Investigating the functional role of cellular phenotypes in melanoma targeted therapy

Final Report

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Introduction

Melanoma is one of the most aggressive and therapy-resistant human cancers. Recent therapies have achieved significant improvements in initial response, manifested by tumor shrinkage. However, 50% of the patients treated with targeted therapy and 41% of the patients treated with immunotherapy relapse and develop a more aggressive form of disease shortly after treatment. Melanoma heterogeneity plays a key role in this phenomenon, in which malignant cells within the same tumor display high transcriptional variation and give rise to drug tolerance. Several studies have tried to characterize melanoma heterogeneity by single cell RNA sequencing (scRNAseq), focusing mainly on the transcriptional landscape of malignant melanoma cells.

The tumor microenvironment is an important contributing factor in disease development and progression. Upon treatment, intercellular communication between malignant and immune cells provides a complex cellular network, which can shift the immune system towards pro-tumorigenic functions and promote malignancy development in melanoma. This communication between immune and malignant cells in the context of melanoma heterogeneity remains to be characterized. In this project, we performed scRNAseq of biopsies from melanoma patients on treatment, in order to elucidate the cellular composition and crosstalk between melanoma and immune cells in resistant and responding patients during the course of targeted therapy. The aim of the present research is to identify new prognostic biomarkers, so as to better predict the optimal therapy type for each patient.

Research progress and results

We performed scRNAseq analysis on biopsies from four melanoma patients before and at different time points after treatment with pharmacological inhibitors of BRAF and MEK (BRAFi/MEKi), which target a signaling pathway frequently hyperactivated in melanoma patients. Two of these patients turned out to be therapy-resistant, one patient displayed complete response and one patient had partial response in the beginning but developed rapid progression and died by the end of the treatment.

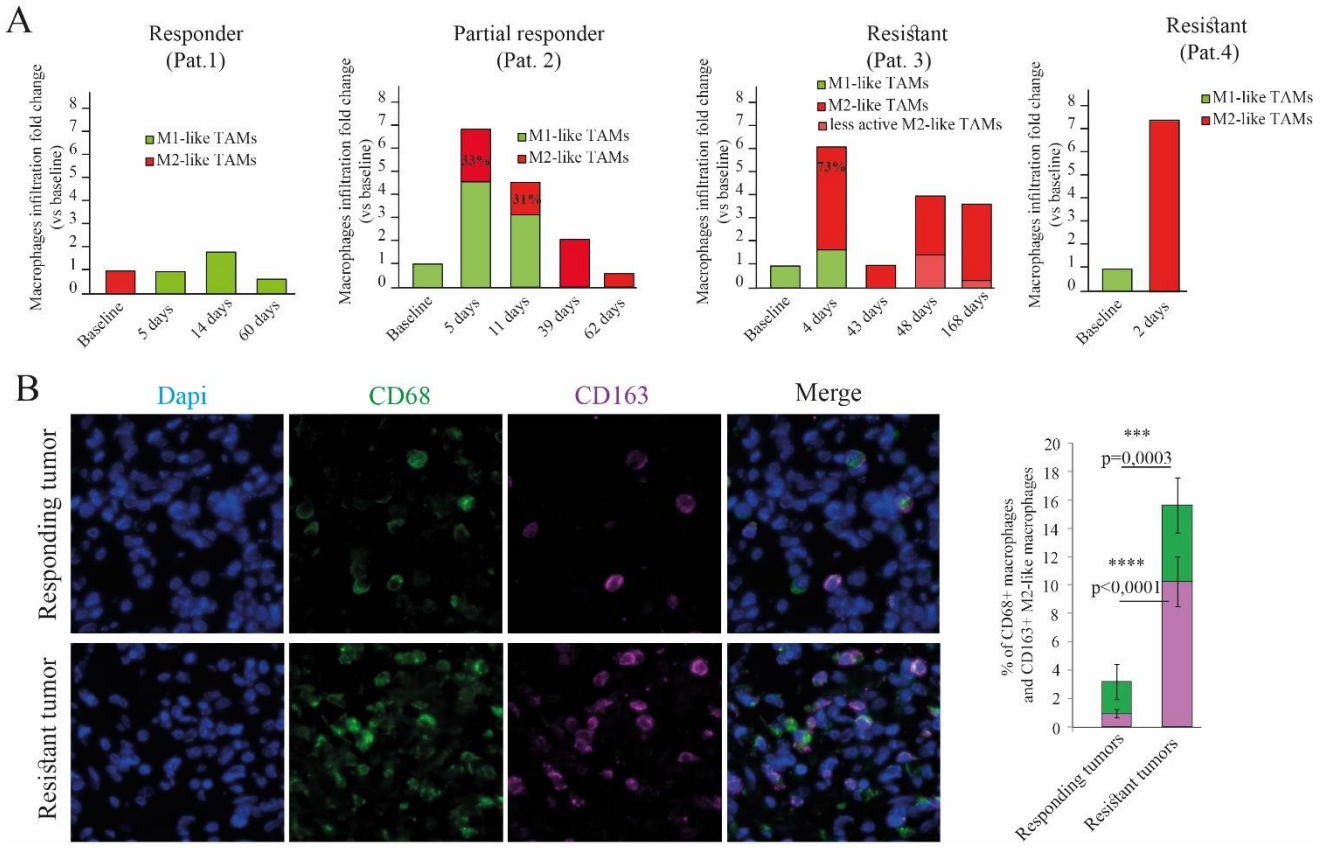
Comparison of cellular landscapes demonstrated strong differences in the phenotype of a particular innate immune cell population, i.e., the so-called tumor-associated macrophages

(TAMs) infiltrating the tumors. Unlike the responding patient, both resistant patients exhibited a strong increase in TAMs exhibiting pro-tumorigenic features ("M2-like") (Figure 1A). These M2-like TAMs appeared shortly after the first treatment and remained at all further analyzed time points. The partially responding patient demonstrated mixed TAM populations at the responding stage of the treatment, with predominant recruitment of anti-tumorigenic macrophages ("M1-like"). However, with disease progression, the phenotype of the TAMs switched to an alternative M2-like phenotype.

To verify a possible correlation between infiltration of pro-tumorigenic TAMs and therapeutic response in additional patients, we performed immunofluorescence staining on previously collected melanoma biopsies taken at responding or resistant tumor stages after BRAFi/MEKi treatment. Our results reveal significantly higher infiltration of total TAMs as well as M2-like TAMs in resistant tumors (Figure 1B), in line with the results of our scRNAseq analysis.

In sum, our findings strongly suggest that disease aggressiveness and therapy resistance in melanoma is associated with emergence of pro-tumorigenic TAMs in patient tumors during targeted therapy. The presence of pro-tumorigenic TAMs might thus serve as a prognostic marker for therapy outcome. Furthermore, targeting of this innate immune cell population might help to prevent therapy resistance formation in melanoma.

Research figures



(A) TAM infiltration and phenotype characterization in responding and resistant patients upon treatment with BRAF/MEK inhibitors. **(B)** Immunofluorescence staining of total CD68+ macrophages and M2-like CD163+ macrophages in melanoma tumors at the resistant or responding stage. 9 tumors at the responding stage and 8 tumors at the resistant stage were analyzed. The green bar shows the percentage of total macrophages in the tumor, and the purple bar indicates the number of M2-like TAMs within total macrophages.

Publications related to the project

We are currently preparing a manuscript related to this project, which we aim to submit for publication in the first half of 2021.