Exploiting the dark matter of single-cell transcriptomes to encompass suppressive myeloid cell differentiation.

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Oncologic context and objectives

Inhibition of the physiologic host-protecting functions of the immune system, commonly designed as **immunosuppression**, can be achieved by many different mechanisms required to maintain self-tolerance and immune homeostasis. These mechanisms of immunosuppression can be triggered in pathological conditions including infectious diseases, intoxication, therapies, surgery, or even aging. In the context of cancer, several immune cells can acquire immunosuppressive function in the Tumor Micro-Environment (TME) leading to the inhibition of anti-tumoral effector T lymphocytes or NK cells. Different subsets of immunosuppressive cells of myeloid origin are capable of promoting tumor escape and progression, including the so-called **Myeloid-derived suppressor cells (MDSC)**, the Tumor Associated Macrophages, regulatory T and B cells, as well as other types including neutrophils (Tie et al. 2022). Recently, the definition of "MDSC" has been a subject of intensive debate, considering their context-dependent nature as heterogeneous populations with varying degree of immaturity and ambiguous phenotypes. They express a combination of core gene expression found in their cellular counterparts, i.e. neutrophils and monocytes, along with suppressive or pro-inflammatory gene expression profiles, emphasizing their context-specific states (Hedge et al. 2021).

MDSCs are involved in many biological processes and in resistance to therapies, particularly in cancers. As recently proposed, targeting those cells thus represents an attractive therapeutic option. While their functions and actions have been extensively studied, the origin and the differentiation paths of these **highly heterogeneous populations** are much less investigated and are not fully resolved. Furthermore, unlike other cells such as regulatory T cells, no master regulator has been identified, so far. To investigate these issues, the **identification of key regulators of their differentiation and their function is essential**.

We argue that focusing research on finding new protein-coding markers reduces the scope of possibilities by considering only the "tip of an iceberg*", that is the transcriptome. Therefore, the aim is to advance our understanding of the acquisition of suppressive states in myeloid cells by comprehensively characterizing their transcriptomic landscape, with a particular focus on the non-coding part. The main objective of the project is thus to **identify lncRNAs that specifically regulate the immunosuppressive activity of "MDSC" populations in solid tumors**. Here, we leverage well annotated scRNA-seq data containing immunosuppressive populations from patients with HCC (Giraud*, Chalopin* et al. 2024) as well as ribo-depleted RNA-seq and scRNA-seq data from an *in vitro* model (Fig. 1 and 3).

* Protein-coding genes make 2% of the human genome

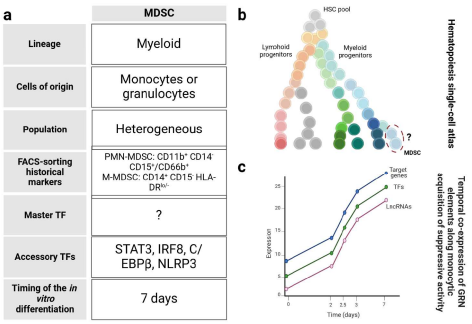


Figure 1: Characteristics of MDSCs and objectives of the study. a) MDSCs were shown to represent very heterogeneous populations within the myeloid lineage. While key transcription factors such as STAT3 have been identified, none singularly drives MDSC differentiation. This suggests the existence of multiple regulatory programs, possibly acting either concurrently or at different temporal stages (Blaye et al. 2022). The specific objectives of this study are to characterize and define the various states of monocytes leading to MDSCs in cancers, HCC, using a single-cell atlas mimicking the hematopoietic system (panel b), and to identify the lncRNAs specifically involved in the acquisition of suppressive activity in monocytes (panel c).

Construction of a hematopoietic single-cell atlas in hepatocellular context

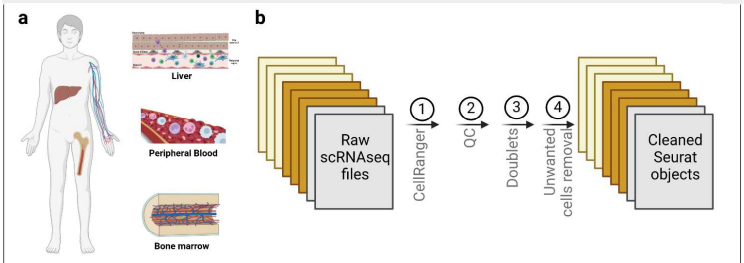


Figure 2. Construction of a single-cell atlas. a) We used dataset that we recently published (Giraud*, Chalopin* et al. 2024), dataset from the HuMoSC model (fig. 3) and publicly available datasets from bone marrow, blood and liver tissues (healthy and HCC). b) Simplified single-cell workflow - after downloading data for **129 samples**, we used CellRanger v7, removed low quality cells, removed doublets based on a consensus protocol and removed non-immune cells based on the expression of non-immune markers. Integration of all 129 samples was performed using Harmony to correct sample-batch effect. This resulted in an atlas containing ~**411,000** cells.

Experimental design to follow the temporal acquisition of immunosuppressive function (*in vitro* culture)

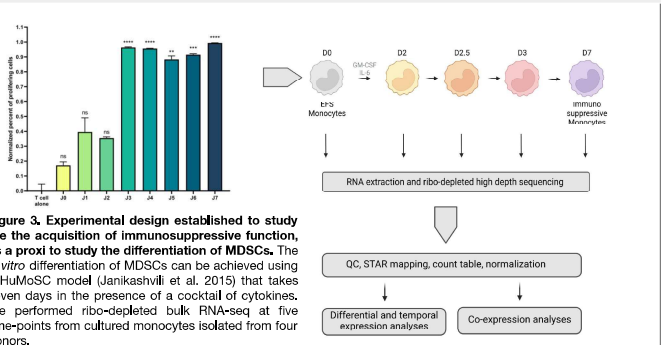
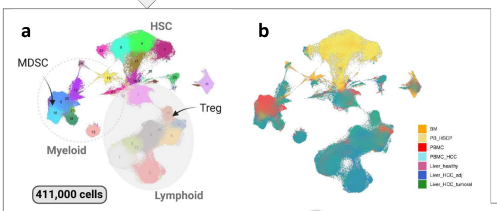


Figure 3. Experimental design established to study the the acquisition of immunosuppressive function, as a proxi to study the differentiation of MDSCs. The *in vitro* differentiation of MDSCs can be achieved using a HuMoSC model (Janikashvili et al. 2015) that takes seven days in the presence of a cocktail of cytokines. We performed ribo-depleted bulk RNA-seq at five time-points from cultured monocytes isolated from four donors.



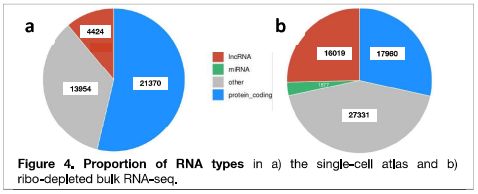


Figure 4. Proportion of RNA types in a) the single-cell atlas and b) ribo-depleted bulk RNA-seq.

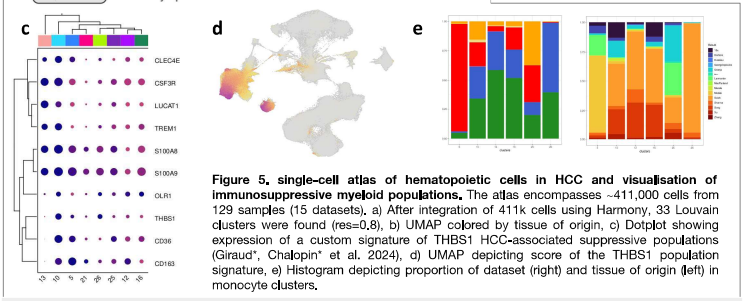


Figure 5. single-cell atlas of hematopoietic cells in HCC and visualisation of immunosuppressive myeloid populations. The atlas encompasses ~411,000 cells from 129 samples (15 datasets). a) After integration of 411k cells using Harmony, 33 Louvain clusters were found (res=0,8), b) UMAP colored by tissue of origin, c) Dotplot showing expression of a custom signature of THBS1 HCC-associated suppressive populations (Giraud*, Chalopin* et al. 2024), d) UMAP depicting score of the THBS1 population signature, e) Histogram depicting proportion of dataset (right) and tissue of origin (left) in monocyte clusters.

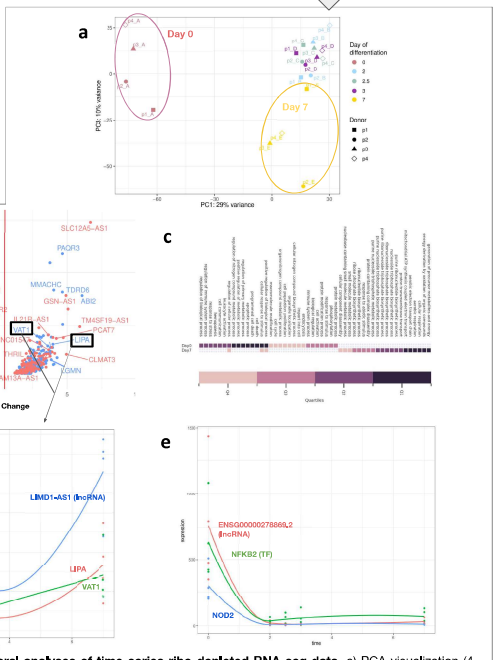


Figure 6. Differential and temporal analyses of time-series ribo-depleted RNA-seq data. a) PCA visualization (4 donors, 5 time-points), b) Differential expression analyses between Day 0 and Day 7, c) Enrichment analyses from DE genes visualized using orsum package, d) Temporal expression of LIPA, VAT1 and LIMD1-AS1 (lncRNA) identified in the same WGCNA module. LIPA (lipid metabolism gene) and VAT1 (involved in antigen uptake) were also found as top DE genes in Day 7. Together with two other co-expressed genes, namely CSTB and CTSB, they were identified in immunosuppressive mo-macs infiltrating NSCLC (Park et al. 2023), and e) Temporal expression of NOD2, its transcription factor (TF) NFKB2 and the lncRNA ENSG00000278869.2 identified in the same WGCNA module.

Identification of MDSC populations in the single-cell atlas

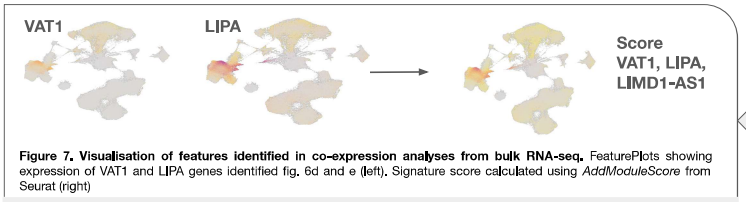


Figure 7. Visualisation of features identified in co-expression analyses from bulk RNA-seq. FeaturePlots showing expression of VAT1 and LIPA genes identified fig. 6d and e (left). Signature score calculated using *AddModuleScore* from Seurat (right)

scRNA-seq expression of genes identified in RNA-seq

Gene-lncRNA regulation through the temporal acquisition of suppressive function

Tie et al. 2022, J Hematol Oncol.
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Giraud*, Chalopin* et al. 2024, Cell Reports.
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Park et al. 2023, Nature Immunol.

Acknowledgment
Aurélien Barré and Alexis Groppi
Emmanuel Saliba and Tom Gräfenhan