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Tyk2 expression and its signaling enhances the invasiveness of prostate cancer cells

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Abstract

Protein tyrosine kinase plays a central role in the proliferation and differentiation of various types of cells. One of these protein kinases, Tyk2, a member of the Jak family kinases, is known to play important roles in receptor signal transduction by interferons, interleukins, growth factors, and other hormones. In the present study, we investigated Tyk2 expression and its role in the growth and invasiveness of human prostate cancer cells. We used a small interfering RNA targeting Tyk2 and an inhibitor of Tyk2, tyrphostin A1, to suppress the expression and signaling of Tyk2 in prostate cancer cells. We detected mRNAs for Jak family kinases in prostate cancer cell lines by RT-PCR and Tyk2 protein in human prostate cancer specimens by immunohistochemistry. Inhibition of Tyk2 signaling resulted in attenuation of the urokinase-type plasminogen activator-enhanced invasiveness of prostate cancer cells *in vitro* without affecting the cellular growth rate. These results suggest that Tyk2 signaling in prostate cancer cells facilitate invasion of these cells, and interference with this signaling may be a potential therapeutic pathway.

Keywords: Tyk2; Jak; Prostate cancer; Invasion; Metastasis

Prostate cancer arises as a consequence of an imbalance between cell division and differentiation. The proliferation, differentiation, growth, and apoptosis of normal and malignant cells are regulated by many different cytokines and growth factors. Protein tyrosine kinases (PTKs) play a central role in the proliferation and differentiation of various types of cells. They participate in the cellular responses to growth factors, and activation of their protein kinase activity is critical for the transmission of mitogenic signals. In a previous study, to explore the function of PTKs in the developing prostate gland, we screened for PTKs expressed in CD44-positive cells from the developing mouse prostate. CD44 is a cell surface glycoprotein receptor and this signaling regulates several important biologic processes including lymphocyte homing and activation, hematopoiesis, and tumor progression and metastasis [1]. In addition, CD44 is expressed during mouse prostate development but not in the adult prostate. Treatment with neutralizing antibodies to CD44 inhibits androgen-stimulated ductal branching morphogenesis in serum-free organ cultures of the mouse prostate [2]. Therefore, CD44 is one of the markers of early progenitor cells in prostate tissues. Using CD44 as a cell surface marker, we isolated several PTK genes including Tyk2 from CD44-positive prostate cells [3].

To date, four mammalian members of the Jak family have been identified, namely, Tyk2, Jak1, Jak2, and Jak3. Previous reports from other laboratories have also demonstrated that Jaks are expressed in a human prostate cancer xenograft model and in bone marrow metastases [4,5]. Extensive studies over the last few years have suggested

Abbreviations: Jak, Janus kinase; RT-PCR, reverse transcriptase-polymerase chain reaction; SH2, Src homology 2; Stat, signal transducers and activators of transcription.

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that Jak kinases play important roles in the responses to interferons, interleukins, growth factors, hormones, and urokinase-type plasminogen activator (uPA), also known as urokinase [6,7]. Activation of Jaks leads to the tyrosine phosphorylation of receptors, producing docking sites for various SH2-containing signaling molecules including Stat proteins [8]. It is well documented that Stats are over-activated in some malignancies. For example, Stat3 activity is elevated in prostate cancer [9]. Accumulating evidence for constitutive activation of various Stats and other oncoproteins in different cancers strongly suggests that Jak kinases play critical roles in the pathogenesis of many human neoplastic diseases [6].

In this study, we examined the expression and biological significance of Tyk2 in prostate cancer. We show that Tyk2 is involved in uPA-induced cell invasion, which is a measure of the malignant potential of prostate cancer cells. Blockade of Tyk2 signaling by a small interfering RNA (siRNA) or by the PTK inhibitor tyrphostin A1 significantly suppresses the invasiveness of human prostate cancer cells into Matrigel. Our results demonstrate that activation of the Tyk2 signaling pathway is important for the enhancement of prostate cancer cell invasiveness by uPA. Thus, the Tyk2 signaling pathway may be a worthwhile target for therapeutic intervention in prostate cancer.

Materials and methods

Cell culture and reagents. Three human prostate cancer cell lines LNCaP, PC-3, DU145, and TSU-Pr1 (bladder cancer cell line) and MCF-7 (breast cancer cell line) were used in this study. The cells were routinely maintained in RPMI 1640 supplemented with 10% fetal calf serum, 100 units/ml penicillin, and 100 μg/ml streptomycin. Cell growth was assessed using a colorimetric proliferation assay employing the tetrazolium 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2*H* tetrazolium (MTS). Each day, MTS was added, and the absorbance at 490 nm was measured on microplate reader after a 60-min incubation at 37 °C. The siRNA duplex targeting Tyk2 and a control siRNA were purchased from Santa Cruz Biotechnology (Santa Cruz, CA). For transfection with siRNAs, DU145 cells were placed in Opti-MEM I (Invitrogen, Carlsbad, CA) and then transfected using oligofectamine (Invitrogen). Tyrphostin A1 (Sigma–Aldrich, St. Louis, MI), an inhibitor of Tyk2 PTK activity, was used at 100 μM, and the effect of uPA (R&D Systems, Minneapolis, MN) was examined at a concentration of 5 nM.

RT-PCR analyses. For RNA expression analysis, total RNA was extracted from cells using RNA Bee (Tel-Test, Friendswood, TX) according to the manufacturer's protocol. The RNA was then treated with DNAse I to remove contaminating DNA and then reverse transcribed using an oligo-dT primer and Super-Scriptβ reverse transcriptase (Invitrogen) in a volume of 25 μl. The primer sequences were as follows: human Jak1, 5′-AAGTGATGTCCTTACCACA-3′ and 5′-AGCAGCCACAC TCAGGTTCT-3′; human Jak2, 5′-GAGCCTATCGGCATGGAATA-3′ and 5′-ATATCTAACACTGCCATCCC-3′; human Jak3, 5′-CAAACAC CACTCCCTGTCCT-3′ and 5′-TGGGGGTGTTCCTGAAGTAG-3′; Tyk2, 5′-GGATGGCCAGGGGCAGTAAG-3′ and 5′-GGATCTCCTC CTCGGTCGAC-3′; prostate-specific antigen, 5′-GGTCGGCACAGCC TGTTTCA-3′ and 5′-CCACGATGGTGTCCTTGATC-3′; β-actin, 5′-GACTACCTCATGAAGATCCT-3′ and 5′-GCGGATGTCCACGTCA CACT-3′. The resulting cDNA was subjected to PCR.

Immunoblot analyses. The cells were washed twice with cold PBS, and lyzed on ice in 2× sample buffer (125 mmol/L Tris, pH 6.8, 4% SDS, 10% 2-β mercaptoethanol, 20% glycerol, 0.06% bromophenol blue). The cell lysates were boiled for 3 min and resolved by 10% SDS–PAGE. Proteins were transferred onto a PVDF membrane (Bio-Rad, Hercules, CA), and immunoblotting was performed using rabbit anti-human Tyk2 antibody (Santa Cruz Biotechnology; 1:1000) or rabbit anti-human β-tubulin antibody (Santa Cruz Biotechnology) as an internal loading control. Goat anti-rabbit antibody conjugated by HRP (Bio-Rad, 1:3000 dilution) were used as a secondary antibody. Immunoreactive proteins were visualized with ECL detection reagents (Amersham Biosciences, Piscataway, NJ).

Immunohistochemistry. Serial 4-mm-thick sections were deparaffinized in three changes of xylene and rehydrated through a graded series of ethanol decreasing from 100% to 70%. The sections were immersed in citrate buffer (pH. 6.0) and autoclaved at 120 °C for 5 min and then placed in 3% hydrogen peroxide in methanol for 20 min at room temperature to block endogenous peroxidase activity. Nonspecific protein binding was blocked by incubating the section for 30 min–1 h in 5% goat serum. Next, the sections were incubated overnight at 4 °C in polyclonal rabbit Tyk2 antibody (Santa Cruz Biotechnology). Sections were then processed for immunohistochemistry using the EnVisionTM+ system (DAKO, Denmark). We examined 70 samples from prostate cancer patients.

Matrigel invasion assay. Membrane inserts (8-μm pore size) for 24-well transwell plates were prepared by coating with Matrigel basement membrane matrix (BD Biosciences, San Jose, CA) according to the manufacturer's instructions. DU145 cells were placed in the upper chamber at a density of 2×10^4 cells/insert. Medium containing 5 nM uPA (R&D Systems, Minneapolis, MN) was added to the lower chamber as a chemoattractant. To inhibit the invasion, 200 nM of Tyk2 siRNA/200 nM oligofectamine or 100 μM of tyrphostin A1 was added to the medium. After 24 h, the upper surface of the inserts was wiped with cotton swabs, and the inserts were stained with Trypan blue. Cells that migrated through the Matrigel and the filter pores to the lower surface were counted in five random high-power fields per insert using a light microscope.

Results

Expression analyses of Tyk2 in prostate cancer cell lines and tissues

We first analyzed the expression of Jaks in MCF-7, LNCaP, PC-3, TSU-Pr1 and DU145 cells by RT-PCR (Fig. 1A). Amplified products for four members of the Jak family, Tyk2, Jak1, Jak2, and Jak3, were clearly detected in these cell lines. We also found that prostate-specific antigen was expressed by LNCaP cells which is androgen sensitive prostate cancer cell line as previously described [3]. Although we tested one androgen sensitive prostate cancer cell line LNCaP, there was no difference of the expression level of Jaks mRNAs between androgen sensitive cells and insensitive cells. We next examined the expression of Tyk2 in human prostate cancer tissue specimens by immunohistochemistry with rabbit anti-human Tyk2 antiserum. The reactivity for Tyk2, shown as brown color, was higher in cancerous than in noncancerous glands in the same field (Fig. 1B). The staining for Tyk2 was heterogeneous and predominately located in the cytoplasm of prostate cancer cells. Of 70 samples from prostate cancer patients, Tyk2 staining was clearly detected in 17 (24.3%). Further studies are needed to accurately determine the correlation between Tyk2 expression in the cancerous tissues and clinical and pathological variables.