Article

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Analyzing Differences in Hematological and Immunological Characteristics Related to Common Gene Mutations in Myelodysplastic Syndromes

Jianing Yu[1], Xiaohuan Peng[1,2], Rui Wang[1], Jun Bai[2], Yanhong Li[2], Liansheng Zhang[1,2,*], Lijuan Li[1,2,*]

[1]Department of Hematology, Lanzhou University Second Hospital, Lanzhou University, 730000 Lanzhou, Gansu, China
[2]Key Laboratory of the Hematology of Gansu Province, Lanzhou University Second Hospital, Lanzhou University, 730000 Lanzhou, Gansu, China
*Correspondence: doctorzhanglsh@sina.com (Liansheng Zhang); doctorjuan@sina.com (Lijuan Li)
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Background: Genetic mutations play a crucial role in the development and progression of myelodysplastic syndromes (MDS), impacting the immune microenvironment and influencing the choice of treatment regimen, as well as the efficacy and prognosis of patients. The objective of this study was to examine variations in hematological and immunological characteristics associated with common gene mutations in MDS patients and establish a foundation for the precise treatment of MDS.

Methods: The hematological, immunological, and other clinical features of 71 recently diagnosed MDS patients from January 1, 2019, to July 31, 2023, were retrospectively analyzed. These patients were categorized based on their gene mutations, and the variances in hematological and immunological characteristics among distinct groups were compared.

Results: Hematological variances were observed among different gene mutation groups. Specifically, platelet counts in the splicing factor 3B subunit 1 (*SF3B1*) mutation group were notably higher compared to the wild-type group ($p = 0.009$). Conversely, in the additional sex combs like 1 (*ASXL1*) mutation groups, monocyte ratios were significantly elevated in comparison to the wild-type group ($p = 0.046$), and in the ten-eleven translocation 2 (*TET2*) mutation group, lymphocyte ratios were significantly lower ($p = 0.022$). Additionally, the leukocyte ($p = 0.005$), neutrophil ratio ($p = 0.002$), and lymphocyte ratio ($p = 0.001$) were significantly higher in the Runt-related transcription factor 1 (*RUNX1*) mutation group. Regarding immunological distinctions, the Natural Killer (NK) cell ratio demonstrated a significant increase in the *SF3B1* mutation group ($p = 0.005$). Moreover, the *TET2* mutation group exhibited a significantly higher Interleukin-8 (IL-8) level ($p = 0.017$). In contrast, the U2 small nuclear RNA auxiliary factor 1 (*U2AF1*) group displayed significantly lower levels of IL-1β ($p = 0.033$), IL-10 ($p = 0.033$), and Tumour Necrosis Factor-α (TNF-α) ($p = 0.009$).

Conclusion: Distinct variations exist in the immune microenvironment of MDS associated with different genetic mutations. Further studies are imperative to delve into the underlying mechanisms that drive these differences.

Keywords: myelodysplastic syndromes; gene mutations; immune microenvironment; lymphocyte subsets; cytokines

Introduction

Myelodysplastic syndromes (MDS) are hematological neoplasms that originate from hematopoietic stem cells, characterized by pathological hematopoiesis, and associated with a high risk of transformation to acute myeloid leukemia (AML) [1]. Various mutations in both driver and non-driver genes have been identified in association with MDS. Common mutations include splicing factor 3B subunit 1 (*SF3B1*), ten-eleven translocation 2 (*TET2*), additional sex combs like 1 (*ASXL1*), Serine/arginine-rich splicing factor 2 (*SRSF2*), Runt-related transcription factor 1 (*RUNX1*), U2 small nuclear RNA auxiliary factor 1 (*U2AF1*), tumor suppressor p53 (*TP53*), Stromal antigen 2 (*STAG2*), and enhancer of zeste homolog 2 (*EZH2*) [2]. Studies have demonstrated that the development of MDS involves both genetic mutations and immune disorders, contributing to the heterogeneity in clinical features and the immune microenvironment. This variability, in turn, leads to diverse treatment options and outcomes [3]. Driver mutations in distinct cellular pathways can induce different MDS phenotypes, and the majority of patients exhibit complex combinations of mutations, contributing to the high clinical heterogeneity of the disease [1].

The relationship between genetic mutations and immune disorders is intricate and variable. Gene mutations can directly or indirectly influence the immune microenvironment, affecting cytokine levels and immune cell numbers. For instance, *TET2* mutations have been shown to inhibit Natural Killer (NK) cell function in MDS patients, resulting in reduced expression of key molecules such as Tumour Necrosis Factor-α (TNF-α) [4]. Conversely, the

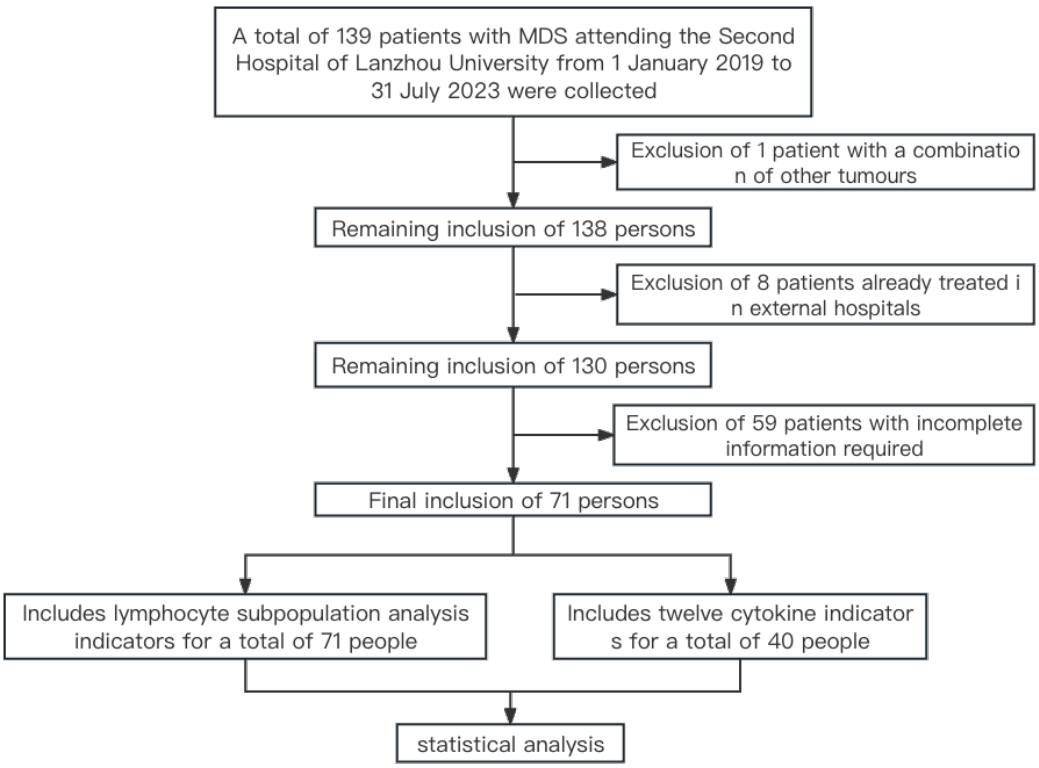

Fig. 1. Flowchart of data collection. The figure was produced via the online website https://www.processon.com (ProcessOn v1.0.8, Beijing Damaidi Information Technology Co., Ltd., Beijing, China).

immune microenvironment also influences gene expression and function. Immune molecules like cytokines can induce the expression of genes involved in inflammation and immune responses, contributing to the development of autoimmune diseases and cancer [5]. This study aims to deepen the understanding of the disease by analyzing the clinical features of MDS patients and investigating the relationship between gene mutations and hematological and immunological features.

Information and Methodology

Research Object

This study included a total of 139 patients with a confirmed diagnosis of MDS who were treated at the Second Hospital of Lanzhou University from January 1, 2019, to July 31, 2023. Patients with a combination of other neoplasms, those previously treated at an external hospital, and those with incomplete clinical features were excluded (Fig. 1). The study received approval from the Ethics Committee of the Lanzhou University Second Hospital (2023A-704), and we affirm that the study adheres to the 1964 Declaration of Helsinki and its subsequent amendments. Informed consent was obtained from all participants in the study.

Inclusion Criteria

(1) The patient received a definitive diagnosis of MDS based on the 2006 Vienna Minimum Diagnostic Criteria for MDS [6]. (2) The patient underwent a comprehensive bone marrow examination, including cytomorphology, histopathology, flow immunophenotyping, cytogenetic, and molecular genetics testing; Immunological examinations, such as lymphocyte subsets and cytokine analysis.

Exclusion Criteria

(1) Concurrent autoimmune diseases, a history of organ transplantation, and other immunodeficiencies; (2) Presence of symptoms related to infections, such as bacterial, fungal, viral, and atypical pathogens in the week preceding the consultation; (3) Previous coexistence with other hematological disorders (e.g., acute myeloid leukemia, hemolytic anemia, aplastic anemia, immune thrombocytopenia, multiple myeloma, lymphoma, phagocytosis syndromes), as well as a history of systemic tumor radiotherapy, chemotherapy, or other related treatments; (4) Concomitant severe dysfunction of organs such as the heart, brain, liver, kidneys, and others; (5) Pregnancy or lactation in women.

Table 1. Experimental Instruments.

Main instruments	Manufacturer
Ultra-clean bench (BSC-1500IIA2-X)	BIOBASE, Jinan, China
Cryogenic refrigerator (4 °C, 20 °C) (DW-86L728J)	Qingdao Haier Electric Co., Qingdao, China
FACS Canto flow cytometer	BD Bioscience, San Jose, CA, USA
Flow Sampling Tube	BD Bioscience, San Jose, CA, USA
Various types of micropipettes (L-1000XLS+, etc)	RAININ Company, Oakland, CA, USA
Pipette tips (TP-200, etc)	Wuhan Servicebio Technology Co., Ltd. Wuhan, China
Multi-tube automatic balancing centrifuge (5810R)	Eppendorf, Hamburg, Germany
Rapid mixer (MIX-2500)	Hangzhou Yooning Instrument Co., Ltd. Hangzhou, China
Disposable sterile pipette (BS-XG-03)	Biosharp, Beijing, China
Automatic electrochemical analyser (cobas e801)	Roche, Basel, Switzerland

Table 2. Experimental reagents.

Reagent name	Manufacturer	Item number
APC-labelled Mouse Anti-Human CD4 Antibody	BD Bioscience, San Jose, CA, USA	662965
PE-labelled Mouse Anti-Human CD8 Antibody	BD Bioscience, San Jose, CA, USA	662965
FITC-labelled Mouse Anti-Human CD3 Antibody	BD Bioscience, San Jose, CA, USA	662965
PerCP-labelled Mouse Anti-Human CD45 Antibody	BD Bioscience, San Jose, CA, USA	662965
PE-labelled Mouse Anti-Human CD16+CD56 Antibody	BD Bioscience, San Jose, CA, USA	662965
APC-labelled Mouse Anti-Human CD19 Antibody	BD Bioscience, San Jose, CA, USA	662965
Erythrocyte Lysate	BD Bioscience, San Jose, CA, USA	662965
Phosphate Buffered Saline (PBS)	MultiSciences Biotech Co., Ltd, Hangzhou, China	70-PS0021
Human 12 Cytokines Detection Kit (Flow Fluorescence Method)	CELLGENE BIOTECH CO.,LTD, Hangzhou, China	P010100403

Methodology

Collection of General Information and Hematological Characteristics

This encompasses gender, age, white blood cell count (WBC), neutrophil ratio (NE%), lymphocyte ratio (LY%), monocyte ratio (MO%), red blood cell count (RBC), hemoglobin (HGB), and platelet count (PLT).

Collection of Immunological Characteristics

Plasma cytokine levels, encompassing Interleukin (IL)-1β, IL-2, IL-4, IL-5, IL-6, IL-8, IL-10, IL-12p70, TNF-α, Interferon (IFN)-γ, IFN-α, and IL-17A, were assessed using flow cytometry and the flow microsphere cytometric bead array (CBA). Lymphocyte subset levels, including total T lymphocyte subsets, $CD4^+$ T lymphocytes, $CD8^+$ T lymphocytes, $CD4^+/CD8^+$, total B lymphocytes, and NK cells, were also examined. The experimental instruments are detailed in Table 1 and the experimental reagents are detailed in Table 2.

Flow Microsphere Assay to Detect Peripheral Blood Levels of IL-1β, IL-2, IL-4, IL-5, IL-6, IL-8, IL-10, IL-12p70, TNF-α, IFN-γ, IFN-α, IL-17A.

(1) Specimen Collection: A total of 2 mL of early morning, fasting venous blood samples were collected from the MDS patients included in the study using serum tubes. The upper layer of serum was subsequently extracted through centrifugation at 4000 r for 10 minutes.

(2) Calibrator Dilution Procedure: Transfer the quantitative calibrator to the upper sample tube, designating this tube as the highest concentration. Take 2 mL of sample dilution and add it to the centrifuge tube containing the calibrator, allowing it to stand for 15 minutes at room temperature. Utilize a pipette to blow and mix the calibrator thoroughly. Prepare 11 sample tubes and label them in sequential dilution order as 1:2, 1:4, 1:8, 1:16, 1:32, 1:64, 1:128, 1:256, 1:512, 1:1024, 1:2048. Add 300 µL of the sample dilution to each tube. Next, transfer 300 µL of liquid from the highest concentration calibrator tube to the 1:2 tube, and pipette the calibrator. After blowing and mixing, aspirate 300 µL of liquid from the 1:2 tube into the 1:4 tube, blow and mix, and repeat these steps until reaching the 1:2048 tube.

(3) Preparation of Capture Microsphere Suspension: Calculate the number of microsphere suspensions to be captured using the formula: n = 25 µL × (number of test samples + 10 standards + 1 negative control). Centrifuge the capture microsphere mixture at a low speed of 200 g for 5 minutes, discard the supernatant, and replenish the volume of the microsphere buffer with the volume of the discarded supernatant. Vortex the mixture thoroughly to ensure complete mixing, and incubate for 15 minutes at room temperature, shielded from light. After mixing, take 25 µL of the microsphere suspension, ensuring it is well-mixed, and transfer it into all sample tubes, including calibration and sample tubes.

(4) Transfer 25 µL of the calibrator diluted according to the concentration gradient into the calibrator tube. Similarly, take 25 µL of the test sample into the sample tube. Vortex and mix both tubes thoroughly.

(5) Add 25 µL of fluorescence detection reagent to all experimental tubes. Gently shake the tubes and incubate them for 2.5 hours at room temperature, keeping them away from light.

(6) Add 1 mL of Phosphate Buffered Saline (PBS) to each tube, then centrifuge at 200 g for 5 minutes. Discard the supernatant after centrifugation.

(7) Add 100 µL of PBS into each tube, re-suspend the cells, mix thoroughly, and proceed with the machine testing.

Flow Cytometry for Total T Lymphocyte Subsets, $CD4^+$ T Lymphocytes, $CD8^+$ T Lymphocytes, $CD4^+/CD8^+$, Total B Lymphocytes, NK-Cells ratio.

(1) Label two 12 × 75 mm flow tubes as A and B;

(2) Pipette 20 µL of BD Multitest CD3/CD8/CD45/CD4 Reagent into the bottom of tube A;

(3) Pipette 20 µL of BD Multitest CD3/CD16+CD56/CD45/CD19 Reagent into the bottom of tube B;

(4) Pipette 50 µL of well-mixed whole blood specimen (EDTA anticoagulated) into the bottom of tubes A and B;

(5) Cover the tubes with caps, shake gently, and incubate at room temperature for 15 minutes, away from light;

(6) Add 450 µL of BD Multitest Hemolysin diluted to 1× (1:9 deionized water/BD Multitest 10×concentrate) to tubes A and B;

(7) Cover the tubes with caps, mix gently by shaking, and then incubate at room temperature for 15 minutes, protected from light. Finally, run the assay.

MDS Gene Mutation Collection and Grouping

Gene mutation testing was conducted by either Hyster Laboratories or Goldwyn Medical, with the study encompassing 24 genes commonly associated with MDS. The detection of gene mutations utilized next-generation sequencing technology, and patients' gene mutations were identified through panel sequencing.

For each patient, 2 mL of bone marrow fluid was collected, and single nucleated cells were isolated. Genomic DNA extraction followed, and all genes targeted for detection were simultaneously captured using probe capture library construction technology. The constructed library underwent testing on the Illumina sequencing machine (NovaSeq6000/HiSeq2500, San Diego, CA, USA, etc.), with an average sequencing depth of 2000×. Recorded gene mutations for all patients were of the point mutation type, excluding nonsense mutations. Mutations were further categorized into mutant and wild-type groups based on the presence or absence of the grouped genes.

Table 3. Patient characteristics.

	MDS patients containing lymphocyte subsets	MDS patients containing Plasma cytokine
Number of cases	71	40
Gender n (%)		
Male	32 (45.1%)	19 (47.5%)
Female	39 (54.9%)	21 (52.5%)
Age (M, QR)	(54, 26)	(54, 23)
Number of mutations n (%)		
0	28 (39.4%)	16 (40.0%)
1	25 (35.2%)	12 (30.0%)
2	12 (16.9%)	8 (20.0%)
3	3 (4.2%)	1 (2.5%)
>3	3 (4.2%)	3 (7.5%)

MDS, myelodysplastic syndromes; M, QR, median and quartile range.

Statistical Processing

Statistical analysis was performed using SPSS 26.0 software (IBM Corp., Armonk, NY, USA). For measurement data, the Wilcoxon rank-sum test was applied, and the results were presented as the median and quartile range (M, QR). Count data were analyzed using the chi-square test, with data presented as frequencies and percentages (n, %). A significance level of $p < 0.05$ was considered indicative of a statistically significant difference.

Results

Basic Characteristics of Patients

This study included a total of 71 patients diagnosed with MDS during their initial assessment at our hospital. Among them, results for lymphocyte subset levels were available for all 71 patients, while plasma cytokine levels were obtained for 40 patients. Table 3 presents the basic characteristics of the MDS patients.

Frequency of Gene Distribution

In this study, a total of 12 types of gene mutations, including *ASXL1*, *SF3B1*, *TET2*, *TP53*, *U2AF1*, *RUNX1*, *SETBP1* etc., were identified in more than 2 cases among the included patients (Fig. 2). Notably, the number of mutation cases for *ASXL1*, *SF3B1*, *TET2*, *TP53*, *U2AF1*, and *RUNX1* exceeded 5. Further analysis was conducted on patients with mutations in these six genes. The analysis encompassed hematological indicators (including WBC, NE%, LY%, MO%, RBC, HGB, and PLT) and immunological indicators (including total T lymphocyte subsets, $CD4^+$ T lymphocytes, $CD8^+$ T lymphocytes, $CD4^+/CD8^+$, total B lymphocytes, NK cells, IL-1β, IL-2, IL-4, IL-5, IL-6, IL-8, IL-10, IL-12p70, TNF-α, IFN-γ, IFN-α, and IL-17A). Only positive results are presented below.

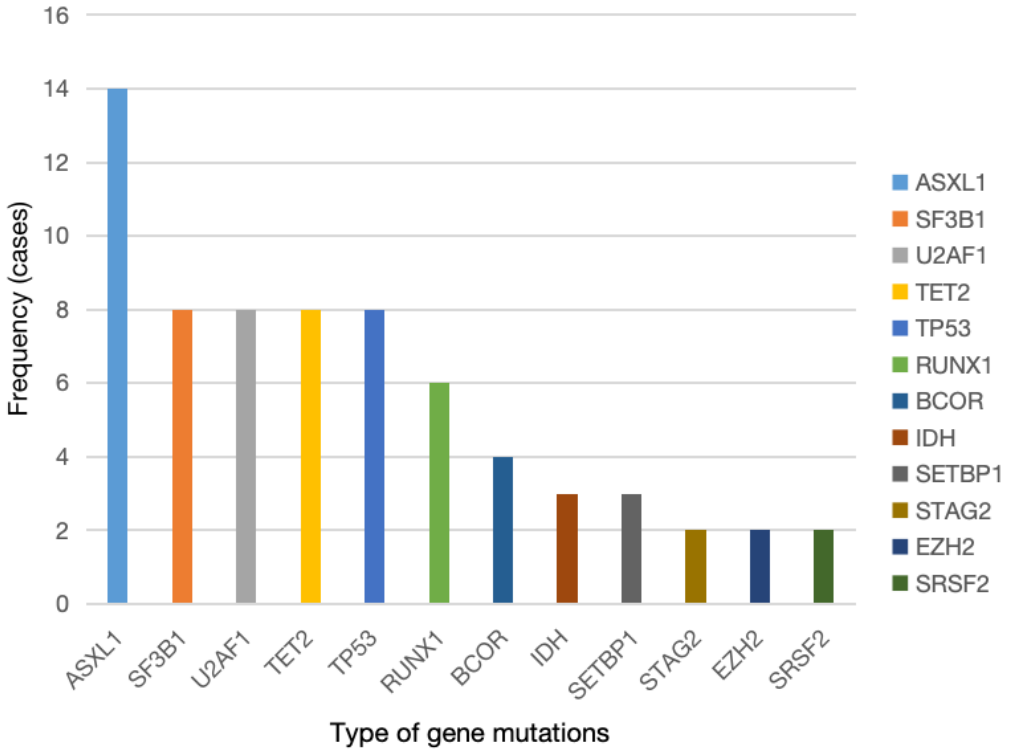

Fig. 2. Type and frequency of gene mutations. *ASXL1*, additional sex combs like 1; *SF3B1*, splicing factor 3B subunit 1; *TET2*, ten-eleven translocation 2; *TP53*, tumor suppressor p53; *U2AF1*, U2 small nuclear RNA auxiliary factor 1; *RUNX1*, Runt-related transcription factor 1; *BCOR*, Boot Camp of the Rockies; *STAG2*, Stromal antigen 2; *IDH*, isocitrate dehydrogenase; *SETBP1*, SET binding protein 1; *EZH2*, enhancer of zeste homolog 2; *SRSF2*, Serine/arginine-rich splicing factor 2. The figure was produced by Word for Mac (16.18 (181014), Microsoft, Redmond, WA, USA).

Results of Hematological Profiling

In the *ASXL1* mutation group (n = 14), MO% was significantly higher compared to the wild-type group (n = 57) (p = 0.046). In the *SF3B1* mutation group (n = 8), PLT was significantly higher than in the wild-type group (n = 63) (p = 0.009), and WBC was also significantly elevated (p = 0.033). LY% was significantly lower in the *TET2* mutation group (n = 8) compared to the wild-type group (n = 63) (p = 0.022). Additionally, WBC (p = 0.005), NE% (p = 0.002), and LY% (p = 0.001) were significantly higher in the *RUNX1* mutation group (n = 6) compared to the wild-type group (n = 65). The results of hematological profiling in other mutation groups did not show statistical significance ($p > 0.05$) (Table 4).

Lymphocyte Subsets and Cytokine Analysis Results

In the *SF3B1* mutant group (n = 8), the proportion of NK cells was significantly higher compared to the wild-type group (n = 63) (p = 0.005). In the *TET2* mutant group (n = 6), IL-8 levels were significantly higher than in the wild-type group (n = 34) (p = 0.017). For the *U2AF1* mutant group (n = 6), IL-1β levels were significantly lower compared to the wild-type group (n = 34) (p = 0.033), and both IL-10 (p = 0.033) and TNF-α levels (p = 0.009) were significantly lower. The results of immunological characterization in other mutant groups did not show statistical significance ($p > 0.05$) (Table 5). It is important to note that due to the limited sample size of 40 patients in the Plasma cytokine levels group, the *TET2* and *U2AF1* groups included only 40 patients in the analysis.

Discussion

With the advancement of molecular biology techniques, our understanding of MDS has significantly deepened. However, the factors influencing the development of MDS are not fully elucidated, and research indicates that 85%–90% of patients harbor at least one somatic cell mutation [7]. Genetic mutations play a pivotal role in the diagnosis and treatment of MDS, and the treatment strategies and prognosis vary depending on the specific mutations. Common gene mutations in MDS can be categorized into transcription factors (e.g., *RUNX1*, ETS variant 6 (*ETV6*), GATA binding protein 2 (*GATA2*)), RNA splicing (e.g., *SF3B1*, *SRSF2*, *U2AF1*, zinc finger CCCH-type, RNA binding motif and serine/arginine-rich 2 (*ZRSR2*)), DNA methylation genes (e.g., DNA (cytosine-5-)- methyltransferase 3 alpha (DNMT3A), isocitrate dehydrogenase (*IDH*) 1/2, *TET2*), histone modification genes (e.g., *ASXL1*,

Table 4. Results of haematological characterization.

Genetics	Mutant group (M, QR)	Wild-type group (M, QR)	Z/χ^2	p
ASXL1 (n)	14	57		
Gender (M/F)	8/6	24/33	1.027	0.311
Age (M, QR)	(68, 29)	(53, 28)	–2.342	0.019*
MO%	(0.09, 0.09)	(0.06, 0.05)	–0.058	0.046*
SF3B1 (n)	8	63		
Gender (M/F)	6/2	26/37	3.262	0.071
Age (M, QR)	(56.5, 20)	(54, 27)	–0.628	0.530
WBC (10^9/L)	(4.20, 4.95)	(2.37, 1.91)	–2.128	0.033*
PLT (10^9/L)	(181.50, 202.75)	(44.00, 115.00)	–2.601	0.009**
TET2 (n)	8	63		
Gender (M/F)	2/6	30/33	1.467	0.226
Age (M, QR)	(57, 27)	(54, 26)	–0.337	0.736
LY%	(0.25, 0.31)	(0.45, 0.22)	–2.283	0.022*
RUNX1 (n)	6	65		
Gender (M/F)	2/4	30/35	0.365	0.546
Age (M, QR)	(67, 24)	(54, 25)	–1.148	0.258
WBC (10^9/L)	(6.51, 3.97)	(2.37, 1.67)	–2.688	0.005**
NE%	(0.69, 0.16)	(0.43, 0.18)	–2.875	0.002**
LY%	(0.17, 0.20)	(0.45, 0.21)	–3.113	0.001**

Note: * indicates $p < 0.05$ and ** indicates $p < 0.01$. MO%, monocyte ratio; WBC, white blood cell count; PLT, platelet count; NE%, neutrophil ratio; LY%, lymphocyte ratio.

Table 5. Results of immunological characterization.

Genetics	Mutant group (M, QR)	Wild-type group (M, QR)	Z/χ^2	p
SF3B1 (n)	8	63		
Gender (M/F)	6/2	26/37	3.262	0.071
Age (M, QR)	(56.5, 20)	(54, 27)	–0.628	0.530
NK cell ratio	(16.40, 8.10)	(9.40, 9.46)	–2.783	0.005**
TET2 (n)	6	34		
Gender (M/F)	1/5	18/16	2.691	0.101
Age (M, QR)	(57, 30)	(54, 23)	–0.144	0.926
IL-8 (pg/mL)	(128.55, 1667.55)	(18.63, 32.16)	–2.348	0.017*
U2AF1 (n)	6	34		
Gender (M/F)	4/2	15/19	1.040	0.308
Age (M, QR)	(57.5, 45)	(54, 17)	–0.569	0.592
IL-1β (pg/mL)	(0.00, 0.45)	(0.77, 2.59)	–2.158	0.033*
IL-10 (pg/mL)	(1.16, 1.48)	(2.44, 2.07)	–2.102	0.033*
TNF-α (pg/mL)	(0.14, 0.53)	(1.15, 2.64)	–2.526	0.009**

Note: * indicates $p < 0.05$ and ** indicates $p < 0.01$. NK, Natural Killer; IL, Interleukin; TNF-α, Tumour Necrosis Factor-α.

Boot Camp of the Rockies (*BCOR*), *EZH2*), DNA repair (e.g., *TP53*), and others [2]. This study demonstrates that investigating the hematological and immunological characteristics associated with common gene mutations and analyzing the impact of genetic alterations on the tumor microenvironment can enhance the selection of therapeutic options and provide insights for assessing prognosis.

It has been observed that genetic mutations in MDS patients can influence leukocyte expression. For instance, the *ASXL1* mutation has been identified to inhibit the transcriptional process of granulosa progenitor cells, resulting in reduced neutrophil production [8]. In our study, we found a significantly higher MO% in the *ASXL1* mutation group. Consistent with the findings of Chee L *et al*. [3], increased monocyte levels were associated with high-risk MDS and poor treatment outcomes, suggesting a potentially poor prognosis in patients with *ASXL1* mutations. The authors hypothesize that *ASXL1* gene mutations may contribute to elevated monocyte levels in the immune microenvironment of MDS, ultimately influencing disease development. However, as of now, there is no existing literature reporting on this aspect, so further research is required.

In our study, the RUNX1 mutation group exhibited elevated WBC and NE%, aligning with similar findings

in previous research [9]. However, it's noteworthy that RUNX1 gene deletion typically leads to impaired myeloid differentiation and blockade of granulocyte differentiation [10–12]. This inconsistency with our study's results may be attributed to several factors, including the self-renewal capacity of tumor cells with limited differentiation ability, the existence of potential undiscovered pathways and genetic alterations, and the diverse roles played by RUNX1 in the survival and maintenance of hematopoietic stem cells at different maturity levels [13].

Erythropoiesis stands out as a significant and long-standing clinical manifestation of MDS [14]. Different mutations in MDS mediate different mechanisms that result in anemia. In particular, ineffective erythropoiesis is commonly observed in patients with refractory anemia with ring sideroblasts a majority of whom carry mutations in the *SF3B1* gene [15]. For the remaining patients, anemia is attributed to hypoproliferative red lineage. Previous studies have indicated that *SF3B1* mutations primarily impact red lineage maturation [16]. The results of the present study did not reveal a significant difference in HGB in the *SF3B1* mutant group compared with the wild-type group. This lack of significance may be attributed to an insufficient sample size for inclusion in the analysis.

Moreover, genetic mutations in MDS patients also impact the number and function of platelets (PLT). For instance, patients with initial DNMT3A mutations were reported to have higher PLT counts compared to those with DNMT3A wild-type [17]. On the other hand, mutations in the *ASXL1* and RUNX1 genes may be associated with reduced PLT, and thrombocytopenia has been identified as a poor prognostic factor for MDS [18]. The data from our study revealed that, except for the *SF3B1* mutation group, all other mutation groups exhibited reduced PLT. Several reasons may account for this observation: (1) thrombocytopenia caused by gene mutations; (2) excessive platelet destruction due to abnormal immune microenvironment post-disease onset; and (3) diminished platelet production stemming from megakaryocyte differentiation or maturation disorders.

Dysregulation of innate and adaptive immune systems, along with inflammatory signaling in the bone marrow microenvironment and hematopoietic cells, plays a pivotal role in the development and progression of MDS [19]. This immune system dysregulation within the tumor microenvironment facilitates the immune escape of tumor cells, allowing the expansion of malignant clones. Certain gene mutations have been identified to be involved in immune signaling, such as the spliceosome mutations *U2AF1* and *SF3B1*, both of which induce the activation of inflammatory immune signaling [20,21]. TP53 mutations are also implicated in immune dysregulation in MDS, promoting immune escape in tumor cells [22].

MDS patients with mutations in the TP53 gene exhibited elevated expression of immune checkpoint molecules, including PD-L1 on HSC and TIM3 on myeloid-derived suppressor cells [23,24]. The TP53 mutant MDS microenvironment adopts an immunosuppressive phenotype, potentially serving as a major driver of poor prognosis.

Abnormalities in immune cell numbers and function are associated with the progression of MDS [19]. The data from our study revealed a higher proportion of NK cells in the *SF3B1* mutant group compared to the wild-type group, possibly contributing to the better prognosis observed in *SF3B1* mutant patients. No significant abnormalities in the number of immune cells were identified in the other mutant groups.

Previous research has reported the presence of NK cell maturation disorders in MDS patients, leading to dysfunction [4,7,25,26]. This underscores the involvement of NK cells in the immune surveillance of MDS cells and their impact on the body's anti-tumor immune response.

Abnormal cytokine expression plays a complex and crucial role in the immune dysregulation observed in MDS. Overexpression of TNF-α, IFN-γ, IL-6, and IL-8 has garnered significant interest, and their mechanism of action may impact HSPC proliferation and differentiation by influencing the activation of the nuclear factor kappa-B (NF-κB) pathway [27].

In our study, a significant elevation of IL-8, a pro-inflammatory cytokine, was observed in the *TET2* mutant group. The pathogenesis of MDS driven by mutations leading to high IL-8 expression is closely associated with inflammation, and previous research has demonstrated that splicing factor mutations in MDS can induce inflammation [28].

Conversely, a noteworthy reduction in the expression of IL-1β, IL-10, and TNF-α was observed in the *U2AF1* mutant group compared to the wild-type group. It was found that *U2AF1* mutation induced Interleukin-1 receptor-associated kinase 4 (IRAK4) isoforms, activating the innate immune pathway in myeloid malignancies [21]. Similarly, *SF3B1* mutations also lead to the expression of IRAK4 isoforms in MDS and inflammatory cytokine production [20]. This suggests a potential link between genetic alterations and chronic innate immune signaling in MDS, aligning with the results of this study. A more comprehensive investigation of the immune microenvironmental differences associated with various genetic mutations and the positive or negative role of inflammation in disease development can contribute to further refining the treatment of MDS.

Conclusion

In conclusion, the analysis of variations in hematological and immunological characteristics among different gene mutation groups has revealed distinct differences in the immune microenvironment of MDS associated with various genetic mutations. Gene mutations exhibit a close relationship with the immune microenvironment, and scru-

tinizing the alterations in the immune microenvironment prompted by different gene mutations can aid in identifying novel therapeutic targets and refining treatment regimens, ultimately enhancing the efficacy and prognosis for patients. Given the small sample size in the present study, further validation in a larger sample is imperative to analyze changes in the immune microenvironment in the bone marrow and ascertain potential underlying mechanisms.

Availability of Data and Materials

Data supporting the results of this study are available from the corresponding author [Liansheng Zhang] upon reasonable request.

Author Contributions

JY designed the study, collected the data, analyzed the data, and wrote the manuscript; XP designed the study and wrote the manuscript; RW acquired the data and wrote the manuscript; JB was responsible for the methodological design of the study and wrote the manuscript; YL was responsible for the design and completion of the experimental portion of the study and the writing of the associated content; LZ conceived of the study and revised the article; LL conceived of the study and revised the article. All of the above authors approved the final published version of the article and agreed to take responsibility for all aspects of the work and to ensure that issues related to the accuracy or completeness of any part of the work were properly investigated and resolved.

Ethics Approval and Consent to Participate

The study was approved by the Ethics Committee of the Lanzhou University Second Hospital (2023A-704), and we affirm that the study adheres to the 1964 Declaration of Helsinki and its subsequent amendments. Informed consent was obtained from all participants in the study.

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Conflict of Interest

The authors declare no conflict of interest.

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