A Novel Spatial Profiling Approach Reveals Distinct Fibroblast and Immune Architectures in Invasive Pleomorphic versus Classic Lobular Carcinomas

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Background

Invasive Lobular carcinomas (ILCs) comprise a histologically and biologically diverse group of breast cancers. While classic invasive lobular carcinomas (ICLCs) are typically characterized by cohesive, infiltrative growth and immune-excluded microenvironments, pleomorphic invasive lobular carcinomas (IPLCs) display a combination of different histological patterns. IPLCs are characterized by tumor cells typically present with enlarged, irregularly contoured nuclei, pronounced hyperchromasia and pleomorphism, high mitotic activity, and a cytoplasm that appears eosinophilic and faintly granular. Several studies describe a rapid clinical course marked by early relapse, higher recurrence rates, and reduced overall survival in IPLCs. The role of cancer-associated fibroblasts (CAFs) in lobular carcinoma, and particularly the disparities between these two subtypes, remains poorly understood. Research into this area presents a significant opportunity to improve treatment strategies for aggressive subtypes of this tumor. Based on our previous findings of differential CAF cell densities in five phenotypes between ICLCs and IPLCs, we aimed to investigate the spatial relationships and distribution patterns within these phenotypes, as the spatial organization of fibroblasts and immune cells in these histologic subtypes remains underexplored.

Methods

Twelve primary ILC surgical specimen whole slide sections (IPLCs: cases 7–12; ICLCs: cases 13–18) were obtained from departmental archives through IRB protocols approved by the Institutional Review Board at The University of Texas MD Anderson Cancer Center. These cases were collected based on the WHO diagnostic criteria which is based on histology to distinguish the pleomorphic ILC cases and the status of E-Cadherin IHC (negative) to diagnose the case as ILC. Only the cases which had at least 30% of stromal content by microscopic analysis of hematoxylin and eosin-stained sections by two independent pathologists were included in the study. All the cases did not receive neoadjuvant therapy at the time of biopsy. We performed high-dimensional multiplex immunofluorescence (mIF) spatial analysis using a comprehensive fibroblast-focused panel (including FAP, S100A4, Thy1, A-SMA) and immune markers (CD45) were used to characterize tumor (CK+), fibroblast, and immune compartments. Spatial clustering and neighborhood analysis were conducted using the Spatial Image Analysis of Tissues (SPIAT) R package v 4.5.1, to define the microenvironmental architecture.

Results

IPLCs demonstrated marked spatial heterogeneity, with fragmented CK+ tumor regions interspersed among stromal and immune elements. Fibroblast landscapes in IPLCs were notably diverse, featuring abundant hybrid subtypes (e.g., S100+Thy1+, A-SMA+FAP+) and frequent tumor-stroma integration within shared neighborhoods. These dynamic fibroblast populations supported active extracellular matrix (ECM) remodeling, potentially facilitating invasion and contributing to partial immune engagement. CD45+ immune cells were consistently more prominent in IPLCs, forming discrete immune-enriched clusters and mixed neighborhoods, suggesting an immune-intermediate or partially inflamed phenotype.

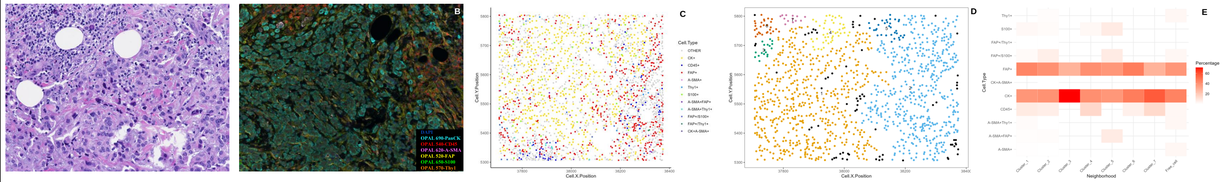


Figure 1. IPLC. A) H&E, 20x. B) mIF Composite, 20x. C) Cell categories. D) Cluster composition. E) Neighborhood composition.

In contrast, ICLCs displayed large, cohesive CK+ tumor regions with minimal fragmentation and low spatial heterogeneity. Fibroblast subtypes were simpler and predominantly peripherally distributed, and they rarely formed hybrid phenotypes. The stroma primarily acted as structural support, with limited ECM remodeling and minimal immune modulation. CD45+ cells were sparse and diffusely scattered, consistent with an immune-excluded ("cold") microenvironment..

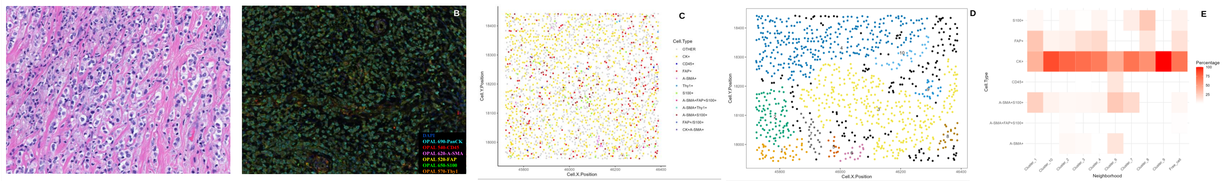


Figure 2. ICLC. A) H&E, 20x. B) mIF Composite, 20x. C) Cell categories. D) Cluster composition. E) Neighborhood composition.

Conclusions

While descriptive, our data reveal profound differences in the spatial microenvironments of IPLC and ICLCs. IPLCs exhibit a dynamic, heterogeneous stroma characterized by integrated fibroblast-tumor interactions and increased immune infiltration, aligning with their aggressive clinical behavior. In contrast, ICLCs maintain a cohesive tumor architecture with simplified, peripheral stroma and sparse immune presence.

These insights highlight the significance of spatial context as an innovative and informative strategy in comprehending ILC biology and suggest that stromal and immune features may inform therapeutic strategies, including potential vulnerability to stroma- or immune-targeted interventions in IPLCs.

References

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