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

#4135

Qingyan Au • Jun Fang • Arezoo Hanifi • Anna Juncker-Jensen • Judy Kuo • Eric Leones • Flora Sahafi • RaghavKrishna Padmanabhan • Nicholas Hoe • Josette 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 an immunologically permissive 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). In solid tumors, these different myeloid cell populations are well characterized and extensively studied. However, in hematological 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 (Sun H. et al. Int J Hematol. 2015). Significantly higher numbers of G-MDSC and M- MDSC were present at diagnosis in classic Hodgkin lymphoma (cHL) (Romano A. et al. Br J Haematol. 2015). The accumulation of TAMs was also reported to be associated with poor prognosis in cHL (Steidl C. et al. N Engl J Med. 2010). Collectively, these results indicate that the tumor-resident myeloid cells play an important clinical role, thus highlighting the need for monitoring and comprehensive characterization of various myeloid subsets in hematological malignancies, especially in the tumor FFPE sections.

Herein, we report an analysis of MDSCs and 'protumoral' M2 macrophages using MultiOmyx hyperplexed immunofluorescence (IF) assay in 9 clinical samples diagnosed with HL. MultiOmyx is a proprietary multi 'omic' technology that enables detection and visualization of up to 60 biomarkers on a single 4µM FFPE slide (Gerdes MJ. et al. PNAS 2013). The HL FFPE sections were stained with a 13-marker panel including Arginase 1, CD11b, CD14, CD15, CD16, CD33, CD68, CD163, HLA-DR, CD3, CD4, CD8 and FOXP3. We observed that both M-MDSC (Fig 2A, characterized as CD11b+CD14+CD15-CD33+HLA-DR-) and G-MDSC (Fig 2B, identified as CD11b+CD14- CD15+CD33+HLA-DR-) accumulated within the TME in all 9 HL samples, with higher frequency of G-MDSCs over M- MDSCs. Arg1 expression was detected exclusively in G-MDSC population (Fig 2C). The data also revealed an abundant M2 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. 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.

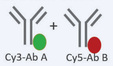
Correlation study was also performed to determine if significant correlations exist between MDSCs and TAMs and how these immunosuppressive myeloid cells are related to the Regulatory T cells (Tregs, CD3+CD4+FOXP3+) in HL samples. In addition to 9 HL samples, the same 13-plexed panel was used to characterize the myeloid cell population from AML patients and diffuse large B cell lymphoma (DLBCL) patients.

TAMs and MDSCs are emerging as potential biomarkers for prognosis of cancer as well as therapeutic targets. As demonstrated in this study, MultiOmyx 13-plexed panel has the potential to monitor the changes of immunosuppressive myeloid cells in response to immune modulating drugs such as MDSC- targeting drugs (e.g. PDE-5 inhibitors, COX-2 inhibitors), TAM-targeting agents (e.g. anti-CSF1R) and combination therapy in treatment of lymphoma and leukemia.

Overview of Assay Workflow

1. Acquire Background

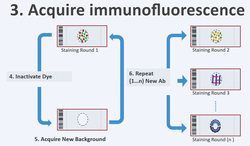
2. Stain Slide



Phenotype	Co-expressions
T helper cells	CD3+CD4+
T cytotoxic cells	CD3+CD8+
T regulatory cells	CD3+CD4+FoxP3+
Myeloid cells	CD11b+CD33+
M1 TAMs	CD68+HLA-DR+
M2 TAMs	CD68+CD163+
M-MDSC	CD11b+CD14+CD15-HLA-DR-
G-MDSC	CD11b+CD15+CD14-HLA-DR-

Table 1. Phenotyping by MultiOmyx 13-plexed panel. Cell surface markers associated with cell subsets analyzed in the study.

3. Acquire immunofluorescence



MultiOmyx Multiplexed IF Assay

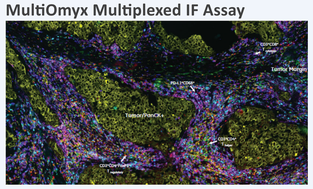


Figure 1. Assay Workflow. For MultiOmyx IF study, slides were prepared and stained using MultiOmyx multiplexing IF staining protocol. For each round of staining, conjugated fluorescent antibodies were applied to the slide, followed by imaging acquisition of stained slides. The dye was erased, enabling a second round of staining with another pair of fluorescent antibodies.

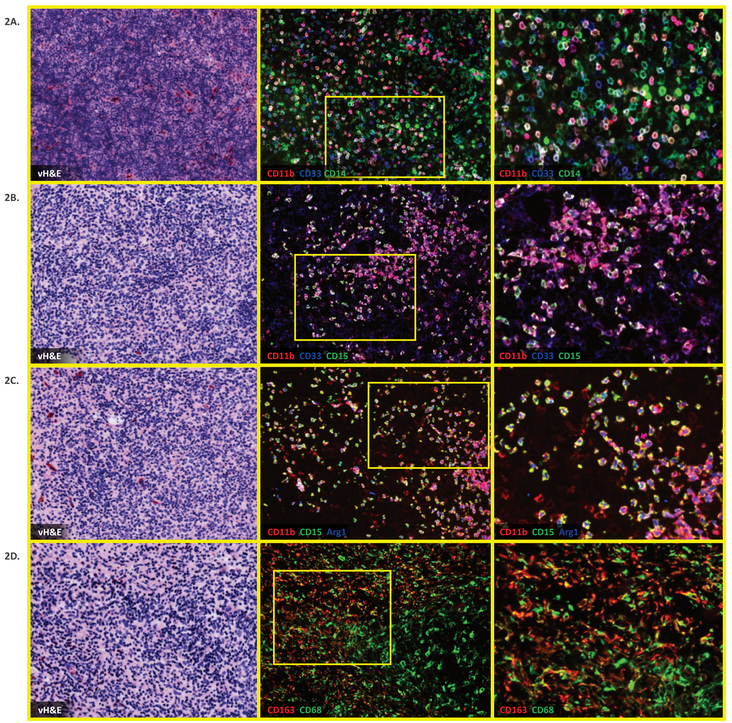
Conclusions

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.

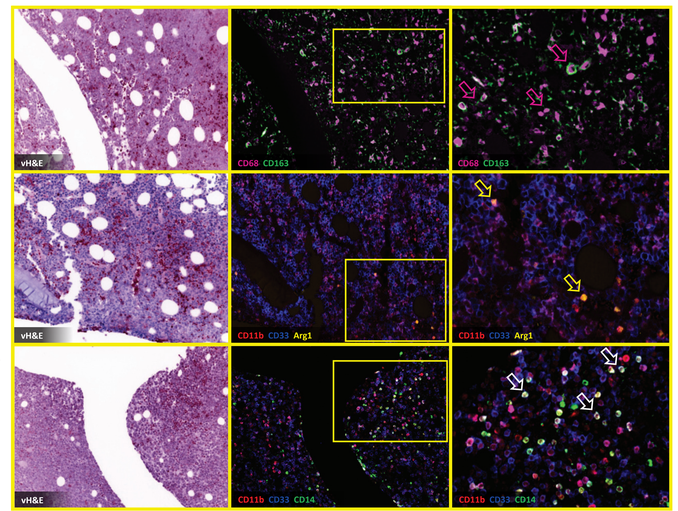
- M2 TAM, G-MDSC and M-MDSC in different types of hematological malignancies were characterized by 13-plexed MultiOmyx assay. The IF color overlaid images shown in Figure 2A-I, provide examples of unambiguous classification of different subtypes of myeloid cells.
- Both M-MDSC and G-MDSC accumulated in HL samples, with higher frequency of G-MDSCs over M-MDSCs. Arg1 expression was detected exclusively in G-MDSC population.
- Pearson correlation was used to study positive and negative correlations between different subtypes of myeloid cells in HL samples. There is a significant positive correlation between T cytotoxic cells to M-MDSCs ($p<0.05$) in the 9 HL patients in the study.
- Nearest neighbor analysis indicates that Tregs are in closer proximity to M2 TAMs than MDSCs. Tregs and M2 TAMs are spatially more close to G-MDSCs than M-MDSCs in the 9 HL patient samples used in the study.

Characterizations of MDSCs and TAMs in HL, AML and DLBCL Using MultiOmyx IF Assay

Representative Images from Hodgkin Lymphoma Patients



Representative Images from Acute Myeloid Leukemia Patients



Representative Images from Diffuse Large B-cell Lymphoma Patients

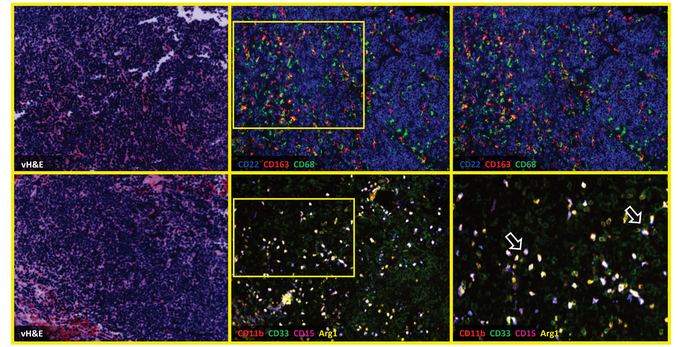


Figure 2. Characterization of MDSCs and TAMs in HL, AML and DLBCL using MultiOmyx hyperplexed IF assay. In each row, vH&E image (simulated HE image from fluorescent signals) for the selected HL region of interest (ROI) is shown on the left; color overlaid image representing different subtypes of myeloid cells are shown in the middle; the higher magnification of the middle image is shown on the right. (A) Representative image for M-MDSCs. CD11b+CD33+CD14+ M-MDSC cells are in white. (B) Representative image for G-MDSCs. CD11b+CD33+CD15+ G-MDSC cells are in white. (C) Representative image for Arg1-expressing G-MDSC. Arg-1 expressing G-MDSCs are the white cells positive for CD11b, CD15 and Arg1. (D) Representative images for M2 macrophages. The M2 macrophages co-expressing CD68 and CD163 are in yellow. The CD68+CD163- M1 macrophages are in green. (E) Representative image for M2 macrophages. The magenta arrows indicate examples of M2 macrophages. (F) Representative image for Arginase expressing MDSCs, indicated by the yellow arrows. (G) Representative image for M-MDSCs, indicated by the white arrows. (H) Representative image for M2 macrophages. B cells are identified by CD22. (I), Representative image for Arginase expressing G-MDSCs, indicated by the white arrows.

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

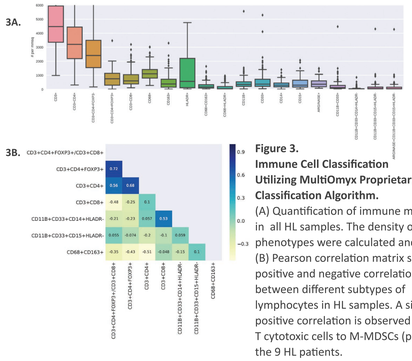
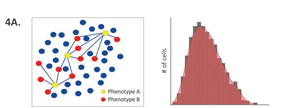


Figure 3. Immune Cell Classification Utilizing MultiOmyx Proprietary Cell Classification Algorithm. (A) Quantification of immune markers in all HL samples. The density of phenotypes were calculated and plotted. (B) Pearson correlation matrix showed positive and negative correlations between different subtypes of lymphocytes in HL samples. A significant positive correlation is observed between T cytotoxic cells to M-MDSCs ($p<0.05$) in the 9 HL patients.



5 Nearest Neighbor Analysis

Average distance of 5 nearest "B" neighbors to "A"

Figure 4. Nearest Neighbor Spatial Analysis of Immune Suppressive Cells. (A) The average of the distance of the 5 nearest neighbors from any given phenotypes is calculated. (B) Spatial correlations of Tregs and MDSCs to M2 TAMs. (C) Spatials correlation of Tregs, M2 TAMs and G-MDSCs to M-MDSCs. (D) Spatial correlations of Tregs, M2 TAMs and M-MDSCs to G-MDSCs. (E) Spatial correlations of M2 TAMs and MDSCs to Tregs.