Characterization of Myeloid-Derived Suppressor Cells and Tumor Associated Macrophages Using MultiOmyx® Hyperplexed Immunofluorescence Assay in Hodgkin Lymphoma

Qingyu An • Jun Fang • Arezoie Hamih • Anna Juncker-Jensen • Judy Xu • Eric Leones • Flora Saha • RaghavKrishna Padmanabhan • Nicholas Hoe • Joëtta William

Neogenomics Laboratories, Aliso Viejo, CA

INTRODUCTION

Tumor microenvironment (TME) consists of heterogeneous subsets of myeloid cells which plays a crucial role in promoting cancer development and metastasis. Tumor associated macrophages (TAMs) and myeloid derived suppressor cells (MDSCs) all contribute to the immunosuppressive microenvironment for cancer cells. On basis of surface markers expression, MDSC can be further subdivided into granulocytic MDSC (G-MDSC, polymorphonuclear MDSC) and monocytic MDSC (M-MDSC) [1]. In solid tumors, these different myeloid cell populations are well characterized and extensively studied. However, in hematologic malignancies the role of myeloid cell subsets has been less studied. A recent study showed an increase in MDSC in the bone marrow (BM) at time of diagnosis in acute myeloid leukemia (AML) patients [2]. Importantly higher numbers of G-MDSC and M-MDSC were present at diagnosis in classic Hodgkin lymphoma (cHL) [3]. The accumulation of TAMs was also reported to be associated with poor prognosis in cHL [4, 5]. Collectively, these results indicate that the tumor-resident myeloid cells play an important role in clinical practice, thus highlighting the need for monitoring and comprehensive characterization of various myeloid subsets in hematological malignancies, especially in the tumor microenvironment (TME).

CHARACTERIZATIONS OF MDSCS AND TAMs IN HL, AML AND DLBCL USING MULTIOMYX IF ASSAY

In this study, MultiOmyx 13-plexed panel was utilized to characterize different subtypes of myeloid cells in HL, AML and DLBCL samples. MultiOmyx proprietary algorithm was used to perform cell classification and spatial analysis in HL samples.

- M2 TAM: G-MDSC and M-MDSC in different types of hematological malignancies were characterized by 13-plexed MultiOmyx assay. The IF color overlaid images shown in Figure 2A-I provide examples of unambiguous classification of different subtypes of myeloid cells. Both M-MDSC and G-MDSC accumulated in HL samples, with higher frequency of G-MDSC over M-MDSC. Arg1 expression was detected exclusively in G-MDSC population. Pearson correlation was used to study positive and negative correlations between different subsets of tumor-resident myeloid cells. Correlation analysis was performed to determine if significant correlations exist between MDSCs and TAMs and how tumor-resident myeloid cells correlate to the establishment of an immunosuppressive TME. Using the MultiOmyx proprietary algorithm, which takes into account the staining patterns, we quantified the counts and density of different tumor-resident myeloid subsets and study the spatial correlations between different subset of tumor-resident myeloid cells.

- T regulatory cells (Tregs) and M2 TAMs are spatially more close to G-MDSCs than M-MDSCs in the 9 HL patient samples used in the study.

- Nearest neighbor analysis indicates that Tregs are in closer proximity to M2 TAMs than MDSCs. The data also revealed an abundant Arg1 macrophages (Fig 2D, characterized as CD68+CD163+) present in all HL samples. The detection of both MDSCs and M2 macrophages in HL samples supports the hypothesis that these cells contribute to the establishment of an immunosuppressive TME.

- CD68+CD163+ M2 TAMs are in green. (E) Representative image for G-MDSC. CD11b+CD33+CD15+ Arg1-expressing G-MDSCs are the white cells positive for CD11b, CD15 and Arg-1. (F) Spatial correlations of G-MDSCs to G-MDSCs. (G) Spatial correlations of G-MDSCs to TAMs. (H) Spatial correlations of G-MDSCs to Tregs. (I) Spatial correlations of TAMs to G-MDSCs. (J) Spatial correlations of TAMs to Tregs. (K) Spatial correlations of Tregs to G-MDSCs and TAMs. (L) Spatial correlations of Tregs to MDSCs and TAMs.

OVERVIEW OF ASSAY WORKFLOW

1. Acquire Background 2. Stain Slide 3. Acquire immunofluorescence

Figure 2. Characterization of MDSCs and TAMs in HL, AML and DLBCL using MultiOmyx IF Assay.

Quantification and Nearest Neighbor Spatial Analysis of the Immunosuppressive Cells in HL

Figure 3. Nearest Neighbor Spatial Analysis of Immunosuppressive Cells in HL.

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