THE ROLE OF IMMUNE MICROENVIRONMENT IN DETERMINING THE ORGAN-SPECIFIC HOMING OF METASTASES

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Introduction Metastasis to different organs or tissues may require distinct sets of regulators which may influence the homing and growth of tumour cells to specific secondary sites. Under the hypothesis that the immune microenvironment of the different niches may play an important role in this process, we have categorised metastatic samples from different primary tumours based on their immune profile.

Material and methods Gene expression data from metastatic samples with different primary tumour origin (n=342) were downloaded from open repository GEO. Samples were scored using different gene expression profiles and characterised on the basis of their immune and stromal infiltration and activation of immune response pathways (Immunophenoscore, MCPcounter, ESTIMATE; among others). Resultant scores were analysed for statistical differences with ANOVA test. Multivariate analysis was used for clustering the samples based in their immune-features.

Results and discussions As expected, significant differences were found between the immune profiles of samples metastasizing in distinct organs. For instance, breast cancer metastasis in lung showed a much higher immunogenic score than breast metastasis in brain (p=5e-4), suggesting a different immune microenvironment modulation. Also in breast, significant differences have been found in cell lineages infiltration, lung metastasis being the ones with the highest T cell component (p=0.002) and liver metastasis the ones with the lowest infiltration of endothelial cells (p=0.005). Moreover, in other cancer types like melanoma, samples showed differences among different metastatic locations. Interestingly, when comparing metastatic samples originating from different primary tumour, a high concordance among secondary tumours in immune scores were found, specifically in brain metastasis. These results suggest that cells needs to share similar molecular profiles to evade the immune surveillance and growth in a specific niche, independently of their origin.

Conclusion Metastases from the same primary tumour growing in different organs show differences in their immune profile. However, those samples from different primary origin but growing in the same secondary organ shared a characteristic immune profile. These results suggest that immune system plays a role in determining the organ-specific homing of metastasis.

A WORKFLOW FOR OPTIMISED ISOLATION AND ANALYSIS OF TUMOUR INFILTRATING IMMUNE SUBPOPULATIONS

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Introduction Syngeneic mouse tumour models are widely used to analyse tumour immunology due to their fully competent immune-repertoire and have paved the way for novel immuno-therapy agents in multiple tumour entities. However, the amount and composition of tumour infiltrating leukocytes (TIL) is highly variable. This complicates targeted analysis, in particular for small leukocyte subpopulations that may not be analysed properly or lost in the background noise. When working with large cohort sizes, immune-phenotyping by flow cytometry is time consuming and highly work intensive. We have developed improved workflows combining automated tissue dissociation with novel TIL specific isolation reagents to allow for more accurate and faster analysis of TILs and TIL subpopulations.

Material and methods We have developed novel TIL specific enrichment reagents for the magnetic cell sorting (MACS) based isolation directly from dissociated tumour tissue. The composition of these populations before and after separation was analysed by flow cytometry. The developed tools were combined with optimised tissue dissociation and flow panels to establish comprehensive workflows.

Results and discussions We have established workflows combining optimised and automated tissue dissociation using the gentleMACS platform with TIL specific isolation to improve and accelerate downstream analysis. Isolation of TIL was improved by developing new CD45-, CD4+, CD8+, and pan T cell enrichment reagents for MACS-based isolation directly from dissociated tumour tissue. Applying this workflow, CD45+TIL were enriched to purities above 80%-90% with high yields (>70%) from divers syngeneic mouse tumours. This allowed for rapid and reliable analysis even of small TIL subpopulations. Moreover, by using the newly developed T cell reagents, T cells were enriched to purities higher than 90%, allowing for a reliable analysis of rare T cell subpopulations.

Importantly, while TIL or T cell enrichment significantly reduced analysis time and reagent costs in immune subset analysis, the composition of infiltrating cells was not affected, excluding the risk of introducing a bias by this method.

Conclusion We have developed improved workflows for the isolation of generic TIL and T cells from mouse tumours reducing time and costs of downstream analysis while standardising and enhancing the detection and quantification of immune cell subpopulations. CD45+TILs, pan-, CD4+ or CD8+ T cells can directly be isolated from dissociated mouse tumours and analysed for subpopulations.

HIGH-THROUGHPUT SINGLE-CELL T-CELL RECEPTOR PROFILING BY SMART TECHNOLOGY

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Introduction Single-cell T-cell receptor (scTCR) clonotype analysis permits the determination of the specific TCR alpha-beta (α/β) chain pairing expressed on each cell. This pairing information allows researchers to gain insight into Tcell heterogeneity and plasticity, determine the contribution of the pairing to antigen specificity of the individual TCR, and design therapeutics. Here we employ a 5’-RACE-like approach
and SMART technology, in conjunction with two novel next-generation sequencing (NGS) library preparation kits, using the same primer pairs, to capture full-length variable regions of TCR-α and -β chains.

**Material and methods** Method 1, using the SMARTer Human scTCRα/β Profiling Kit, permits NGS library preparation of FACS-sorted cells in 96-well plates. Method 2 is an adaptation of the process above that scales to ~1200 single cells using the SMARTer ICELL8 Single-Cell System, which enables single-cell isolation and nanoliter PCR in a nanowell chip.

**Results and discussions** We present data showing α/β pairing from Jurkat, CCRF, PBMCs, and CD4 +T cells. In addition to the sensitivity of this method, the ability to pool the cDNA from 96 wells into 12 sequencing libraries adds to the ease of use. Consistent with immunology reports, unstimulated CCRF-CEM cells examined with this kit expressed a TCR-β chain, but not a TCR-α chain.

scTCR profiling with SMARTer ICELL8 Single-Cell System as a proof of principle of scTCRα/β was performed on Jurkat cells and CCRF-CEM cells.

Jurkat cells and CCRF-CEM cells were processed using an ICELL8 chip preprinted with barcoded oligos. Paired TCRα/β Jurkat clonotypes were detected in 77% and 87% cells in mixed and single cell populations, respectively.

**Conclusion** The ability of the core biochemistry and PCR components of these kits to be used with either FACS-sorted cells in 96-well plates or >1,000 cells in novel ICELL8 chips (in development) points to the general utility and scalability of this approach in understanding paired scTCR clonotype diversity.

**PO-392 GALECTIN-9 IS REQUIRED FOR DENDRITIC CELL FUNCTION AND DIRECTLY CONTRIBUTES TO DC-MEDIATED ANTI-TUMOUR IMMUNITY**

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**Introduction** Dendritic cells (DCs) express a variety of cell surface receptors aimed at facilitating recognition, uptake and presentation of tumour antigens, which allows for the initiation of a potent anti-tumour immune response. Galectin-mediated interactions on DC-plasma membrane are emerging as potent modulators of cellular organisation although the underlying mechanisms are ill defined. We discovered that galectin-9 (gal-9) is required for DC phagocytosis and migration indicating that gal-9 controls tumour immune surveillance.

**Material and methods**

**Results and discussions** We discovered a novel interaction between Galectin-9 and the phagocytic receptor DC-SIGN and demonstrated that galectin-9 is essential for DCs to take up antigens. Atomic force microscopy experiments uncovered that Galectin-9 directly controls plasma membrane stiffness via the reorganisation of the actin cytoskeleton.

Moreover, we observed that galectin-9 is essential for chemokine-driven DC migration and for their capacity to react to melanoma cells, which may have direct implications for tumour immune surveillance. Supporting this hypothesis, we observed that the expression of galectin-9 is down-regulated in DCs upon co-culturing with melanoma tumour cells.

**Conclusion** In summary, our work identified Galectin-9 as a novel plasma membrane organiser in DCs through reorganisation of the actin cytoskeleton that underlies plasma membrane rigidity. Furthermore, our data postulates galectin-9 as a key modulator of DC function, with implications in the ability of DCs to initiate an anti-tumour response. Based on our findings, we hypothesise that loss of galectin-9 impairs DCs to migrate and respond to tumours hampering anti-tumour immunity, thus facilitating tumour immune escape.