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Induction of steroid sulfatase expression in PC-3 human prostate cancer cells by insulin-like growth factor II

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HIGHLIGHTS

- IGF-II induces STS gene expression in PC3 human prostate cancer cells.
- Induction of steroid sulfatase occurs through PI 3-kinase/Akt-NFκB pathway.
- IGF-II activates STS promoter activity.
- IGF-II induces 17β-HSD1 and 3 causing estrone and β-estradiol production.

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ABSTRACT

Human steroid sulfatase (STS) plays an important role in regulating the formation of biologically active estrogens and may be a promising target for treating estrogen-mediated carcinogenesis. The molecular mechanism of STS gene expression, however, is still not clear. Growth factors are known to increase STS activity but the changes in STS expression have not been completely understood. To determine whether insulin-like growth factor (IGF)-II can induce STS gene expression, the effects of IGF-II on STS expression were studied in PC-3 human prostate cancer cells. RT-PCR and Western blot analysis showed that IGF-II treatment significantly increased the expression of STS mRNA and protein in concentration- and time-dependent manners. To understand the signaling pathway by which IGF-II induces STS gene expression, the effects of specific PI3-kinase/Akt and NF-κB inhibitors were determined. When the cells were treated with IGF-II and PI3-kinase/Akt inhibitors, such as LY294002, wortmannin, or Akt inhibitor IV, STS expression induced by IGF-II was significantly blocked. Moreover, we found that NF-κB inhibitors, such as MG-132, bortezomib, Bay 11-7082 or Nemo binding domain (NBD) binding peptide, also strongly prevented IGF-II from inducing STS gene expression. We assessed whether IGF-II activates STS promoter activity using transient transfection with a luciferase reporter. IGF-II significantly stimulated STS reporter activity. Furthermore, IGF-II induced expression of 17β-hydroxysteroid dehydrogenase (HSD) 1 and 3, whereas it reduced estrone sulfotransferase (EST) gene expression, causing enhanced estrone and β-estradiol production. Taken together, these results strongly suggest that IGF-II induces STS expression via a PI3-kinase/Akt–NF-κB signaling pathway in PC-3 cells and may induce estrogen production and estrogen-mediated carcinogenesis.

1. Introduction

Steroid sulfatase (STS) is the enzyme responsible for the hydrolysis of aryl (estrone sulfate) as well as alkyl steroid sulfates (dehydroepiandrosterone sulfate) (Reed et al., 2005). Estrone sulfate is considered an important source for the formation of active estrogens such as estrone or 17β-estradiol that can stimulate tumor growth (Purohit and Foster, 2012; Reed et al., 2005). Metabolic activation of estrone by STS has been suggested to be a major factor in estrogen-dependent carcinogenesis. STS also regulates the production of 5-androstenediol from dehydroepiandrosterone sulfate and subsequent reduction of dehydroepiandrosterone by 17β-hydroxysteroid dehydrogenase (HSD) 1. 5-Androstenediol is a ligand of the estrogen receptor and it strongly stimulated hormone-dependent growth of breast cancer cells and carcinogen-induced

Abbreviations: EST, estrone sulfotransferase; HSD, 17β-hydroxysteroid dehydrogenase; IGF, insulin-like growth factor; IL-6, interleukin 6; NBD, nemo binding domain; STS, steroid sulfatase; TNF, tumor necrosis factor.

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mammary tumors in rodent (Adams et al., 1981). STS is primarily expressed in steroidogenic and steroid-responsive tissues such as placenta, prostate, testis, ovary, breast, and adrenal gland although STS appears to be ubiquitous in small quantities to nearly all tissues. STS expression has been considered significantly in estrogen-dependent breast or endometrial tumors (Dao et al., 1974; Utsumi et al., 2000; Pasqualini et al., 1986). In prostate cancer, STS expression has also been considered important for in situ androgen production (Naitoh et al., 1989; Pasqualini et al., 1996; Nakamura et al., 2006). However, the possibility that STS may play an important role in estrogen production in human prostate cancers was also suggested (Naitoh et al., 1989). Previously, the status of aromatase, 17β-HSD, and STS in prostate cancer cells were studied and they were actually involved in estrogen production and metabolism in cultured prostate cancer cells (Nakamura et al., 2006).

The functional importance of STS is underlined by recessive X-linked ichthyosis, a keratinization disorder, which is caused by deletions or point mutations in the STS gene leading to deficient activity (Hernandez-Martin et al., 1999; Ghosh, 2004). Recently, it is suggested that STS gene may play a role in susceptibility for attention deficit hyperactivity disorder, the most common children behavioral disorder (Brookes et al., 2008; Kent et al., 2008).

Because of the postulated role of STS on estrogen and androgen generation in tumor cells, STS is considered a target enzyme for preventing estrogen- and androgen-dependent cancers. Various STS inhibitors showing potent inhibition of STS activity in vitro and in vivo have been developed and a Phase I and II clinical trial with 667 COUMATE in postmenopausal women with hormone-dependent breast cancer were performed although the results were not quite successful (Geisler et al., 2011; Maltais and Poirier, 2011; Purohit and Foster, 2012).

Although expression and activity of STS is increased in malignant tumors, little is known about the regulation of STS expression. A previous report suggested that tumor necrosis factor (TNF)-α and interleukin 6 (IL-6) may increase STS enzyme activity via a post-translational modification or by increasing substrate availability in MCF-7 cells and indicated that STS activation can occur independently of STS promoters (Newman et al., 2000). However, treatment of human promyeloid cells with retinoids or 1α, 25-dihydroxyvitamin D3 increased STS mRNA and activity (Hughes et al., 2001, 2008). Our recent study clearly showed that tumor necrosis factor (TNF)-α increases STS expression at the transcriptional level in PC-3 human prostate cancer cells (Suh et al., 2011).

Insulin-like growth factors such as IGF-I and IGF-II are peptide growth factors with functional similarity with insulin. These factors promote cancer cell proliferation and may play important roles in development of prostate cancer cells as well as breast cancer cells (De León et al., 1992; Russell et al., 1998). Mitogenic effects of IGFs are mediated through binding with IGF-I receptor and activation of PI-3 kinase/Akt signaling pathway. Interestingly, it was suggested that transcription of IGF-II is regulated by estrogen and IGF-II signaling can stimulate estrogen-mediated gene expression (Lee et al., 1994). Previous studies in our laboratory demonstrated that the IGF-II showed a strong induction of STS expression whereas IGF-I had little effect on STS expression.

In these studies, the effect of IGF-II on STS expression in PC-3 human prostate cancer cells was studied to determine whether IGF-II is able to regulate STS gene transcription at the transcriptional level. For this purpose, we have constructed a STS promoter-luciferase reporter to measure STS promoter activity for the first time. Because IGF-II induces cell survival signals through activation of the PI3-kinase/Akt and its downstream target NF-κB, evidence for the involvement of PI3-kinase/Akt and NF-κB signal pathway in STS expression by IGF-II has also been determined.

2. Materials and methods

2.1. Reagents

Human recombinant IGF-II was purchased from PeproTech (Rocky Hill, NJ). RPMI 1640 medium, penicillin and streptomycin were obtained from Welgene (Daegu, Korea). Fetal bovine serum (FBS) or enhanced chemiluminescence detecting reagent was from Thermo Scientific. Charcoal stripped FBS and piceatannol were from Sigma (St. Louis, MO). LY294002, MG-132, PD168393, Akt inhibitor IV, and Bay 11-7082 were obtained from Calbiochem (San Diego, CA). Bortezomib was obtained from Selleck (West Paterson, NJ). SB203580 was obtained from Tocris (Ellisville, MO). Wortmannin was obtained from Enzo Life Science (Plymouth Meeting, PA). M-MLV reverse transcriptase or RNase inhibitor (RNasin) was purchased from Promega (Madison, WI). Ex Taq polymerase or SYBR Premix Ex Taq was obtained from TaKaRa Bio (Shiga, Japan). Goat polyclonal antibody for STS and horseradish peroxidase (HRP)-conjugated mouse anti-goat IgG were purchased from Abcam (Cambridge, UK). Other chemicals and reagents were of the highest quality commercially available.

2.2. Cell culture

Cell lines were obtained from American Type Culture Collection (ATCC, Manassa, VA) and cultured in RPMI 1640 medium supplemented with 10% (v/v) heat-inactivated FBS, 100 unit/ml penicillin, and 100 μg/ml streptomycin. Cells were maintained at 37 °C in a humidified atmosphere of 5% CO_2.

2.3. RT-PCR and quantitative real-time PCR

Total RNA was extracted using Ribospin™ (GeneALL, Seoul, Korea). Total RNA (500 ng) was transcribed at 37 °C for 1 h in a volume of 20 μl containing 5× RT buffer, 10 mM dNTPs, 40 units of RNase inhibitor, 200 units of M-MLV reverse transcriptase, and 100 pmole of oligo-dT primer. Subsequently, 0.8 μl of the reaction mixture from each samples was amplified with 10 pmole of each oligonucleotide primers, 0.2 mM dNTPs, 1.5 mM $MgCl_2$ and 1.25 units of *Ex Taq* DNA polymerase in a final volume of 25 μl. PCR was performed as follows: one cycle of 95 °C for 2 min, followed by 35 cycles of denaturation at 95 °C for 10 s, annealing at 61 °C for 15 s, and extension at 72 °C for 15 s. Human STS cDNA was amplified using a sense primer (5′-CCTCCTACTGTTCTTTCTGTGGG-3′) and an antisense primer (5′-GGTCGATATTGGGAGTCCTGATA-3′). Human estrogen sulfotransferase gene (SULT1E1) was amplified using a sense primer (5′-ATGGTGGCTGGTCATCCAAAT-3′) and an antisense primer (5′-ATCCTCTTTCAGGTCTTCGTAGA-3′). Human HSD17B1 gene was amplified using a sense primer (5′-ACCTTCCACCGCTTCTACCA-3′) and an antisense primer (5′-GAACGT CGCCGAACACTTC-3′). Human HSD17B3 gene was amplified using a sense primer (5′-CGGACGCTGGAAAAACTAGAG-3′) and an antisense primer (5′-AGCATTCCGACATTGTTGACTAA-3′). Human HSD17B5 gene was amplified using a sense primer (5′-TCTGGGATCTCAACGAGACAA-3′) and an antisense primer (5′-TGGAACTCAAAA ACCTGCACG-3′). Human HSD17B7 gene was amplified using a sense primer (5′-AGCCTGAATCTCTCAATCCTCT-3′) and an antisense primer (5′-GCA GTGTCTTCATCTAGGTCCA-3′). Human HSD17B12 gene was amplified using a sense primer (5′-CTTGACCATCTATTCTGCAACCA-3′) and an antisense primer (5′-CTTGCT CCTATACTCCTCATGGA-3′). Human GAPDH was amplified using a sense primer (5′-TGAACGGGAAGCTCACTGG-3′) and an antisense primer (5′-TCCACCACCCTGTTGCTGTA-3′). The number of amplification cycles was optimized in preliminary experiments to ensure that the PCR did not reach a plateau. PCR products were subjected to a 2% (w/v) agarose gel electrophoresis, and analyzed by ChemiDoc XRS (Bio-Rad, Hercules, CA). Quantitative real-time PCR was performed using SYBR® Premix Ex Taq (Takara) and the MyiQ2 Two-Color Real-Time PCR detection system (Bio-Rad). The PCR analyses for human STS was done as follows: after an initial denaturation at 95 °C for 10 min, the amplification was done by denaturation at 95 °C for 10 s, annealing at 58 °C for 15 s, and extension at 72 °C for 15 s for 40 cycles.

2.4. Western blot

Cells were solubilized with ice-cold lysis buffer (pH 7.4) containing 25 mM HEPES, 1% Triton X-100, 50 mM NaCl, 1 mM EDTA, 1 mM EGTA, 1 mM PMSF, and 1 μg/ml leupeptin. Extracted proteins (30 μg) were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) on 10% polyacrylamide gels, and were electrophoretically transferred onto polyvinylidene difluoride (PVDF) membrane. Membranes were blocked with 5% (w/v) nonfat dried milk in Tris-buffered saline for 1 h. Membranes were then incubated overnight with anti-STS polyclonal antibodies as a 1:1000 dilution in 5% (w/v) nonfat dried milk in Tris-buffered saline containing 0.1% Tween-20 at 4 °C. The membrane was incubated for 2 h with a HRP-conjugated mouse anti-goat IgG antibody. Proteins were visualized by enhanced chemiluminescence detection and the band intensity was analyzed by ChemiDoc XRS densitometer and quantified by Quantity One software (Bio-Rad). Protein concentrations were estimated using the bichinchoninic acid method according to the supplier's recommendation (Thermo) using bovine serum albumin as a standard.