Digital pathology of cutaneous malignant melanoma by spatial transcriptomics

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BACKGROUND: Cutaneous malignant melanoma (CMM) is characterized by extensive inter- and intratumoral genetic heterogeneity including tumor cell interactions with the stroma and the immune system. Insights into CMM biology have led to successful development of novel targeted therapies and immunological checkpoint blocking agents.

HYPOTHESIS: We aim to analyze sequential tumor biopsies from stage IV CMM patients by using spatial transcriptomics (ST) to identify clinically useful prognostic and predictive biomarkers of response and durable benefit from these therapies.

METHODS: Current methods for gene expression analysis typically provide either the full complexity of the transcriptome or positional information for a few expressed genes. By applying the emerging technique ST, this enables transcriptome analysis with spatial resolution by combining massively parallel RNA-sequencing with a histologic imaging. To achieve this, HTX-staining and subsequent imaging is performed on a microarray before the tissue is treated to permeabilize the cells. The mRNA diffuses and hybridizes onto poly(thymine)-stretches of arrayed primers, labeled with positional barcodes. Each position has currently the size of roughly ten cells, though the aim is to reach single cell resolution in the near future. A reverse transcription reaction is performed on the object slide to obtain barcoded cDNA from mRNA molecules. The cDNA is sequenced and a transcriptome with exact positional information is obtained. The sequencing data is visualized together with the tissue image using a ST viewer.

RESULTS: Differential expression analyses within and across CMM metastases reveal tumor heterogeneity and we aim to identify different transcriptome profiles associated with CMM-specific prognosis as well as outcome of treatment with novel targeted therapies and immunological checkpoint blocking agents. To achieve this, fresh frozen tumor samples collected before/during therapy and at disease progression from stage IV CMM patients are being analyzed.
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