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Characterization of the Molecular Signature of Human Monocytes in Aging and Myelodysplastic Neoplasms

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Increased life expectancy has led to an increased risk for aging-associated disorders, including cardiovascular diseases and malignancies [1, 2]. Low-grade chronic inflammation, myeloid cell predominance, and dysfunction of adaptive immunity characterize aging, whereas skewed hematopoiesis results in increased risk for myeloid malignancies, such as myelodysplastic neoplasms (MDS) and chronic myelomonocytic leukemia (CMML) [1]. MDS is characterized by an aging-related heterogeneous group of clonal disorders of hematopoietic progenitors [3], with bone marrow inflammation playing an important role in disease progression [4]. CMML shares common features with myeloproliferative neoplasms and MDS, including common molecular aberrations, but it is distinguished by persistent clonal monocytosis [5].

Monocytes are key players in inflammation, characterized by plasticity, playing diverse roles during inflammation, acting either as a source of cytokines or driving the resolution of inflammation [6]. During aging, monocytes and macrophages have decreased capacity for apoptotic cell clearance and produce increased amounts of inflammatory cytokines [7]. Regarding clonal hematopoiesis, they release increased levels of IL-1β [1], further associated with increased prevalence of cardiometabolic disorders in preclinical clonal hematopoiesis [1] or MDS [8].

To study immune cell populations in blood in aging and MDS, we performed deep immunophenotyping of peripheral blood mononuclear cells (PBMCs) from nine young adults, nine older individuals, and nine patients with MDS (Table S1), using cytometry by time-of-flight (CyTOF). We identified 23 clusters of cells, including T, B, NK, dendritic cells (DCs), and monocytic cells (Figure 1A). Increased proportions of clusters characterized as naïve $CD4^+$ and $CD8^+$ T cells ($CCR7^+CD45RA^+CD27^+IL\text{-}7Ra^+$), $CD161^{hi}CD8^+$T cells, and plasmacytoid DCs (pDCs) were observed in PBMCs from young individuals compared to older individuals. On the other hand, the proportion of effector memory $CD8^+$ T cell ($CD45RO^+CD45RA^-CCR7^-$), $CD57^+CD4^+$ and $CD57^+CD8^+$ T effector cell ($CCR7^-$) clusters ($T4_{eff}CD57^+$ and $T8CD57^+$), and a cluster of monocytes ($CD14^{dim}CD38^{dim}$) increased in older individuals (Figure 1B,C). Regarding MDS, an increased proportion of the classical monocyte cluster ($CD14^+CD38^+$) was observed (Figure 1C). Principal component analysis (PCA) demonstrated a separation of samples derived from young adults, mainly attributed to the differences in the proportion of $T8CD57^+$, $T4CD57^+$ clusters (Figure 1D), whereas

Abbreviations: CMML, chronic myelomonocytic leukemia; DCs, dendritic cells; DEGs, differentially expressed genes; MDS, myelodysplastic neoplasms; PBMCs, peripheral blood mononuclear cells.
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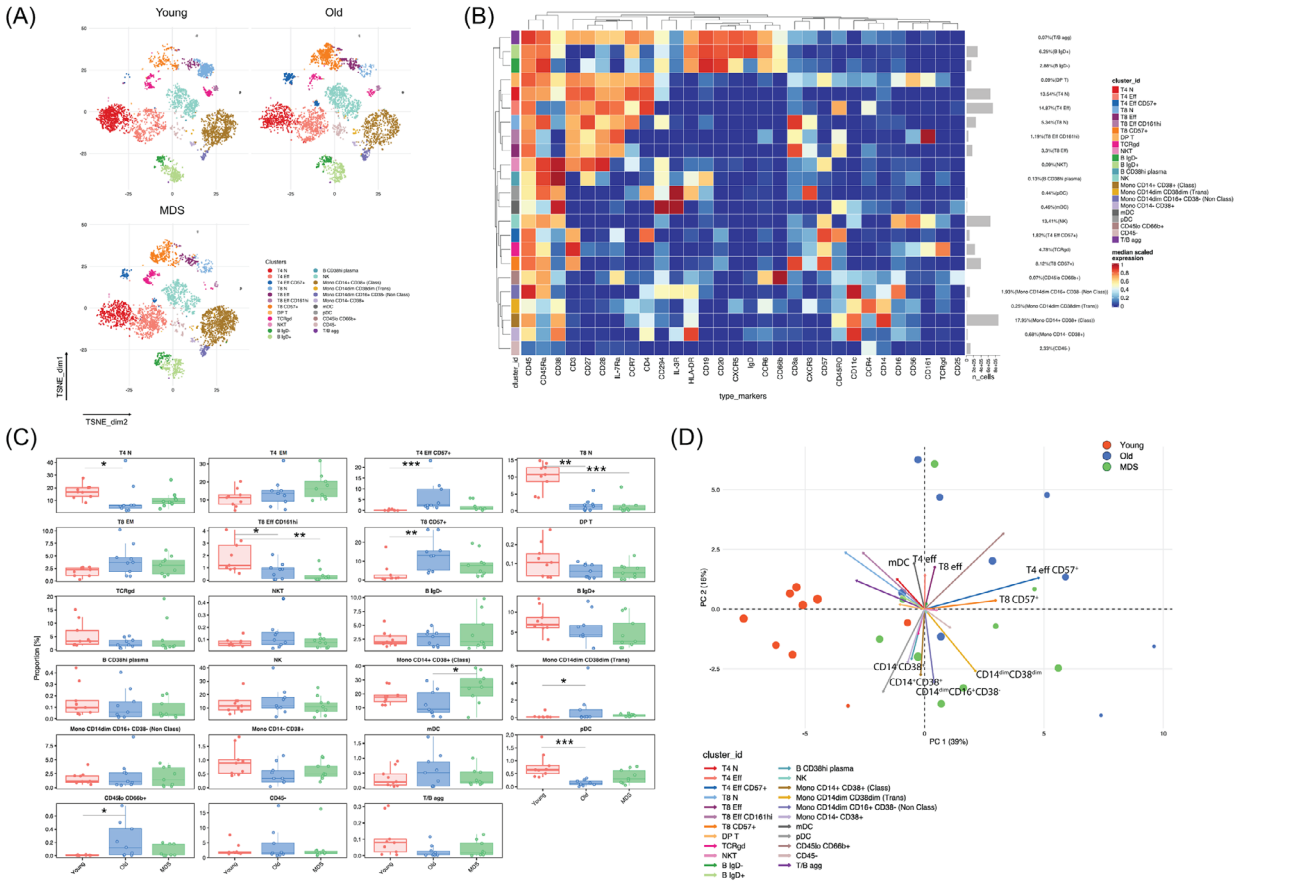

FIGURE 1 | Unsupervised immunophenotyping of PBMCs by CyTOF. (A) t-SNE plot visualizing cluster assignments of cell subtypes. (B) Heatmap showing the expression of markers for each cluster. (C) Box plots showing the percentage of each immune cell subset. (D) PCA among young and old healthy donors and MDS patients. $N = 9$ samples per group. Samples were analyzed in three batches. Agg = aggregates; DP double positive = $CD4^+CD8^+$; Eff = effector; mDC = myeloid dendritic cells; Mono = monocytes; N = naïve; NK = natural killer; pDC = plasmacytoid dendritic cells; T4/8 = CD4/8 T cells; TCRgd = T cell receptor gamma delta. $^*p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$.

the other two groups were separated on the basis of the percentage of myeloid DC (mDCs), $CD4^+$T effector, expressing memory markers ($CD4^+CD45RO^+CCR7^+CD28^+$ $T4_{eff}$), $CD8^+$T effector cells ($CD8^+CD45RO^+CCR7^{lo}CD28^+$ $T8_{eff}$), and monocytic clusters (Figure 1D).

We then focused on monocytes, performing unsupervised clustering based on the expression of markers expressed on $CD14^+$ monocytes. The samples from the older and MDS groups were partitioned in different clusters (Figure S1A). The expression levels of CCR6, CD45RA, IL-3R, and CD294 were increased in old subjects (Figure S1B). Using flow cytometry, we observed an increased frequency of $CD14^+CD16^+$ intermediate/transitional cells in samples from older controls and in the proportion of $CD14^{dim}CD16^+$ nonclassical monocytes in MDS (Figures S1C,D and Table S2). Additionally, CD206 expression was increased in older subjects compared to patients with MDS (Figure S1E,F), whereas no difference was observed in CD163, CD86, or CD80 expression (Figure S3).

We then studied the molecular phenotype of isolated $CD14^+$ cells that include monocytes and DC subsets, from young and older individuals and patients with MDS using RNAseq (Figure 2A). We detected 1087 upregulated differentially expressed genes (DEGs) and 790 downregulated in young individuals compared to old control subjects (Table S3). KEGG pathway analysis showed that DEGs upregulated in young adults were enriched in RNA polymerase and ribosome biogenesis pathways, whereas the downregulated DEGs were enriched in phagocytosis, pathogen clearance, complement and coagulation cascades, and cytokine signaling pathways (Figure 2B). We identified several DEGs upregulated in cells from older individuals, including genes encoding Fc gamma receptors (*FCGR1A, FCGR2A, FCGR2B, FCGR3A, FCGR3B*), integrin subunits (*ITGA1, ITGA2B, ITGB3, ITGB5*), and the chemokines *CXCL1* and *CXCL8*, and *CSF1* (Figure 2C). GSEA demonstrated a significant correlation of several inflammatory gene sets (Figure 2D).

When we compared older subjects and MDS patients, 104 DEGs were downregulated, and 66 were upregulated in MDS (Table S3). DEGs upregulated in MDS were associated with antigen presentation and phagosome formation, whereas those downregulated to inflammation, including TNF and IL-17 signaling pathways (Figure 2E). Furthermore, GSEA exhibited enrichment of the IFN-α response pathway in MDS (Figure 2F,G), whereas enrichment of inflammatory pathways was observed in control group. DEGs downregulated in MDS included *CXCL1, CXCL8*, *CXCR1*, *S100A8, S100A9*, and *SELL*, and the upregulated *CD209*

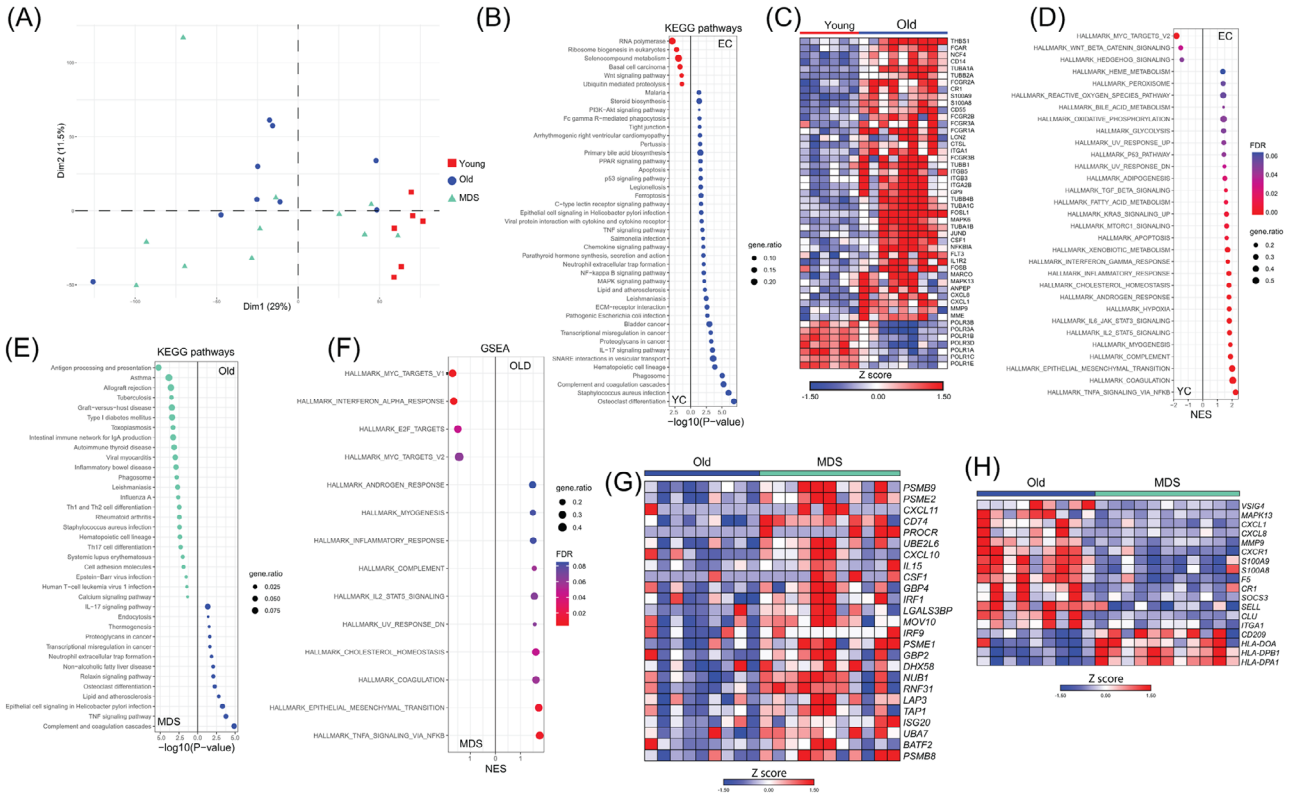

FIGURE 2 | Transcriptomic analysis of isolated CD14$^+$ cells. (A) PCA shows that young healthy donors ($n = 6$) segregate along the first dimension, Dim1, compared to old healthy donors ($n = 9$) and MDS patients ($n = 11$). Moreover, old healthy donors segregate along the second dimension, Dim2, compared to MDS patients. (B) Pathway analysis of differentially expressed genes of young (YC) compared to old healthy donors (EC) using KEGG database. (C) Heatmap depicting the differentially expressed genes of the highly enriched pathways from KEGG database. (D) Enriched pathways from young healthy donors compared to old healthy donors using GSEA pre-ranked analysis. (E) Pathway enrichment analysis of differentially expressed genes of old healthy donors compared to MDS, using KEGG database. (F) Enriched pathways from old healthy donors compared to MDS patients using GSEA pre-ranked analysis. (G) Heatmap illustrating differentially regulated targets of IFN-α response pathways. (H) Heatmap depicting representative differentially expressed genes of the pathways generated by the EnrichR analysis. Analysis was performed in a single batch.

and the class II major histocompatibility complex genes *HLA-DOA, HLA-DPA1*, and *HLA-DPB1* were included in the antigen presentation pathway (Figure 2H).

To discern whether the molecular signature of CD14$^+$ cells in MDS differs from that of CMML, we utilized previously published RNAseq data from CD14$^+$ cells from CMML patients (Gene Expression Omnibus database GSE135902) [9]. PCA displayed a distinct segregation of samples from CMML patients from the samples of their control group (Figure S4A). We detected 2104 DEGs that were significantly upregulated and 978 that were downregulated in CMML (Figure S4B). Comparing DEGs in MDS and CMML datasets, both compared with the respective control group (Figure S4C), we detected 49 common DEGs, showing mostly contrasting expression patterns (Figure S4D). Finally, we compared the pathways generated by GSEA and observed that CD14$^+$ cells from CMML displayed enrichment of inflammatory pathways, including TNF signaling, inflammatory response, and IL6/JAK/STAT3 signaling, further distinguishing the molecular signature of CD14$^+$ cells in CMML compared to MDS (Figure S4E).

Chronic inflammation in aging is the result of chronic stimulation of the innate immune system [1]. Regarding its effect on monocyte function, an increased production of inflammatory cytokines by macrophages was previously reported [7]. To this direction, we have observed significant upregulation of genes associated with inflammatory signatures in cells from older individuals.

We also demonstrated that the transcriptome of monocytes from patients with MDS shows a negative association with inflammatory pathways compared to age-matched individuals and patients with CMML. This is in line with the recently reported underrepresentation of inflammatory pathways in bone marrow mDCs and monocytes in MDS, as shown by microarray transcriptional analysis [10]. Interestingly, transcriptomic data in monocytes from patients with CMML showed increased representation of inflammatory pathways [9], providing evidence that despite the common pathogenic features, there are major differences in immune cell function in CMML and MDS.

Author Contributions

I.M. designed the study and supervised the project. I.K., H.H., A.C., and K.L. contributed to the study design and edited the manuscript. C.M.R., M.G., C.K., A.F., and N.P. conducted experiments. C.M.R., A.T., and G.V.

analyzed data. I.M. and C.M.R. wrote the manuscript. P.P., M.P., C.M., T.S., D.D., and E.L. recruited the samples.

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Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

RNA-seq data have been deposited to the EGA database (EGAS00001007676). All other data are available from the corresponding author upon request.

Peer review

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Supporting Information

Additional supporting information can be found online in the Supporting Information section.

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