

The effect of androgen stimulation on the Ishikawa endometrial cancer cell line

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Introduction

Endometrial cancer (EC) is the 4th most common cancer in women in the UK. It accounted for approximately 97,000 deaths worldwide in 2020, with more than 400,000 newly diagnosed patients. While unopposed oestrogen is thought to be the main culprit risk factor for the pathogenesis of EC, hyperandrogenaemia has also been reported to increase the risk of EC. This effect is hypothesised to be secondary to peripheral aromatisation in adipose tissues into oestrogen however, the direct role of androgen on the development of EC has not been thoroughly investigated.

Aims

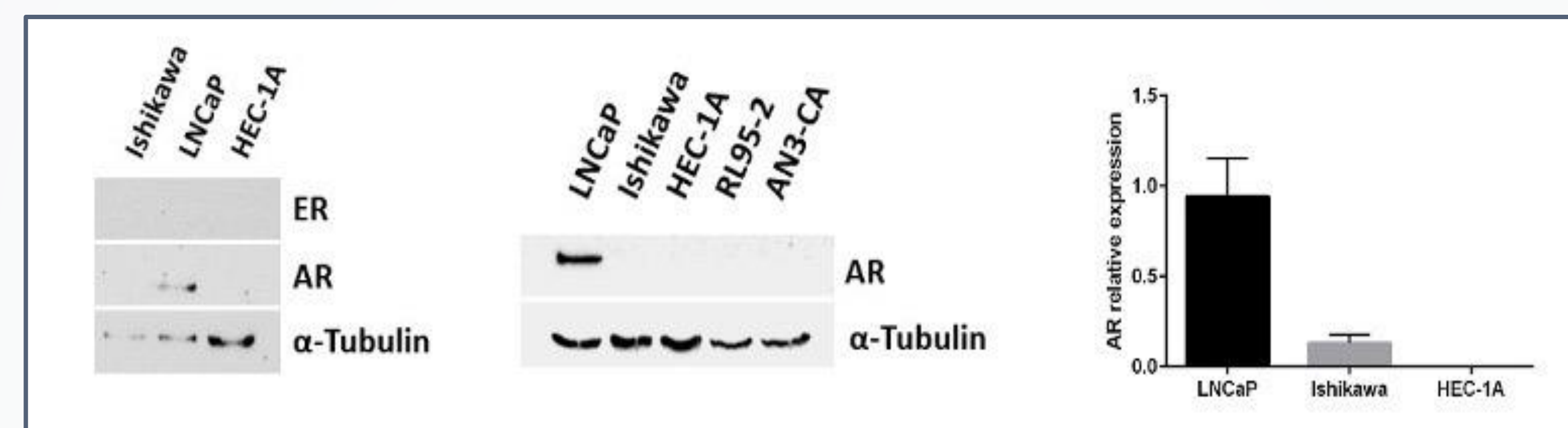
To study the effect of natural and synthetic androgen stimulation on the Ishikawa cancer cell line.

Methods

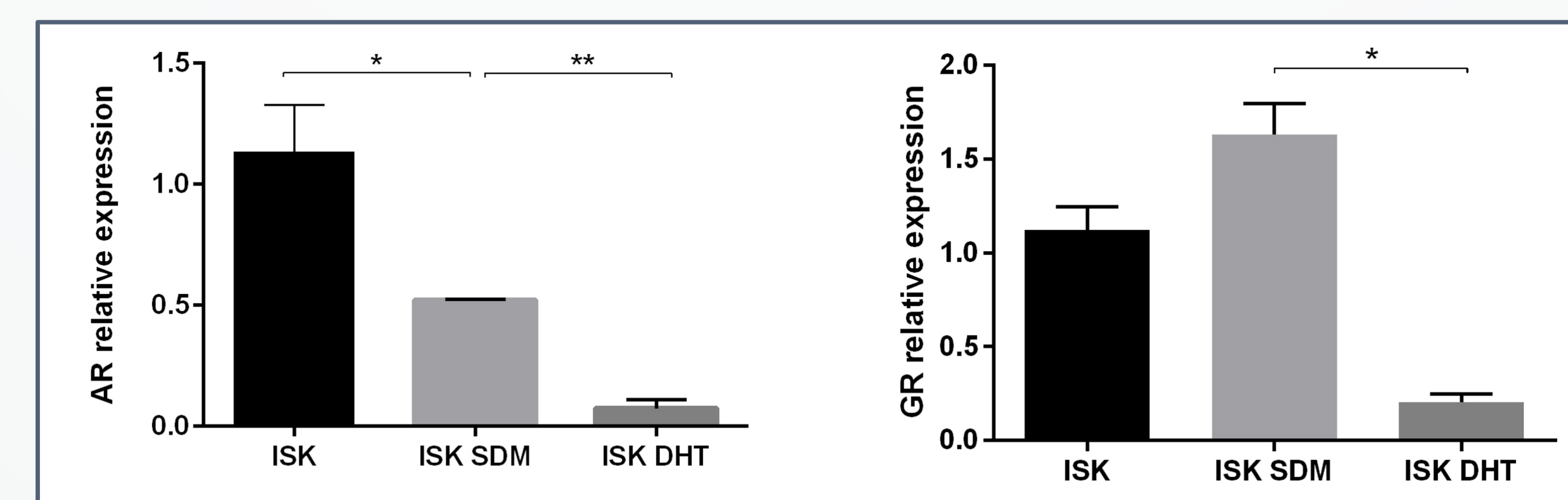
Ishikawa cells were maintained in standard cell culture media, steroid-depleted media (SDM) or SDM supplemented with 10nM of dihydrotestosterone (DHT). To assess the effect of DHT exposure on the expression of Androgen Receptor (AR), western blot and reverse transcription quantitative polymerase chain reaction (RT-qPCR) were performed. SRB assay was utilised to examine the proliferative effects of 5 different doses of either DHT or the synthetic androgen (R1881) alongside vehicle control across 5 days to study the impact of androgen stimulation on cell proliferation.

Results

AR protein levels in various EC cell lines were undetectable via western blot, compared to the prostate cell line (LNCaP) as a positive control. In contract, qPCR showed detectable levels of AR mRNA in Ishikawa cells, albeit at approximately 7-fold less than the LNCaP cells.

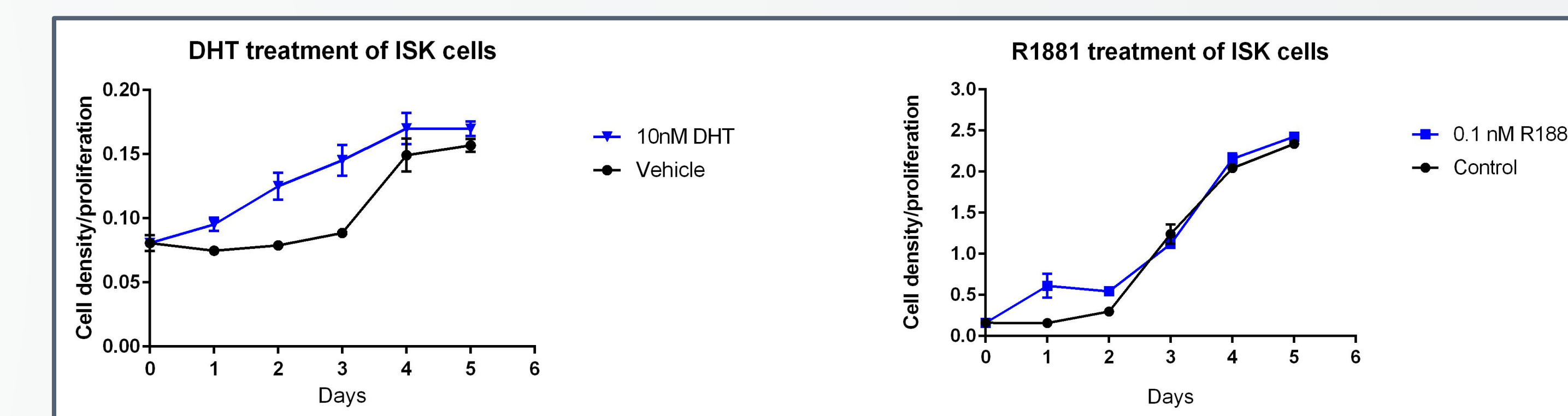


qPCR showed a decreased level of the AR transcripts in the Ishikawa cell line after steroid depletion and further decline following DHT treatment. Glucocorticoid receptor (GR) levels showed an insignificant increase in the mRNA levels following steroid depletion, however the addition of DHT caused a statistically significant 5-fold reduction in GR mRNA expression.



Results (continued)

SRB proliferation assay showed statistically significant increase in proliferation in response to DHT stimulation, with the most significance seen with 10nM DHT dose. The peak of proliferation difference between 10nM DHT treatment and vehicle control was seen on both Day 2 and 3. Treatment with R1881 also showed increase in cellular proliferation, albeit less significant difference when compared to controls.



Conclusion

Our provisional data showed an increase in proliferation in response to DHT stimulation with optimal dose of 10nM and treatment duration of 2-3 days. Treatment with R1881 showed a less significant rise in proliferation, particularly on Day 1. While our results are still provisional, they open the door for more investigation of the role of targeting AR in treating EC

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