The Mechanistic Effects and Clinical Applications of Various Derived Mesenchymal Stem Cells in Immune Thrombocytopenia

Yue He Dexiang Ji Wei Lu Guoan Chen

Department of Hematology, The First Affiliated Hospital of Nanchang University, Nanchang, China

Keywords

Mesenchymal stem cells · Immune thrombocytopenia · Mechanism · Clinical application

Abstract

Immune thrombocytopenia (ITP) is an acquired autoimmune disorder characterized by persistent thrombocytopenia resulting from increased platelet destruction and a loss of autoimmune tolerance. The pathogenesis of ITP is highly complex. Although ITP may be effectively controlled with currently available medications in some patients, a subset of cases remain refractory. The application of mesenchymal stem cells (MSCs) for human hematopoietic stem cell transplantation has increasingly demonstrated that MSCs modulate innate or adaptive immunity, thus resulting in a tolerant microenvironment. Functional defects and immunomodulatory disorders have been observed after the use of bone marrow mesenchymal stem cells (BM-MSCs) from patients with ITP. Here, we summarize the underlying mechanisms and clinical applications of various derived MSCs for ITP treatment, focusing on the main mechanisms underlying the functional defects and immune dysfunction of BM-MSCs from patients with ITP. Functional effects associated with the

activation of the p53 pathway include decreased activity of the phosphatidylinositol 3 kinase/Akt pathway and activation of the TNFAIP3/NF- κ B/SMAD7 pathway. Immune dysfunction appears to be associated with an impaired ability of BM-MSCs to induce various types of immune cells in ITP. At present, research focusing on MSCs in ITP remains in preliminary stages. The application of autologous or exogenous MSCs in the clinical treatment of ITP has been attempted in only a small case study and must be validated in larger-scale clinical trials.

© 2021 S. Karger AG, Basel

Introduction

Immune thrombocytopenia (ITP) is a hemorrhagic disease characterized by skin, mucosal, or visceral bleeding; reduced platelet count and survival time; development of bone marrow megakaryocytes; and maturation disorders. ITP is less prevalent in men than in women of childbearing age, and it accounts for 1 in 3 cases involving hemorrhagic disease [1]. ITP in adults frequently exhibits a chronic course. Approximately, 10–20% of ITPs in adults are refractory, showing resistance to treatment.

Table 1. Characterization of MSCs from BM, AT, and UCB

	BM-MSCs	UC-MSCs	ASCs
Shape	Spindle	Spindle	Spindle
Cell proliferation	*	***	**
Clonality	**	***	*
Senescence	**	*	***
Differentiation ability	**	***	**
Anti-inflammatory ability	*	***	*
Expression of genes associated with osteogenic differentiation	***	***	*
Expression of genes associated with angiogenesis	*	***	*
CD49	—	—	+
CD54	—	—	+
CD106	+	+	—
Phenotype	Expression of CD29, CD44, CD73, CD90, CD105 Lack of expression of CD14, CD19, CD34, CD45, CD79a, HLA-DR		

+, positive; —, negative; BM-MSCs, bone marrow mesenchymal stem cells; UC-MSCs, umbilical cord-derived mesenchymal stem cells; ASCs, adipose tissue-derived mesenchymal stem cells. * The comparative ability or expression of various derived MSCs is lowest. ** The comparative ability or expression of various derived MSCs is moderate. *** The comparative ability or expression of various derived MSCs is highest.

Environmental and genetic factors, along with abnormal immune regulation, are associated with the occurrence of ITP. Accumulating evidence shows that genetic predisposition factors may play an important role in the pathogenesis of ITP. Studies on inpatients with ITP have identified polymorphisms in major histocompatibility complex [2], Fcγ receptor [3], transcription factors [4], chemokines [5], and pro- and anti-inflammatory cytokines together with their receptors; these polymorphisms are often identified in ITP cohorts, specifically during the chronic course of the disease [6, 7]. Environmental factors also play crucial roles in ITP. Previous reports have described the involvement of several viral infections in the ITP immune process, including human immunodeficiency virus, hepatitis C virus, Epstein-Barr virus, and cytomegalovirus [8–10]. Interestingly, the protein sequences of these viruses are homologous to those of the viral protein GPIIb/IIIa, some of which can be identified by anti-platelet antibodies. In 2012, Grimaldi-Bensouda et al. [11] found that *Helicobacter pylori* decreases the expression of Fcγ-RIIb on monocytes and that this process plays a mechanistic role in ITP. The immunological pathogenesis of ITP is complex and includes dysfunction of natural killer cells, macrophages, and dendritic cells (DCs, which have roles in the innate immune response) and B lymphocytes and T lymphocytes (which have roles in the adaptive immune response).

Mesenchymal stem cells (MSCs) have the potential to differentiate, and their immunomodulatory characteristics can be used for transplantation and the treatment of autoimmune diseases. In ITP, the biological and immunological characteristics of MSCs are defective, thus indicating that the immune dysfunction of MSCs is associated with ITP pathogenesis [12–14]. Consequently, the administration of autologous or exogenous MSCs to patients with ITP has provided new options for the clinical treatment of ITP, particularly for refractory cases.

The Characteristics of MSCs Derived from Various Origins

MSCs have been identified in virtually all organs and tissues. MSCs are normally derived from the mesoderm and ectoderm of many different types of tissues and organs. MSCs exhibit high plasticity, low immunogenicity, and multidirectional differentiation ability. They can also act in a paracrine manner through multiple bioactive factors, and they pose a low risk of teratogenesis and tumorigenesis [15].

The major sources of MSCs are the human umbilical cord, bone marrow, and adipose tissue. As shown in Table 1, a variety of derived MSCs share identical biological properties but differ in various aspects, including their proliferation, immunophenotype, differentiation poten-

Table 2. Difference between ITP-MSCs and normal BM-MSCs

	BM-MSCs	ITP-MSCs
Shape	Spindle	Larger and flattened
Apoptotic cells	Normal round and regular nuclei	Fragmentation and condensation of nuclei
Apoptotic rate	Normal	Up
Cell proliferation	Normal	Down
Senescence	Normal	Up
Mitochondrial function	Normal	MMP↓, Bcl-2/Bax↓, P16↑, caspase-9 ↑, caspase-3↑
Phenotype	Expression of CD105, CD73, CD90 Lack of expression of CD14, CD19, CD34, CD45, HLA-DR	
Differentiation ability	Similar differentiation	

BM-MSCs, bone marrow mesenchymal stem cells; ITP-MSCs, BM-MSCs in ITP.

tial, and gene expression profile [16, 17]. Table 1 shows some of the verified immune phenotypes. However, some controversy remains regarding certain immune phenotypes, including CD106, Stro-1, and CD34 [18, 19]. Umbilical cord-derived mesenchymal stem cells (UC-MSCs) have the strongest differentiation ability. However, whether adipose tissue-derived mesenchymal stem cells (ASCs) or bone marrow mesenchymal stem cells (BM-MSCs) exhibit the strongest differentiation remains a matter of debate. Data supporting both arguments have been reported [15, 20]. Compared with BM-MSCs, ASCs have been reported to exhibit a stronger adipogenic differentiation ability and a weaker osteogenic differentiation ability [21]. However, other studies have not replicated this finding [22, 23], possibly because MSCs from different sources vary in their responses to different stimuli. Alternatively, the conditions most suitable for the differentiation of BM-MSCs may not be suitable for ASCs. In addition, ASCs significantly inhibit the differentiation of peripheral blood mononuclear cells into DCs and the expression of co-stimulatory molecules on the surfaces of mature monocyte-derived DCs [24].

BM-MSCs in ITP

Mechanisms Underlying the Functional Deficiency and Immunomodulatory Dysfunction of ITP-MSCs

BM-MSCs are pluripotent stem cells. As early as 1996, Friedenstein et al. [25] reported that these particular stem cells have multidirectional differentiation potential. In 2005, Lazarus et al. [26] applied BM-MSCs to human hematopoietic stem cell transplantation, thus providing a better understanding of the role of these cells in regulat-

ing the immune system and inflammation. BM-MSCs provide an effective cell therapy for diseases of the immune system, including graft-versus-host disease [27], systemic lupus erythematosus [28], rheumatoid arthritis [29], autoimmune encephalomyelitis, and multiple sclerosis [30, 31]. ITP-MSCs cannot proliferate routinely and are defective in immunomodulation. Exogenous BM-MSCs have been shown to reverse this defect of proliferation and immunomodulation in both in vitro cytological experiments and in vivo animal experiments [32, 33]. Substantial differences exist between BM-MSCs and BM-MSCs in ITP (ITP-MSCs) in terms of their appearance, proliferation, apoptosis, senescence, and differentiation (Table 2) [14, 34, 35]. To better explore the mechanisms underlying the impairment of ITP-MSCs, Zhang et al. [35] have used microarray technology to identify key differentially expressed genes (740 genes and 32 miRNAs) in BM-MSCs from unaffected participants and patients with ITP. They found that cellular stress response, unfolded protein response damage, and DNA transcription are all involved in ITP-MSCs [35]. However, in using this approach, the authors might potentially have missed some genes. Therefore, the occurrence of ITP-MSCs may involve the action of certain unknown genes.

Mechanisms of Apoptosis

Three mechanisms of apoptosis exist in ITP-MSCs (Fig. 1). The first mechanism involves activation of the p53 pathway. The expression of p53 and p21 is significantly upregulated in ITP-MSCs; p53 translocates to the nucleus, where it acts as a transcription factor, thus promoting the expression of caspase-9 and the activation of caspase-3 [14]. Platelet-derived growth factor-BB (PDGF-BB) belongs to the family of vascular endothelial

Fig. 1. Mechanism of apoptosis in ITP-MSCs.

growth factors. In the past, the roles of PDGF-BB focused on stimulating angiogenesis, stabilizing neovascularization, mobilizing MSCs, promoting vascular healing, promoting tissue repair, and protecting numerous cell types from apoptosis [36–38]. PDGF-BB protects ITP-MSCs against apoptosis, senescence, and immunoregulatory deficiency via the p53/p21 pathway [14]. Cannabinoid receptor 2 mediates the anti-inflammatory and immunomodulatory properties of human BM-MSCs. Studies have confirmed that the combination of cannabinoid receptor 2 stimulation and dexamethasone attenuates apoptosis via Bcl-2, thus restoring the immunomodulatory properties of ITP-MSCs [12]. The second mechanism involves a decrease in the activity of the phosphatidylinositol 3 kinase (PI3K)/Akt pathway. miR-98-5p is associated with defective ITP-MSCs in a mouse model and has been found to negatively regulate the PI3K/Akt pathway via insulin-like growth factor 2 (IGF-2). All-trans retinoic acid (ATRA) is a bioactive metabolite of vitamin A that plays important roles in cell proliferation, cell differentiation, apoptosis, and embryonic development. ATRA has been found to protect MSCs from apoptosis by downregulating the PI3K/Akt pathway [34]. miR-98-5p upregulates p53 and induces apoptosis in ITP-MSCs by inhibiting β -transducer repeat sequence protein (β -TrCP)-dependent p53 ubiquitination [34]. The third mechanism involves the TNFAIP3/NF- κ B/

SMAD7 pathway. Alpha-induced protein3 (TNFAIP3) acts as a negative-feedback regulator of NF- κ B activation, and its expression is diminished in ITP-MSCs. TNF- α activates the NF- κ B signaling pathway in ITP-MSCs through a series of regulatory mechanisms involving NF- κ B kinase subunit beta (IKK β) kinase and TNFAIP3. Other mechanisms involve phosphorylation changes in NFKBIA and the nuclear translocation and phosphorylation of the NF- κ B subunit RelA. Apoptosis is ultimately induced in ITP-MSCs through the activation of the NF- κ B signaling pathway and the attenuation of TGF- β signaling pathway activity in response to high expression of Smad7 [13].

Immune Deficiency

The pathogenesis of ITP involves humoral immunity and includes increased platelet destruction by autoantibodies directed against platelet glycoproteins. The inhibition of anti-glycoprotein IIb-IIIa peptides presented by self-reactive CD4⁺ T cells recognizing antigen-presenting cells is the key link between activation and maintenance of the response of pathogenic anti-platelet autoantibodies [14]. DCs from patients with ITP promote B-cell proliferation and antibody production via the B-lymphocyte stimulator [39].

In order to better understand the immune disorders of ITP-MSCs, the effects of ITP-MSCs on 7 immune cells

Fig. 2. Mechanism of regulation of immune cell dysfunction in ITP-MSCs.

are summarized in Figure 2. CD4⁺ CD25⁺ Treg cells are important immunomodulatory cells. Patients with ITP exhibit defects in the quantity and function of CD4⁺ CD25⁺ Treg cells [14]. CD4⁺ CD25⁺ Treg cells suppress the proliferation of Treg cells and downregulate the expression of Foxp3 at the mRNA level. In cell experiments, ITP-MSCs have been shown to inhibit the secretion of IL-2 and IFN- γ by helper T cells (Th1 cells), thus promoting the secretion of IL-4 and IL-10 by Th2 cells and impairing the ability to induce inactive Treg cells [40]. Exogenous BM-MSCs effectively increase the platelet count, decrease the proliferation of Treg cells, diminish the ratio of inhibitory cytokines (TGF- β 1 and IL-10)/Treg cells, and restore the expression of Foxp3 [32]. Defects have also been reported in the number and function of suppressor T (Ts) cells in patients with ITP. CD8⁺CD28[−] T cells are a subset of Ts cells. Deficiency in CD28, a co-stimulatory factor for the activation and proliferation of T cells, may diminish immune function [41]. Ts cells up-regulate the expression of the leukocyte immunoglobulin-like receptors B2 and B4, thus inhibiting immune activation by inducing tolerant antigen-presenting cells (such as DCs). Ts cells also directly inhibit the activation of T cells by secreting the inhibitory cytokine interleukin-10 (IL-10) and by contacting cells, thereby inhibiting immune activation [42]. ATRA preconditioning promoted BM-MSCs' induction of Ts-cell percentages and UC-

MSCs efficiently improved ITP Ts-cell numbers and dysfunction [43].

DCs, the most powerful antigen-presenting cells, play major roles in initiating and controlling the size and quality of the acquired immune response. DCs induce peripheral tolerance via mechanisms such as the secretion of the clonal deletion of autoreactive T cells, soluble factors, and feedback control of Tregs. BM-MSCs are capable of modulating the T-cell responses indirectly by promoting the tolerogenic properties of DCs [44]. ITP-MSCs are also deficient in the ability to induce CD34(+)-regDCs. The number of megakaryocyte (MK) cells in patients with ITP is significantly lower than that in BM-MSCs when CD34⁺ DC cells are co-cultured with BM-MSCs, thus indicating an impaired ability of ITP-MSCs to support the differentiation of megakaryocytes [13]. ATRA has been shown to repair the deficiency of CD34(+)-regDCs induced by MSCs in ITP via the Notch-1/Jagge-1 pathway [40]. As an immunomodulator, thalidomide restores the regulatory effect of MSC-induced DC in patients with ITP by up-regulating Oct3/4 and TGF- β 1 and by downregulating caspase-8 and caspase-10 [45]. Moreover, Nestin(+) BM-MSCs are involved in the occurrence of ITP via perturbing the MK distribution and via the CXCL12/CXCR4 axis [46]. Unfortunately, studies on the interaction between ITP-MSCs and natural killer cells have yet to be performed.

Others

The occurrence of ITP may be associated with single-nucleotide polymorphisms, nucleotides, autophagy, the exocrine system, and complement activation. ITP-MSCs contain several G/C polymorphisms in the pre-miR-146a sequence, which lead to decreased production of miR-146a [47]. The underlying mechanism has been demonstrated to involve the downregulation of BM-MSCs by the expression of miRNAs in ITP-MSCs [47]. Studies have investigated the crosstalk between autophagy and platelets, although little is known about the relationship between autophagy and ITP-MSCs. Autophagy defects occur in ITP through 2 pathways: the deletion of autophagy-related gene 7 (ATG7) and signaling abnormalities caused by the overexpression of the mechanistic target of rapamycin kinase [48]. Members of these pathways, such as CD41 and CD61, are considered important markers of hematopoietic stem cells because they affect the differentiation of megakaryocytes and ultimately limit the function and number of platelets. The differentiation of bone marrow hematopoietic stem cells into megakaryocytes is blocked by impaired autophagy, although the effect of BM-MSCs on autophagy remains unknown. Impaired autophagy could potentially influence the proliferation and apoptosis of BM-MSCs and negatively influence differentiation. These interesting possibilities are worthy of further exploration. The exocrine bodies of MSCs modulate immune-related processes by carrying the bioactive substances of mother cells. At present, the use of the exocrine body instead of MSCs has mainly focused on immune diseases such as multiple sclerosis, systemic lupus erythematosus, graft-versus-host disease, and type 1 diabetes [49–51]. However, little is known about the exocrine bodies of BM-MSCs in ITP. The enhancement of peripheral complement activation is a pathogenic factor underlying ITP. During the deposition of complement, the number of BM-MSCs decreases, and this is accompanied by dysfunction, including diminished function and increased apoptosis. ATRA protects complement-positive MSCs from dysfunction and apoptosis by upregulating DNA hypermethylation of the IL-1 β promoter, which is helpful in restoring the thrombopoietic niche [52].

The Clinical Application of BM-MSCs in Patients with ITP

We investigated the clinical application of stem cells by searching the US National Library of Medicine database for relevant clinical trials published through February 2021 (<http://www.clinicals.gov>). We identified 981 studies by using the keyword “MSCs” and 187 studies by using the

keyword “BM-MSCs.” Most studies involving “BM-MSCs” had completed phase 1 (safety) and phase 2 (efficacy) trials using allogeneic and autologous bone marrow stem cells. Phase 3/4 trials had been completed for acute leukemia, cardiovascular disease, rheumatoid arthritis, spinal cord injury, and other diseases. BM-MSCs account for only 0.01% of all mononuclear cells and exhibit a low proliferation ability. Challenges clearly remain in implementing BM-MSC-based therapy. Several problems are associated with amplification, including genetic instability, senescence, and transformation [53]. In the past, BM-MSCs were usually amplified through 4 methods: co-culture with related cell growth factors, drug stimulation, physical and mechanical stimulation, and the editing of genes associated with cell proliferation. Traditional methods have been unable to alleviate the problem of poor proliferation. However, the development of 3D culture methods has improved the ability to amplify BM-MSCs. The BM-MSCs amplified through this method are superior to those produced by traditional culture methods in terms of secretion and immunogenicity, and they also have been found to improve the body weight, spleen index, platelet index, and bone marrow homing ability in a mouse model of ITP [54]. Cytological experiments performed in vitro have also shown that BM-MSCs regulate the balance of Th1/Th2 cell activity in ITP, thereby inhibiting the secretion of IL-2 and IFN- γ by Th1 cells and promoting the secretion of IL-4 by Th2 cells, which then upregulate CD4 $^{+}$ CD25 $^{+}$ T cells and induce immune tolerance [55]. In vivo, BM-MSCs have been found to increase platelet count in a mouse model of ITP. BM-MSCs have also demonstrated excellent immunomodulatory ability for treating refractory ITP; these cells effectively regulate the balance of immune cells and reshape functional activity in refractory cases [56]. Unfortunately, attempts have not yet been made to treat patients with ITP with exogenous BM-MSCs.

UC-MSCs in ITP

The umbilical cord is an easily accessible source from which MSCs can be easily isolated and amplified. The newly derived UC-MSCs exhibit a more favorable immunogenic and stronger immunosuppressive potential than mature BM-MSCs [57]. In vitro studies have shown that platelet counts are increased in patients with ITP after treatment with UC-MSCs. Two main mechanisms underlie these observations. First, UC-MSCs increase platelet production by increasing the level of thrombopoietin,

downregulating the expression of co-stimulatory molecules (CD80, CD40L, and Fas), stimulating Th1/Th2/Treg imbalance, and reversing megakaryocyte dysfunction [58]. Second, UC-MSCs suppress the proliferation of autoreactive B and T lymphocytes, thus destroying autologous platelets and regulating the secretion of anti-platelet antibodies [59].

The efficacy of UC-MSCs in the treatment of ITP has been further confirmed by clinical case reports. For example, Mu et al. [60] have reported a unique case of severe Henoch-Schonlein purpura complicated by thrombocytopenia in a 12-year-old boy whose bone marrow sections showed MK maturation disorder. After the transplantation of Huc-MSCs, the patient's platelet count gradually increased to $15 \times 10^2/\text{L}$. Another 4 patients with chronic refractory ITP received Huc-MSCs transplantation, 3 of whom achieved complete remission. Within 12 months of follow-up, 1 patient achieved complete remission. Within 24 months after Huc-MSC transplantation, no serious adverse events were reported [61]. Consequently, UC-MSCs may offer a new therapeutic approach for treating patients with ITP. However, further work is needed to investigate the long-term efficacy and safety profile of this approach.

ASCs in ITP

In 2009, Fang et al. [62] reported successful treatment of chronic ITP with human ASCs. The patient achieved complete, hormone-independent platelet remission but relapsed 3 months later. Nonetheless, this study has provided insight into the role of ASCs in ITP. ASCs are easily attached to plastic culture bottles and expanded in vitro [63]. These cells can also differentiate into multiple cell lines and thus have the potential to repair, maintain, or strengthen various tissues. ASCs are easily obtained from adipose tissue through minimally invasive and safe surgical methods. Therefore, autologous ASCs have high potential for application in regenerative medicine and the treatment of organs that have been damaged by injury and disease. ASCs may also provide an efficient therapeutic option for the treatment of hematological diseases, such as ITP and refractory pure red cell aplastic anemia [64]. In murine transplantation models, the levels of PLT increase in TP mice treated with ASCs; the levels of Th1 (IL-2 and IFN- γ) and Th17 (IL-17) decrease, while the levels of Th2 (IL-4 and IL-10) and Th3 (TGF- β 1) increase [33]. These results provide scientific evidence supporting the transplantation of ASCs.

Conclusion

Herein, we have described the underlying mechanisms and clinical applications of 3 sources of MSCs in ITP. The deficiency and immune tolerance of ITP-MSCs are critical factors in the pathogenesis of ITP. However, understanding of the mechanisms and treatment of patients with ITP remains limited. Whether MSCs can effectively regulate the balance of immune cells, improve functional activity, and enhance immune tolerance in patients with refractory ITP remain to be elucidated. In vitro cell experiments, animal experiments, and clinical case reports indicate that these 3 methods are effective. Unfortunately, the benefit for patients is rather limited as at present, owing to the lack of a means to identify the patients most likely to benefit from this regimen. We propose that BM-MSCs are most suitable for patients with ITP because of the ability of these cells to proliferate, differentiate, and regulate the immune system. If a more effective method can be devised to amplify BM-MSCs, these cells may become the best option for clinical treatment in the future.

Conflict of Interest Statement

The authors declare that they have no competing interests.

Funding Sources

This work was supported by the International Collaboration Fund from the National Science and Technology Committee of China (No. 2011DFA32820), the Innovation Fund Project in Jiangxi Province (No. YC2020-B031), and the National Natural Science Fund Project (No. 81460037).

Author Contributions

Yue He and Wei Lu drafted the manuscript, Dexiang Ji revised the manuscript, and Guoan Chen critically revised the manuscript. All authors approved the final version and are responsible for the accuracy and integrity of all aspects of the manuscript.

References

- Purnamawati K, Ong JA, Deshpande S, Tan WK, Masurkar N, Low JK, et al. The importance of sex stratification in autoimmune disease biomarker research: a systematic review. *Front Immunol*. 2018;9:1208.
- Nomura S, Matsuzaki T, Ozaki Y, Yamaoka M, Yoshimura C, Katsura K, et al. Clinical significance of HLA-DRB1*0410 in Japanese patients with idiopathic thrombocytopenic purpura. *Blood*. 1998;91(10):3616–22.
- Amorim DM, Silveira VS, Scrideli CA, Queiroz RG, Tone LG. Fcγ receptor gene polymorphisms in childhood immune thrombocytopenic purpura. *J Pediatr Hematol Oncol*. 2012;34(5):349–52.
- Zhang D, Zhang X, Ge M, Xuan M, Li H, Yang Y, et al. The polymorphisms of T cell-specific TBX21 gene may contribute to the susceptibility of chronic immune thrombocytopenia in Chinese population. *Hum Immunol*. 2014;75(2):129–33.
- Ku FC, Tsai CR, Der Wang J, Wang CH, Chang TK, Hwang WL. Stromal-derived factor-1 gene variations in pediatric patients with primary immune thrombocytopenia. *Eur J Haematol*. 2013;90(1):25–30.
- Pehlivan M, Okan V, Sever T, Balci SO, Yilmaz M, Babacan T, et al. Investigation of TNF-α, TGF-β1, IL-10, IL-6, IFN-γ, MBL, GPIIb/IIIa, and IL1A gene polymorphisms in patients with idiopathic thrombocytopenic purpura. *Platelets*. 2011;22(8):588–95.
- Rocha AM, De Souza C, Rocha GA, De Melo FF, Saraiva IS, Clementino NC, et al. IL1RN VNTR and IL2-330 polymorphic genes are independently associated with chronic immune thrombocytopenia. *Br J Haematol*. 2010;150(6):679–84.
- Zhang W, Nardi MA, Borkowsky W, Li Z, Karpatsin S. Role of molecular mimicry of hepatitis C virus protein with platelet GPIIb/IIIa in hepatitis C-related immunologic thrombocytopenia. *Blood*. 2009;113(17):4086–93.
- Bettaieb A, Oksenhendler E, Duedari N, Bierling P. Cross-reactive antibodies between HIV-gp120 and platelet gpIIIa (CD61) in HIV-related immune thrombocytopenic purpura. *Clin Exp Immunol*. 1996;103(1):19–23.
- Boughton BJ, Simpson AW, Bolt C, Buchan A, McLeish P. Platelet membrane glycoprotein IIb/IIIa has sequence homologies with human virus proteins and synthetic viral peptides inhibit Anti-GPIIb/IIIa antibodies in autoimmune thrombocytopenic purpura. *Platelets*. 1995;6(2):75–82.
- Grimaldi-Bensouda L, Michel M, Aubrun E, Leighton P, Viallard JF, Adoue D, et al. A case-control study to assess the risk of immune thrombocytopenia associated with vaccines. *Blood*. 2012;120(25):4938–44.
- Rossi F, Tortora C, Palumbo G, Punzo F, Argenziano M, Casale M, et al. CB2 receptor stimulation and dexamethasone restore the anti-inflammatory and immune-regulatory properties of mesenchymal stromal cells of children with immune thrombocytopenia. *Int J Mol Sci*. 2019;20.
- He Y, Xu LL, Feng FE, Wang QM, Zhu XL, Wang CC, et al. Mesenchymal stem cell deficiency influences megakaryocytopoiesis through the TNFAIP3/NF-κB/SMAD pathway in patients with immune thrombocytopenia. *Br J Haematol*. 2018;180(3):395–411.
- Zhang JM, Feng FE, Wang QM, Zhu XL, Fu HX, Xu LP, et al. Platelet-Derived growth Factor-BB protects mesenchymal stem cells (MSCs) derived from immune thrombocytopenia patients against apoptosis and senescence and maintains MSC-Mediated immunosuppression. *Stem Cells Transl Med*. 2016;5(12):1631–43.
- Bianco P, Robey PG, Simmons PJ. Mesenchymal stem cells: revisiting history, concepts, and assays. *Cell Stem Cell*. 2008;2(4):313–9.
- Jin HJ, Bae YK, Kim M, Kwon SJ, Jeon HB, Choi SJ, et al. Comparative analysis of human mesenchymal stem cells from bone marrow, adipose tissue, and umbilical cord blood as sources of cell therapy. *Int J Mol Sci*. 2013;14(9):17986–8001.
- Kern S, Eichler H, Stoeve J, Klüter H, Bieback K. Comparative analysis of mesenchymal stem cells from bone marrow, umbilical cord blood, or adipose tissue. *Stem Cells*. 2006;24(5):1294–301.
- Rada T, Reis RL, Gomes ME. Distinct stem cells subpopulations isolated from human adipose tissue exhibit different chondrogenic and osteogenic differentiation potential. *Stem Cell Rev Rep*. 2011;7(1):64–76.
- Zuk PA, Zhu M, Ashjian P, De Ugarte DA, Huang JI, Mizuno H, et al. Human adipose tissue is a source of multipotent stem cells. *Mol Biol Cell*. 2002;13(12):4279–95.
- De Ugarte DA, Morizono K, Elbarbary A, Alfonso Z, Zuk PA, Zhu M, et al. Comparison of multi-lineage cells from human adipose tissue and bone marrow. *Cells Tissues Organs*. 2003;174(3):101–9.
- Sakaguchi Y, Sekiya I, Yagishita K, Muneta T. Comparison of human stem cells derived from various mesenchymal tissues: Superiority of synovium as a cell source. *Arthritis Rheum*. 2005;52(8):2521–9.
- Bochev I, Elmadjian G, Kyurkchiev D, Tzvetanov L, Altankova I, Tivchev P, et al. Mesenchymal stem cells from human bone marrow or adipose tissue differently modulate mitogen-stimulated B-cell immunoglobulin production in vitro. *Cell Biol Int*. 2008;32(4):384–93.
- De Ugarte DA, Alfonso Z, Zuk PA, Elbarbary A, Zhu M, Ashjian P, et al. Differential expression of stem cell mobilization-associated molecules on multi-lineage cells from adipose tissue and bone marrow. *Immunol Lett*. 2003;89(2–3):267–70.
- Ivanova-Todorova E, Bochev I, Mourdjeva M, Dimitrov R, Bukarev D, Kyurkchiev S, et al. Adipose tissue-derived mesenchymal stem cells are more potent suppressors of dendritic cells differentiation compared to bone marrow-derived mesenchymal stem cells. *Immunol Lett*. 2009;126(1–2):37–42.
- Friedenstein AJ, Piatetzky-Shapiro II, Petrakova KV. Osteogenesis in transplants of bone marrow cells. *J Embryol Exp Morphol*. 1966;16(3):381–90.
- Lazarus HM, Koc ON, Devine SM, Curtin P, Maziarz RT, Holland HK, et al. Cotransplantation of HLA-identical sibling culture-expanded mesenchymal stem cells and hematopoietic stem cells in hematologic malignancy patients. *Biol Blood Marrow Transplant*. 2005;11(5):389–98.
- Le Blanc K, Rasmusson I, Sundberg B, Götherström C, Hassan M, Uzunel M, et al. Treatment of severe acute graft-versus-host disease with third party haploidentical mesenchymal stem cells. *Lancet*. 2004;363(9419):1439–41.
- Sun L, Akiyama K, Zhang H, Yamaza T, Hou Y, Zhao S, et al. Mesenchymal stem cell transplantation reverses multiorgan dysfunction in systemic lupus erythematosus mice and humans. *Stem Cells*. 2009;27(6):1421–32.
- Augello A, Tasso R, Negrini SM, Cancedda R, Pennesi G. Cell therapy using allogeneic bone marrow mesenchymal stem cells prevents tissue damage in collagen-induced arthritis. *Arthritis Rheum*. 2007;56(4):1175–86.
- Liang J, Zhang H, Hua B, Wang H, Wang J, Han Z, et al. Allogeneic mesenchymal stem cells transplantation in treatment of multiple sclerosis. *Mult Scler*. 2009;15(5):644–6.
- Zappia E, Casazza S, Pedemonte E, Benvenuto F, Bonanni I, Gerdoni E, et al. Mesenchymal stem cells ameliorate experimental autoimmune encephalomyelitis inducing T-cell anergy. *Blood*. 2005;106(5):1755–61.
- Zhang P, Zhang G, Liu X, Liu H, Yang P, Ma L. Mesenchymal stem cells improve platelet counts in mice with immune thrombocytopenia. *J Cell Biochem*. 2019.
- Xiao J, Zhang C, Zhang Y, Zhang X, Zhao J, Liang J, et al. Transplantation of adipose-derived mesenchymal stem cells into a murine model of passive chronic immune thrombocytopenia. *Transfusion*. 2012;52(12):2551–8.
- Wang Y, Zhang J, Su Y, Wang C, Zhang G, Liu X, et al. MiRNA-98-5p targeting IGF2BP1 induces mesenchymal stem cell apoptosis by modulating PI3K/Akt and p53 in immune thrombocytopenia. *Mol Ther Nucleic Acids*. 2020;20:764–76.
- Zhang JM, Zhu XL, Xue J, Liu X, Long Zheng X, Chang YJ, et al. Integrated mRNA and miRNA profiling revealed deregulation of cellular stress response in bone marrow mesenchymal stem cells derived from patients with immune thrombocytopenia. *Funct Integr Genomics*. 2018;18(3):287–99.

- 36 Hollinger JO, Hart CE, Hirsch SN, Lynch S, Friedlaender GE. Recombinant human platelet-derived growth factor: biology and clinical applications. *J Bone Joint Surg Am.* 2008;90 Suppl 1:48–54.
- 37 Alvarez RH, Kantarjian HM, Cortes JE. Biology of platelet-derived growth factor and its involvement in disease. *Mayo Clin Proc.* 2006;81(9):1241–57.
- 38 Fiedler J, Röderer G, Günther KP, Brenner RE. BMP-2, BMP-4, and PDGF-bb stimulate chemotactic migration of primary human mesenchymal progenitor cells. *J Cell Biochem.* 2002;87(3):305–12.
- 39 Yu H, Liu Y, Han J, Yang Z, Sheng W, Dai H, et al. TLR7 regulates dendritic cell-dependent B-cell responses through BlyS in immune thrombocytopenic purpura. *Eur J Haematol.* 2011;86(1):67–74.
- 40 Xu LL, Fu HX, Zhang JM, Feng FE, Wang QM, Zhu XL, et al. Impaired function of bone marrow mesenchymal stem cells from immune thrombocytopenia patients in inducing regulatory dendritic cell differentiation through the Notch-1/Jagged-1 signaling pathway. *Stem Cells Dev.* 2017;26(22):1648–61.
- 41 Cortesini R, LeMaout J, Ciubotariu R, Cortesini NS. CD8+CD28– T suppressor cells and the induction of antigen-specific, antigen-presenting cell-mediated suppression of Th reactivity. *Immunol Rev.* 2001;182:201–6.
- 42 Reiser J, Banerjee A. Effector, memory, and dysfunctional CD8(+) t cell fates in the anti-tumor immune response. *J Immunol Res.* 2016;2016:8941260.
- 43 Li H, Guan Y, Sun B, Dou X, Liu X, Xue F, et al. Role of bone marrow-derived mesenchymal stem cell defects in CD8(+) CD28(–) suppressor T-lymphocyte induction in patients with immune thrombocytopenia and associated mechanisms. *Br J Haematol.* 2020.
- 44 Osorio F, Fuentes C, López MN, Salazar-Onfray F, González FE. Role of dendritic cells in the induction of lymphocyte tolerance. *Front Immunol.* 2015;6:535.
- 45 Falco P, Bertola A, Bringhen S, Cavallo F, Boccadoro M, Palumbo A. Successful management of immune thrombocytopenic purpura with thalidomide in a patient with multiple myeloma. *Hematol J.* 2004;5(5):456–7.
- 46 Wang M, Feng R, Zhang JM, Xu LL, Feng FE, Wang CC, et al. Dysregulated megakaryocyte distribution associated with nestin(+) mesenchymal stem cells in immune thrombocytopenia. *Blood Adv.* 2019;3:1416–28.
- 47 Jazdzewski K, Murray EL, Franssila K, Jarzab B, Schoenberg DR, de la Chapelle A. Common SNP in pre-miR-146a decreases mature miR expression and predisposes to papillary thyroid carcinoma. *Proc Natl Acad Sci U S A.* 2008;105(20):7269–74.
- 48 Sun R-J, Shan N-N. Megakaryocytic dysfunction in immune thrombocytopenia is linked to autophagy. *Cancer Cell Int.* 2019;19(1):59.
- 49 Zhang S, Chu WC, Lai RC, Lim SK, Hui JH, Toh WS. Exosomes derived from human embryonic mesenchymal stem cells promote osteochondral regeneration. *Osteoarthritis Cartilage.* 2016;24(12):2135–40.
- 50 Kordelas L, Rebmann V, Ludwig AK, Radtke S, Ruesing J, Doeppner TR, et al. MSC-derived exosomes: a novel tool to treat therapy-refractory graft-versus-host disease. *Leukemia.* 2014;28(4):970–3.
- 51 Mokarizadeh A, Delirez N, Morshedi A, Mosayebi G, Farshid AA, Mardani K. Microvesicles derived from mesenchymal stem cells: potent organelles for induction of tolerogenic signaling. *Immunol Lett.* 2012;147(1–2):47–54.
- 52 Zhu X, Wang Y, Jiang Q, Jiang H, Lu J, Wang Y, et al. All-trans retinoic acid protects mesenchymal stem cells from immune thrombocytopenia by regulating the complement-interleukin-1 β loop. *Haematologica.* 2019;104(8):1661–75.
- 53 Neri S. Genetic stability of mesenchymal stromal cells for regenerative medicine applications: a fundamental biosafety aspect. *Int J Mol Sci.* 2019;20(10):2406.
- 54 Gong X, Sun D, Li Z, Shi Q, Li D, Ju X. Three-dimensional culture of umbilical cord mesenchymal stem cells effectively promotes platelet recovery in immune thrombocytopenia. *Biol Pharm Bull.* 2020;43(7):1052–60.
- 55 Tao Y, Song D, Zhang F, Ren S, Zhang H, Sun L. Transplantation of bone-marrow-derived mesenchymal stem cells into a murine model of immune thrombocytopenia. *Blood Coagul Fibrinolysis.* 2017;28(8):596–601.
- 56 Zhang D, Li H, Ma L, Zhang X, Xue F, Zhou Z, et al. The defective bone marrow-derived mesenchymal stem cells in patients with chronic immune thrombocytopenia. *Autoimmunity.* 2014;47(8):519–29.
- 57 Romanov YA, Svintsitskaya VA, Smirnov VN. Searching for alternative sources of post-natal human mesenchymal stem cells: candidate MSC-like cells from umbilical cord. *Stem Cells.* 2003;21(1):105–10.
- 58 Ma L, Zhou Z, Zhang D, Yang S, Wang J, Xue F, et al. Immunosuppressive function of mesenchymal stem cells from human umbilical cord matrix in immune thrombocytopenia patients. *Thromb Haemost.* 2012;107(5):937–50.
- 59 Qiu ZY, Yang SG, Chen ZP, Zhao QJ, Chen XL, Zhou ZP, et al. [Mesenchymal stem cells derived from human umbilical cord tissue modulate the secretion of antiplatelet antibody from splenocytes of ITP patients in vitro]. *Zhongguo Shi Yan Xue Ye Xue Za Zhi.* 2008;16(6):1372–5.
- 60 Mu K, Zhang J, Gu Y, Li H, Han Y, Cheng N, et al. Cord-derived mesenchymal stem cells therapy for liver cirrhosis in children with refractory Henoch-Schönlein purpura: A case report. *Medicine.* 2018;97:e13287.
- 61 Wang X, Yin X, Sun W, Bai J, Shen Y, Ao Q, et al. Intravenous infusion umbilical cord-derived mesenchymal stem cell in primary immune thrombocytopenia: a two-year follow-up. *Exp Ther Med.* 2017;13(5):2255–8.
- 62 Fang B, Song YP, Li N, Li J, Han Q, Zhao RC. Resolution of refractory chronic autoimmune thrombocytopenic purpura following mesenchymal stem cell transplantation: a case report. *Transplant Proc.* 2009;41(5):1827–30.
- 63 Li Q, Zhao F, Li Z, Duan X, Cheng J, Zhang J, et al. Autologous fractionated adipose tissue as a natural biomaterial and novel one-step stem cell therapy for repairing articular cartilage defects. *Front Cell Dev Biol.* 2020;8:694.
- 64 Ebrahimian TG, Pouzoulet F, Squiban C, Buard V, André M, Cousin B, et al. Cell therapy based on adipose tissue-derived stromal cells promotes physiological and pathological wound healing. *Arterioscler Thromb Vasc Biol.* 2009;29(4):503–10.