Spatially resolved analysis of tumor microenvironment revealed biologically driven subgroups with distinct clinical outcome in invasive lobular carcinoma

Matteo Serra[1], Mattia Rediti[1], Laetitia Collet[1], Frédéric Lifrange[2], Nicola Occelli[1], David Venet[1], Xiaoxiao Wang[1], Delphine Vincent[1], Ghizlane Rouas[1], Ligia Craciun[2], Denis Larsimont[2], Miikka Vikkula[3], François P Duhoux[4], Laurence Buisseret[1], Françoise Rothé[1], Christos Sotiriou[1]

[1]Breast Cancer Translational Research Laboratory J-C Heuson, Institut Jules Bordet, Université Libre de Bruxelles, Brussels, Belgium, [2] Laboratoire d'Anatomie Pathologique, Institut Jules Bordet, Université Libre de Bruxelles, Brussels, Belgium, [3] Human Molecular Genetics, de Duve Institute, Université Catholique de Louvain, Brussels, Belgium, [4] Department of Medical Oncology, Institut Roi Albert II, Cliniques universitaires Saint-Luc, UCLouvain, Brussels, Belgium

LAY ABSTRACT

In this work, we aimed to study tumor microenvironment heterogeneity in ILC. We performed spatial transcriptomics (ST) on 43 frozen tumor samples obtained from patients with estrogen receptor-positive, HER2-negative ILC. ST is a technique that allows us to sequence the RNA of a slice of tissue by keeping the spatial information of the RNA expression, thanks to special spots that are able to capture the RNA of the tissue. Hematoxylin/eosin sections relative to each sample were morphologically annotated by assigning a cell type (label) to each cell and structure present in the slide. We performed clustering analysis in each sample (at the spot level), identifying groups of spots (across all our patients) sharing common characteristics from their gene expression point of view. The information coming from the spot level clustering analysis and the morphological annotation was merged and used as input for a clustering analysis at the patient level. We identified four groups of patients inside our cohort, and we annotated them using both morphological and gene expression data. The groups were named as: proliferative (P), normal-stroma enriched (NSE), metabolic (M) and metabolic-immune enriched (MIE). We validated our findings in an external cohort (METABRIC), and we observed the same biological differences that we observed in our dataset among the four groups. Moreover, we observed differences in disease outcome between groups (with NSE showing better and M and P worse outcome for relapse-free survival). Of note, two of the three groups associated to worse disease outcome (M and MIE) were related to metabolism and not to proliferation, showing an important implication of metabolism in the biology of ILC.

OBJECTIVES

- To characterize the **spatial transcriptome heterogeneity** of lobular BC including its tumor microenvironment
- To interrogate whether spatial transcriptomics may improve the **prediction of the risk of recurrence** in lobular breast cancer

BACKGROUND

- Invasive lobular breast carcinoma (ILC) represents **15%** of all invasive breast cancers (BC)
- Understudied subtype
- Characterized by **late relapse**
- Loss of cell adhesion and typical "single file" pattern of the cells (Fig. 1)
- The tumor microenvironment (TME) is the set of **normal cells, molecules and blood vessels** that surround and feed a tumor cell (Fig 2.)
- A tumor can change its TME, and the TME can affect how a tumor growths and spreads
- Interaction between cancer cells and TME plays a role in **defining prognosis** in BC

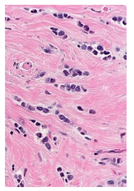

Figure 1.

CONCLUSION

- Morphological annotation and clustering analysis revealed a high level of **inter- and intra-patient heterogeneity**, both in terms of morphology and gene expression
- This heterogeneity allowed us to **identify four groups of patients** (classes)
- These four groups showed **different biological features and different disease outcome** in our dataset
- Those differences (both biological and in terms of survival) were observed also in METABRIC
- Since 2 of these 4 groups were related to increased metabolism, **metabolism seems to be a key feature in ILC biology**

MATERIALS AND METHODS

Spatial transcriptomics

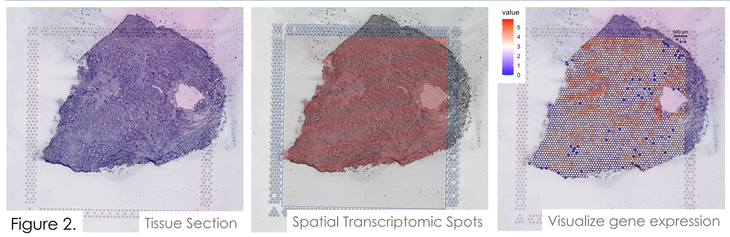

Figure 2. Tissue Section | Spatial Transcriptomic Spots | Visualize gene expression

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

Cons:
- Lower resolution than single cell RNA-seq

Data

- Spatial transcriptomics (ST – Fig. 3) was performed on **43 ILC primary frozen tumor** samples (HR+, HER2-) coming from patients with long term follow up (Table 1.)

	ST cohort	Grade			Tumor stage		Nodal status		Disease relapse	
	Tot	G1	G2	G3	T1	T2-3	N0	N+	No	Yes
N. of samples	43	5	34	4	24	19	30	13	34	9

Table 1.

- Public microarray expression lobular datasets (**METABRIC, n = 122**) as validation set

Computational analysis

Annotation of H&E slides relative to ST samples → Co-occurrence analysis (at the spot-level) between tumor and all the other TME cell types → Clustering of ST spots (based on gene expression) of integrated samples → Integration of annotation, co-occurrence and clustering analyses to build a ILC classification → Validation of our classification in external ILC microarray cohort (METABRIC)

RESULTS

Morphological annotation and co-occurrence analysis

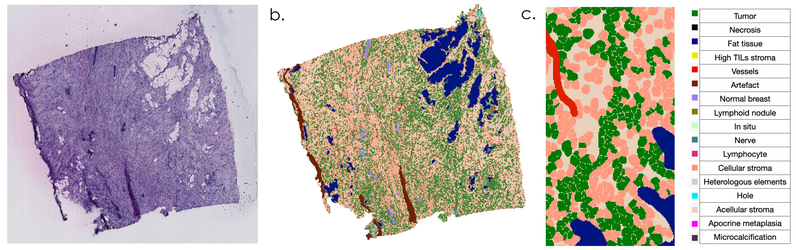

Figure 3.

- Level of co-occurrence (CO) between tumor spots and each cell type in the TME was computed (for each cell type) as in Formula 1, 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 spots containing tumor

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

Formula 1.

Clustering analysis

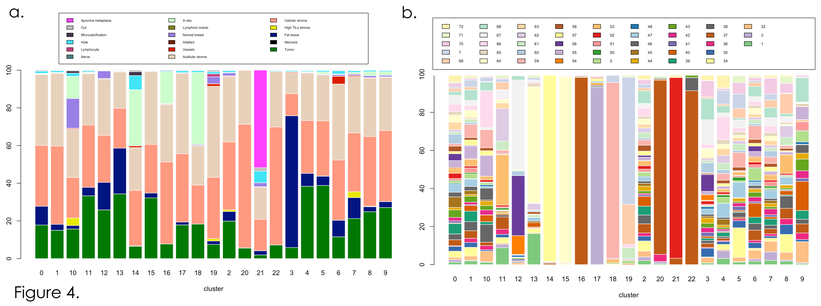

Figure 4.

- 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. 4a,b)

ILC classification and external validation

Morphological annotation *(% of each morph. class per sample)*

Co-occurrence *(CO for each morph. class with tumor, per sample)*

Spot-level clustering *(% of each cluster per sample)*

→ intNMF

	NORMAL-STROMA ENRICHED (NSE)	PROLIFERATIVE (P)	METABOLIC (M)	METABOLIC-IMMUNE ENRICHED (MIE)
Morphology	Fibroblasts, in situ	Tumor cells	Acellular stroma, vessels	Adipose tissue, vessels
GSEA	Stroma-related pathways	Proliferation-related pathways	Metabolic-related pathways	Metabolic and immune-related pathways

Figure 5.

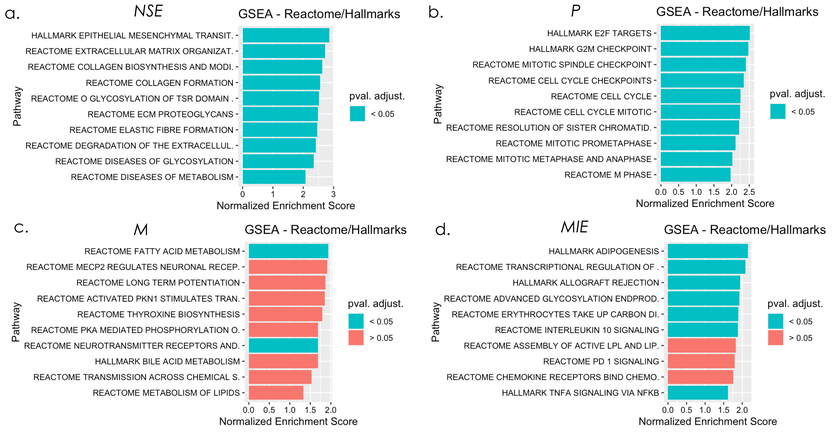

Figure 6.

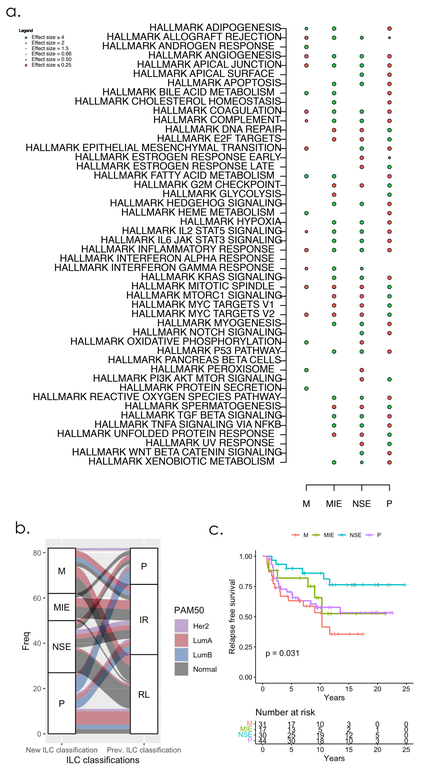

Figure 7.

- Information coming from morphological annotation, co-occurrence analysis and spot-level clustering (summarizing RNA-seq information) was merged and used to feed a clustering algorithm based on NMF (intNMF) to obtain a patient-level classification
- Four classes of patients were identified and annotated using both morphology and gene set enrichment analysis (GSEA, Fig. 5)
- Differences in terms of enriched pathways (Hallmarks and Reactome) between groups are shown in Fig. 6
- To validate our findings, we derived four gene signatures (from differential expression analysis between samples pseudo-bulks) related to the four groups of patients. These signatures allowed us to retrieve the same groups in external microarray cohort (METABRIC)
- In the METABRIC, the four groups showed the same biological differences observed in our cohort (Fig. 7a)
- No concordance was found between our classification, PAM50 and previous ILC classification – proliferative (P), immune-related (IR), reactive-like (RL) subtypes - (Ciriello et al. 2015. Cell, Fig. 7b).
- Survival analysis performed in METABRIC showed that NSE was associated to longer relapse-free survival (Fig. 7c) compared to the other subtypes

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*

ACKNOWLEDGMENTS AND CONTACTS

Acknowledgments: Fond de la Recherche Scientifique, Télévie, Association Jules Bordet, Breast Cancer Research Foundation. **Contact:** matteo.serra@hubruxelles.be