E-cadherin suppression supports transcriptional programs of lineage plasticity in early ILC tumorigenesis

Maggie Musick[1], Chinasa A. Ufondu[2], Julie H. Ostrander[3], Matthew J. Sikora[1]

1.Department of Pathology, University of Colorado Anschutz Medical Campus

2.Department of Pharmacology, Molecular Pharmacology and Therapeutics (MPaT), University of Minnesota, Twin Cities

3.Masonic Cancer Center, University of Minnesota, Twin Cities

University of Colorado Anschutz Medical Campus

Lay Abstract

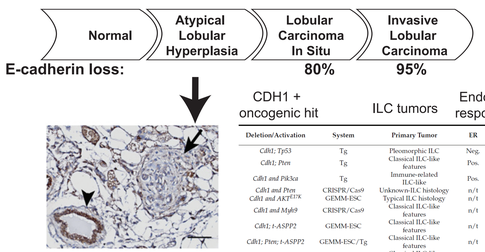
E-cadherin loss occurs in ~95% of invasive lobular carcinoma (ILC) and ~80% of lobular carcinoma in situ (LCIS), but our understanding of how E-cadherin loss promotes ILC initiation in human mammary cells is limited. To better understand the cellular processes that are affected by E-cadherin loss, we are investigating how the reduction in E-cadherin expression in healthy human mammary epithelial cells (HMECs) leads to a tumor-promoting state. So far, we have used HMECs derived from three donors and have seen similar shifts toward a tumor-promoting state. We have also observed differences between the HMECs which could help us determine what types of cells within the breast are more susceptible to becoming ILC. Further investigation of these similarities and differences could help us to find new biomarkers that can be used to improve current risk assessment and treatment strategies for LCIS and ILC.

Technical Abstract

E-cadherin loss, a hallmark of invasive lobular carcinoma (ILC), is an early and nearly universal event in ILC and associated precursor lesions (e.g. lobular carcinoma in situ, LCIS). However, ILC tumorigenesis, i.e. the progression from atypical lobular hyperplasia (ALH) to LCIS to ILC is not well understood, which confounds patient risk evaluation and treatment. Studies to date strongly support that E-cadherin has direct tumor suppressor roles that are pivotal to breast tumorigenesis, but mechanistic studies in human cells are limited. To model early ILC tumorigenesis, we use human mammary epithelial cells (HMECs), combining various modes of E-cadherin suppression with other candidate oncogenic hits, to determine how E-cadherin inhibition or loss remodels the HMEC genome to facilitate ILC tumorigenesis. In RNA-seq of three independent HMEC models (HMEC lines 122, 153, and 184, with CCND1 over-expression), we found that antibody inhibition of E-cadherin increases gene expression signatures associated with transformation and cancer phenotypes. For example, the hallmark signature of epithelial-mesenchymal transition (EMT) was activated in all three models (q=5.09E-8, 1.52E-18, 4.65E-13). Notably, the increased EMT signatures upon E-cadherin inhibition are accompanied by coordinate shifts in gene expression toward a more mesenchymal phenotype (e.g. increased SNAI2, VIM, FN1, ZEB1), and a basal or myoepithelial-like phenotype (e.g. decreased EPCAM / increased ITGA6). Initial experiments with E-cadherin knock down by siRNA have shown similar changes in EMT gene expression by qPCR. Parallel studies in CDH1-knockout HMEC models are underway to compare how E-cadherin suppression relates to complete loss-of-function observed in most clinical ILC. Taken together, E-cadherin suppression shifts pre-cancerous HMEC cells toward a more myoepithelial-like (i.e. dedifferentiated) gene expression phenotype, supporting E-cadherin loss remodeling the cellular transcriptome to facilitate ILC tumorigenesis.

E-cadherin Loss in Early ILC Tumorigenesis

Figure 1: E-cadherin loss is a hallmark of ILC



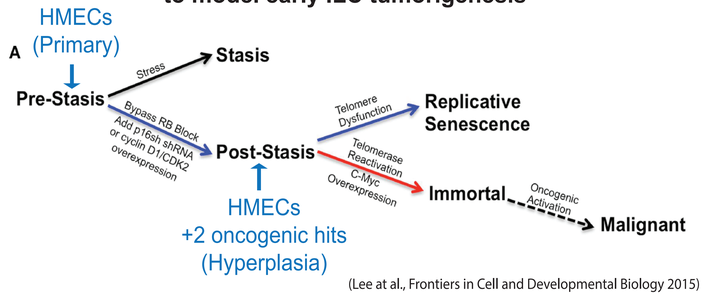
(Jonkers 2006)

Deletion/Activation	System	Primary Tumor	ER	Tumor Onset (Weeks)	Laboratory/Institute	Ref.
Cdh1; Tp53	Tg	Pleomorphic ILC	Neg.	20–32	Prof. Jos Jonkers/NKI	[25]
Cdh1; Pten	Tg	Classical ILC-like features	Pos.	8–16	Prof. Jos Jonkers/NKI	[125]
Cdh1 and Pik3ca	Tg	Immune-related ILC-like	Pos.	5–12	Prof. Sean E. Egan/U of T	[126]
Cdh1 and Pten	CRISPR/Cas9	Unknown/ILC histology	n/t	28	Prof. Jos Jonkers/NKI	[127]
Cdh1 and AKT^{E17K}	GEMM-ESC	Typical ILC histology	n/t	n/s	Prof. Jos Jonkers/NKI	[127]
Cdh1 and Myb9	CRISPR/Cas9	Classical ILC-like features	n/t	n/s	Prof. Jos Jonkers/NKI	[128]
Cdh1; t-ASPP2	GEMM-ESC	Classical ILC-like features	n/t	9–15	Prof. Jos Jonkers/NKI	[128,129]
Cdh1; Pten; t-ASPP2	GEMM-ESC/Tg	Classical ILC-like features	n/t	5–9	Prof. Jos Jonkers/NKI	[129]
Cdh1; t-MYPT1	GEMM-ESC	Classical ILC-like features	n/t	10–16	Prof. Jos Jonkers/NKI	[128,129]
Cdh1; Pten; t-MYPT1	GEMM-ESC/Tg	Classical ILC-like features	n/t	5–8	Prof. Jos Jonkers/NKI	[129]
Cdh1; Trp1	GEMM-ESC	Classical ILC-like features	n/t	76	Prof. Jos Jonkers/NKI	[130]

(Sflomos 2021)

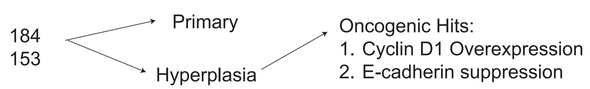
Using HMECs as an Early ILC Tumorigenesis Model

Figure 2: Using Human Mammary Epithelial Cells (HMECs) to model early ILC tumorigenesis



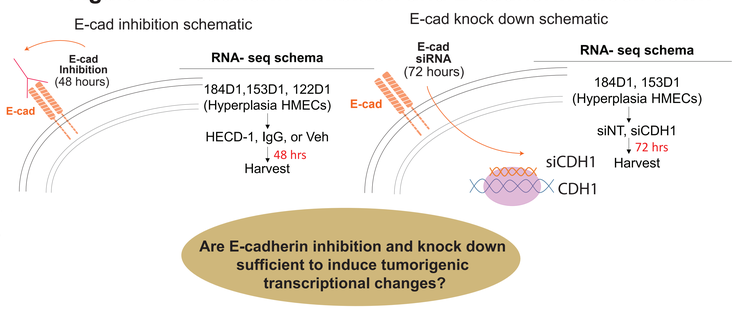
(Lee at al., Frontiers in Cell and Developmental Biology 2015)

2 HMEC Models:



E-cadherin Suppression Methods

Figure 3: E-cadherin inhibition and E-cadherin knock down



E-cadherin suppression is differentially associated with EMT

Figure 4: E-cadherin inhibition and E-cadherin genetic suppression are significantly and differentially associated with EMT following fSGEA analysis

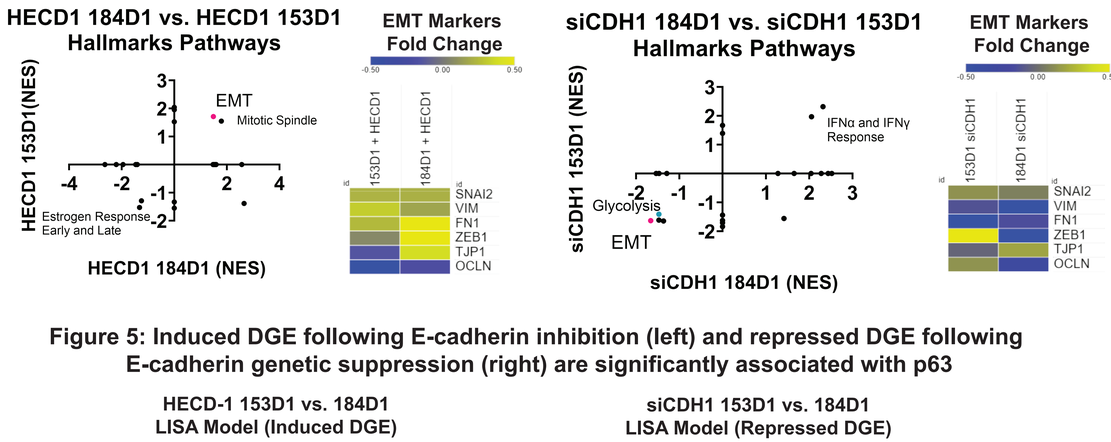


Figure 5: Induced DGE following E-cadherin inhibition (left) and repressed DGE following E-cadherin genetic suppression (right) are significantly associated with p63

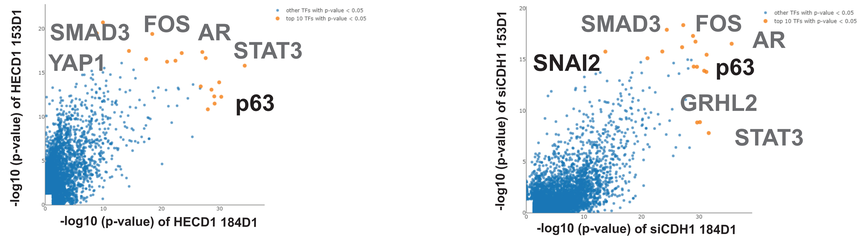
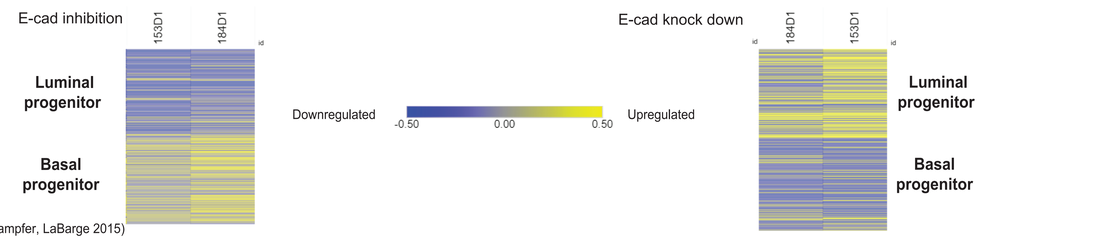


Figure 6: Opposite shifts in luminal vs. basal progenitor signatures



(Stampfer, LaBarge 2015)

Conclusion

Conclusion:

- E-cadherin inhibition and E-cadherin knock down are differentially associated with EMT
- E-cadherin inhibition induces a transcriptional shift toward a dedifferentiated state in hyperplasia-like HMECs
- E-cadherin knock down in HMECs resembles an ILC precursor transcriptional phenotype

Acknowledgements

Sikora Lab

Ostrander Lab, Univ. of Minnesota

NIH NATIONAL CANCER INSTITUTE T32CA190216-06

American Cancer Society

TheoryLab Milburn Family