

## RESEARCH HIGHLIGHT

# Driver or passenger - roles of the glucocorticoid receptor in castration resistance prostate cancers

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In North America, prostate cancer is the most prevalent cancer in men and the second leading cause of their cancer deaths. With the rate of new cases rising each year, prostate cancer poses a heavy burden on both the economy and society. While the first line of treatment for metastatic prostate cancer is androgen deprivation therapy, it has become evident that tumors eventually become castration resistant. One of the proposed mechanisms by which tumors overcome androgen deprivation therapy is through the expression and activation of glucocorticoid receptors. However, whether the glucocorticoid receptor functions as a key driver for castration resistant progression or a biomarker reflecting androgen receptor activity remains elusive. In our recent study, we utilized tissue microarrays and multiple prostate cancer xenograft and cell models to investigate the roles of the glucocorticoid receptor during castration resistant progression. As a result, we determined that the expression of the glucocorticoid receptor is inversely correlated with androgen receptor activity and is not associated with castration resistant phenotypes. In addition, we identified a negative androgen responsive element in the promoter region of the glucocorticoid receptor gene through chromatin immunoprecipitation analysis combined with DNA sequencing technology. We showed that the androgen receptor interacted directly to this response element to exert suppressive effects on the transcription of the glucocorticoid receptor gene. In conclusion, the androgen receptor negatively regulates the expression of the glucocorticoid receptor and can potentially serve as a biomarker to monitor prostate tumor progression.

**Keywords:** glucocorticoid receptor; androgen receptor; prostate tumor

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## Castration-Resistant Prostate Cancer

Prostate cancer (PCa) is the most prominent cancer in men and the second leading cause of their cancer death in North America [1]. With the incidences of PCa rising each year, PCa care costs increase in parallel posing a heavy burden on both the economy and society [2, 3]. Even with new androgen deprivation therapy (ADT) for metastatic PCa patients, most

tumors recur on average 19 months after initial treatment [4]. Once at this stage, no curative therapy is available [4,5]. Therefore, it is important to investigate the molecular mechanisms by which PCa cells develop resistance to ADT in PCa patients.

Primarily, the growth and survival of PCa cells depend on androgen [6, 7]. Thus, for several decades, the recognized

principal treatment for metastatic PCa is ADT that either inhibits androgen synthesis and/or blocks the activation of the androgen receptor (AR) [7-9]. Although PCa patients initially respond well to ADT, the tumors eventually become castration resistant PCa (CRPC) [4, 5, 10, 11]. Despite the extremely low levels of circulating androgens, AR activity continues to be functional in most CRPC cells [4, 9]. Even resistance to newest generation anti-androgen drugs such as Enzalutamide in metastatic tumors is inevitable through many proposed mechanisms such as gain-of-function mutations, amplifications, or post-transcriptional modifications of the AR gene [12,13]. In addition, emerging evidence shows that a subtype of CRPC tumors such as neuroendocrine tumors is completely AR negative and able to bypass androgen deprivation [13]. One of the recently proposed mechanisms by which CRPC tumors bypass AR blockade is through the activation and overexpression of the glucocorticoid receptors (GR) [14,15].

### **The Roles of Glucocorticoid Receptor in Prostate Cancers**

The role of GR in PCa tumors has been controversial in several studies and whether it acts as a driver in CRPC progression remains to be determined. In previous studies, it was reported that GR had anti-proliferative functions in both *in vivo* and *in vitro* prostate cancer models [16-19]. Contrary to these studies, others have shown that GR acted as a positive regulator for cancers in promoting rapid tumor progression [15]. However, corticosteroids are common drugs administered to PCa patients undergoing ADT, chemo-, and radiation-therapy for side effects such as inflammation and pain relief [20]. It had been reported in multiple clinical trials that CRPC patients who were administered corticosteroids post-ADT treatments usually showed symptomatic improvements and lower PSA levels through a feedback inhibitory mechanism triggered by exogenous glucocorticoids on androgen synthesis [21-23]. Thus, many questions arise and remain unanswered for GR functions in PCa. It is critical to elucidate the roles of GR in PCa with the consideration of several different aspects such as tumor heterogeneity to understand multi-functional properties of GR signaling.

Recent studies proposed that GR conferred resistance to anti-androgens through bypassing AR signaling blockade in LNCaP xenograft models [14]. GR shows high protein sequence homology to the AR in their DNA binding domains [24]. It was reported that over 50% of AR and GR targeted genes including PSA were commonly regulated by both receptors [14]. As a result, it was proposed that GR might have a similar functional role as AR in continuously driving AR-targeted gene expressions in tumors undergoing ADT

[14]. In addition, clonal selection of LNCaP xenografts after long-term Enzalutamide treatment showed a gain of GR expression, further supporting that GR may compensate the inactivated AR signaling in CRPC tumors [14]. Moreover, the capacity of GR to drive aggressive phenotypes of CRPC was supported by the observation that rapid tumor progression was correlated with higher GR expression in LNCaP xenografts and human metastatic tumors [14].

### **Androgen Receptor Signaling Negatively Regulates GR Expression in Prostate Cancers**

In our recent study, we showed that there was an inverse correlation between AR activity and GR protein expression during PCa progression [25]. This conclusion was drawn from a series of immunohistochemistry studies performed on human PCa tissue microarrays as well as various xenograft tumor models. Pathological scoring of GR expression in PCa tissues showed increases in GR protein levels under ADT treatment. However, GR levels dropped to pre-ADT levels when the tumors progressed into the CRPC stage. Although the C4-2 xenografts showed a heterogeneous AR positivity, AR negative cells were always GR positive, further supporting the conclusion that AR has a suppressive effect on GR expression. Collectively, our results demonstrate that GR expression is suppressed by AR signaling.

### **GR Expression is Not Correlated with Aggressive Phenotypes of Prostate Cancer Tumors**

In addition, we showed that GR expression was comparatively similar in tumors characterized by different Gleason scores [25]. Lower GR expression was observed in castration resistant patient biopsies, patient derived xenografts, and C4-2 xenografts. Through these multiple patient samples and tumor models, we concluded that reduced expression of the GR was associated with more aggressive CRPC phenotypes. In several CRPC patient biopsies, GR was highly expressed while both AR and PSA expressions were negative. These findings indicate that, at least in these tumors, elevated GR expression could not compensate for the AR in driving AR-targeted gene expression such as PSA. These results provide further support that CRPC tumors may develop a different mechanism that is independent of AR signaling. Furthermore, some of these tumors presented neuroendocrine morphological changes [13]. Together, these results show that the GR does not substitute the AR in driving CRPC progression.

### **A Novel Mechanism in which Androgen Receptor Signaling Inhibits Glucocorticoid Expression**

Furthermore, our study provided new evidence indicating a novel mechanism by which AR-signaling impedes on the transcriptional activity of the GR gene [25]. We performed ChIP-seq assays in different PCa cell lines cultured in either androgen depleted medium or medium containing DHT, an androgen agonist. A DNA fragment containing a negative androgen responsive element (nARE) on the GR gene was precipitated by AR antibodies at about 160Kbp upstream of the transcription initiation site. Moreover, we determined through gel-shift assay analysis that AR association with the nARE region was direct and dependent on the presence of DHT.

Finally, we confirmed that GR transcriptional repression mediated by AR was dependent on the presence of the nARE [25]. Site mutagenesis of the nARE abolished AR-mediated repression of GR promoter activity. AR recruitment to the GR gene caused reduction of Histone 3 acetylation at not only the nARE region, but also the exon 1 and 2 of the GR gene, suggesting that the nARE can recruit AR to suppress GR gene transcription via a long-range regulatory mechanism. Interestingly, endogenous GR expression inhibited by liganded-AR has a greater repressive effect than our luciferase assays, suggesting that other cis-elements and protein factors between GR gene and nARE may also be involved in regulating GR transcription. This observation may, in part, explain why a portion of PCa tumors do not show an inverse correlation between GR expression and AR signaling.

### Concluding Remarks

In summary, our studies as well as studies from others consensually agree that the AR signaling in PCa cells negatively regulates GR expression. However, our study demonstrates that increased GR expression is not associated with a more aggressive tumor phenotypes. Rather, GR expression reflects the AR-signaling activity in prostate tumors and can potentially serve as a biomarker for the effectiveness of AR signaling blockade. The increased expression of GR in some CRPC tumors may suggest that these tumors may no longer possess active AR signaling and/or no longer require the AR signaling for survival. Moreover, we identified a nARE located near the promoter region of the GR gene that can recruit AR to repress the transcriptional activity of the GR. In conclusion, GR can potentially be a biomarker to monitor the activity of AR signaling in PCa patients.

### Conflict of Interest

The authors declare that there is no conflict of interest.

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