# Functional interaction between the von Hippel-Lindau protein and Androgen receptor in ccRCC


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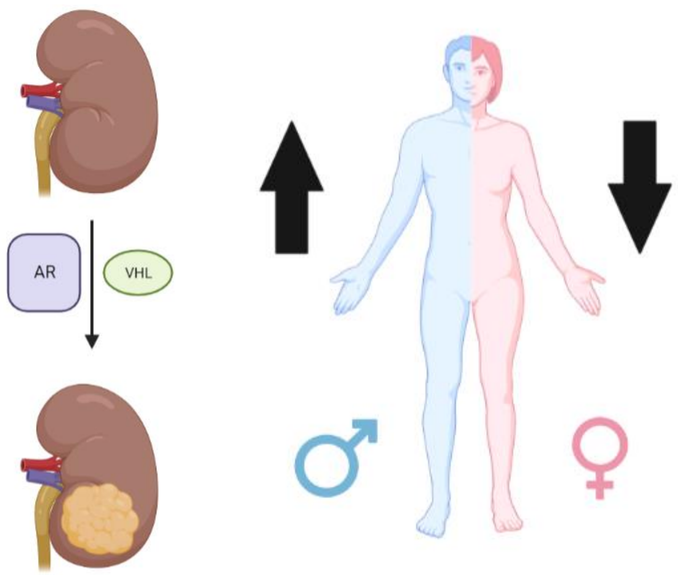
## BACKGROUND

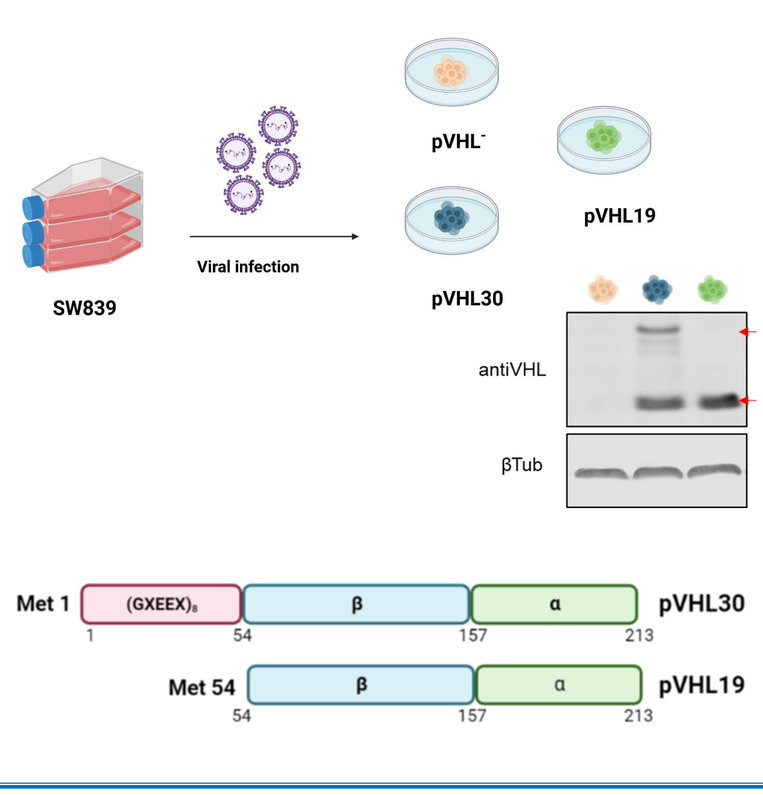
More than 80% of clear cell renal cell carcinomas (ccRCC) are linked to loss of function (LOF) mutations of the von Hippel-Lindau protein (pVHL). Males have a higher risk of developing renal cancer compared to females, implying a role for androgens in the etiology of the disease. The main mediator of androgen signaling is Androgen Receptor (AR). AR and pVHL have been reported to structurally and functionally interact with each other. However, how the functional relationship between AR and pVHL is regulated and how it impacts ccRCC gender bias is unknown. In this study, we used molecular, cellular and biochemical analysis to characterize pVHL/AR interaction in different ccRCC cell models.

## AIM

**To elucidate whether the interplay between AR and pVHL has a role in ccRCC sex discrepancy**




## EXPERIMENTAL MODEL




## RESULTS

### PPi network predicts a direct interaction AR-pVHL

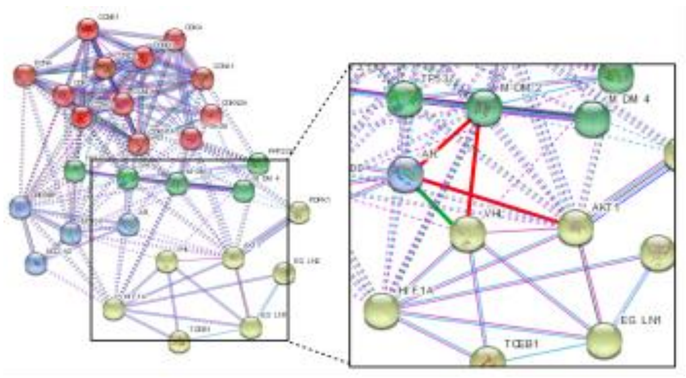



**Figure 1. Protein-protein interaction network centered around AR and pVHL** Connections between nodes represent experimental evidence while bubbles are for proteins forming the network. Proteins are grouped by shared biological pathways and colored accordingly. A green line is used to underline the novel association between AR and pVHL.

### Both pVHL isoforms interact with AR

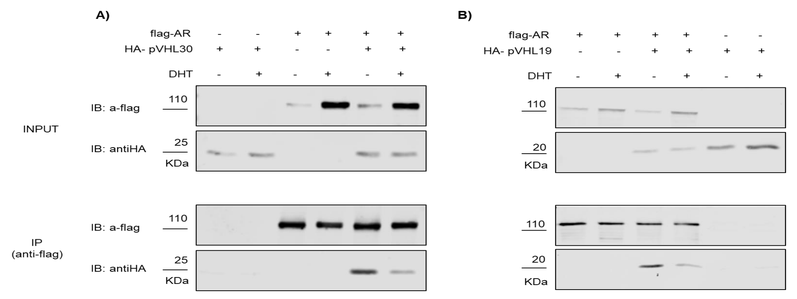



**Figure 2. Co-ip experiments in HEK293T cells showing protein-protein interactions between AR and pVHL isoforms A)** AR and pVHL30 (full-length isoform) – **B)** AR and pVHL19 (shorter isoform) As reported in panels A and B these physical associations are regulated by androgens binding suggesting that AR in its unliganded state forms a complex with pVHL which detach upon DHT treatment (n=3).

### pVHL doesn't change AR protein accumulation

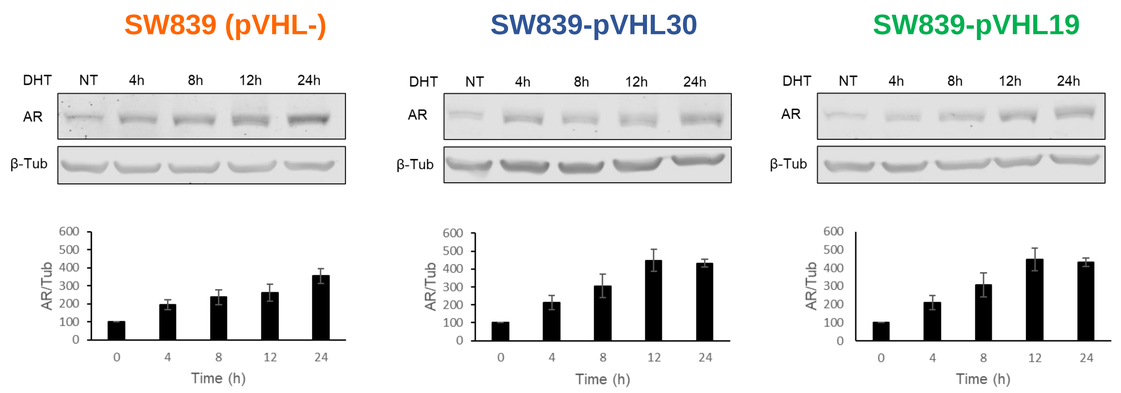



**Figure 3. Analysis of AR accumulation upon DHT treatment in ccRCC cells** pVHL30 and pVHL19 do not influence AR protein accumulation. Indeed after 24h of treatment, AR levels in all three cell lines are similar (n=3).

### pVHL influences AR protein turnover in an isoform-specific manner

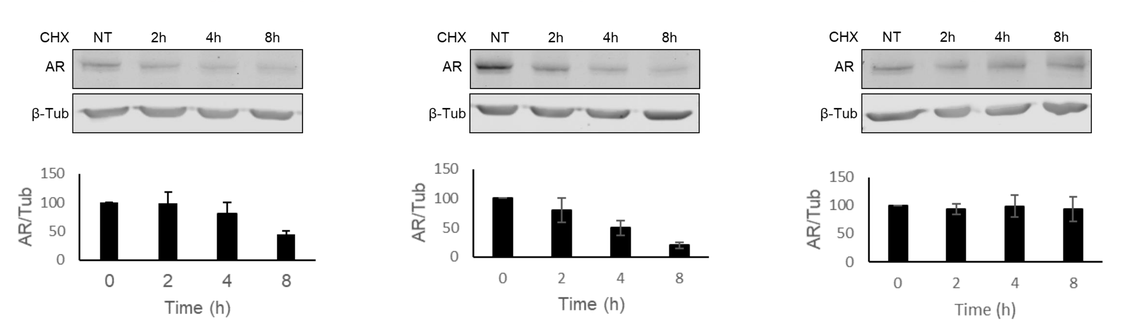



**Figure 4. Turnover experiments in ccRCC cell lines showing pVHL isoform-specific impact on AR stability** pVHL affects AR protein stability in an isoform-specific manner. Indeed, pVHL30 seems to increase the AR protein turnover, while pVHL19 lacks this function yielding to AR stabilization (n=3).

### pVHL effects on cell proliferation are reduced by AR activation

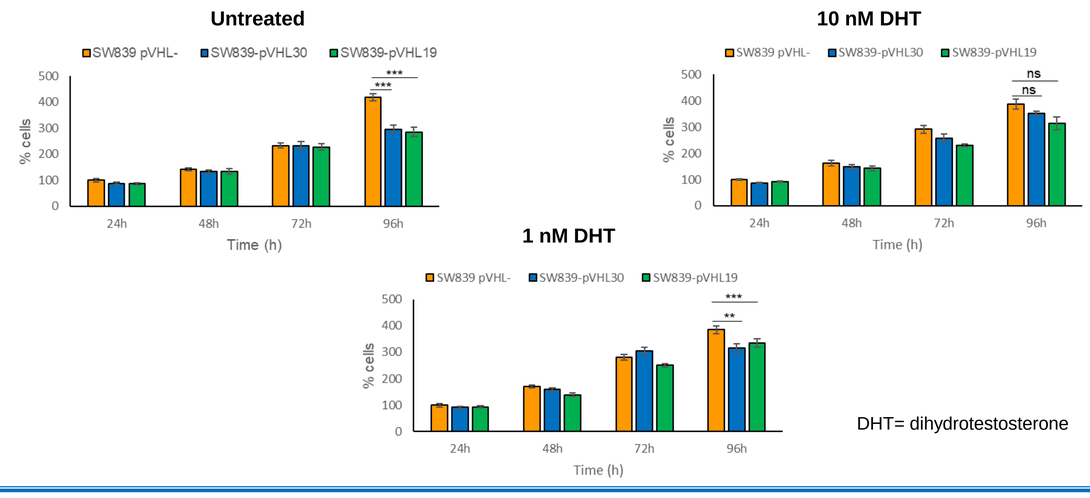



**Figure 5. Proliferation assays measuring both pVHL and androgens effect on ccRCC cell lines** pVHL isoforms expression decrease ccRCC cell proliferation which is recovered by AR activation upon treatment with 10 nM DHT. This finding suggests that AR in its active state may act as pro-proliferative factor in renal cancer (n=3).

DHT= dihydrotestosterone

## CONCLUSIONS

To sum up:

- pVHL isoforms interact with AR regulating its protein stability in an isoform-specific fashion: pVHL30 increases the AR protein turnover while pVHL19 lacks this function leading to AR stabilization;
- pVHL doesn't change AR accumulation upon DHT treatment;
- pVHL expression in RCC cells reduces cell proliferation which is recovered by AR activation upon ligand binding.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