P2-21-01 - Decoding Inter- and Intra-Tumor Heterogeneity in Lobular Breast Cancer Using Spatial Transcriptomics and Clustering Analysis

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BACKGROUND

Lobular breast cancer

- Invasive lobular breast carcinoma (ILC) represents around **15%** of all invasive breast cancers (BC)
- Characterized by **late relapse**
- Loss of cell adhesion and typical "single file" pattern of the cells (**Fig. 1**)
- Frequent mutation of *CDH1*, **PIK3CA**, *PTEN*, *AKT1*

Fig. 1.
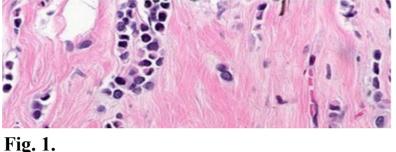
Tumor microenvironment

- The tumor microenvironment (TME) is the set of **normal cells, molecules and blood vessels** that surround and feed a tumor cell
- A tumor can influence its TME during evolution, and the TME can affect how a tumor grows and spreads
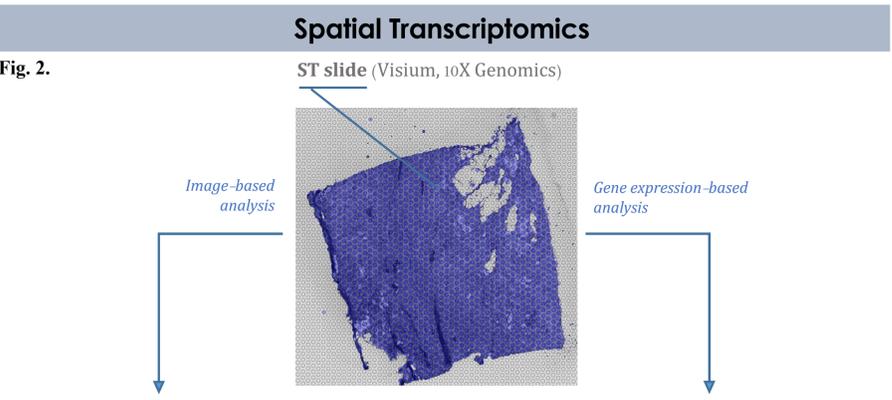
OBJECTIVES

- 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

CONCLUSIONS

- Different tumor clusters characterised by different hallmarks were present in the same tumor, highlighting **intra-patient heterogeneity**
- **Inter-patient heterogeneity** was highlighted by the sample-specificity of some other tumor clusters
- **Differences in the spatial organisation of the clusters** were associated with **differences in disease outcome** in our dataset, with a higher spatial heterogeneity (higher level of disorganisation) of tumor clusters being associated with disease relapse
- Our results revealed a substantial inter- and intra-patient heterogeneity of ILC both at the tumor and TME levels. Different tumor clusters characterized by specific hallmarks were associated to specific clinical features and disease outcome, offering novel perspectives for optimized ILC care
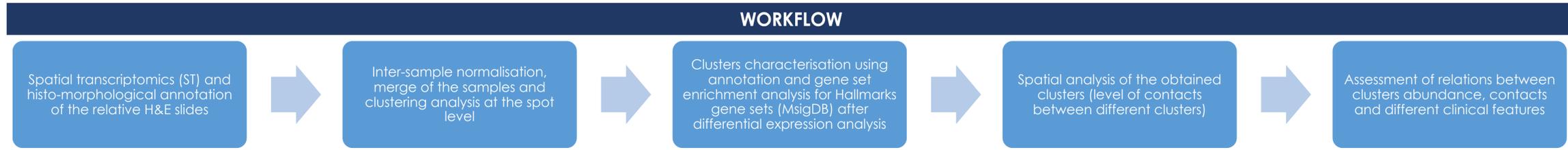
METHODS

Spatial Transcriptomics

Fig. 2.

ST slide (Visium, 10X Genomics)

Image-based analysis

Gene expression-based analysis

500 µm

Histo-morphological annotation

H&E slide

Semi-automated annotation

Annotated H&E slide

analysis

RNA sequencing (spot level)

value

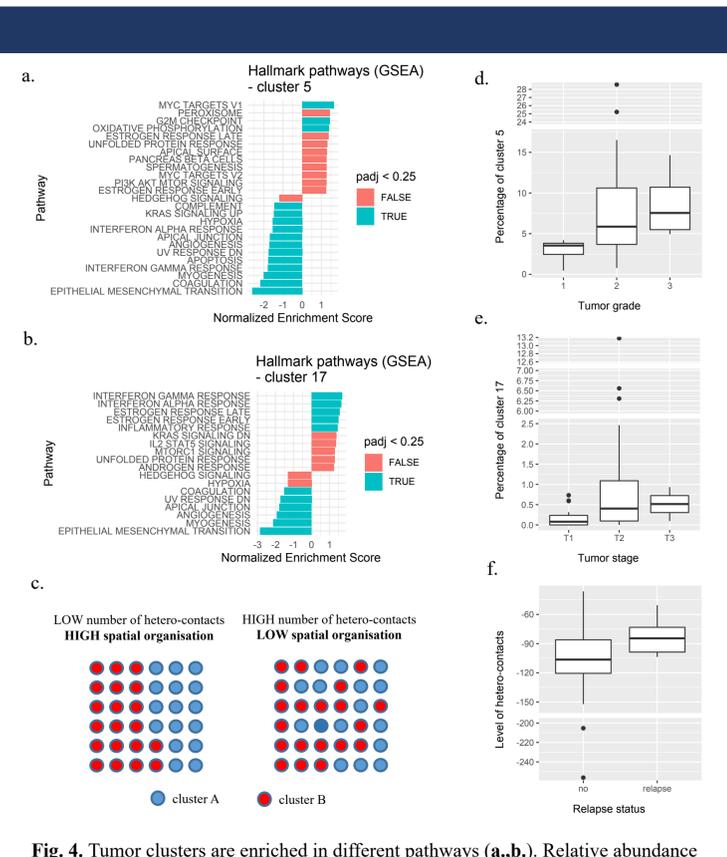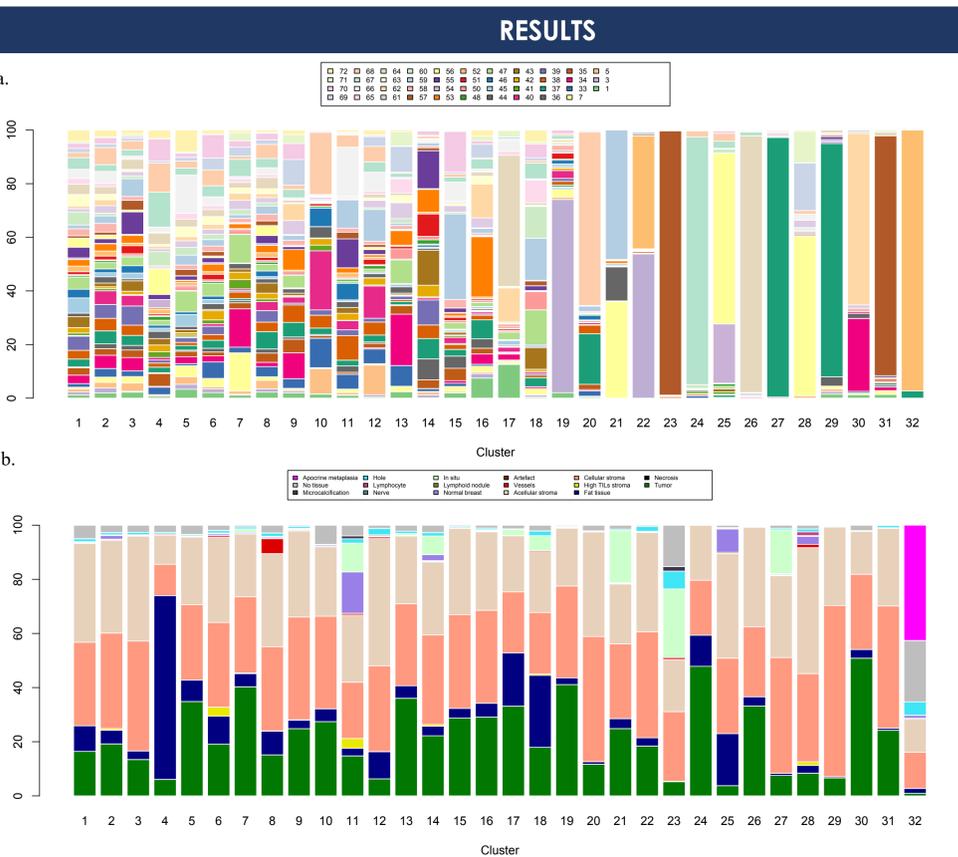*Clustering analysis*

Clusters of spots (based on gene expression)

Tumor, Necrosis, Fat tissue, High TILs stroma, Vessels, Artefact, Normal breast, Lymphoid nodule, In situ, Nerve, Lymphocyte, Cellular stroma, Heterologous elements, Hole, Acellular stroma, Apocrine metaplasia, Microcalcification

seurat_clusters: 1–32

WORKFLOW

- Spatial transcriptomics (ST) and histo-morphological annotation of the relative H&E slides
- Inter-sample normalisation, merge of the samples and clustering analysis at the spot level
- Clusters characterisation using annotation and gene set enrichment analysis for Hallmarks gene sets (MsigDB) after differential expression analysis
- Spatial analysis of the obtained clusters (level of contacts between different clusters)
- Assessment of relations between clusters abundance, contacts and different clinical features

RESULTS

Table 1.

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

- Spatial transcriptomics (ST – **Fig. 2**) was performed on **43 ILC primary frozen tumor** samples (HR+, HER2-) coming from patients with long term follow up (**Table 1.**)
- **32 clusters** (at the spot level) were identified across all the samples. All the clusters representing normal structures were shared across samples. Some stroma and tumor clusters were also shared, while other ones were sample-specific (**Fig. 3 a.**)
- Clusters were annotated as **"tumor"** clusters if the percentage of tumor from annotation (**Fig. 2**) inside the cluster was higher than the average of our cohort (>29%, **Fig. 3 b.**)
- Different tumor clusters were enriched in **different pathways** and present in different samples (**Fig. 4 a.,b.,c.**). Different tumor clusters inside the same sample were observed as well
- A tumor cluster enriched in **MYC targets, G2M checkpoint and oxidative phosphorylation** was more represented in samples with **higher tumor grade** (p=0.016, **Fig. 4 d.**), while tumor cluster enriched in **interferon alfa and gamma** pathways was more present in samples with **higher tumor stage** (p=0.006, **Fig. 4 e.**)
- The number of contacts between different clusters (**spatial disorganization**) was higher in samples coming from **patients who experienced disease relapse** (**Fig. 4 c., f.**)

Fig. 3. Subdivision of each cluster (different bars) across all the samples (colors of the bars, **a.**). Percentage of annotations of the spots that constitute the different clusters (**b.**)

Hallmark pathways (GSEA) - cluster 5

Hallmark pathways (GSEA) - cluster 17

padj < 0.25 FALSE TRUE

Normalized Enrichment Score

LOW number of hetero-contacts **HIGH spatial organisation**

HIGH number of hetero-contacts **LOW spatial organisation**

cluster A — cluster B

Fig. 4. Tumor clusters are enriched in different pathways (**a.,b.**). Relative abundance of clusters between clinical features (**d.,e.**). Example of different level of spatial heterogeneity (**c.**) and differences in spatial heterogeneity between clinical features (**f.**)

REFERENCES

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