Title: Drivers of estrogen-induced invasive lobular carcinoma.

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Estrogen receptor (ER) expression and E-cadherin (CDH1) loss are the two major molecular hallmarks of invasive lobular carcinoma (ILC) of the breast; however, it is unclear how ER signaling and E-cadherin loss interact during ILC tumor formation. Furthermore, prior research suggests that ER may have a unique role in ILC initiation and progression. We hypothesize that E-cadherin loss alters global expression and ER activity in human mammary epithelial cells (HMECs) to promote ILC tumorigenesis.

To test the effect of E-cadherin loss during estrogen-driven tumorigenesis, we established a CRISPR/Cas9 approach for knocking out CDH1 in primary HMECs. Since primary HMECs did not show robust ER expression, we also re-expressed ER using lentiviral transduction of ESR1. We observed decreased ER phosphorylation at the Serine 167 site in the CDH1 knockout (KO) cells, suggesting impaired ER signaling. We also performed RNAseq from two independent CDH1 targeting gRNAs vs non-targeting gRNA (NTG) control (-/+ ER). When comparing the two CDH1 gRNAs to NTG, 2882 shared genes were differentially regulated. Analysis of these shared genes showed the enrichment of estrogen response and cell cycle pathways and repression of epithelial-mesenchymal transition and glycolysis in the CDH1 KO sample, corresponding to human ILC data. Metabolic studies showed decreased glucose uptake in the KO cells; we also observed a decrease in glycolysis and an increase in the mitochondrial ATP production rate in the KO cells compared to control in a Seahorse™ ATP rate assay.

In addition, we hope to identify mediators that drive estrogen-induced tumorigenesis using the mouse mammary intraductal (MIND) model, which better recapitulates the progression of ILC and response to estrogen in vivo. We injected control and CDH1 KO cells into the mammary glands of adult female NSG mice, with estrogen supplemented in their drinking water, and assessed for engraftment using bioluminescence imaging (BLI). BLI suggests enhanced engraftment of CDH1 KO cells compared to CDH1 wild-type HMECs and a survival advantage for HMECs expressing ESR1. Notably, Cell-titre-glo™ survival assay showed increased survival with cMyc and myrAKT oncogenes in KO cells, suggesting oncogenic cooperation with E-cadherin loss. Understanding the unique interactions between E-cadherin loss and ER in ILC could lead to novel therapeutic approaches for the prevention and treatment of ILC.