Spatial sequencing of T cell repertoire in breast cancer

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Background

- Analysis of T-cell repertoire (TCR) has been proposed as a new approach to better understand the adaptive immune response in cancer diseases.

- Next generation sequencing of the TCR has proven to be a powerful approach to characterize this repertoire. In oncology, this could lead to a better understanding of tumor immune environment and allow to select and follow patients who benefit from immunomodulatory therapies. However, current TCR sequencing in tumor tissues is based on bulk RNA or DNA extraction, which does not provide neither the functional information at the clonotype level nor the spatial context.

- Here, we explore a new method which provides the spatial localization of individual T cell clonotypes in tumor samples.

Materials and Methods

Frozen tumor sections (12 µm) were positioned on a 10x Visium Spatial Gene Expression slide, and mRNA was captured in spatial spots of 55 µm diameter. Each transcript was tagged with a Unique Molecular Identifier (UMI) and a spatial barcode during an in situ reverse transcription. Starting from the generated cDNA, the TRB genes were amplified using a multiplex PCR with a set of primers positioned on the *TRBV* genes (Fig 1). TRB libraries were sequenced on the Illumina MiSeq platform. Analyzed samples were 3 mm diameter Tru-Cut tumor biopsies collected before and during neoadjuvant treatment of locally advanced breast cancer patient included in a clinical trial (NCT03356860).

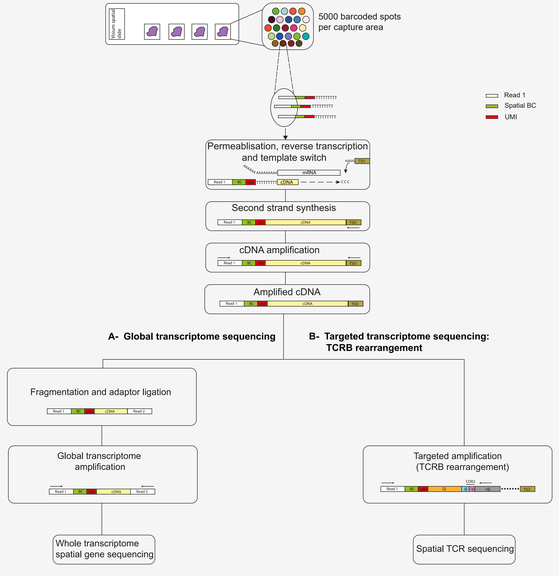


Figure 1: A general overview of the whole transcriptome and TCR spatial sequencing workflow.

Results

A- Global transcriptome sequencing

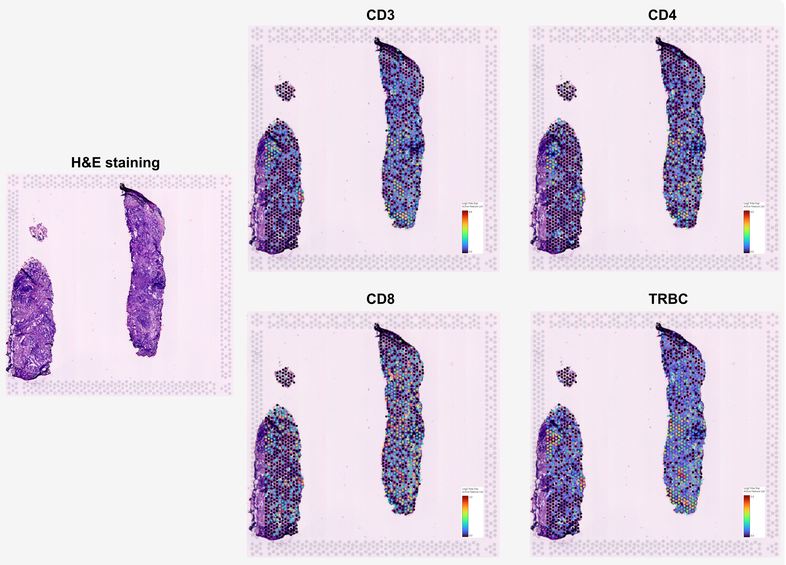
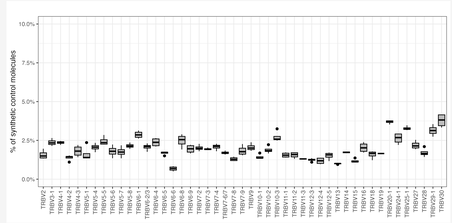


Figure 2: Illustration of the global transcriptome sequencing results. Fresh frozen OCT embedded breast cancer biopsy was H&E stained, imaged and then processed according to the Visium Spatial Gene Expression workflow. For illustration purposes, the gene expression of T cell markers (CD3, CD4, CD8) and TRBC within the tissue is shown. 10x Visium whole transciptome spatial gene expression workflow allows the identification of T cell localization within the tissue but it does not allow to profile theTCRB rearrangement (*TRBV-CDR3-TRBJ*) that characterize a TCR clonotype .

B- Targeted transcriptome sequencing: TCRB rearrangment

Figure 3: To amplify TCRB rearrangements, a set of multiplex primers was evaluated in a synthetic TRBV repertoire to validate the amplification of all functional *TRBV* genes (based on the IMGT catalog). Adjustement of primers proportions in the multiplex mix and usage of Unique Molecules Identifier (UMI) reduce amplification bias during NGS library setup and allows absolute quantification of sequenced TCRB molecules. *TRBV* genes distribution in synthetic control libraries replicates are shown.



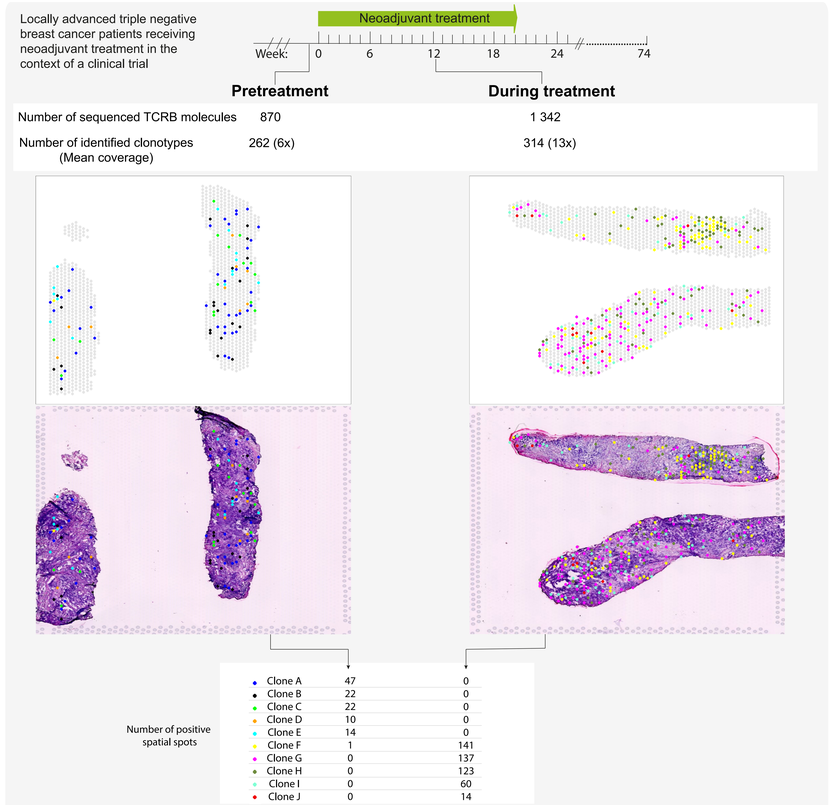


Figure 4: Fresh Frozen OCT embedded biopsies collected before and 12 weeks after neoadjuvant treatment of locally advanced breast cancer patient were processed according to the targeted transcriptome sequencing (TCRB rearrangment) workflow. The number of sequenced TCRB molecules and distinct T cell clonotypes was determined (a T cell clonotype is defined by a productive TCR rearrangment (*TRBV-CDR3-TRBJ*). Non productive rearrangements or those for which *TRBV* or *TRBJ* genes were not identified were excluded. The spatial distribution in the tumor for the 5 most represented clonotypes (top 5) in pre-treatment and the top 5 clonotypes during-treatment biopsies are shown in the biopsy mappings. Overlap of the top 5 most represented clonotypes over time, before and during paclitaxel treatment, is shown. We observed a dynamic change in the TCR repertoire composition as well as their spatial distribution during treatment.

Conclusions

This proof of concept work demonstrates the feasibility of spatial TCR sequencing in tumor biopsies, providing a new method to explore the spatial organization of T cells clonotypes in the tumor microenvironment, explore T cells functional profiles at the clonal level and follow modifications during tumor development and treatments.

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