Association of the Immune Microenvironment and Race in the Carolina Breast Cancer Study

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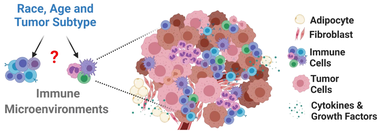
Introduction

- Black women suffer 40% higher mortality from breast cancer (BC) compared to non-Hispanic white women, even within BC subtypes (Richardsen *et* al., 2016; Cary *et al*., 2006; O'Brien *et al*., 2010; Desantis *et al*., 2015).
- The tumor immune microenvironment is becoming increasingly recognized as an additional and important mediator of tumor initiation, progression and response to therapy.
- Using gene expression as a surrogate measure of tumor infiltrating lymphocytes (TILs), multiple studies have found that high levels of TILs positively predict response to therapy in triple negative (TNBC) and HER2-positive BC(Adams *et al*., 2014; Denkart *et al*., 2010; Loi *et al*., 2014).
- However, immune response in BC has never been studied in a diverse, population-based study. As a result, differences in the BC immune microenvironment by race are poorly understood.

Study Objective

To characterize immune microenvironments of BC in a diverse patient population and evaluate how they vary with regard to molecular, clinical and epidemiological variables, including race and age.



Study Population and Assay

The Carolina Breast Cancer Study (CBCS)

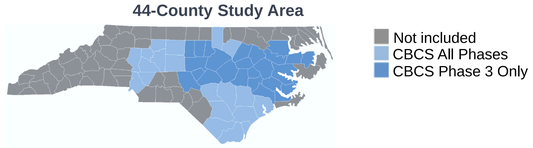
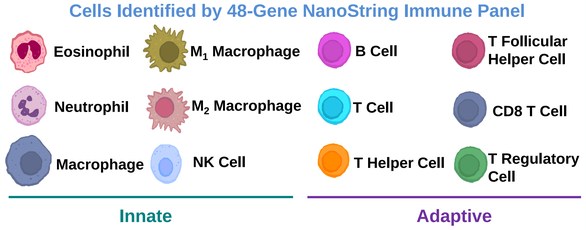


Figure 1. The Carolina Breast Cancer Study (CBCS) is a three-phase population-based study that enrolled approximately 5300 women across 44 North Carolina counties. The CBCS oversampled black and young (<50) women to allow for the evaluation of biological and socioeconomic risk factors that contribute to racial disparities in BC.

NanoString RNA Expression Profiling

Cells Identified by 48-Gene NanoString Immune Panel



Methodology

Study Cohort

- 1952 BC tumors from 1030 Black and 922 non-Black participants from CBCS phases 1-3.
- Race in our study was self-reported and represents a social construct.
- RNA was extracted from FFPE tumor blocks and gene expression was measured using the NanoString RNA expression assay.

Immune Phenotypes

- Consensus clustering was used to classify tumors into expression-based immune microenvironment phenotypes.
- TILS were quantified from tissue microarrays (TMAs) by histopathology review and a validated computer-trained lymphocyte algorithm (Olsson *et al*., *unpublished*).

Statistical Analysis

- Relative frequency differences (RFDs) and 95% confidence intervals (95% CI) were used to estimate associations between immune phenotypes and other variables.
- Immune cell scores were calculated as the median expression of all genes in each signature, and compared across covariates of interest using generalized linear models adjusting for age, race and tumor subtype.

Results

Global Immune Phenotypes in the CBCS

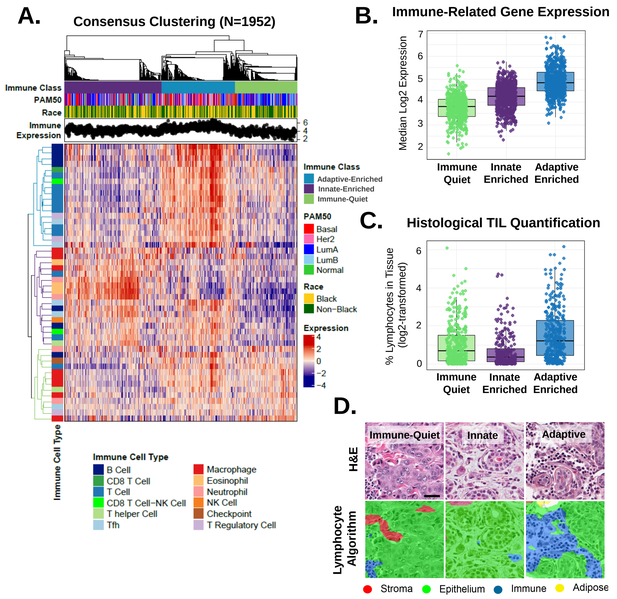


Figure 2. Global immune classes correspond to distinct phenotypes enriched for features related to adaptive-enriched, innate-enriched and immune-quiet microenvironments. A) Consensus clustering using 48-gene immune panel groups CBCS tumors into three immune phenotypes that correlated with B) median overall immune gene expression levels. C) Histological quantification of TILs from tumor slides revealed significantly more TILS in adaptive-enriched tumors than innate-enriched and immune-quiet tumors. D) Representative H&E images and algorithm tissue classification of adaptive-enriched, innate-enriched and immune-quiet tumor sections. Scale Bar = 25μm.

Results

Association of Immune Phenotypes with Patient and Tumor Characteristics

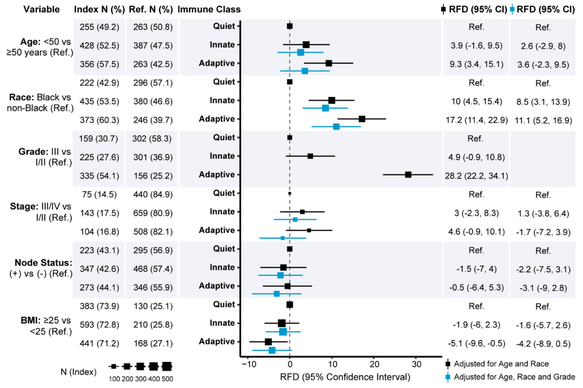


Figure 3. Adaptive-enriched class associates with young age and high tumor grade, while both Innate-Enriched and Adaptive Enriched classes associate with Black race. RFD: relative frequency difference; 95% CI: 95% Confidence Interval; BMI: body mass index. Referent group = Immune-Quiet

Association of Immune Phenotypes and both Clinical and Intrinsic Tumor Subtypes

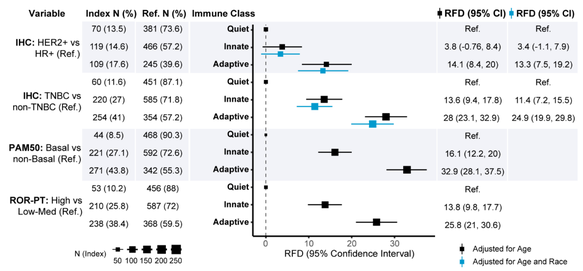


Figure 4. Adaptive-enriched and Innate-enriched classes associate with TNBC, the basal-like subtype, and high risk of recurrence scores.
RFD: relative frequency difference; HR: Hormone receptor; ROR-PT: risk of recurrence score incorporating subtype, proliferation score and tumor size; TNBC: triple negative breast cancer. Referent group = Immune-Quiet

Immune Cell-Specific Expression Differences by Age

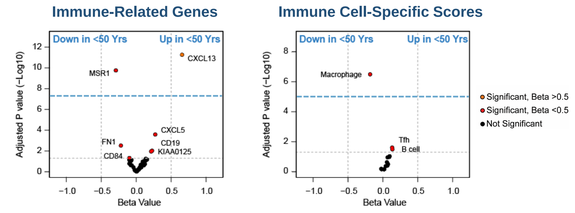


Figure 5. Immune cell marker-specific expression differs between younger and older participants. Volcano plots reveal significantly increased expression of B cell markers, but decreased macrophage markers in patients <50 years old. However, after additional adjustment for tumor subtype, only markers above the blue-dashed line remained statistically significant.

Results

Immune Cell-Specific Expression Differences by Race and Tumor Subtype

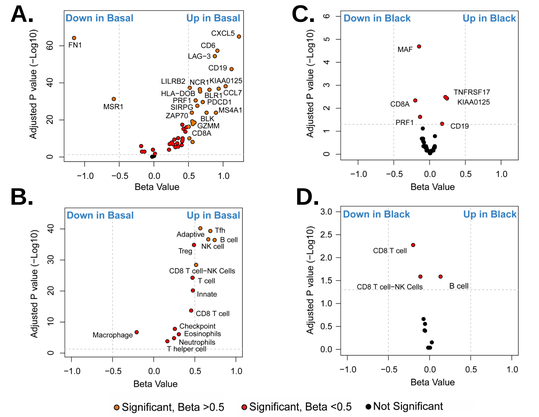


Figure 6. Immune cell marker-specific expression differ across subtype and race. A) Volcano plots of basal-like vs non basal-like tumors illustrate significant differences in immune related genes and (B) immune cell scores, with most immune markers being increased in basal-like tumors relative to non-basal tumors. C) Volcano plots display differentially expressed immune genes by race, with D) significantly elevated B Cell scores and decreased CD8 T cell scores in tumors from black women relative to non-black women, even after adjustments for age and tumor subtype.

Conclusions

1. Identified three global immune phenotypes in the CBCS based on expression related to an adaptive-enriched, innate-enriched or immune-quiet microenvironment.
   - These groups were correlated with median overall immune gene expression and TIL measurements from H&E tissue sections.
2. Adaptive and Innate-enriched immune classes were strongly associated with high ROR-PT scores, TNBC, the basal-like intrinsic subtype and Black race.
3. Differential expression analysis revealed that immune marker gene expression is highest in the basal-like BC subtype. With respect to race, CD8 T cell markers were significantly decreased in tumors from black women relative to non black women, while B cell markers were elevated.
4. Our data suggest that immune response is intricately related to race and tumor subtype, and suggest that race-specific differences in immune cell distributions exist within breast tumors.
5. This North Carolina study represents the largest cohort to evaluate the role of race and the immune microenvironment. The results of this work provide critical information to help clinicians close the health disparities gap while highlighting the importance of racial diversity in impactful clinical trial design both in North Carolina and beyond.

Acknowledgements

The authors would like to thank CBCS participants and staff who generously gave their time to these studies. This work was supported by the Graduate Training Program in Translational Medicine (AMH); Komen Career Catalyst Grant (CCR16376756) (KAH); P50 CA058223 and U01 CA179715 (MAT); P30 ES010126, and U54 CA156733 (MAT); Susan G. Komen for the cure (CBCS).