overexpression of CXCL10 inhibited cell proliferation of the transfected cells by 30%–40% in serum-limited medium (1% FCS in RPMI1640 medium). CXCR3 expression was significantly induced by the overexpression of CXCL10 as determined by RT-PCR and fluorescence-activated cell sorting (FACS). Overexpression of CXCL10 inhibited cell proliferation by up-regulation of CXCR3 receptor. CXCL10 may be potentially useful in the treatment of prostate cancer.

Acknowledgment

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