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UNRAVELING THE PHENOTYPIC HETEROGENEITY IN MELANOMA AT SINGLE-CELL LEVEL THROUGH IMAGING-BASED SPATIAL PROTEOMICS

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Background: Melanoma originates from the uncontrolled proliferation of melanocytes and is the deadliest form of skin cancer. Early-stage melanoma can be successfully treated with surgical resection; however, this approach is not suitable for late-stage tumors, which are also resistant to classical anticancer therapies. Immune checkpoint inhibitors and targeted therapy have significantly improved the survival rate of patients with advanced melanoma. However, the effectiveness of both therapeutic options is often impaired by innate and acquired resistance.
Melanoma cells are characterized by great heterogeneity and possess the capacity to rearrange the transcriptomic programs, in response to environmental conditions.
Therapy-driven inflammation has been proposed as a condition that promotes the switching of melanoma cells to a dedifferentiated phenotype, characterized by the loss of melanoma differentiation antigens. This switch allows melanoma cells to evade the anti-tumor immune response and to invade tissues.

Aim: Our study aims to characterize the complexity of the melanoma tumor microenvironment, considering the spatial context. Specifically, we investigated how the absence of macroscopic pigmentation relates with melanocytic differentiation marker expression, at single cell level, and whether immune infiltration depends on the spatial organization of differentiated or dedifferentiated melanoma cells.

Patients and methods: To achieve this, we performed imaging-based proteomics experiments (PhenoCycler®) on FFPE samples from 38 patients with melanoma. Samples included primary tumors and metastases, 17 of which were annotated by pathologists as pigmented, and 21 as unpigmented.

The spatial complexity of the tumor microenvironment was analyzed by designing panels to detect melanoma cells in all their differentiation stages, immune population, epithelia, stroma and vessels.
The differentiation stages of melanoma cells was identified based on the expression of melanoma associated antigens and transcription factors (Melan-A, Tyrosinase, gp100, MITF, SOX10) and dedifferentiation markers (NGFR, Axl).
The macroscopic pigmentation status was compared to the protein expression of differentiation markers and to immune infiltration. Cellular neighborhood analysis was also performed, using a newly developed Python workflow (SPACEc).

Results and conclusions: Here, we present a novel and detailed characterization of the melanoma microenvironment, contributing to a deep understanding of the phenotypic heterogeneity in the spatial context. This provides insights into the mechanisms of therapy resistance and immune evasion, ultimately guiding the development of more effective, personalized treatment strategies.