Check for updates

Improved therapeutic consistency and efficacy of CD317+ MSCs through stabilizing TSG6 by PTX3

Shaoquan Shi[1,2†], Si Chen[3†], Bowei Liang[3†], Yumeng Li[1,2], Qi Ma[1,2], Meiqi Li[1,2], Jingting Zhang[1,2], Lan Yao[1,2] and Jianyong Xu[1,2*]

Abstract

Background Previously, we have demonstrated that the batch variations of human platelet lysate (conventional MSC expansion medium) induce MSC heterogeneity and therapeutic inconsistency. On the other hand, the MSCs expanded with chemical defined medium have improved therapeutic consistency.

Methods In the current study, we studied the MSC subpopulation composition and variation in different types and batches of MSC expansion medium with scRNA-seq analysis.

Results MSCs expanded with different batches of media have higher levels of heterogeneity from the perspective of cell subpopulation composition at transcriptome levels and therapeutic inconsistency. The CD317+ subpopulation has enhanced immune suppression activities. And the percentage of CD317+ MSCs within MSCs is tightly correlated with its immune suppression activities, and also contributes to the heterogeneity and therapeutic inconsistency of MSCs. the CD317+ MSCs have increased expression levels of PTX3, which might stabilize the TSG6 protein and improve the therapeutic effects

Conclusions Thus, purifying CD317+ MSCs is one efficient strategy to reduce MSC heterogeneity and increase the therapeutic consistency of MSCs.

Keywords Mesenchymal stem/stromal cells, MSCs, CD317, Therapeutic consistency, Therapeutic efficacy

†Shaoquan Shi, Si Chen and Bowei Liang contributed equally to this work and share first authorship.

*Correspondence:
Jianyong Xu
xujianyong820915@163.com
[1] Shenzhen Key Laboratory of Reproductive Immunology for Peri-Implantation, Shenzhen Zhongshan Institute for Reproduction and Genetics, Shenzhen Zhongshan Obstetrics & Gynecology Hospital, Fuqiang Avenue 1001, Shenzhen 518060, Guangdong, People's Republic of China
[2] Guangdong Engineering Technology Research Center of Reproductive Immunology for Peri-Implantation, Shenzhen 518000, People's Republic of China
[3] Shenzhen University Medical School, Shenzhen University, Shenzhen 518000, People's Republic of China

Background

Mesenchymal stem/stromal cells (MSCs) are a diverse cell population found in various tissues like bone marrow, umbilical cord, teeth, and adipose tissue. Unlike other adult stem cells or fully differentiated cells, MSCs primarily function to sense and respond to changes in their microenvironment. They adapt to factors like oxygen levels, cytokines, and extracellular matrix composition and respond by modifying the extracellular matrix, recruiting immune cells, and releasing bioactive molecules like cytokines. Their immunomodulatory properties make them valuable in conditions involving immune system dysregulation, such as autoimmune diseases [1]. Additionally, MSCs promote tissue repair and angiogenesis,

Shi *et al. Stem Cell Research & Therapy* (2024) 15:92

Page 2 of 15

making them promising for treating diseases like cardiovascular disorders, diabetes, and neurological conditions. Both pre-clinical and clinical studies support their therapeutic potential, positioning MSCs as a key candidate for advancing medical treatments across various diseases with ongoing research expected to further enhance their clinical use [1–5].

After the initial demonstration of MSCs, extensive research spanning decades has been dedicated to exploring their therapeutic applications. Regrettably, despite the fast growth of clinical trials, only a limited number have successfully evolved into practical therapeutic products. The significant challenge of cell heterogeneity stands as a formidable obstacle in the pursuit of anticipated clinical outcomes [1, 2, 4, 6, 7]. This heterogeneity in MSCs can be attributed to a range of factors, including donor conditions such as age, gender, health status, and genetic background, along with tissue origin and the specific strategies employed for MSC isolation and expansion, encompassing variables like digestion enzymes, matrix proteins, cell culture medium, and passage numbers. These factors collectively contribute to the complexity of achieving consistent and predictable results in MSC-based therapies [2, 6–11].

In pursuit of strategies to mitigate MSC heterogeneity and improve therapeutic consistency, the approach of isolating homogeneous subpopulations guided by specific markers has gained prominence for its potential to yield more predictable clinical outcomes [2, 4, 6]. To date, several distinct MSC subpopulations have been developed, each characterized by its unique functions. These functions encompass the regulation of cell adhesion [12–17], facilitation of regeneration [18–23], and modulation of the immune system [11, 24, 25].

In the realm of immune modulation, specific MSC subpopulations have been explored, including TNFAIP6$^+$ MSCs and CD200$^+$ MSCs. TNFAIP6$^+$ MSCs are responsive to inflammatory environments, secreting anti-inflammatory cytokines like TNFAIP6, which leads to immune suppression and reduces MSC death induced by activated immune cells. Surviving MSCs express more anti-inflammatory cytokines, further enhancing their immune suppression capabilities [11, 24]. The therapeutic effects have been validated in the mouse model of acute inflammation [11]. However, the levels of TSG-6 mRNA are negatively correlated with their potential for osteogenic differentiation in vitro and poorly correlated with other criteria for evaluating human MSCs [24]. CD200$^+$ subpopulations have demonstrated enhanced immune suppression in a mouse skin allograft model, potentially related to CD200's involvement in dendritic cell and macrophage immune responses, although further validation is required. CD200 is down-regulated

during differentiation [26]. And its expression is absent in umbilical cord blood-derived MSCs and minimal in adipose-derived MSCs [26–28]. Therefore, current research on immune regulation by MSC subpopulations remains limited and more MSC specific markers need to be identified.

The emergence and development of high-throughput technologies have revolutionized various fields of life sciences [29–31]. Within the realm of high-throughput technologies, single-cell RNA sequencing (scRNA-seq) has emerged as a significant innovation in the MSC field, garnering widespread interest in recent years. By unraveling the gene expression profiles of individual cells within a population, this technique unveils the remarkable intricacies of cellular diversity and heterogeneity, ushering in a revolutionary breakthrough in cellular biology research [32, 33]. Unlike traditional bulk RNA sequencing methods, scRNA-seq offers precise insights into cell functions and types, all while overcoming the challenges posed by sample heterogeneity [33–35]. Through scRNA-seq, various new subpopulations of MSCs have been unveiled. These include CMKLR1$^+$ MSCs with enhanced immune suppression capabilities [36], LRRC75A$^+$ MSCs producing increased levels of VEGF [37], and S100A9$^+$ and F3$^+$ MSCs demonstrating superior regenerative properties [38–40].

Previously, we have demonstrated that the batch variations of human platelet lysate (conventional MSC expansion medium) induce MSC heterogeneity and therapeutic inconsistency [9]. On the other hand, the MSCs expanded with chemical defined medium have improved therapeutic consistency [9]. To further investigate whether the MSC subpopulation variations are involved in the induction of MSC heterogeneity and therapeutic inconsistency by the batch variation of MSC expansion medium, in the current study, we studied the MSC subpopulation composition and variation in different types and batches of MSC expansion medium with scRNA-seq analysis. Our data revealed that the CD317$^+$ MSCs have improved immune suppression activities and therapeutic effects in the mouse model of IBD (inflammatory bowel disease). Furthermore, the percentage of CD317$^+$ MSCs could be an efficacy predictor of its therapeutic effects in vivo.

Methods

Human MSCs isolation, expansion and purification

This study was approved by the ethics committee of Shenzhen Zhongshan Urology Hospital and Shenzhen University, and followed the tenants of the Declaration of Helsinki. The human umbilical cord derived MSCs were isolated, expanded and characterized as described previously [8–11]. Briefly, the MSCs were isolated from

Shi *et al. Stem Cell Research & Therapy*   (2024) 15:92

Page 3 of 15

human umbilical cords (donor age: 28 years old) after the tissue was minced and digested with 1 mg/mL collagenase B (STEMCELL Technologies). MSCs were expanded with chemical defined medium NBVbe we developed [10] or different batches of PL plus 2 U/ml heparin [9]. The MSCs were characterized with Stem-Pro® Adipogenesis Differentiation Kit (Gibco), Stem-Pro® Osteogenesis Differentiation Kit (Gibco), StemPro® Chondrogenesis Differentiation Kit (Gibco) according to instructions. The $CD317^+$ and $CD317^-$ MSCs were purified with BD FACSAria SORP cell sorter (BD Biosciences) after staining with anti-CD317-PE (Thermo Fisher Scientific) or IgG-PE. For PTX3 overexpression, the coding region sequence was cloned into the lentiviral vector (pLVX-Puro) as described before [8]. For shRNA construction, target sequences were cloned into the lentivirus pLKO.1-puro vector as described before [41]. Lentivirus production and cell infection were performed as described previously [8]. Target sequences of PTX3 were listed in Additional file 1: Table S1.

Single-cell RNA-seq and analysis

Human MSCs were prepared for scRNA-seq (single-cell RNA sequencing) at passage 6 at 90% confluence. Cells were detached with TrypLE and resuspended in HBSS containing 0.04% BSA at the concentration of 1×10^6 cells/mL. Samples (1×10^6 cells for each sample) were sequenced with the Illumina NovaSeq 6000 System (paired-end mode) after library construction with 10×Genomics Chromium platform. Sequencing data were analyzed using Seurat packages in R (v 4.0.0) after being processed with 10xGenomics pipeline Cell Ranger (v2.1.0).

$CD317^+$/$CD317^-$ MSC purification

Human MSCs were prepared for cell purification at passage 3. The MSCs were detached with TrypLE and stained with anti-CD317-PE (Thermo Fisher Scientific) or IgG-PE. Then, the $CD317^+$ and $CD317^-$ MSCs were purified with the BD FACSAria SORP cell sorter (BD Biosciences).

MSC-PBMC co-culture

The MSC-PBMC co-culture was performed as described previously with modifications [8, 9, 11]. Briefly, the human PBMCs (peripheral blood mononuclear cells) were isolated with the Ficoll-Paque® Plus (Merk). The PBMCs were stained with the CellTrace™ CFSE (ThermoFisher), stimulated with Dynabeads® Human T-Activator CD3/CD28 (Thermo Fisher Scientific) for 24 h, and co-cultured with purified $CD317^+$ or $CD317^-$ MSCs (20×10^4 PBMCs vs 5×10^4 MSCs) for 48 h. The

fluorescence signal was detected with flow cytometer (BD Accuri™ C6 Plus).

Mouse model of IBD (full name)

The mice (C57BL/6J, male, 8 weeks old) were purchased from the Guangdong Medical Laboratory Animal Center and maintained in specific pathogen-free conditions. This study has been reported in line with the ARRIVE guidelines 2.0 and approved by the Animal Research Ethics Committee of the School of Medicine, Shenzhen University. The mouse model of IBD (inflammatory bowel disease) was induced by providing 3% dextran sodium sulfate (DSS) in the drinking water with refreshed every two days. After 7 days treatment, the drinking water was refreshed without DSS. The disease activity index (DAI) was estimated as described before [42]. Mice were divided into 3 groups of eight mice each as follows: Group I, mice transplanted with PBS intravenously; Group II, mice transplanted with $CD317^+$ MSCs (1×10^6 cells/mouse) intravenously; Group III, mice transplanted with $CD317^-$ MSCs (1×10^6 cells/mouse) intravenously. Serum levels of cytokines were measured with ELISA kits as described before [11]. Quantitative PCR (qPCR) was performed as described before after total RNA extraction and reverse transcription [8, 11]. The primer sequences were listed in Additional file 1: Table S1.

Tissue analysis

Mice were anesthetized with isoflurane by using the anesthesia system (R550, RWD Life Science) and euthanized with overdose CO_2. The hematoxylin and eosin (HE) analysis of the colon tissue was performed as described previously [8, 11]. Histology score was estimated as described before [43]. Immunohistochemistry staining of CD45 was performed as described before [11].

Statistics

Data are shown as mean ± SEM (standard error of the mean) and analyzed with SPSS software for Windows (version 26, SPSS Inc). Student *t*-test was applied to the two groups comparison. One-way ANOVA analysis was applied to the multiple group comparison with normal data distribution, parametric test and Tukey Post Hoc tests. $P < 0.05$ indicates statistical significance.

Results

We have demonstrated previously that the MSC expansion medium could induce the heterogeneity and therapeutic inconsistency of MSCs [9]. To further explore the underlying mechanisms, the MSCs expanded with two batches of conventional MSC culture medium containing human platelet lysate (hPL) and two batches of full chemical defined medium were subject to single-cell RNA

Shi *et al. Stem Cell Research & Therapy*   (2024) 15:92

Page 4 of 15

sequencing (scRNA-seq, Additional file 1: Table S2). There totally 19 different MSC subpopulations were detected with non-linear dimensionality reduction analysis with UMAP (uniform manifold approximation and projection) (Fig. 1A, Additional file 1: Table S3). And their distribution and composition vary significantly among MSCs expanded with different batches of hPL, while they are more stable in the chemical defined medium (Fig. 1B, C). Correlation and heatmap analysis showed that the Subpopulation 0, 2, 3, 6, 8, 9, 12, 13, 15, and 16 were clustered together; Subpopulation 1, 5, 7, 10, and 11 were clustered together; and Subpopulation 4, 14, 17 were clustered together (Fig. 1D–F). Therefore, batch-to-batch variations of hPL-based media could induce MSC heterogeneity from the perspective of subpopulation composition.

The MSCs expanded with PL1, CDM1, and CDM2 have higher immune suppression activities than the MSCs expanded with PL2 [9]. To identify the MSC subpopulation responsible for immune suppression, a panel of immune related genes were plotted (Additional file 1: Table S4). Among them, the gene CD317, interferon-α inducible protein 6 (IFI6), intercellular adhesion molecule 1 (ICAM1), TNF Superfamily Member 4 (TNFSF4), and CD200 had the similar expression pattern with their immune suppression activities (Fig. 2). The expression of CD317 was restricted to a subset of MSCs with high expression levels (Fig. 2). Furthermore, within the hTERT immortalized human bone marrow MSC colonies, it has been demonstrated that the MSCs from the CD317$^+$ colony have up-regulated mRNA levels of immunosuppressive genes than the CD317$^-$ MSCs in vitro [44]. And our recent investigation also indicated that the CD317$^+$ MSCs have enhanced immunological anti-inflammatory activities [45]. Therefore, the CD317$^+$ and CD317$^-$ MSCs were purified with fluorescence-activated cell sorting (FACS) from MSCs expanded with hPL. And the MSC-PBMC co-culture assay showed that the CD317$^+$ MSCs have stronger immune suppression activities than the CD317$^-$ MSCs (Fig. 3A) [45]. To further confirm these findings, the CD317$^+$ and CD317$^-$ MSCs were transplanted into the mouse model of IBD. The CD317$^+$ MSCs showed improved therapeutic effects from the perspectives of body weight (Fig. 3B), DAI (disease activity index, Fig. 3C), colon length (Fig. 3D, E), and histology scoring (Fig. 3F, G). Indeed, the CD317$^+$ MSCs had significantly stronger immune suppression activities, from the perspectives of reducing spleen weight (Fig. 4A, B), CD45$^+$ lymphocytes infiltration (Fig. 4C), and the expression levels of pro-inflammatory cytokine IL-6, IL-1β, TNF-α, and IFN-γ (Fig. 4D, E). Interestingly, the CD317$^+$ MSCs also significantly up-regulated the anti-inflammatory cytokine IL-10, TGF-β, and TSG6 (Fig. 4D, E). Therefore,

the CD317$^+$ MSCs had stronger immune suppression activities and improved therapeutic effects than CD317$^-$ MSCs both in vitro and in vivo.

As we demonstrated before, MSCs expanded with different batches of hPL had different levels of immune suppression activities (Fig. 5A). And the MSCs expanded with chemical defined medium had comparable levels of immune suppression activities (Fig. 5A). Furthermore, the MSCs derived from different donors also had different levels of immune suppression activities (Fig. 5A). Interestingly, the percentage of CD317$^+$ MSCs within these MSCs also varied significantly and had the similar pattern with the immune suppression activities (Fig. 5A, B). Indeed, the correlation analysis showed that the percentage of CD317$^+$ MSCs within MSCs expanded with different batches of hPL was positively correlated with the ratio of lymphocytes proliferation suppressed (Fig. 5C). In the mouse model of IBD, the percentage of CD317$^+$ MSCs was also negatively correlated with the histology score (Fig. 5D) and serum level of TNF-α (Fig. 5E). In contrast, the CD317$^+$ MSCs purified from the MSCs expanded with different batches of hPL had the similar level of immune suppression activities in vitro and in vivo (Fig. 5F–H). Therefore, the percentage of CD317$^+$ MSCs within MSCs is tightly correlated with its immune suppression activities, and also contributes to the heterogeneity and therapeutic inconsistency of MSCs. Purifying CD317$^+$ MSCs is one efficient strategy to reduce MSC heterogeneity and increase the therapeutic consistency of MSCs.

CD317, also known as BST2 (bone marrow stromal cell antigen 2) or Tetherin, is involved in immunological responses to virus infection and production [46]. The functions and the underlying mechanisms of CD317 in MSCs remain largely unknown. To reveal the underlying mechanisms, the transcriptomes of CD317$^+$ MSCs and CD317$^-$ MSCs were analyzed (Additional file 1: Table S5). GO analysis showed that the up-regulated genes in the CD317$^+$ MSCs were mainly involved in functions such as extracellular matrix modification and wound healing (Fig. 6A). Among the top 20 up-regulated genes in CD317$^+$ MSCs (Fig. 6B), the gene PTX3 is potentially interesting. PTX3 (Pentraxin 3), also known as TSG-14 (tumor necrosis factor inducible gene 14), is a soluble pattern recognition molecule which plays critical roles in inflammation and tissue regeneration [47]. Indeed, knocking-down PTX3 in CD317$^+$ MSCs significantly reduced the immune suppression activities (Fig. 6C), while overexpressing PTX3 in CD317$^-$ MSCs improved their immune suppression activities (Fig. 6D).

It has been demonstrated that tumor necrosis factor stimulated gene 6 (TSG6) is the major effector contributing to the therapeutic effects of MSCs in treating IBD
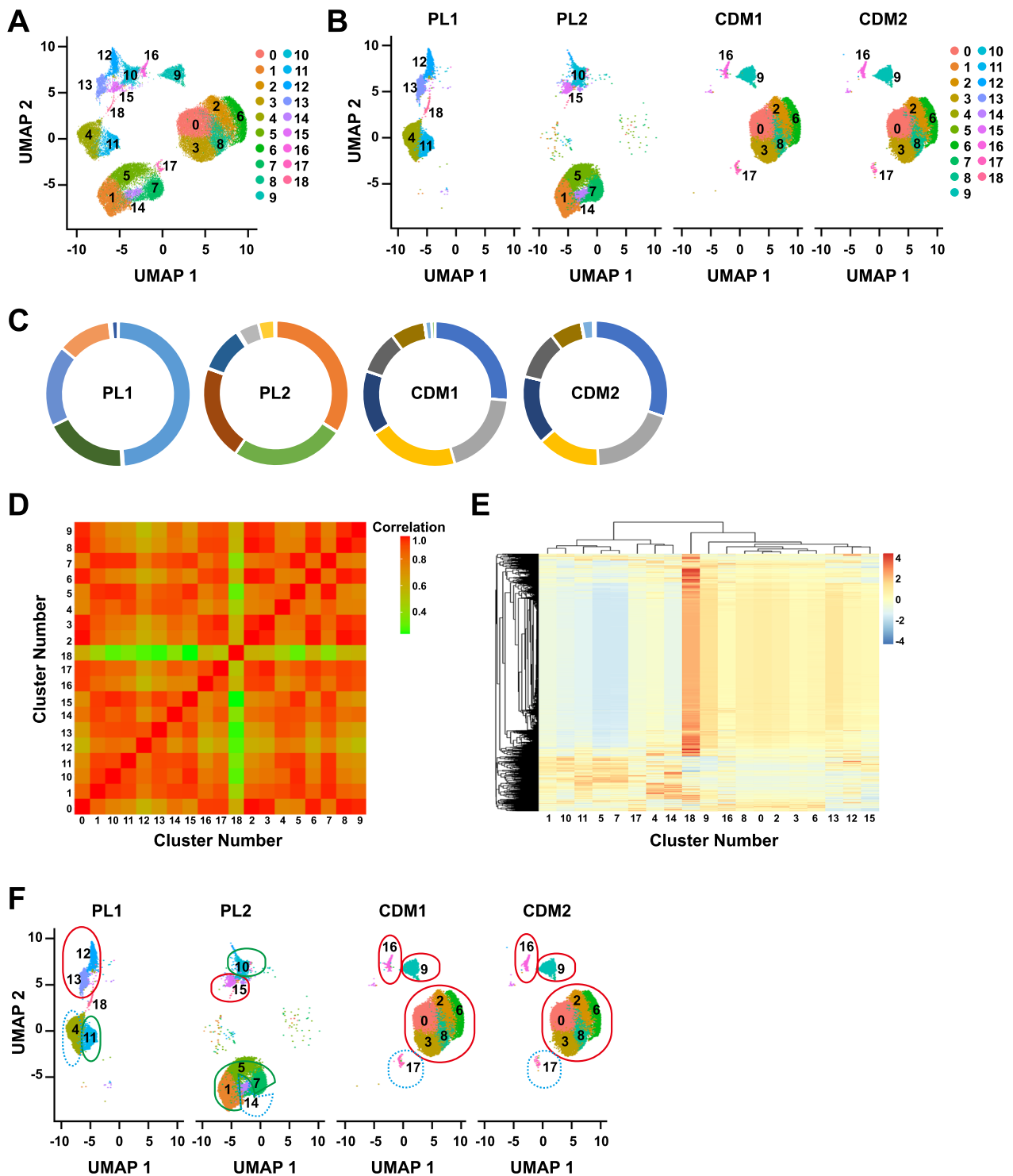
Fig. 1 scRNA-Seq of MSCs. The MSCs expanded with two batches of conventional MSC culture medium containing PL (human platelet lysate, PL1 and PL2) and full chemical defined medium (CDM1 and CDM2) were subject to single-cell RNA sequencing (scRNA-seq). **A** Cell cluster identification via non-linear dimensionality reduction analysis with UMAP after integrating 4 samples. **B** Subpopulation distribution and constitution in MSCs expanded with different media. **C** Percentage of different clusters in MSCs expanded with different media. **D** Correlation analysis of different MSC clusters. **E** Clustering analysis of different MSC clusters. **F** The more correlated clusters were labelled with colors
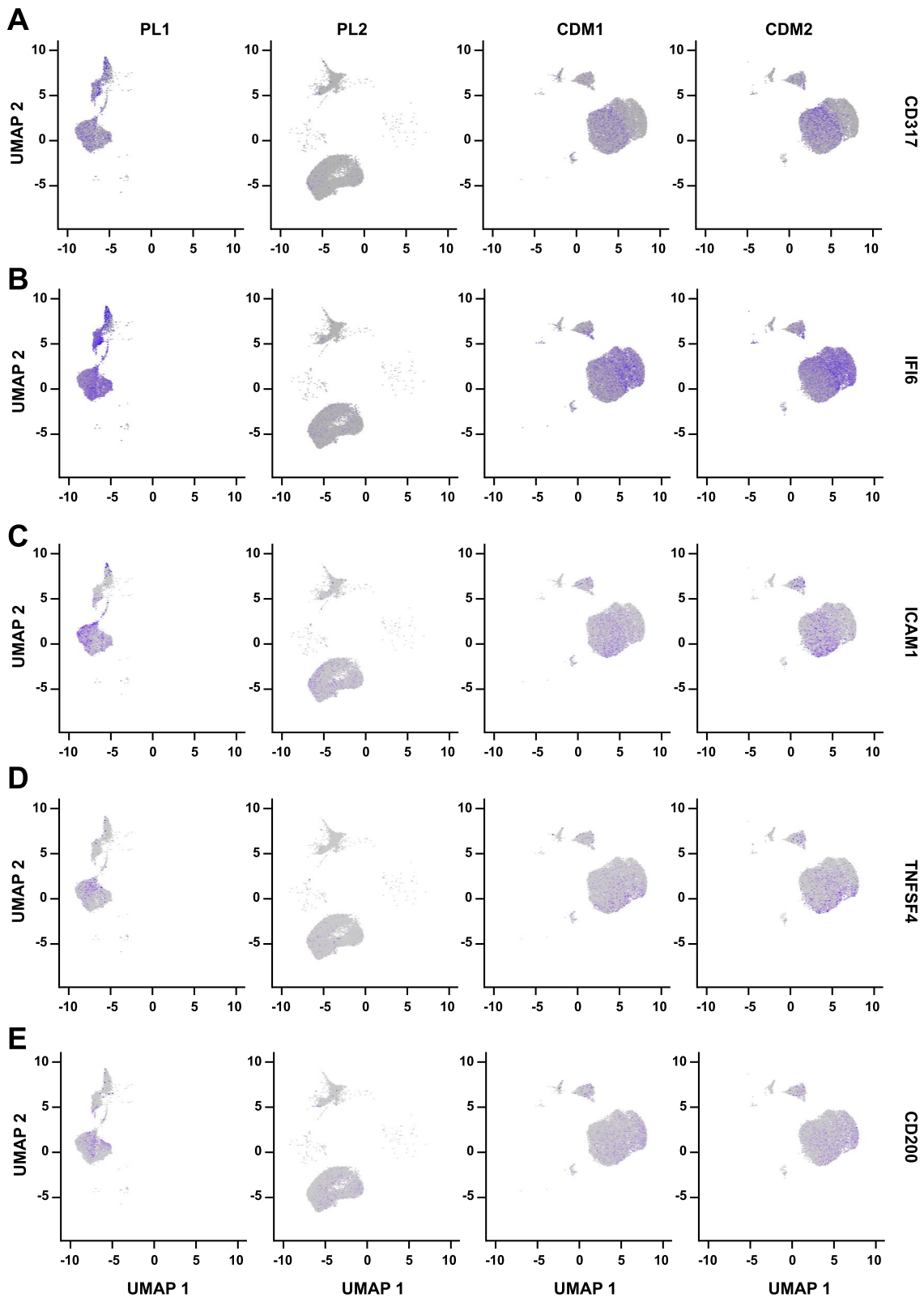
Shi *et al. Stem Cell Research & Therapy*   (2024) 15:92

Page 6 of 15

Fig. 2 MSC Marker Identification. Feature plotting the gene CD317 (**A**), IFI6 (**B**), ICAM1 (**C**), TNFSF4 (**D**), and CD200 (**E**) on transcriptome of MSCs expanded with different batches of expansion media. IFI6, interferon-α inducible protein 6; ICAM1, intercellular adhesion molecule 1; TNFSF4, TNF Superfamily Member 4
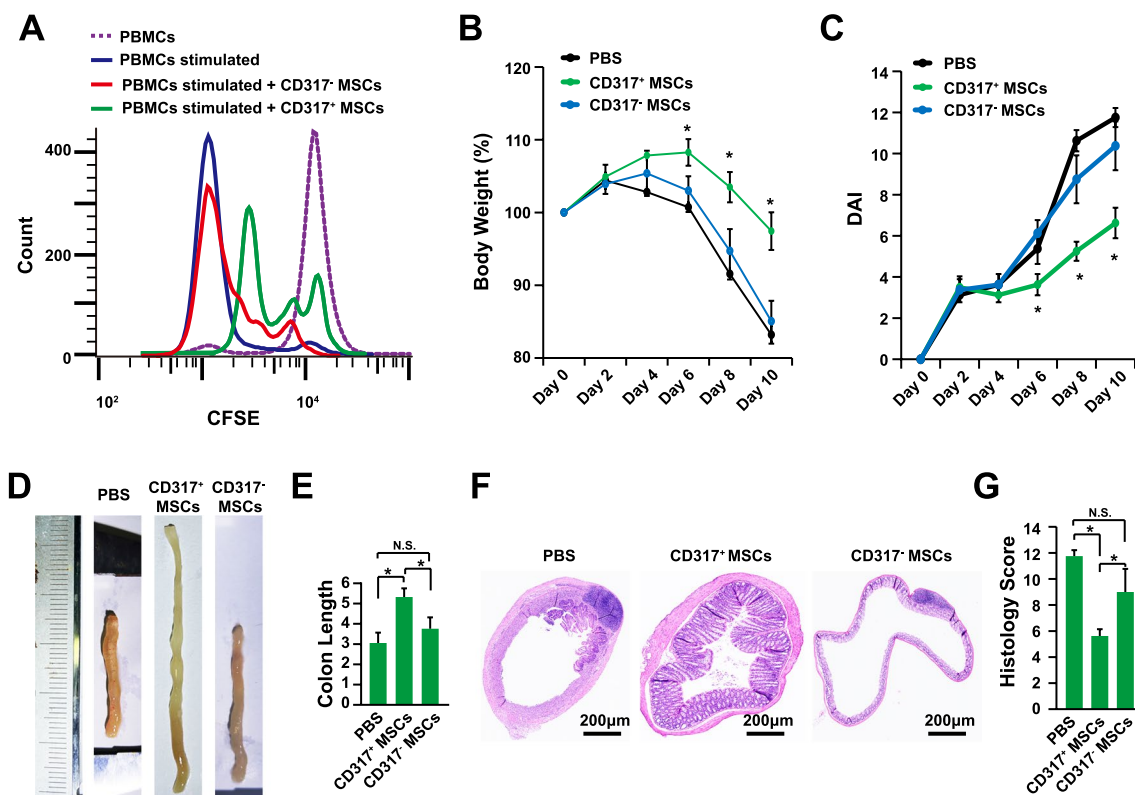
Fig. 3 Improved Therapeutic Effects of CD317⁺ MSCs. **A** PBMC proliferation assay after coculture with CD317⁺ or CD317⁻ MSCs. **B** Body weight of IBD mice transplanted with CD317⁺ MSCs, CD317⁻ MSCs, or negative control PBS (n = 8). **C** DAI (disease activity index) of IBD mice transplanted with CD317⁺ MSCs, CD317⁻ MSCs, or negative control PBS (n = 8). **D** Representative figures of colon length. **E** Colon length of IBD mice transplanted with CD317⁺ MSCs, CD317⁻ MSCs, or negative control PBS (n = 8). **F** Representative figures of HE staining of colon tissues after 10 days post DSS stimulation. **G** Histology score of IBD mice transplanted with CD317⁺ MSCs, CD317⁻ MSCs, or negative control PBS (n = 8). *Indicates $P < 0.05$

with strong immune suppression capabilities [1, 11, 48–51]. Furthermore, TSG6 has been demonstrated as a biomarker to predict the therapeutic efficacy of MSCs in vivo [24]. PTX3 could bind to TSG6 directly [52, 53]. Therefore, we wondered that whether PTX3 could regulate the expression or activity of TSG6. Data showed that neither knocking-down PTX3 in CD317⁺ MSCs nor overexpressing PTX3 in CD317⁻ MSCs could regulate the mRNA levels of TSG6 (Fig. 7A, B). However, in the mouse model of IBD, knocking-down PTX3 in CD317⁺ MSCs reduced the serum level of TSG6 and therapeutic effects (Fig. 7C), while overexpressing PTX3 in CD317⁻ MSCs increased the serum level of TSG6 and therapeutic effects (Fig. 7D), indicating that the PTX3 might regulate the protein stability of TSG6. Indeed, co-transfusion of PTX3 and TSG6 significantly delayed the clearance of TSG6 in vivo (Fig. 7E). Therefore, we proposed the underlying mechanism might be that the increased expression level of PTX3 in the CD317⁺ MSCs stabilize the TSG6 protein, which improves the therapeutic effects (Fig. 7F).

In summary, MSCs expanded with different batches of media have higher levels of heterogeneity from the perspective of cell subpopulation composition at transcriptome levels and therapeutic inconsistency. The CD317⁺ subpopulation has enhanced immune suppression activities. And the percentage of CD317⁺ MSCs within MSCs is tightly correlated with its immune suppression activities, and also contributes to the heterogeneity and therapeutic inconsistency of MSCs. Purifying CD317⁺ MSCs is one efficient strategy to reduce MSC heterogeneity and increase the therapeutic consistency of MSCs. The CD317⁺ MSCs have increased expression levels of PTX3, which might stabilize the TSG6 protein and improve the therapeutic effects.

Discussion

Inflammatory bowel disease (IBD) is attracting more scientists to investigate its underlying mechanisms and therapeutic approaches, due to its critical roles in the development of colorectal cancer (CRC) with increasing prevalence [54, 55]. CRC has been the third most
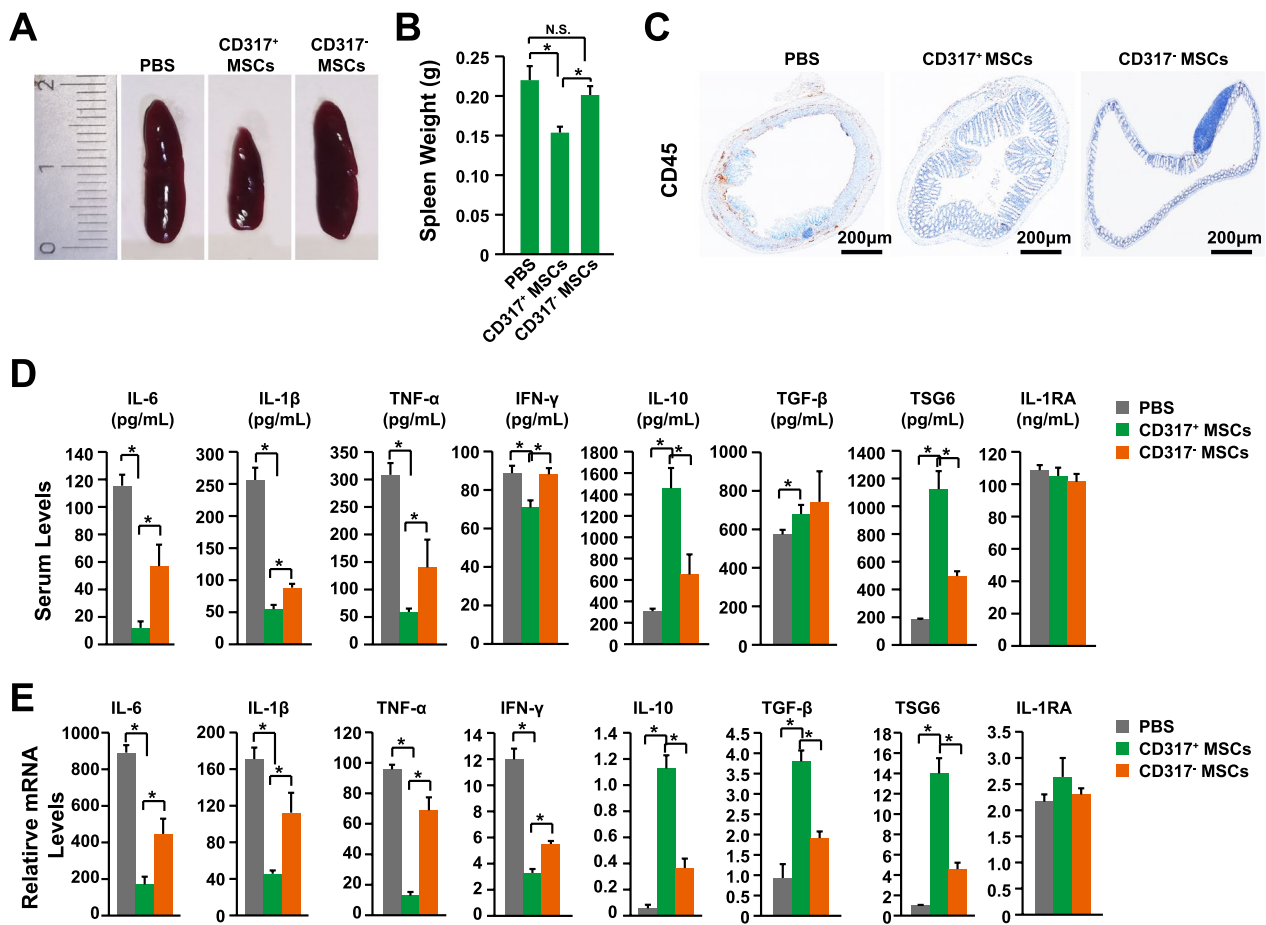
Fig. 4 Enhanced Immune-suppression Activities of CD317^{+} MSCs. **A** Representative figures of spleen of IBD mice transplanted with CD317^{+} MSCs, CD317^{−} MSCs, or negative control PBS. **B** Spleen weight of IBD mice transplanted with CD317^{+} MSCs, CD317^{−} MSCs, or negative control PBS (n = 8). **C** Representative figures of CD45 staining of colon tissues after 10 days post DSS stimulation. **D** Serum levels of IL-6, IL-1β, TNF-α, IFN-γ, IL-10, TGF-β, TSG6, and IL-1RA were determined via ELISA (n = 8). **E** mRNA levels of IL-6, IL-1β, TNF-α, IFN-γ, IL-10, TGF-β, TSG6, and IL-1RA in colon tissues were determined via qPCR (n = 8). *Indicates $P < 0.05$

common cancer diagnosis and the third leading cause of cancer-related deaths for both men and women. It's worth noting that when looking at overall cancer-related deaths, CRC takes the second spot, and it's the top cause of cancer-related deaths among men under 50 years old [56]. The pathogenesis of IBD involves intricate immune mechanisms [57, 58]. The standard treatment is limited. A significant breakthrough in recent years has been the introduction of TNF-α inhibitors, which have offered substantial, long-term relief and enhancement of the condition for the majority of IBD patients [59]. However, in clinical trials, up to 40% of patients were observed to have a primary non-response to TNF-α inhibitors, and about 23–46% of patients experienced secondary loss of response after one year of treatment [60]. This suggests that IBD still requires new treatment strategies.

IBD is a kind of inflammation related disease [57, 58]. In light of this, the immunological basis of the

IBD implies that MSCs-based cell therapies can be a rational therapeutic modality to alleviate IBD pathological signs due to their capacity to moderate inflammatory responses [61–63]. A growing body of proof suggests MSCs as a promising therapeutic tool for IBD treatment mainly due to their immunomodulatory and anti-inflammatory attributes [64–71]. And its clinical efficacy has been confirmed in clinical trials [61–63, 72–75]. Regrettably, few of them have successfully evolved into practical therapeutic products. The significant challenge of cell heterogeneity stands as a formidable obstacle in the pursuit of anticipated clinical outcomes [1–7]. Among different strategies to reduce the MSC heterogeneity and improve the therapeutic efficacy and consistency [2, 6, 8–10], purifying homogenous MSC populations with enhanced biological functions is one promising approach [2, 4, 6].
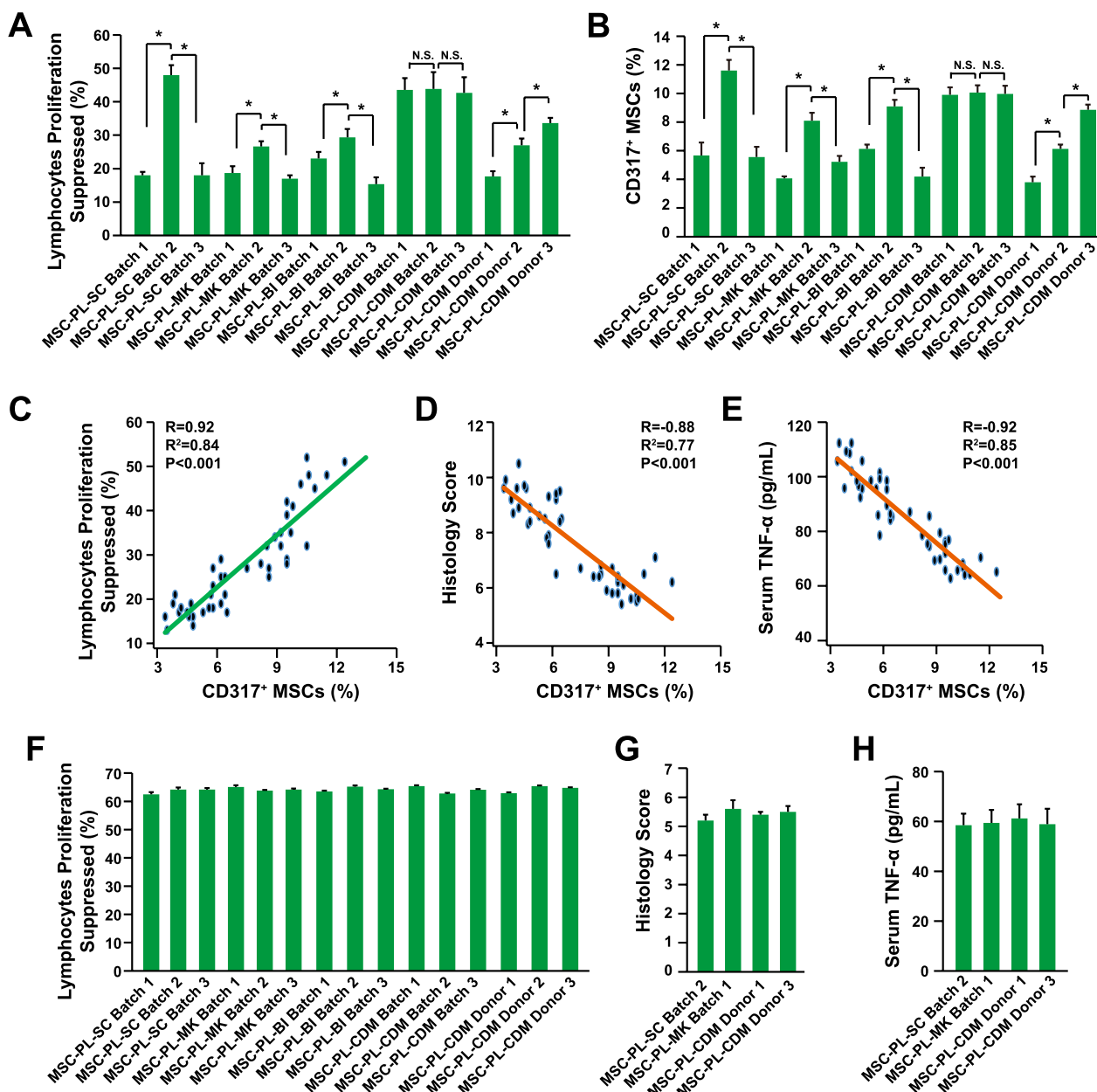
Shi et al. Stem Cell Research & Therapy   (2024) 15:92

Page 9 of 15

Fig. 5 Improved Therapeutic Consistency and Efficacy of CD317^{+} MSCs. The MSCs were expanded with different batches of media or from different donors **A** PBMC proliferation assay after coculture with MSCs (n = 3). **B** Percentage of CD317^{+} MSCs (n = 3). **C** Correlation analysis between lymphocytes proliferation suppressed by MSCs and the percentage of CD317^{+} MSCs within MSCs. **D** Correlation analysis between histology score in IBD mice treated with MSCs and the percentage of CD317^{+} MSCs within MSCs. **E** Correlation analysis between serum levels of TNF-α in IBD mice treated with MSCs and the percentage of CD317^{+} MSCs within MSCs. **F** PBMC proliferation assay after coculture with CD317^{+} MSCs (n = 3). **F** PBMC proliferation assay after coculture with CD317^{+} MSCs (n = 3). **G** Histology score in IBD mice treated with CD317^{+} MSCs (n = 8). **H** Serum levels of TNF-α in IBD mice treated CD317^{+} MSCs (n = 8). *Indicates *P* < 0.05

We have demonstrated previously that the MSC expansion medium could induce the heterogeneity and therapeutic inconsistency of MSCs [9]. Therefore, in the current study, we studied the MSC subpopulation composition and variation in different types and batches of MSC expansion medium with scRNA-seq analysis. The data have shown that the MSCs are very heterogenous from the perspective of subpopulation distribution and constitution. And their distribution and constitution vary significantly among MSCs expanded with different
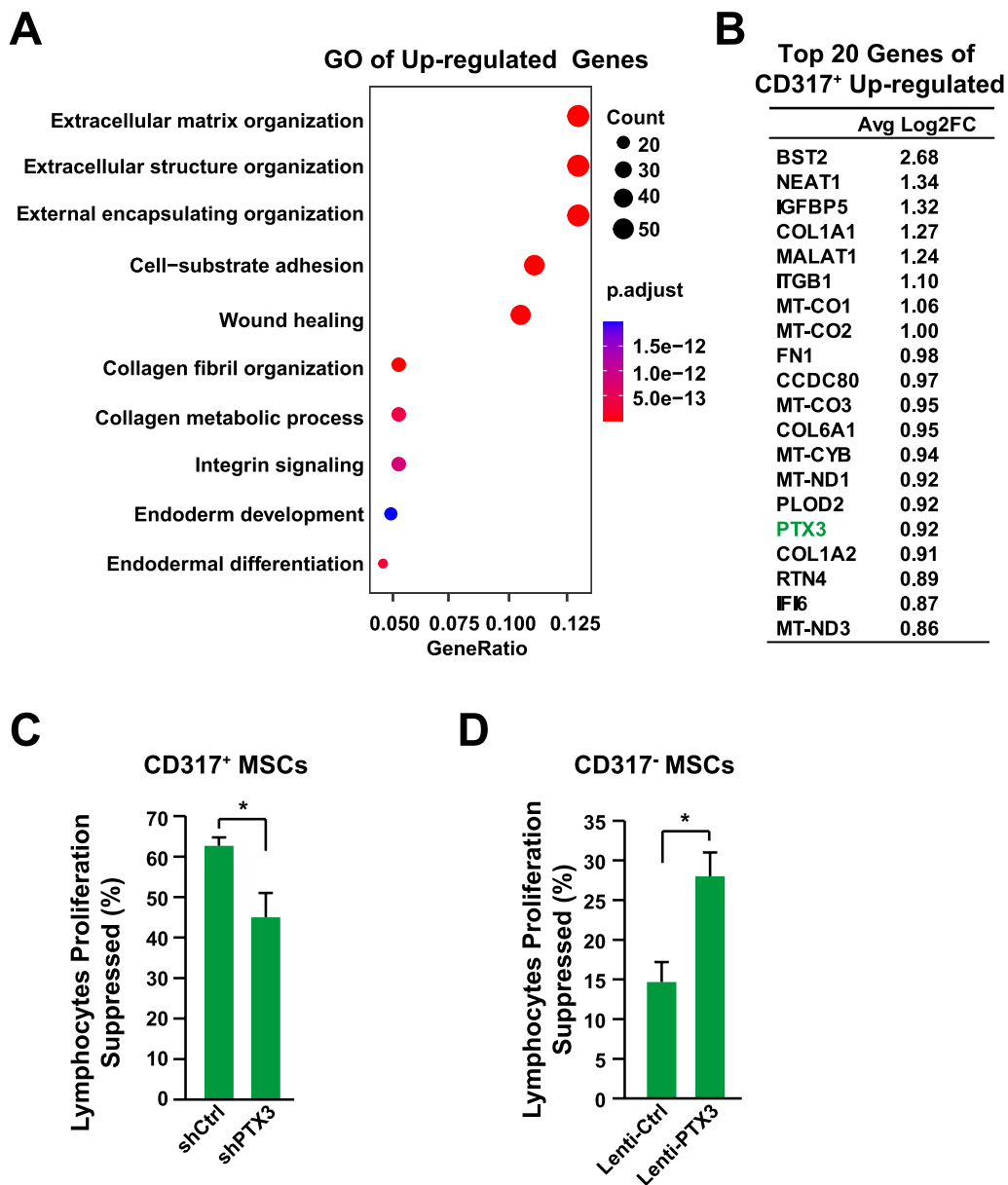
Shi *et al. Stem Cell Research & Therapy*   (2024) 15:92

Page 10 of 15

A

GO of Up-regulated Genes

B

Top 20 Genes of CD317⁺ Up-regulated

	Avg Log2FC
BST2	2.68
NEAT1	1.34
IGFBP5	1.32
COL1A1	1.27
MALAT1	1.24
ITGB1	1.10
MT-CO1	1.06
MT-CO2	1.00
FN1	0.98
CCDC80	0.97
MT-CO3	0.95
COL6A1	0.95
MT-CYB	0.94
MT-ND1	0.92
PLOD2	0.92
PTX3	0.92
COL1A2	0.91
RTN4	0.89
IFI6	0.87
MT-ND3	0.86

C

CD317⁺ MSCs

D

CD317⁻ MSCs

Fig. 6 PTX3 Mediates Immune-suppression Function of CD317⁺ MSCs. **A** GO enrichment analysis of up-regulated genes in CD317⁺ MSCs. **B** Top 20 up-regulated genes in CD317⁺ MSCs. **C** PBMC proliferation assay after coculture with CD317⁺ MSCs knocking-down PTX3 (n = 3). **D** PBMC proliferation assay after coculture with CD317⁻ MSCs overexpressing PTX3 (n = 3). GO, gene ontology. *Indicates $P < 0.05$

batches of hPL, while they are more stable in the chemical defined medium. To identify the MSC subpopulation responsible for immune suppression, a panel of immune related genes were analyzed. Among them, the CD317⁺ MSCs represent a MSC subpopulation with enhanced immune suppression activities and improved therapeutic effect in the mouse model of IBD.

CD317 is a transmembrane glycoprotein that plays a role in inhibiting virus replication and regulating the immune system. CD317 expression is induced by type I and type II interferons in response to viral infections, and plays a role in regulating NF-κB signaling, which is important for the host's inflammatory response to viruses [46]. It has been demonstrated that the CD317⁺ colony, within the hTERT immortalized human bone marrow MSC colonies, has up-regulated mRNA levels of immunosuppressive genes than the CD317⁻ MSCs in vitro [44]. And the CD317⁺ circulating progenitors had higher regenerative potentials [76, 77]. However, it's worth noting that freshly isolated CD317⁻ MSCs from human bone
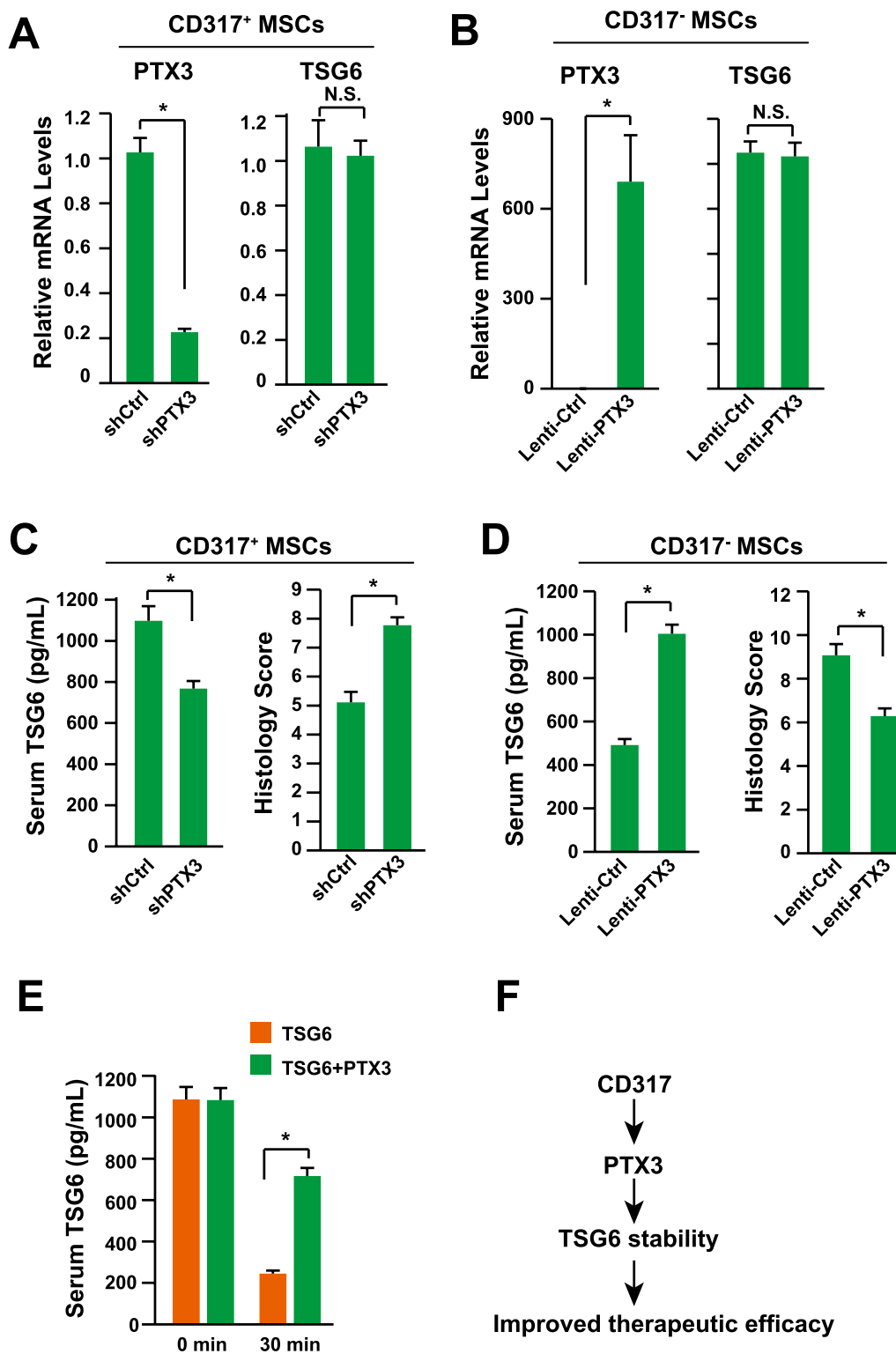
Fig. 7 PTX3 Stabilizes TSG6. **A** The mRNA levels of PTX3 and TSG6 were determined via qPCR after knocking-down PTX3 in CD317^{+} MSCs (n = 3). **B** The mRNA levels of PTX3 and TSG6 were determined via qPCR after over-expressing PTX3 in CD317^{−} MSCs (n = 3). **C** Serum level of TSG6 and histology score in IBD mice treated with CD317^{+} MSCs knocking-down PTX3 (n = 8). **D** Serum level of TSG6 and histology score in IBD mice treated with CD317^{−} MSCs over-expressing PTX3 (n = 8). **E** Serum level of TSG6 after intravenously injection of 1 μg TSG6 protein and 1 μg PTX3 protein (n = 8). **F** Proposed potential mechanism of upregulating PTX3 in CD317^{+} MSCs. *Indicates $P < 0.05$

Shi *et al. Stem Cell Research & Therapy*   (2024) 15:92

Page 12 of 15

marrow show better immune suppression abilities than CD317[77] MSCs [77]. The discrepancy might result from tissue origin (umbilical cord vs bone marrow), expansion medium (platelet vs fetal bovine serum), and cell population (primary vs immortalized and clonal selected).

Correlation analysis showed that the percentage of CD317^{+} MSCs within MSCs expanded with different batches of hPL was positively correlated with their immune suppression activities in vitro, and also their therapeutic effects in vivo. In contrast, the CD317^{+} MSCs purified from the MSCs expanded with different batches of hPL had the similar level of immune suppression activities. Therefore, the percentage of CD317^{+} MSCs within MSCs is tightly correlated with its immune suppression activities, and also contributes to the heterogeneity and therapeutic inconsistency of MSCs. Purifying CD317^{+} MSCs is one efficient strategy to reduce MSC heterogeneity and increase the therapeutic consistency of MSCs.

Transcriptomic analysis and function validation indicate that the gene PTX3 contributes to the immune suppression function of CD317^{+} MSCs. PTX3 is a soluble glycoprotein, responding to inflammatory cues by peripheral blood leukocytes and myeloid DCs, notably induced by IL-1β, TNF-α, and microbial components [47]. Furthermore, the PTX3 is up-regulated in the IBD development, indicating its important role in IBD [47]. PTX3 contributes to the M1-M2 switch of macrophage mediated by MSCs [78]. Knocking-down PTX3 significantly reduced the immune suppression function of MSCs and their therapeutic effect, indicating a potential marker for improving the therapeutic effects of MSCs [78, 79]. Moreover, the PTX3 and TSG6 are coordinated expressed under inflammation stresses [80], and they two interact with each other [52, 53, 81, 82]. And TSG6 is the major effector contributing to the therapeutic effects of MSCs in treating IBD with strong immune suppression capabilities [1, 11, 48–51]. Furthermore, TSG6 has been demonstrated as a biomarker to predict the therapeutic efficacy of MSCs in vivo [24]. Our data also showed here that the increased expression level of PTX3 in the CD317^{+} MSCs stabilize the TSG6 protein, which improves the therapeutic effects.

TSG6 is a relatively small protein exhibiting a wide range of activities. Its diverse functions include regulating immune and stromal cell activities, contributing to extracellular matrix formation and remodeling, and controlling the association of matrix molecules with cell surface receptors and signaling factors, such as chemokines and bone morphogenetic proteins (BMPs). It is upregulated in response to inflammation and is produced by various cell types, including MSCs. Another unique function is its enzymatic activity in catalyzing the covalent modification of non-sulfated GAG hyaluronan (HA) with heavy chains (HCs) from proteoglycans, leading to the formation of HC-HA complexes, which are essential in processes like ovulation, fertilization, and inflammation, where they either confer tissue protection or contribute to pathological processes [48]. The most studied function of TSG6 in MSCs is its immune suppression activities [49, 50, 83–95]. And our previous investigation indicates that the purified TSG6^{+} mouse MSCs have enhanced immune suppression activities and improved therapeutic effects in the mouse model of acute inflammation [11]. TSG6 is involved in the therapeutic effects of MSCs in treating IBD, such as mucosal barrier recovery through activating endogenous stem cells [96–99], and immune modulation [49, 50, 97, 100–102]. Lacking TSG6 makes mice more vulnerable to IBD development, resulting from dysregulated HA deposit [50, 103]. In addition, TSG6 stimulates a macrophage phenotypic shift from M1 to M2, having an important role in alleviating DSS-induced colitis [50, 100].

Conclusions

In conclusion, MSCs expanded with different batches of media have higher levels of heterogeneity from the perspective of cell subpopulation composition at transcriptome levels and therapeutic inconsistency. The CD317^{+} subpopulation has enhanced immune suppression activities. And the percentage of CD317^{+} MSCs within MSCs is tightly correlated with its immune suppression activities, and also contributes to the heterogeneity and therapeutic inconsistency of MSCs. Purifying CD317^{+} MSCs is one efficient strategy to reduce MSC heterogeneity and increase the therapeutic consistency of MSCs. The CD317^{+} MSCs have increased expression levels of PTX3, which might stabilize the TSG6 protein and improve the therapeutic effects.

Supplementary Information

Availability of data and materials
The scRNA-seq dataset has been deposited into the China National Center for Bioinformation (https://www.cncb.ac.cn/) with the accession BioProject No. PRJCA024438.

Declarations

Ethics approval and consent to participate
This study was approved by the ethics committee of Shenzhen Zhongshan Obstetrics & Gynecology Hospital (formerly Shenzhen Zhongshan Urology Hospital, No. 202000062, Aug18, 2020) and adhered to the tenants of the Declaration of Helsinki. The patients provided written informed consent for the use of samples. The mouse study was approved by the Animal Research Ethics Committee of the School of Medicine, Shenzhen University (No. A202200064, Feb23, 2022). Title of the approved project for both animal and human studies: purification and characterization of the regulatory MSC subpopulation.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

References

1. Jiang W, Xu J. Immune modulation by mesenchymal stem cells. Cell Prolif. 2020;53(1): e12712.
2. Levy O, Kuai R, Siren EMJ, Bhere D, Milton Y, Nissar N, et al. Shattering barriers toward clinically meaningful MSC therapies. Sci Adv. 2020;6(30): eaba6884.
3. Soliman H, Theret M, Scott W, Hill L, Underhill TM, Hinz B, et al. Multipotent stromal cells: one name, multiple identities. Cell Stem Cell. 2021;28(10):1690–707.
4. Krampera M, Le Blanc K. Mesenchymal stromal cells: putative microenvironmental modulators become cell therapy. Cell Stem Cell. 2021;28(10):1708–25.
5. Hoang DM, Pham PT, Bach TQ, Ngo ATL, Nguyen QT, Phan TTK, et al. Stem cell-based therapy for human diseases. Signal Transduct Target Ther. 2022;7(1):272.
6. Dunn CM, Kameishi S, Grainger DW, Okano T. Strategies to address mesenchymal stem/stromal cell heterogeneity in immunomodulatory profiles to improve cell-based therapies. Acta Biomater. 2021;133:114–25.
7. Costa LA, Eiro N, Fraile M, Gonzalez LO, Saa J, Garcia-Portabella P, et al. Functional heterogeneity of mesenchymal stem cells from natural niches to culture conditions: implications for further clinical uses. Cell Mol Life Sci. 2021;78(2):447–67.
8. Xu J, Chen J, Li W, Lian W, Huang J, Lai B, et al. Additive therapeutic effects of mesenchymal stem cells and IL-37 for systemic lupus erythematosus. J Am Soc Nephrol. 2020;31(1):54–65.
9. Xu J, Lian W, Wu H, Wang X, Chen J, Yang L, et al. Improved therapeutic consistency and efficacy of mesenchymal stem cells expanded with chemically defined medium for systemic lupus erythematosus. Cell Mol Immunol. 2020;17(10):1104–6.
10. Xu J, Lian W, Chen J, Li W, Li L, Huang Z. Chemical-defined medium supporting the expansion of human mesenchymal stem cells. Stem Cell Res Ther. 2020;11(1):125.
11. Li L, Yang L, Chen X, Chen X, Diao L, Zeng Y, et al. TNFAIP6 defines the MSC subpopulation with enhanced immune suppression activities. Stem Cell Res Ther. 2022;13(1):479.
12. Harkness L, Zaher W, Ditzel N, Isa A, Kassem M. CD146/MCAM defines functionality of human bone marrow stromal stem cell populations. Stem Cell Res Ther. 2016;7:4.
13. Wangler S, Menzel U, Li Z, Ma J, Hoppe S, Benneker LM, et al. CD146/MCAM distinguishes stem cell subpopulations with distinct migration and regenerative potential in degenerative intervertebral discs. Osteoarthr Cartil. 2019;27(7):1094–105.
14. Al BM. Comparison of the migration potential through microperforated membranes of CD146+ GMSC population versus heterogeneous GMSC population. Stem Cells Int. 2021;2021:5583421.
15. Bowles AC, Kouroupis D, Willman MA, Perucca Orfei C, Agarwal A, Correa D. Signature quality attributes of CD146(+) mesenchymal stem/stromal cells correlate with high therapeutic and secretory potency. Stem Cells. 2020;38(8):1034–49.
16. Wei Y, Zhang L, Chi Y, Ren X, Gao Y, Song B, et al. High-efficient generation of VCAM-1(+) mesenchymal stem cells with multidimensional superiorities in signatures and efficacy on aplastic anaemia mice. Cell Prolif. 2020;53(8): e12862.
17. Jia Y, Wang A, Zhao B, Wang C, Su R, Zhang B, et al. An optimized method for obtaining clinical-grade specific cell subpopulations from human umbilical cord-derived mesenchymal stem cells. Cell Prolif. 2022;55: e13300.
18. Colletti E, El Shabrawy D, Soland M, Yamagami T, Mokhtari S, Osborne C, et al. EphB2 isolates a human marrow stromal cell subpopulation with enhanced ability to contribute to the resident intestinal cellular pool. FASEB J. 2013;27(6):2111–21.
19. Zhao H, Feng J, Seidel K, Shi S, Klein O, Sharpe P, et al. Secretion of shh by a neurovascular bundle niche supports mesenchymal stem cell homeostasis in the adult mouse incisor. Cell Stem Cell. 2014;14(2):160–73.
20. Kramann R, Goettsch C, Wongboonsin J, Iwata H, Schneider RK, Kuppe C, et al. Adventitial MSC-like cells are progenitors of vascular smooth muscle cells and drive vascular calcification in chronic kidney disease. Cell Stem Cell. 2016;19(5):628–42.
21. Shi Y, He G, Lee WC, McKenzie JA, Silva MJ, Long F. Gli1 identifies osteogenic progenitors for bone formation and fracture repair. Nat Commun. 2017;8(1):2043.
22. Cassandras M, Wang C, Kathiriya J, Tsukui T, Matatia P, Matthay M, et al. Gli1(+) mesenchymal stromal cells form a pathological niche to promote airway progenitor metaplasia in the fibrotic lung. Nat Cell Biol. 2020;22(11):1295–306.
23. Magallanes J, Liu NQ, Zhang J, Ouyang Y, Mkaratigwa T, Bian F, et al. A new mouse model of post-traumatic joint injury allows to identify the contribution of Gli1+ mesenchymal progenitors in arthrofibrosis and acquired heterotopic endochondral ossification. Front Cell Dev Biol. 2022;10: 954028.
24. Lee RH, Yu JM, Foskett AM, Peltier G, Reneau JC, Bazhanov N, et al. TSG-6 as a biomarker to predict efficacy of human mesenchymal stem/progenitor cells (hMSCs) in modulating sterile inflammation in vivo. Proc Natl Acad Sci USA. 2014;111(47):16766–71.
25. Zhu Y, Yang Y, Zhang Y, Hao G, Liu T, Wang L, et al. Placental mesenchymal stem cells of fetal and maternal origins demonstrate different therapeutic potentials. Stem Cell Res Ther. 2014;5(2):48.
26. Delorme B, Ringe J, Gallay N, Le Vern Y, Kerboeuf D, Jorgensen C, et al. Specific plasma membrane protein phenotype of culture-amplified and native human bone marrow mesenchymal stem cells. Blood. 2008;111(5):2631–5.
27. Pietila M, Lehtonen S, Tuovinen E, Lahteenmaki K, Laitinen S, Leskela HV, et al. CD200 positive human mesenchymal stem cells suppress TNF-alpha secretion from CD200 receptor positive macrophage-like cells. PLoS ONE. 2012;7(2): e31671.
28. Najar M, Raicevic G, Jebbawi F, De Bruyn C, Meuleman N, Bron D, et al. Characterization and functionality of the CD200-CD200R system during mesenchymal stromal cell interactions with T-lymphocytes. Immunol Lett. 2012;146(1–2):50–6.
29. Metzker ML. Sequencing technologies—the next generation. Nat Rev Genet. 2010;11(1):31–46.

Shi *et al. Stem Cell Research & Therapy*   (2024) 15:92

Page 14 of 15

30. Shen X, Zhao Y, Wang Z, Shi Q. Recent advances in high-throughput single-cell transcriptomics and spatial transcriptomics. Lab Chip. 2022;22(24):4774–91.

31. Zhang Z, Wu S, Stenoien DL, Paša-Tolić L. High-throughput proteomics. Annu Rev Anal Chem. 2014;7:427–54.

32. Jovic D, Liang X, Zeng H, Lin L, Xu F, Luo Y. Single-cell RNA sequencing technologies and applications: a brief overview. Clin Transl Med. 2022;12(3): e694.

33. Papalexi E, Satija R. Single-cell RNA sequencing to explore immune cell heterogeneity. Nat Rev Immunol. 2018;18(1):35–45.

34. Kolodziejczyk AA, Kim JK, Svensson V, Marioni JC, Teichmann SA. The technology and biology of single-cell RNA sequencing. Mol Cell. 2015;58(4):610–20.

35. Shapiro E, Biezuner T, Linnarsson S. Single-cell sequencing-based technologies will revolutionize whole-organism science. Nat Rev Genet. 2013;14(9):618–30.

36. Xie Z, Yu W, Ye G, Li J, Zheng G, Liu W, et al. Single-cell RNA sequencing analysis of human bone-marrow-derived mesenchymal stem cells and functional subpopulation identification. Exp Mol Med. 2022;54(4):483–92.

37. Miura T, Kouno T, Takano M, Kuroda T, Yamamoto Y, Kusakawa S, et al. Single-cell RNA-Seq reveals LRRC75A-expressing cell population involved in VEGF secretion of multipotent mesenchymal stromal/stem cells under ischemia. Stem Cells Transl Med. 2023;12(6):379–90.

38. Sun C, Wang L, Wang H, Huang T, Yao W, Li J, et al. Single-cell RNA-seq highlights heterogeneity in human primary Wharton's jelly mesenchymal stem/stromal cells cultured in vitro. Stem Cell Res Ther. 2020;11(1):149.

39. Chen P, Tang S, Li M, Wang D, Chen C, Qiu Y, et al. Single-cell and spatial transcriptomics decodes Wharton's Jelly-derived mesenchymal stem cells heterogeneity and a subpopulation with wound repair signatures. Adv Sci. 2023;10(4): e2204786.

40. Chen Y, Ouyang Y, Li Z, Wang X, Ma J. S100A8 and S100A9 in cancer. Biochim Biophys Acta Rev Cancer. 2023;1878(3): 188891.

41. Xu JY, Lee YK, Ran X, Liao SY, Yang J, Au KW, et al. Generation of Induced cardiospheres via reprogramming of skin fibroblasts for myocardial regeneration. Stem Cells. 2016;34(11):2693–706.

42. Alex P, Zachos NC, Nguyen T, Gonzales L, Chen TE, Conklin LS, et al. Distinct cytokine patterns identified from multiplex profiles of murine DSS and TNBS-induced colitis. Inflamm Bowel Dis. 2009;15(3):341–52.

43. Dieleman LA, Palmen MJ, Akol H, Bloemena E, Pena AS, Meuwissen SG, et al. Chronic experimental colitis induced by dextran sulphate sodium (DSS) is characterized by Th1 and Th2 cytokines. Clin Exp Immunol. 1998;114(3):385–91.

44. James S, Fox J, Afsari F, Lee J, Clough S, Knight C, et al. Multiparameter analysis of human bone marrow stromal cells identifies distinct immunomodulatory and differentiation-competent subtypes. Stem cell reports. 2015;4(6):1004–15.

45. Song J, Ma Q, Li Y, Wang X, Chen S, Liang B, et al. CD317(+) MSCs expanded with chemically defined media have enhanced immunological anti-inflammatory activities. Stem Cell Res Ther. 2024;15(1):2.

46. Tiwari R, de la Torre JC, McGavern DB, Nayak D. Beyond tethering the viral particles: immunomodulatory functions of tetherin (BST-2). DNA Cell Biol. 2019;38(11):1170–7.

47. Wu Q, Cao F, Tao J, Li X, Zheng SG, Pan HF. Pentraxin 3: a promising therapeutic target for autoimmune diseases. Autoimmun Rev. 2020;19(12): 102584.

48. Day AJ, Milner CM. TSG-6: a multifunctional protein with anti-inflammatory and tissue-protective properties. Matrix Biol. 2019;78–79:60–83.

49. Yang S, Liang X, Song J, Li C, Liu A, Luo Y, et al. A novel therapeutic approach for inflammatory bowel disease by exosomes derived from human umbilical cord mesenchymal stem cells to repair intestinal barrier via TSG-6. Stem Cell Res Ther. 2021;12(1):315.

50. Sala E, Genua M, Petti L, Anselmo A, Arena V, Cibella J, et al. Mesenchymal stem cells reduce colitis in mice via release of TSG6, independently of their localization to the intestine. Gastroenterology. 2015;149(1):163-76.e20.

51. Yang H, Feng R, Fu Q, Xu S, Hao X, Qiu Y, et al. Human induced pluripotent stem cell-derived mesenchymal stem cells promote healing via TNF-alpha-stimulated gene-6 in inflammatory bowel disease models. Cell Death Dis. 2019;10(10):718.

52. Noone DP, Dijkstra DJ, van der Klugt TT, van Veelen PA, de Ru AH, Hensbergen PJ, et al. PTX3 structure determination using a hybrid cryoelectron microscopy and AlphaFold approach offers insights into ligand binding and complement activation. Proc Natl Acad Sci USA. 2022;119(33): e2208144119.

53. Salustri A, Garlanda C, Hirsch E, De Acetis M, Maccagno A, Bottazzi B, et al. PTX3 plays a key role in the organization of the cumulus oophorus extracellular matrix and in in vivo fertilization. Development. 2004;131(7):1577–86.

54. Kaplan GG, Windsor JW. The four epidemiological stages in the global evolution of inflammatory bowel disease. Nat Rev Gastroenterol Hepatol. 2021;18(1):56–66.

55. Shah SC, Itzkowitz SH. Colorectal cancer in inflammatory bowel disease: mechanisms and management. Gastroenterology. 2022;162(3):715-30.e3.

56. Siegel RL, Wagle NS, Cercek A, Smith RA, Jemal A. Colorectal cancer statistics, 2023. CA Cancer J Clin. 2023;73(3):233–54.

57. de Souza HS, Fiocchi C. Immunopathogenesis of IBD: current state of the art. Nat Rev Gastroenterol Hepatol. 2016;13(1):13–27.

58. Kim HS, Shin TH, Lee BC, Yu KR, Seo Y, Lee S, et al. Human umbilical cord blood mesenchymal stem cells reduce colitis in mice by activating NOD2 signaling to COX2. Gastroenterology. 2013;145(6):1392-1403.e8.

59. Nielsen OH, Ainsworth MA. Tumor necrosis factor inhibitors for inflammatory bowel disease. New Engl J Med. 2013;369(8):754–62.

60. Ben-Horin S, Kopylov U, Chowers Y. Optimizing anti-TNF treatments in inflammatory bowel disease. Autoimmun Rev. 2014;13(1):24–30.

61. Molendijk I, Bonsing BA, Roelofs H, Peeters KC, Wasser MN, Dijkstra G, et al. Allogeneic bone marrow-derived mesenchymal stromal cells promote healing of refractory perianal fistulas in patients with Crohn's disease. Gastroenterology. 2015;149(4):918-27.e6.

62. Panés J, García-Olmo D, Van Assche G, Colombel JF, Reinisch W, Baumgart DC, et al. Expanded allogeneic adipose-derived mesenchymal stem cells (Cx601) for complex perianal fistulas in Crohn's disease: a phase 3 randomised, double-blind controlled trial. Lancet. 2016;388(10051):1281–90.

63. Duijvestein M, Vos AC, Roelofs H, Wildenberg ME, Wendrich BB, Verspaget HW, et al. Autologous bone marrow-derived mesenchymal stromal cell treatment for refractory luminal Crohn's disease: results of a phase I study. Gut. 2010;59(12):1662–9.

64. Nikolic A, Simovic Markovic B, Gazdic M, Randall Harrell C, Fellabaum C, Jovicic N, et al. Intraperitoneal administration of mesenchymal stem cells ameliorates acute dextran sulfate sodium-induced colitis by suppressing dendritic cells. Biomed Pharmacother. 2018;100:426–32.

65. Chao K, Zhang S, Qiu Y, Chen X, Zhang X, Cai C, et al. Human umbilical cord-derived mesenchymal stem cells protect against experimental colitis via CD5(+) B regulatory cells. Stem Cell Res Ther. 2016;7(1):109.

66. Forte D, Ciciarello M, Valerii MC, De Fazio L, Cavazza E, Giordano R, et al. Human cord blood-derived platelet lysate enhances the therapeutic activity of adipose-derived mesenchymal stromal cells isolated from Crohn's disease patients in a mouse model of colitis. Stem Cell Res Ther. 2015;6(1):170.

67. Ye Y, Zhang X, Su D, Ren Y, Cheng F, Yao Y, et al. Therapeutic efficacy of human adipose mesenchymal stem cells in Crohn's colon fibrosis is improved by IFN-γ and kynurenic acid priming through indoleamine 2,3-dioxygenase-1 signaling. Stem Cell Res Ther. 2022;13(1):465.

68. Qiu Y, Guo J, Mao R, Chao K, Chen BL, He Y, et al. TLR3 preconditioning enhances the therapeutic efficacy of umbilical cord mesenchymal stem cells in TNBS-induced colitis via the TLR3-Jagged-1-Notch-1 pathway. Mucosal Immunol. 2017;10(3):727–42.

69. Fan H, Zhao G, Liu L, Liu F, Gong W, Liu X, et al. Pre-treatment with IL-1β enhances the efficacy of MSC transplantation in DSS-induced colitis. Cell Mol Immunol. 2012;9(6):473–81.

70. Fu Y, Ni J, Chen J, Ma G, Zhao M, Zhu S, et al. Dual-functionalized MSCs that express CX3CR1 and IL-25 exhibit enhanced therapeutic effects on inflammatory bowel disease. Mol Ther. 2020;28(4):1214–28.

71. Xu C, Feng C, Huang P, Li Y, Liu R, Liu C, et al. TNFα and IFNγ rapidly activate PI3K-AKT signaling to drive glycolysis that confers mesenchymal stem cells enhanced anti-inflammatory property. Stem Cell Res Ther. 2022;13(1):491.

72. Ouboter L, Barnhoorn M, Plug L, van Pel M, Zwaginga JJ, Roelen D, et al. DOP64 endoscopically injected allogeneic mesenchymal stromal cells

Shi *et al. Stem Cell Research & Therapy*   (2024) 15:92

Page 15 of 15

alter the mucosal immune cell compartment in patients with ulcerative proctitis. J Crohn's Colitis. 2021;15(Supplement_1):S098–9.

73. Ouboter L, Barnhoorn M, van Pel M, Zwaginga JJ, Hawinkels L, Maljaars J, et al. P363 locally injected allogeneic bone marrow-derived mesenchymal stromal cells for the treatment of refractory proctitis: clinical results of a phase IIa trial. J Crohn's Colitis. 2021;15(Supplement_1):S381.

74. Dige A, Hougaard HT, Agnholt J, Pedersen BG, Tencerova M, Kassem M, et al. Efficacy of injection of freshly collected autologous adipose tissue into perianal fistulas in patients with Crohn's disease. Gastroenterology. 2019;156(8):2208-16.e1.

75. Forbes GM, Sturm MJ, Leong RW, Sparrow MP, Segarajasingam D, Cummins AG, et al. A phase 2 study of allogeneic mesenchymal stromal cells for luminal Crohn's disease refractory to biologic therapy. Clin Gastroenterol Hepatol. 2014;12(1):64–71.

76. Lo Sicco C, Reverberi D, Villa F, Pfeffer U, Quarto R, Cancedda R, et al. Circulating healing (CH) cells expressing BST2 are functionally activated by the injury-regulated systemic factor HGFA. Stem Cell Res Ther. 2018;9(1):300.

77. Kay AG, Fox JM, Hewitson JP, Stone AP, Robertson S, James S, et al. CD317-positive immune stromal cells in human "Mesenchymal Stem Cell" populations. Front Immunol. 2022;13: 903796.

78. Kim M, Kwon JH, Bae YK, Kim GH, Um S, Ha J, et al. Soluble PTX3 of human umbilical cord blood-derived mesenchymal stem cells attenuates hyperoxic lung injury by activating macrophage polarization in neonatal rat model. Stem Cells Int. 2020;2020:1802976.

79. Cappuzzello C, Doni A, Dander E, Pasqualini F, Nebuloni M, Bottazzi B, et al. Mesenchymal stromal cell-derived PTX3 promotes wound healing via fibrin remodeling. J Invest Dermatol. 2016;136(1):293–300.

80. Maina V, Cotena A, Doni A, Nebuloni M, Pasqualini F, Milner CM, et al. Coregulation in human leukocytes of the long pentraxin PTX3 and TSG-6. J Leukocyte Biol. 2009;86(1):123–32.

81. Tseng SC, He H, Zhang S, Chen SY. Niche regulation of limbal epithelial stem cells: relationship between inflammation and regeneration. Ocul Surf. 2016;14(2):100–12.

82. Fossati G, Pozzi D, Canzi A, Mirabella F, Valentino S, Morini R, et al. Pentraxin 3 regulates synaptic function by inducing AMPA receptor clustering via ECM remodeling and β1-integrin. EMBO J. 2019;38(1): e99529.

83. Abraham C, Cho JH. Inflammatory bowel disease. N Engl J Med. 2009;361(21):2066–78.

84. Zhang S, Fang J, Liu Z, Hou P, Cao L, Zhang Y, et al. Inflammatory cytokines-stimulated human muscle stem cells ameliorate ulcerative colitis via the IDO-TSG6 axis. Stem Cell Res Ther. 2021;12(1):50.

85. Wan Y, Song M, Xie X, Chen Z, Gao Z, Wu X, et al. BMSCs regulate astrocytes through TSG-6 to protect the blood–brain barrier after subarachnoid hemorrhage. Mediators Inflamm. 2021;2021:5522291.

86. Tian J, Kou X, Wang R, Jing H, Chen C, Tang J, et al. Autophagy controls mesenchymal stem cell therapy in psychological stress colitis mice. Autophagy. 2021;17(9):2586–603.

87. Huang Q, Cheng X, Luo C, Yang S, Li S, Wang B, et al. Placental chorionic plate-derived mesenchymal stem cells ameliorate severe acute pancreatitis by regulating macrophage polarization via secreting TSG-6. Stem Cell Res Ther. 2021;12(1):337.

88. Gu G, Lv X, Liu G, Zeng R, Li S, Chen L, et al. Tnfaip6 secreted by bone marrow-derived mesenchymal stem cells attenuates TNBS-induced colitis by modulating follicular helper T cells and follicular regulatory T cells balance in mice. Front Pharmacol. 2021;12: 734040.

89. Wan YM, Li ZQ, Zhou Q, Liu C, Wang MJ, Wu HX, et al. Mesenchymal stem cells alleviate liver injury induced by chronic-binge ethanol feeding in mice via release of TSG6 and suppression of STAT3 activation. Stem Cell Res Ther. 2020;11(1):24.

90. Li Q, Song WJ, Ryu MO, Nam A, An JH, Ahn JO, et al. TSG-6 secreted by human adipose tissue-derived mesenchymal stem cells ameliorates severe acute pancreatitis via ER stress downregulation in mice. Stem Cell Res Ther. 2018;9(1):255.

91. Shin TH, Kim HS, Kang TW, Lee BC, Lee HY, Kim YJ, et al. Human umbilical cord blood-stem cells direct macrophage polarization and block inflammasome activation to alleviate rheumatoid arthritis. Cell Death Dis. 2016;7(12): e2524.

92. Wang S, Lee JS, Hyun J, Kim J, Kim SU, Cha HJ, et al. Tumor necrosis factor-inducible gene 6 promotes liver regeneration in mice with acute liver injury. Stem Cell Res Ther. 2015;6:20.

93. Kota DJ, Wiggins LL, Yoon N, Lee RH. TSG-6 produced by hMSCs delays the onset of autoimmune diabetes by suppressing Th1 development and enhancing tolerogenicity. Diabetes. 2013;62(6):2048–58.

94. Choi H, Lee RH, Bazhanov N, Oh JY, Prockop DJ. Anti-inflammatory protein TSG-6 secreted by activated MSCs attenuates zymosan-induced mouse peritonitis by decreasing TLR2/NF-kappaB signaling in resident macrophages. Blood. 2011;118(2):330–8.

95. Lee RH, Pulin AA, Seo MJ, Kota DJ, Ylostalo J, Larson BL, et al. Intravenous hMSCs improve myocardial infarction in mice because cells embolized in lung are activated to secrete the anti-inflammatory protein TSG-6. Cell Stem Cell. 2009;5(1):54–63.

96. Xu J, Wang X, Chen J, Chen S, Li Z, Liu H, et al. Embryonic stem cell-derived mesenchymal stem cells promote colon epithelial integrity and regeneration by elevating circulating IGF-1 in colitis mice. Theranostics. 2020;10(26):12204–22.

97. Yang H, Feng R, Fu Q, Xu S, Hao X, Qiu Y, et al. Human induced pluripotent stem cell-derived mesenchymal stem cells promote healing via TNF-α-stimulated gene-6 in inflammatory bowel disease models. Cell Death Dis. 2019;10(10):718.

98. Sémont A, Mouiseddine M, François A, Demarquay C, Mathieu N, Chapel A, et al. Mesenchymal stem cells improve small intestinal integrity through regulation of endogenous epithelial cell homeostasis. Cell Death Differ. 2010;17(6):952–61.

99. Sheng H, Shao J, Townsend CM Jr, Evers BM. Phosphatidylinositol 3-kinase mediates proliferative signals in intestinal epithelial cells. Gut. 2003;52(10):1472–8.

100. Song WJ, Li Q, Ryu MO, Ahn JO, Bhang DH, Jung YC, et al. TSG-6 released from intraperitoneally injected canine adipose tissue-derived mesenchymal stem cells ameliorate inflammatory bowel disease by inducing M2 macrophage switch in mice. Stem Cell Res Ther. 2018;9(1):91.

101. Chen PM, Liu KJ, Hsu PJ, Wei CF, Bai CH, Ho LJ, et al. Induction of immunomodulatory monocytes by human mesenchymal stem cell-derived hepatocyte growth factor through ERK1/2. J Leukocyte Biol. 2014;96(2):295–303.

102. Li N, Zhang Y, Nepal N, Li G, Yang N, Chen H, et al. Dental pulp stem cells overexpressing hepatocyte growth factor facilitate the repair of DSS-induced ulcerative colitis. Stem Cell Res Ther. 2021;12(1):30.

103. Albtoush N, Queisser KA, Zawerton A, Lauer ME, Beswick EJ, Petrey AC. TSG6 hyaluronan matrix remodeling dampens the inflammatory response during colitis. Matrix Biol. 2023;121:149–66.

Publisher's Note