Assessing E-cadherin loss on Estrogen Receptor activity in Human Mammary Epithelial Cell Models

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Lay Abstract

ILC are uniquely estrogen-driven among breast cancers, as nearly all ILC are estrogen receptor α (ER)-positive and increased breast cancer risk after hormone-replacement therapy is most strongly linked to ILC. Furthermore, clinical, epidemiological, and laboratory studies suggest that ER function is unique in ILC cells. Since ~95% of ILC tumors show E-cadherin loss, we aim to study how this loss affects ER using human mammary epithelial cells (HMECs) derived from donor tissue. We developed HMECs with decreased E-cadherin expression and injected them into the mammary glands of female mice. We then monitored the mice to see if the human cells survived and were incorporated into the mouse mammary gland. Our goal is to mimic the ILC phenotype both in human cells and mice to study how ER impacts the initiation and progression of the disease. This study could lead to new therapeutic approaches for preventing and treating ILC.

Introduction

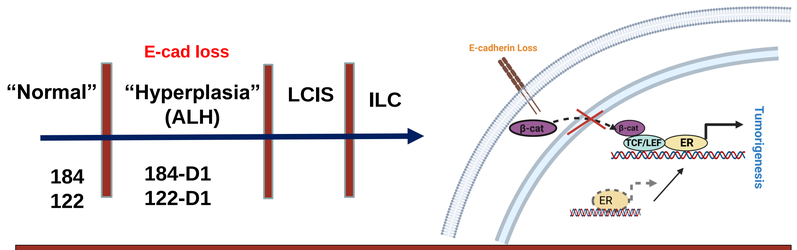
- E-cadherin (*CDH1* gene) loss is a hallmark of ILC and occurs in ~95% of cases.
- Novel models have been developed to better mimic and study the initiation and progression of ILC tumors such as the mouse mammary intraductal model (MIND).
- Preliminary studies suggest that estrogen drives ILC tumorigenesis *in vivo* and that ER inhibition in ILC alters the function of the TCF/LEF transcriptional factors.
- We propose to study estrogen-driven ILC initiation and progression by modeling CDH1 loss in human mammary epithelial cells (HMECs).

Hypothesis

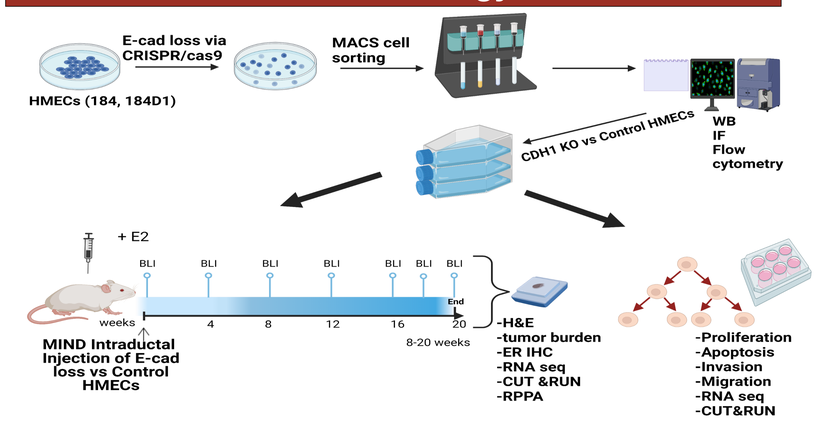
We hypothesize that E-cadherin loss affects ER through alteration of DNA binding and transcriptional activity to enhance ER-driven ILC progression.

Aim

To test the effects of E-cadherin loss on ER activity in HMEC models.



Methodology



Results

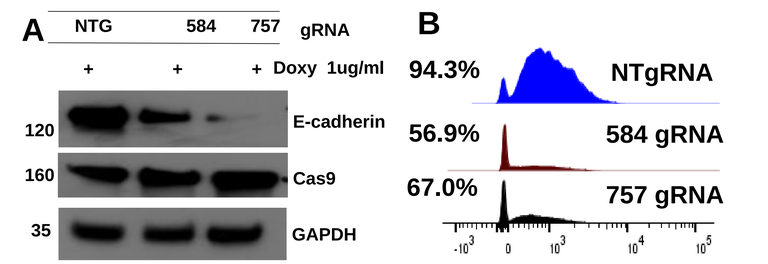


Figure 1: A) Western blot analysis showing CRISPR/cas9 CDH1 Knockout in 184AA3 lysates. Proteins: E-cadherin, Cas9 with GAPDH as loading control B) Flow cytometry analysis showing decrease in % E-cadherin expression following CRISPR/cas9 knockout.

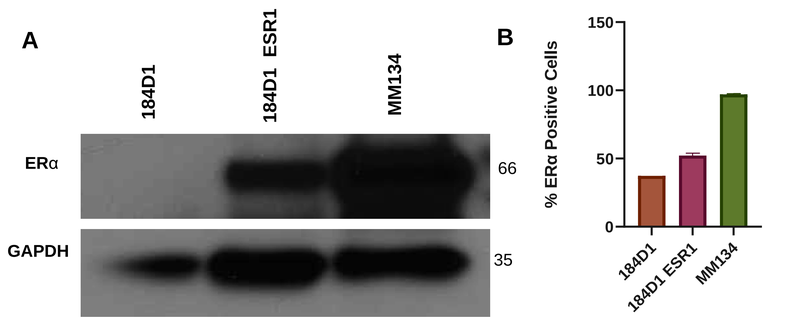


Figure 2: ESR1 overexpression in 184D1 cells A) Western blot . Proteins: **ER**α, GAPDH as loading control B) Bar graph showing % ERα positive cells quantified using flow cytometry.

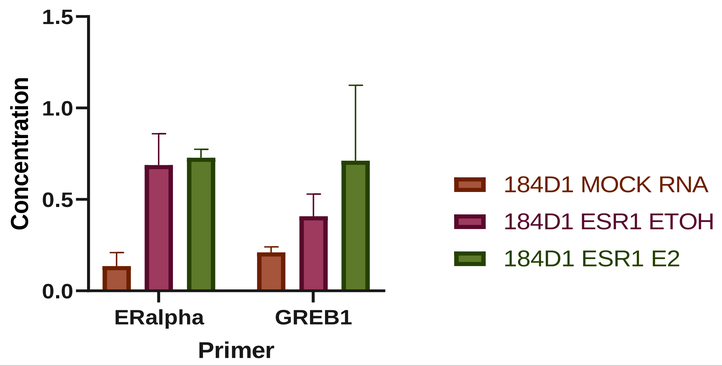


Figure 3: qPCR on mock and ESR1 overexpressed 184D1 cells after treatment with 10nM E2 for 24hrs. Bar graphs show mRNA expression levels of ERα and GREB1.

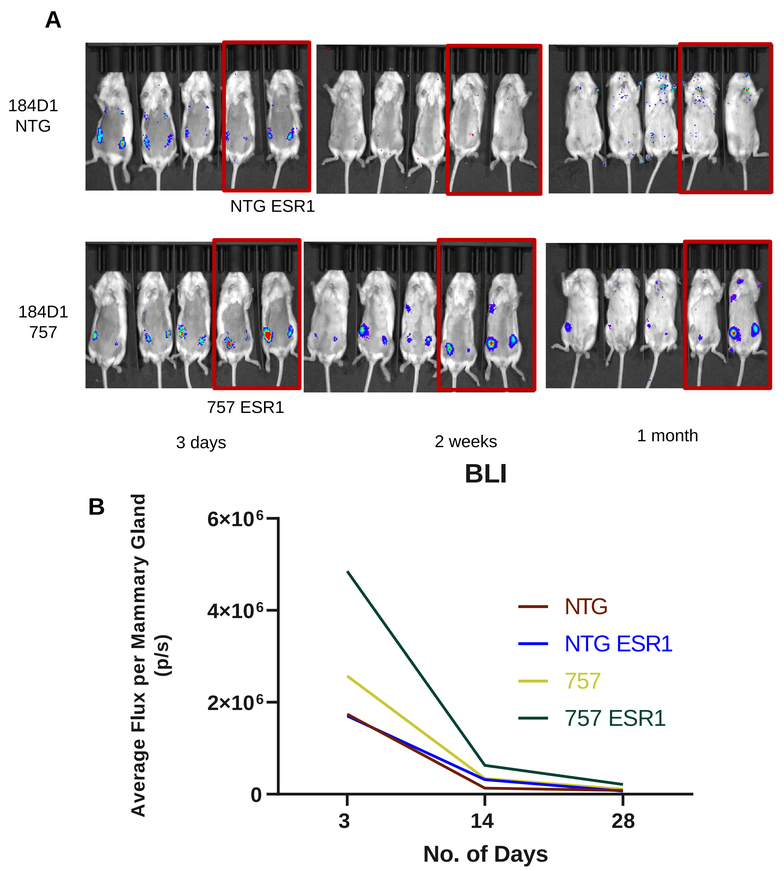


Figure 4 :Bioluminescence imaging following intraductal injection suggests increased engraftment of HMECs with CDH1-757. All mice were injected with 50,000 HMECs (184D1 NTG or 184D1-757) into the #3 and #4 mammary glands. BLI was performed 3 days, 2 weeks and 1 month post injection. B) Graph showing the quantified Bioluminescence per mammary gland in photons per second.

Impact

- Understanding how E-cadherin loss remodels ER activity will identify unique estrogen/ILC association.
- Studying ILC tumorigenesis models in an ER+ context can define mechanisms of initiation and progression.
- Improve individualized risk assessment, limit over-treatment, and build new research on ILC-specific treatment and prevention strategies

Future Directions

- Assess ER reprogramming in Estrogen-treated CDH1 KO cells using RNA seq.
- IHC and IF on mammary tissues to evaluate engraftment of HMECs
- Proliferation, invasion and migration assays in CDH1 KO vs Control HMECs.

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