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# Involvement of Interleukin-6 and Androgen Receptor Signaling in Pancreatic Cancer

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**Abstract**

Pancreatic cancer remains one of the "difficult-to-treat" cancers. Signaling of androgen receptor (AR), one of the nuclear receptors, in the pancreas may be related to carcinogenesis. Higher interleukin-6 (IL-6) levels have been observed in pancreatic cancer patients. It is also well known that IL-6 affects the AR signaling pathway and that AR is important for cell migration activities. We demonstrated that IL-6 enhances the phosphorylation of STAT3 and MAPK, which in turn enhances AR-mediated transcription in pancreatic cancer cell lines. This activity was blocked by a pharmacological inhibitor of the JAK/STAT signaling pathway, AG490, and one of the MAPK signaling pathways, U0126. IL-6 also enhances pancreatic cancer cell migration in the presence of AR. This activity is blocked by AR-siRNA. IL-6 acts as a positive regulator in AR signaling, providing further insight into the progression of pancreatic carcinogenesis.

**Keywords**

androgen receptor, STAT3, IL-6, pancreatic cancer

## Introduction

Pancreatic cancer represents a major health problem worldwide, showing steadily increasing incidence rates. Pancreatic cancer, although infrequent, has an exceptionally high mortality rate, making it 1 of the 4 or 5 most common causes of cancer mortality in developed countries.[1] As examples, it is the fifth, fourth, and third most common cause of cancer mortality in Japan, the United States, and Israel, respectively.[2-5] Despite the high mortality rate associated with pancreatic cancer, its etiology is poorly understood. In Japan, the mortality rate of pancreatic cancer in men is higher than in women. On average, women live longer than men, so the mortality number of pancreatic cancer is higher in men than in women at less than 80 years of age in Japan.[3] The significance of androgen receptor (AR) expression in pancreatic cancer has been studied extensively.[6,7] These issues prompted us to investigate AR-mediated signaling in pancreatic cancer.

AR is a ligand-dependent transcription factor that belongs to the nuclear receptor superfamily.[8,9] The transcriptional activation function of AR is reported to be associated not only with sexual development in men but also with human carcinogenesis.[10] AR has a modular structure containing an N-terminus that harbors transcriptional activation domain(s), a central DNA-binding domain, and a C-terminal ligand-binding domain. Binding of androgen to the ligand-binding domain induces a conformational change in AR and leads to the shuttling of the receptor from the cytoplasm to the nucleus, where it forms a homodimer and is required for the androgen response element (ARE). ARE, the AR-specific DNA sequence, is present in regulatory elements on target genes, such as vascular endothelial growth factor (VEGF) and transforming growth factor beta-1, and regulates the growth and proliferation of hepatocytes.[11,12]

Interleukin-6 (IL-6) is a well-studied proinflammatory cytokine with numerous links to a variety of malignancies. IL-6 has been noted to be overexpressed in patients with pancreatic cancer.[13] Serum levels of IL-6 are directly associated with decreased performance and increased mortality rates in pancreatic cancer. For those with IL-6 levels greater than 5.2 pg/mL, there is a 0% survival rate of 1 year and an average survival length of 4.6 months, compared with a 49% survival rate and survival length of 10.9 months for patients with IL-6 levels less than 5.2 pg/mL.[14] Two subunits compose the IL-6 receptor: the α-subunit, a ligand-specific 80-kDa protein with a short intracellular domain, and GP130, a 130-kDa protein. The extracellular domain of GP130 has an analogy with other cytokines, and the internal domain of GP130 transmits signals to the cytoplasm, resulting in activation of Janus kinases (JAKs) and mitogen-activated protein kinase (MAPK).[15]

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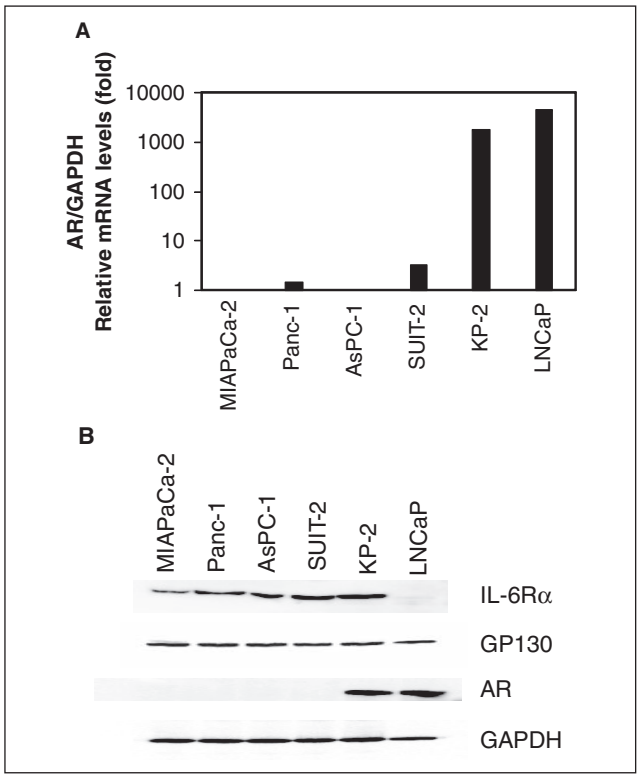


**Figure 1.** AR and IL-6R expression in pancreatic cancer cell lines. (**A**) Relative expression of AR mRNA. RNAs from MIAPaCa-2, Panc-1, AsPC-1, SUIT-2, KP-2 cells, and LNCaP (AR-positive) cells were subjected to real-time RT-PCR for AR and GAPDH gene expression. AR/GAPDH mRNA in MIAPaCa-2 was set as 1-fold. The results were based on the relative expression level of the housekeeping gene GAPDH as an internal control. (**B**) IL-6Rα, GP130, AR, and GAPDH protein expression. Total cell lysates (5 μg) of MIAPaCa-2, Panc-1, AsPC-1, SUIT-2, KP-2, and LNCaP were subjected to 10% SDS-PAGE for immunoblotting with specific antibodies against IL-6Rα (Santa Cruz Biotechnology), GP130 (Cell Signaling Technology), AR (Santa Cruz Biotechnology), and GAPDH (Santa Cruz Biotechnology).

Members of the signal transducer and activator of transcription (STAT) family of transcription factors have been implicated in transformation, tumor cell survival, invasion, and metastasis.[16] Activation of STAT3, in particular, has been detected in diverse malignancies, including those of the pancreas.[17] STAT3 is also constitutively activated in immune cells in the tumor microenvironment.[18] JAK 1 activates STAT3 by phosphorylating Ser-727 and Tyr-705.[19-22] Both serine and tyrosine phosphorylations are necessary for full activation of STAT3.[23]

IL-6 induces phosphorylation of MAPK and activation of the AR N-terminal domain (NTD).[24] RAS-MAPK pathways are known to play important roles in the oncogenesis and progression of pancreatic cancer.[25] Activation of the AR N-terminal domain by IL-6 via STAT3 has been reported.[24] Both pathways are known to enhance the transactivation of AR in the presence of ligand.[24,26]

In the present study, we have shown that IL-6 enhances AR activity in the presence of androgen in pancreatic cancer cell lines. IL-6 increases STAT3 phosphorylation at Tyr-705 and ERK1 phosphorylation, which in turn activate AR. We have also observed that IL-6 enhances pancreatic cancer cell migration and the expression of androgen-responsive genes (ARGs) in the presence of androgen. Thus, it is clear that AR activation through the activation of STAT3 and MAPK by IL-6 plays an important role in pancreatic carcinogenesis.

## Results

*Pancreatic cancer cell lines variably express androgen receptor*. To investigate the expression of AR in pancreatic cancer cell lines, RNAs were extracted from 5 pancreatic cancer cells. LNCaP cells were used as positive control, and QRT-PCR assay was performed. AR/GAPDH mRNA in MIAPaCa-2 was set as 1-fold. The resulting data revealed that AR was expressed in pancreatic cancer KP-2 cells at a substantially high level (1,350-fold), in SUIT-2 cells at a moderate level (3.73-fold), and in Panc-1 cells at a low level (1.43-fold), compared with the AR-positive prostate cancer cell line LNCaP (4,380-fold). AR expression was detected much less in AsPC-1 (0.094-fold) (Fig. 1A). These results were also confirmed at the protein level. AR protein expressions were weaker in pancreatic cancer cell lines than in the positive control of LNCaP (Fig. 1B).

*Pancreatic cancer cells have IL-6 receptors*. Cytokine receptors from the IL-6 receptor family are comprised of ligand-specific α chains and a common signaling chain, GP130, which is also required for high-affinity binding. Next, we investigated the expression of IL-6 receptor upstream of both the JAK/STAT3 and MAPK cascades. An appropriately sized band was detected with anti-IL-6 receptor-α antibodies in pancreatic cancer cell line lysates. Anti-GP130 antibodies also detected the band in these cell lysates (Fig. 1B). It was revealed that IL-6 receptor-α was faint in LNCaP cells, but almost all pancreatic cancer cells expressed both IL-6 receptor-α and GP130.

*Androgen receptor-mediated signaling involves JAK/STAT3 and MAPK signaling pathways in pancreatic cancer cells*. AR is activated by the JAK/STAT3 and MAPK signaling pathways in prostate cancer and hepatocellular carcinoma.[24,27] We postulate that specific alternative pathways, which affect AR and its transcriptional activities in a ligand-independent manner, are activated in pancreatic cancer. We used specific inhibitors for JAK/STAT3 (AG490) and MAPK (U0126) signaling pathways to test the involvement of downstream kinases in AR activation. MIAPaCa-2 cells and SUIT-2 cells were cotransfected with the AR expression vector and ARE reporter vectors and treated with the specific inhibitors in the presence of 10 nM DHT. The reporter activity in MIAPaCa-2 was significantly reduced in the presence of

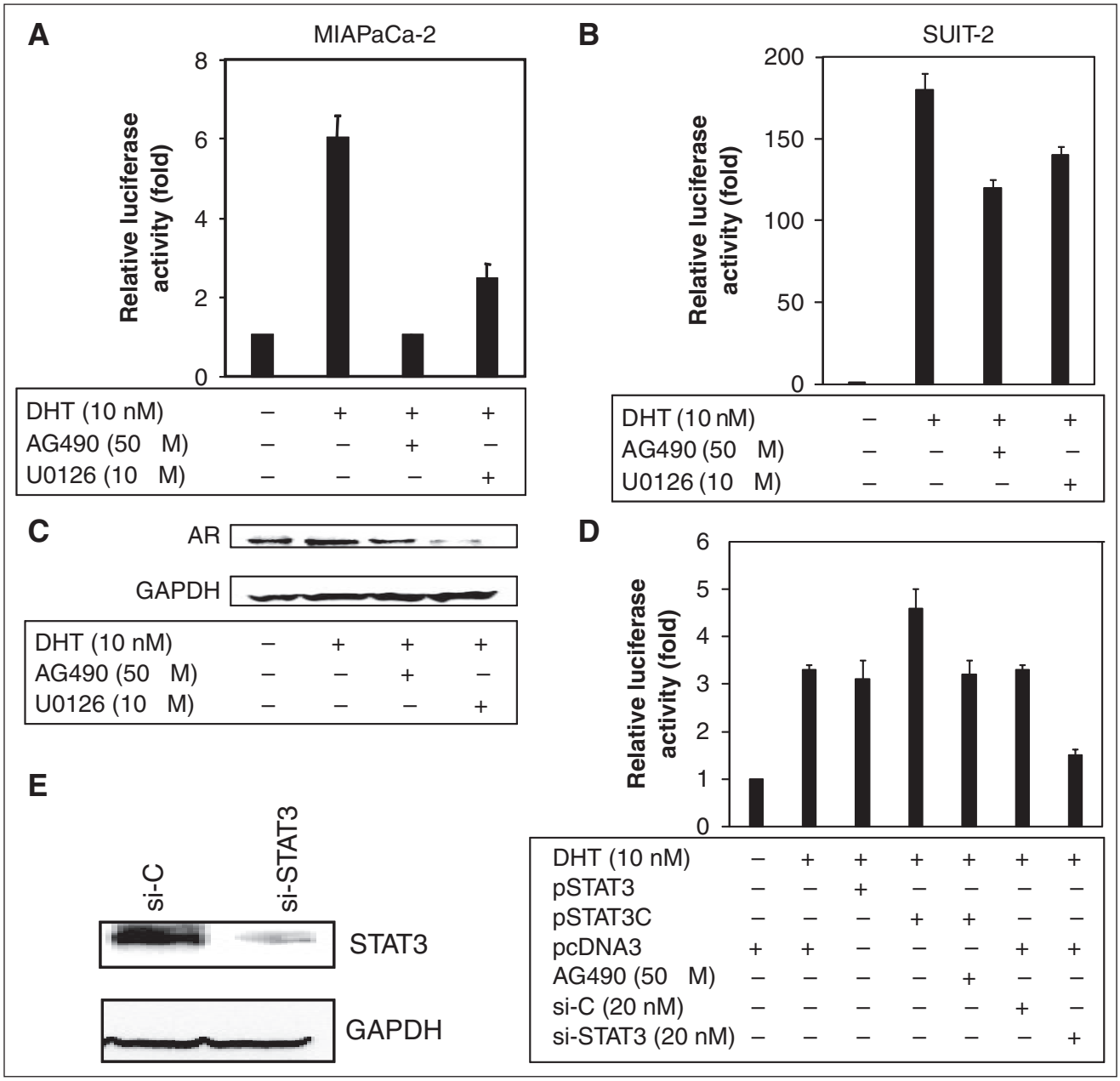


**Figure 2.** Involvement of STAT3 and MAPK in AR activation in pancreatic cancer cells. MIAPaCa-2 cells and SUIT-2 cells were cotransfected with pSG5-AR and pARE(4)-Luc. Cells were treated with 10 nM DHT with or without 50 μM AG490 or 10 μM U0126. AR transactivation was blocked by AG490 or U0126 in MIAPaCa-2 (**A**) and SUIT-2 (**B**). Luciferase activity was measured and is shown as an average value from 3 independent experiments (mean ± standard deviation [SD]). Relative luciferase activity of untreated control was set as 1. (**C**) Western blot analysis of the AR and GAPDH. AR expression was affected by U0126 more than by AG490 in MIAPaCa-2. (**D**) The gain-of-function mutant of STAT3, STAT3C, activated AR. MIAPaCa-2 cells were cotransfected with pSG5-AR, pARE(4)-Luc, pcDNA, pcDNA-STAT3, or pcDNA-STAT3C. siRNA against STAT3 (si-STAT3) and control siRNA (si-C) were also used to determine the specificity. Luciferase activity was measured and shown as an average value from 3 independent experiments (mean ± SD). STAT3C is the constitutively activated form of STAT3. (**E**) Knockdown of STAT3 expression in MIAPaCa-2 cells. Lysates from MIAPaCa-2 cells after both control siRNA (si-C) and siRNA targeting against STAT3 (si-STAT3) treatments were immunoblotted with antibodies against STAT3 or GAPDH.

AG490 ($P = 0.000095$) and partially reduced in the presence of U0126 ($P = 0.00076$) (Fig. 2A). Similar results were also obtained in SUIT-2 cells (AG490 [$P = 0.000038$]; U0126 [$P = 0.00038$]) (Fig. 2B). Therefore, these results indicate that both JAK/STAT3 and MAPK activations might be involved in AR transactivation in pancreatic cancer. We also examined the expression of AR by Western blotting. U0126 completely inhibited the expression of AR compared to control, but AG490 did not (Fig. 2C). There were discrepancies between reporter assays and the expression of

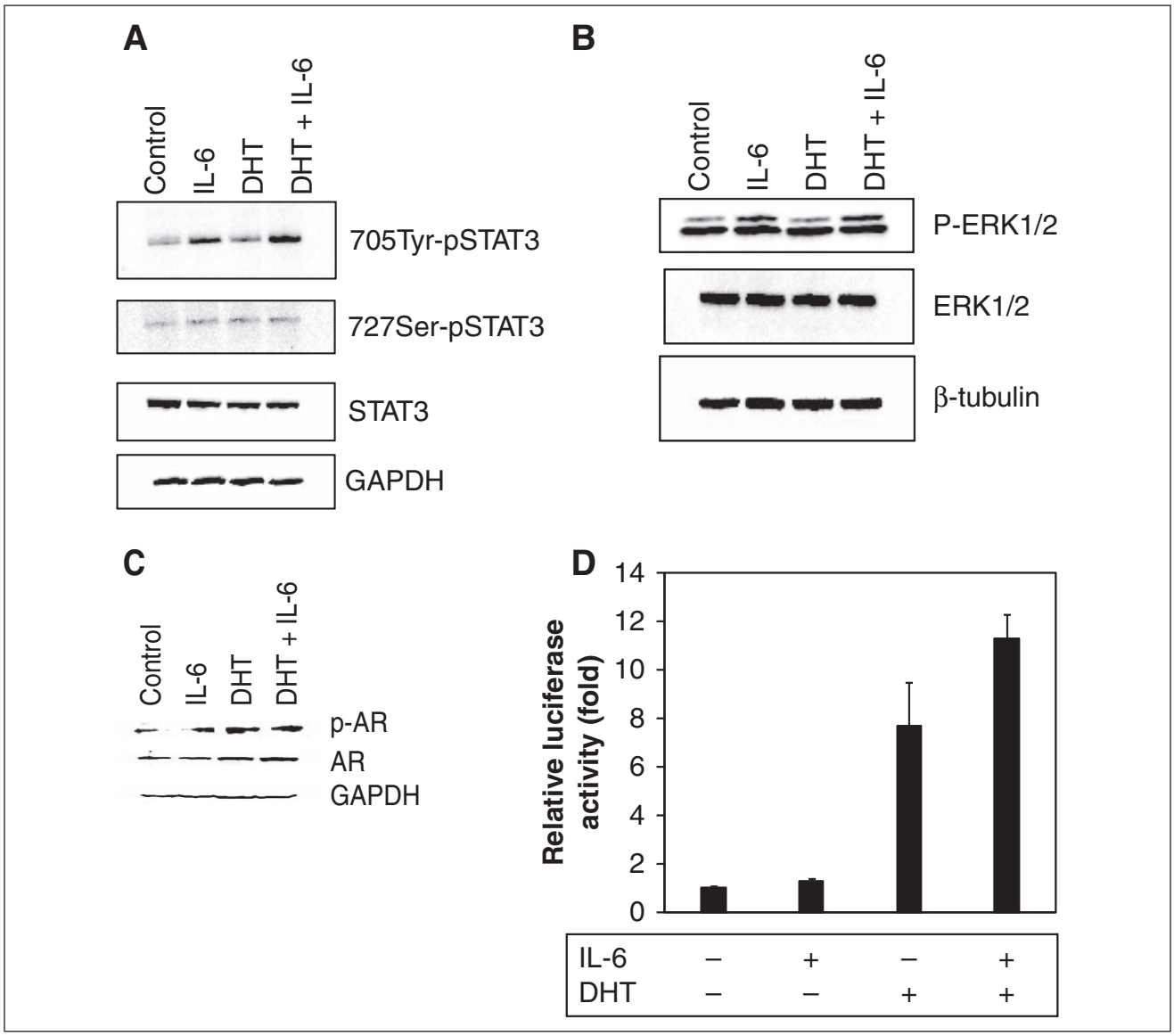


**Figure 3.** IL-6 augments the phosphorylation status of STAT3 and ERK (**A**, **B**). IL-6 does not affect the phosphorylation status of AR at Ser-210 (**C**) but affects the activation of AR (**D**). MIAPaCa-2 cells were treated with or without 50 ng/mL IL-6 and/or 10 nM 5α-androgen-17β-ol-3-one (DHT). (**A**) The phosphorylation status of STAT3 and total STAT3 was detected by Western blot analysis using anti-phospho-STAT3 (pTyr-705 or pSer-727) or anti-STAT3 antibody. (**B**) The phosphorylation status of ERK1/2 and total ERK1/2 was detected by Western blot analysis using anti-phospho-ERK1/2 (pThr-202 and pTyr-204) or anti-ERK1/2 antibody. (**C**) Phosphorylation status of AR (p-AR) in untreated AR-overexpressing MIAPaCa-2 cells (control) and AR-overexpressing MIAPaCa-2 cells treated with or without IL-6 and/or DHT. The phosphorylation status of AR and total AR was detected by Western blot analysis using anti-phospho-AR (pSer-210) or anti-AR antibody. (**D**) Activation of AR in the presence or absence of IL-6 and/or DHT. MIAPaCa-2 cells were cotransfected with pSG5-AR and pARE(4)-Luc. Cells were treated with 10 nM DHT with or without 50 ng/mL IL-6. Twenty-four hours after cotransfection, luciferase activity was measured and shown as an average value from 3 independent experiments (mean ± SD). Relative luciferase activity of untreated control was set as 1.

AR. It is possible that the inhibition of MAPK and JAK/STAT pathways induced the stability of AR protein rather than the augmentation of transcriptional machinery. It seems that JAK/STAT3 signaling plays an important role in the activation of AR, but not through phosphorylation of AR. Bromberg *et al.*[28] demonstrated that a constitutively activated form of STAT3, STAT3C, which has 2 sustained cysteine residues within the COOH-terminal loop of the SH2 domain, resulted in a spontaneous transcriptionally active dimer. Additional experiments using STAT3C clearly demonstrated the activation potential of STAT3C against AR in the presence of androgen ($P = 0.0054$) (Fig. 2D). But AG490 only partially blocks this potential of STAT3C, meaning that the STAT3 pathway is important for AR activation (Fig. 2D). To determine the specificity, we also used siRNA against STAT3 (si-STAT3) in these experiments, and knockdown of STAT3 led to the inhibition of AR translational activity (Fig. 2D and 2E).

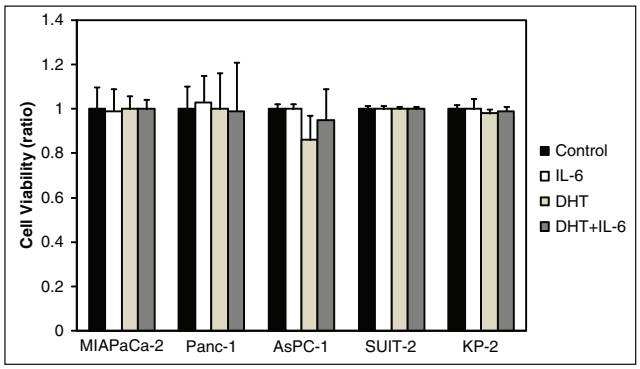


**Figure 4.** DHT and/or IL-6 did not affect pancreatic cell growth. To evaluate cell growth and cell viability 24 hours after drug treatment, dimethylthiazol carboxymethoxyphenyl sulfophenyl tetrazolium (MTS) assay was performed using the CellTiter 96 Aqueous One-Solution Cell Proliferation Assay Kit (Promega). MIAPaCa-2, Panc-1, AsPC-1, SUIT-2, and KP-2 cells were incubated with or without IL-6 (100 ng/mL) and/or DHT (10 nM) for 24 hours. In each cell line, untreated control was set as 1.

*IL-6 augments phosphorylations of Tyr705-STAT3 and ERK1/2 in pancreatic cancer cells*. We investigated the phosphorylation status of STAT3 and ERK1/2 to understand how these signals affect AR gene transactivation in the presence of IL-6 in MIAPaCa-2. Our results demonstrated significant phosphorylation of Tyr705-STAT3 in the presence of IL-6 and DHT, compared with that in the presence of only IL-6 (Fig. 3A). However, phosphorylation of Ser727-STAT3 in the presence of IL-6 and DHT was similar to that in the presence of only IL-6. As for ERK1/2, we also observed significant phosphorylation in the presence of IL-6 (Fig. 3B). Overall, the phosphorylation of Tyr705 in STAT3 appeared to be important for the enhancement of AR activity (Fig. 3A).

*IL-6 does not affect phosphorylation of Ser-210 AR but affects ARE activation*. There was no apparent difference between the presence and absence of IL-6 alone (Fig. 3C). The phosphorylation level of AR was not increased upon IL6 or IL-6/DHT treatment even though the total amount of AR was significantly increased. However, the combination of DHT plus IL-6 induced additional activation of ARE, compared with no drugs ($P = 0.000059$), IL-6 alone ($P = 0.000066$), or DHT alone ($P = 0.038$) (Fig. 3D), consistent with previous reports of the existence of ligand-independent activation of AR.[24]

*IL-6 is important for AR-positive pancreatic tumor cell migration in the presence of androgen*. We analyzed cell proliferation in 3 AR-positive pancreatic cancer cell lines. Cell proliferation was not decreased in the presence of IL-6 and/or DHT as compared with untreated cells (Fig. 4). To evaluate the possible effects on pancreatic cancer cells, an *in vitro* wound-healing scratch assay was performed to assess cell mobility/migration in the presence or absence of IL-6 and/or DHT (Fig. 5A-E). Cell migration was measured as a ratio compared to that of untreated cells. In the 3 AR-positive cell lines, KP-2, SUIT-2, and Panc-1, DHT positively affected cell migration (Fig. 5A-C). In KP-2 ($P = 0.00032$) and SUIT-2 ($P = 0.056$), IL-6 tended to increase cell mobility in the presence of DHT, but in its absence, no effects of IL-6 were observed (Fig. 5A and 5B). On the other hand, DHT, either with or without IL-6, failed to induce any positive cell migration in AsPC-1 and MIAPaCa-2 cells, which expressed less AR (Fig. 5D and 5E).

*AR is important for pancreatic tumor cell migration promoted by IL-6 in the presence of androgen*. We also performed a wound-healing scratch assay of KP-2 cells with AR knockdown by siRNA (Fig. 5F-H). The migration of cells in AR knockdown cells and control siRNA-transfected cells was $32.6 \pm 8.14$ and $331 \pm 38.2$ per area, respectively ($P = 0.0056$). Knockdown of AR decreased tumor cell migration, consistent with recent findings that AR knockout neutrophils demonstrated reduced migration activities[29] and that the lack of STAT3 could impair neutrophil migration.[30]

## Discussion

Our results showed that pancreatic cancer cells variably express AR, supporting the previous study that the expression level of ARs in pancreatic cancer cells is higher than that of normal pancreatic cells.[6] Recent reports of STAT3 activation in epithelial tumors also underscore the importance of IL-6 signaling and the inflammatory response in tumorigenesis, providing an opportunity for therapeutic intervention.[31,32] In this study, we investigated the regulation of IL-6–inducible AR activation in pancreatic cancer cell lines in the presence of DHT. The expression of AR was at low levels in MIAPaCa-2, consistent with a previous study.[33] We used a reporter assay for studies of AR activation in this cell line. Our results demonstrated that kinase inhibitors for JAK/STAT3 and MAPK pathways downregulated AR activation in the presence of DHT. IL-6 is an inflammatory cytokine, and its receptor molecules, IL-6 receptor-α and GP-130, are located upstream of the JAK/STAT3 and MAPK pathways. In the absence of DHT, IL-6 does not lead to the activation of AR (Fig. 3D).

Our observations agree with earlier reports suggesting that IL-6 can enhance AR transactivation via STAT3 and MAPK signaling pathways in prostate cancer cells.[24] Together, these results indicate that the positive effects of IL-6 on AR activation could lead to pancreatic carcinogenesis *in vivo*. We examined the effects of IL-6 and/or DHT on the phosphorylation of STAT3, ERK1/2, and AR. The effects of both IL-6 and DHT administration on STAT3 or ERK1/2 phosphorylation were the same as that of IL-6 alone, and the effects of both IL-6 and DHT on AR phosphorylation were the same as that of DHT alone. However, the effects of both

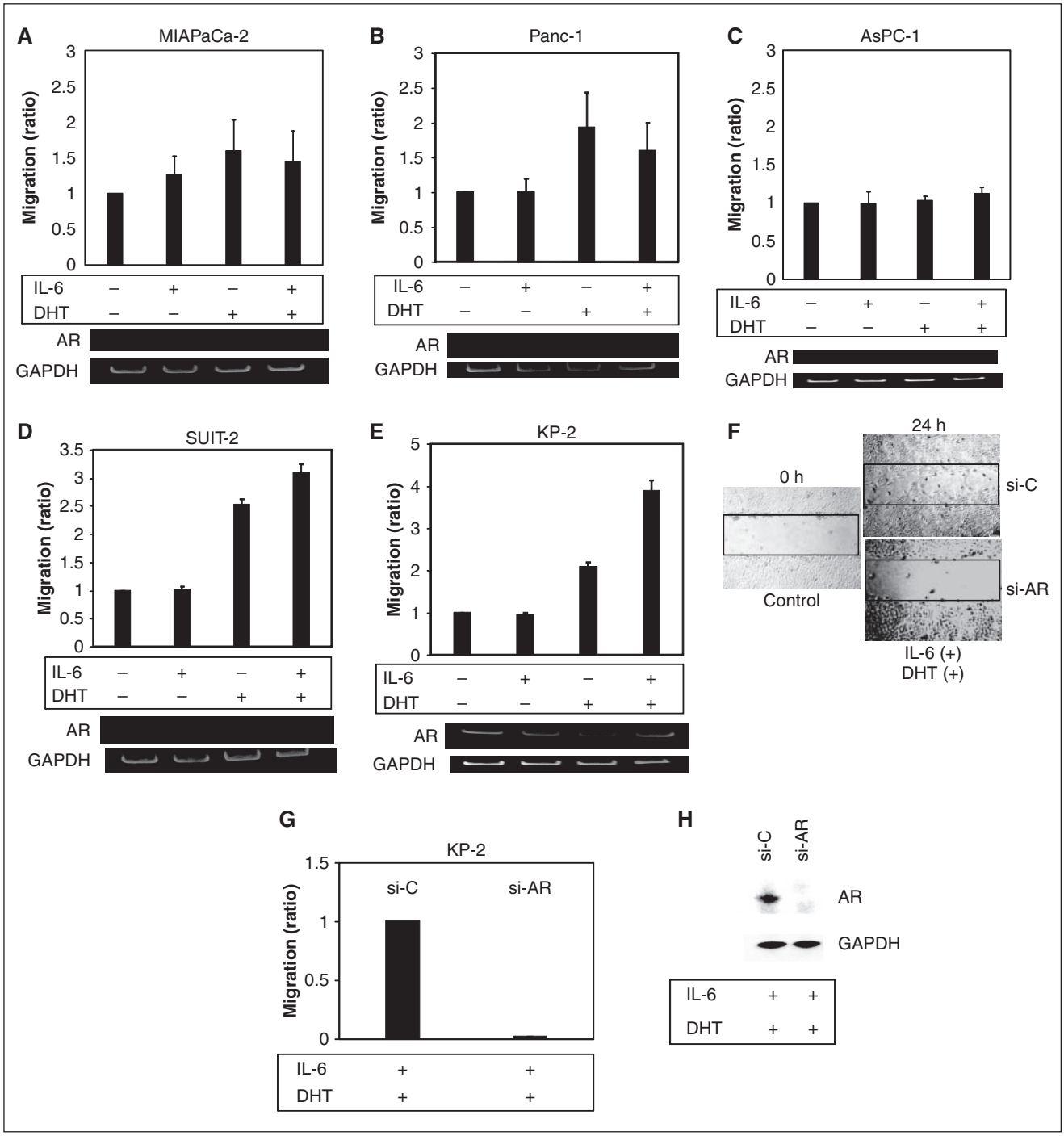


**Figure 5.** Migration of pancreatic cancer cell lines was investigated using a wound-healing scratch assay (**A-E**). Knockdown of AR suppressed migration of pancreatic cancer cells in the presence of IL-6 and DHT (**F-H**). KP-2 (**A**), SUIT-2 (**B**), Panc-1 (**C**), AsPC-1 (**D**), and MIAPaCa-2 (**E**) cells were subjected to wound-healing scratch assay. Cells were incubated with or without IL-6 (100 ng/mL) and/or DHT (10 nM) for approximately 24 hours. The migration thus observed was presented as a ratio of migration, considering migration in untreated control as 1. Scion images were used for analysis. The error bars represent mean ± SD of 3 independent experiments. Results of semiquantitative RT-PCR analysis for AR and GAPDH mRNAs are shown in the lower part below each graph (**A-E**). KP-2 cells were transfected with 10 nM AR siRNA (si-AR) or 10 nM control siRNA (si-C) (Thermo Fisher Scientific Dharmacon), subjected to wound-healing scratch assay, and incubated with IL-6 (100 ng/mL) and DHT (10 nM) for 24 hours. (**F**) Light microscopy pictures show KP-2 cells transfected with 10 nM si-C or 10 nM si-AR and incubated with 100 ng/mL IL-6 and 10 nM DHT at 0 hours (left panel) and 24 hours later (right panels). (**G**) The migration observed was presented as a ratio of migration, considering migration in transfection with si-C as 1. The error bars represent mean ± SD of 3 independent experiments. (**H**) Knockdown of AR expression in KP-2 cells. Whole cell lysates from KP-2 cells after si-C or si-AR treatment were immunoblotted with antibodies against AR or GAPDH.

IL-6 and DHT on AR activation were stronger than that of IL-6 or DHT alone. This accounts for the activation of AR that was enhanced by IL-6, occurring after the phosphorylation of STAT3 or MAPK in pancreatic cancer cells. Ueda *et al*.[24] reported a direct interaction between amino acids 234 to 558 of the AR N-terminal domain and STAT3 following IL-6 treatment. Our results (Figs. 2 and 3) also suggest that STAT3 plays an important role in the activation of AR in a way other than its phosphorylation.

Pancreatic cancer and its cell lines are reported to produce IL-6. One of the complications of pancreatic cancer is chronic pancreatitis, in which a higher production of IL-6 is seen.[13] The IL-6/STAT3 pathway plays an important role in pancreatic carcinogenesis as well as in that of other sites.[34] IL-6 is also the major cytokine-inducing transcription of human C-reactive protein (CRP), which proved to be a strong independent predictor of survival.[35,36]

Consistent with the physiological role of androgens as a modulator of cellular proliferation as well as of response to wounds and wound healing, genes associated with these processes were enriched among androgen-responsive genes (ARGs) and primary AR target genes.[11,12,27] We found that several were ARGs modulated by IL-6 in the presence of DHT (data not shown). Future studies will reveal the in-depth function of other migration-related molecules. In conclusion, our studies also provided evidence that IL-6 can interfere with AR signaling, which could be a critical mechanism for cell invasion in pancreatic cancer.

## Materials and Methods

*Cell lines, plasmids, transfections, and reagents*. Human pancreatic cancer cell lines (MIAPaCa-2, Panc-1, AsPC-1, SUIT-2, and KP-2) and LNCaP prostate cancer cell lines were maintained in RPMI 1640 medium and Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum.[37] The plasmid-expressing human AR protein (pSG5-AR)[38] was a kind gift from Professor J.T. Isaacs, and the AR response element–directed luciferase reporter plasmid (pARE(4)-Luc) was previously described.[39] The plasmids pSTAT3 and pSTAT3C were kind gifts from Professor A. Yoshimura.[40]

Synthetic human IL-6 and 5α-androgen-17β-ol-3-one (DHT) were purchased from Wako Pure Chemical Industries Ltd. (Osaka, Japan) and Sigma-Aldrich (St. Louis, MO), respectively. The specific kinase inhibitors AG490 and U0126 were used in this study (Calbiochem, San Diego, CA). The reagents were prepared by dissolving them in DMSO or ethanol and added to cultured cells at proper dilutions.

*Quantitative PCR (Q-PCR) assay*. cDNA was generated by reverse transcription using the PrimeScript RT reagent kit (Perfect Real Time) (Takara, Otsu, Japan). Reactions were determined with the SYBR Green I detection chemistry system using an ABI Step One real-time PCR system (Applied Biosystems, Foster City, CA).[27,41] GAPDH was used as a control gene for normalization, and data were analyzed by the comparative threshold cycle method.[42]

*Immunoblot analysis*. Cells were harvested using sodium dodecyl sulfate sample buffer. Proteins were subjected to electrophoresis on 10% polyacrylamide gel and transferred onto a nitrocellulose membrane. The membrane was probed with an antibody for STAT3, phospho-(Tyr-705)-STAT3, phospho-(Ser-727)-STAT3 (Cell Signaling Technology, Danvers, MA), AR, phospho-AR, IL-6 receptor-alpha (IL-6Rα), GP130, or GAPDH (Santa Cruz Biotechnology, Santa Cruz, CA). Proteins were visualized using an enhanced chemiluminescent ECL Western blot substrate (Pierce, Rockford, IL) scanned by an image analyzer (LAS-1000, Fuji Film, Tokyo, Japan).

*Luciferase assay*. MIAPaCa-2 cells in 6-well plates were transfected with 2 μg plasmid DNA using Effectene (Qiagen, Hilden, Germany), following the manufacturer's instructions. Cells were incubated in RPMI 1640 plus 10% FCS after transfection and were then treated with 10 nM DHT, 50 ng/mL IL-6, or its control solvent ethanol in RPMI 1640 without FCS on the second day for 24 hours. At 48 hours posttransfection, cells were lysed with reporter lysis buffer (Promega, Madison, WI), and luciferase activity was determined using a luminometer (ATTO Technology, Amherst, NY).

*Cell viability assay*. To evaluate cell growth and cell viability 24 hours after drug treatment, dimethylthiazol carboxymethoxyphenyl sulfophenyl tetrazolium (MTS) assay was performed using the CellTiter 96 Aqueous One-Solution Cell Proliferation Assay Kit (Promega).

*Wound-healing scratch assay*. Cells were grown on 35-mm cell culture dishes until confluence. A p-200 pipette tip was used to scratch the dish, and the serum-free medium with or without IL-6 and/or DHT was replaced to remove the floating cells.[43] Up to 24 hours after scratching, the cells were observed by microscopy (Eclipse TE2000-U, Nikon, Tokyo, Japan). Migration was quantified by counting the number of cells that moved into the reference area. The migration observed was presented as a ratio, considering migration in the untreated control as 1. Scion images were used for analysis.

*siRNA*. The siRNAs si-STAT3 and si-AR were named on the basis of their target genes, STAT3 and AR, respectively. Si-C was negative control siRNA. They were ON-TARGETplus siRNAs purchased from Thermo Fisher Scientific Inc. (Waltham, MA). Approximately $1 \times 10^6$cells/well were placed in 6-well plates (Iwaki Glass, Tokyo, Japan) 24 hours prior to transfection. Cells were transfected with 20 to 50 nM

siRNA using TransMessenger Transfection Reagent (Qiagen) or Effectene Transfection Reagent (Qiagen) according to the manufacturer's protocol. Twenty-four hours later, cells were treated with or without IL-6 and/or DHT. At the same time, wound-healing scratch assay was performed. In another case, 48 hours after transfection, whole cell lysates were collected for immunoblotting analysis.

*Statistical analysis*. All statistical analyses were performed using DA Stats software (O. Nagata, Nifty Serve: PAF01644). Statistical analyses were done using a 2-tailed Student *t* test or Welch *t* test for paired data.

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## Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the authorship and/or publication of this article.

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