ImmunoTools *special* Award 2024

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Epigenetic-Metabolic Axis Influencing Macrophage Polarization in the Colorectal Cancer Tumor Microenvironment

Deciphering cell-cell interactions in the tumor microenvironment is crucial for understanding immunosuppressive mechanism and identification of novel therapeutic approaches that could complement immune checkpoint therapy in non-responsive colorectal cancer patients.

Macrophages, being a critical component of the tumor microenvironment, possess a dual capability in regulating immunosuppressive circuits. Their functional reprogramming towards an anti-tumorigenic phenotype has been acknowledged as a valuable approach in synergizing with immune checkpoint inhibition. The phenotypical diversity of macrophages is significantly shaped by the interaction between epigenetic modifications and metabolic processes originating from microenvironmental cues. Consequently, our main objective is to pinpoint the epigenetic mechanisms influencing the immunometabolic characteristics of macrophages in physiological tissue as opposed to the tumor microenvironment and to investigate the possibility of using epigenetic drugs to successfully alter the phenotype of tumor-suppressive macrophages.

To faithfully model the physiological versus the malignant colon microenvironment, we have established a system containing patient-derived fibroblasts and organoid lines isolated from matched tumor and adjacent healthy colon tissues, co-cultured with human macrophages. Given the substantial macrophage heterogeneity observed *in vivo*, we plan to use multi-omics single-cell technology and determine transcriptional and epigenetic programs of different cell populations.

While single-cell RNA sequencing is a powerful tool for profiling gene expression at the transcriptomic level, its limitation lies in the inability to directly measure protein expression. Therefore, to accurately identify distinct macrophage polarization populations, it is crucial to

validate and assess protein markers associated with different polarization states. Utilizing the ImmunoTools set of flow cytometry antibodies, we aim to validate and assess protein markers associated with different macrophage polarization states, ensuring accurate identification of distinct populations within our system. This methodology will facilitate the assessment of how well our enhanced *in vitro* triple culture system replicates the heterogeneity found in the initial tumor in comparison to normal colon tissue and enhance setting a rationale behind utilizing our model to study and model cell-cell interactions within the tumor microenvironment in *in vitro* settings.