Increased levels of circulating granulocytic myeloid-derived suppressor cells in lumbar disc herniation

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Received November 11, 2022; Accepted March 10, 2023

DOI: 10.3892/etm.2023.12066

Abstract. Myeloid-derived suppressor cells (MDSCs) expand when the body undergoes inflammatory diseases and chronic diseases. However, its role in intervertebral disc degeneration remains unclear. The present study aimed to characterize specific subsets of MDSCs as potential indicators of disease progression in patients with lumbar disc herniation (LDH). The Gene Expression Omnibus (GEO) database was used to analyze the changes in granulocyte MDSCs (G-MDSCs). Peripheral blood samples were collected from 40 patients with LDH and 15 healthy controls, and flow cytometry was used to characterize different subsets of MDSCs. All subjects underwent lumbar spine magnetic resonance imaging. Then, t-distributed stochastic neighborhood embedding and FlowSOM were used to analyze the data obtained by CytoFlex. The correlation between circulating MDSCs and the clinicopathological stage of LDH was then further analyzed. The GEO database predicted that G-MDSCs were highly expressed in patients with LDH. The frequency of circulating G-MDSCs increased with Pfirrmann stage III and IV, while the percentage of mononuclear MDSCs (M-MDSCs) only increased. Patient age and sex did not correlate with the frequency of circulating G-MDSCs and M-MDSCs. The results of the computer algorithm analysis were consistent with those of our manual gating. The present study showed that the occurrence of LDH led to changes in the MDSC subpopulation in the circulating peripheral blood of patients, and the frequency of circulating G-MDSCs in patients with clinical stage III and IV LDH increased with the degree of degeneration. The determination of G-MDSCs can be used as an auxiliary examination item for LDH.

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Key words: myeloid-derived suppressor cells, lumbar disc herniation, autoimmunity, inflammatory

Introduction

Lower back pain (LBP) is a common clinical problem that affects 70~85% of the population worldwide. Chronic pain seriously affects the quality of life and mental state of patients, which in turn incurs a substantial social burden (1). LBP is commonly caused by lumbar disc herniation (LDH) (2). Anatomically, the intervertebral disc (IVD) is a complex tissue composed of three parts: the central nucleus pulposus (NP), the peripheral annulus fibrosus (AF), and two cartilage endplates (CEPs) (3,4). During IVD formation, NP tissue is captured by the surrounding AF and CEPs to isolate the NP from the immune system. Traditionally, the intervertebral disc is identified as an immune-privileged organ (5). Degeneration or trauma of the intervertebral disc can lead to the exposure of antigen components to circulating blood, resulting in an abnormal autoimmune response and chronic inflammation, accompanied by low back pain and sciatica (6,7). Therefore, intervertebral disc degeneration (IVDD) is an autoimmune disease and is accompanied by the inflammatory response that constantly exacerbates the process of degeneration (8). Furthermore, when the body is under pathological conditions, such as autoimmune diseases, tumors, inflammation or infections, these factors not only can block the normal differentiation of immature myeloid cells (IMCs), but also induce them to become a bone marrow-derived inhibitor myeloid-derived suppressor cells (MDSCs), which are massively recruited and activated in the peripheral blood, bone marrow or tumor tissue (9). Therefore, this study seeks to explore the specific changes in MDSCs in LDH.

MDSCs are immunoregulatory cells, and they prevent uncontrolled inflammation and maintain homeostasis of immune response in the early tissue damage (10). However, in chronic inflammation and extensive tissue damage caused

by severe trauma or chronic virus infection, the normal differentiation of MDSCs is interrupted by pathological processes, resulting in their prolonged expansion and enhanced immunosuppressive function (11). Under these conditions, MDSCs become chronic inflammatory cells that impair protective immune responses, which lead to an inability to clear infection, increased tissue damage from autoimmune responses, and a failure to restore tissue homeostasis and regulate tissue repair (12). Therefore, MDSCs play a dual role in autoimmunity. On the one hand, these differences can be explained by the different autoimmune models and phenotypes used in various studies and strategies for identifying MDSCs (13). On the other hand, these contradictory findings may also indicate that the dual contribution to protecting and worsening the disease depends on the severity and stage of the disease.

MDSCs are characterized by a lack of lineage markers (B cells, T cells, and natural killer cells), low or negative expression of HLA-DR, and expression of monocytes and the myeloid marker CD33 (14). According to the different surface markers, MDSCs can be divided into granulocyte MDSCs, or polymorphonuclear MDSCs (G-MDSCs: HLA-DR$^-$ Lin$^-$ CD33$^+$ CD15$^+$ CD14$^-$), and mononuclear MDSCs (M-MDSCs: HLA-DR$^{-/low}$ CD14$^+$). In addition, there is also a group of phenotypically immature MDSCs called known as early MDSCs (eMDSCs: HLA-DR$^-$ Lin$^-$ CD33$^+$ CD14$^-$ CD15$^-$) in human peripheral blood mononuclear cells. The frequency of MDSCs in patients with LDH, the MDSC subpopulations, and their clinical relevance remain unclear to date.

At present, the gold standard for the diagnosis of LDH is magnetic resonance imaging (MRI) followed by surgery, and conservative treatment, such as bed rest, functional exercise, and physical therapy, is the primary therapeutic option (15). Both these treatments are aimed at individual symptom relief and supportive treatment, not radical recovery of LDH. The main problems with using MRI for diagnosis are that the procedure is time-consuming, results in potential radiation exposure, lacks predictive capability and is not cost-effective.

This study aims to find an immune marker that is a potential target for the treatment of LDH. We have undertaken a comprehensive flow cytometry-based study to investigate the phenotype of circulating MDSCs in patients with LDH. We hope to identify potential subtypes that could serve as an auxiliary examination item for LDH and therapeutic targets to prevent the progression of IVDD.

Materials and methods

Study population. This study was approved by the Institutional Review Board (Ethical batch number: PJ 2022-02-16). From June 2021 to June 2022, 40 patients with LDH (Pfirrmann classification) with complete clinical data were recruited at the First Affiliated Hospital of Anhui Medical University, China (Table I). Fifteen healthy donors (HD) were used as controls, and they also underwent MRI to rule out LDH. Written informed consent was obtained before blood sampling.

LDH was confirmed in these patients using MRI. All subjects had no history of underlying diseases such as infections, tumors, and chronic inflammation known to affect the level of MDSCs. It is worth mentioning that all the samples were collected at patient's first visit. Because of the persistence of the intervertebral disc injury, we could not study the effect of the length of LDH history on MDSCs. Our inclusion criteria were as follows:(1) patients who were hospitalized and diagnosed with lumbar disc herniation in the First Affiliated Hospital of Anhui Medical University; (2) peripheral blood samples for all patients obtained at their initial visit; (3) patients with low back pain or lower limb pain and numbness as the main clinical symptoms; (4) magnetic resonance imaging showing degenerative changes in lumbar intervertebral discs; (5) no conditions affecting the structure of the spine; and (6) no previous lumbar spine surgery or other treatments that would deform the lumbar spine.

Furthermore, our study used the following strict exclusion criteria: (1) patients with severe scoliosis; (2) patients with a recent history of lumbar spine trauma or fracture; (3) patients with nondegenerative or chronic infectious diseases of the lumbar spine; (4) patients with diseases of the autoimmune system or metabolic diseases; (5) patients who had previously or were currently on immunoadaxis; (6) patients who had used glucocorticoids in the last 3 months; and (7) patients who had used NSAIDs within the last 2 weeks.

GEO database analysis. The Gene Expression Omnibus (GEO), a publicly available NCBI genomics database of high-throughput gene expression data, was thoroughly queried for all datasets involving studies of MDSCs. The eligibility criteria for analysis were as follows: (1) studies with IVDD tissue samples; (2) studies with information on the technology and platform utilized; and (3) studies that included normal groups as controls. Based on these criteria, two datasets for G-MDSCs were downloaded from the repository; no dataset for M-MDSCs was found. Principal component analysis (PCA) was performed on the datasets for dimensionality reduction and quality control. If the quality of a particular sample was insufficient, it was excluded from subsequent analysis.

MRI classification. MRI has proven to be an extremely valuable tool in the assessment of normal and pathological spinal anatomy. It is commonly used to assess the containment of discal material by the outer fibers of the AF and posterior longitudinal ligaments. The German Siemens 3.0T MRI scanner was used to perform lumbar intervertebral disc MRI examinations on the subjects. Patients were placed in the conventional supine position, and the scan sequences included fat-suppressed T1, T2 and DW I transverse coronal and sagittal three-dimensional scan sequences. All scanned images were processed and transferred to the PACS image reading system, and two deputy chief physicians of the Department of Orthopedics used the Pfirrmann grading system (10,16) to classify the lumbar intervertebral disc degeneration based on the lumbar MRI images. The degree of LDH was evaluated according to the morphologic structure of the responsible disc and assigned one of five stages (I-V) (criteria presented in Table II). The control group was identified as stage I.

Whole blood staining and MDSC immunophenotyping. Peripheral blood (2-3 ml) was collected from patients and processed within 6 h. For lysing of the red blood cells (RBCs), 10x RBC lysis buffer was diluted to 1x working concentration with deionized water. The 1x solution was then warmed

Table I. Breakdown of demographics between patients with stages I-IV LDH.

Pfirrmann stage	n	Age (years)	Sex (male/female)
Stage I	15	43.53±19.26	7/8
Stage II	14	44.43±16.73	6/8
Stage III	15	46.60±16.13	8/7
Stage IV	11	46.73±16.26	5/6
F/χ^2	-	0.832	0.347
P-value	-	0.483	0.951

to 25˚C before use. Then, 50.0 ml 1x RBC lysis buffer was added to each 50 ml centrifuge tube containing up to 2.5 ml whole blood. Each tube was gently vortexed immediately after adding the lysate, before incubation at 25˚C in the dark for 10-15 min. Thereafter, the tubes were centrifuged at 350 x g for 5 min, and the supernatant was aspirated without disturbing the precipitate. The pellet was resuspended in an appropriate amount of PBS buffer and washed once. For antibody labelling, the live cells were counted and resuspended in PBS buffer at a concentration of 5-10x10^6 cells/ml, and then 100 μl cell suspension was dispensed into respective 2 ml Eppendorf tubes.

To determine the frequency and phenotype of MDSCs in fresh whole blood, five surface markers were combined, including the lineage-specific mixtures (CD3/CD19/CD56/CD14/CD20/CD16)-APC (Biolegend, Cat: 348803, 20 μl/test), HLA-DR-APC-Cy7 (Biolegend, Cat: 307617, 5 μl/test), CD33-PerCP-Cy5.5 (Biolegend, Cat: 303413, 5 μl/test), CD15-FITC (Biolegend, Cat: 323003, 5 μl/test) and CD14-PE (Biolegend, Cat: 301805, 5 μl/test). Incubation with recommended concentration of fluorescent primary antibodies were performed for 15-20 min on ice in the dark. Then, the solution was centrifuged at 350 x *g* for 5 min and washed 2x with at least 2 ml of PBS buffer. Afterwards, the cell pellet was resuspended in 0.5 ml of cell staining buffer. As the sample was fresh peripheral blood, no viability staining solution was added to exclude dead cells. Finally, flow cytometry analysis was performed on a CytoFLEX flow cytometer (Beckman Coulter, Brea, CA, USA), and FlowJo v10.7.2 (TreeStar, Ashland, OR, USA) was used to analyze the data.

Data analysis

Cytometry data auxiliary analysis of G-MDSCs. We used manual gating and automated cluster analysis methods for exploratory analysis. Data analysis of flow cytometry is traditionally performed through manual gating, which includes a visual inspection of two-dimensional scatterplots to identify known cell populations. However, this technique suffers from several major limitations, including subjectivity, operator bias, difficulty in detecting unknown cell populations, and difficulty in reproducibility. Therefore, we additionally used clustering and dimensionality reduction as an algorithmic method to reduce subjectivity and bias. The dimensionality reduction algorithm, t-distributed stochastic neighborhood embedding (t-SNE) was used to visualize the high-dimensional single cell data. Furthermore, FlowSOM was used as an automatic analysis method to perform cluster analysis on single biological samples, multiple samples based on each sample, or combined data from multiple samples. The detected clusters (cell populations) were then analyzed individually or compared between samples.

We obtained consistent results with the manual gating by performing the computer algorithm. The Barnes-Hut implementation of t-SNE by the 'tsne' package of R with 1,000 iterations, a perplexity parameter of 30, a trade-off θ of 0.5, and the 'FlowSOM' package of R with 8 meta clusters were used to perform the dimensionality reduction algorithm. Each island represents a group of similar cells, and from the results, we can see directly that the number of G-MDSCs increased with the increasing clinical LDH stage.

Statistics. Distribution analysis was performed by the D'Agostino-Pearson test on Prism v8.0.2 (GraphPad, San Diego, CA, USA). Unpaired parametric student's t-test was used to determine the statistical significance between observations and groups. Assuming that the number of MDSCs did not follow a normal distribution, comparisons between different groups were made using the Mann-Whitney U test. Spearman's test was used to assess the correlation between circulating MDSCs and lumbar disc herniation stage. The diagnostic accuracy of biomarkers was determined by receiver operating characteristic (ROC) curve analysis, using GraphPad Prism v8.0.2. Statistical analyses were performed on Prism v8.0.2 (GraphPad, San Diego, CA, USA) and SPSS version 13.0 for Windows (SPSS Inc., Chicago, IL, USA). Differences were considered statistically significant at two-tailed $P<0.05$.

Results

GSE database predicts changes in the proportion of MDSC subtypes. The gene expression sequencing results of the GSE56081 dataset were collected from the public platform GEO of the NCBI, including the NP of 5 patients with lumbar disc degeneration and 5 control NP tissues. In addition, referring to the GSE24102_GRANULOCYSTIC_MDSC_VS_NEUTROPHIL_UP differential gene set (using a series of up-regulated genes in G-MDSCs as a reference), we used the ssGSEA method to score 10 patients in the GSE56081 cohort to obtain the infiltration of G-MDSCs in the NP according to the condition of each patient. The corresponding sequencing data of M-MDSCs were not found. We demonstrated that compared with the NP of normal patients, the G-MDSCs infiltration score of patients with lumbar disc degeneration was significantly higher (0.692 vs. 0.211, P=0.01) (Fig. 1).

The gating of different MDSC subtypes in blood from patients. Using five-color flow cytometric analysis, we aimed to explore all three subsets of circulating MDSCs: HLA-DR^- Lin^- $CD33^+$ $CD14^-$ $CD15^-$ immature MDSCs (a), HLA-DR^- Lin^- $CD33^+$ $CD15^+$ $CD14^-$ G-MDSCs (b), and HLA-$DR^{-/low}$ $CD14^+$ M-MDSCs (c) in whole blood from patients with LDH and age-matched healthy controls. IVDDs were divided into stages I-IV (Fig. 2), while no stage V cases-the most severe condition-were observed in our samples. The frequency of circulating MDSCs was calculated as a percentage of single-cells. Representative flow cytometric data of a healthy donor and a patient with stage IV is shown in Fig. 3A-B.

Table II. Pfirrmann grading scale standard.

Pfirrmann scale	Structure	Margin between nucleus and annular	Signal intensity	Height of disc
I	Homogenius, white	Clear	High signal	Normal
II	Inhomogenius or gray horizontal line	Clear	High signal	Normal
III	Inhomogenius, gray	Unclear	Medial signal	Normal to slightly decreased
IV	Inhomogenius, gray to dark	Vanish	Low signal	Normal to obviously decreased
V	Inhomogenius, dark	Vanish	Low signal	Collapse

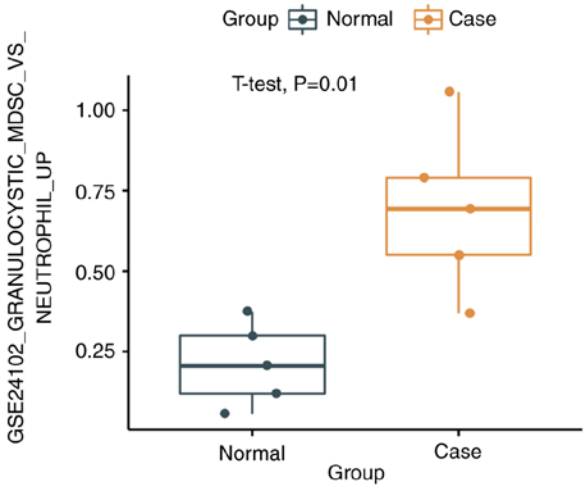

Figure 1. Gene expression prediction of lumbar disc degeneration patients in GSE24102. The G-MDSC cell infiltration score of patients with lumbar disc degeneration was significantly increased (0.692 vs. 0.211; P=0.01). G-MDSC, granulocyte myeloid-derived suppressor cells.

The frequency of G-MDSCs increased in the blood of patients with stage III and IV. A significantly higher level of circulating G-MDSCs was observed in patients with LDH that in controls (mean 0.6805 vs. 0.2180%, P=0.0004) (Fig. 4A). When patients were further divided by the Pfirrmann stage, a direct correlation between the LDH stage and percentage of circulating G-MDSCs was noted (Spearman's r=0.718, P<0.001). There was a significant difference in the level of G-MDSCs between patients with stage IV LDH and controls (P<0.0001), as well as stage III and controls (P<0.0001) but there was no significant difference between stage II and controls (P=0.1156) (Figs. 4B and 5). The receiver operating characteristic (ROC) curve (area=0.8642, P<0.0001) also suggested that G-MDSCs had clinical diagnostic potential (Fig. 4C).

No difference in the frequency of circulating M-MDSCs between patients and HD. The frequency of circulating M-MDSCs was not significantly different between patients with IVDD and controls (mean 2.147 vs. 2.286%, P=0.6755) (Fig. 4D). The level of M-MDSCs appeared to increase but was not significant between stage II and HD (P=0.5468), between stages III and IV (P=0.7347), and between stage III and controls (P=0.7326) (Fig. 4E). The ROC curve (area=0.5517, P=0.5580) of M-MDSCs did not show clinical diagnostic potential (Fig. 4F).

For immature MDSCs, the analysis showed that these HLA-DR$^-$ Lin$^-$ CD33$^+$ cells in peripheral blood samples almost all expressed CD15$^+$. Therefore, in the peripheral blood of patients with LDH, the phenotype of immature MDSCs was virtually non-existent.

Discussion

Due to the acceleration of population aging, IVDD will become a topic of interest in the future. LDH mainly manifests as LBP and nerve root pain, which is related to the rupture of AF, NP, and nerve fiber stimulation, affecting ~9% of the global population (11). Intervertebral disc degeneration is a complex pathological phenomenon caused by heredity, aging, malnutrition, loading history and autoimmunity, but the mechanism has not yet been clarified (17,18). Gertzbein *et al* (19) identified a potential role for autoimmunity in IVDD, pointing to components such as proteoglycans in the NP as a major cause of chronic inflammatory responses. Bobechko *et al*'s (20) research also supported the role of autoimmunity in IVDD by implanting autologous NP into the ears of rabbits, resulting in local lymphadenopathy, which is proved that NP has a self-antigen function. Starkweather *et al* (21) demonstrated that herniated NP tissue or disc fragments in the epidural space activated the neuroimmune system, inducing the release of a high number of inflammatory cells, including macrophages, T cells, and a few monocytes from the degenerating disc (22). Wang *et al* (23) also discovered that when the human immune system is exposed to intervertebral disc tissue, macrophage infiltration and the release of IL-6, interleukin 1β (IL-1β), and TNF are related to the subsequent autoimmune response. In addition, monocyte chemoattractant protein 1 (MCP-1) released from the intervertebral disc is able to amplify macrophage infiltration and inflammatory responses, which indicates that the immune response is involved in the pathogenesis of IVDD (24).

Although some patients can be relieved by conservative treatment, persistent and severe pain often necessitates surgery. The first case of spontaneous resorption of the lumbar IVD in myelography was demonstrated in 1945 by Key *et al* (25). Since then, increasing reports have confirmed that LDH may spontaneously disappear with time without surgery (26,27), which should be considered by patients who choose not to have surgery. The mechanism of phagocytosis is also the most supported by clinical research. Previous studies also showed that neovascularization and macrophage infiltration were required for spontaneous absorption of herniated discs (28,29).

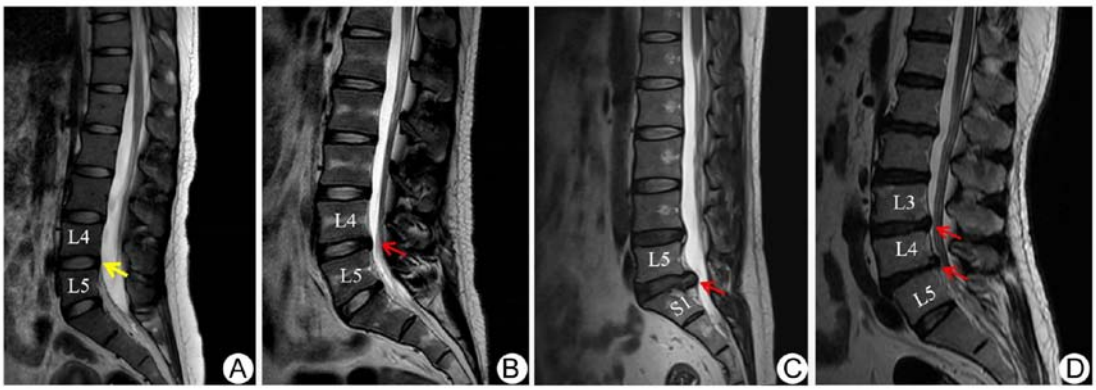

Figure 2. Magnetic resonance imaging Pfirrmann grading. (A) HD: L4-5 IVD of patient with Pfirrmann stage I. The IVD has a uniform high signal, and the NP and AF are clearly separated (yellow arrow). (B) Stage II patient: L4-5 IVD with horizontal low signal bands but a clear border between the NP and the AF (red arrow). (C) Stage III patient: L5-S1 IVD with a gray NP signal and an unclear boundary with the AF, but no significant change in height (red arrow). (D) Stage IV patient: L4-5 IVD with a gray NP signal, and a significantly reduced the height of the IVD (red arrow). IVD, intervertebral disc; NP, nucleus pulposus; AF, peripheral annulus fibrosus.

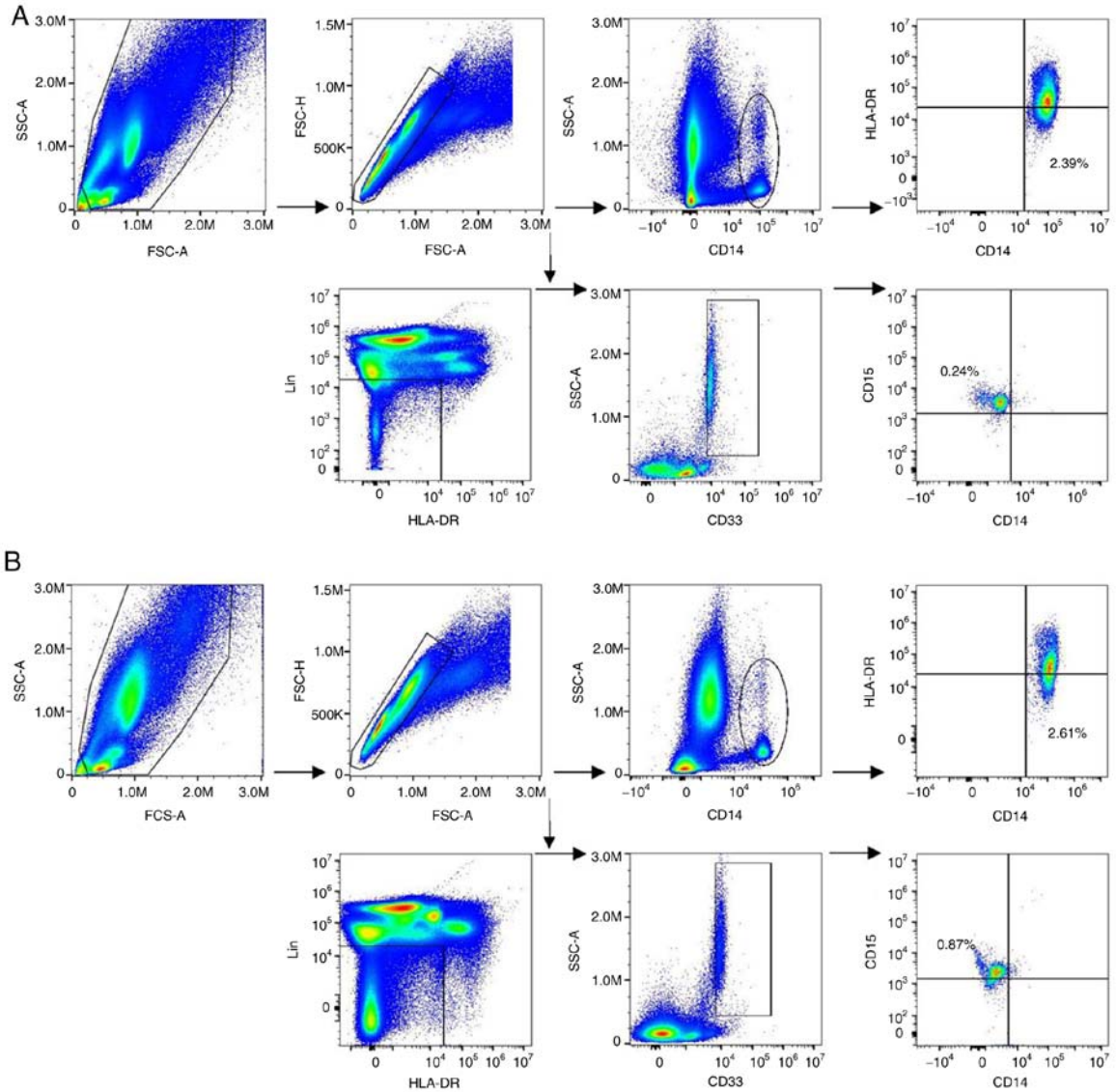

Figure 3. Identification and characterization of different MDSC subtypes in blood from patients with LDH. (A) Frequency of G-MDSCs and M-MDSCs were 0.24 and 2.39%, respectively, in a healthy donor. Gating strategy for MDSCs: For immature MDSCs and G-MDSCs, the acquired cells were first gated on the non-expression of Lin and HLA-DR. Within this population, the fraction of cells expressing both CD33 and SSC was determined. The expression of CD15 was further explored in this fraction. For M-MDSCs, SSC vs. CD14 was first gated to isolate the monocytes cell populations, and then HLA-DR vs. CD14 was plotted for M-MDSCs. (B) Frequency of G-MDSCs and M-MDSCs were 0.87 and 2.61%, respectively, in a patient with stage IV LDH. MDSC, myeloid-derived suppressor cells; LDH, lumbar disc herniation; G-MDSC, granulocyte MDSCs.

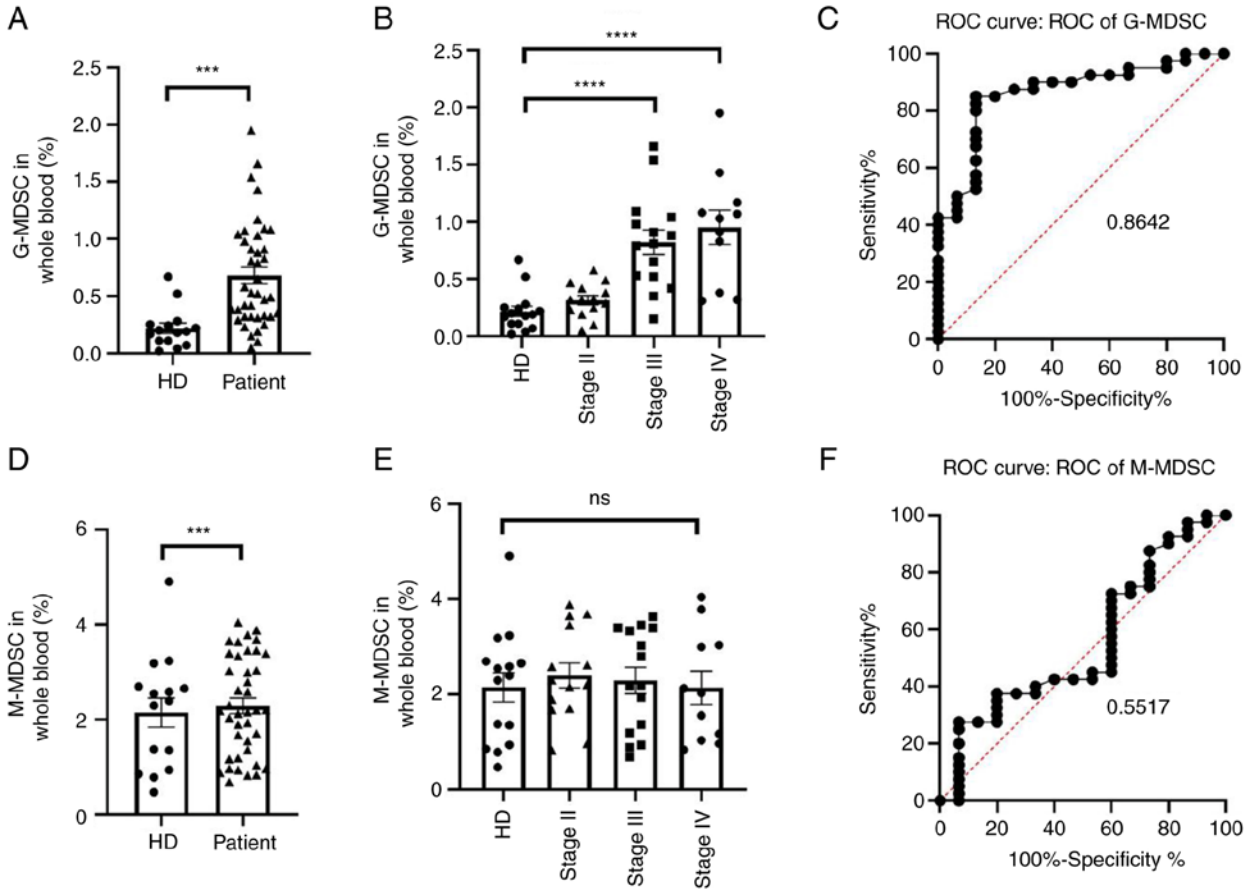

Figure 4. Level of G-MDSCs and M-MDSCs in patients with LDH. (A) Frequency of G-MDSCs is significantly different between HD and patients. (B) Frequency of G-MDSCs increased with increasing clinical LDH stage. (C) ROC curve of patients with LDH and the frequency of G-MDSCs. (D) Frequency of M-MDSCs is not significantly different between healthy donors and patients. (E) Frequency of M-MDSCs is not significantly different between HD and patients in different stages. (F) ROC curve of patients with LDH and the frequency of M-MDSCs. ***P<0.001 and ****P<0.0001. MDSC, myeloid-derived suppressor cells; LDH, lumbar disc herniation; G-MDSC, granulocyte MDSCs; M-MDSCs, mononuclear MDSCs; ROC, receiver operating curve.

Takada *et al* (30) observed that mechanical hyperalgesia in a rat intervertebral disc model was substantially correlated with macrophage infiltration and the up-regulation of TNF-α, IL-6, IL-8, and COX-2. Arai *et al* (31) proposed that the spontaneous absorption of IVDs might be a consequence of the phagocytic activity of T cells and macrophages. These studies show that macrophages and T cells seem to play an important role in this pathological process. Furthermore, the inflammatory signal from macrophages as well as T cells can mature and activate MDSCs (32). MDSCs in turn inhibit the T cell cycle and immune checkpoints, down-regulate T cell receptors, and recruit regulatory T cells. They also suppress the activity of other immune cells through the production of ROS, RNS, degradation of L-arginine, and the production of anti-inflammatory factors, such as TGF-β and IL-10 (25,33). These factors further hinder the reabsorption process.

In our study, we provided a new basis for the conservative treatment of patients with LDH. We analyzed and correlated the proportion of MDSCs in the peripheral blood of patients to determine the MDSC subgroup distribution in the different stages of LDH. We were unable to collect patients with stage V, because the stage is so severe that it's rare. We showed that the proportion of G-MDSCs increased with LDH progression, but no correlation was found with early-stage II. This may be on the one hand because stage II patients were hospitalized rarely, and we did not collect enough samples; on the other hand, this likely indicated that the proportion of G-MDSCs is not obvious during early IVD injury. It may be that AF integrity is still retained during this stage, and the effect of tearing is either non-existent or negligible. This also confirms that the proportion of MDSCs increases after trauma. For M-MDSCs, although their proportion of it increased to a certain extent, it was not statistically significant. Related researches have shown that extensive tissue damage caused by trauma or surgery can lead to the release of bone marrow-derived cells, including MDSCs (34). Interestingly, MDSCs could play a repair role in the early stage of trauma and aggravate the disease in the later stage of chronic inflammation (35). This suggests that MDSCs may play a different role in the progression of the disease. However, trauma can stimulate the expression of T-helper 2 (Th_2) lymphocytes. Th_2 cytokines can boost the expression of arginase-1 in MDSCs and lead to arginine deficiency, which further inhibits the function of lymphocytes, such as T cells, macrophages, and natural killer cells (36,37), in turn hindering the progress of NP reabsorption. In this study, the G-MDSCs obtained by our subtyping analysis were from phenotype $CD33^+$. A study by Fultang *et al* (38) showed that the CD33 humanized monoclonal antibody gemtuzumab ozogamicin has made clinical progress in human trials, which could be used to target and reduce the inhibitory effect of MDSCs on cellular

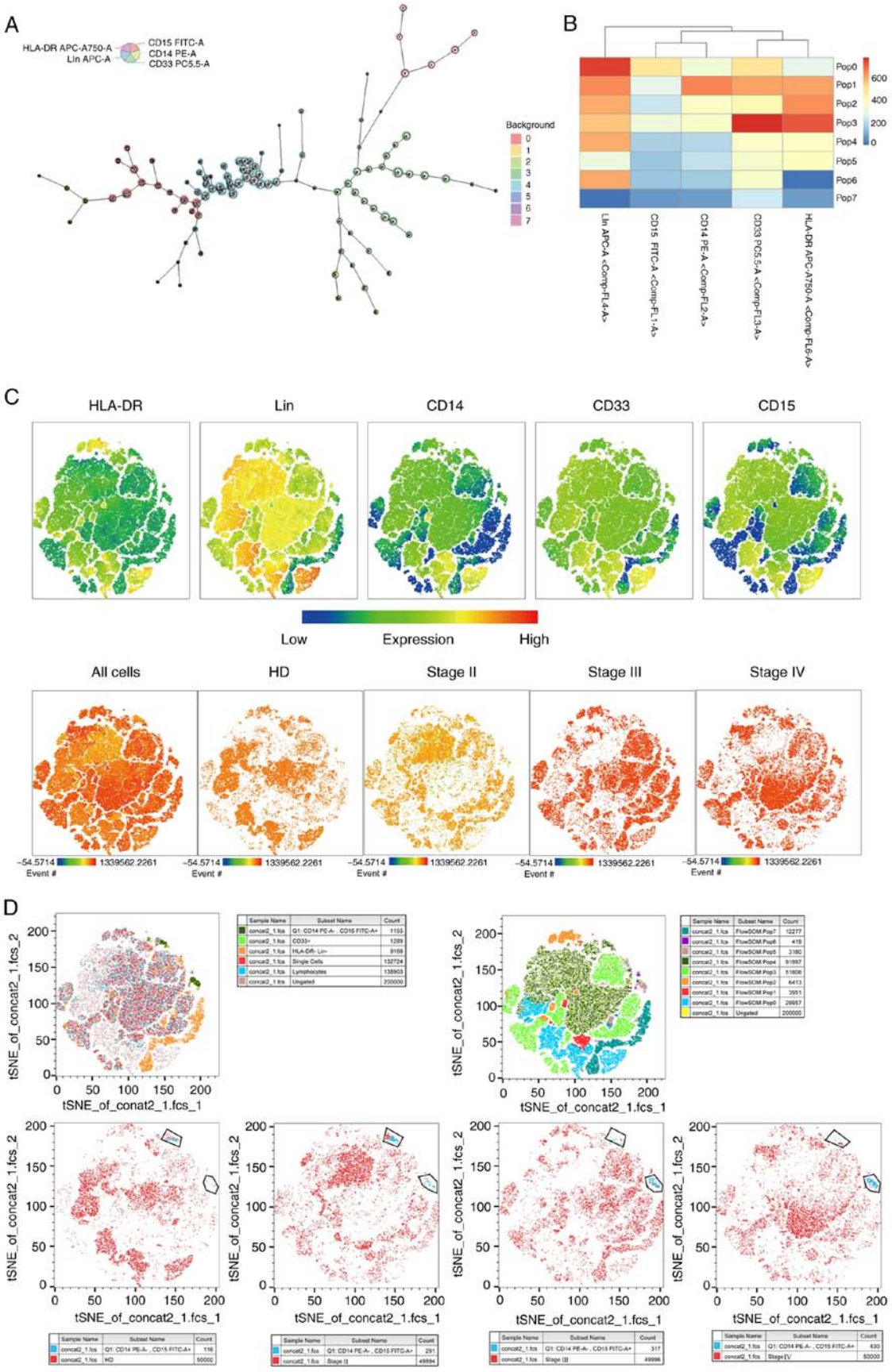

Figure 5. FlowSOM and t-SNE analysis of G-MDSCs. (A) MDSC subsets were presented as a minimum spanning tree of 100 nodes from a self-organizing map method (containing healthy donor and Stage II, III and IV for 4 cases) using FlowSOM. Each node includes phenotypically similar cells, and the size of the node indicates the number of cell events. (B) Cluster overview and abundance of per Pop were showed by FlowSOM. Referring to the expression of markers, Pop5 was the most similar to G-MDSCs. (C) Heatmap of t-SNE. The markers expression of Pops were intuitively presented. (D) Combining t-SNE with FlowSOM automatic gating compared to t-SNE with manual gating analysis. t-SNE, t-distributed stochastic neighbourhood embedding; MDSC, myeloid-derived suppressor cells; G-MDSC, granulocyte MDSCs.

immunity. It may promote NP reabsorption. Therefore, the use of this drug may aid in the treatment of LDH in the future.

To avoid the partial loss of G-MDSCs in the PBMCs during the Ficoll density gradient separation process and a deviation in MDSC subgroup analysis (39), we lyzed red blood cells in the whole blood to obtain and analyze the percentage of circulating MDSCs in this study. In addition, we used the cocktail lineage (CD3/CD19/CD56/CD14/CD20/CD16) to exclude the non-specific staining of a large number of neutrophils in the whole blood. Negative selection of CD16 antibody can filter mature neturophils out. CD3/CD19/CD56 antibodies negatively select mature lymphocytes and CD14 antibody negatively selects mononuclear cells. These technical points make our research results more accurate. Furthermore, we were not able to obtain many samples because we used strict criteria to ensure that the patient only had LDH. Finally, it is worth mentioning that because intervertebral disc degeneration is a long-term and complex process, the current animal models of intervertebral disc degeneration are mostly caused by mechanical damage (40), and there is no model that is very consistent with the upright characteristics of the human spine, our research has not yet involved animal-level verification. However, we believe that exciting models will be developed in the near future.

In conclusion, the peripheral blood MDSC subset changes significantly after lumbar disc degeneration, and our findings provide new clinical evidence for the autoimmune and inflammatory theory of disc degeneration. Our study shows that an increasing proportion of circulating G-MDSCs is associated with stage III or IV in patients with LDH. The detection of peripheral blood MDSC subpopulation can be used as an auxiliary examination project for lumbar disc degenerative diseases. This study has several limitations that need to be emphasized, such as the limited number of samples, the absence of authoritative animal model and control tissue of normal IVD, and a lack of pathological examination. There is also a lack of detailed research on MDSC function, and further in-depth researches are needed. Lumbar disc degeneration is a multi-factor co-operation, continuous progression of chronic inflammatory process, and autoimmunity plays an important role in its pathogenesis and symptom development. By elucidating the mechanism of IVDD, the development of drugs for the immune system may be a new idea for the treatment of low back pain. New discoveries to further promote the autoimmune research on IVDD will have great benefits to society.

Acknowledgements

Not applicable.

Funding

This work was supported by grants from the National Natural Science Foundation of China (grant nos. 31701162, 8197102295 and U1732157).

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Author's contributions

BS, WH and JD contributed to the conception, design and interpretation of the study. HZ and CL performed the experiments and wrote the manuscript. HZ, CS and FH were responsible for the tissue preservation collection, statistical analysis and bioinformatics analysis in preparation of figures and tables. HZ and CL confirm the authenticity of all the raw data. All the authors participated in revision of the manuscript. All authors have read and approved the final manuscript.

Ethics approval and consent to participate

The present study was approved by The Committee on Medical Ethics of The First Affiliated Hospital of Anhui Medical University and a waiver of informed consent was granted (approval no. PJ 2022-02-16).

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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