GLUCOCORTICOID RECEPTOR ACTIVATION INHIBITS TUMOR CELL GROWTH WHILE INCREASING METASTATIC CHARACTERISTICS IN MODELS OF INVASIVE LOBULAR BREAST CANCER

Poster #23

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Lay abstract

Invasive lobular breast carcinoma (ILC) is a highly prevalent subtype of breast cancer. Our understanding of metastatic spread poses an unmet clinical challenge. The stress steroid hormone known as cortisol (ie glucocorticoids) contributes to ILC biology through interaction with the hormone receptor glucocorticoid receptor. The aim of this study is to understand how previous findings of glucocorticoid receptor activity contribute to processes observed in ILC such as cellular growth and migration (ie metastasis). Hormonal homeostasis driven by the body's stress response is an integral part of ILC tumor biology which we hypothesize that activation above baseline may promote enhanced invasion and metastatic spread. Thus, understanding the contributions of glucocorticoids to these processes will significantly improve current therapeutic intervention strategies.

Scientific Abstract

Background: Estrogen receptor (ER)-positive invasive lobular breast cancer (ILC) is the second most common histological subtype of breast cancer. Genotypic and phenotypic characteristics include e-cadherin (*CDH1*) loss that is associated with "single file" tumor architecture. Metastatic pattern is characterized by discontinuous tumor cell foci invading serosal surfaces. ER is a driver of ILC relapse and anti-estrogen treatment can reduce early disease progression to metastasis.

Findings: In this study we examined isogenic GR+ and GR- ILC models and found that GR activation resulted in reduced activity of the "Estrogen-mediated S phase entry" gene expression pathway and associated decreased ILC cell proliferation. GR-mediated gene expression data also suggested that GR activation contributes to increased ILC integrin expression. GR expression also is associated with more efficient mesothelial cell clearance while a GR+ versus GR- xenograft MIND model revealed both decreased primary mammary gland tumor growth and increased distal metastasis to bone.

Conclusions: GR activity in ILC reduces ILC cell proliferation gene expression pathways associated with G1/S phase entry while increasing gene expression pathways associated with the metastatic cascade.

Future studies: Selective GR modulation and its potential role in inhibiting ILC metastasis will be evaluated.

Background & Objectives

Objective: Determine how GR affects ILC tumor biology and metastatic phenotypes of disease

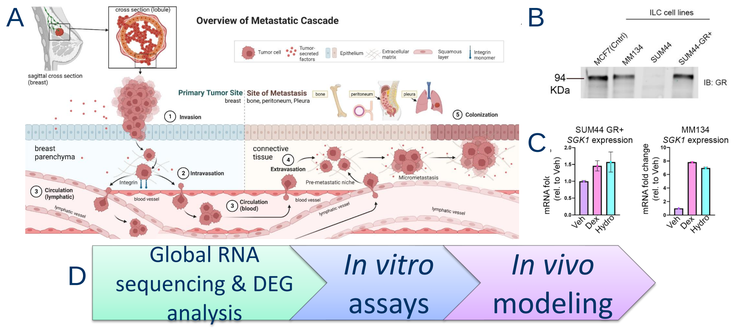
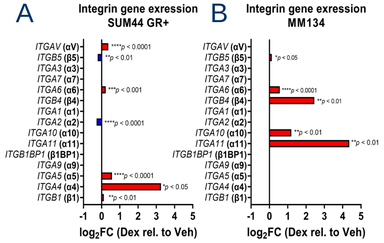


Figure 1. A) Schematic illustration of metastatic cascade. B) Immunoblot detection of GR in ILC cell lines MDA-MB-134-VI (MM134), SUM44, SUM44 ectopically expressing GR, and control cell like MCF7. C) Quantitative PCR of GR target gene, *SGK1*, after ligand induction with Dex (synthetic), Hydrocortisone (serum cortisol), or vehicle in SUM44-GR+ (left) or MM134 (right) cells. D) Illustration of experimental steps

GR activity increases migratory gene expression pathways, integrins, and cell-ECM adherence

Table 1. DEG analysis for SUM44-GR+ and MM134 in 100nM Dex vs Vehicle conditions.

Ingenuity Pathway Analysis		Activation z-score	
Cell proliferation		Dex vs Veh	*p*-values
Estrogen-mediated S phase entry	SUM44	−1.6	$3.09x10^{-4}$
	MM134	−1.6	$1.02x10^{-4}$
ECM adhesion (metastasis) Integrin signaling	SUM44	+1.6	$5.75x10^{-3}$
	MM134	ns	
Epithelial Mesenchymal Transition	SUM44	+0.7	$1.02x10^{-4}$
	MM134	+1.7	$1.41x10^{-2}$



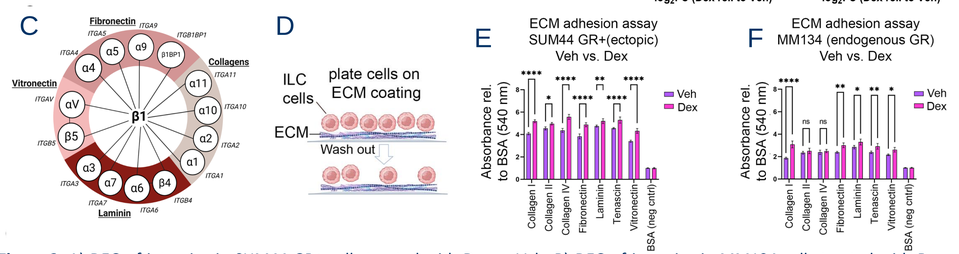


Figure 2. A) DEG of integrins in SUM44-GR+ cells treated with Dex vs Veh. B) DEG of integrins in MM134 cells treated with Dex vs. Veh. C) Schematic illustration of integrin gene names, associated proteins, and associated ECM substrates. D) Schematic illustration of cell-ECM adherence assay. E) Cell-ECM adherence assay for SUM44-GR+ cells and MM134 cells (F) comparing ligand activation with Dex vs. Veh.

GR activity increases mesothelial clearance and adherence to serosal surfaces

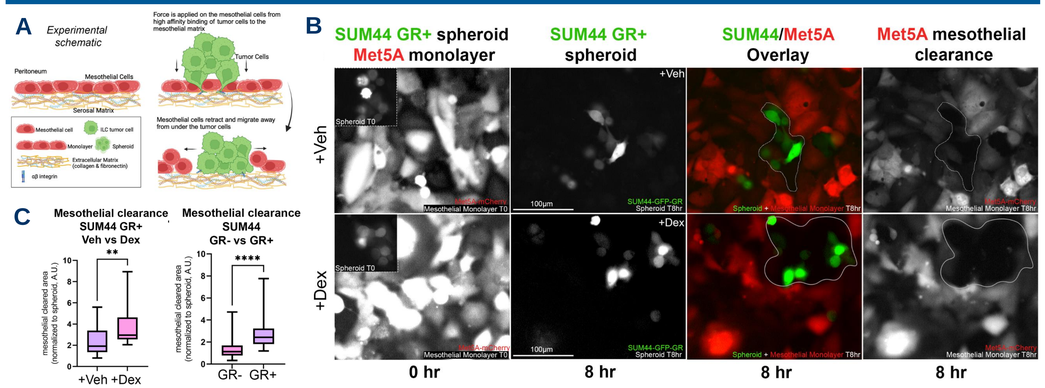


Figure 3. A) Schematic illustration of mesothelial clearance assay adapted from Brugge lab. B) Timelapse of mesothelial clearance assay with SUM44-GR+ cells treated with 100 nM Dexamethasone. C) Quantification of mesothelial clearance efficacy in SUM44-GR+ cells treated with Dex vs. Veh (left) and SUM44 GR- vs SUM44-GR+ cells (right).

GR reduces localized mammary gland growth/proliferation and increases distal bone mets

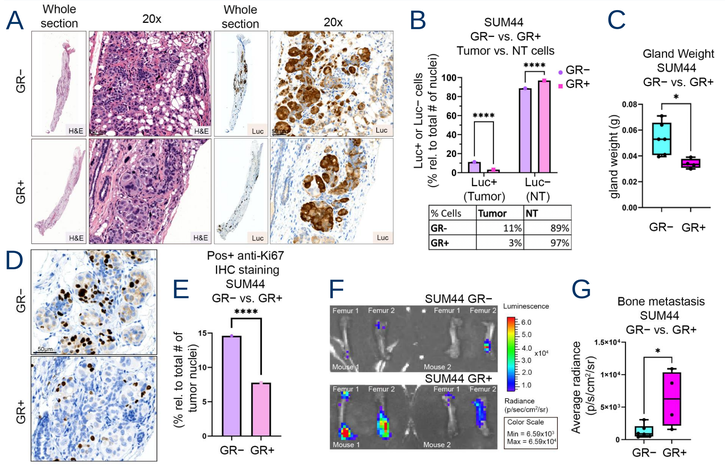


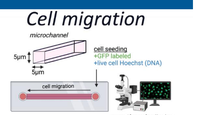
Figure 4. A) H&E staining (left) and anti-luciferase antibody staining (right) of mammary gland tissue xenografted with SUM44 (top) or SUM44-GR+ (bottom) cells quantified in B. C) Gland weight of mice engrafted with SUM44 or SUM44-GR+ cells after resection 113 days post injection. D) Ki67 staining of SUM44 (top) or SUM44-GR+ cells 113 days post injection and proliferation index quantified in E. F) Ex vivo bioluminescence of mice femurs 113 days post injection in sum44 (top) or sum44-GR+ (bottom) cells quantified in G.

Conclusions

- GR activation of nuclear function decreases cell proliferation and ILC cell growth at xenografted site
- GR activation increases migratory gene expression pathways, integrin gene expression, and mesothelial clearance
- GR activation underlies ILC cell-ECM preference through integrin gene expression

Future Directions

- Investigate how GR activation affects microchannel migration
- MIND model growth/metastasis across multiple ILC cell lines



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