Comparative spatial profiling of the melanoma microenvironment pre- and post-immunotherapy resistance

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Background

Immunotherapy has transformed melanoma treatment. Anti-PD-1 (programmed cell death-1) monotherapy and the combination of anti-PD-1 and anti-CTLA-4 (cytotoxic T-lymphocyte antigen-4) are the current standard of care for patients with metastatic melanoma.

However, around **50% of patients fail to response or develop resistance**. Understanding the spatial organization of cells within the tumour microenvironment is critical to reveal mechanisms driving resistance.

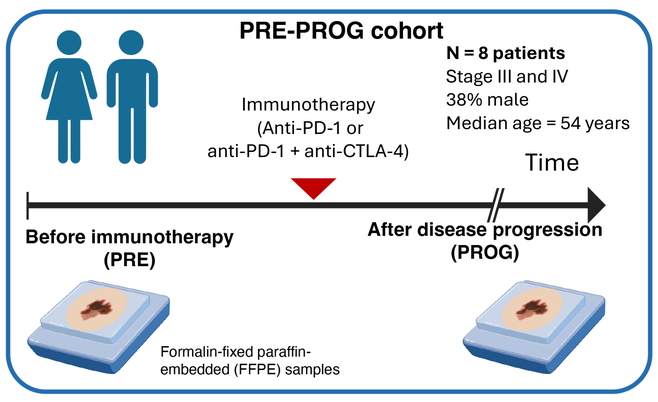
Using Xenium In Situ spatial transcriptomics, we mapped how gene expression, cellular niches and intercellular communication change before treatment and after immunotherapy resistance.

Objectives

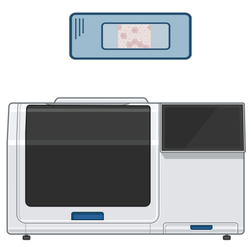
- Identify the changes in gene expression, cellular composition, and spatial distribution of cells In the tumour microenvironment between two time points: before immunotherapy and after resistance develops.
- Identify shared and patient-specific changes in gene expression and spatial features associated with immunotherapy resistance.
- Investigate the signalling pathways and specific ligand-receptor interactions driving immunotherapy resistance.

Methods

Patient cohort



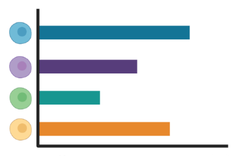
Profiling of spatially-resolved single-cell gene expression



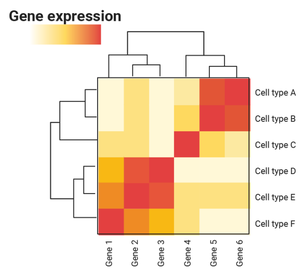
480 targeted gene panel
Xenium In Situ
(10x Genomics) platform

Analysis of changes in gene expression and spatial distribution

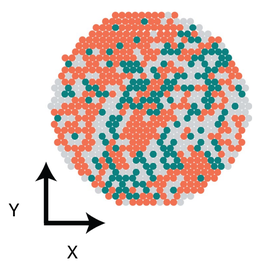
Cell type composition



Comparison of gene expression

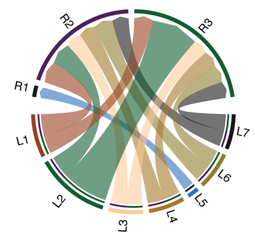


Characterization of spatial niches



Cell-cell communication

Ligand-receptor interactions

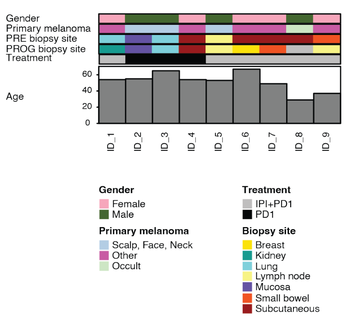


The analysis of gene expression and spatial niches was carried out with Seurat v5.3.0 (doi.org/10.1038/s41587-023-01767-y). CellChat v2.1.2 was used to analyze cell-cell communication (doi.org/10.1038/s41596-024-01045-4)

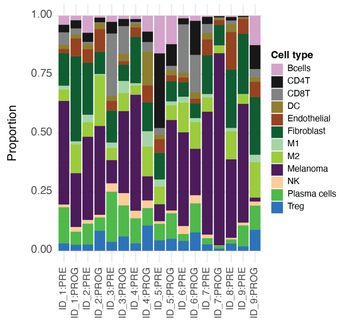
Results

Melanoma progression (PROG) is characterized by increased immunosuppression and the activation of pathways that support survival and proliferation

Overview of patients' clinical features

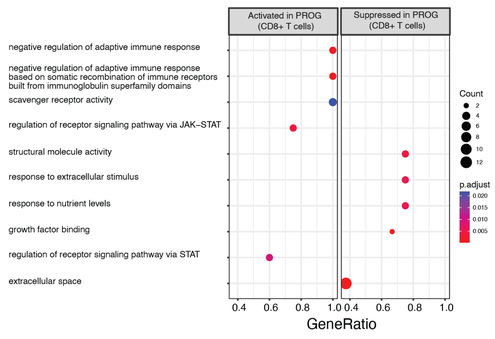


Proportion of cells of each cell type by sample



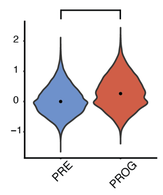
We annotated 12 main cell types across matched samples for 8 patients, plus a pre-treatment sample for ID_8. The proportion of cells by cell type was heterogeneous across patients and time points, with melanoma cells making a mean of 33% across all samples (2-82%).

Immune suppression of CD8+ T cells in the PROG stage

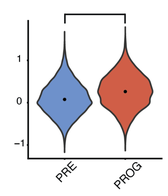


The Gene Set Enrichment Analysis showed that multiple GO biological processes related to immune activity are suppressed in the PROG stage compared to pre-treatment in CD8+T cells.

mTORC1 signalling

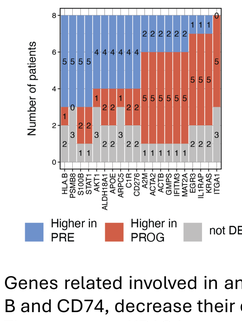


PI3K/AKT/mTOR signalling

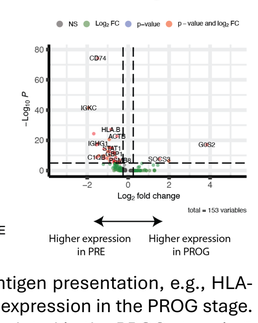


The mTORC1 and PI3K/AKT/mTOR signalling pathways are consistently upregulated in melanoma cells in the PROG stage (N = 8 patients). These signalling pathways support the survival, growth and proliferation of melanoma cells.

Most frequent DE genes in melanoma cells between PRE and PROG



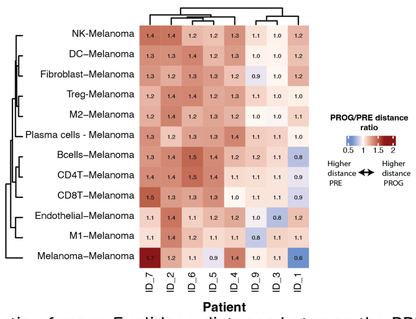
DE genes in dendritic cells in the PRE and PROG stages



Genes related involved in antigen presentation, e.g., HLA-B and CD74, decrease their expression in the PROG stage. The oncogene KRAS is upregulated in the PROG stage in 5 out of 8 patients (p adjusted < 0.05, LogFC > 0.25)

Cellular niche organization and composition

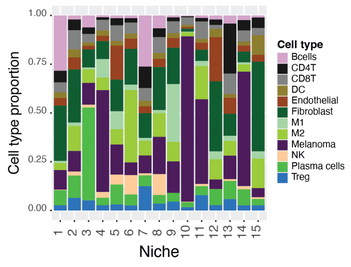
Melanoma cells are generally closer to immune cells before treatment



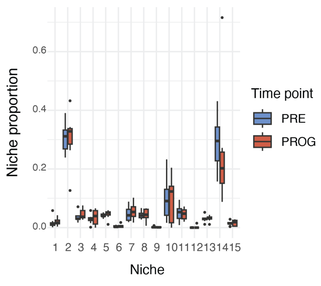
The ratio of mean Euclidean distance between the PRE and PROG stages is shown

Most cells are located in melanoma-rich and fibroblast-rich niches in the PRE and PROG stages

Cell type proportion across niches



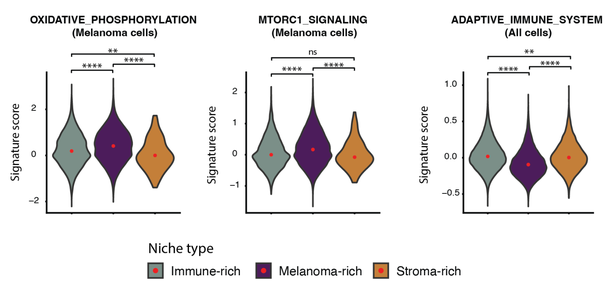
Niche proportion by time point



Niche proportion across samples

Spatial niches were defined based on local cell-type composition across the samples in each time point. The optimal number of niches (N = 15) was selected where inter-niche cell type composition distance was maximized. The most abundant clusters in both the PRE and PROG stages are dominated by melanoma cells (niches 10 and 14) or by fibroblasts (niche 2).

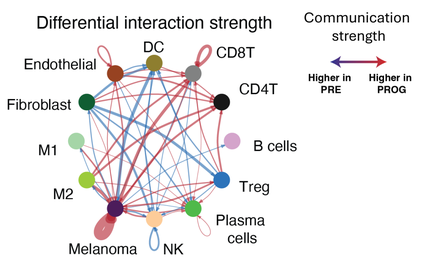
Melanoma-rich niches show increased oxidative phosphorylation and lower immune signalling



The gene expression of melanoma cells in melanoma-dominated niches show increased oxidative phosphorylation and mTORC1 signalling, consistent with increased metabolic and proliferative activity. In contrast, these niches exhibited decreased immune activity

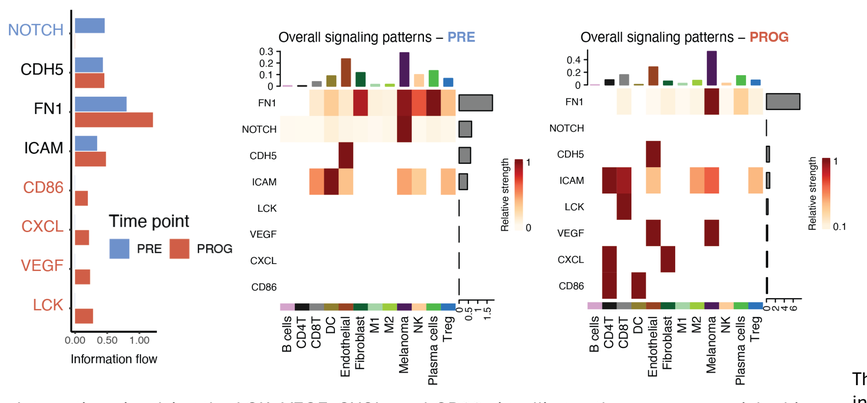
Different signalling pathways are active in the PRE and PROG stages

Switch in communication between cell types before treatment and after developing resistance



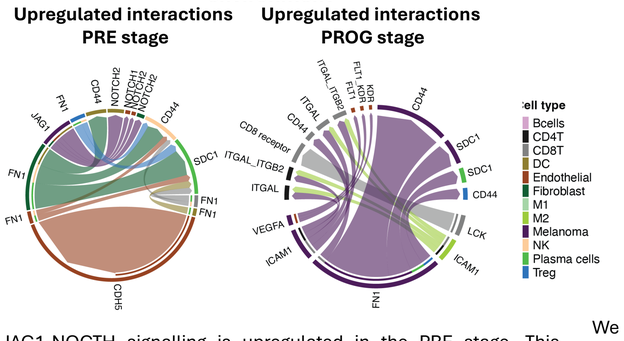
The net plot shows the changes in overall communication between cell types across all patients. Melanoma, endothelial and CD8+T cells show an overall increased communication in the PROG stage. The interaction between melanoma cells is much stronger in the PROG stage, consistent with the higher distance from other cell types.

Differential cell-cell communication in the PRE and PROG stages



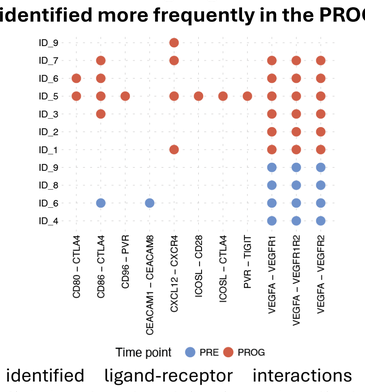
Interactions involving the LCK, VEGF, CXCL, and CD86 signalling pathways were enriched in the PROG stage across all patients. The CXCL pathway includes CXCL12-CXCR4, interaction that has been linked to progression and enhanced invasion (doi.org/10.1016/j.cyto.2024.156629).

Upregulation of JAG1-NOCTH communication in the PRE stage suggest an immunosuppressive environment



The JAG1-NOCTH signalling is upregulated in the PRE stage. This interaction has been associated with an immunosuppressive microenvironment and poor prognosis (doi.org/10.3389/fonc.2023.1091488). In the PROG stage, the VEGFA signalling targeting FLT suggests active angiogenesis.

Potential drug target interactions are identified more frequently in the PROG stage



We identified ligand-receptor interactions involving potential drug target-proteins in each patient and time point. Overall, more interactions are observed in the PROG stage. Patients 5, 6 and 7 show CTLA4 interactions in the PROG stage despite receiving anti-CTLA4 immunotherapy.

Conclusions

- We profiled the spatial gene expression of matched samples collected before treatment (PRE) and after disease progression (PROG) from 8 advanced melanoma patients using the Xenium In Situ platform.
- The PROG stage is characterized by increased immune suppression and proliferation.
- Genes related to antigen presentation are frequently downregulated in the PROG stage.
- The analysis of gene expression showed increased proliferation and oxidative phosphorylation and decreased immune activity in melanoma-dominated niches.
- The JAG1-NOCTCH was upregulated in the PRE stage and has been linked to an immunosuppressive microenvironment. We identified CXCL12-CXCR4 in the PROG stage, which has been associated with enhanced invasion and proliferation.
- Cell-cell interactions involving potential drug target genes were more frequently found in the PROG stage.

Acknowledgements

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