Upregulation of the immune checkpoint protein B7-H3 is associated with an immune suppressive environment in progression from in situ to invasive lobular breast cancer

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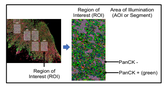
Abstract

Invasive lobular breast cancer (ILC) is an understudied subtype of breast cancer with late recurrence, metastasis to serosal surfaces, such as the peritoneum, and dismal long-term outcome.

We utilized digital spatial profiling of genes and proteins to interrogate mechanisms controlling the transition from in situ to invasive lobular breast cancer at the molecular level. We discovered that the immune checkpoint protein B7-H3 is upregulated in ILC tumor cells and cells in the tumor microenvironment (TME). B7-H3 may play a role in tumor cell invasion and immune cell evasion. Outside of the basement membrane tumor cells interact with integrins, collagens and other extracellular matrix proteins. B7-H3 is important for tumor cell proliferation and activation of downstream cancer-associated pathways. B7-H3 is also expressed by antigen presenting cells and fibroblasts that play a role in creating an immune-suppressed environment.

Methods



The ILC FFPE tissue sections were stained with four morphology markers: Fluorescent antibody markers for DNA (SYTO13), Pan cytokeratin (Cy3), CD45 (Texas Red) (pseudo-colored yellow). For the RNA assays, a RNAscope probe for *NR3C1* (GR) tagged with Cy5. For the protein assays, a Cy5 labeled antibody was used.

Selection of regions of interest (ROIs), segmentation for PanCK and statistical analyses were conducted within the GeoMx DSP Analysis Suite Version 2.4.0.421. Spatial deconvolution to obtain cell type abundances was performed using a plug-in tool from nanoString and the tumor/immune cell matrix (https://pubmed.ncbi.nlm.nih.gov/35046414/). Pathway analyses were performed using Gene Set Enrichment Analyses (GSEA)

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RNA expression changes in ILC vs LCIS

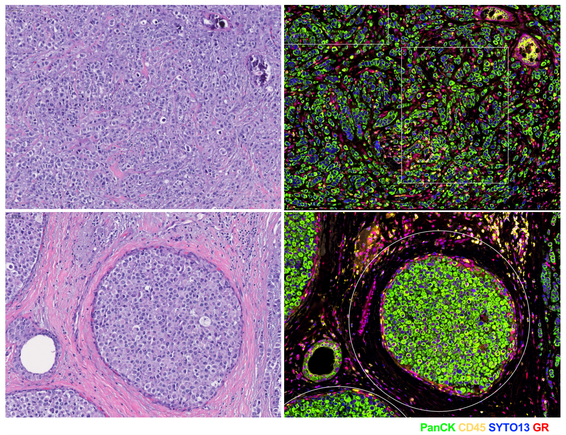


Figure 1. H&E (left) and fluorescent images (right) showing invasive ILC (top) and in situ LCIS (bottom) regions of the **same tumor**

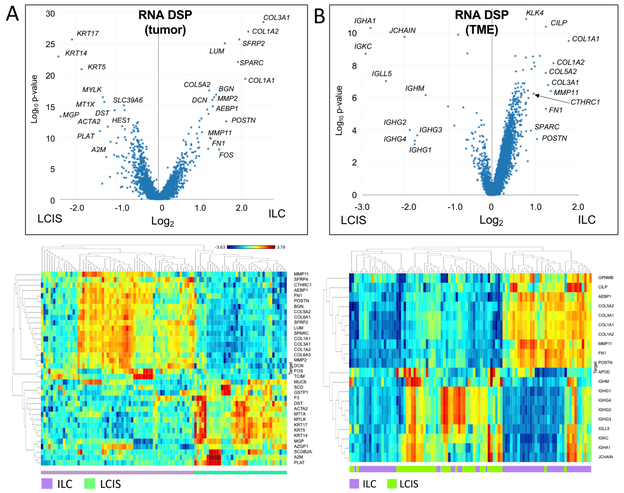


Figure 2. Volcano plots (top) and heat maps (bottom) from LMM analysis of genes differentially expressed between regions of invasive (ILC) vs in situ (LCIS) tumor cells (**A**) or TME (**B**). Analyses were performed separately for tumor or TME

Protein expression reflects a suppressed tumor immune microenvironment in ILC

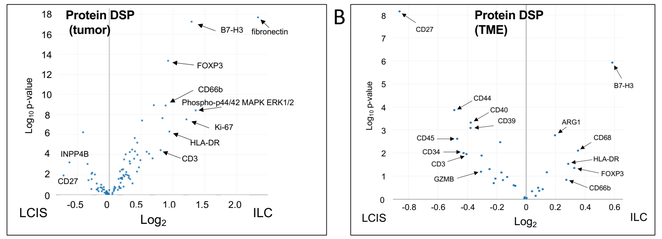


Figure 3. Linear mixed model (LMM) analyses were performed to test differential protein expression between regions of ILC vs in situ LCIS tumor cells (**A**) or TME (**B**)

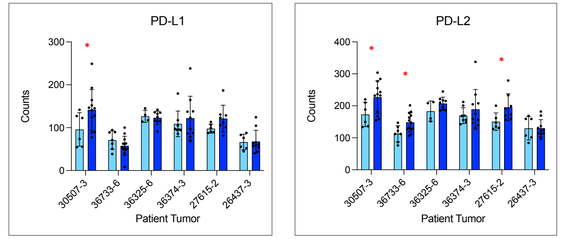


Figure 5. Expression of B7 family checkpoint proteins PD-L1 and PD-L2 in LCIS and ILC measured by GeoMx DSP in tumor cells

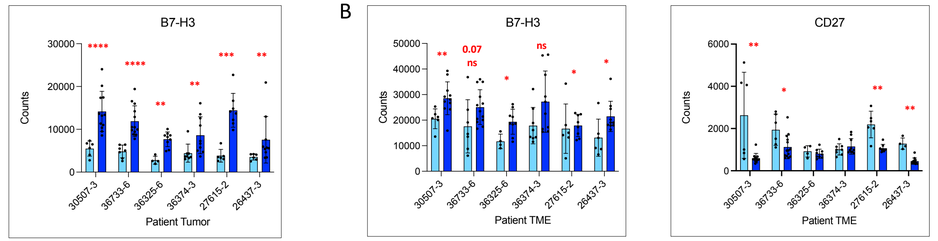


Figure 6. Expression of CD27 protein in LCIS and ILC lesions within the same tumor measured by GeoMx DSP in TME

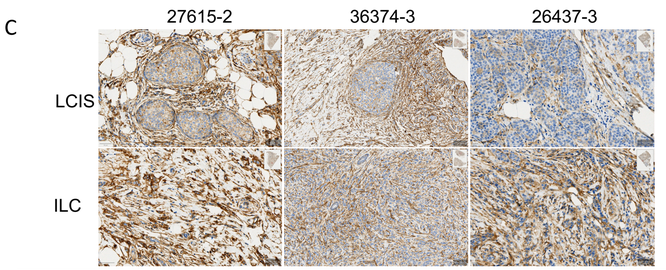
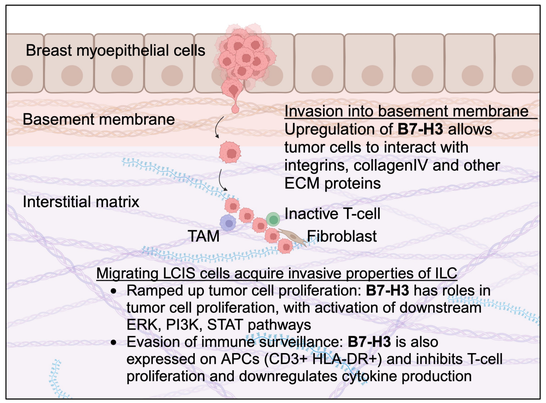


Figure 4. Expression of B7-H3 protein in LCIS and ILC lesions within the same tumor measured by GeoMx DSP in tumor cells (**A**) and TME (**B**) and IHC for CD276 (B7-H3) (**C**)

Case number	Classic or Pleomorphic	Ki-67%	ER %
30507-3	P	5	100/3+
37633-6	C	<1	100/3+
36325-6	C	5	80/2-3+
36374-3	P	15	70/1-2+
27615-2	P	nd	20/2+
26437-3	C	nd	70/2-3+

Conclusions

Model for LCIS to ILC transition



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