Liu *et al. Journal of Translational Medicine* (2022) 20:499
https://doi.org/10.1186/s12967-022-03709-9

Journal of Translational Medicine

LETTER TO THE EDITOR Open Access

Single-cell RNA sequencing identifies the properties of myelodysplastic syndrome stem cells

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Letter to editor,

Myelodysplastic syndromes (MDS) are heterogeneous clonal diseases characterized by cytopenia caused by ineffective hematopoiesis and high risk of transformation into acute myeloid leukemia (AML). At present, the pathogenesis of MDS has not been elucidated. MDS is a group of stem cell diseases. The abnormal proliferation and blockade in differentiation of hematopoietic stem cells (HSCs) result in cytopenia and leukemic transformation. HSCs architectures in MDS can also predict therapeutic reaction [1]. Therefore, the high-resolution analysis of HSCs is of great significance. We investigated the properties of MDS stem cells by single-cell RNA sequencing (scRNA-seq). Lineage negative (Lin^-) cells from bone marrow aspirates of 5 patients with MDS and 2 patients with secondary AML (sAML) were sorted out for scRNA-seq (Additional file 1: Additional methods, Additional file 2: Table S1). The scRNA-seq data of bone marrow mononuclear cells from 17 healthy donors (HDs) were downloaded from the Gene Expression Omnibus database (GSE120221) [2].

A total of 65,509 Lin^- hematopoietic cells were analyzed. After dimension reduction, clustering, and visualization, we identified HSC/multipotent progenitor (MPP) populations and conducted an in-depth analysis. We investigated the properties of MDS stem cells by analyzing the differentially expressed genes (DEGs) between patients and HDs. We found that genes associated with neutrophil granule, such as *MPO*, *AZU1*, *DEFA3*, had elevated expression in MDS patients compared with HDs (Fig. 1A and 1B). This is consistent with the tendency of predominantly myeloid differentiation trajectories of HSCs in MDS. We identified that the down-regulated DEGs in HSCs/MPPs were enriched in ribosome, translation, mRNA catabolic process, Th17 cell differentiation and antigen processing and presentation in MDS patients (Fig. 1C), which supported the opinion that MDS stem cells were in a relatively static state and had impaired immune function. The progression of MDS to AML usually bases on acquired mutations [3]. Our results suggested that abnormal proliferation, RNA metabolism and ribosome biogenesis also exist in MDS stem cells during leukemic transformation (Fig. 1D - F).

It is noteworthy that ribosomal genes were widely down-regulated in HSCs/MPPs of MDS/sAML patients compared with those of HDs (Fig. 1G and H), which suggested that abnormal ribosome biogenesis may be involved in the pathogenesis of MDS and leukemic transformation. The Cancer Genome Atlas (TCGA) database analysis also showed decreased expression of some ribosomal genes in AML (Fig. 1I). The results of quantitative real-time PCR verification showed that the expression of *RPL31* and *RPL21* mRNA in $CD34^+$ cells of patients with LR-MDS (n = 40), HR-MDS

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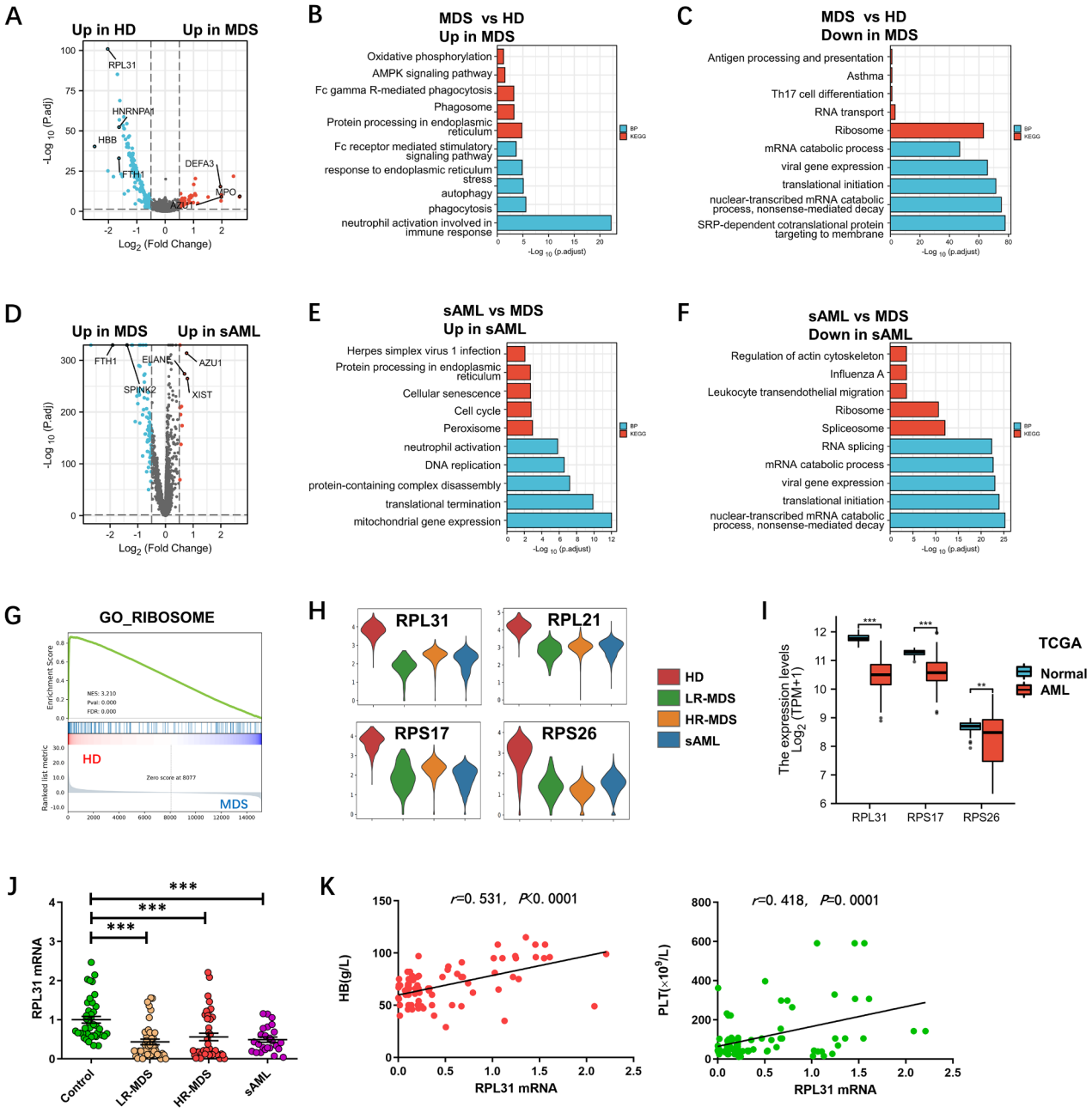

Fig. 1 Analyses of aberrantly expressed genes and functional enrichment in myelodysplastic syndromes (MDS) stem cells by single-cell RNA sequencing **A** Volcano plot of differentially expressed genes (DEGs) in hematopoietic stem cells (HSCs)/multipotent progenitors (MPPs) between MDS patients and healthy donors (HDs). **B** Functional enrichment bar chart of up-regulated DEGs in HSCs/MPPs of MDS patients compared with HDs. Red: Kyoto Encyclopedia of Genes and Genomes (KEGG) gene sets, blue: Biological process (BP). **C** Functional enrichment bar chart of down-regulated DEGs in HSCs/MPPs of MDS patients compared with HDs. **D** Volcano plot of DEGs in HSCs/MPPs between secondary acute myeloid leukemia (sAML) and MDS patients. Red plot: up-regulated in sAML patients, blue plot: up-regulated in MDS patients. **E** Functional enrichment bar chart of up-regulated DEGs in HSCs/MPPs of sAML patients compared with MDS patients. **F** Functional enrichment bar chart of down-regulated DEGs in HSCs/MPPs of sAML patients compared with MDS patients. **G** Gene sets enrichment analysis (GSEA) showed decreased expression of ribosomal genes in HSCs/MPPs of MDS patients compared with HDs. **H** The violin plots of expression of ribosomal genes in HSCs/MPPs of HDs and patients with lower-risk MDS (LR-MDS), higher-risk MDS (HR-MDS) and sAML. **I** The Cancer Genome Atlas (TCGA) database analysis showed the expression of several ribosomal genes in AML patients. **J** Quantitative real-time PCR (qPCR) analyses of *RPL31* mRNA in $CD34^+$ hematopoietic stem and progenitor cells from bone marrow of controls (n = 40), LR-MDS (n = 40), HR-MDS (n = 40) and sAML patients (n = 25). Significant difference is analyzed using analysis of variance. ***, $P < 0.001$. **K** Analyses of correlation between *RPL31* mRNA and hemoglobin (HB), platelet (PLT) in MDS patients (n = 80)

(n = 40) and sAML (n = 25) were significantly lower than those of control (n = 40) ($P < 0.001$) (Fig. 1J, Additional file 3: Fig. S1A) and positively correlated with the levels of hemoglobin and platelet (Fig. 1K, Additional file 3: Fig. S1B).

Hematopoietic homeostasis depends on the balance between self-renewal and differentiation of HSCs. Increasing genome data showed that abnormal expression of some crucial regulatory genes may break the homeostasis and eventually lead to hematological malignant diseases. Our results revealed MDS stem cells have abnormal proliferation, ribosome biogenesis, RNA metabolism and impaired immune function. The expression of ribosomal genes was down-regulated in MDS stem cells. Abnormal ribosome biogenesis and regulation of translation was frequent in myeloid diseases. Gene mutations of ribosomal protein could lead to Diamond-Blackfan Anemia, Schwachman Diamond Syndrome, Congenital Dyskeratosis and some other diseases, all of which have defects in hematopoiesis. In addition, the dyserythropoiesis in MDS with isolated del(5q) is related to the loss of heterozygosity of *RPS14* gene. Haploinsufficiency of *RPS14* leads to activation of innate immune system and p53 pathway, excessive apoptosis of erythroblasts and megaloblastic anemia. Study by Saha et al.[4] showed that the expression of ribosomal genes in leukemia stem cells (LSCs) was down-regulated compared with normal HSCs, which was consistent with the results in our study. The biosynthesis of proteins in HSCs is lower than that in committed progenitor cells and differentiated cells [5]. LSCs may have similar mechanisms to survive and resist to chemotherapy. Therefore, abnormal ribosome biogenesis is an important feature during development and progression of MDS and may be also related to therapeutic resistance.

Abbreviations

DEG	differentially expressed gene
HD	healthy donor
HSC	hematopoietic stem cell
HR-MDS	higher-risk myelodysplastic syndrome
LSC	leukemia stem cell
Lin^-	Lineage negative
LR-MDS	lower-risk myelodysplastic syndrome
MPP	multipotent progenitor
MDS	myelodysplastic syndrome
sAML	secondary acute myeloid leukemia
scRNA-seq	single-cell RNA sequencing
TCGA	The Cancer Genome Atlas

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12967-022-03709-9.

Supplementary Material 1

Supplementary Material 2

Supplementary Material 3

Acknowledgements

We would like to thank Christopher S Hourigan for providing single-cell RNA sequencing data of HDs.

Authors' contributions

YL conceived the study, designed and performed research, analyzed data and wrote the manuscript. HN and NS designed and performed research, performed bioinformatics and statistical analyses. WZ and LL supervised the study, analyzed and interpreted data. HW, RF and ZS conceived and supervised the study, interpreted the data and revised the manuscript. All authors discussed the results and approved the final version of the manuscript.

Funding

This research was funded by the National Natural Science Foundation of China (No. 81970116, 81800123), the Zhao Yicheng (Youth Incubation) Fund of Tianjin Medical University General Hospital (No. ZYYFY2017026), Tianjin Key Medical Discipline(Specialty) Construction Project.

Availability of data and materials

The raw data reported in this study are deposited in the NCBI Sequence Read Archive under bioproject No.PRJNA720840.

Declarations

Ethics approval and consent to participate

The study was conducted according to the guidelines of the Declaration of Helsinki, and approved by the Ethics Committee of Tianjin Medical University General Hospital (NO. IRB2021-WZ-051, April 2021). Informed consent was obtained from all subjects involved in the study.

Consent for publication

The authors affirm that human research participants provided informed consent for publication.

Competing interests

The authors declare that they have no competing interests.

Received: 14 October 2022 / Accepted: 17 October 2022
Published online: 03 November 2022

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