Development of Murine Models to Evaluate the Impact of Glucocoritcoid Receptor (GR) Inhibition on Chemotherapy Response

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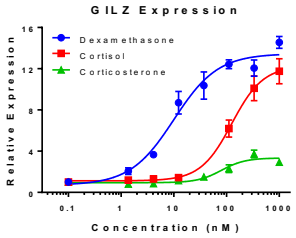
ABSTRACT

The Glucocorticoid Receptor (GR) is a member of the nuclear receptor superfamily of ligand-activated transcription factors. GR is activated by its endogenous steroid hormone ligand, cortisol, and by synthetic glucocorticoids such as dexamethasone. In preclinical studies, glucocorticoids have been reported to confer resistance to antimetabolites, taxanes and platinum compounds in lung, prostate, bladder, renal, ovarian and triple negative breast cancers, and to antiandrogen therapies in prostate cancer. Therefore, a molecule that inhibits GR activation could attenuate the development of resistance to cancer therapeutics. 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 enhances the efficacy of chemotherapeutic agents under conditions supporting GR activation. In humans, the predominant glucocorticoid is cortisol, while in mice the predominant glucocorticoid is corticosterone. Corticosterone is a potent agonist of murine GR, but a weak agonist of human GR. When human tumor cells are grown in mice, GR is not active in the tumor cells due to the lack of circulating cortisol. Therefore it is necessary to provide exogenous cortisol to fully activate GR. Using xenograft models of ovarian and 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 is effective at restoring the response to chemotherapy in these models. These results demonstrate the therapeutic potential of GR inhibitors in combination with clinically utilized chemotherapeutics. Furthermore, the in vivo models developed provide a milieu for testing and developing GR inhibitors.

Results

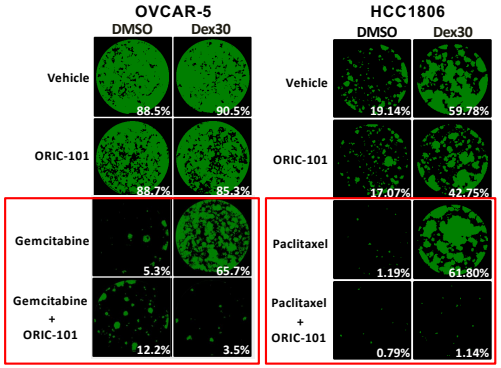
Differential Activation of Human GR by Various Glucocorticoids



The synthetic glucocorticoid, dexamethasone, and the predominant human glucocorticoid, cortisol, robustly activate endogenous GR target gene expression in OVCAR-5 ovarian cancer cells . In contrast, the major murine glucocorticoid, corticosterone, is a very weak agonist of human GR.

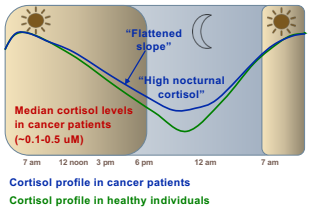
RESULTS

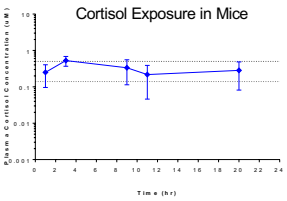
GR Activation Protects Against Chemotherapy in Ovarian and Breast Cancer Cell Lines



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. ORIC-101, a novel GR inhibitor, blocks dex-mediated chemoprotection. Numbers in white indicate percent confluency.

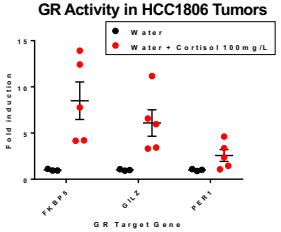
Administration of Cortisol in Drinking Water Results in Circulating Cortisol Levels Similar to Cancer Patients





Plasma cortisol concentration in mice after 20 days of chronic administration of 100 mg/L cortisol in the drinking water. Mean concentrations ranged from 0.184 +_0.266 to 0.474 +_ 0.318 µM. These levels were consistent with reported mean plasma cortisol levels in cancer patients throughout the day, which range from 0.1 to 0.5 µM.

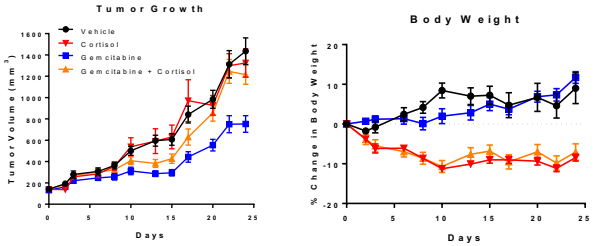
GR is Active in Tumors Grown in Mice on Cortisol Water



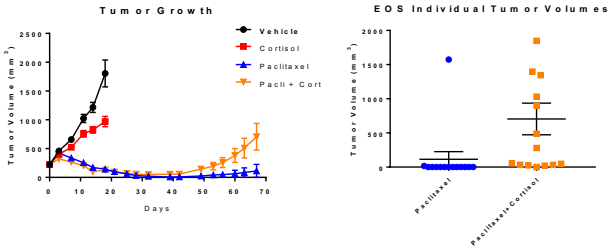
Expression of endogenous GR target genes is significantly increased tumors grown in mice whose drinking water is supplemented with 100 mg/L cortisol relative to mice drinking unsupplemented water.

RESULTS

Efficacy of Chemotherapy is Diminished in Tumors Grown Under Conditions Simulating Cortisol Levels in Cancer Patients



Tumor growth curves (left) and body weights (right) for mice with established OVCAR-5 ovarian tumors grown in the presence or absence of cortisol. The efficacy of gemcitabine was significantly diminished in tumors grown under conditions simulating human cortisol levels. Cortisol treatment resulted in a sustained 10% body weight loss. This degree of cortisol-induced weight loss is consistently observed across studies in naïve and tumor bearing mice, but is manageable with dietary supplements.



Tumor growth curves (left) and end of study tumor volumes (right) for mice with established HCC1806 breast tumors grown in the presence or absence of cortisol. The efficacy of paclitaxel was significantly diminished in tumors grown under conditions simulating human cortisol levels. Tumor volumes on Day 68 in mice receiving paclitaxel + cortisol were 513.43% larger than the volume in mice receiving paclitaxel alone.

CONCLUSIONS

- GR activation by physiological levels of ligand protects tumor cells against chemotherapy in vitro
- Administration of cortisol in the drinking water results in circulating cortisol levels in mouse similar to those found in cancer patients
- Chemotherapy response is attenuated in tumors grown in cortisol-treated mice

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