Contribution of the GR-LEDGF/p75 Axis to Prostate Cancer Chemoresistance

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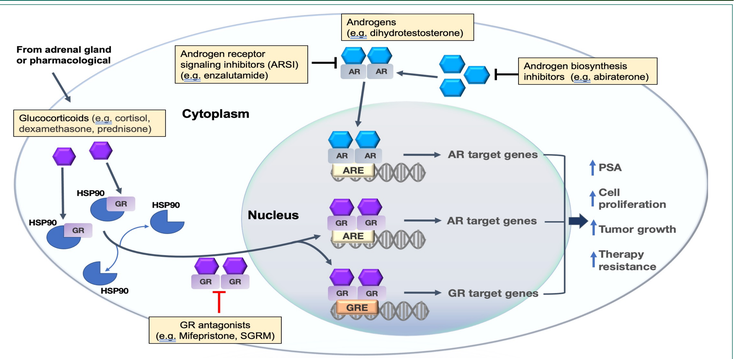
INTRODUCTION

Prostate cancer (PCa) is the second leading cause of cancer deaths in U.S. men, disproportionally affecting men of African ancestry (AA) such as African American, Afro-Caribbean, and West-African. Advanced PCa is treated with anti-androgen therapy and chemotherapy using the drug docetaxel (DTX). Glucocorticoids are administered to PCa patients during therapy and have been recently implicated in PCa resistance to anti-androgen therapy via an "androgen receptor (AR) bypass" mechanism, and in resistance to chemotherapy. This may be critical to AA men with PCa since they have chronically elevated endogenous glucocorticoid levels compared to Caucasian American (CA) men. These high glucocorticoid levels are associated with cumulative life stressors linked to low socioeconomic status such as living in areas with poverty, racism, crowded living, high crime, and low access to quality healthcare. Potentially, these chronically high levels may predispose AA men with PCa to increased therapy resistance.

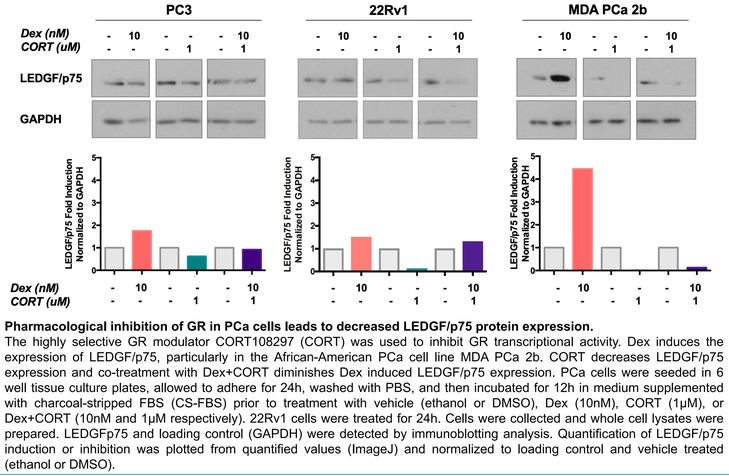
Glucocorticoids bind to the glucocorticoid receptor (GR) to exert their actions, but the GR-mediated mechanisms of chemoresistance are unknown, as well as their possible contribution to PCa mortality disparities. Our group has previously reported that glucocorticoids upregulate the chemoresistance-associated protein LEDGF/p75 in PCa cells and identified consensus GR binding sites in the promoter region of the gene encoding this protein. Here we explore the **hypothesis** that GR transcriptionally regulates and interacts with LEDGF/p75 to enhance chemoresistance in PCa cells.

FIG 1. THE GR "BYPASS" IN PROSTATE CANCER



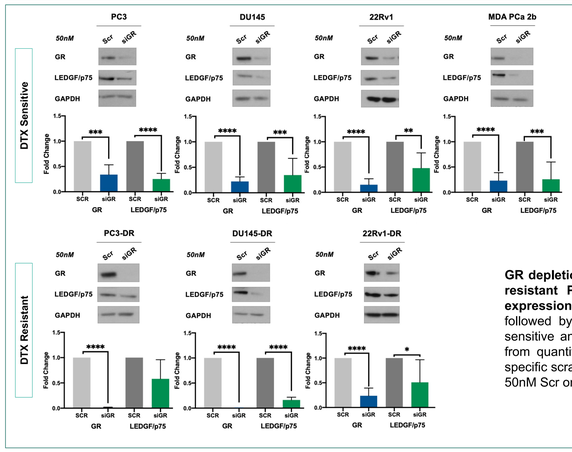
The interplay between AR and GR in the context of PCa. AR and GR belong to the same family of nuclear receptors involved in the transcriptional regulation of genes associated with prostate cell proliferation, tumor growth, and therapy resistance. They bind to androgen receptor elements (ARE) or glucocorticoid response elements (GRE) in the promoter regions of AR- and GR-target genes. Blockade of AR with anti-androgen drugs temporarily reduces PCa growth but leads to the activation of GR, which in turn "bypasses" AR signaling and activates both AR- and GR- target genes by binding to their promoter AREs and GREs. This GR activation leads to increased expression of genes that promote prostate tumor growth and chemotherapy resistance.

FIG 2. LEDGF/p75 EXPRESSION DECREASES AFTER GR BLOCKADE WITH CORT108297 IN PCa CELLS



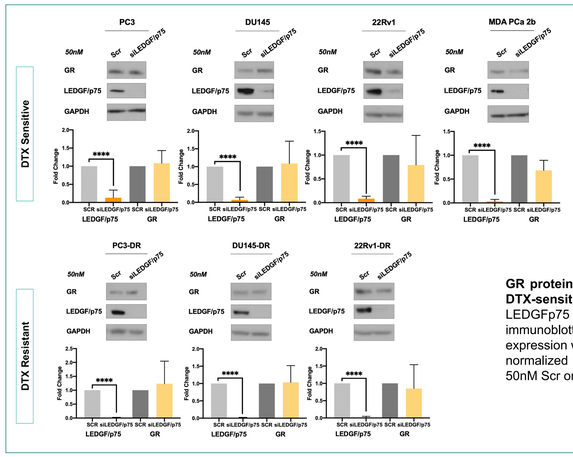
Pharmacological inhibition of GR in PCa cells leads to decreased LEDGF/p75 protein expression. The highly selective GR modulator CORT108297 (CORT) was used to inhibit GR transcriptional activity. Dex induces the expression of LEDGF/p75, particularly in the African-American PCa cell line MDA PCa 2b. CORT decreases LEDGF/p75 expression and co-treatment with Dex+CORT diminishes Dex induced LEDGF/p75 expression. PCa cells were seeded in 6 well tissue culture plates, allowed to adhere for 24h, washed with PBS, and then incubated for 12h in medium supplemented with charcoal-stripped FBS (CS-FBS) prior to treatment with vehicle (ethanol or DMSO), Dex (10nM), CORT (1μM), or Dex+CORT (10nM and 1μM respectively). 22Rv1 cells were treated for 24h. Cells were collected and whole cell lysates were prepared. LEDGFp75 and loading control (GAPDH) were detected by immunoblotting analysis. Quantification of LEDGF/p75 induction or inhibition was plotted from quantified values (ImageJ) and normalized to loading control and vehicle treated (ethanol or DMSO).

FIG 3. LEDGF/p75 EXPRESSION DECREASES AFTER GR SILENCING IN PCa CELLS



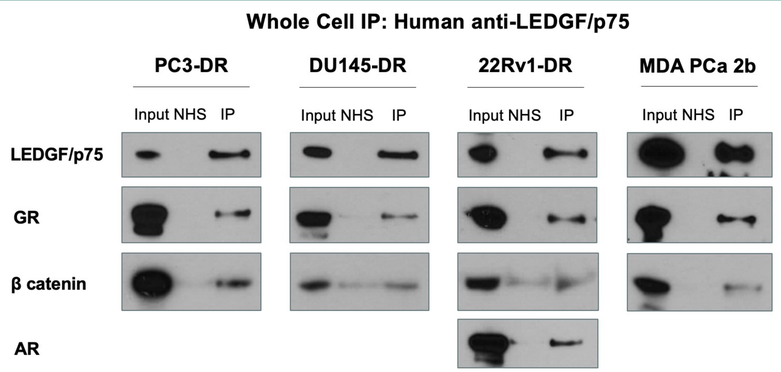
GR depletion via gene silencing (siRNA) in DTX-sensitive and -resistant PCa cells leads to decreased LEDGF/p75 protein expression. Transient knockdown of GR (siGR) was performed followed by immunoblotting detection of GR in docetaxel (DTX)-sensitive and -resistant cells. LEDGF/p75 expression was plotted from quantified values (ImageJ software) and normalized to non-specific scrambled control (Scr). Transfections were performed using 50nM Scr or 50nM total tri-silencer siRNA against GR for 72h.

FIG 4. GR EXPRESSION IS NOT ALTERED UPON LEDGF/p75 SILENCING IN PCa CELLS



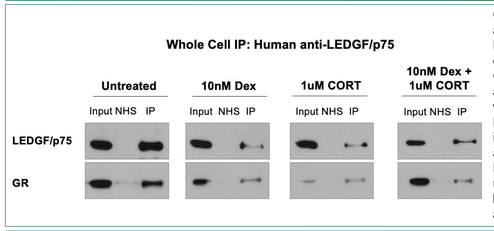
GR protein levels are not altered after LEDGF/p75 depletion in DTX-sensitive and -resistant cells. Transient knockdown of LEDGFp75 (siLEDGF/p75) was performed followed by immunoblotting detection of LEDGF/p75 in PCa lysates. GR expression was plotted from quantified values (ImageJ software) and normalized to non-specific Scr. Transfections were performed using 50nM Scr or 50nM siRNA-LEDGF/p75 for 72h.

FIG 5. LEDGF/p75, GR, AR, AND β-CATENIN INTERACT IN DTX-SENSITIVE AND -RESISTANT PCa CELLS



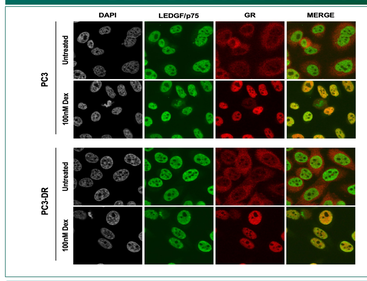
LEDGF/p75, GR, AR and β-catenin interact in DTX-sensitive and -resistant PCa cells. Immunoprecipitations were performed using a human serum containing autoantibodies specific to LEDGF/p75. IP was confirmed by immunoblotting with rabbit monoclonal antibodies specific for either GR, LEDGF/p75, β-catenin, or AR. Normal human serum (NHS) was used as negative control for IP and whole cell lysates collected from IP reactions were used as input (1% of IP).

FIG 6. GR BLOCKADE WITH CORT108297 DOES NOT AFFECT THE LEDGF/p75-GR INTERACTION



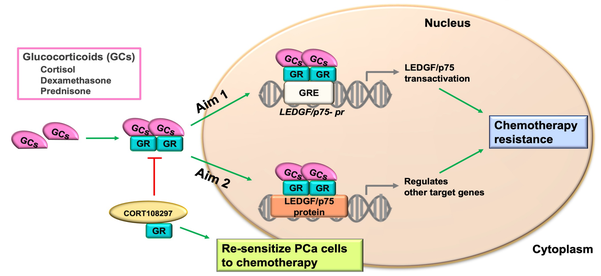
GR blockade with CORT108297 does not affect the LEDGF/p75-GR interaction in DTX-resistant PC3-DR PCa cells. PC3-DR cells were treated with Dex (10nM) for 1hr, CORT (1μM) for 6hr, or Dex+CORT (10nM and 1μM respectively). Immunoprecipitations were performed using specific human anti-LEDGF/p75 serum. IP was confirmed by immunoblotting with rabbit monoclonal antibodies specific for either GR or LEDGF/p75. Normal human serum (NHS) was used as negative control for IP and whole cell lysates collected from IP reactions were used as input (1% of IP).

FIG 7. LEDGF/p75 AND GR COLOCALIZE IN DTX-SENSITIVE AND -RESISTANT PCa CELLS



LEDGF/p75 colocalizes with GR in PC3 and PC3-DR cells. Cells were treated with 100nM Dex for 30 mins. Human anti-LEDGF/p75 antibody displays the dense fine speckled nuclear pattern detected with FITC-labeled secondary anti-human antibody (green). GR was co-incubated with human anti-LEDGF/p75 autoantibodies and detected with rhodamine–labeled secondary antibody (red). Merged images show the yellow staining usually seen in colocalization. Nuclear staining was detected with DAPI. Confocal microscopy was used for imaging.

FIG 8. GR/LEDGFp75 CONTRIBUTION TO PCa CHEMORESISTANCE



Proposed conceptual model for GR-LEDGF/p75 axis contribution to PCa chemotherapy resistance. Glucocorticoids bind to GR in the cytoplasm and this complex translocates into the cell nucleus where transactivation of the LEDGF/p75 gene occurs at the promoter (pr) region. Activated GR also interacts with the LEDGF/p75 protein as part of the RNA polymerase II complex in active chromatin sites to transcriptionally regulate other target genes. These events contribute to chemotherapy resistance. Targeting GR with its antagonist CORT108297 abolishes its interaction with LEDGF/p75 and transcriptional regulation, leading to re-sensitization of chemoresistant PCa cells to docetaxel therapy.

CONCLUSIONS

- GR inhibition decreased LEDGF/p75 expression, but the opposite was not observed, suggesting that LEDGF/p75 is a target gene of GR.
- LEDGF/p75, GR and β-catenin co-immunoprecipitated in DTX-sensitive and -resistant PCa cells, and this interaction is not altered by CORT108297 treatment.
- Endogenous LEDGF/p75 and GR co-localized in the nucleus of chemoresistant PCa cells, confirming their interaction.
- Further studies are being conducted to determine the molecular mechanisms by which the LEDGF/p75-GR interaction contributes to PCa chemoresistance, and its potential role in PCa mortality disparities.

ACKNOWLEDGEMENTS

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