

Bacterial Lipopolysaccharides Induce Steroid Sulfatase Expression and Cell Migration through IL-6 Pathway in Human Prostate Cancer Cells

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Abstract

Steroid sulfatase (STS) is responsible for the conversion of estrone sulfate to estrone that can stimulate growth in endocrine-dependent tumors such as prostate cancer. Although STS is considered as a therapeutic target for the estrogen-dependent diseases, cellular function of STS are still not clear. Previously, we found that tumor necrosis factor (TNF)- α significantly enhances steroid sulfatase expression in PC-3 human prostate cancer cells through PI3K/Akt-dependent pathways. Here, we studied whether bacterial lipopolysaccharides (LPS) which are known to induce TNF- α may increase STS expression. Treatment with LPS in PC-3 cells induced STS mRNA and protein in concentration- and time-dependent manners. Using luciferase reporter assay, we found that LPS enhanced STS promoter activity. Moreover, STS expression induced by LPS increased PC-3 tumor cell migration determined by wound healing assay. We investigated that LPS induced IL-6 expression and IL-6 increased STS expression. Taken together, these data strongly suggest that LPS induces STS expression through IL-6 pathway in human prostate cancer cells.

Key Words: Steroid sulfatase, Lipopolysaccharides, Interleukin-6, Tumor cell migration, PC-3

INTRODUCTION

Steroid sulfatase (STS) is responsible for the enzymatic hydrolysis of steroid sulfates such as estrone sulfate (E1S) and dehydroepiandrosterone sulfate (DHEAS) to their unconjugated, biologically active forms (Reed *et al.*, 2005). DHEA can be converted to 5-androstene-3 β , 17 β -diol, which has been shown to have weak estrogenic properties. However, due to the high concentration of this steroid, it can stimulate the growth of breast cancer cells *in vitro* and *in vivo* (Aidoo-Gyamfi *et al.*, 2009). E1S is a steroid conjugate present in high concentrations in tissue and blood in women with breast cancer. E1S is considered an important source for the formation of active estrogens which is able to be produced by STS activity (Aidoo-Gyamfi *et al.*, 2009).

Sulfated estrogens are unable to bind to estrogen receptors and thus they are inactive as hormones. In other words, sulfation changes the polarity of the hydrophobic steroid ring and then is used for hormone transport (Kriz *et al.*, 2008). Furthermore, sulfated steroid conjugates play a role as a reservoir of active hormones or their precursors via STS action.

Metabolic activation of E1S has been suggested to play a

major role in mammary carcinogenesis (Chang, 2011). STS is involved in the formation of active estrogens such as E1 or E2. A high proportion, approximately 40% of breast cancers are hormone-dependent and it is the E2 that is suggested to be important in the initiation, promotion and progression of this disease (Ahmed *et al.*, 2002). Moreover, STS expression is increased in malignant breast tumors and has prognostic importance (Utsumi *et al.*, 2000; Pasqualini, 2009).

Just like breast tumor, estrogens are locally synthesized by aromatase in human prostate cancer (Härkönen and Mäkelä, 2004). Various types of 17 β -hydroxysteroid dehydrogenase (17 β -HSD) also have been reported to be expressed at least in some human prostate cancers (Koh *et al.*, 2002). These findings above all suggest that *in situ* estrogen production and metabolism are involved in human prostate cancer (Koh *et al.*, 2002). However, *in situ* or intratumoral production of estrogens has remained relatively unknown compared to that of androgens (Nakamura *et al.*, 2005).

Besides 17 β -HSD type I and aromatase, STS belongs to the most important targets for potential endocrine therapy in humans (Suzuki *et al.*, 2005). Inhibitors of steroid sulfatase are being developed as a novel therapy for hormone-dependent

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breast cancer in postmenopausal women (Stanway *et al.*, 2007). It maintains the equilibrium between sulfated and unconjugated steroids whose effects in breast cancer development are, in many cases, completely different (Reed *et al.*, 2005).

Chronic inflammation has been linked to the development of cancer. As a potent inflammatory stimulus, lipopolysaccharides (LPS), a cell wall constituent of Gram negative bacteria, induce the release of a wide range of cytokines and growth factors from immune cells (Wilson *et al.*, 2002; Li *et al.*, 2012). In addition, LPS can alter cytokine levels by stimulating inflammatory cells in the tumor microenvironment to induce tumor cell proliferation, promote angiogenesis, and facilitate invasion and metastasis (Schetter *et al.*, 2010). Recently, some reports have demonstrated that LPS can exert direct effects on tumor cells *in vivo* and *in vitro* (Wang *et al.*, 2003). Although recently explored roles of STS in many pathophysiological processes in cancers prompted efforts for preparation of efficient STS modulators, the cellular function of STS is still not clear.

Induction of STS expression may require mRNA or protein synthesis. However, which factors can directly affect the expression of STS has not been examined in detail in human prostate cancer. As shown in former studies, LPS is known to stimulate STS expression in monocytes and macrophages (Guha and Mackman, 2001). Macrophage-borne STS converts DHEAS to DHEA. It has been shown that DHEA, through receptor-mediated mechanisms similar of steroid hormones, represents an important regulator of interleukin-2 (IL-2) production and the TH1 immune response (Daynes *et al.*, 1990).

Inflammatory cytokines such as IL-6 and IL-1 β regulate proliferation of breast cancer cells through estrogen production by steroid-catalyzing enzymes including STS and aromatase (Honma *et al.*, 2002). IL-6 enhances the activity of steroid catalyzing enzymes (Purohit *et al.*, 1996; Reed and Purohit, 1997).

LPS released from gram-negative bacteria binds to LPS binding protein (LBP), and then the LPS-LBP complex binds to CD14 on the surface of neutrophils, monocytes and macrophages. These complexes may stimulate cytokine expression including IL-1 β and IL-6 (Okada *et al.*, 1999).

In these studies, we explored the effect of LPS on STS expression in PC-3 human prostate cancer cells to elucidate whether LPS is able to regulate transcription of the STS gene. We also studied LPS-induced STS expression enhances tumor cell migration. As IL-6 is an important contributor to the effects of LPS in inflammation (Wright *et al.*, 1993), evidence for the involvement of IL-6 pathway in STS expression by LPS has also been determined.

MATERIALS AND METHODS

Reagents

Rabbit polyclonal antibody for STS was purchased from Abcam (Cambridge, UK). Antibodies against GAPDH were purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA). Bacterial lipopolysaccharides were purchased from Sigma-Aldrich (St. Louis, MO, USA). Enhanced chemiluminescence (ECL) detecting reagent was from Thermo Scientific (Rockford, IL, USA). Dual-Luciferase Reporter Assay kit was purchased from Promega (Madison, WI, USA). Ex taq polymerase was obtained from TaKaRa Bio (Shiga, Japan). Other chemicals and reagents were of the highest quality commercially available.

Cell culture

Human prostate cancer PC-3 cells and HeLa cells were obtained from Korean Society Cell Bank (KCLB). Cells were grown in RPMI 1640 medium supplemented with 10% heat-inactivated FBS, 100 units/ml penicillin, and 100 μ g/ml streptomycin. Cells were maintained at 37°C in a humidified atmosphere of 5% CO₂. After incubation, the cells were harvested by scrapping and solubilized in 50 mM Tris-HCl (pH 8.0) containing 150 mM NaCl, 1% nonidet P-40, 1 mM PMSF, 1 μ g/ml aprotinin, and 1 μ g/ml leupeptin. Cells were centrifuged at 1,000 \times g for 4 min at 4°C and the pellets were resuspended and stored in -70°C.

Transient transfection of siRNA

Cells were harvested at a density of 5 \times 10⁵ cells per 6-well plate. Transfection was carried out using the Neon™ transfection system (Invitrogen, Carlsbad, CA), and 1 \times 10⁶ cells were transfected with 50 nM STS siRNA (Thermo Scientific, Rockford, IL, USA). Following microporation, cells were cultured in 100-mm dishes with antibiotic-free RPMI for 24 h.

RNA isolation, reverse transcription and RT-PCR

After transfection, 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 μ l containing 5 \times RT buffer, 10 mM dNTPs, 40 units of RNase inhibitor, 200 units M-MLV reverse transcriptase and 100 pmole of oligo-dT primer. Subsequently, 0.8 μ l of the reaction mixture from each samples was amplified with 10 pmole of each oligonucleotide primers, 0.2 mM dNTPs, 1.5 mM MgCl₂ and 1.25 units of Ex taq polymerase in a final volume of 25 μ l. PCR was performed as follows: One cycle of 95°C for 2 min, followed by 35 cycles of denaturation at 95°C for 10 sec, annealing at 58°C for 15 sec, and extension at 72°C for 15 sec. 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, USA). The primers used for human STS were 5'- CCT CCTACT GTT CTT TCC GTG GG-3' (sense) and 5'- GGT CGA TAT TGG GAG TCT TGA TA-3' (antisense). The primers used for human IL-6 were 5'-GCA CTG GCA GAA AAC AAC CT-3' (sense) and 5'-CAG GGG TGG TTA TTG CAT CT-3' (antisense).

Western blot analysis

Following transfection, whole cell lysates were prepared and protein concentration was determined using BCA Protein Assay Reagents. Cellular extracts (20 μ g) were separated on 10% SDS-PAGE at 100 V and transferred onto 0.45 μ m PVDF membrane. Nonspecific binding was blocked with 5% nonfat milk in TBS-T for 1 h at room temperature. Primary antibody was used at a 1:1000 dilution. Secondary antibody was used in a 1:5000 dilution. The incubation of primary antibodies was done at 4°C for overnight incubation. Secondary antibodies were done at 4°C for 2 h. Proteins were visualized by an ECL method and the band intensity was analyzed by ChemiDoc XRS densitometer system and quantified by Quantity One software (Bio-Rad).

Luciferase assay

Cells (1.5 \times 10⁴ cells/well) were transfected with 0.4 μ g of STS luciferase reporter plasmid according to the manufac-

turer's protocol using Neon™ transfection system (Invitrogen). pRL-CMV (Promega) was cotransfected for internal control. Cells were treated with LPS (10 µg/µl) for 24 h. Cells were lysed with the lysis buffer and then the luciferase activities were measured consecutively using the Dual Luciferase Assay System (Promega) with Synergy™ H1 hybrid microplate reader (Biotek, Winooski, VT, USA).

Wound healing assay

Cells (1×10⁶ cells/well) were cultured in 6-well cell culture plate. Cells were washed with PBS and treated with mitomycin C (25 µg/ml) for 30 min. One mm wide scratch was made across the cell layer using a sterile pipette tip. Plates were photographed after indicated time.

Statistical analysis

Statistical analysis was performed by using one-way analysis of variance, followed by Dunnett's Multiple Comparison *t*-test using Graph-Pad Prism Software (GraphPad Software Inc., San Diego, CA) when appropriate. The difference was considered statistically significant at *p*<0.05.

RESULTS

Effects of LPS on STS expression in prostate cancer cells

To elucidate whether LPS regulate STS gene expression in PC-3 cells, the mRNA and protein expression of STS were

measured by RT-PCR and Western blot, respectively. PC-3 cells were grown for 24 h in the presence of increasing concentrations of LPS (0, 0.1, 1 or 10 µg/ml). As shown in Fig. 1A, when cells were treated with LPS, STS mRNA expression was significantly enhanced in a concentration-dependent manner. In agreement with increasing mRNA levels, levels of STS protein were also significantly induced by LPS (Fig. 1A). We compared the time courses for the induction of STS mRNA and protein expression in LPS-treated cells (Fig. 1B). At 1 µg/ml of LPS, the mRNA level of STS was ~2.2-fold higher than that of control. Similarly, we found ~2.3-fold induction of STS expression after 24 h treated with LPS (1 µg/ml). When cells were treated with LPS (10 µg/ml), the induction of STS was shown after 6 h of treatment. Induction of STS mRNA was plateaued after 48 h treatment (Fig. 1B).

Effects of LPS on activation of STS promoter in cervical cancer cells

To examine whether induction of STS expression by LPS is related to activation of promoter region of STS, luciferase assay was performed. HeLa cells were selected because it has higher transfection efficiency of STS reporter plasmid. HeLa cells were transfected with STS reporter plasmid (pGL3-STs2 luc). After transfection, cells were treated with LPS (10 µg/ml) for 24 h and luciferase activity was determined. As shown in Fig. 2, the results of luciferase assay indicated ~1.5-fold induction in STS transcription by LPS.

Effects of LPS-induced STS expression on tumor cell migration in prostate cancer cells

To elucidate whether LPS-induced STS expression causes PC-3 cell migration and invasion, we determined the effect of LPS on tumor cell migration using a wound healing assay. To determine the role of STS on tumor cell migration, cells were treated with STS siRNA (50 nM) for 48 h to knockdown STS expression selectively. Only 22.2% of the injury line was removed by STS knockdown although almost all of the injury line was closed by scrambled control. When STS knock-downed cells were treated with LPS (10 µg/ml), 74.0% of the injury line was recovered by LPS (Fig. 3). These results show that STS expression induced by LPS may cause PC-3 cell migration.

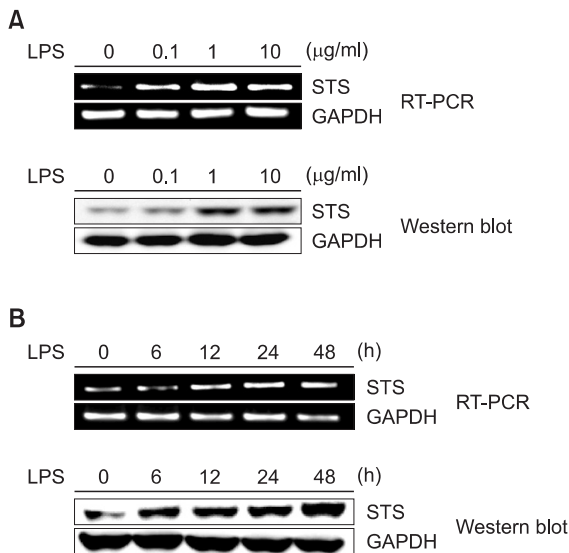


Fig. 1. LPS induce STS mRNA and protein expression in PC-3 cells. (A) Concentration-dependency. PC-3 cells were treated with various concentrations of LPS (0, 0.1, 1 or 10 µg/ml) for 24 h. The protein and mRNA level of STS was determined using RT-PCR and Western blot. Total RNA was isolated and human STS genes were amplified with specific primers. The PCR products were separated on a 2% agarose gel. Expression of GAPDH mRNA was determined as a RNA control. Total cellular lysates were prepared for Western blot analysis using antibody against STS. GAPDH was used as a loading control. (B) Time-dependency. Cells were treated with 10 µg/ml LPS for 0, 6, 12, 24 or 48 h. The protein and mRNA level of STS was determined using RT-PCR and Western blot.

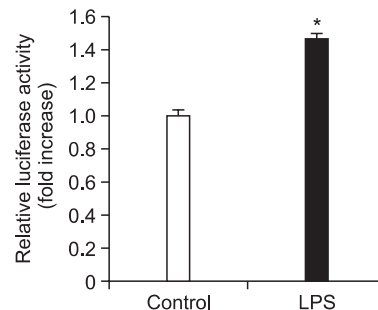


Fig. 2. LPS induce STS promoter activity in HeLa cells. HeLa cells were transiently transfected with pGL3-STs2luc and pRL-CMV plasmids. At 24 h after transfection, cells were incubated at 37°C with 10 µg/ml of LPS for 24 h. Luciferase activity was measured and expressed as the mean ± S.D. of four separate experiments. **p*<0.05 compared with untreated control cells. The relative firefly luciferase activity, normalized by the renilla luciferase activity, is shown.

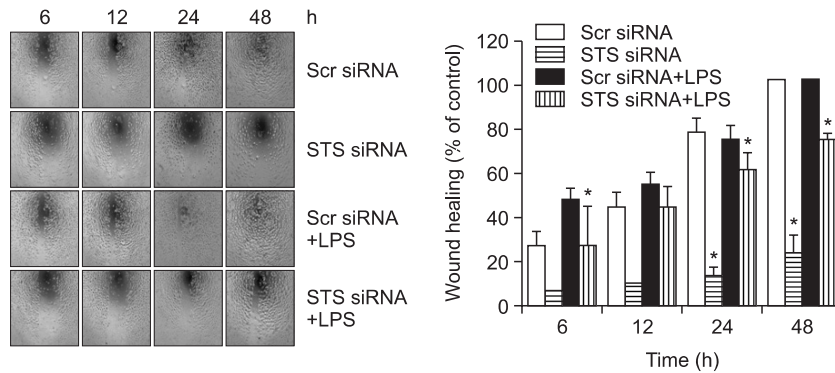


Fig. 3. LPS-induced STS expression enhances tumor cell migration in PC-3 cells. PC-3 cells were transiently transfected with STS siRNA (50 nM) for 48 h. After that, cells were incubated at 37°C with 10 µg/ml LPS for 24 h. An injury line was created, and the area devoid of cells was measured. The gap closure percentages were calculated as (area at 0-72 h)/(area at 0 h) and expressed as the mean ± S. E. of two separate experiments. **p*<0.05 compared with untreated control cells.

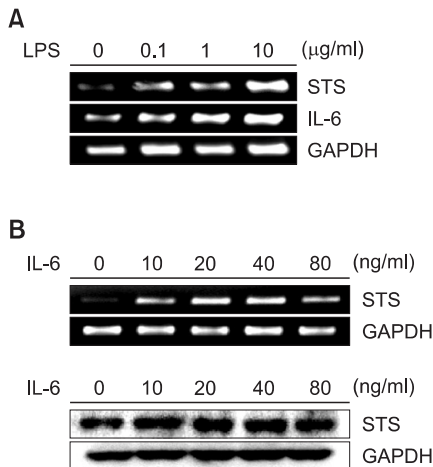


Fig. 4. IL-6 induces STS expression in PC-3 cells. (A) PC-3 cells were treated with various concentrations of LPS (0, 0.1, 1 or 10 µg/ml) for 24 h. Total RNA was isolated and human STS and IL-6 genes were amplified with specific primers. The PCR products were separated on a 2% agarose gel. Expression of GAPDH mRNA was determined as a RNA control. (B) PC-3 cells were treated with various concentrations of IL-6 (0, 10, 20, 40 or 80 ng/ml) for 16 h. Total RNA was isolated and human STS genes were amplified with specific primers. The PCR products were separated on a 2% agarose gel. Expression of GAPDH mRNA was determined as a RNA control. Total cellular lysates were prepared for Western blot analysis using antibody against STS. GAPDH was used as a loading control.

IL-6 involvement in LPS-induced STS expression in prostate cancer cells

To elucidate how LPS induce STS expression, we studied whether LPS-induced STS expression correlates with the IL-6 pathway. LPS enhance the release of inflammatory cytokines including IL-6. Previous report showed that various cytokines such IL-6 and IL-1β enhance the activity of STS (Honma *et al.*, 2002). We determined that LPS induced IL-6 and STS mRNA expression in PC-3 cells in concentration-dependent manner (Fig. 4A). To confirm that IL-6 induces STS expression, the mRNA and protein levels of STS were measured by RT-PCR and Western blot. PC-3 cells were grown for 16 h in the pres-

ence of increasing concentrations of IL-6 (0, 10, 20, 40 or 80 ng/ml). IL-6 increased levels of STS mRNA and protein in a concentration-dependent manner (Fig. 4B). These data indicated that released IL-6 by LPS treatment may transduce intracellular signal to induce STS expression.

DISCUSSION

STS has an important role in regulating estrogen biosynthesis that is required for tumor growth and survival within hormone-dependent cancers such as prostate and breast cancers. STS is the only well-known enzyme in human cancer cells that is capable to desulfate E1S and DHEAS as an important step in the conversion of these precursors to active forms (Nussbaumer and Billich, 2005). STS expression is increased in malignant breast tumors and has prognostic importance. STS immunoreactivity was strongly correlated with tumor size (Suzuki *et al.*, 2003). Research into the control and inhibition of this enzyme has been stimulated by its important role in supporting the growth of hormone-dependent tumors of the breast and prostate. STS is also known to be involved in many physiological functions, such as regulating part of the immune response and some aspects of cognitive function (Reed, 1995; Reed and Purohit, 1997).

The STS pathway is responsible for the production of various steroids with estrogenic properties, namely 5-androstenediol (Adiol), from DHEAS and subsequent reduction of DHEA by 17β-HSD1 (Purohit *et al.*, 2011; Purohit and Foster, 2012). Adiol, although an androgen, can bind to the ER and has been shown to stimulate the proliferation of a number of ER-positive breast cancer cells in an ER-dependent manner (Phan *et al.*, 2011). Unrestricted production of Adiol can occur via the STS pathway and may promote tumor progression.

In prostate cancer cells, STS expression and their possible roles associated with *in situ* estrogen metabolism are significant. Although increased level of STS was often observed in prostate and breast cancer cells, the direct stimulator of STS expression was not established.

LPS which are known to induce TNF-α stimulates STS expression in monocytes and macrophages (Schmidt *et al.*, 2000). LPS is viewed as critical stimuli in inflammation-associated cancer and promotes tumor metastasis (Lu *et al.*, 2006;

Finzi *et al.*, 2009). There were significantly increased lung metastases in animals that received an equivalent LPS injection. Triggering of TLR4 by LPS, induced tumor promotion by the induction of proliferation, activation of NF- κ B, p65 binding to DNA, and resistance to cytotoxicity accompanied by the increased production of proinflammatory cytokines (IL-6 and IL-8), VEGF, and GM-CSF (Bao *et al.*, 2011). These factors are known to promote the development of myeloid-derived suppressor cells as well as tumor progression.

In this study, we demonstrated whether human STS gene expression might be controlled by LPS. Our data clearly show that LPS are able to induce STS mRNA expression in time- and concentration-dependent manners. To elucidate LPS directly induce STS by activating STS promoter, we performed luciferase assay. LPS treatment showed ~1.5 fold higher luciferase activity than control.

LPS stimulate the lung adenocarcinoma A549 cell proliferation especially 24-hour treatment. The ratio of cells in G2/M cell cycle treated with LPS (100 ng/ml) was significant higher than non-treated groups (Hattar *et al.*, 2012). LPS induce macrophages to migrate from inflamed tissues to lymph nodes using the adhesion molecule Mac-1. LPS promote prostaglandins concomitant to eliciting macrophage migration (Tajima *et al.*, 2008). LPS enhanced the cell adhesion and migration of MDPC-23 cells, a process that requires TLR4, suggesting that TLR4 may play a pivotal role for tooth repair and regeneration (Park *et al.*, 2011). To elucidate whether LPS induces tumor cell migration in PC-3 cells, we performed wound healing assay. Suppression of migration by STS knockdown was recovered by LPS. These results indicated that LPS-induced STS expression increased tumor cell migration.

Innate immune responses triggered by LPS involve the coordinated production of a multitude of inflammatory mediators, especially IL-6 (Sawa *et al.*, 2008; Greenhill *et al.*, 2011). We studied that LPS-induced STS expression are mediated by IL-6 pathway. We confirmed that LPS induced IL-6 expression and IL-6 enhanced STS expression. Because multiple TLR ligands like LPS induce an IL-6 transcriptional response via STAT3 signal pathway, LPS mediated STS expression may be caused by TLR signal pathway (Mäkelä *et al.*, 2009; Walton *et al.*, 2009). The detailed mechanisms of LPS-induced STS expression need to be studied.

In summary, our present study suggests an important mechanism and function of STS expression in PC-3 cells. Our results imply that LPS induces STS expression and tumor cell migration through IL-6 pathway. Future studies need to be conducted to identify the effect of LPS-induced STS expression and to confirm whether LPS induces STS expression and tumor cell migration in other cancer cells and *in vivo*.

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REFERENCES

Ahmed, S., Owen, C. P., James, K., Sampson, L. and Patel, C. K. (2002) Review of estrone sulfatase and its inhibitors—an important new target against hormone dependent breast cancer. *Curr. Med.*

Chem. **9**, 263-273.

Aidoo-Gyamfi, K., Cartledge, T., Shah, K. and Ahmed, S. (2009) Estrone sulfatase and its inhibitors. *Anticancer Agents Med. Chem.* **9**, 599-612.

Bao, H., Lu, P., Li, Y., Wang, L., Li, H., He, D., Yang, Y., Zhao, Y., Yang, L., Wang, M., Yi, Q. and Cai, Z. (2011) Triggering of toll-like receptor-4 in human multiple myeloma cells promotes proliferation and alters cell responses to immune and chemotherapy drug attack. *Cancer Biol. Ther.* **11**, 58-67.

Chang, M. (2011) Dual roles of estrogen metabolism in mammary carcinogenesis. *BMB Rep.* **44**, 423-434.

Daynes, R. A., Dudley, D. J. and Araneo, B. A. (1990) Regulation of murine lymphokine production *in vivo*. II. Dehydroepiandrosterone is a natural enhancer of interleukin 2 synthesis by helper T cells. *Eur. J. Immunol.* **20**, 793-802.

Finzi, L., Shao, M. X., Paye, F., Housset, C. and Nadel, J. A. (2009) Lipopolysaccharide initiates a positive feedback of epidermal growth factor receptor signaling by prostaglandin E2 in human biliary carcinoma cells. *J. Immunol.* **182**, 2269-2276.

Greenhill, C. J., Rose-John, S., Lissiala, R., Ferlin, W., Ernst, M., Hertzog, P. J., Mansell, A. and Jenkins, B. J. (2011) IL-6 trans-signaling modulates TLR4-dependent inflammatory responses via STAT3. *J. Immunol.* **186**, 1199-1208.

Guha, M. and Mackman, N. (2001) LPS induction of gene expression in human monocytes. *Cell. Signal.* **13**, 85-94.

Härkönen, P. L. and Mäkelä, S. I. (2004) Role of estrogens in development of prostate cancer. *J. Steroid Biochem. Mol. Biol.* **92**, 297-305.

Hattar, K., Savai, R., Subtil, F. S., Wilhelm, J., Schmall, A., Lang, D. S., Goldmann, T., Eul, B., Dahlem, G., Fink, L., Schermuly, R. T., Banat, G. A., Sibellius, U., Grimminger, F., Vollmer, E., Seeger, W. and Grandel, U. (2012) Endotoxin induces proliferation of NSCLC *in vitro* and *in vivo*: role of COX-2 and EGFR activation. *Cancer Immunol. Immunother.* [Epub ahead of print]

Honma, S., Shimodaira, K., Shimizu, Y., Tsuchiya, N., Saito, H., Yanaiharu, T. and Okai, T. (2002) The influence of inflammatory cytokines on estrogen production and cell proliferation in human breast cancer cells. *Endocr. J.* **49**, 371-377.

Koh, E., Noda, T., Kanaya, J. and Namiki, M. (2002) Differential expression of 17 β -hydroxysteroid dehydrogenase isozyme genes in prostate cancer and noncancer tissues. *Prostate* **53**, 154-159.

Kriz, L., Biciková, M. and Hampl, R. (2008) Roles of steroid sulfatase in brain and other tissues. *Physiol. Res.* **57**, 657-668.

Li, H., Yuan, X., Tang, J. and Zhang, Y. (2012). Lipopolysaccharide disrupts the directional persistence of alveolar myofibroblast migration through EGF receptor. *Am. J. Physiol. Lung Cell. Mol. Physiol.* **302**, L569-579.

Lu, H., Ouyang, W. and Huang, C. (2006) Inflammation, a key event in cancer development. *Mol. Cancer Res.* **4**, 221-233.

Mäkelä, S. M., Strengell, M., Pietilä, T. E., Osterlund, P. and Julkunen, I. (2009) Multiple signaling pathways contribute to synergistic TLR ligand-dependent cytokine gene expression in human monocyte-derived macrophages and dendritic cells. *J. Leukoc. Biol.* **85**, 664-672.

Nakamura, Y., Suzuki, T., Nakabayashi, M., Endoh, M., Sakamoto, K., Mikami, Y., Moriya, T., Ito, A., Takahashi, S., Yamada, S., Arai, Y. and Sasano, H. (2005) In situ androgen producing enzymes in human prostate cancer. *Endocr. Relat. Cancer* **12**, 101-107.

Nussbaumer, P. and Billich, A. (2005) Steroid sulfatase inhibitors: their potential in the therapy of breast cancer. *Curr. Med. Chem. Anticancer Agents* **5**, 507-528.

Okada, H., Ohtsuka, H., Kon Nai, S., Kirisawa, R., Yokomizo, Y., Yoshino, T. and Rosol, T. J. (1999) Effects of lipopolysaccharide on production of interleukin-1 and interleukin-6 by bovine mammary epithelial cells *in vitro*. *J. Vet. Med. Sci.* **61**, 33-35.

Park, J. H., Kwon, S. M., Yoon, H. E., Kim, S. A., Ahn, S. G. and Yoon, J. H. (2011) Lipopolysaccharide promotes adhesion and migration of murine dental papilla-derived MDPC-23 cells via TLR4. *Int. J. Mol. Med.* **27**, 277-281.

Pasqualini, J. R. (2009) Breast cancer and steroid metabolizing enzymes: the role of progestogens. *Maturitas* **65 Suppl 1**, S17-21.

Phan, C. M., Liu, Y., Kim, B. M., Mostafa, Y. and Taylor, S. D. (2011) In-

- hibition of steroid sulfatase with 4-substituted estrone and estradiol derivatives. *Bioorg. Med. Chem.* **19**, 5999-6005.
- Purohit, A. and Foster, P. A. (2012) Steroid sulfatase inhibitors for estrogen- and androgen-dependent cancers. *J. Endocrinol.* **212**, 99-110.
- Purohit, A., Wang, D. Y., Ghilchik, M. W. and Reed, M. J. (1996) Regulation of aromatase and sulphatase in breast tumour cells. *J. Endocrinol.* **150 Suppl**, S65-71.
- Purohit, A., Woo, L. W. and Potter, B. V. (2011) Steroid sulfatase: a pivotal player in estrogen synthesis and metabolism. *Mol. Cell. Endocrinol.* **340**, 154-160.
- Reed, M. J. (1995) The discriminant-function test: a marker of Th1/Th2 cell cytokine secretion and breast tumour oestrogen synthesis. *Mol. Med. Today* **1**, 98-103.
- Reed, M. J. and Purohit, A. (1997) Breast cancer and the role of cytokines in regulating estrogen synthesis: an emerging hypothesis. *Endocr. Rev.* **18**, 701-715.
- Reed, M. J., Purohit, A., Woo, L. W., Newman, S. P. and Potter, B. V. (2005) Steroid sulfatase: molecular biology, regulation, and inhibition. *Endocr. Rev.* **26**, 171-202.
- Sawa, Y., Ueki, T., Hata, M., Iwasawa, K., Tsuruga, E., Kojima, H., Ishikawa, H. and Yoshida, S. (2008) LPS-induced IL-6, IL-8, VCAM-1, and ICAM-1 expression in human lymphatic endothelium. *J. Histochem. Cytochem.* **56**, 97-109.
- Schetter, A. J., Heegaard, N. H. and Harris, C. C. (2010) Inflammation and cancer: interweaving microRNA, free radical, cytokine and p53 pathways. *Carcinogenesis* **31**, 37-49.
- Schmidt, M., Kreutz, M., Löffler, G., Schölmerich, J. and Straub, R. H. (2000) Conversion of dehydroepiandrosterone to downstream steroid hormones in macrophages. *J. Endocrinol.* **164**, 161-169.
- Stanway, S. J., Delavault, P., Purohit, A., Woo, L. W., Thuriereau, C., Potter, B. V. and Reed, M. J. (2007) Steroid sulfatase: a new target for the endocrine therapy of breast cancer. *Oncologist.* **12**, 370-374.
- Suzuki, T., Miki, Y., Nakamura, Y., Moriya, T., Ito, K., Ohuchi, N. and Sasano, H. (2005) Sex steroid-producing enzymes in human breast cancer. *Endocr. Relat. Cancer* **12**, 701-720.
- Suzuki, T., Nakata, T., Miki, Y., Kaneko, C., Moriya, T., Ishida, T., Akina-ga, S., Hirakawa, H., Kimura, M. and Sasano, H. (2003) Estrogen sulfotransferase and steroid sulfatase in human breast carcinoma. *Cancer Res.* **63**, 2762-2770.
- Tajima, T., Murata, T., Aritake, K., Urade, Y., Hirai, H., Nakamura, M., Ozaki, H. and Hori, M. (2008) Lipopolysaccharide induces macrophage migration via prostaglandin D(2) and prostaglandin E(2). *J. Pharmacol. Exp. Ther.* **326**, 493-501.
- Utsumi, T., Yoshimura, N., Takeuchi, S., Maruta, M., Maeda, K. and Harada, N. (2000) Elevated steroid sulfatase expression in breast cancers. *J. Steroid Biochem. Mol. Biol.* **73**, 141-145.
- Walton, K. L., Holt, L. and Sartor, R. B. (2009) Lipopolysaccharide activates innate immune responses in murine intestinal myofibroblasts through multiple signaling pathways. *Am. J. Physiol. Gastrointest. Liver Physiol.* **296**, G601-611.
- Wang, J. H., Manning, B. J., Wu, Q. D., Blankson, S., Bouchier-Hayes, D. and Redmond, H. P. (2003) Endotoxin/lipopolysaccharide activates NF-kappa B and enhances tumor cell adhesion and invasion through a beta 1 integrin-dependent mechanism. *J. Immunol.* **170**, 795-804.
- Wilson, J. W., Schurr, M. J., LeBlanc, C. L., Ramamurthy, R., Buchanan, K. L. and Nickerson, C. A. (2002) Mechanisms of bacterial pathogenicity. *Postgrad. Med. J.* **78**, 216-224.
- Wright, R. M., Holladay, C. S. and Spangelo, B. L. (1993) Lipopolysaccharide induces interleukin-6 release from rat peritoneal macrophages in vitro: evidence for a novel mechanism. *Circ. Shock* **41**, 131-137.