SPOT LIGHT

Breast cancer disparities and tumor biology

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How Imaging Mass Cytometry transformed one lab's discovery of the intrinsic behavior of a tumor

Breast cancer mortality is more than 40% higher for African American women in the US than Caucasian women. Yet, the cancer's incidence across race is very similar.

Melissa Davis, PhD, is Scientific Director of the International Center for the Study of Breast Cancer Subtypes (ICSBCS) and Assistant Professor of Cell and Developmental Biology at Weill Cornell Medical College. She is working to uncover the various components that contribute to cancer mortality disparities between races.

While multiple factors play a role in cancer survival, tumor characteristics are believed to account for almost a quarter of racial differences. Early research on variations in tumor biology among breast cancer patients of different races labeled hormone receptor status as a differentiating factor. To take this further, Davis explains, "The triple-negative subtype in breast cancer lacks expression of hormone receptors that are the basis of common targeted therapies and has the highest rate in non-Hispanic black patients. It is important that we identify what molecular markers underly this subtype beyond hormone receptor status or genomic profiling, which can be impractical in a clinical setting."

A look into African history explains much about the origins of the triple-negative subtype and what other related phenotypes might be present in women with this diagnosis. Davis focuses on the African diaspora, commonly used to describe the mass dispersion of African people during the transatlantic slave trade, recruiting patients for sample collection from Africa, the Caribbean islands and North America.

Africa has the lowest incidence of breast cancer in the world, yet the highest mortality rate due to the prevalence of triple-negative diagnoses. This component of African ancestry spreads to black Americans in the US and black South Africans, with similar trends in West Africa, all coinciding with the transatlantic slave trade across the Americas and the Caribbean.

Exploring the DARC phenotype

Davis and her lab aim to find molecular markers and functional differences that might explain the frequency of the triple-negative subtype in these populations. They are currently focused on one particular allele, the Duffy-null allele of the Duffy antigen receptor for chemokines (DARC) gene, that arose in sub-Saharan Africa and plays a role in immune response regulation. It was fixed into this population because it confers resistance to malaria.

Its global distribution is vast, with over 70% of Africans carrying the allele in America alone, and tracks with movement along the transatlantic slave trade. Duffy-null carriers are also erythrocytic-null, expressing the gene only in endothelial

cells. This is an important feature because it results in recruitment of immune cells into inflamed areas through the circulatory system. In a tumor, this would influence the microenvironment in an organ site, such as in breast cancer.

Recently, the Davis lab discovered that tumors expressing this gene also exhibit higher levels of inflammatory chemokines and infiltrating immune cells. Owing to this chemokine recruitment, a high level of DARC expression in a tumor is associated with significant survival benefits in all subtypes.

Imaging DARC with immunohistochemistry

In the effort to uncover how the DARC gene phenotype bestows a survival advantage, a look at gene signatures correlated with DARC status identified a gene subset that tended to be more highly expressed in DARC-positive tumors. Davis used these as a template to learn more at the single-cell level with traditional immunohistochemistry (IHC).

The goal was to determine whether these markers and the patterns of these markers correlate synchronously across the tumor space by compartmentalizing features and quantifying cell types. However, IHC quickly became its own challenge, with the inability to multiplex marker staining on single sections. The initial work was tedious but yielded results after months of simple cell identification. The team saw a higher rate of infiltrating CD3+ cells. Davis focused in on 20-plus markers, but with IHC, this would become exponentially more difficult. The solution was Imaging Mass Cytometry™ (IMC™).

"If we were going to describe and characterize a tumor phenotype based on heterogeneity, we needed a better platform. We turned to IMC with the Hyperion™ Imaging System at the Englander Institute for Precision Medicine to define spatial distinctions characterizing the DARC immune tumor type," said Davis. "We were able to multiplex up to 30 markers as our target number of conjugated antibodies on a single slide and acquire this data in a way where we could quantify the markers and do computational analyses."

Moving forward with IMC

A first step was careful consideration of the regions of interest (ROIs). With a need to compare populations, it was important to collaborate with a pathologist who could demarcate areas of interest to capture the stroma as well as the solid tumor space and to ensure that the captured area was comparable across different patient populations.

IMC comparison of the tumor types showed that in the DARC-negative tumors, immune cell markers isolated to the stromal area with less infiltration into solid tumor space. Conversely, images of DARC-positive tumors

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showed more infiltration into the solid tumor space and increased association with endothelial cells and vascular structure. Differences in tumor architecture suggest that infiltration occurs because DARC-positive tumors are more vascularized. These findings blend nicely with prior bulk-tumor RNA-seq data indicating that the spatial distribution of immune cells is variable between tumors.

"And we can apply this to other diseases, such as cancer, that are highly reliant on an immune response."

"These images provided a view of the distribution of markers and relationships of cell populations across DARC-positive and DARC-negative tumors. Quantifying behavior at a single-cell level with IMC offers more information about what is happening across the architecture and connects these findings to clinical implications in breast cancer," clarifies Davis.

IMC made it easy to identify subpopulations of cells present only in a DARC-high vs. a DARC-low environment by combining all ROIs to view non-overlapping subsets. Davis found one subset of cells in DARC-positive tumors that co-expressed immune cell and mesenchymal markers, mapping these vimentin-positive immune cells as infiltrating into the solid tumor area and indicating a distinct DARC-regulated tumor microenvironment. Further study will include functional characterization of these subpopulations using models of DARC tumors in mice and *ex vivo* patient-derived organoids.

Initial clinical implications of these phenotypes for survival advantage are promising. Next steps in this project will be to correlate DARC phenotypes in tissues with clinical outcome and clinical annotation and to stratify populations by subtype.

Imaging Mass Cytometry empowered Davis not only to discover what cell populations are present in these tumors and where they are located but, simultaneously, also to detail the intricate behavior and interactions occurring among cells and how these actions affect clinical outcome in triple-negative breast cancer patients.

"Why is this important in the context of disparities? We know from research on human evolution that immune cells originating from people of African descent compared to European descent have completely different responses to the same bacterial pathogen. This indicates that evolutionary consequences or natural events, like the types of pathogens present in Europe or in Africa, have elicited unique immune responses," Davis concludes.

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“And we can apply this to other diseases, such as cancer, that are highly reliant on an immune response. Consider implications on therapeutics, and whether tumor cell subtypes could identify subpopulations in which immunotherapies are effective and predict treatment response based on these patterns.”

Watch Melissa Davis’ webinar on this topic:
Characterizing distinctions in DARC-related Tumor immune MicroEnvironment (DARC TiME)

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