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Unique protein expression profiles discriminate ex vivo differentiated monocytic MDSCs from TAMs.

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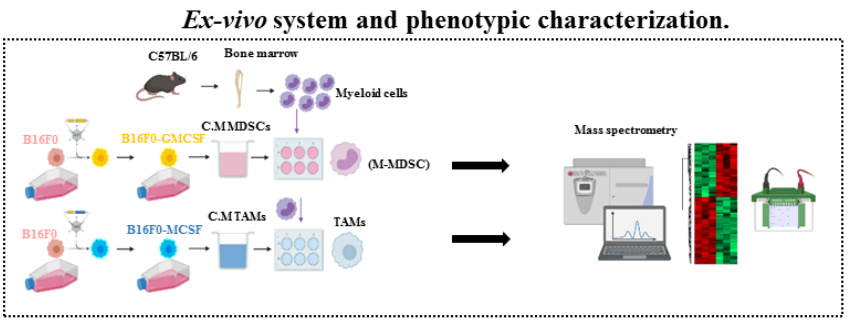
Graphical Abstract



Abstract.

Detailed knowledge of tumor biology is necessary for tumor reprogramming. Tumor associated macrophages (TAMs) and monocytic myeloid suppressor cells (m-MDSCs) are major tumor-promoting cells within the tumor microenvironment (TME). Differentiation of m-MDSC and TAMs is shaped by tumor microenvironment. Several factors have been shown to drive m-MDSC differentiation into TAMs, indicating that they are two different populations. However, TAMs and m-MDSC closely related functions, phenotypic similarities and differentiation plasticity contributes to the confusion over their ontogeny and differential characteristics.

Here, we revealed the key differences between m-MDSC and TAMs, focused on differential pathways by high-throughput proteomics.

Introduction *(optional)*

The role of myeloid cells in tumors has sparked increasing interest since they are relevant modulators of TME. Indeed, myeloid cells constitute the major component of TME. Therefore, it is important to identify the molecular signatures associated to cancer-promoting myeloid cells. But the great plasticity of these cells leads to a confusion of their cellular identity. As in the case of TAMs and m-MDSCs, both share many features, and they may also have common monocytic precursors. Hence, it is crucial to identify the main molecular differences between m-MDSC and TAMs.

Materials and Methods *(optional)*

We used an *ex vivo* differentiation system for MDSCs and TAM by modelling the tumor microenvironment from C57BL/6J mouse bone marrow cells in conditioning medium (1). Monocytic MDSCs were purified using the myeloid-derived suppressor cell isolation kit (Miltenyi Biotec; Bergisch, Germany) according to the manufacturer's recommendations. An experiment of mass spectrometry-based quantitative (shotgun) proteomics was performed. Functional proteomic interactome networks were constructed with STRING and classified by gene ontology and KEGG pathways Tfacts algorithm (https://www.tfacts.org/TFactS-new/TFactS-v2/index1.html; accessed on 5 May 2021) was used to identify potentially activated/de-activated transcription factors using the indicated differential proteomes. Targeted proteins were evaluated using western blot.

Results and Discussion *(optional)*

A total of 1536 proteins were uniquely identified. Of these, 336 were differentially regulated supporting the evidence for these myeloid populations are distinct from each other (Figure 1a). The differential proteome in TAMs and m-MDSCs exhibited different regulating networks of lysosomal metabolism and leukocyte transendothelial migration respectively (Figure 1b,c).

Kinases were selected from the proteomes of TAMs and m-MDSCs and their expression compared to identify profiles separating both populations. MAPK3 was up-regulated in m-MDSCs compared to TAMs (Figure 1d). Both TAM and m-MDSC have strongly upregulated phosphorylation on S727. Moreover, strong increase in STAT3 Y705 phosphorylation was observed in TAM versus both monocytic (Figure 1d). To identify transcription factor profiles that could discriminate between the two populations, the Tfacts algorithm was used with the m-MDSC differential proteome. HIF1-alpha was predicted as a principal transcription factor upregulated in m-MDSC compared to TAMs, which was confirmed by western blot.

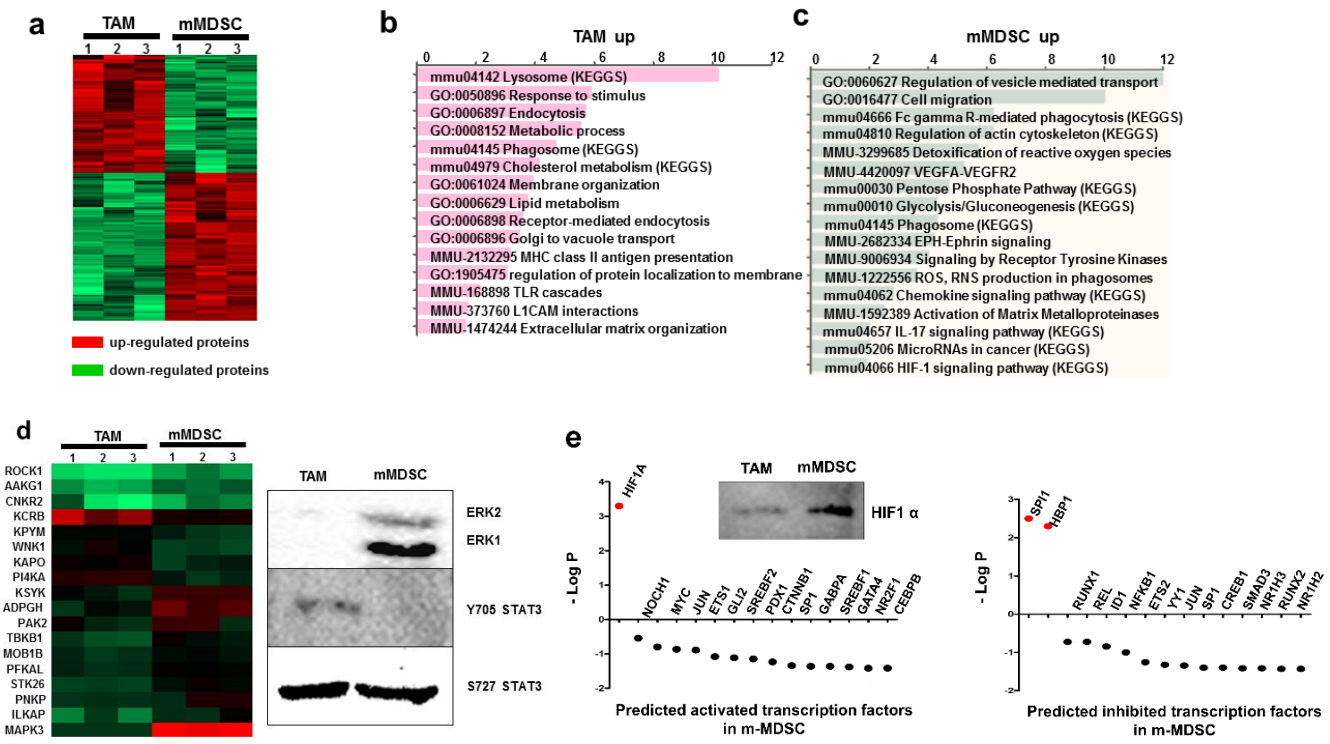


Figure 1. Differential proteomes between TAM and m-MDSCs discriminate both populations. (**a**) Heat map of the differentially expressed proteins ($p < 0.01$) between the indicated samples with 3 independent biological triplicates of TAM and m-MDSC cultures. Red and green, up and down-regulated proteins, respectively. (**b**) The bar graphs represent the enrichment of the differentially pathways in the TAM proteome, as analysed by gene ontology and KEGGs pathways. (**c**)Same as (**b**) but using the differentially up-regulated proteome in monocytic MDSCs. (**d**) Heat map representing the differential expression of the indicated kinases identified in the TAM and m-MDSC proteomes. Kinase expression in both subsets. (**d**) Western blots of the indicated kinases in TAMs and m-MDSCs as shown. (**e**) The dot plot graphs represent the probability of each indicated transcription factor to be either activated (left graph) or inhibited (right graph) in m-MDSCs compared to TAMs using the Tfacts algorithm. Transcription factors with statistical significance of association to the differential m-MDSC proteome according to probability ($p < 0.05$) and false discovery rates ($p < 0.05$) are highlighted in red. The western blot shows HIF1-alpha expression in TAMs or monocytic MDSCs as indicated.

Conclusions *(optional)*

In the present study, we identified differences in proteomic signatures between myeloid cells modelling M-MDSCs and TAMs. These analyses shed light on the differences between the m-MDSC and TAMs identifying each cell type with unique proteomic fingerprints.

References *(mandatory)*

1) Liechtenstein T, Perez-Janices N, Gato M, et al. A highly efficient tumor-infiltrating MDSC differentiation system for discovery of anti-neoplastic targets, which circumvents the need for tumor establishment in mice. Oncotarget. 2014;5(17):7843-7857. doi:10.18632/oncotarget.2279

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