

A novel Glucocorticoid Receptor (GR) antagonist overcomes GR-mediated chemoresistance in Triple Negative Breast Cancer

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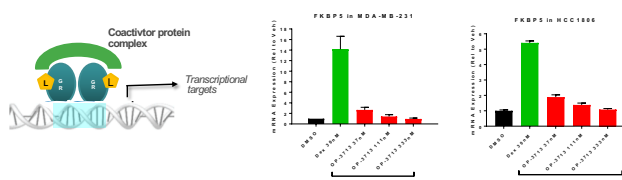
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ABSTRACT

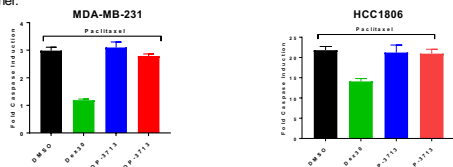
The Glucocorticoid Receptor (GR) is a member of the nuclear receptor superfamily of ligand-activated transcription factors that is activated by its endogenous steroid hormone ligand cortisol, and by synthetic glucocorticoid drugs such as dexamethasone. Several preclinical studies have shown that GR mediates resistance to both targeted therapies and conventional chemotherapies in epithelial cancers, such as prostate, bladder, renal, ovarian and triple negative breast cancers (TNBC) (Gassler et al. 2005, Li et al. 2017, Zhang et al. 2007). In TNBC, both GR activation and a disrupted cortisol secretion cycle are associated with poor prognosis, chemotherapy resistance, and increased recurrence (Pan et al. 2011, Skor et al. 2013). Therefore a molecule that inhibits GR activation could attenuate the development of resistance to chemotherapy, the standard of care for patients with advanced TNBC. We are developing novel GR inhibitors that effectively block GR transcriptional activity in cells by competing for ligand binding and blocking GR-Coactivator interactions. In vitro, GR inhibition by OP-3713 blocks transcription of GR-mediated target genes and enhances the efficacy of chemotherapeutic agents in TNBC cells under GR activation by dexamethasone. In humans, the predominant glucocorticoid is cortisol, while in mice the predominant glucocorticoid is corticosterone (Siswanto et al 2008), which is a potent agonist of murine GR but a weak agonist of human GR. Therefore it is necessary to provide exogenous cortisol to fully activate GR. Using xenograft models of triple negative breast cancer, we find that tumors grown in mice with physiologically relevant circulating cortisol levels are significantly less sensitive to standard of care chemotherapy than those grown in the absence of cortisol. Inhibition of GR by OP-3713 prevents tumor relapse following chemotherapy treatment. We have begun to elucidate the mechanisms by which GR mediates chemoresistance in TNBC and its reversal by OP-3713. Our findings indicate the important role of GR as a mediator of resistance in TNBC and demonstrate the therapeutic potential of GR inhibitors in combination with clinically utilized chemotherapeutics.

Results

OP-3713 blocks GR transcriptional activity and restores apoptosis induced by chemotherapy in TNBC cells in vitro



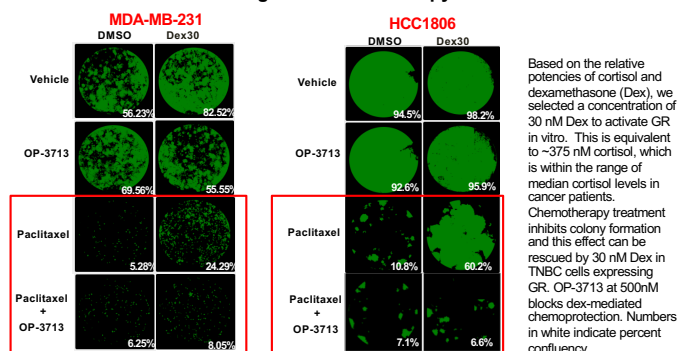
The synthetic glucocorticoid dexamethasone (Dex) robustly activates endogenous GR target gene expression, such as FKBP5, in MDA-MB-231 and HCC1806 TNBC cells. OP-3713 blocks FKBP5 gene induction in a dose-dependent manner.



MDA-MB-231 and HCC1806 cells were treated with 100 nM paclitaxel in the presence or absence of 30 nM Dexamethasone (Dex30) and 500 nM OP-3713. Dex treatment protects cells from chemotherapy-induced apoptosis, and this effect is reversed by OP-3713.

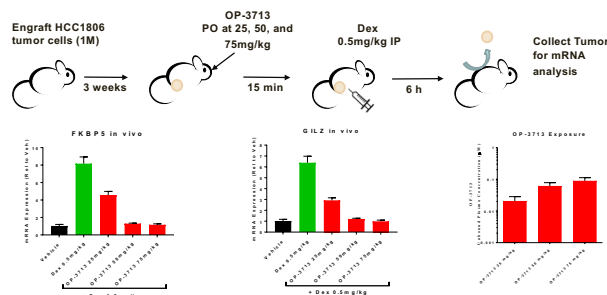
RESULTS

GR Activation Protects Against Chemotherapy in TNBC Cancer Cells



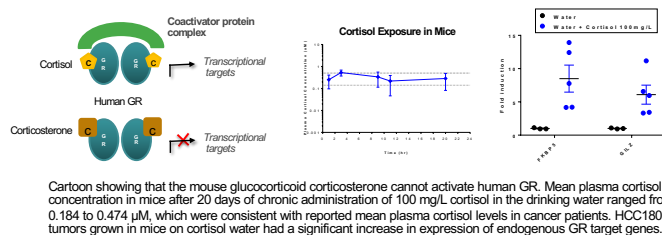
Based on the relative potencies of cortisol and dexamethasone (Dex), we selected a concentration of 30 nM Dex to activate GR in vitro. This is equivalent to ~375 nM cortisol, which is within the range of median cortisol levels in cancer patients. Chemotherapy treatment inhibits colony formation and this effect can be rescued by 30 nM Dex in TNBC cells expressing GR. OP-3713 at 500nM blocks dex-mediated chemoprotection. Numbers in white indicate percent confluency.

OP-3713 blocks GR transcriptional activity in TNBC xenografts in vivo



Schematic of a PKPD study to measure the effect of OP-3713 on Dexamethasone-induced target gene expression in HCC1806 xenografts. Dexamethasone at 0.5mg/kg robustly activates endogenous expression levels of two established GR target genes, FKBP5 and GILZ, in HCC1806 xenografts in vivo and OP-3713 blocks this GR transcriptional activity in a dose-dependent manner.

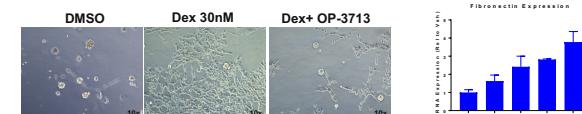
GR is Active in Tumors Grown in Mice on Cortisol Water



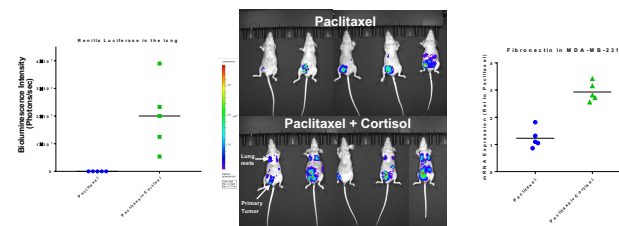
Cartoon showing that the mouse glucocorticoid corticosterone cannot activate human GR. Mean plasma cortisol concentration in mice after 20 days of chronic administration of 100 mg/L cortisol in the drinking water ranged from 0.184 to 0.474 μ M, which were consistent with reported mean plasma cortisol levels in cancer patients. HCC1806 tumors grown in mice on cortisol water had a significant increase in expression of endogenous GR target genes.

RESULTS

GR accelerates invasion and metastases in MDA-MB-231 cells

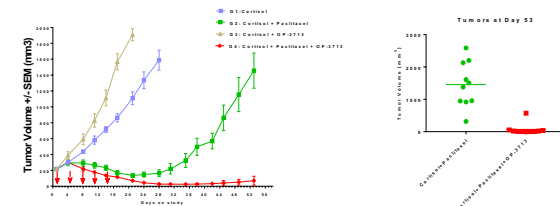


MDA-MB-231 cells grown in a 3D matrigel culture assay for 7 days form significant invasive protrusions in the presence of Dex at 30nM. This effect is reversed by OP-3713. Long-term treatment of these cells with Dex in vitro induces the expression of EMT genes, such as Fibronectin.



Bioluminescence imaging at Day 60 of mice with established MDA-MB-231 tumors expressing Renilla luciferase shows that mice that received paclitaxel (Q3Dx5) in the presence of cortisol at 100mg/L have a much higher incidence of lung metastasis at relapse compared to mice treated with paclitaxel alone, and their primary tumors express higher levels of EMT genes, such as Fibronectin.

OP-3713 prevents tumor relapse after chemotherapy in Conditions Simulating Cortisol Levels in Cancer Patients



Tumor growth curves and end of study tumor volumes for mice with established HCC1806 TNBC tumors grown in the presence of 100mg/L cortisol. OP-3713 was significantly efficacious in inhibiting the relapse of HCC1806 tumors after paclitaxel dosing (Q3Dx5 as shown by the red arrows). The tumor incidence on Day 53 in mice receiving paclitaxel + cortisol was 100% compared to 11% in mice treated with paclitaxel + cortisol + OP-3713.

CONCLUSIONS

- GR inhibition by OP-3713 blocks transcription of GR-mediated target genes under GR activation by dexamethasone both in vitro and in vivo
- GR activation by physiological levels of ligand protects tumor cells against chemotherapy in vitro and this effect is reversed by OP-3713
- GR activation in MDA-MB-231 significantly enhances invasion and metastasis
- GR inhibition by OP-3713 prevents the relapse of TNBC tumors after chemotherapy