Chung Shan Medical Journal 2019; 30:67-76

Review Article

The Role of Mesenchymal Stem Cells in Severe Aplastic Anemia: from Bench to Bedside

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Severe aplastic anemia (SAA), a rare but potentially life-threatening disease, is the paradigm of bone marrow failure syndromes. Mesenchymal stem cells (MSCs) are crucial in providing the specialized bone marrow microenvironment required for hematopoiesis. Deficiency in the bone marrow microenvironment is a potential factor for the development of SAA. In this review article, we describe the association of MSCs and SAA, focusing mainly on the alterations in MSCs from patients with SAA. Further, we address the benefits of MSC infusion in SAA animal models and in human clinical applications.

Key words: bone marrow failure, mesenchymal stem cells, microenvironment, severe aplastic anemia

Introduction

Severe aplastic anemia (SAA), a rare but potentially life-threatening disease, is the paradigm of bone marrow (BM) failure syndromes. While there have been significant advances in the management of this disease, the etiology of SAA has not been completely elucidated. Mesenchymal stem cells (MSCs), first described by Friedenstein in 1966 [1], play an important role in providing the specialized BM microenvironment needed for survival and differentiation of hematopoietic stem cells (HSCs). Deficiency in or dysfunction of the BM microenvironment could predispose patients to the development of SAA.

In this review article, we describe the association of MSCs and SAA, focusing mainly on the alterations in MSCs from patients with SAA. We address the benefits of MSC infusion in SAA animal models and in human clinical applications.

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Mesenchymal Stem Cells

There are three main cellular systems in the BM: hematopoietic, endothelial, and stromal [2]. The stromal cell system, first proposed by Owen in 1985 [3], provides the essential microenvironment for hematopoiesis in the BM. MSCs, a natural component of stromal BM, constitute a small percentage of cells, about one in 3.4×10^4, that

support hematopoiesis [4]. As the so-called stem cells, MSCs maintain a level of self-renewal and are capable of differentiating into a variety of mesenchyme-lineage cells. In the BM, MSC-derived stromal cells establish an appropriate scaffold and a complex network of cytokines, growth factors, adhesion molecules, and extracellular matrix components [5,6]. Many studies have demonstrated the positive effects of MSCs on in vitro HSC expansion [7-11]. In animal models, infusion of MSCs has been shown to enhance HSC engraftment during transplantation [11-14].

While BM remains a traditional source of MSCs, they can be isolated from a variety of adult and fetal tissues, including peripheral blood, adipose tissue, dental pulp, placenta, amniotic fluid, amniotic membrane, Wharton's jelly of umbilical cord, and cord blood [15-22]. Generally, the frequency of MSCs is higher in fetal tissues. Despite inconsistent definitions among investigators, MSCs are characterized by their in vitro growth pattern, expressions of specific surface markers, and multipotent differentiation potential according to the International Society for Cellular Therapy criteria [23]. Having a high proliferative capacity, MSCs possess a spindle-shaped fibroblastic morphology in vitro. In addition, they express a panel of surface markers positive for mesenchymal antigens (CD105 and CD73) and adhesion molecules (CD29, CD44, CD106, and CD90) and negative for hematopoietic antigens (CD34, CD45, CD14, CD11b, CD19, CD79α, and HLA-DR). With a broad differentiation potential, the most unique property for identifying MSCs is their capacity for tri-lineage mesenchymal differentiation into bone, fat, and cartilage.

MSCs possess profound immunomodulatory effects. Many studies have demonstrated that MSCs can mediate immunomodulatory effects by interacting with immune cells from both innate (dendritic cells and natural killer cells) and adaptive (T cells and B cells) systems [24-33]. Along with modifying the secretion of various cytokines, MSCs can orchestrate the direct cell-to-cell contact and microenvironment between the interacting populations [34,35]. Moreover, MSCs express HLA-class I, but not class II molecules. Many in vitro and in vivo studies have demonstrated that MSCs can escape recognition by the alloreactive immune system [35-37]. Due to low immunogenicity, MSCs possess great utility in clinical cell-based therapy.

Severe Aplastic Anemia

SAA is a rare but potentially life-threatening disease with an annual incidence of one to six per million [38-42]. The incidence is higher in Asia than in the West [43, 44]. SAA is characterized by pancytopenia resulting from hypocellular BM without infiltration or fibrosis. The criteria for SAA diagnosis include BM cellularity of less than 25% with at least two of the following conditions: absolute neutrophil count less than 0.5×10^9/L, platelet count less than 20×10^9/L, and reticulocyte count less than 1% [38,45-47].

SAA is considered heterogeneous in origin. First described by Ehrlich as an "empty" appearance of the BM in a pregnant woman [48], precipitating factors have been investigated based on patient history. A variety of environmental and host factors have also been described. However, a specific cause cannot be identified in most patients, and this is referred to as idiopathic SAA. Immune-mediated HSC destruction is the most widely accepted pathogenesis of idiopathic SAA [44]. The impact of T cell attack on BM has been demonstrated in vitro and in vivo [49-54]. However, non-immune etiopathogenesis has been inferred from a failure to respond to immunosuppressive therapy (IST). Primary HSC deficiencies, including decrease in number and dysfunction, are potential factors associated with the development of SAA [55-58]. More recently, deficiency in or dysfunction of the BM microenvironment has been considered a factor in hematopoietic impairment in SAA patients.

SAA was almost uniformly fatal until the 1970s. Significant advances have been made in the management of this disease in recent decades. Definitive therapies for SAA are IST and HSC transplantation (HSCT), with comparable long-term survival for both modalities [59]. HSCT is considered the first choice for patients under 40 with a matched sibling donor, although this treatment is still limited by the long-term complications of graft-versus-host

disease and secondary malignancies [44,45]. Overall survival is 85-90% with best results in younger patients. Survival rate is only 50% for those over 40 [60]. IST is indicated for those 40 and over and those under 40 without a matched sibling donor. Immunosuppressive agents used in the treatment of SAA exert their action by reducing hematopoietic suppression from autoreactive immune cells [44]. Most specialists use an antithymocyte globulin-based regimen in combination with cyclosporine, which produces hematologic recovery in about 60-70% of patients [59]. As the outcome of unrelated donor HSCT is still not as favorable as that of a matched sibling donor, it is not recommended as first-line therapy for SAA, even in younger patients [59].

Alterations in Mesenchymal Stem Cells from Patients with Severe Aplastic Anemia

Insufficiency in the BM microenvironment, of which MSCs are an important element, could be a potential factor for hematopoietic impairment in SAA. Many studies have been conducted to investigate alterations in MSCs in patients with SAA. In vitro, SAA MSCs fail to grow complete confluent stromal layers to maintain HSCs [61-63]. Using basic properties as indicators [23], we found that SAA MSCs have poor potential for proliferation and differentiation [64]. In addition, the apoptotic rate of SAA MSCs is high [65]. The cellular elements in the BM microenvironment markedly decrease in patients with SAA, suggesting an association between impaired HSC niches and SAA [66]. Deficiency in immunomodulatory abilities of SAA MSCs, including suppression of CD4 (+) T cell proliferation, promotion of regulatory T cell expansion, and regulation of release of cytokines, has been demonstrated, indicating loss of immunoprotection in SAA BM [67,68]. Taken together, these findings provide evidence for functionally abnormal microenvironment in SAA BM, which may result from defects in MSCs.

Aberrant gene expression profiles have been found in SAA MSCs on microarray assay [65,69]. In addition, there is downregulation of *CXCL12* gene, which is important in the regulation of signaling pathways involving HSC survival, proliferation, adhesion, and migration [70-72], in SAA MSCs [69]. A low expression level of *FGF2* gene, which can preserve long-term repopulating ability of HSCs [73], has also been found in SAA MSCs [74]. Downregulation of *GATA-2* and overexpression of *PPARγ* gene in SAA MSCs explain the fatty marrow replacement in patients with SAA [75]. In addition to aberrant gene expressions, SAA MSCs have been found to overexpress membrane-bound IL-15, stimulating T cell proliferation [76]. These findings regarding the defects of SAA MSCs further confirm their abnormal biological properties and provide significant evidence for the possible mechanism of the destruction of the BM microenvironment in SAA.

Effects of Mesenchymal Stem Cell Infusion in Animal Models of Severe Aplastic Anemia

Several animal studies have been conducted to evaluate the potential effects of MSC infusion on SAA. In mice with irradiation-induced SAA, MSC infusion ameliorates cytopenia in the peripheral blood [77-80], enhances hematopoietic recovery in the BM [77-79,81], decreases apoptosis of BM cells [77-79], and improves survival [77,78,81]. The anti-apoptotic effects of MSCs are mediated through the PI3K/Akt pathway [79]. In addition to attenuating radiation-induced hematopoietic toxicity, MSCs provide immunoprotection by alleviating lymphocyte-mediated CFU-GM inhibition, enhancing regulatory T cell expansion, modulating the expression of T cell chemokine receptors, and skewing the Th1/Th2 balance toward anti-inflammatory Th2 polarization [81].

In mice with irradiation-induced SAA, co-infusion of MSCs with autologous HSCs facilitates hematopoietic reconstitution. The effect of MSCs is dose-dependent and associated with increased homing of transplanted HSCs, indicating that MSCs act as HSC carriers to assist in their migration and homing to BM niches [82]. To mimic the pathogenesis of immune-mediated HSC destruction in SAA, a radiation- and immunity-induced mouse SAA model was used to exam the role of MSCs in HSCT. Co-infusion of HSCs and MSCs was better than HSCT alone, with improved survival and increased hemoglobin levels in the

peripheral blood [83]. Despite different origins of MSCs, the above studies demonstrate benefits of MSC infusion in SAA animal models, with and without HSC co-infusion.

Clinical Applications of Mesenchymal Stem Cells in Severe Aplastic Anemia

Clinical applications of MSCs are evolving rapidly. With the capacity to differentiate into various connective tissue lineages, MSCs have been widely used in tissue repair and regeneration [84]. With the potential for hematopoietic support and immunomodulation in the BM microenvironment, MSCs can be used effectively and safely for enhancement of engraftment [85-88], prevention and treatment of graft failure [88,89], and management of graft-versus-host disease in HSCT [90-92].

SAA is a BM failure syndrome associated with immune-mediated HSC destruction and microenvironmental insufficiency in the BM. As previously described, MSCs are a promising therapy. In 2003, Fouillard et al. first reported one patient with idiopathic SAA who failed to respond to initial IST and was treated with MSC infusions. Recovery of the stromal niche and hematopoiesis in the BM was noted [93]. Later, a clinical trial using MSCs to treat refractory SAA was conducted [94]. Six of the 18 patients (33%) showed complete or partial responses to MSC treatment. A possible mechanism by which MSCs promote hematopoietic recovery is increase in regulatory T cells. In 2015, Cle et al. reported the results of a phase 1/2 trial, adding MSC infusions to the standard second-line treatment with IST for SAA [95]. Two of nine (22%) patients achieved partial hematologic responses at 6 months after therapy. The discrepancy in responses to MSC treatment in SAA may be explained by the diversity of the disease itself (e.g., patient age, etiology, severity, stage) and that of treatment methods (e.g., previous therapies, MSC dose per infusion, number of MSC infusions).

HSCT is a definitive therapy for SAA, especially for those refractory to first-line IST. However, there are significant risks of graft failure and graft-versus-host disease in allogeneic HSCT for SAA. With the potential for hematopoietic support and immunomodulation, additive MSC infusions may be beneficial in HSCT for SAA. Accordingly, we co-infused umbilical cord-derived MSCs into two patients with refractory SAA during unrelated donor HSCT [96]. Both patients achieved rapid hematopoietic recovery without severe infusion-related side effects. Consistent with our results, Si et al. reported that 37 patients with SAA who received HSCT and subsequent MSC infusion demonstrated prompt HSC homing and engraftment [97].

Further Clinical Considerations

Here, we address the association of MSCs with SAA and the use of MSCs in SAA animal models and patients with SAA. MSCs appear to be a promising therapy for SAA, but several important issues need to be addressed in terms of their clinical application. The first is the quality of MSCs for clinical use, such as available sources, the convenience of obtaining MSCs, the quality control of in vitro-culturing, and the appropriate passage number. The second is the use of MSCs for SAA, including disease status, patient characteristics, optimum cell dose, number of infusions, and concurrent treatment (HSCs, immunosuppressants). Finally, long-term safety needs to be assessed in future studies, although no severe short-term adverse effects of MSC infusion have been observed.

Acknowledgments

This study was supported by the Taiwan Ministry of Science and Technology (MOST 107-2314-B-040-019) and the Taiwan Ministry of Health and Welfare Clinical Trial and Research Center of Excellence (MOHW105-TDU-B-212-133019).

References

1. Friedenstein AJ, Piatetzky-Shapiro II, Petrakova KV: Osteogenesis in transplants of bone marrow cells. *Journal of Embryology and Experimental Morphology* 1966, 16:381-390.
2. Deans RJ, Moseley AB: Mesenchymal stem cells: biology and potential clinical uses. *Exp*

Hematol 2000, 28:875-884.
3. Owen M: Lineage of osteogenic cells and their relationship to the stromal system. *Bone and mineral research* 1985, 3:25.
4. Wexler SA, Donaldson C, Denning-Kendall P, Rice C, Bradley B, Hows JM: Adult bone marrow is a rich source of human mesenchymal 'stem' cells but umbilical cord and mobilized adult blood are not. *Brit J Haematol* 2003, 121:368-374.
5. Lazennec G, Jorgensen C: Concise review: adult multipotent stromal cells and cancer: risk or benefit? *Stem Cells* 2008, 26:1387-1394.
6. Chao YH, Wu HP, Chan CK, Tsai C, Peng CT, Wu KH: Umbilical cord-derived mesenchymal stem cells for hematopoietic stem cell transplantation. *J Biomed Biotechnol* 2012, 2012:759503.
7. Ito Y, Hasauda H, Kitajima T, Kiyono T: Ex vivo expansion of human cord blood hematopoietic progenitor cells using glutaraldehyde-fixed human bone marrow stromal cells. *J Biosci Bioeng* 2006, 102:467-469.
8. da Silva CL, Goncalves R, Crapnell KB, Cabral JMS, Zanjani ED, Almeida-Porada G: A human stromal-based serum-free culture system supports the ex vivo expansion/maintenance of bone marrow and cord blood hematopoietic stem/progenitor cells. *Exp Hematol* 2005, 33:828-835.
9. Li N, Feugier P, Serrurrier B, Latger-Cannard V, Lesesve J-F, Stoltz J-F, Eljaafari A: Human mesenchymal stem cells improve ex vivo expansion of adult human CD34+ peripheral blood progenitor cells and decrease their allostimulatory capacity. *Exp Hematol* 2007, 35:507-515.
10. Van Overstraeten-Schlogel N, Beguin Y, Gothot A: Role of stromal-derived factor-1 in the hematopoietic-supporting activity of human mesenchymal stem cells. *Eur J Haematol* 2006, 76:488-493.
11. Carrancio S, Blanco B, Romo C, et al.: Bone marrow mesenchymal stem cells for improving hematopoietic function: an in vitro and in vivo model. *PLoS ONE* 2011, 6:e26241.
12. Almeida-Porada G, Flake AW, Glimp HA, Zanjani ED: Cotransplantation of stroma results in enhancement of engraftment and early expression of donor hematopoietic stem cells in utero. *Exp Hematol* 1999, 27:1569-1575.
13. Almeida-Porada G, Porada CD, Tran N, Zanjani ED: Cotransplantation of human stromal cell progenitors into preimmune fetal sheep results in early appearance of human donor cells in circulation and boosts cell levels in bone marrow at later time points after transplantation. *Blood* 2000, 95:3620-3627.
14. Noort WA, Kruisselbrink AB, in't Anker PS, et al.: Mesenchymal stem cells promote engraftment of human umbilical cord blood-derived CD34(+) cells in NOD/SCID mice. *Exp Hematol* 2002, 30:870-878.
15. Minguell JJ, Erices A, Conget P: Mesenchymal stem cells. *Exp Biol Med* 2001, 226:507-520.
16. Campagnoli C, Roberts IA, Kumar S, Bennett PR, Bellantuono I, Fisk NM: Identification of mesenchymal stem/progenitor cells in human first-trimester fetal blood, liver, and bone marrow. *Blood* 2001, 98:2396-2402.
17. in 't Anker PS, Noort WA, Scherjon SA, et al.: Mesenchymal stem cells in human second-trimester bone marrow, liver, lung, and spleen exhibit a similar immunophenotype but a heterogeneous multilineage differentiation potential. *Haematologica* 2003, 88:845-852.
18. Guillot PV, Gotherstrom C, Chan J, Kurata H, Fisk NM: Human first-trimester fetal MSC express pluripotency markers and grow faster and have longer telomeres than adult MSC. *Stem Cells* 2007, 25:646-654.
19. Wang HS, Hung SC, Peng ST, et al.: Mesenchymal stem cells in the Wharton's jelly of the human umbilical cord. *Stem Cells* 2004, 22:1330-1337.
20. Zuk PA, Zhu M, Mizuno H, et al.: Multilineage cells from human adipose tissue: implications for cell-based therapies. *Tissue Eng* 2001, 7:211-228.
21. Antonucci I, Stuppia L, Kaneko Y, et al.: Amniotic fluid as a rich source of mesenchymal stromal cells for transplantation therapy. *Cell*

Transplant 2011, 20:789-795.
22. Kim J, Lee Y, Kim H, et al.: Human amniotic fluid-derived stem cells have characteristics of multipotent stem cells. *Cell Proliferation* 2007, 40:75-90.
23. Dominici M, Le Blanc K, Mueller I, et al.: Minimal criteria for defining multipotent mesenchymal stromal cells. *Cytotherapy* 2006, 8:315-317.
24. Bartholomew A, Sturgeon C, Siatskas M, et al.: Mesenchymal stem cells suppress lymphocyte proliferation in vitro and prolong skin graft survival in vivo. *Exp Hematol* 2002, 30:42-48.
25. Prevosto C, Zancolli M, Canevali P, Zocchi MR, Poggi A: Generation of CD4+ or CD8+ regulatory T cells upon mesenchymal stem cell-lymphocyte interaction. *Haematologica* 2007, 92:881-888.
26. Corcione A, Benvenuto F, Ferretti E, et al.: Human mesenchymal stem cells modulate B-cell functions. *Blood* 2006, 107:367-372.
27. Ramasamy R, Fazekasova H, Lam EWF, Soeiro I, Lombardi G, Dazzi F: Mesenchymal stem cells inhibit dendritic cell differentiation and function by preventing entry into the cell cycle. *Transplantation* 2007, 83:71-76.
28. Di Nicola M, Carlo-Stella C, Magni M, et al.: Human bone marrow stromal cells suppress T-lymphocyte proliferation induced by cellular or nonspecific mitogenic stimuli. *Blood* 2002, 99:3838-3843.
29. Glennie S, Soeiro I, Dyson PJ, Lam EWF, Dazzi F: Bone marrow mesenchymal stem cells induce division arrest anergy of activated T cells. *Blood* 2005, 105:2821-2827.
30. Sotiropoulou PA, Perez SA, Gritzapis AD, Baxevanis CN, Papamichail M: Interactions between human mesenchymal stem cells and natural killer cells. *Stem Cells* 2006, 24:74-85.
31. Jiang XX, Zhang Y, Liu B, Zhang SX, Wu Y, Yu XD, Mao N: Human mesenchymal stem cells inhibit differentiation and function of monocyte-derived dendritic cells. *Blood* 2005, 105:4120-4126.
32. Beyth S, Borovsky Z, Mevorach D, et al.: Human mesenchymal stem cells alter antigen-presenting cell maturation and induce T-cell unresponsiveness. *Blood* 2005, 105:2214-2219.
33. Rasmusson I, Le Blanc K, Sundberg B, Ringden O: Mesenchymal stem cells stimulate antibody secretion in human B cells. *Scand J Immunol* 2007, 65:336-343.
34. English K: Mechanisms of mesenchymal stromal cell immunomodulation. *Immunol Cell Biol* 2013, 91:19-26.
35. Pelagiadis I, Dimitriou H, Kalmanti M: Biologic characteristics of mesenchymal stromal cells and their clinical applications in pediatric patients. *J Pediatr Hematol Oncol* 2008, 30:301-309.
36. Jones BJ, McTaggart SJ: Immunosuppression by mesenchymal stromal cells: from culture to clinic. *Exp Hematol* 2008, 36:733-741.
37. Aggarwal S, Pittenger MF: Human mesenchymal stem cells modulate allogeneic immune cell responses. *Blood* 2005, 105:1815-1822.
38. Montane E, Ibanez L, Vidal X, et al.: Epidemiology of aplastic anemia: a prospective multicenter study. *Haematologica* 2008, 93:518-523.
39. Maluf EMCP, Pasquini R, Eluf JN, Kelly J, Kaufman DW: Aplastic anemia in Brazil: incidence and risk factors. *Am J Hematol* 2002, 71:268-274.
40. Mary JY, Baumelou E, Guiguet M: Epidemiology of aplastic anemia in France: a prospective multicentric study. *Blood* 1990, 75:1646-1653.
41. Davies SM, Walker DJ: Aplastic anaemia in the Northern Region 1971-1978 and follow-up of long term survivors. *Clin Lab Haematol* 1986, 8:307-313.
42. Szklo M, Sensenbrenner L, Markowitz J, Weida S, Warm S, Linet M: Incidence of aplastic anemia in metropolitan Baltimore: a population-based study. *Blood* 1985, 66:115-119.
43. Young NS, Kaufman DW: The epidemiology of acquired aplastic anemia. *Haematologica* 2008, 93:489-492.
44. Young NS, Calado RT, Scheinberg P: Current concepts in the pathophysiology and treatment of aplastic anemia. *Blood* 2006, 108:2509-2519.
45. Davies JK, Guinan EC: An update on the

management of severe idiopathic aplastic anaemia in children. *Brit J Haematol* 2007, 136:549-564.
46. Kurre P, Johnson FL, Deeg HJ: Diagnosis and treatment of children with aplastic anemia. *Pediatr Blood Cancer* 2005, 45:770-780.
47. Camitta BM, Thomas ED, Nathan DG, et al.: Severe aplastic anemia: a prospective study of the effect of early marrow transplantation on acute mortality. *Blood* 1976, 48:63-70.
48. Ehrlich P: Ueber einem Fall von Anämie mit Bemerkungen über regenerative Veränderungen des Knochenmarks. *Charité-Annalen* 1888, 13:300-309.
49. Dubey S, Shukla P, Nityanand S: Expression of interferon-gamma and tumor necrosis factor-alpha in bone marrow T cells and their levels in bone marrow plasma in patients with aplastic anemia. *Ann Hematol* 2005, 84:572-577.
50. Hara T, Ando K, Tsurumi H, Moriwaki H: Excessive production of tumor necrosis factor-alpha by bone marrow T lymphocytes is essential in causing bone marrow failure in patients with aplastic anemia. *Eur J Haematol* 2004, 73:10-16.
51. Chen J, Lipovsky K, Ellison FM, Calado RT, Young NS: Bystander destruction of hematopoietic progenitor and stem cells in a mouse model of infusion-induced bone marrow failure. *Blood* 2004, 104:1671-1678.
52. Bloom ML, Wolk AG, Simon-Stoos KL, Bard JS, Chen J, Young NS: A mouse model of lymphocyte infusion-induced bone marrow failure. *Exp Hematol* 2004, 32:1163-1172.
53. Sloand E, Kim S, Maciejewski JP, Tisdale J, Follmann D, Young NS: Intracellular interferon-gamma in circulating and marrow T cells detected by flow cytometry and the response to immunosuppressive therapy in patients with aplastic anemia. *Blood* 2002, 100:1185-1191.
54. Risitano AM, Maciejewski JP, Green S, Plasilova M, Zeng W, Young NS: In-vivo dominant immune responses in aplastic anaemia: molecular tracking of putatively pathogenetic T-cell clones by TCR beta-CDR3 sequencing. *Lancet* 2004, 364:355-364.
55. Rizzo S, Scopes J, Elebute MO, Papadaki HA, Gordon-Smith EC, Gibson FM: Stem cell defect in aplastic anemia: reduced long term culture-initiating cells (LTC-IC) in CD34+ cells isolated from aplastic anemia patient bone marrow. *Hematol J* 2002, 3:230-236.
56. Ball SE, Gibson FM, Rizzo S, Tooze JA, Marsh JC, Gordon-Smith EC: Progressive telomere shortening in aplastic anemia. *Blood* 1998, 91:3582-3592.
57. Scopes J, Daly S, Atkinson R, Ball SE, Gordon-Smith EC, Gibson FM: Aplastic anemia: evidence for dysfunctional bone marrow progenitor cells and the corrective effect of granulocyte colony-stimulating factor in vitro. *Blood* 1996, 87:3179-3185.
58. Marsh JC, Chang J, Testa NG, Hows JM, Dexter TM: The hematopoietic defect in aplastic anemia assessed by long-term marrow culture. *Blood* 1990, 76:1748-1757.
59. Scheinberg P, Young NS: How I treat acquired aplastic anemia. *Blood* 2012, 120:1185-1196.
60. Marsh JCW: Treatment of acquired aplastic anemia. *Haematologica* 2007, 92:2-5.
61. Holmberg LA, Seidel K, Leisenring W, Torok-Storb B: Aplastic anemia: analysis of stromal cell function in long-term marrow cultures. *Blood* 1994, 84:3685-3690.
62. Hotta T, Kato T, Maeda H, Yamao H, Yamada H, Saito H: Functional changes in marrow stromal cells in aplastic anaemia. *Acta Haematologica* 1985, 74:65-69.
63. Juneja HS, Gardner FH: Functionally abnormal marrow stromal cells in aplastic anemia. *Exp Hematol* 1985, 13:194-199.
64. Chao YH, Peng CT, Harn HJ, Chan CK, Wu KH: Poor potential of proliferation and differentiation in bone marrow mesenchymal stem cells derived from children with severe aplastic anemia. *Ann Hematol* 2010, 89:715-723.
65. Li J, Yang S, Lu S, et al.: Differential gene expression profile associated with the abnormality of bone marrow mesenchymal stem cells in aplastic anemia. *PLoS ONE* 2012, 7:e47764.
66. Wu L, Mo W, Zhang Y, et al.: Impairment of

hematopoietic stem cell niches in patients with aplastic anemia. *Int J Hematol* 2015, 102:645-653.

67. Bacigalupo A, Valle M, Podesta M, et al.: T-cell suppression mediated by mesenchymal stem cells is deficient in patients with severe aplastic anemia. *Exp Hematol* 2005, 33:819-827.
68. Li J, Lu S, Yang S, et al.: Impaired immunomodulatory ability of bone marrow mesenchymal stem cells on CD4(+) T cells in aplastic anemia. *Results Immunol* 2012, 2:142-147.
69. Chao YH, Wu KH, Chiou SH, et al.: Downregulated CXCL12 expression in mesenchymal stem cells associated with severe aplastic anemia in children. *Ann Hematol* 2015, 94:13-22.
70. Mishima S, Nagai A, Abdullah S, et al.: Effective ex vivo expansion of hematopoietic stem cells using osteoblast-differentiated mesenchymal stem cells is CXCL12 dependent. *Eur J Haematol* 2010, 84:538-546.
71. Lataillade JJ, Clay D, Bourin P, et al.: Stromal cell-derived factor 1 regulates primitive hematopoiesis by suppressing apoptosis and by promoting G(0)/G(1) transition in CD34(+) cells: evidence for an autocrine/paracrine mechanism. *Blood* 2002, 99:1117-1129.
72. Broxmeyer HE, Kohli L, Kim CH, et al.: Stromal cell-derived factor-1/CXCL12 directly enhances survival/antiapoptosis of myeloid progenitor cells through CXCR4 and G(alpha) i proteins and enhances engraftment of competitive, repopulating stem cells. *Journal of Leukocyte Biology* 2003, 73:630-638.
73. Yeoh JSG, van Os R, Weersing E, et al.: Fibroblast growth factor-1 and -2 preserve long-term repopulating ability of hematopoietic stem cells in serum-free cultures. *Stem Cells* 2006, 24:1564-1572.
74. Jiang Sy, Xie Xt, Jiang H, Zhou Jj, Li Fx, Cao P: Low expression of basic fibroblastic growth factor in mesenchymal stem cells and bone marrow of children with aplastic anemia. *Pediatr Hematol Oncol* 2014, 31:11-19.
75. Xu Y, Takahashi Y, Wang Y, et al.: Downregulation of GATA-2 and overexpression of adipogenic gene-PPARgamma in mesenchymal stem cells from patients with aplastic anemia. *Exp Hematol* 2009, 37:1393-1399.
76. Wenxin L, Jinxiang F, Yong W, Wenxiang L, Wenbiao S, Xueguang Z: Expression of membrane-bound IL-15 by bone marrow fibroblast-like stromal cells in aplastic anemia. *Int Immunol* 2005, 17:429-437.
77. Hu KX, Sun QY, Guo M, Ai HS: The radiation protection and therapy effects of mesenchymal stem cells in mice with acute radiation injury. *Brit J Radiol* 2010, 83:52-58.
78. Gan J, Meng F, Zhou X, et al.: Hematopoietic recovery of acute radiation syndrome by human superoxide dismutase-expressing umbilical cord mesenchymal stromal cells. *Cytotherapy* 2015, 17:403-417.
79. Zhang J, Zhou S, Zhou Y, et al.: Adipose-derived mesenchymal stem cells (ADSCs) with the potential to ameliorate platelet recovery, enhance megakaryopoiesis, and inhibit apoptosis of bone marrow cells in a mouse model of radiation-induced thrombocytopenia. *Cell Transplant* 2016, 25:261-273.
80. Shim S, Lee SB, Lee JG, Jang WS, Lee SJ, Park S, Lee SS: Mitigating effects of hUCB-MSCs on the hematopoietic syndrome resulting from total body irradiation. *Exp Hematol* 2013, 41:346-353.e342.
81. Qiao S, Ren H, Shi Y, Liu W: Allogeneic compact bone-derived mesenchymal stem cell transplantation increases survival of mice exposed to lethal total body irradiation: a potential immunological mechanism. *Chin Med J* 2014, 127:475-482.
82. Fernandez-Garcia M, Yanez RM, Sanchez-Dominguez R, et al.: Mesenchymal stromal cells enhance the engraftment of hematopoietic stem cells in an autologous mouse transplantation model. *Stem Cell Res Ther* 2015, 6:165.
83. Li J, Chen H, Lv YB, et al.: Intraperitoneal injection of multiplacentas pooled cells treatment on a mouse model with aplastic anemia. *Stem Cells Int* 2016, 2016:3279793.
84. Caplan AI: Adult mesenchymal stem cells for tissue engineering versus regenerative

medicine. *J Cell Physiol* 2007, 213:341-347.
85. Wu KH, Sheu JN, Wu HP, Tsai C, Sieber M, Peng CT, Chao YH: Cotransplantation of umbilical cord-derived mesenchymal stem cells promote hematopoietic engraftment in cord blood transplantation: a pilot study. *Transplantation* 2013, 95:773-777.
86. Lazarus HM, Koc ON, Devine SM, 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, 11:389-398.
87. Macmillan ML, Blazar BR, DeFor TE, Wagner JE: Transplantation of ex-vivo culture-expanded parental haploidentical mesenchymal stem cells to promote engraftment in pediatric recipients of unrelated donor umbilical cord blood: results of a phase I-II clinical trial. *Bone Marrow Transplant* 2009, 43:447-454.
88. Le Blanc K, Samuelsson H, Gustafsson B, et al.: Transplantation of mesenchymal stem cells to enhance engraftment of hematopoietic stem cells. *Leukemia* 2007, 21:1733-1738.
89. Ball LM, Bernardo ME, Roelofs H, et al.: Cotransplantation of ex vivo expanded mesenchymal stem cells accelerates lymphocyte recovery and may reduce the risk of graft failure in haploidentical hematopoietic stem-cell transplantation. *Blood* 2007, 110:2764-2767.
90. Wu KH, Chan CK, Tsai C, et al.: Effective treatment of severe steroid-resistant acute graft-versus-host disease with umbilical cord-derived mesenchymal stem cells. *Transplantation* 2011, 91:1412-1416.
91. Le Blanc K, Rasmusson I, Sundberg B, et al.: Treatment of severe acute graft-versus-host disease with third party haploidentical mesenchymal stem cells. *Lancet* 2004, 363:1439-1441.
92. Ringden O, Uzunel M, Rasmusson I, et al.: Mesenchymal stem cells for treatment of therapy-resistant graft-versus-host disease. *Transplantation* 2006, 81:1390-1397.
93. Fouillard L, Bensidhoum M, Bories D, et al.: Engraftment of allogeneic mesenchymal stem cells in the bone marrow of a patient with severe idiopathic aplastic anemia improves stroma. *Leukemia* 2003, 17:474-476.
94. Xiao Y, Jiang ZJ, Pang Y, et al.: Efficacy and safety of mesenchymal stromal cell treatment from related donors for patients with refractory aplastic anemia. *Cytotherapy* 2013, 15:760-766.
95. Cle DV, Santana-Lemos B, Tellechea MF, et al.: Intravenous infusion of allogeneic mesenchymal stromal cells in refractory or relapsed aplastic anemia. *Cytotherapy* 2015, 17:1696-1705.
96. Chao YH, Tsai C, Peng CT, Wu HP, Chan CK, Weng T, Wu KH: Cotransplantation of umbilical cord MSCs to enhance engraftment of hematopoietic stem cells in patients with severe aplastic anemia. *Bone Marrow Transplant* 2011, 46:1391-1392.
97. Si Y, Yang K, Qin M, et al.: Efficacy and safety of human umbilical cord derived mesenchymal stem cell therapy in children with severe aplastic anemia following allogeneic hematopoietic stem cell transplantation: a retrospective case series of 37 patients. *Pediatr Hematol Oncol* 2014, 31:39-49.