Journal of Cancer Research and Clinical Oncology (2019) 145:2293–2301
https://doi.org/10.1007/s00432-019-02993-1

ORIGINAL ARTICLE – CANCER RESEARCH

Dihydrotestosterone promotes kidney cancer cell proliferation by activating the STAT5 pathway via androgen and glucocorticoid receptors

Sahyun Pak[1] · Wansuk Kim[2] · Yunlim Kim[3,4] · Cheryn Song[4] · Hanjong Ahn[4]

Received: 19 January 2019 / Accepted: 5 August 2019 / Published online: 10 August 2019

Abstract

Purpose Androgen receptors (ARs) are expressed on a variety of cell types, and AR signaling plays an important role in tumor development and progression in several cancers. This in vitro study evaluated the effect of dihydrotestosterone (DHT) on the proliferation of renal cell carcinoma (RCC) cells in relation to AR status.

Methods Steroid hormone receptor expression was evaluated using RT-PCR and Western blotting. The effect of DHT on cell proliferation and STAT5 phosphorylation was evaluated in RCC cell lines (Caki-2, A498, and SN12C) and primary RCC cells using cell viability assays and Western blotting. ARs and glucocorticoid receptors (GRs) were knocked down with small interfering RNAs before assessing changes in cell proliferation and STAT5 activation.

Results DHT treatment promoted cell proliferation and increased STAT5 phosphorylation regardless of AR status. The AR antagonist bicalutamide reduced kidney cancer cell proliferation, regardless of AR status. AR and GR knockdown blocked STAT5 activation and reduced cell proliferation in all RCC cell lines. In patient-derived primary cells, DHT enhanced cell proliferation and this effect was diminished by treatment with the AR antagonists bicalutamide and enzalutamide and the GR antagonist mifepristone.

Conclusion DHT promotes cell proliferation through STAT5 activation in RCC cells, regardless of AR status. DHT appears to utilize the AR and GR pathways to activate STAT5, and the inhibition of AR and GR showed antitumor activity in RCC cells. These data suggest that targeting AR and GR may be a promising new approach to the treatment of RCC.

Keywords Kidney neoplasms · Androgen receptor · Glucocorticoid receptor · STAT5 transcription factor

Introduction

Kidney cancer is among the ten most frequently diagnosed cancers in the USA in both males and females, with an estimated 64,000 new cases in 2017 (Siegel et al. 2017). Renal cell carcinoma (RCC) accounts for 90% of kidney cancer cases, which is associated with a 5 year survival rate of 74%. Metastatic RCC is diagnosed in approximately one-third of patients with kidney cancer, and distant metastatic progression has been documented in approximately one quarter of patients who underwent nephrectomy for localized kidney cancer (Choueiri and Motzer 2017). Significant improvements in survival have been achieved in patients with RCC following the availability of therapeutic agents, such as those targeting the vascular endothelial growth factor (VEGF) or the mammalian target of rapamycin (mTOR) pathway. However, almost all patients will develop resistance to both VEGF-targeted and mTOR-targeted therapies and the

Sahyun Pak and Wansuk Kim contributed equally to this work.

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s00432-019-02993-1) contains supplementary material, which is available to authorized users.

✉ Hanjong Ahn
hjahn@amc.seoul.kr

[1] Department of Urology, Center for Urologic Cancer, National Cancer Center, Goyang, Korea

[2] Department of Urology, Inje University Busan Paik Hospital, Busan, Korea

[3] Asan Institute for Life Science, Asan Medical Center, Seoul, Korea

[4] Department of Urology, Asan Medical Center, University of Ulsan College of Medicine, 88 Olympic-ro 43-gil, Songpa-gu, Seoul 05505, Korea

prognosis of metastatic RCC remains poor, with a 5 year survival rate of just 8%. Therefore, new therapeutic targets for RCC are urgently required (Choueiri and Motzer 2017; Jonasch et al. 2014).

As the incidence of RCC is approximately 1.7 times higher in men than in women (Bray et al. 2018; Siegel et al. 2017), the role of sex hormones and their receptors in the pathogenesis of RCC has been investigated (Lucca et al. 2015; Stone 2014). Steroid hormone receptors are nuclear hormone receptors of the NR3 class, with endogenous agonists that are either 3-hydroxysteroids or 3-ketosteroids (Alexander et al. 2015). The hormonal ligand-binding domains of these receptors share a similar tertiary structure (Whitfield et al. 1999). Androgen receptors (ARs) are expressed in various cell types, and AR signaling plays an important role in tumor development and progression in many cancers (Chang et al. 2014). Although several studies report an association between AR activity and the development and progression of RCC, the role of AR in RCC remains the subject of some debate (He et al. 2014a, b; Langner et al. 2004; Lee et al. 2017; Wang et al. 2017; Zhao et al. 2016; Zhu et al. 2014).

The Janus kinase (JAK) signal transducer and activator of transcription (STAT) pathway is associated with interferon-α (IFNα), IFNγ, and interleukin-6 (IL-6)-mediated signaling (Yu et al. 2014). STAT5, along with STAT3, is an oncogenic downstream mediator of the JAK-STAT pathway and is involved in the pathogenesis of various cancers, particularly hematopoietic cancers (Wingelhofer et al. 2018; Yu et al. 2014). Previous studies showed that the activation of STAT5 signaling promotes cancer cell proliferation and survival in conjunction with other core cancer pathways (Vogelstein and Kinzler 2004; Wingelhofer et al. 2018). In addition, cross communication between STAT5 and AR signaling has been documented (Song et al. 2014; Tan et al. 2008). The nuclear localization and transcriptional activity of STAT5 is increased by synergism with the AR (Ferbeyre and Moriggl 2011).

This study was designed to evaluate the effect of dihydrotestosterone (DHT) on the proliferation of RCC cells in relation to AR status.

Materials and methods

Cell culture, reagents, and antibodies

RCC cell lines were used in this in vitro study; Caki-2 and A498 were obtained from the American Type Culture Collection, and SN12C cells were obtained from the Korean Cell Line Bank. The cells were maintained in DMEM or RPMI 1640 (Invitrogen, Carlsbad, CA) with 10% fetal bovine serum (FBS; GIBCO, Grand Island, NY), 100 units/ml penicillin, and 100 µg/ml streptomycin in a 5% CO_2 atmosphere at 37 °C. For androgen-induced proximity studies, the cells were cultured in phenol red-free medium containing 10% charcoal-stripped serum (Invitrogen) for 24 h. The charcoal-stripped serum has reduced hormone levels and is ideally suited for androgen signaling studies.

After obtaining informed consent, clear cell RCC tissue was obtained from ten patients who underwent radical or partial nephrectomy at the Asan Medical Center. Prior to obtaining the tissue samples, patients had received no therapy for RCC (additional patient information is provided in Supplementary Table 1). All procedures were approved by the Institutional Review Board of the Asan Medical Center (no. 2014-0957). Tumor specimens were prepared by mincing and digestion in RPMI containing 1 mg/ml type I collagenase (Sigma Aldrich, St. Louis, MO) for 1 h at 37 °C. Cells were washed with medium containing 10% FBS to inactivate the collagenase and then with PBS to remove the FBS. The cells were plated and maintained in RPMI containing 10% FBS in a 5% CO_2 atmosphere at 37 °C. Only low-passage-number cells (< passage 3–5) were used in the experiments. Mycoplasma testing was performed using a PCR-based e-mycoplasma test kit (iNtRON Biotechnology, Seongnam, Korea).

Anti-phosphoSTAT5 (Cell Signaling, Danvers, MA), anti-AR (Cell Signaling), and anti-ACTB (Santa Cruz Biotechnology, Santa Cruz, CA) antibodies were used for Western blot analysis; 5-α-DHT and the AR antagonist bicalutamide (Casodex, CDX) were purchased from Sigma (St. Louis, MO) and dissolved in ethanol to prepare 10% stock solutions.

Cell viability assay

Cell viability was determined using the CellTiter-Glo Luminescent Cell Viability assay (Promega, Madison, WI). In brief, 2000 cells were seeded in 96-well plates and cultured at 37 °C overnight prior to drug treatment. After 72 or 96 h, the plates were incubated with the reagent at room temperature for 10 min to stabilize the luminescence signal; results were assessed using a MicroLumatPlus LB luminometer microplate reader (Berthold Technologies, Bad Wildbad, Germany). All plates included control wells containing cell-free medium to determine background luminescence. Data represent the percentage of untreated cells [(treatment value − blank)/vehicle value − blank)] and are expressed as the mean ± standard error of the mean (SEM) of at least three experiments.

Cell viability was determined by trypan blue staining; 20 µl of the cell suspension was mixed with 20 µl of trypan blue solution and transferred to a hemocytometer twin chamber. Viable cells (trypan blue-positive) were counted, and the percentage of viable cells was calculated as the number of

trypan blue-positive cells in the treatment group divided by that in the untreated control group. Results were analyzed using GraphPad Prism® version 5.00 (GraphPad Software, San Diego, CA).

Western blot analysis

RCC cells were cultured in DMEM or RPMI 1640 without phenol red containing 1% charcoal-stripped FBS for 18–24 h. The cells were treated with sodium meta-arsenite at various concentrations for different periods and subsequently lysed. Protein concentrations were measured using the Bradford protein assay (Bio-Rad, Hercules, CA). Equal amounts of proteins were subjected to SDS-PAGE and electrophoretically transferred to a PVDF membrane. The membranes were incubated with the primary antibodies and subsequently with the secondary antibody conjugated with peroxidase. Signals were detected using the chemiluminescent detection system (Millipore, Bedford, MA). Membranes were stripped and re-probed with ACTB antibody to confirm equal protein loading.

RNA interference experiments

Cells were cultured to 60% confluency 24 h prior to transfection with small interfering RNA (siRNA). The cells were transiently transfected with AR-specific siRNA and non-specific control siRNA using HiPerfect transfection reagent (Qiagen, Valencia, CA). After 72 h, the cells were harvested and cell extracts were screened for AR expression by Western blot analysis and real-time reverse transcription-PCR (qRT-PCR). The cells showing the most efficient AR knockdown were used for further experiments, including AR and p-STAT5 localization by immunofluorescence, as previously described.

Real-time reverse transcription-PCR analysis

Total RNA was extracted from samples using TRIzol reagent (Invitrogen). Total RNA (2 µg) from each sample was subjected to reverse transcription using the SuperScript II First-Strand cDNA Synthesis Kit (Invitrogen), and the cDNAs were subjected to real-time PCR analysis for AR and PSA expression. Real-time PCR reactions were carried out using the ABI 7000 sequence detector system (Applied Biosystems, Foster City, CA). Primers were designed as follows: androgen receptor forward, 5′-CAGTGGATGGGC TGAAAAAT-3′; reverse, 5′-AGCGTCTTGAGCAGGATG T-3′; glucocorticoid receptor (GR) forward, 5′-GCGATG G TCTCAGAAACCAAAC-3′; reverse, 5′-GCAGAGGAT AACTTCCTCTGTAAT CTC-3′; ERα forward, 5′-CCGGCA TTCTACAGGCCAAA-3′; reverse, 5′-GCGAGTCTCCTT GGCAGATTC-3′; ACTB forward, 5′-CACCACCATGTA C CCTGGCA-3′; reverse, 5′–9 ACATCTGCTGGAAGG TGGAC-3′. The cycler was programmed with the following conditions: (a) initial denaturation at 94 °C for 2 min, followed by 35 cycles of (b) 94 °C for 40 s, (c) annealing of the primer template at 58 °C for 40 s, and (d) extension at 72 °C for 40 s. PCR amplification efficiency and linearity for each gene including targeted and control genes were tested.

Results

DHT increases kidney cancer cell proliferation and antiandrogen treatment reduce the proliferation, regardless of AR status

The level of endogenous expression of steroid hormone receptors, including ARs, GRs, estrogen receptors, and progesterone receptors, was assessed in RCC cell lines (Caki-2, A498, and SN12C). Western blotting showed that ARs were strongly expressed in Caki-2 and A498 cells, while GRs were strongly expressed in SN12C cells (Fig. 1a). To determine the relative steroid hormone receptor mRNA expression levels, real-time RT-PCR analysis was performed in RCC cells. Endogenous AR mRNA expression was markedly higher in Caki-2 cells than in A498 or SN12C cells, while GR mRNA levels were higher in SN12C cells than in Caki-2 or A498 cells (Fig. 1b).

Cell viability assays showed that DHT treatment increased cell proliferation in both AR-positive (Caki-2 and A498) and AR-negative (SN12C) RCC cells in a concentration-dependent manner (Fig. 1c). The interaction between STAT5, AR, and GR has been well documented (Bianchi et al. 2000; Stöcklin et al. 1996; Tan et al. 2008). In the current study, a concentration-dependent increase in phosphorylated STAT5 was seen in all RCC cells treated with DHT (Fig. 1d).

Evaluation of cells treated with DHT (0, 0.1, 1 nM) in the presence or absence of 1 nM CDX showed that CDX reduced the proliferation of Caki-2, A498, and SN12C cells (Fig. 2a). Western blotting showed that CDX treatment induced a decrease in STAT5 phosphorylation and AR expression in Caki-2, A498, and SN12C cells (Fig. 2b).

Knockdown of AR and GR blocks the growth of RCC cells treated with DHT

We investigated whether knockdown of AR and GR with siRNA affects the growth of RCC cells treated with or without DHT. RCC cells were transfected with 2 µM siRNA control, siRNA AR, siRNA GR, and a combination of siRNA AR and siRNA GR for 72 h, followed by 1 nM DHT. Cell viability assays showed that knockdown of AR or GR reduced cell proliferation in AR-positive cells (Caki-2 and

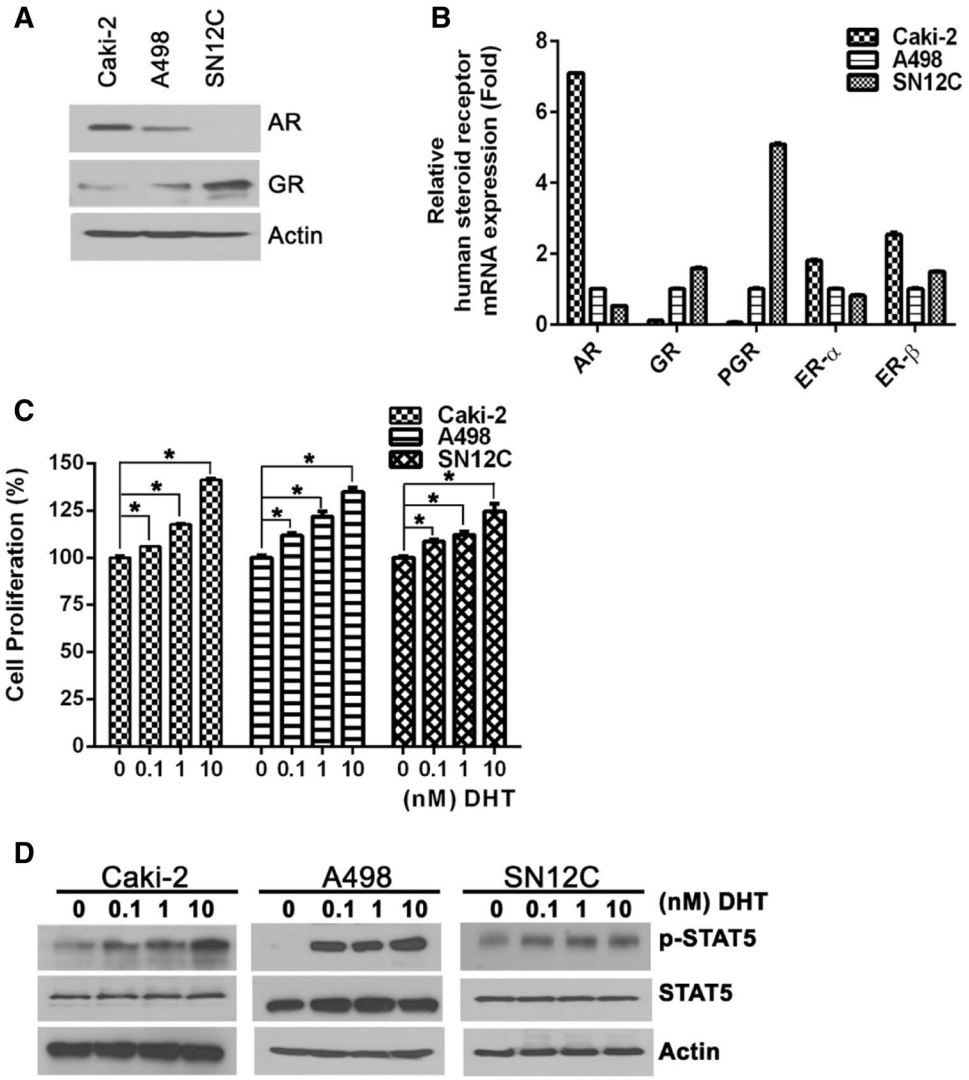

Fig. 1 Effect of DHT on cell growth, STAT5 phosphorylation and multiple human steroid receptor basal mRNA and protein expression levels in different RCC cell lines. *AR* androgen receptor, *GR* glucocorticoid receptor, *PGR* progesterone receptor, *ER* estrogen receptor. **a** Human RCC cell line lysates were evaluated by Western blot for human AR and actin protein expression. **b** qRT-PCR analysis of multiple steroid receptor mRNA, relative to ACTB in human renal cell carcinoma cell lines. **c** RCC cell lines were treated with DHT 0.1–10 nM. Cell viability was determined using trypan blue staining. Results are expressed ± SEM of three independent experiments. $^*p < 0.05$ by one-way ANOVA. **d** Western blot analysis was performed using the antibodies specified

A498; Fig. 3a). In SN12C cells, siRNA GR reduced cell proliferation; however, siRNA AR did not significantly decrease cell proliferation. Simultaneous knockdown of AR and GR resulted in a significant reduction in cell viability versus the knockdown of AR or GR alone.

Western blotting showed that AR and GR expression was effectively knocked down by siRNAs (Fig. 3b). Consistent with the results of the cell viability assay, STAT5 phosphorylation was markedly decreased after AR or GR knockdown (Fig. 3b and Supplementary Fig. 2). Taken together, these results suggest that DHT enhances RCC cell proliferation through STAT5 activation via the AR and GR pathways.

AR and GR expression is also associated with primary RCC cell proliferation

The relative mRNA expression of AR and GR in primary RCC cell cultures was determined using qRT-PCR (Fig. 4a). Western blot analysis was performed in four cell samples treated with 0.1–10 nM DHT for 24 h (Fig. 4b). In primary cells from patients 2, 3, and 4, AR and phosphorylated STAT5 expression increased after DHT treatment in a concentration-dependent manner. Similarly, DHT treatment increased GR and phosphorylated STAT5 expression in primary cells from patients 1, 2, and 4. Optical microscopy showed that DHT induced cell proliferation in a primary RCC cell line. The effects of CDX, the AR signaling inhibitor enzalutamide (MDV3100), and the GR antagonist mifepristone (RU486) were determined in one primary cell sample that showed expression of both AR and GR (patient 4). Cells were treated with 10 nM DHT for 24 h in the presence or absence of 1 μM CDX, 10 μM MDV3100, and 10 μM RU486 (Fig. 4d). Cell viability assays showed that CDX, MDV3100, and RU486 had antitumor activity against primary RCC cells.

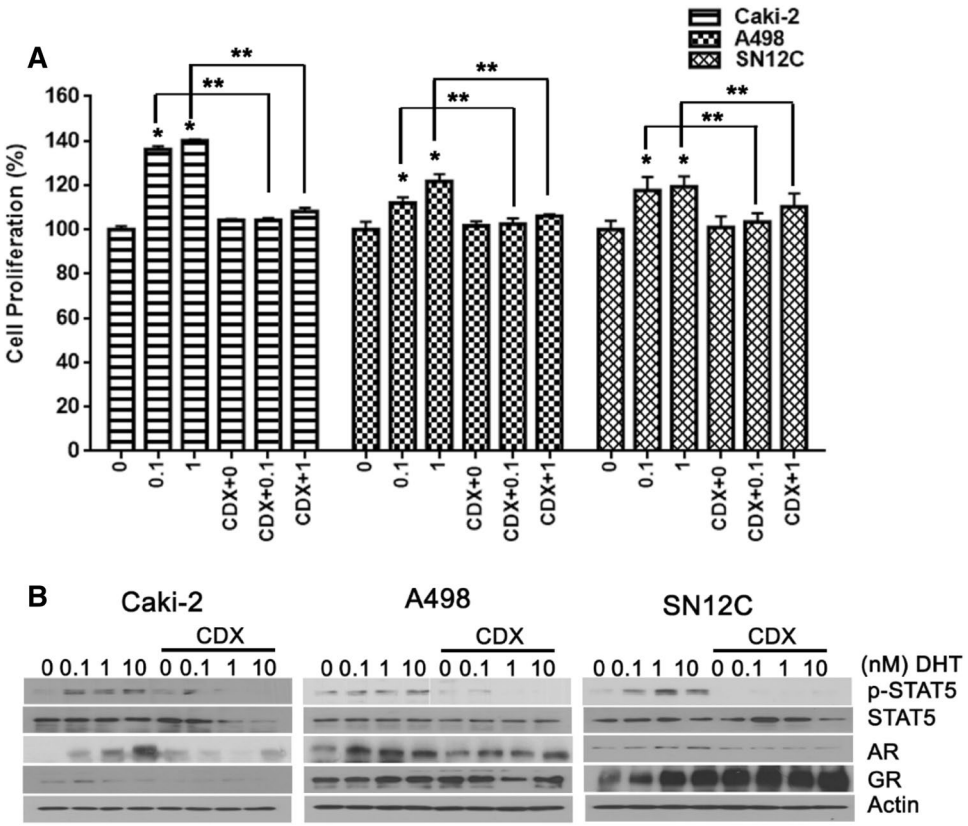

Fig. 2 The effect of DHT (after AR blocking) on cell growth, STAT5 phosphorylation, and AR expression in human RCC cell lines. **a** RCC cell lines were treated with DHT (0, 0.1, 1 nM) in the presence or absence of 1 nM CDX (bicalutamide, AR antagonist). Cell viability was determined by trypan blue staining. Results are expressed ± SEM of three independent experiments. **b** RCC cell lines were treated with DHT (0, 0.1, 1, 10 nM) in the presence or absence of 1 nM CDX. Western blot analysis was performed using the antibodies specified

Discussion

This study demonstrates that DHT promotes the growth of RCC cells by activating the STAT5 pathway, regardless of AR status. DHT appears to utilize the AR and GR pathways to activate STAT5. These data demonstrate the potential antitumor activity of AR and GR inhibition in the treatment of RCC.

Androgens and their receptors are considered to play an important role in RCC development as the incidence of RCC is approximately 1.7 times higher in men than in women (Chang et al. 2014; He et al. 2014a, b; Langner et al. 2004; Lee et al. 2017; Lucca et al. 2015; Stone 2014; Wang et al. 2017; Zhao et al. 2016; Zhu et al. 2014). However, RCC is not considered to be a sex hormone-dependent cancer and smoking, obesity, hypertension, end-stage renal disease, and alcohol intake are key risk factors. Among these, cigarette smoking and end-stage renal disease are more common in men than in women (Lucca et al. 2015). Several studies report that AR expression is associated with tumor stage, grade, and prognosis (Ha et al. 2015; He et al. 2014b). ARs are expressed in the proximal and distal tubules of normal human kidneys (Zhao et al. 2016), and immunohistochemical studies have also found 15–40% of RCC tissues to be AR-positive (Ha et al. 2015; Langner et al. 2004; Zhu et al. 2014).

STAT proteins are transcription factors that are crucial in mediating nearly all cytokine-driven signaling. Of the STAT protein family, STAT5 is the most important for cancer progression, along with STAT3 (Yu et al. 2014). STAT5 is involved in promoting cell cycle progression and cellular transformation, and preventing apoptosis (Bromberg and Darnell 2000). In addition, the STAT pathway has been shown to have an oncogenic role in various hematologic and solid tumors, including RCC (Buettner et al. 2002; Horiguchi et al. 2002; Li et al. 2013; Song et al. 2007; Xin et al. 2009). The interaction between activated STAT5 and liganded AR has been well documented (Hoang et al. 2015; Song et al. 2014; Tan et al. 2008). Consistent with this, the current study found that DHT treatment increased STAT5 phosphorylation and AR knockdown decreased STAT5 activation in AR-positive RCC cell lines and primary RCC cells. Interestingly, we found that DHT promotes RCC cell growth not only in AR-positive cells, but also in AR-negative cells. Increased STAT5 phosphorylation and cell proliferation was observed in AR-negative SN12C cells after DHT treatment in a concentration-dependent manner, indicating that STAT5 activation by DHT may play a role in the progression of RCC.

The GR mediates the action of glucocorticoids, which regulates cellular differentiation, growth, immune responses, inflammation, and metabolism. However, the association

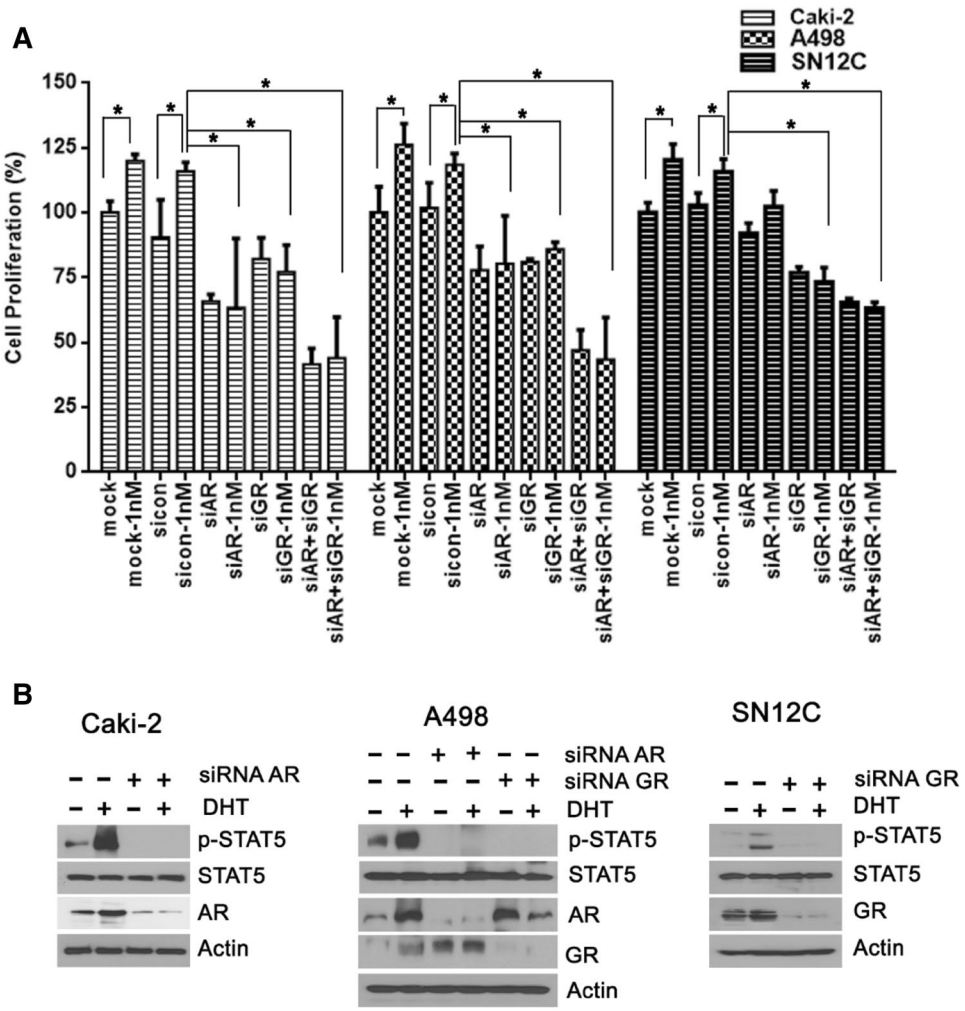

Fig. 3 The effect of DHT (after knockdown of GR and AR) on cell growth, STAT5 phosphorylation, and GR and AR expression in Caki-2, A498, and SN12C cells. **a** Cells were transfected with 2 μM siRNA control, siRNA AR, siRNA GR, or both for 72 h, followed by 1 nM DHT. Cell viability was measured with CellTiter-Glo. Results are expressed ± SEM of three independent experiments. **b** Western blot analysis was performed using the antibodies specified

between RCC and GRs is largely unknown. A previous immunohistochemical study showed GR expression to be present in the glomeruli and proximal tubules of the normal kidney cortex and in 66% of clear cell RCC cases (Yakirevich et al. 2011). In addition, GR expression has been associated with the aggressiveness of RCC (Yakirevich et al. 2011). AR and GR share structural and functional properties as members of the steroid receptor superfamily of ligand-regulated transcription factors (Song et al. 2014; Yakirevich et al. 2011), leading to the hypothesis that DHT can cross-bind to GR. The GR bypass model of AR pathway blockade was proposed by Arora et al. to explain the mechanism of acquired resistance to AR-targeted therapy in patients with castration-resistant prostate cancer (Arora et al. 2013). They found that GR binds to, and regulates, a subset of AR targets. In addition, we previously reported that DHT enhances cell proliferation through STAT5 activation via the GR pathway in castration-resistant prostate cancer cells devoid of ARs (Song et al. 2014). The results of the current study are consistent with the previous observations in prostate cancer cells, showing that DHT appears to utilize the GR pathway in RCC cells to activate the STAT5 pathway. In this study, GR was increased in AR-negative SN12C cell and primary RCC cells treated with DHT. We presume that excessive DHT drives GR activation (Nikolić et al. 2015; Song et al. 2014). The increase in GR was accompanied by an increase in the level of phosphorylated STAT5, suggesting that DHT-bound GR induced STAT5 activation. We observed that, while growth stimulation by DHT through GRs was less effective than through ARs, it was sufficient to significantly increase the cell population (34.3% in Caki-2, 25.0% in A498, and 15.7% in SN12C).

The results obtained in the current study showed that the knockdown of AR or GR with siRNAs decreased cell proliferation and attenuated the effects of DHT in RCC cell lines. AR and GR antagonists also blocked the growth of primary cells from clear cell RCC patients. These results suggest that targeting AR and GR expression may represent a promising new treatment approach for patients with RCC. Currently, radical or partial nephrectomy is the only curative treatment for localized RCC. However, medical treatment is required in approximately one-third of patients who

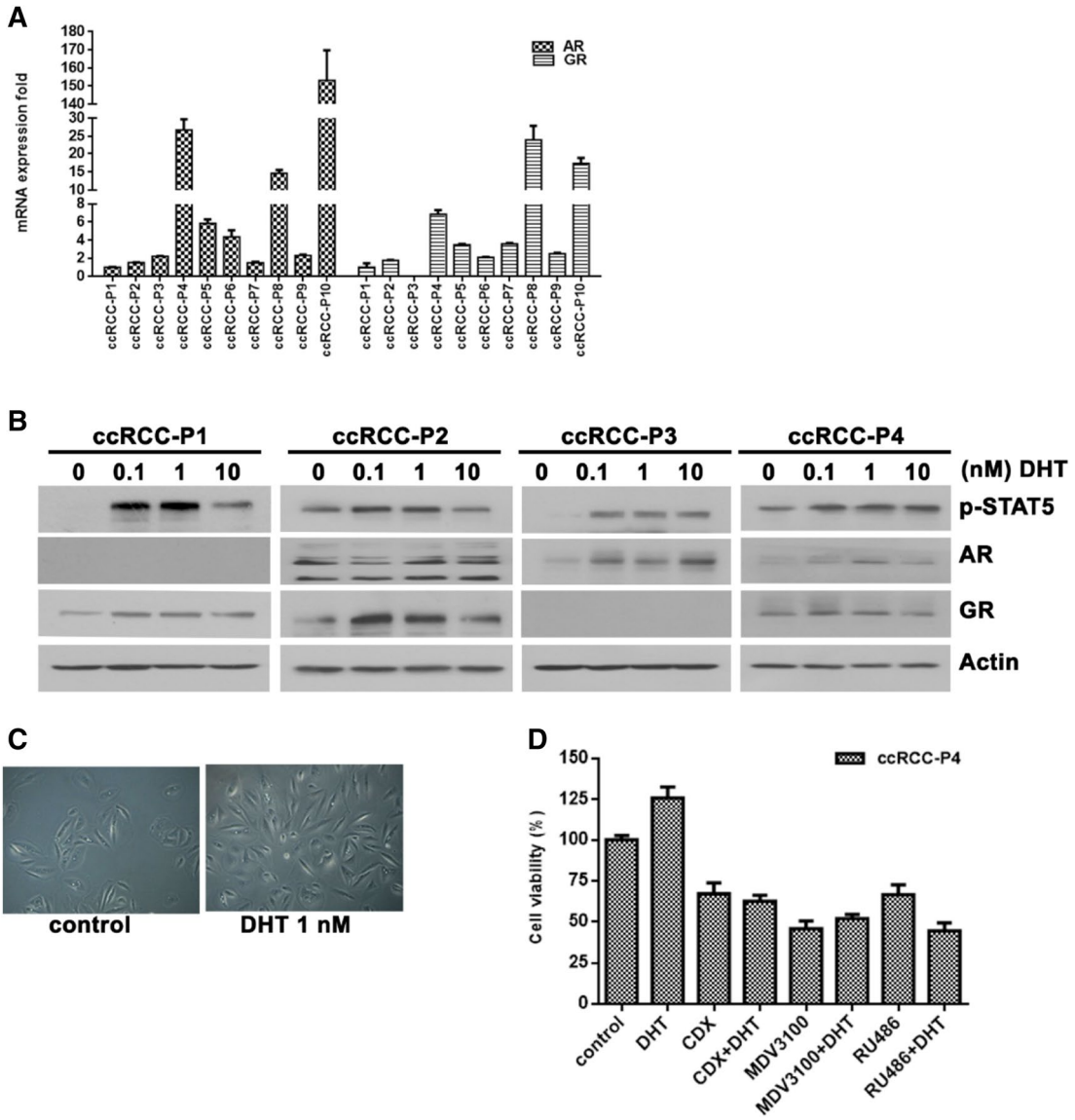

Fig. 4 DHT induces STAT5 activation in human primary renal cell carcinoma cells. **a** Relative mRNA expression levels of AR and GR in primary renal cell carcinoma cells, which were normalized to the expression levels in one primary cell sample from a female patient (patient 1). **b** Human primary renal cell carcinoma cells from patients treated with 0.1–10 nM DHT for 24 h. Western blot analysis was carried out using the antibodies indicated. **c** Cells from a patient (P4) treated with 1 nM DHT for 24 h; images were obtained using an optical microscope. **d** Cells were treated with 10 nM DHT for 24 h in the presence or absence of 1 μM CDX, 10 μM MDV3100, and 10 μM RU486

are initially diagnosed with RCC and a quarter of patients who have undergone surgical resection of RCC. In the past, nonspecific immunotherapy with the cytokines IFNα and IL-2 was used as a standard treatment option for patients with RCC (Choueiri and Motzer 2017). The availability of VEGF- and mTOR-targeted therapies expanded the range of treatment options and prolonged progression-free survival and overall survival. However, almost all patients will eventually develop resistance to these therapies. While new targeted agents and immune checkpoint inhibitors have shown promising efficacy (Choueiri et al. 2016; Motzer et al. 2015), alternative therapeutic targets are required. The clinical evaluation of JAK-STAT signaling inhibitors has been initiated in ongoing trials in a range of other cancers (Johnson et al. 2018), and the results presented here highlight the potential importance of also targeting the JAK-STAT5 pathway in RCC. In addition, a growing number of studies have demonstrated that inhibition of JAK-STAT signaling downregulates programmed cell death protein 1 (PD-1) and/or PD-L1 expression, suggesting that JAK-STAT signaling modulation may have therapeutic potential in combination with immune checkpoint inhibitors (nivolumab or pembrolizumab) to

improve efficacy (Atsaves et al. 2017; Austin et al. 2014; Johnson et al. 2018; Zhang et al. 2016).

In summary, our findings indicate that DHT promotes cell proliferation through STAT5 activation in RCC cells. This stimulatory effect was seen regardless of the AR status of RCC cells. DHT appears to utilize the AR and GR pathways to activate STAT5, and inhibition of ARs and GRs shows antitumor activity in RCC cell lines and primary cells. Together, these results suggest that targeting AR and GR may represent a promising new approach to the treatment of RCC.

Acknowledgements This study was supported by a Grant (W2014-012) from the Asan Institute for Life Sciences, Asan Medical Center, Seoul, Korea.

Compliance with ethical standards

Conflict of interest The authors declare no conflict of interest.

References

Alexander SP et al (2015) The concise guide to PHARMACOLOGY 2015/16: nuclear hormone receptors. Br J Pharmacol 172:5956

Arora VK et al (2013) Glucocorticoid receptor confers resistance to antiandrogens by bypassing androgen receptor blockade. Cell 155:1309–1322. https://doi.org/10.1016/j.cell.2013.11.012

Atsaves V et al (2017) PD-L1 is commonly expressed and transcriptionally regulated by STAT3 and MYC in ALK-negative anaplastic large-cell lymphoma. Leukemia 31:1633

Austin JW, Lu P, Majumder P, Ahmed R, Boss JM (2014) STAT3, STAT4, NFATc1, and CTCF regulate PD-1 through multiple novel regulatory regions in murine T cells. J Immunol 192(10):4876–4886. https://doi.org/10.4049/jimmunol.1302750

Bianchi M, Meng C, Ivashkiv LB (2000) Inhibition of IL-2-induced Jak-STAT signaling by glucocorticoids. Proc Natl Acad Sci 97:9573–9578

Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A (2018) Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin 68:394–424

Bromberg J, Darnell JE (2000) The role of STATs in transcriptional control and their impact on cellular function. Oncogene 19:2468

Buettner R, Mora LB, Jove R (2002) Activated STAT signaling in human tumors provides novel molecular targets for therapeutic intervention. Clin Cancer Res 8:945–954

Chang C, Lee S, Yeh S, Chang T (2014) Androgen receptor (AR) differential roles in hormone-related tumors including prostate, bladder, kidney, lung, breast and liver. Oncogene 33:3225

Choueiri TK, Motzer RJ (2017) Systemic therapy for metastatic renal-cell carcinoma. N Engl J Med 376:354–366

Choueiri TK et al (2016) Cabozantinib versus everolimus in advanced renal cell carcinoma (METEOR): final results from a randomised, open-label, phase 3 trial. Lancet Oncol 17:917–927

Ferbeyre G, Moriggl R (2011) The role of Stat5 transcription factors as tumor suppressors or oncogenes. Biochimica Et Biophysica Acta (BBA) Rev Cancer 1815:104–114

Ha Y-S, Lee GT, Modi P, Kwon YS, Ahn H, Kim W-J, Kim IY (2015) Increased expression of androgen receptor mRNA in human renal cell carcinoma cells is associated with poor prognosis in patients with localized renal cell carcinoma. J Urol 194:1441–1448

He D et al (2014a) ASC-J9 suppresses renal cell carcinoma progression by targeting an androgen receptor-dependent HIF2α/VEGF signaling pathway. Can Res 74:4420–4430

He D et al (2014b) New therapy via targeting androgen receptor → HIF-2α → VEGF signals with ASC-J9® to suppress renal cell carcinoma progression. Cancer Res 2681:2013

Hoang DT et al (2015) Inhibition of Stat5a/b enhances proteasomal degradation of androgen receptor liganded by antiandrogens in prostate cancer. Mol Cancer Ther 14:713–726

Horiguchi A, Oya M, Shimada T, Uchida A, Marumo K, Murai M (2002) Activation of signal transducer and activator of transcription 3 in renal cell carcinoma: a study of incidence and its association with pathological features and clinical outcome. J Urol 168:762–765

Johnson DE, O'Keefe RA, Grandis JR (2018) Targeting the IL-6/JAK/STAT3 signalling axis in cancer. Nat Rev Clin Oncol 15:234

Jonasch E, Gao J, Rathmell WK (2014) Renal cell carcinoma. BMJ 349:g4797

Langner C, Ratschek M, Rehak P, Schips L, Zigeuner R (2004) Steroid hormone receptor expression in renal cell carcinoma: an immunohistochemical analysis of 182 tumors. J Urol 171:611–614

Lee GT et al (2017) Intracrine androgen biosynthesis in renal cell carcinoma. Br J Cancer 116:937

Li S et al (2013) Icaritin inhibits JAK/STAT3 signaling and growth of renal cell carcinoma. PLoS One 8:e81657

Lucca I, Klatte T, Fajkovic H, De Martino M, Shariat SF (2015) Gender differences in incidence and outcomes of urothelial and kidney cancer. Nat Rev Urol 12:585

Motzer RJ et al (2015) Nivolumab versus everolimus in advanced renal-cell carcinoma. N Engl J Med 373:1803–1813

Nikolić M et al (2015) Possible involvement of glucocorticoids in 5α-dihydrotestosterone-induced PCOS-like metabolic disturbances in the rat visceral adipose tissue. Mol Cell Endocrinol 399:22–31

Siegel RL, Miller KD, Jemal A (2017) Cancer statistics, 2017. CA Cancer J Clin 67:7–30

Song C, Jun S-Y, Hong J-H, Ahn H (2007) Transforming growth factor-β downregulates interleukin-2-induced phosphorylation of signal transducer and activator of transcription 5 in human renal cell carcinoma. J Cancer Res Clin Oncol 133:487–492

Song C, Kim Y, Min GE, Ahn H (2014) Dihydrotestosterone enhances castration-resistant prostate cancer cell proliferation through STAT5 activation via glucocorticoid receptor pathway. Prostate 74:1240–1248

Stöcklin E, Wissler M, Gouilleux F, Groner B (1996) Functional interactions between Stat5 and the glucocorticoid receptor. Nature 383:726

Stone L (2014) Androgen receptor—a new target in renal cell carcinoma? Nat Rev Urol 11:425–426

Tan S-H et al (2008) Transcription factor Stat5 synergizes with androgen receptor in prostate cancer cells. Can Res 68:236–248

Vogelstein B, Kinzler KW (2004) Cancer genes and the pathways they control. Nat Med 10:789

Wang K, Sun Y, Tao W, Fei X, Chang C (2017) Androgen receptor (AR) promotes clear cell renal cell carcinoma (ccRCC) migration and invasion via altering the circHIAT1/miR-195-5p/29a-3p/29c-3p/CDC42 signals. Cancer Lett 394:1–12

Whitfield GK, Jurutka PW, Haussler CA, Haussler MR (1999) Steroid hormone receptors: evolution, ligands, and molecular basis of biologic function. J Cell Biochem 75:110–122

Wingelhofer B et al (2018) Implications of STAT3 and STAT5 signaling on gene regulation and chromatin remodeling in hematopoietic cancer. Leukemia 32(8):1713–1726. https://doi.org/10.1038/s41375-018-0117-x

Xin H, Zhang C, Herrmann A, Du Y, Figlin R, Yu H (2009) Sunitinib inhibition of Stat3 induces renal cell carcinoma tumor cell apoptosis and reduces immunosuppressive cells. Cancer Res. https://doi.org/10.1158/0008-5472.Can-08-4323

Yakirevich E et al (2011) Expression of the glucocorticoid receptor in renal cell neoplasms: an immunohistochemical and quantitative reverse transcriptase polymerase chain reaction study. Hum Pathol 42:1684–1692

Yu H, Lee H, Herrmann A, Buettner R, Jove R (2014) Revisiting STAT3 signalling in cancer: new and unexpected biological functions. Nat Rev Cancer 14:736

Zhang N, Zeng Y, Du W, Zhu J, Shen D, Liu Z, Huang J-A (2016) The EGFR pathway is involved in the regulation of PD-L1 expression via the IL-6/JAK/STAT3 signaling pathway in EGFR-mutated non-small cell lung cancer. Int J Oncol 49:1360–1368

Zhao H, Leppert JT, Peehl DM (2016) A protective role for androgen receptor in clear cell renal cell carcinoma based on mining TCGA data. PLoS One 11:e0146505

Zhu G et al (2014) The expression and evaluation of androgen receptor in human renal cell carcinoma. Urology 83:510.e519–510.e524