FULLY AUTOMATED SPATIAL MULTI-OMICS ANALYSIS TO MAP THE TUMOR MICROENVIRONMENT WITH SINGLE-CELL RESOLUTION

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Background The tumor and its microenvironment are distinguished by highly heterogeneous cell types in dynamic evolution. In the past decades, the adoption of single-cell RNA sequencing technologies improved our understanding of intra- and inter-patient variations, refining cancer diagnosis and targeted therapy, while still lacking spatial context information. The spatial analysis of single-cell gene expression provides previously missing information on cell interactions, crucial for innovative treatment opportunities. However, the large-scale implementation of these assays is hampered by the lack of automated and user-friendly workflow solutions.

Here, we aim at automating an in situ transcriptomic assay and integrating it with a proteomic workflow on the COMET™ platform for easy and comprehensive mapping of the tumor microenvironment.

Methods COMET™ is a microfluidic-based instrument capable of automated sequential immunofluorescence (seqIF™) assays. The in situ RNA detection was based on DNA padlock probe hybridization and ligation, followed by rolling circle amplification and gene detection with fluorophore-tagged probes. A panel of probes targeting various immune-oncology genes was designed and the gene expression was evaluated in combination with a high-plex seqIF™ analysis.

Results The protocol for in situ RNA detection was automated and optimized on COMET™, resulting in specific detection of multiple transcripts (cell-type specific markers, transcription factors, secreted cytokines) on various tumor samples, with a signal quality that allows downstream computational analysis of gene expression signals. The transcriptomic assay was then integrated with a seqIF™ workflow for the co-detection of several target proteins for tumor microenvironment characterization on the same tissue section (figure 1). Double RNA and protein analysis allowed to examine the expression of biomarkers for which no good antibody clones are available, validation of new antibodies by detecting RNA and protein co-localization, in addition to the identification of the cellular source of secreted molecules. Compared to the manual protocol, the combined automated workflow resulted in a drastic time reduction being based on iterative fast cycles of detection and imaging of two transcripts or proteins each, lasting approximately 30 and 40 minutes, respectively.

Conclusions We showed here that in situ spatial transcriptomics assays can be fully automated and combined with spatial proteomics on the COMET™ platform for a multi-omics approach with advantages in terms of time and complexity reduction with respect to manual protocols. This combinatorial automated detection of RNAs and proteins of pivotal biomarkers provides a powerful new tool for a simpler and better mapping of the tissue spatial context.

REFERENCES

Abstract 38 Figure 1 Automated and simultaneous in situ detection of RNA and protein. RNA (ACTB, RPLP0, JUN, MYC) and protein (panCK and Vimentin) targets are co-detected on a human melanoma frozen section with a fully automated workflow on the COMET™ platform. Background autofluorescence was subtracted and brightness adjusted for visualization purposes.