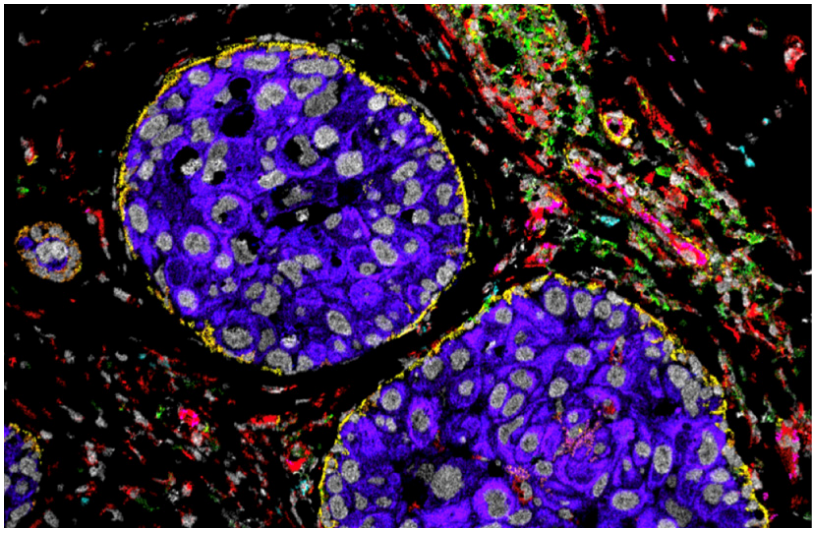
CASE STUDY

Breast Cancer:
MIBI Spatial Proteomic Signatures of Progression

Risom *et al.*, *Cell* 2022 Vol. 185(2):299-310



HIGHLIGHTS

- MIBI spatial proteomics revealed signatures for progression to invasive breast cancer
- Transition to invasive forms was marked by coordinated shifts in location/function of key cell populations
- Predictive power relied heavily on spatial features enabled by MIBI

ABSTRACT

Ductal carcinoma *in situ* (DCIS) is a pre-invasive lesion that is thought to be a precursor to invasive breast cancer (IBC). DCIS comprises approximately 20% of newly diagnosed breast cancer cases, and unlike IBC, is not life-threatening. However, if left untreated, up to half of DCIS patients will develop IBC within 10 years, so clinical management has trended towards presuming all patients are progressors and treating them with surgery, radiation therapy, and pharmacological interventions. Thus, understanding the central biological features in DCIS that drive the transition to IBC is a critical unmet need for guiding appropriate patient care.

This case study describes research at the Stanford University School of Medicine to understand how the tumor microenvironment (TME) changes with transition to IBC. They used Multiplexed Ion Beam Imaging (MIBI™) and a 37-plex antibody staining panel to analyze over 79 clinically annotated surgical resections covering the full spectrum of breast cancer progression.

CASE STUDY

MIBI Spatial Proteomic Signatures of Breast Cancer Progression

The need to better understand signs of progression to invasive breast cancer

It's a decades-old mystery among oncologists: why do some women diagnosed with non-invasive breast tumors progress to invasive breast cancer while others do not? Understanding this — and being able to predict accurately which women fall into which category — would allow clinicians to provide more aggressive treatment to those who need it most, and spare many patients painful and debilitating treatment that is unnecessary.

Recently, scientists at Stanford University and other institutions collaborated to answer this question using a unique combination of valuable tools: Washington University's archival breast tissue collection, clinically annotated with more than a decade of cancer progression data; multiplexed ion beam imaging (MIBI™) technology for high-dimensional detection of spatially resolved proteins; and a machine learning approach to make sense of the tremendous amount of data generated.

Based on their work, the team discovered highly predictive elements associated with progression or non-progression in women diagnosed with ductal carcinoma *in situ* (DCIS). Their efforts have paved the way for future development of a prognostic test that could be a huge leap forward for the 60,000 women diagnosed with DCIS each year in the U.S. alone.

Characterizing DCIS progression

In the paper describing their work, lead author Tyler Risom, senior author Michael Angelo, and collaborators discuss the current challenge that inspired this project. While extensive genomic studies have been performed to find biomarkers associated with progression from DCIS to invasive breast cancer, to date none of

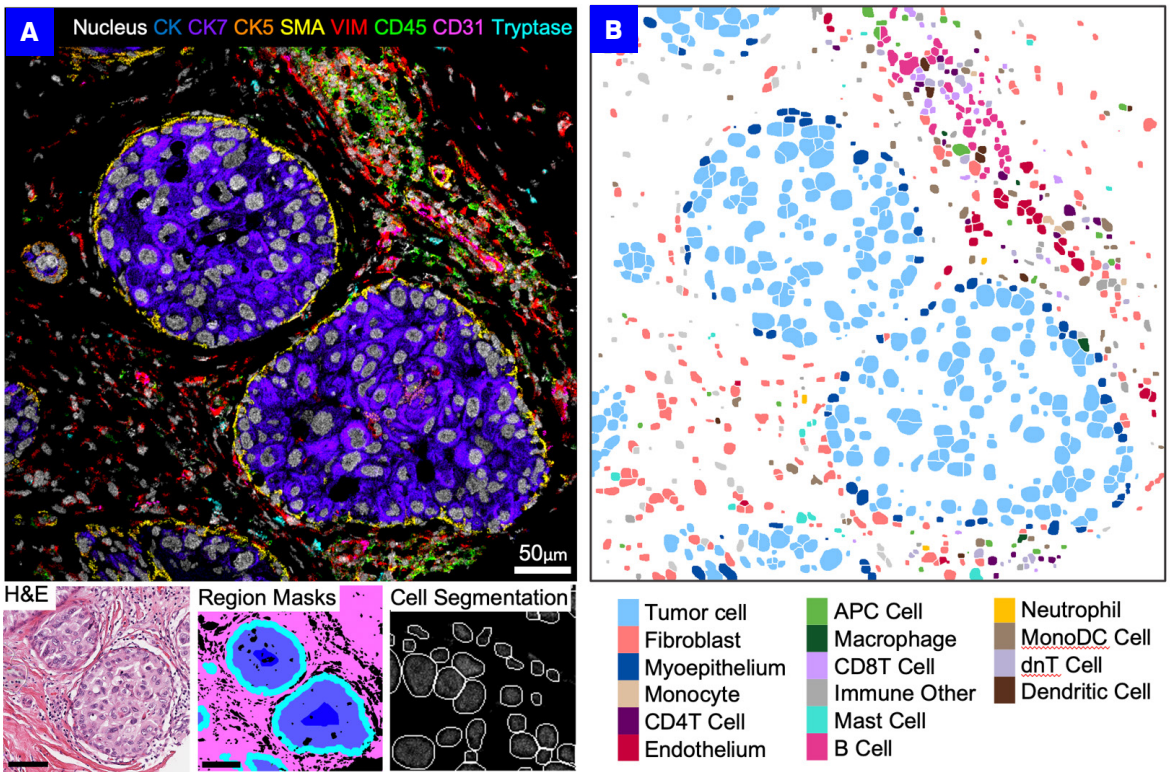


FIGURE 1. Single-cell phenotype map and spatial atlas of a section of DCIS tissue. **A.** MIBI image overlay of a DCIS tumor. The corresponding H&E image; tissue region masks marking stroma (pink); and cell segmentation example are also shown. **B.** Cell phenotype map of the same field of view.

those results has provided a clear, consistent biological explanation for patients' different outcomes.

"In light of this uncertainty, clinical management has trended towards treating all patients presumptively as progressors with surgery, radiation therapy, and pharmacological interventions that carry risks for therapy-related adverse events," the authors report. They posited that spatial proteomics data, which can produce more actionable data related to phenotype, might yield new answers and possibly even help guide treatment decisions.

The team turned to the Washington University Resource Archival Human Breast Tissue cohort, a treasure trove of FFPE samples that includes patients diagnosed with DCIS and records of their outcomes over 11 years, including which patients progressed to invasive breast cancer. Because DCIS is diagnosed based on its hallmark structure — a very specific organization of tumor, stromal, and myoepithelial cells — the scientists were eager to deploy MIBI spatial proteomics technology to resolve the precise cell location, composition and function using 37 proteins of interest.

They used this approach to characterize 79 samples featuring normal breast, DCIS, or invasive breast cancer. Then they implemented machine learning tools to identify and map 16 different cell populations and their varied spatial parameters across the samples. Overall, they measured more than 400 features in each sample and incorporated these into a classifier to determine which ones, if any, could be clearly linked to progression patterns.

How MIBI technology made a difference

The team used MIBI technology, a high-definition spatial proteomics platform commercialized by Ionpath, because of its ability to generate a highly multiplexed image and spatially resolved single-cell profile of the tissue microenvironment.

"With so many diverse cell types in the stroma, myoepithelium, and immune component, the multiplex capability of MIBI technology was one of its most useful advantages over other technologies," Risom says. "Typically the multiplex capability of approaches like multiplex immunofluorescence falls short of being able to look at all those factors at once and show us their spatial organization and localization."

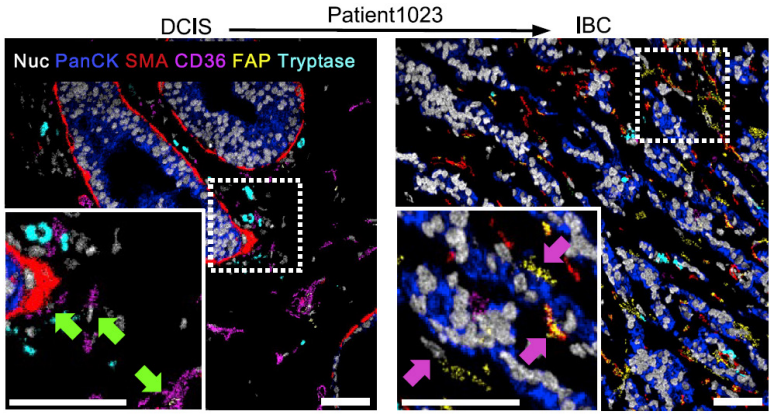


FIGURE 2. Representative MIBI image overlays showing the primary DCIS diagnosis (left) and invasive recurrence (right) from patient 1023. MIBI imaging enabled observation of structural and functional coordination in the tumor stroma that was correlated with disease progression.

The fact that it can be used with archival FFPE samples was also critical. "That allowed us to look at this very special, very powerful patient cohort," Risom adds.

A unique feature of DCIS made its spatial organization of particular interest to scientists: its tumor cells are held at bay by a very thin boundary of myoepithelial cells. When that boundary fails, it gives cancer the opportunity to invade surrounding breast tissue. But studying these cells has been a major challenge. "There are so few of them and they're stretched so thin," Risom says. Using a pixel-based clustering approach with MIBI technology, his team was able to profile the myoepithelium as a layer of tissue. "We were able to isolate the myoepithelial layer and assess its thickness and continuity around the tumor based on how many nuclei or cells are within a certain amount of space," Risom says.

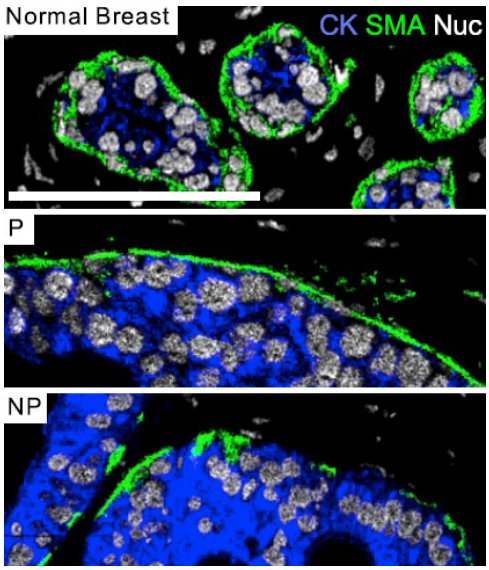


FIGURE 3. Zoomed in areas of MIBI overlays show differences in myoepithelial integrity in normal breast tissue, a DCIS progressor tumor (P), and a non-progressor tumor (NP).

Key discoveries and next steps

By mining the MIBI data and using more than 400 features to train a machine learning classifier, the scientists made huge strides in differentiating between women who progressed to invasive breast cancer and those who didn't. Key features that were strongly associated with progression or non-progression were then assessed on samples not used to train

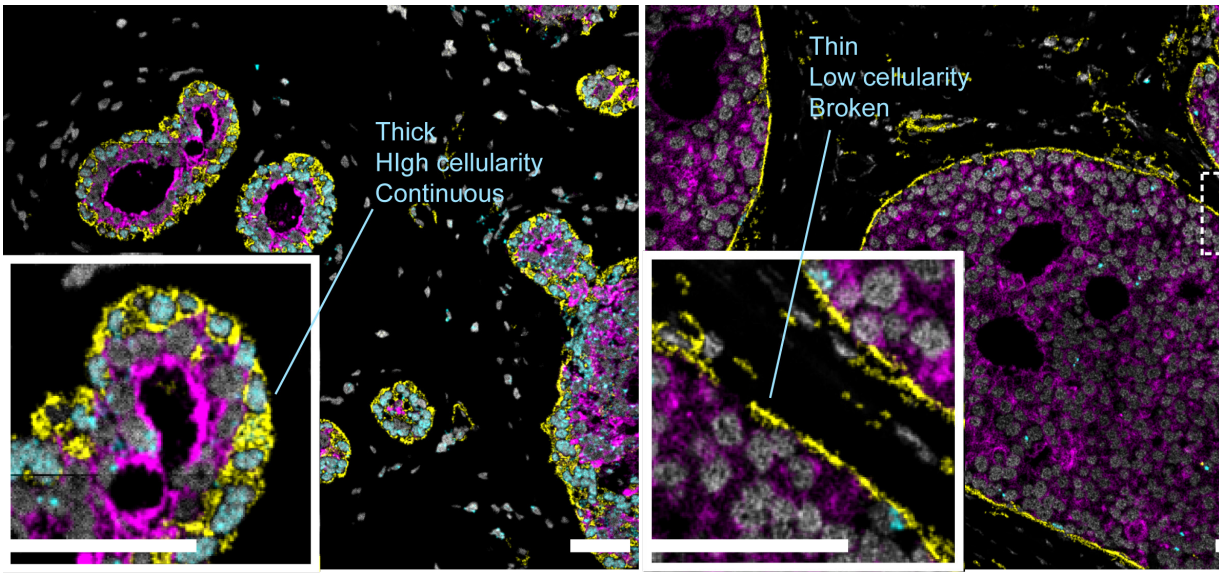


FIGURE 4. MIBI overlays of normal (left) and DCIS (right) tissue. Differences in thickness, cellularity, and integrity of "myoepithelial layer" can be observed.

the classifier, demonstrating that it had "high accuracy in identifying women who would progress," Risom says. "The number of features is really what gave the classifier its power. If you only had four-color immunofluorescence, you couldn't get to 433 features."

One of those defining features was an E-cadherin high myoepithelial phenotype that appears to be highly enriched in patients whose DCIS later progressed to invasive disease. "It was really prominent, and it took the resolution and multiplexing powers of MIBI to be able to isolate that thin membrane and spot the expression state," Risom says.

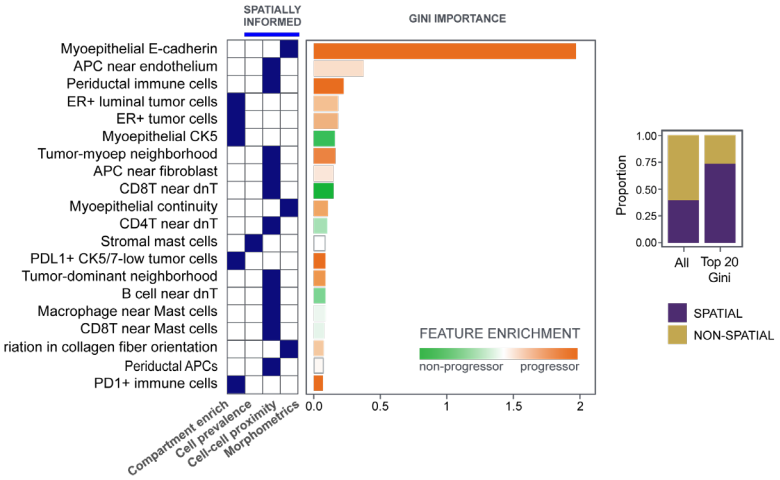


FIGURE 5. Of the top features correlated to disease progression, spatial features outnumbered non-spatial features —highlighting the utility and importance of spatial analysis.

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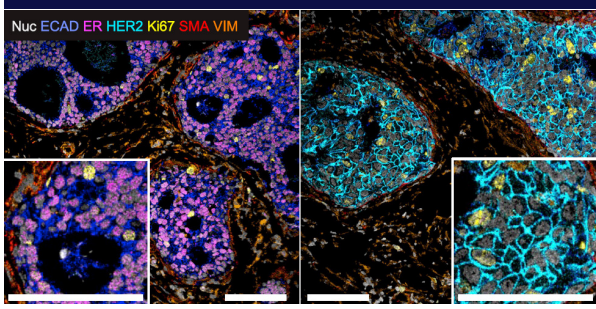

Other features, many of them structural, were also associated with progression. All of these candidates will be followed up in future studies in the Angelo lab to better understand their link to disease state. The Angelo lab has also initiated studies on larger patient cohorts to verify the predictive power of a MIBI-based diagnostic test with the ultimate goal of solving the elusive challenge of predicting the DCIS progression and informing appropriate patient treatment.

This work was done as part of the Human Tumor Atlas Network. Once released, the full results will be available there.

Risom, T. *et al.*, Transition to invasive breast cancer is associated with progressive changes in the structure and composition of tumor stroma. *Cell* 2022(2):299-310.

doi: https://doi.org/10.1016/j.cell.2021.12.023

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