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 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, MSK6A, PSEN, AKT1

Tumor microenvironment

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

METHODS

Spatial Transcriptomics

Fig. 2. ST slide (Virtual, x5 Grosissement) of the relative H&E slides

Histo-morphological annotation

Clustering analysis

RNA sequencing (spot level).

Spatial transcriptomics (ST) and Pathology were used to characterize the spatial heterogeneity of ILC including tumor microenvironment.

RESULTS

Table 1. ST cohort Grade Tumor stage

<table>
<thead>
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<th>N. of samples</th>
<th>Total</th>
<th>G1</th>
<th>G2</th>
<th>G3</th>
<th>N1</th>
<th>N2</th>
<th>N3</th>
<th>T1</th>
<th>T2</th>
<th>T3</th>
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<tbody>
<tr>
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<td>13</td>
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<td>7</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
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</tbody>
</table>

Spatial transcriptomics (ST - Fig. 2) was performed on 40 ILC primary frozen tumor samples (HR+: HR-) coming from patients with long term follow up (Table 1).

32 clusters at the spot level were identified across the samples. All the clusters representing normal structures were shared across samples. Some stroma and tumor clusters were also shared, while others were sample specific (Fig. 2a).

Clusters were annotated as "tumor" clusters if the percentage of tumor from annotation (Fig. 2b) inside the cluster was higher than the average of our cohort (p<0.05, Fig. 3a)

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 was observed in MYC targeted, G3M checkpoint and oxidative phosphorylation was more represented in samples with higher tumor grade (p<0.016, Fig. 4 d), while tumor cluster enriched in interleukin and growth 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 f).

CONCLUSIONS

OBJECTIVES

• To characterize the spatial transcriptomics heterogeneity of ILC including tumor microenvironment
• To investigate whether spatial transcriptomics may improve the prediction of the risk of relapse in lobular breast cancer

WORKFLOW

Inter-sample normalization, merge of the samples and clustering analysis of the spot level.

Clustering characterization using annotation and gene set enrichment analysis (Fig. 3b) other differential expression analysis

Spatial analysis of the obtained clusters ( differentiation between different clusters) Assessment of relations between different clusters and different clinical features

ACKNOWLEDGMENTS AND CONTACTS

REFERENCES


Fig. 1. Annotated ST slide (Virtual, x5 Grosissement) of the relative H&E slides.

Fig. 2. Spatial transcriptomics (ST) and Pathology were used to characterize the spatial heterogeneity of ILC including tumor microenvironment.

Fig. 3. Subdivision of each cluster (different boxes) across all the slides (colors of the boxes, a.c). Percentage of annotations of the spots that constitute the different clusters (b).

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