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Cross-Talk between Signal Transducer and Activator of Transcription 3 and Androgen Receptor Signaling in Prostate Carcinoma Cells

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Interleukin 6 (IL-6) plays important roles in the immune system, hematopoiesis, as well as the growth of various tumors. Androgens are important in the initiation and progression of prostate cancer and their effects are mediated by androgen receptor (AR). Here we present a molecular mechanism for the effects of IL-6 on prostate cancer cells through a cross-talk between IL-6 and AR signaling pathways. IL-6-induced activation of signal transducer and activator of transcription 3 (STAT3) was augmented by AR in the presence of dihydrotestosterone (DHT). In addition, DHT treatment augmented endogenous STAT3-mediated gene expression by IL-6. Conversely, DHT-induced AR activity was increased by IL-6, and a dominant negative form of STAT3 inhibited AR activation. In contrast, DHT-mediated enhancement of STAT3 activation was inhibited by flutamide, an AR antagonist. We provide evidence that these activities are due to direct physical interactions between STAT3 and AR in prostate cancer cells.

Key Words: **IL-6; androgen receptor (AR); signal transducer and activator of transcription 3 (STAT3); cross-talk; coactivator.**

Interleukin-6 (IL-6) is a pleiotropic cytokine that regulates immune and inflammatory responses (1, 2). IL-6 also acts as a growth regulator in many malignant tumors, including prostate carcinoma (3, 4). Both IL-6 and IL-6 receptor are expressed in prostate carcinoma cell lines (3). IL-6 also stimulates the growth of prostate carcinoma cells as a paracrine or autocrine growth factor (4). The receptors for the IL-6 family of cytokines share the gp130 molecule through which signals are generated, although the cytoplasmic region of gp130 does not contain any catalytic domain. Instead, the Janus kinase (Jak) family of protein kinases constitutively associate with gp130 and are activated by the IL-6 family of cytokines (5), leading to the tyrosine-phosphorylation and activation of signal transducer and activator of transcription (STAT) family of transcription factors.

One member of the STAT family of proteins is STAT3 which is mainly activated by IL-6 family of cytokines, epidermal growth factor, and leptin (2, 5). Like other members of the STAT family, STAT3 is tyrosine-phosphorylated by Jak kinases, then forms a dimer and translocates into the nucleus to activate target genes (6, 7). It has been shown that the activated STAT3 can mediate cellular transformation (8, 9) and constitutively active STAT3 is found in bone marrow mononuclear cells from patients with multiple myelomas (10).

Androgen receptor (AR) mediates the effects of androgens and plays a central role in prostate cancer progression (11). In the beginning stages of the disease, the growth of prostate cancer is dependent on androgens. This is the basis for androgen ablation therapy which results in the involution of the tumor (12). However, in most cases, it progresses to an androgen-independent phenotype at which time there is no curative therapy available (13).

AR activates transcription through interaction with androgen response elements (AREs) that are in the vicinity of the androgen responsive genes, such as prostate specific antigen (PSA), tissue kallikrein 4 (KLK4), and probasin (PB) (11). Recent studies have docu-

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mented that various signaling cascades, such as those initiated by ErbB2 and IL-6, can also activate AR and the transcription of its target genes (14–16). In addition, a recent report demonstrated that IL-6 activates AR-mediated gene expression through STAT3 (17).

In this study, we have examined the molecular basis for the cross-talk between IL-6 and AR signaling cascades and found that it occurs by direct physical and functional interactions between STAT3 and AR in prostate cancer cells.

MATERIALS AND METHODS

Reagents and antibodies. Human recombinant IL-6 was a kind gift from Ajinomoto (Tokyo, Japan). Human recombinant LIF was purchased from INTERGEN (Purchase, NY). Dihydrotestosterone (DHT) was purchased from Wako Chemicals (Osaka, Japan). Flutamide was purchased from Sigma (St. Louis, MO). HA-tagged STAT3 or Jak1, -285PB-LUC (18), STAT3-LUC (19), FLAG-tagged STAT3 and DN-STAT3 in pEFBOS (20), C/EBPδ cDNA (21) and STAT3-C (8) were kindly provided by Dr. J. N. Ihle (St. Jude SRH, Memphis, TN), Dr. J. Palvimo (University of Helsinki, Finland), Dr. T. Hirano (Osaka Univ., Osaka, Japan), Dr. S. Akira (Osaka Univ., Osaka, Japan), and Dr. J. F. Bromberg (Rockefeller Univ., New York, NY), respectively. C-terminal deletion mutants of AR have previously been described (22). Anti-HA, anti-STAT3 and anti-AR antibodies were purchased from Santa Cruz Biotechnology (Santa Cruz). Anti-FLAG M2 was purchased from Upstate Biotechnology (Lake Placid, NY).

Cell culture, transfections, and luciferase assays. The human prostate carcinoma cell line LNCaP was maintained as described previously (23). Before stimulation, the cells were cultured for 24 h in RPMI 1640 containing 2% TCM (ICN) followed by treatment with IL-6 and/or DHT (23). LNCaP cells ($2–2.5 \times 10^5$ in a 6-cm dish) were transfected by using LipoTAXI (Stratagene) following the manufacturer's instructions. 293T cells were transfected in DMEM containing 1% FCS by the standard calcium precipitation protocol. Luciferase assay was performed as described (24). The cells were harvested 48 h after transfection and lysed in 200 μl of PicaGene Reporter Lysis Buffer (Tokyo Ink, Tokyo, Japan) and assayed for luciferase and β-galactosidase activities according to the manufacturer's instructions. Luciferase activities were normalized to the β-galactosidase activities.

Northern blot analysis. LNCaP cells were maintained as described above. After serum starvation, cells (1×10^7) were treated with IL-6 (100 ng/ml) and/or DHT (10^{-8} M) for 3 or 6 h. Total RNAs were prepared by using Iso-Gen (Nippon Gene) and used in Northern analysis according to the established procedures. A nylon membrane (Hybond N^+, Amersham Pharmacia Biotech) and radiolabelled cDNA probes were used, where indicated.

Immunoprecipitation and immunoblotting. The immunoprecipitation and Western blotting were performed as described previously (24). Cells were harvested and lysed in lysis buffer (50 mM Tris–HCl, pH 7.4, 0.15 M NaCl, containing 0.5% NP-40, 1 μM sodium orthovanadate, 1 μM phenylmethylsulfonyl fluoride and 10 μg/ml each of aprotinin, pepstatin and leupeptin).

The immunoprecipitates from cell lysates were resolved on 5–20% SDS–PAGE and transferred to Immobilon filter (Millipore, Bedford, MA). The filters were then immunoblotted with each antibody. Immunoreactive proteins were visualized using an enhanced chemiluminescence detection system (Amersham Pharmacia Biotech).

RESULTS AND DISCUSSION

Androgens Stimulate IL-6-Induced Gene Expression in LNCaP Cells

To examine the molecular basis of the cross-talk between IL-6 and androgen signaling pathways in prostate cancer cells, we first assessed changes in tyrosine-phosphorylation of STAT3, which trigger its activation, in LNCaP cells. To that end, LNCaP cells were either left untreated or treated with IL-6, and their cell extracts were prepared and subjected to immunoprecipitation using an anti-STAT3 antibody. The immunoprecipitates were then used in Western analysis with an antibody against phospho-tyrosine residues. As shown in Fig. 1A, STAT3 was tyrosine-phosphorylated by IL-6 treatment in LNCaP cells (Fig. 1A). There were similar amounts of STAT3 present in extracts prepared from untreated cells compared with IL-6-treated cells. These data show that IL-6 treatment results in characteristic STAT3 activation in LNCaP cells.

To examine whether androgens have any effect on IL-6-induced transcriptional activation of cellular genes, we carried out Northern analysis on RNA samples prepared from LNCaP cells which were induced by IL-6 and/or androgens. As a target for IL-6/STAT3-mediated gene expression, we analyzed the expression of C/EBPδ (CCAAT/enhancer binding protein δ) which is a regulator of acute-phase response genes in hepatocytes (25). As shown in Fig. 1B, IL-6 induced C/EBPδ expression in LNCaP cells which was dramatically increased in the presence of dihydrotestosterone (DHT), whereas DHT alone did not induce C/EBPδ expression. These data suggest that DHT potentiates IL-6-induced gene expression.

Conversely, to assess whether IL-6 has an effect on androgen regulated gene expression, we examined the expression of KLK4/ARM-1, a recently discovered androgen-regulated gene that belongs to the serine protease gene family (26). The same blot used above in the experiment with C/EBPδ was stripped and reprobed with a KLK4/ARM1 cDNA probe. As shown in Fig. 1B, DHT induced KLK4/ARM-1 mRNA accumulation, but IL-6 alone did not have any effect. In the presence of both DHT and IL-6, KLK4/ARM1 mRNA accumulation was increased approximately twofold compared with DHT alone, whereas there was no significant change in the expression of the housekeeping gene G3PDH. These results suggest that IL-6 potentiates androgen regulated gene expression *in vivo.*

Synergistic Transcriptional Activation by IL-6 and DHT in LNCaP Cells

We then measured the effects of IL-6 and DHT on the activities of STAT3 and AR in a transient transfec-

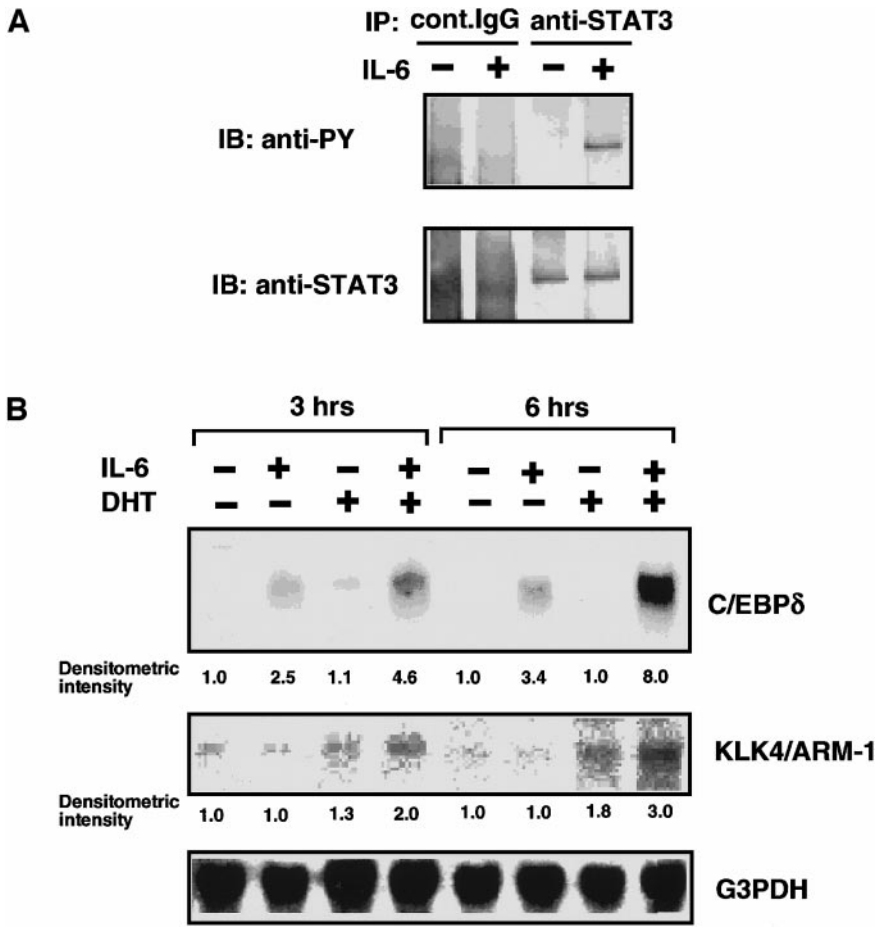

FIG. 1. IL-6-induced tyrosine phosphorylation of STAT3 and synergistic effect of IL-6 and DHT on induction of C/EBPδ and KLK/ARM-1 in LNCaP cells. (A) IL-6-induced tyrosine phosphorylation of STAT3. LNCaP cells (1×10^7) were stimulated with IL-6 (100 ng/ml). Cell lysates were immunoprecipitated with control IgG or anti-STAT3 and immunoblotted with anti-phosphotyrosine antibody (upper panel). The blot was stripped and reprobed with anti-STAT3 (lower panel). (B) Induction of C/EBPδ (upper panel) and KLK4/ARM-1 (middle panel) mRNAs by IL-6 and/or DHT in LNCaP cells. Northern blot analysis of 20 μg of total RNA from LNCaP cells treated with IL-6 (100 ng/ml) and/or DHT (10^{-8} M) for the indicated time. The fold induction of C/EBPδ and KLK4/ARM-1 expression was shown as the densitometric intensity. Glyceraldehyde-3-phosphate dehydrogenase (G3PDH) mRNA is included as a loading control (lower panel).

tion assay. The STAT3-mediated transcriptional responses were measured by using STAT3-LUC, in which the α2-macroglobulin promoter (19) drives expression of the LUC gene. AR activity was monitored by using −285PB-LUC in which a deletion derivative of the probasin promoter drives expression of the LUC gene (18). First, LNCaP cells were transfected with STAT3-LUC and treated with increasing amounts of IL-6 and/or DHT. As shown in Fig. 2A, IL-6 stimulated STAT3-mediated transcription in a dose-dependent manner, whereas DHT did not affect STAT3-LUC activity. When cells were treated with both IL-6 and DHT, 50–60% higher activity was observed compared with that observed with IL-6 alone. These data are consistent with the findings on the activation of endogenous C/EBPδ expression (Fig. 1B).

To assess whether the observed effects were mediated through STAT3 or some other intermediary factors, we used a dominant negative form of STAT3 (DN-STAT3) (20). As expected, DN-STAT3 significantly inhibited IL-6-induced STAT3-LUC expression in a dose-dependent fashion (Fig. 2B). Similarly, DN-STAT3 down-regulated STAT3-LUC expression induced by IL-6 and DHT. These data suggest that the effect of DHT on STAT3-LUC activity is mediated by STAT3.

To assess whether AR may be directly involved in DHT-induced STAT3 activation, we utilized the antiandrogen flutamide that binds to and inhibits AR (27). LNCaP cells were transfected with STAT3-LUC and treated with IL-6 in the absence or presence of DHT and/or flutamide. DHT-induced STAT3 activation was suppressed in the presence of flutamide (Fig. 2C). These results suggest that DHT-induced increase in STAT3-LUC activity is directly mediated by AR in LNCaP cells.

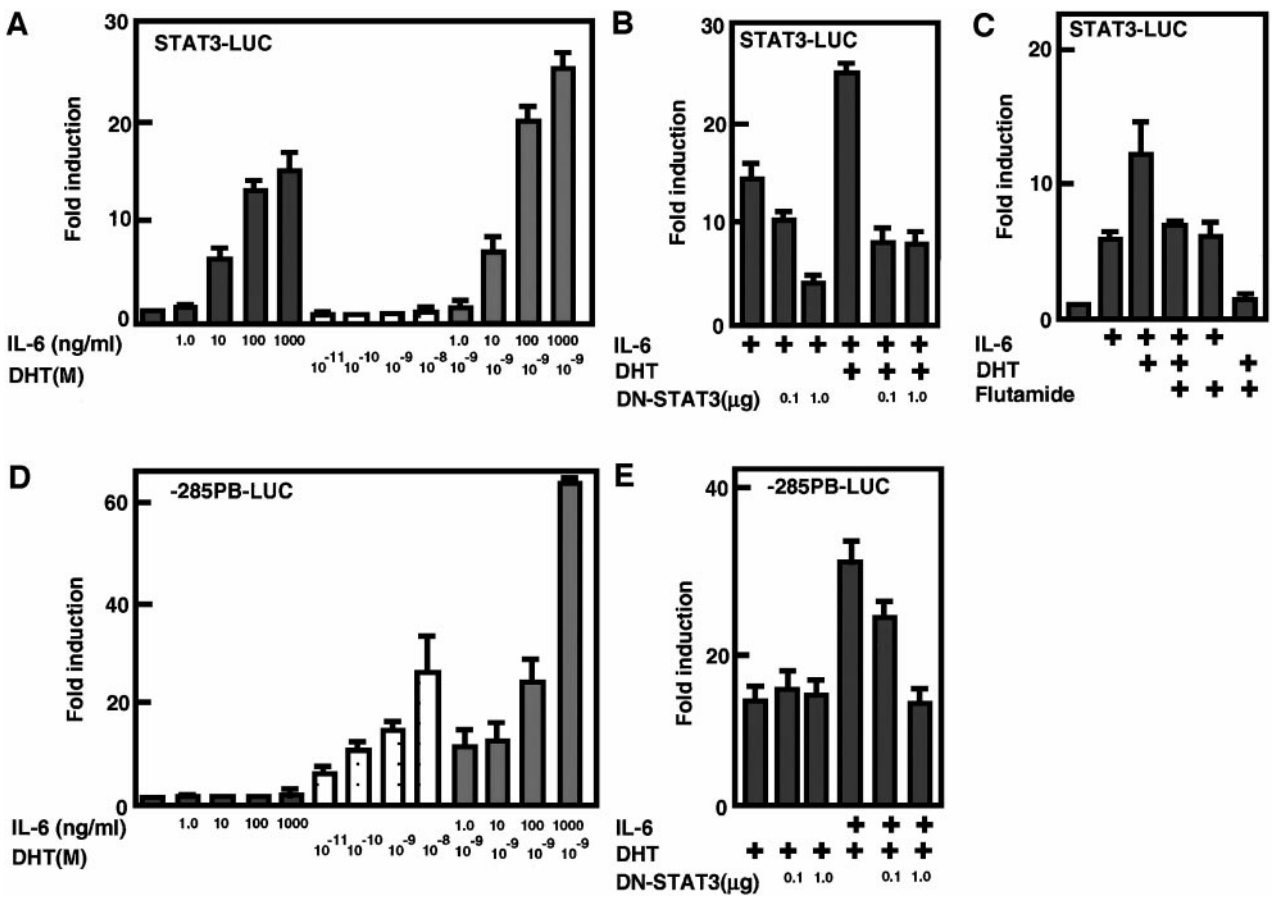

FIG. 2. Synergistic transcriptional activation between IL-6 and DHT in LNCaP cells. LNCaP cells in 6-cm dishes were transfected with STAT3-LUC (1 μg) (A, B, C) or −285PB-LUC (1 μg) (D, E) together with either DN-STAT3 or the pEFBOS empty vector (B, E). Forty-eight hours after transfection, cells were stimulated for an additional 12 h with various concentrations of IL-6 and/or DHT (A, D), 100 ng/ml of IL-6 and 10^{-9} M of DHT with or without DN-STAT3 (B, E), with or without IL-6 (100 ng/ml), DHT (10^{-9} M), and flutamide (10^{-5} M) (C). Cells were harvested and relative luciferase activities were measured. The results are presented as fold induction of luciferase activity from triplicate experiments, and the error bars represent the standard deviations.

We also assessed whether IL-6 has any effect on AR transcriptional activity using the androgen dependent reporter construct −285PB-LUC. LNCaP cells were transfected with −285PB-LUC, and then treated with increasing amounts of IL-6 and/or DHT, and LUC activities were determined. As shown in Fig. 2D, DHT alone stimulated AR-mediated transcription in a dose-dependent manner, whereas IL-6 did not affect −285PB-LUC activity. When cells were treated with both IL-6 and DHT, there was an approximately two-fold higher activity of −285PB-LUC in the presence of IL-6 compared with that observed with DHT alone.

These results suggested that the IL-6 signaling pathway may increase AR transcriptional activity. To assess if STAT3 is involved in this process, we again used DN-STAT3 (20). LNCaP cells were transfected with −285PB-LUC, either alone or in combination with DN-STAT3. Cells were then either treated with DHT alone or DHT plus IL-6 and −285PB-LUC activity was determined. As shown in Fig. 2E, ectopic expression of DN-STAT3 inhibited IL-6-induced enhancement of −285PB-LUC activity.

Cross-Talk between STAT3 and AR Signaling Pathways in 293T Cells

Previous studies have shown that p300/CBP is involved in STAT3- or AR-mediated transcriptional activation (22, 28, 29). To avoid any effects of endogenous p300/CBP and to further delineate the details of the cross-talk between STAT3 and AR signaling pathways, co-transfection assays were performed in the adenovirus transformed embryonic kidney carcinoma cell line 293T, in which endogenous E1A suppresses p300/CBP (30). 293T cells were transfected with STAT3-LUC with or without an expression vector for AR and cells were stimulated with increasing amounts of Leukaemia Inhibitory Factor (LIF) in the absence or presence of DHT. We utilized LIF instead of IL-6 to activate STAT3-LUC in 293T cells, because LIF was shown to

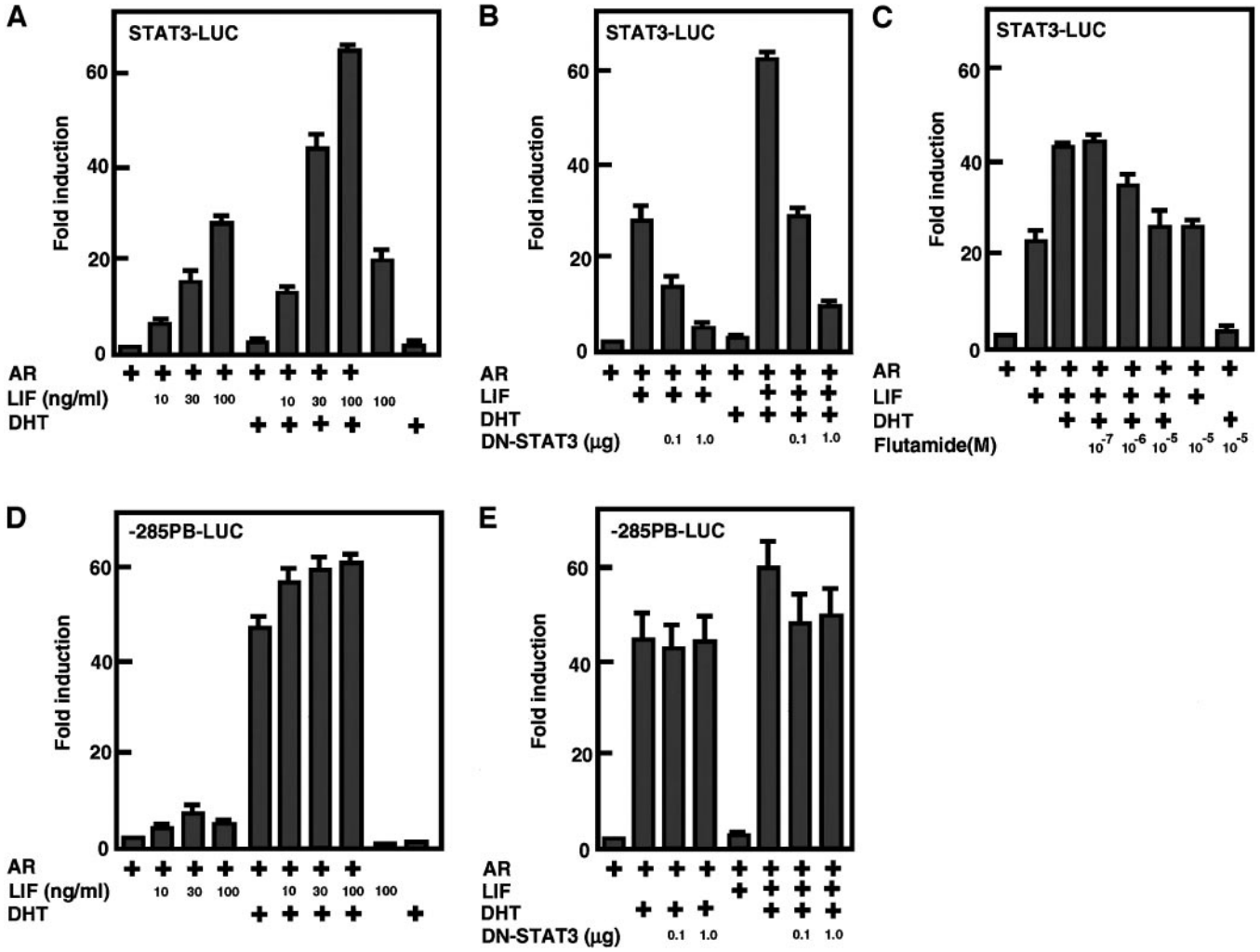

FIG. 3. Reconstitution of cross-talk between STAT3 and AR signaling in 293T cells. 293T cells were transfected with STAT3-LUC (1 μg) (A, B, C) or −285PB-LUC (1 μg) (D, E) together with AR expression construct (1 μg) or the empty pSG5 (1 μg), and/or various dose of DN-STAT3 (B, E), 48 h after transfection, cells were stimulated for 12 h with various concentration of LIF and/or DHT (10^{-9} M) (A, D), or 100 ng/ml of LIF and/or 10^{-9} M of DHT (B, E), or with or without LIF (100 ng/ml) and/or DHT (10^{-9} M), and/or increasing amounts of flutamide as indicated (C), and relative luciferase activities were measured. The results are presented as fold induction of luciferase activity from triplicate experiments, and the error bars represent the standard deviations.

stimulate STAT3-LUC more effectively than IL-6 in these cells (data not shown). As shown in Fig. 3A, STAT3-LUC activity was augmented by increasing amounts of LIF, whereas DHT alone did not have an effect. In the presence of AR, STAT3-LUC was enhanced approximately twofold when cells were simultaneously treated with LIF and DHT. These results indicate that cross-talk between AR and IL-6 signaling occurs in 293T cells similar to that observed in LNCaP cells, suggesting that this process is not mediated by CBP/p300.

To assess whether the increase induced by DHT on STAT3-LUC activity is mediated by STAT3, we utilized DN-STAT3 as in LNCaP cells (Fig. 2B). 293T cells were transfected as described in Fig. 3A in the presence or absence of DN-STAT3. As shown in Fig. 3B, ectopic expression of DN-STAT3 inhibited DHT-induced increase in STAT3-LUC activation in a dose-dependent manner, indicating that it requires intact STAT3.

We then assessed the reverse situation for the effects of STAT3 on AR activity in 293T cells, using −285PB-LUC as a reporter gene. When AR was expressed in 293T cells, DHT strongly increased −285PB-LUC activity (Figs. 3D and 3E). DHT-induced −285PB-LUC activity was modestly augmented by LIF in 293T cells, whereas LIF alone did not affect reporter activity (Fig. 3D).

To assess whether STAT3 is involved in −285PB-LUC activation in 293T cells, we utilized DN-STAT3.

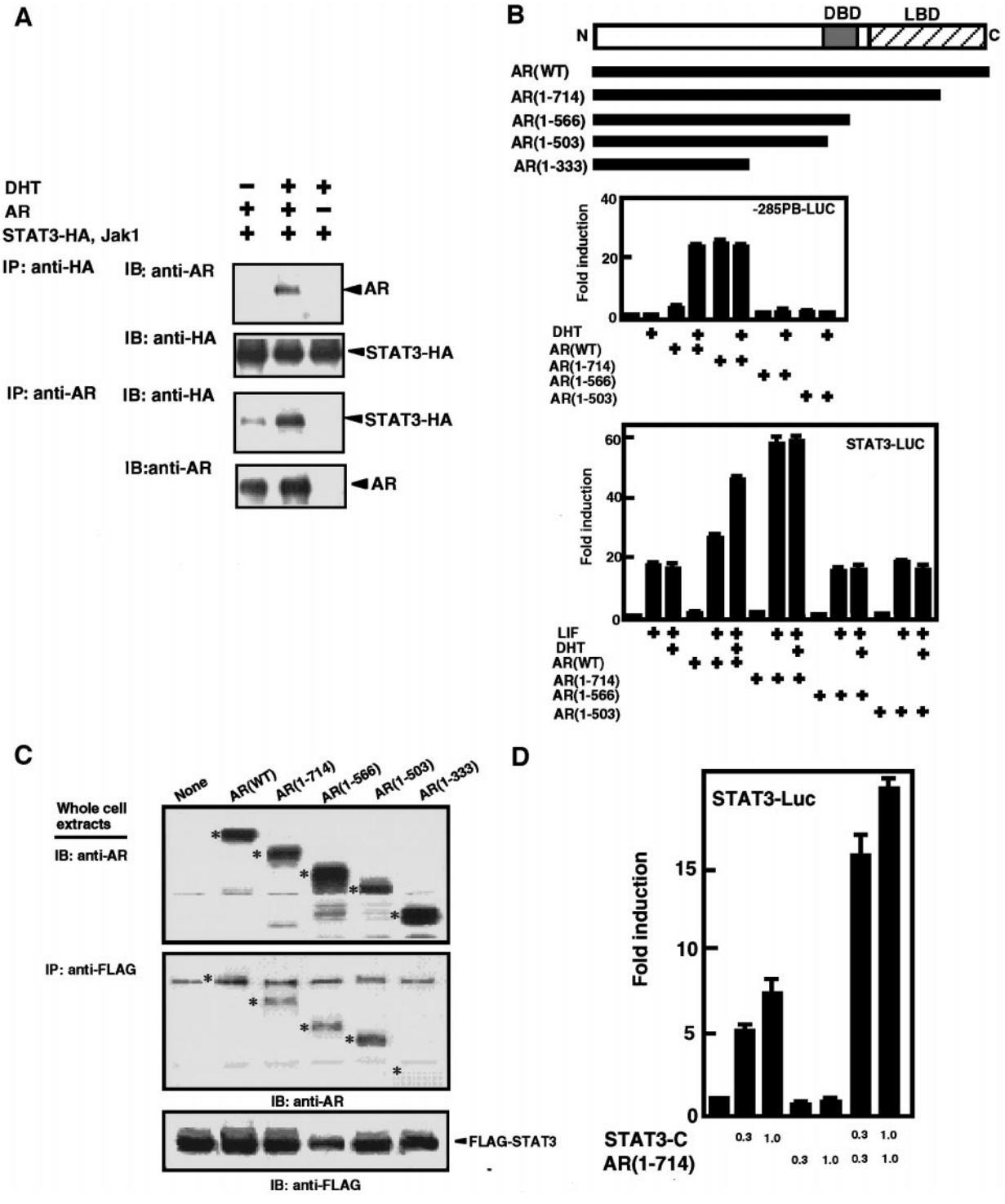

FIG. 4. Interactions between STAT3 and AR. (A) 293T cells (2×10^7) were transfected with AR (10 μg) and HA-tagged STAT3 (5 μg) together with Jak1 (1 μg). Forty-eight hours after transfection, cells were treated with or without DHT (10^{-8} M) for 12 h. Cell lysates were then immunoprecipitated and immunoblotted with anti-HA or anti-AR as indicated. (B) Domain structure of AR and mutant fragments are schematically shown. DBD and LBD indicate DNA-binding domain and the ligand-binding domain, respectively. 293T cells were transfected with STAT3-LUC (1 μg) (upper panel) or −285PB-LUC (1 μg) (lower panel) either in the presence of empty pSG5 (1 μg), wild-type AR (1 μg), or one of the mutants of AR (1 μg) as indicated. Forty-eight hours after transfection, cells were either left untreated or treated with LIF (100 ng/ml) and/or DHT (10^{-9} M), and relative luciferase activities were measured. The results are presented as fold induction of luciferase activity from triplicate experiments, and the error bars represent the standard deviations. (C) Mapping the STAT3 interaction domain of AR. 293T cells were transfected with a series of AR mutants (10 μg) together with wild-type FLAG-STAT3 (5 μg) and Jak1 (1 μg) by calcium

As shown in Fig. 3E, ectopic expression of DN-STAT3 did not significantly change the DHT-induced activation of −285PB-LUC, suggesting that STAT3 is not required for this activity in 293T cells.

To further assess the specificity of AR/DHT function on STAT3 activation 293T cells, we utilized the anti-androgen, flutamide. STAT3-LUC/AR-transfected into 293T cells were treated with LIF in the absence or presence of DHT and/or flutamide. As shown in Fig. 3C, the effect of AR/DHT on STAT3 activation was suppressed by flutamide when cells were treated with increasing concentrations of flutamide. These results indicate that the effect of DHT on STAT3 activation in 293T cells is mediated by AR. Interestingly, the cross-talk between STAT3 and either retinoic acid receptor (RAR) or 1α,25-dihydroxy vitamin D3 receptor (VDR) were not observed in 293T cells (31), suggesting that the potentiation of STAT3 activation in 293T cells is not a general phenomenon for nuclear receptors, but is highly specific for AR.

STAT3 and AR Physically Interact in Vivo

The data described above suggested that there may be direct physical interactions between AR and STAT3. We tested this possibility by coimmunoprecipitation experiments. Expression vectors encoding wild-type AR and Hemagglutinin A epitope (HA)-tagged STAT3 were transiently transfected into 293T cells together with Jak1. After DHT stimulation, cells were lysed and subjected to immunoprecipitation with either an anti-AR monoclonal antibody or anti-HA antibody. Immunoprecipitates were then used in Western analysis. As shown in Fig. 4A, AR and STAT3 were found to be in a complex regardless of which one was immunoprecipitated first. Interestingly, STAT3-AR interactions were substantially increased in the presence of DHT (Fig. 4A). Similar results were obtained in co-immunoprecipitation experiments using LNCaP cells lysates (data not shown).

To delineate the sites in the AR that mediate the protein-protein interactions between STAT3 and AR, coimmunoprecipitation experiments were performed with a series of mutant AR proteins. As shown in Fig. 4B, wild-type AR and AR(1–714) are efficient activators of DHT-induced −285PB-LUC activation, whereas the deletion mutants AR(1–566) and AR(1–503) did not significantly stimulate −285PB-LUC in 293T cells, because they lack a functional DNA-binding domain (DBD). The AR(1–714) deletion mutant increased the basal level of expression of −285PB-LUC even in the absence of DHT, which did not change in response to DHT. These data are in agreement with our previous findings in PC3 cells (22). In parallel with AR activation, both wild-type AR and AR(1–714), but not AR(1–566) and AR(1–503), stimulated STAT3 activation by LIF. Coimmunoprecipitation experiments using these deletion mutant AR revealed that AR deficient in LBD alone, or LBD plus DBD could still interact with STAT3. In contrast, AR(1–333), which encodes only part of the N-terminal domain, lost the ability to interact with STAT3 (Fig. 4C). These data suggest that an intact N-terminus is required for AR to interact with STAT3.

A Constitutively Active AR Augments STAT3 Promoter Activation by an Active Form of STAT3

Several studies demonstrated that IL-6 stimulates AR-mediated gene expression in prostate carcinoma cells via STAT3 as well as ErbB2 (14–17). To show the direct effect of AR on potentiation of STAT3 activation, we used a constitutively active form of STAT3, STAT3-C (8), and constitutive active AR(1–714) as described in Fig. 4B. 293T cells were transfected with STAT3-LUC together with expression vectors for STAT3-C and/or AR(1–714), and the LUC activities were measured. As shown in Fig. 4D, STAT3-LUC activity was stimulated by STAT3-C, whereas AR(1–714) alone did not affect this activity. However, AR(1–714) markedly augmented STAT3-LUC activition by STAT3-C. These results indicate the presence of a direct cross-talk between STAT3-C and AR(1–714) on STAT3 activation in 293T cells.

CONCLUSIONS

We have shown here that AR potentiates IL-6 signaling mediated by STAT3 in prostate cancer cells and that active AR directly associates with and acts as a transcriptional coactivator for STAT3. DHT treatment augmented endogenous STAT3-mediated C/EBPδ gene expression in LNCaP cells by IL-6 as well as STAT3-dependent reporter activity in LNCaP and 293T cells. Potentiation of STAT3 activation by DHT/AR was suppressed by the anti-androgen, flutamide. Conversely, the increase in AR activity by IL-6 was blocked in the

precipitation method. Forty-eight hours after transfection, cells were lysed and immunoprecipitated with anti-FLAG M2. Total cell lysates (20 μg) (upper panel) and the immunoprecipites with anti-FLAG M2 (middle panel) were blotted with anti-AR. The blot was stripped and reprobed with anti-FLAG M2 (lower panel). The asterisks indicate the migration position of the wild-type AR or deletion mutants. (D) 293T cells were transfected with STAT3-LUC (1 μg) together with STAT3-C and/or AR(1–714) expression construct (1 μg) or control vector (1 μg). Forty-eight hours after transfection, cells were harvested and relative luciferase activities were measured. The results are presented as fold induction of luciferase activity from triplicate experiments, and the error bars represent the standard deviations.

presence of dominant negative STAT3. Our results indicate that STAT3 associates with AR in an androgen-dependent manner and the amino terminal domain of AR plays an essential role in STAT3-AR interaction. Recently, we demonstrated that there is a physical interaction between a STAT3 inhibitor, PIAS3 (32), and AR under high stringency conditions when these two molecules were expressed in 293T cells (33). This suggests that endogenous PIAS3 in these cell lines is involved in the interaction of STAT3 and AR. However, such an involvement by PIAS3 does not rule out the potentiation of STAT3 by AR activation, because PIAS3 inhibits the activation of both STAT3 and AR.

A recent report suggested that IL-6 activates AR-mediated gene expression through STAT3 and STAT3 associates with AR in an androgen-independent, but IL-6-dependent manner in LNCaP cells (17). In contrast, our coimmunoprecipitation experiments in ectopically expressed proteins in 293T cells show that AR-STAT3 interactions are highly dependent on androgens. We do not know the basis of this difference. However, the use of different cell lines and source of proteins may be responsible; further work is required to find the reasons for these different results.

Moreover, STAT3 activation enhanced AR-mediated transcription in LNCaP cells as previously described (22), but not in 293T cells. In LNCaP cells, IL-6 may stimulate AR-mediated transcription via other signaling pathways, such as the MAPK cascade modulation of AR activity (34).

It was previously reported that the interaction between STAT3 and GR enhances GR-mediated transcription but not STAT3-mediated transcription, suggesting that STAT3 is a transcriptional coactivator for GR, but not vice versa (35). However, our results suggest another form of interaction between STAT3 and AR in prostate cancer cells which leads to the synergistic transactivation at the STAT3-dependent promoters.

Identification of this novel form of cross-talk between STAT3 and AR in prostate cancer cells may be clinical implications on the progression of androgen- or hormone-sensitive tumors. STAT3 is activated by a variety of growth factors other than IL-6, such as platelet-derived growth factor, epidermal growth factor, growth hormones, and leptin (6, 7). It has been also shown that STAT3 is activated by the ErbB receptor (36). In recent studies, IL-6 was shown to induce tyrosine phosphorylation of ErbB2 receptor via an IL-6 signal transducer, gp130, in prostate carcinoma cells (15). Moreover, ErbB2 is shown to enhance AR activity in prostate carcinoma cells (16). Taken together, IL-6 may play an important role on STAT3 activation via ErbB2 and AR in prostate carcinoma cells.

Conversely, an important way to regulate AR function is thought its interaction with other transcriptional factors. In this regard, the interaction with STAT3 is significant since STAT3 has been recently shown to act as an oncoprotein. Using dominant negative STAT3 molecules, it was found that the v-*src*- or Q205L $G\alpha_o$-mediated cell transformation required STAT3 activation (8, 9). Furthermore, it has been demonstrated that an engineered constitutive active STAT3 acts as a sole transforming agent (8). The existence of constitutive active form of STAT3 was also demonstrated in bone marrow mononuclear cells from patients with multiple myelomas (10). Thus, similar to these examples, STAT3 may be an important regulator of AR function and thereby may have important roles in the progression of prostate cancer. Further understanding of the cross-talk between STAT3 and AR is therefore important as this new information may provide new therapeutic approaches for prostate cancer.

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