PO1-15-03 - Spatially resolved analysis of tumor microenvironment in invasive lobular carcinoma

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Background

- Invasive lobular breast carcinoma (ILC) represents 15% of all invasive breast cancers (BC), but it remains an understudied subtype
- Characterized by late relapse
- Loss of E-cadherin cell adhesion molecule and typical "single file" pattern of the cells
- The tumor microenvironment (TME) is the set of normal cells, molecules and blood vessels that surround and feed a tumor cell
- Interaction between cancer cells and TME plays a role in defining prognosis in BC
- TME of ILC is characterized by low presence of TILs, and higher level of TILs are associated to worse disease outcome

Materials and Methods

Spatial transcriptomics

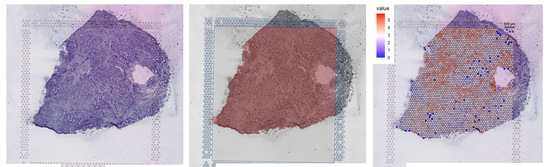


Figure 1.

Pros:
- Spatial information
- Higher resolution than bulk RNA-seq

Cons:
- High cost
- Lower resolution than single-cell

Data

- Spatial transcriptomics (ST - Fig. 1) was performed on 43 ILC primary frozen tumor samples (HR+, HER2-) from patients with long term follow up (Table 1.)

	ST cohort	Grade			Nodal status		Relapse	
	Tot	G1	G2	G3	N0	N+	No	Yes
N. of samples	43	5	34	4	30	13	34	9

Table 1.

- Microarray ILC dataset (METABRIC, ILC = 122) was used as external validation dataset

Objectives

- To characterize the spatial transcriptome heterogeneity of ILC including its tumor microenvironment
- To interrogate whether spatial transcriptomics may improve the prediction of the risk of recurrence in ILC

Results

Morphological annotation and co-occurrence analysis

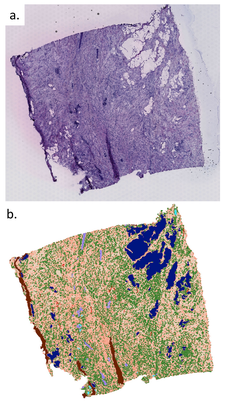


Figure 2.

H&E slides (Fig. 2a) were annotated using QuPath (Fig. 2b) reaching single cell resolution. The level of co-occurrence (CO) between tumor and each cell type in the TME was computed (for each cell type) as:

$$CO = \frac{N.mixed\ spots}{N.tumor\ spots}$$

where "N. mixed spots" is the number of ST spots containing both tumor and the class of interest and "N. tumor spots" is the total number of tumor spots

Clustering analysis

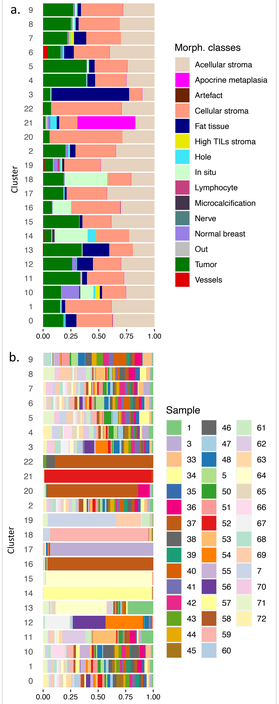
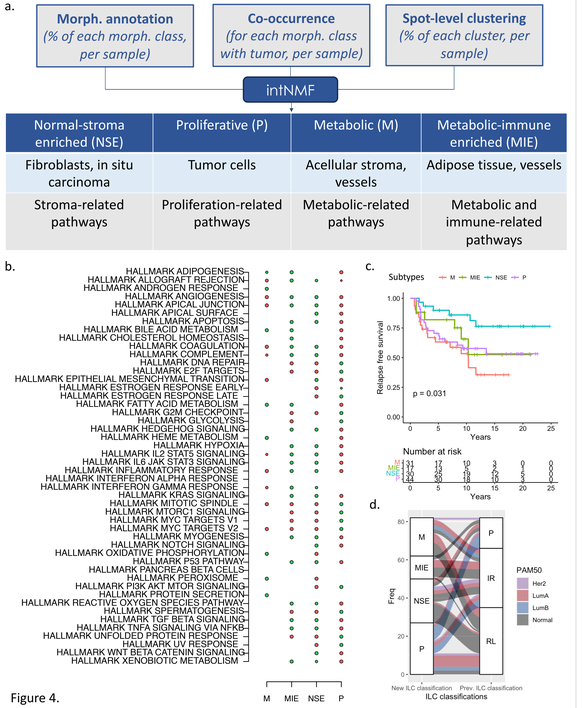


Figure 3.

23 clusters were obtained across all the samples. Some clusters were sample-specific, other clusters were shared between all the samples (mainly normal structures, Fig.3a-b)

ILC classification



Normal-stroma enriched (NSE)	Proliferative (P)	Metabolic (M)	Metabolic-immune enriched (MIE)
Fibroblasts, in situ carcinoma	Tumor cells	Acellular stroma, vessels	Adipose tissue, vessels
Stroma-related pathways	Proliferation-related pathways	Metabolic-related pathways	Metabolic and immune-related pathways

Figure 4.

The information coming from morphological and sequencing analyses was merged and used to obtain a patient-level classification. Four classes were identified, defined by differences in morphology and gene expression (Fig. 4a). Group-specific gene signatures were built, and the four groups were retrieved on METABRIC (Fig. 4b), where NSE group was associated to longer RFI (Fig. 4c). No overlap with PAM50 and previous ILC classification was found (Fig. 4d)

Adipose tissue analysis

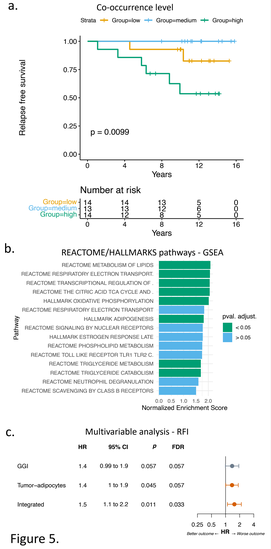


Figure 5.

Higher co-occurrence level of adipose tissue with tumor was associated to worse disease outcome (Fig. 5a). Adipocytes-tumor contact area was enriched in metabolic-related (Fig. 5b) pathways. Such contact area was also enriched in macrophages M2 (from xCell). The gene signature derived from this contact area was associated with bad prognosis in METABRIC, but it wasn't correlated to other prognostic signatures in ILC. Integrated with GGI, our signature showed a high prognostic value (Fig. 5c)

Take-home messages

- ILC is an understudied subtype, with peculiar biological and clinical features
- Spatial transcriptomics enables the analysis of the tumor microenvironment in relation to its composition and organization
- Heterogeneity, both within individual patients (intra-patient) and between different patients (inter-patient), was observed in both morphology and gene expression
- The presence of this heterogeneity enabled the identification of four distinct ILC subgroups: normal-stroma enriched, proliferative, metabolic, metabolic-immune enriched
- These subtypes showed differences in prognosis also in external datasets
- Adipose tissue-tumor cells interactions were associated with worse disease outcome in ILC
- Metabolism seemed to play a key role in ILC biology

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References 1) Ciriello G, et al. 2015. Cell. 2) Desmedt C, et al. 2016. J. Clin. Oncol. 3) Desmedt C, et al. 2018. J. Natl Cancer Inst. 4) NIH - National Cancer Institute. 5) Technical Note – 10x Genomics, (2019, April 30). 6) Parker, et al. 2009. JCO. 7) Sotiriou C, et al. 2006. J Natl Cancer Inst.

Acknowledgments