Mesenchymal stem cells: biological characteristics and clinical application

Yin Hai-bin[1, 2], Tian Xin[2], Yang Chun-hui[2, 3], Gao Xiao-hui[2, 3]

1 *Kunming Medical University, Kunming 650500, Yunnan Province, China*
2 *Department of Hematology and Oncology, Children's Hospital of Kunming Medical University, Kunming 650000,Yunnan Province, China*
3 *Dali College, Dali 671003, Yunnan Province, China*

Abstract

BACKGROUND: The clinical application of mesenchymal stem cells is a hot topic in the research of stem cell transplantation. The safety and effectiveness of mesenchymal stem cells still presents as a huge problem unsolved for its application.

OBJECTIVE: To summarize the biological characteristics of mesenchymal stem cells and prospect of their clinical application.

METHODS: A computer-based online search was conducted in PubMed database from January 1990 to November 2013 and in CNKI database from 2011 to 2013 for the related articles with the key words of "mesenchymal stem cells, separation and culture, surface antigen, inducing and differentiation, clinical application" in English and Chinese.

RESULTS AND CONCLUSION: Mesenchymal stem cells can express a variety of surface antigens, but without specific characteristics. At the same time they have the ability of self-renewal and the potential of multi-directional differentiation into osteocytes, adipocytes, chondrocytes, skeletal muscle cells, and even neurocytes. So, due to their multi-lineage differentiation potential, secreting multiple cytokines and immunomodulatory properties, mesenchymal stem cells have become an attractive candidate for cell therapy in the field of regenerative medicine, hematology, and immunology. Now the clinical trials using mesenchymal stem cells are in Phase III (comparing a newer treatment to the standard or best known treatment), but the cases are rare. Despite this, the mesenchymal stem cells treatment has resulted in many problems that mesenchymal stem cells may favor tumor growth and metastasis, increase in invasive fungal infections and so on. Mesenchymal stem cells have good application prospects in treating several diseases, especially hematologic malignancies. So to figure out how mesenchymal stem cells work *in vivo* as well as how to solve the problems brought by mesenchymal stem cells treatment are the research focus in stem cell transplantation.

Subject headings: stem cells; mesenchymal stem cells; mesenchymal stem cell transplantation; induced pluripotent stem cells; cell culture techniques

Yin HB, Tian X, Yang CH, Gao XH. Mesenchymal stem cells: biological characteristics and clinical application. Zhongguo Zuzhi Gongcheng Yanjiu. 2014;18(14): 2282-2289.

Yin Hai-bin, Studying for master's degree, Kunming Medical University, Kunming 650500,Yunnan Province, China

Corresponding author: Tian Xin, M.D., Chief physician, Master's supervisor, Department of Hematology and Oncology, Children's Hospital of Kunming Medical University, Kunming 650000, Yunnan Province, China

doi:10.3969/j.issn.2095-4344.2014.14.024
[http://www.crter.org]

Accepted: 2014-02-02

INTRODUCTION

Stem cells have the capacity to self-renew and to give rise to cells of various lineages. Thus, in cell-based therapy they play a crucial role. So the hematopoietic stem cell transplantation is being used in treating a lot of diseases, especially leukemia. However, 30%–70% of recipients still suffer from graft versus host disease (GVHD) after hematopoietic stem cell transplantation[1-2]. In addition, in patients who receive radiation therapy, catch-up growth will not be shown and eventually attain reduced final adult height[3]. Mesenchymal stem cells (MSCs) have self-renewal, capacity for multipotent differentiation, properties of tissue repair and unique major histocompatibility complex-unmatched immunosuppression, so they can promote hematopoietic cell engraftment and immune recovery, especially in the prevention and treatment of GVHD. And a growing number of mainly small studies have evaluated the effect of MSC treatment on severe acute GVHD[4-8]. These results are encouraging, but to figure out whether MSCs can be used in other disease, more clinical studies are needed.

DATA AND METHODS

Data sources

Computer-based retrievals were performed by the first author for literatures published in PubMed database (http://www.ncbi.nlm.nih.gov/PubMed/) from January 1990 to November 2013 and in CNKI database (http:// http://www.cnki.net/) from 2011 to 2013 using the key words of "mesenchymal stem cells, separation and culture, surface antigen, inducing and differentiation, clinical

application" in English and Chinese.

Inclusion and exclusion criteria

Inclusion criteria: (1) Randomized controlled trials and quasi-randomized controlled trials; (2) studies concerning MSCs biological characteristics and clinical application. Exclusion criteria: (1) Repetitive research; (2) incomplete information.

Data analysis

Unified inclusion criteria were used for included data, and duplicate data, abstracts, incomplete information and the data from foreign sources were excluded.

Data extraction

A total of 6 505 literatures were initially searched by computer. According to the inclusion and exclusion criteria, 71 articles were screened and included by the authors.

RESULTS

The discovery of MSCs

In 1867, German pathologist Cohnheim suggested the possible existence of stem cells of nonhematopoietic tissues in bone marrow when he was researching wound healing. He injected animals with nonsoluble dye aniline, thus the cells contain the dye will show up at the distal part of the injured tissue. Besides inflammatory cells, these cells included fibroblasts, which were later thought to be derived from the bone marrow, and thus were speculated with functions other than hematogenesis[9]. In 1869, Goujon found red marrow gained the function of osteogenesis after autotransplantation, which was supporting the former speculations. In the middle of 1970, Friedenstein *et al*[10] first reported that a small part of cells in bone marrow could differentiate into osteoid or cartilage-liked clusters during culturing, which was named fibroblast colony-forming unit (CFU-F) or marrow stromal fibroblasts. With the development of research, CFU-F is found to have supporting and inducing functions for hematopoietic cells of bone marrow[11]. Since it derives from bone marrow stroma, it is named bone marrow stromal cells. These cells can form osteoid or cartilage-liked tissue when implanted into the abdominal cavity or kidney capsule, so they are also called bone marrow osteogenic stem cells[12]. Fridenstein et al not only proved the existence of these stem cells, but also developed an easy and possible way of separation and culture *in vitro*. Until today, this is still a typical approach, and has been widely used.

In 1988, Owen *et al* [13] considered bone marrow stromal cell system was developing with the hematopoietic stem cells, and it had the ability of self-renewal and differentiating into cell clusters of all kinds of connective tissues, thus introducing the concept of stromal cell system. So MSCs are multipotent stromal cells and they can differentiate into a variety of cell types[14], including: osteoblasts[15], chondrocytes[16], and adipocytes.

In long-term cultures of bone marrow tissue *in vitro*, stromal cell system mainly includes the following kinds of anchorage-dependent cells: fibroblast-like cells, macrophages, adipocytes, and endothelial cells, in which fibroblast-like cells are thought to be multipotent stromal stem cells. Mark and Ajumdar *et al*, using gradient centrifugation, were able to harvested high purity of fibroblast-like anchorage-dependent cells which took up only 0.001%−0.01% of the total amount of cells, in the layer with the density of 1.077/cm^3. These cells showed up all the features of formal experiments concerning stem cells carried out by Fridenstein *et al*. Since they all eventually differentiated into cells of mesenchymal system, Mark *et al* renamed them as MSCs. However, these fibroblast-like mesenchymal cells are different from real fibroblasts. In a research comparing the two kinds of cells, MSCs could differentiate into specific connective tissue while fibroblasts separated from bone marrow could not in the same inducing condition.

MSCs separation and culture

Bone marrow is the most important source of MSCs. In separation and culture of MSCs from the bone marrow, the usual steps are to separate mononuclear cells of low density, and inoculate the mononuclear cell of low density in the culture media containing selected fetal bovine serum. The anchorage-dependent cells are considered as the primary MSCs *in vitro*. Subculture of MSCs shows dramatic but different potential of proliferation. Some cells can proliferate for more than 15 subcultures while some stop proliferation after four doublings. This phenomenon could be caused by several factors, such as the process of getting bone marrow, the small amounts of MSCs in bone marrow (only 2−5 MSCs in 1×10^6 mononuclear cells in bone marrow), and the ages and health conditions of the donors[17-18]. However, too much subculture could impair or damage the cell functions, mainly shown as the obvious signs of aging or apoptosis[19].

Methods of separation

Nowadays, there are mainly three methods of separating MSCs. (1) Density gradient centrifugation method: According to the different density between MSC and other cells, cells from bone marrow can be separated into three layers using Percoll separation solution. The layer with low density is the layer containing MSCs, which can be used for culture and harvest of the single kind of cells[17]. (2) Fluorescence activated cell sorting method: According to the small volume of MSCs, also the lack of granules, it can be separated using this method. Zohar *et al* [18] separated S cells with small volume, less granules in cytoplasm from periosteum of fetal rats, which could differentiate into chondrocytes and smooth muscle cells of bone. (3) Specific monoclonal antibody separation: Since some stromal precursor cells express surface marks such as CD105, stro-1, the specific monoclonal antibody combined with magnetic separation technology can be used to separate some kinds of cells[20]. Based on present researches, there is still a lack of specific marks in MSCs of bone marrow, so density gradient centrifugation method is widely used for the easy access of equipment and reagent, and the simple

composition of separated cells.

Culture media

The culture media of MSCs are various that Dulbecco's modified Eagle's medium, Ham's nutrient mixture F-12 and RPMI 1640 have all been used. Liu *et al*[21] used low glucose Dulbecco's modified Eagle's medium with different concentrations of fetal bovine serum as the culture medium, and found that the different concentrations of serum greatly affected the purity of MSCs. When the concentration of serum was 10%, the purity of MSCs could reach as high as 95%. While, when the concentration increased to 20%, HLA-DR$^+$ cells would rise accordingly to 36.1%, which indicated that some cells had differentiated into fibroblasts, probably because high concentration of serum could enhance the differentiation of MSCs. Ai *et al* [22] found that the concentrations of fetal bovine serum at 5%−10% and the culture time within 4 to 24 hours are the best for the growth of MSCs. Fu *et al* [23] used a specific culture media Mesencult^{TM}, which is suitable for MSCs' growth, and it is beneficial for MSCs' proliferation while depressing differentiation, so the cells all kept undifferentiated within eight generations.

Surface antigen of MSCs

MSCs express many kinds of surface antigen, but none of them is specific. It expresses the surface marks of mesenchymal cells, endothelial cells and epithelial cells. As a result of the composition of culture medium, the density of cell inoculation and oxygen pressure may all affect the phenotypes of cells. Therefore, there are various relevant reports[24]. However, no matter what method of culture is used, the phenotypes of some cells still remain consistent, such as not expressing CD45 and CD34, which are surface marks of hematopoietic cells, expressing SH2 (CD105), SH3 (CD73) and SH4 (CD73). Up to now, the phenotypes of marrow mesenchymal progenitor cells (before culture) still have not been confirmed yet[20].

The discovery of surface antigens of MSC was mainly through flow cytometry. These antigens can also be found in mesenchymal cells, endothelial cells and epithelial cells, but not in hematopoietic stem cells. At present, there are several CD antigens including CD29, CD44, CD59, CD71, CD90, CD105, CD120a, CD124, CD166, which all express in MSCs; while the surface marks of hematopoietic stem cells include CD3, CD11a, CD14, CD19, CD28, CD33, CD34, CD38, CD45, CD56, CD117, which are negative in MSCs. Also, antigens closely related to allograft rejection such as: HLA-DR, B7-1 (CD80), B7-2 (CD86), CD40 and CD40L are all negative[25].

At present, the surface antigens used to identify cells are mostly chosen from the above antigens. The popular positive marks are CD29, CD44, CD90, CD105, SH2, SH3, STRO-1, negatives are CD14, CD19, CD34, CD45, and allograft rejection associated antigen: HLA-DR, B7-1 (CD80), B7-2 (CD86), CD40, CD40L. Besides, MSCs also express SB-10, a kind of mixed antigens[26]. The results of cytochemistry showed that ANAE and PAS are strongly positive in these cells, while SB and ACP are negative, indicating MSCs have special metabolic characters. Also, approximately 5% of the cells are positive in ALP, indicating that there are different stages of stem cells which are differentiated into osteoblasts.

Inducing and differentiation of MSCs

Inducing in vitro

MSCs have the potential of self-renewal and multi-directional differentiation as a kind of stem cells. In order to inducing the MSCs into bone tissue, Dexamethasone, β-glycerol-phosphoric acid and ascorbate should be contained in the culture medium. The MSCs will gradually form clusters, and the expression of alkaline phosphatase will increase. In this kind of culture condition, evidence of calcium deposition can be found after 1 week, and the amount can keep on increasing in the whole culture process[27]. It has been showed that 1,25-$(OH)_2$-D_3, transforming growth factor-β, interleukin(IL)-6, hyaluronic acid and bone morphogenetic protein-2 all can induce the differentiation of MSCs into bone tissue[28]. The adding of bone morphogenetic protein-1 from cartilages and integrin also can promoting the differentiation[29]. The extent of differentiation of MSCs into cartilages can be told by testing the expression of collagen II in culturing cells. The use of 1-methyl-3-isobutyl xanthine, dexamethasone, insulin and indomethacin can induce MSCs differentiate into adipocytes, with accumulation of lipid vesicles in the cells. These cells can express peroxisome proliferator-activated receptor γ2, lipoprotein lipase and fatty acid-binding protein αP2. Many inducing factors can induce more than 95% of MSCs differentiating into adipocytes, and those induced adipocytes can grow well *in vitro* for at least 3 months, also, the basic culture medium, cell density, growth factors and cell factors all affect the differentiations of MSCs.

Proliferation and differentiation in vivo

Up to now, it is still not so clear about the specific environment for MSCs to direct differentiation *in vivo*, only animal experiments are available. It has been reported that after proliferation of canine MSCs *in vitro*, they are adhered on biological materials, and with the secretion of bone stroma, they can differentiate into bones, which can be used to repair the defects of femoral shafts. After the MSCs of rabbits proliferation *in vitro*, it can reproduce bones and cartilages with the carriers, which can be used to repair cartilage injuries, and tendons as well[30]. When human MSCs of bone marrow is injected to athymic mouse subcutaneously, after 1 month, many kinds of tissues, including bone, cartilage, fat, skeletal muscle and tendons, differentiating from human MSCs, will appear in injection cites.

Cytokine impact on MSCs proliferation

Li *et al* [31] used MTT to observe the impacts of cytokines to MSCs proliferation. They found that interferon-γ, tumor necrosis factor-α, stem cell factor and insulin-like growth factor could dramatically stimulate the proliferation of cells, while IL-4, IL-1 and basic fibroblast growth factor had no

effect on cell growth. Among the former factors, interferon-γ and tumor necrosis factor-α were most effective, which can indicate the important value of MSCs in tissue repair.

Clinical application of MSCs

Recent researches have reveal that: (1) The terminal differentiation of MSCs might exceed the borders of germinal layer, meaning that cells traditionally stemming from middle embryonic layer and then differentiating into mesenchymal cells, would instead transform into parenchymal cells, such as cardiac muscular cells, nervous cells, *etc*.

(2) The karyotype of MSCs remains normal within 12 generations, and telomerase keeps active, meaning that MSCs has a strong ability to proliferate *in vitro*, but are not eternally alive as malignant tumor cells.

(3) MSCs can be harvested from autografts of bone marrow, thus the induced tissue can avoid problems such as tissue matching and immunological rejection when transplanting. In that way, it is possible to use the MSCs as the seeds of tissue engineering, to repair many kinds of injury and defects of tissues, therefore making the repair and restore of tissues and organs possible.

Animal experiments have proven that, through proliferation and inducing *in vitro*, bone marrow MSCs can differentiate into needed cells, which are then implanted in the injured tissues, and the outcomes are good. The most popular used approach is to proliferate the MSCs *in vitro*, and then the cells are combined with scaffolds to form the cell-material complex, and then the complexes are implanted in the defects[32-33]. All the above researches have shown a promising future for application of bone marrow MSCs in the treatment of bone and cartilage injury.

The degenerative changes of some organs may result in some related diseases, such as amyotrophy, myodystrophy[33]. Experiments have proven that MSCs could differentiate into muscular cells both *in vitro* and *in vivo*. So hopefully, they would become regenerating muscle tissues, and thus cure amyotrophy and myodystrophy.

The recent research reveals that MSCs are susceptible to transfection and expression of exogenous genes, thus being considered as a great gene carrier. Researchers also considered that, for diseases caused by gene mutation, they can be treated by introducing normal genes into patients' MSCs, and the cells proliferating *in vitro* and then being injected back to the patients[34].

The hematopoietic function of MSCs has been widely admitted. As a part of bone marrow stromal cells, MSCs act the main cells making up the hemopoietic microenvironment, and also play an important role in hematopoietic regulation. It has been revealed by research that, MSCs cultured *in vitro* are able to excrete cytokines such as IL-6, IL-7, IL-8, IL-11, stem cell growth factor, megakaryocyte colony stimulating factor, Fit-3 conjugant stromal cell-derived growth factor, *etc*. Among them, most cytokines can stimulate the proliferation and differentiation of hematopoietic cells. Meanwhile, MSCs have the ability of differentiating into stromal cells in certain conditions, which can solve the hematopoietic dysfunction caused by deficiency of stromal cells after injury[35].

Researches in recent years have shown that MSCs also played a remarkable role in immune system, for example, negative modulation of unrelated donor can support the proliferation of recipient's hematopoietic stem cells *in vitro*, without activating reactive lymphocyte against donor antigens, meaning that stromal stem cells can down regulate allogeneic immune response, therefore alleviate GVHD[36]. Remberger *et al* [37] discovered that MSCs could have an initial effect on GVHD since patients survived for a longer time and fewer patients died of acute GVHD as compared to the control group.

(4) How do the MSCs run in curing disease? Atsuta *et al* [38] discovered that Fas/Fas-L-induced MM apoptosis plays a crucial role in the MSCs-based inhibition of multiple myeloma growth. Bergfeld *et al* [39] found that the IL-6/STAT3 pathway have been shown to play a central role in control the interaction between MSCs and tumor cells. Hypoxic MSCs with an increased ability can migrate toward tumors through the upregulation of chemokine receptors, such as CXCR4 and CX3CR1[40]. MSCs have inherent tumor-trophic migratory properties, which allow them to serve as vehicles for delivering effective and targeted therapy to isolated tumors and metastatic disease[41]. In addition, Xia *et al* [42] discovered that MSCs not only act as a cell carrier, but also allow the replication of CRAd, significantly enhancing the oncolytic effect and resulting in the augmented tumor inhibition efficiency. However, these may just be only a small part of the action mechanism of MSCs. So, more in-depth studies are needed.

(5) However, the clinical application of MSCs will bring many problems. Not merely is it hard to get enough bone marrow MSCs (BMSCs) expanded for Cell transplantation, but also the growth and differentiation of BMSCs are not an easy thing. Hagmann *et al* [43] discovered that the expression of typical MSC markers depending on the media applied. And it is still unknown which MSC culture is the best in terms of the safety and growth of MSCs. Yamout *et al* [44] discovered that 30% of patients failed to grow an adequate number of BMSCs ($< 2\times10^6$) despite repeated bone marrow aspirations reflecting an inherent deficiency of such cells in the bone marrow of those patients. Why the successful MSC transplantation is so difficult? And whether to get enough MSCs means a successful MSC transplantation? Maybe the quality of MSCs is more important than the quantity. Siegel *et al* [45] found that high-clonogenic BMSCs were smaller, divided more rapidly and more frequent in BMSC preparations from younger, female donors. Therefore, in order to improve curative effect, the younger, female donors are a better choice.

Unfortunately, to improve the quantity and quality of MSCs may be not the biggest challenge for the clinical application of MSCs. The MSC transplantation has some side effects and possible complications including headache[46] and neuropathic pain[47] after parenchymal injection of BMSCs. However, compared with the following those would not be a problem. Negative effects of MSC treatment may result in an increase in invasive fungal infections[37]. In a clinical trial using MSCs to prevent GVHD in patients with hematologic malignancies, MSCs reduced the development of GVHD, but the relapse rate among patients was higher than that in the control group[48]. In general, due to their immunosuppressive actions, MSCs are known to favor tumor growth[49] and metastasis[50]. Studies have reported that MSCs may exert antitumorigenic effects *in vitro* and in a model of Kaposi's sarcoma[51-52]. Since MSCs are supposed to be the progenitor cells of Ewing sarcomas[53], osteoblasts, and fibroblasts, the possibility exists that MSCs may have contributed to the secondary malignancy in these patients. Shalapour *et al* [54] discovered that leukemia cells and MSC have genetic aberrations in common, and that they are clonally related. The endogenous expression of IL-6 and CCL5 by MSCs, have been shown to increase the growth and metastasis of breast cancer cells, respectively[55-56]. However, there have not been significant subsequent reports on the promotion of tumor growth by MSCs in recent years. Some MSCs are able to escape from dividing spontaneously, and these have undergone tumorigenic transformation generating TMC[57]. To use BMSCs in clinical trials, more clinical researches will be needed.

Problems and prospects

Although the techniques of separation and culture of MSCs have been fairly mature, the identification of the cells is by no means satisfactory[58]. For the lack of specific surface antigens, there are always disputations over the isolated MSCs. Identification only based on surface antigens is not cogently enough, and many researchers nowadays believe that the most useful way of identification is differentiation. Whether the cells can be induced into osteocytes, adipocytes and chondrocytes *in vitro* is an important marker to identify MSCs. The typical recognized route is osteogenesis[59-60]. The identification of MSCs is very well-developed in many countries. Except from surface antigens, scholars also test the differentiation of stem cells into adipocytes, chondrocytes, osteoblasts[61], and even hepatocytes and neurocytes[62-63].

In recent years, people have achieved prominent advancements in understanding the biological characters and potential clinical application of MSCs. As mentioned above, BMSCs are highly potent in differentiation, easy to separate, culture and proliferate, convenient to transfect and express exogenous genes. It can alleviate reject reactions due to transplantation. However, many problems remain unsolved, for example, the method of separating effective ingredients of MSCs is still unstable, the specific surface marks of MSCs are unclear, whether MSCs can gain the functions of neurons when differentiated into neurocytes, whether neurocytes can build up precise connections with each other after transplantation, and how long the lifespan of the differentiated cells is. All those questions need to be answered by further research. The present MSCs transplantation has been restricted in firstly inducing differentiation into needed tissues *in vitro*, and then implanting them into the body. The process of inducing differentiation is likely to change the characters of cells. Therefore, once the procedures are widely used in clinical practice, unexpected results may occur. Thus, further research is still needed in many unknown fields about MSCs.

Although the MSCs have been widely used, whether MSCs are officially stem cells has never been completely recognized. MSCs cannot be proven to be stem cells using the definition of haemopoietic stem cells, or strictly proven *in vivo* [64-66]. In single cell level, it can passage and maintain organization interval, while MSCs cannot be transplanted, separated or replanted in recipients that have been through many subcultures. We are neither able to separate MSCs from tissue and learn about their characters, nor to observe their biological features in relatively uncontrolled conditions. The biological connections of MSCs have always been missing.

Before specific markers can be effectively used in separation of MSCs and quantitative analysis system used in identification, the concept of MSCs is merely a hypothesis, since experiments *in vitro* and observations based on which are still not cogently enough. However, the corresponding problem is whether the injection of great amount of cultured MSCs can be transplanted into the bone marrow.

Growing studies have shown clearly that most of the injected MSCs are kept in capillary beds of many tissues, especially lungs[67]. An earlier research showed that one died of respiratory failure at 956 days after implanting stromal cells to dogs. Autopsy found multiple osteogenesis cites produced by the transplants in both of the lungs. Rombouts and Ploemacher[68] found an interesting phenomena in their research on mice, which was the prominent difference in transplantation and homing between "fresh" and cultured MSCs. The "fresh" MSCs were defined as the BM CFU-F of mice, not the cultured ones. In a homogeneous model which underwent sublethal radiation, it was proven that the effective homing to spleen and bone marrow (such as proven by the restoration of CFU-F of donors), was dosage-dependent, while a culture cycle lasting for 24 hours would dramatically decrease the abilities of homing and transplantation of the cells. All those studies have strongly proven that, culture and proliferation could change the characters in homing and transplantation of MSCs, and when immune hinder is absent, stroma cells could be transplanted after "space" is created by radiations. Some studies have indicated that, human MSCs cannot be identified in the bone marrow, only the stromal support from MSC transplantation can be speculated. Another research concerning the promotion of transplantation by allogene or

heterologous allogeneic stromal transplantation was the fetal sheep model[69-70]. In this model, the transplantation of stromal cells was conferred, speculating the transplantation of stromal cells has a “developmental window”. There are no conclusive reports yet about whether infusion of MSCs can enhance bone marrow transplantation or alleviate GVHD. But reports have showed that remarkable effects of bone marrow transplantation and infusion of MSCs could promote the formation of osteogenesis imperfect[71]. Although the odd of successful transplantation of osteoblasts is still not high, the clinical outcome is convincing. Therefore, the osteogenesis imperfect transplantation based on MSCs will achieve better effects in the future[72].

In summary, due to their multi-lineage differentiation potential, secreting multiple biomolecules and immunomodulatory properties, MSCs have become an attractive candidate for cell therapy in the field of regenerative medicine, hematology, and immunology. Now the clinical trials using MSCs are in phase III (comparing a newer treatment to the standard or best known treatment), but cases are rare. So as data about MSCs in clinical application for the disease is limited, a solid conclusion cannot be drawn.

At the same time the clinical applications of MSCs should be careful, and the following problems need to be solved: safety issue, quality control, clinical grade production, autologous or allogeneic MSCs, clinical transition and so on. Allogenic MSCs transplantation may be a better choice to ameliorate chronic myelogenous leukemia[73]. So, special attention is needed to be paid to the clinical application of MSCs.

MSCs-based cell therapy is a potentially useful innovative therapeutic strategy for several diseases and many clinical trials of the potential of MSCs as a therapeutic agent are in progress. Although, there is still a long way to go before using these cells as a routinely applied therapy in clinics. We believe that the MSCs will play a critical role in gene therapy and revolutionize therapies for patients with severe diseases.

REFERENCES

[1] Nash RA, Antin JH, Karanes C, et al. Phase 3 study comparing methotrexate and tacrolimus with methotrexate and cyclosporine for prophylaxis of acute graft-versus-host disease after marrow transplantation from unrelated donors. Blood. 2000;96(6):2062-2068.

[2] Ratanatharathorn V, Nash RA, Przepiorka D, et al. Phase III study comparing methotrexate and tacrolimus (prograf, FK506) with methotrexate and cyclosporine for graft-versus-host disease prophylaxis after HLA-identical sibling bone marrow transplantation. Blood. 1998;92(7):2303-2314.

[3] Chung SJ, Park SW, Kim MK, et al. Growth after hematopoietic stem cell transplantation in children with acute myeloid leukemia. J Korean Med Sci. 2013;28(1): 106-113.

[4] Ringdén O, Uzunel M, Rasmusson I, et al. Mesenchymal stem cells for treatment of therapy-resistant graft-versus-host disease. Transplantation. 2006;81(10):1390-1397.

[5] 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(9419):1439-1441.

[6] Le Blanc K, Frassoni F, Ball L, et al. Mesenchymal stem cells for treatment of steroid-resistant, severe, acute graft-versus-host disease: a phase II study. Lancet. 2008; 371(9624):1579-1586.

[7] von Bahr L, Sundberg B, Lönnies L, et al. Long-term complications, immunologic effects, and role of passage for outcome in mesenchymal stromal cell therapy. Biol Blood Marrow Transplant. 2012;18(4):557-564.

[8] von Bonin M, Stölzel F, Goedecke A, et al. Treatment of refractory acute GVHD with third-party MSC expanded in platelet lysate-containing medium. Bone Marrow Transplant. 2009;43(3):245-251.

[9] Prockop DJ. Marrow stromal cells as stem cells for continual renewal of nonhematopoietic tissues and as potential vectors for gene therapy. J Cell Biochem Suppl. 1998;30-31:284-285.

[10] Friedenstein AJ, Latzinik NW, Grosheva AG, et al. Marrow microenvironment transfer by heterotopic transplantation of freshly isolated and cultured cells in porous sponges. Exp Hematol. 1982;10(2):217-227.

[11] Javazon EH, Beggs KJ, Flake AW. Mesenchymal stem cells: paradoxes of passaging. Exp Hematol. 2004;32(5): 414-425.

[12] Wulf GG, Chapuy B, Trümper L. Mesenchymal stem cells from bone marrow. Phenotype, aspects of biology, and clinical perspectives. Med Klin (Munich). 2006;101(5):408-413.

[13] Owen M, Friedenstein AJ. Stromal stem cells: marrow-derived osteogenic precursors. Ciba Found Symp. 1988;136:42-60.

[14] Beyer Nardi N, da Silva Meirelles L. Mesenchymal stem cells: isolation, in vitro expansion and characterization. Handb Exp Pharmacol. 2006;(174):249-282.

[15] Brighton CT, Hunt RM. Early histological and ultrastructural changes in medullary fracture callus. J Bone Joint Surg Am. 1991;73(6):832-847.

[16] Brighton CT, Hunt RM. Early histologic and ultrastructural changes in microvessels of periosteal callus. J Orthop Trauma. 1997;11(4):244-253.

[17] Yoshii T, Sotome S, Torigoe I, et al. Isolation of osteogenic progenitor cells from trabecular bone for bone tissue engineering. Tissue Eng Part A. 2010;16(3):933-942.

[18] Zohar R, Sodek J, McCulloch CA. Characterization of stromal progenitor cells enriched by flow cytometry. Blood. 1997;90(9):3471-3481.

[19] Bruder SP, Jaiswal N, Haynesworth SE. Growth kinetics, self-renewal, and the osteogenic potential of purified human mesenchymal stem cells during extensive subcultivation and following cryopreservation. J Cell Biochem. 1997;64(2):278-294.

[20] Ratajczak J, Kucia M, Reca R, et al. Quiescent $CD34^+$ early erythroid progenitors are resistant to several erythropoietic 'inhibitory' cytokines; role of FLIP. Br J Haematol. 2003; 123(1):160-169.

[21] Liu X, Wu M, Peng Y, et al. Improvement in poor graft function after allogeneic hematopoietic stem cell transplantation upon administration of mesenchymal stem cells from third-party donors: a pilot prospective study. Cell Transplant. 2013. in press.

[22] Ai G, Su Y, Yan G, et al. The experimental study of bone marrow mesenchymal stem cells on the repair of skin wound combined with local radiation injury. Zhonghua Yi Xue Za Zhi. 2002;82(23):1632-1636.

[23] Fu W, Lu Y, Piao Y. Culture and pluripotentiality of human marrow mesenchymal stem cells. Zhonghua Xue Ye Xue Za Zhi. 2002;23(4):202-204.
[24] Tare RS, Khan F, Tourniaire G, et al. A microarray approach to the identification of polyurethanes for the isolation of human skeletal progenitor cells and augmentation of skeletal cell growth. Biomaterials. 2009;30(6):1045-1055.
[25] In 't Anker PS, Scherjon SA, Kleijburg-van der Keur C, et al. Amniotic fluid as a novel source of mesenchymal stem cells for therapeutic transplantation. Blood. 2003;102(4): 1548-1549.
[26] Peister A, Mellad JA, Larson BL, et al. Adult stem cells from bone marrow (MSCs) isolated from different strains of inbred mice vary in surface epitopes, rates of proliferation, and differentiation potential. Blood. 2004;103(5):1662-1668.
[27] Pittenger MF, Mackay AM, Beck SC, et al. Multilineage potential of adult human mesenchymal stem cells. Science. 1999;284(5411):143-147.
[28] Neman J, Hambrecht A, Cadry C, et al. Clinical efficacy of stem cell mediated osteogenesis and bioceramics for bone tissue engineering. Adv Exp Med Biol. 2012;760: 174-187.
[29] Tsumaki N, Tanaka K, Arikawa-Hirasawa E, et al. Role of CDMP-1 in skeletal morphogenesis: promotion of mesenchymal cell recruitment and chondrocyte differentiation. J Cell Biol. 1999;144(1):161-173.
[30] Young RG, Butler DL, Weber W, et al. Use of mesenchymal stem cells in a collagen matrix for Achilles tendon repair. J Orthop Res. 1998;16(4):406-413.
[31] Li XS, Fan HT, Yuan Y, et al. Modification of in situ cryopreservation of human bone marrow mesenchymal stem cells. Zhongguo Shi Yan Xue Ye Xue Za Zhi. 2003; 11(5):530-533.
[32] El-Menoufy H, Aly LA, Aziz MT, et al. The role of bone marrow-derived mesenchymal stem cells in treating formocresol induced oral ulcers in dogs. J Oral Pathol Med. 2010;39(4):281-289.
[33] Robinson D, Efrat M, Mendes DG, et al. Implants composed of carbon fiber mesh and bone-marrow-derived, chondrocyte-enriched cultures for joint surface reconstruction. Bull Hosp Jt Dis. 1993;53(1):75-82.
[34] Takken T, van den Beuken C, Wulffraat NM, et al. Exercise tolerance in children with juvenile idiopathic arthritis after autologous SCT. Bone Marrow Transplant. 2008;42(5): 351-356.
[35] Koç ON, Gerson SL, Cooper BW, et al. Rapid hematopoietic recovery after coinfusion of autologous-blood stem cells and culture-expanded marrow mesenchymal stem cells in advanced breast cancer patients receiving high-dose chemotherapy. J Clin Oncol. 2000;18(2):307-316.
[36] Frank MH, Sayegh MH. Immunomodulatory functions of mesenchymal stem cells. Lancet. 2004;363(9419): 1411-1412.
[37] Remberger M, Ringdén O. Treatment of severe acute graft-versus-host disease with mesenchymal stromal cells: a comparison with non-MSC treated patients. Int J Hematol. 2012;96(6):822-824.
[38] Atsuta I, Liu S, Miura Y, et al. Mesenchymal stem cells inhibit multiple myeloma cells via the Fas/Fas ligand pathway. Stem Cell Res Ther. 2013;4(5):111.
[39] Bergfeld SA, DeClerck YA. Bone marrow-derived mesenchymal stem cells and the tumor microenvironment. Cancer Metastasis Rev. 2010;29(2):249-261.
[40] Huang YF, Chen MJ, Wu MH, et al. The use of hypoxic cultured mesenchymal stem cell for oncolytic virus therapy. Cancer Gene Ther. 2013;20(5):308-316.
[41] Shah K. Mesenchymal stem cells engineered for cancer therapy. Adv Drug Deliv Rev. 2012;64(8):739-748.
[42] Xia X, Ji T, Chen P, et al. Mesenchymal stem cells as carriers and amplifiers in CRAd delivery to tumors. Mol Cancer. 2011;10:134.
[43] Hagmann S, Moradi B, Frank S, et al. Different culture media affect growth characteristics, surface marker distribution and chondrogenic differentiation of human bone marrow-derived mesenchymal stromal cells. BMC Musculoskelet Disord. 2013;14:223.
[44] Yamout B, Hourani R, Salti H, et al. Bone marrow mesenchymal stem cell transplantation in patients with multiple sclerosis: a pilot study. J Neuroimmunol. 2010; 227(1-2):185-189.
[45] Siegel G, Kluba T, Hermanutz-Klein U, et al. Phenotype, donor age and gender affect function of human bone marrow-derived mesenchymal stromal cells. BMC Med. 2013;11:146.
[46] Mehta T, Feroz A, Thakkar U, et al. Subarachnoid placement of stem cells in neurological disorders. Transplant Proc. 2008;40(4):1145-1147.
[47] Kishk NA, Gabr H, Hamdy S, et al. Case control series of intrathecal autologous bone marrow mesenchymal stem cell therapy for chronic spinal cord injury. Neurorehabil Neural Repair. 2010;24(8):702-708.
[48] Ning H, Yang F, Jiang M, et al. The correlation between cotransplantation of mesenchymal stem cells and higher recurrence rate in hematologic malignancy patients: outcome of a pilot clinical study. Leukemia. 2008;22(3):593-599.
[49] Djouad F, Plence P, Bony C, et al. Immunosuppressive effect of mesenchymal stem cells favors tumor growth in allogeneic animals. Blood. 2003;102(10):3837-3844.
[50] Karnoub AE, Dash AB, Vo AP, et al. Mesenchymal stem cells within tumour stroma promote breast cancer metastasis. Nature. 2007;449(7162):557-563.
[51] Ramasamy R, Lam EW, Soeiro I, et al. Mesenchymal stem cells inhibit proliferation and apoptosis of tumor cells: impact on in vivo tumor growth. Leukemia. 2007;21(2): 304-310.
[52] Khakoo AY, Pati S, Anderson SA, et al. Human mesenchymal stem cells exert potent antitumorigenic effects in a model of Kaposi's sarcoma. J Exp Med. 2006;203(5):1235-1247.
[53] Tirode F, Laud-Duval K, Prieur A, et al. Mesenchymal stem cell features of Ewing tumors. Cancer Cell. 2007;11(5): 421-429.
[54] Shalapour S, Eckert C, Seeger K, et al. Leukemia-associated genetic aberrations in mesenchymal stem cells of children with acute lymphoblastic leukemia. J Mol Med (Berl). 2010;88(3):249-265.
[55] Hall B, Dembinski J, Sasser AK, et al. Mesenchymal stem cells in cancer: tumor-associated fibroblasts and cell-based delivery vehicles. Int J Hematol. 2007;86(1):8-16.
[56] Xishan Z, Guangyu A, Yuguang S, et al. The research on the immuno-modulatory defect of mesenchymal stem cell from Chronic Myeloid Leukemia patients. J Exp Clin Cancer Res. 2011;30:47.
[57] Rubio D, Garcia S, Paz MF, et al. Molecular characterization of spontaneous mesenchymal stem cell transformation. PLoS One. 2008;3(1):e1398.
[58] Javazon EH, Beggs KJ, Flake AW. Mesenchymal stem cells: paradoxes of passaging. Exp Hematol. 2004;32(5):414-425.

[59] Phinney DG, Kopen G, Righter W, et al. Donor variation in the growth properties and osteogenic potential of human marrow stromal cells. J Cell Biochem. 1999;75(3):424-436.
[60] Hudalla GA, Kouris NA, Koepsel JT, et al. Harnessing endogenous growth factor activity modulates stem cell behavior. Integr Biol (Camb). 2011;3(8):832-842.
[61] Riccobono D, Agay D, Scherthan H, et al. Application of adipocyte-derived stem cells in treatment of cutaneous radiation syndrome. Health Phys. 2012;103(2):120-126.
[62] Götherström C, Ringdén O, Westgren M, et al. Immunomodulatory effects of human foetal liver-derived mesenchymal stem cells. Bone Marrow Transplant. 2003; 32(3):265-272.
[63] Lee OK, Kuo TK, Chen WM, et al. Isolation of multipotent mesenchymal stem cells from umbilical cord blood. Blood. 2004;103(5):1669-1675.
[64] Clark BR, Jamieson C, Keating A. Human long-term bone marrow culture. Methods Mol Biol. 1997;75:249-256.
[65] Johansson M, Jansson L, Ehinger M, et al. Neonatal hematopoietic stem cell transplantation cures oc/oc mice from osteopetrosis. Exp Hematol. 2006;34(2):242-249.
[66] Tedersoo L, May TW, Smith ME. Ectomycorrhizal lifestyle in fungi: global diversity, distribution, and evolution of phylogenetic lineages. Mycorrhiza. 2010;20(4):217-263.
[67] Gao J, Dennis JE, Muzic RF, et al. The dynamic in vivo distribution of bone marrow-derived mesenchymal stem cells after infusion. Cells Tissues Organs. 2001;169(1): 12-20.
[68] Rombouts WJ, Ploemacher RE. Primary murine MSC show highly efficient homing to the bone marrow but lose homing ability following culture. Leukemia. 2003;17(1):160-170.
[69] Mackenzie TC, Flake AW. Human mesenchymal stem cells persist, demonstrate site-specific multipotential differentiation, and are present in sites of wound healing and tissue regeneration after transplantation into fetal sheep. Blood Cells Mol Dis. 2001;27(3):601-604.
[70] Lai RC, Chen TS, Lim SK. Mesenchymal stem cell exosome: a novel stem cell-based therapy for cardiovascular disease. Regen Med. 2011;6(4):481-492.
[71] Bobis S, Jarocha D, Majka M. Mesenchymal stem cells: characteristics and clinical applications. Folia Histochem Cytobiol. 2006;44(4):215-230.
[72] Leo AJ, Grande DA. Mesenchymal stem cells in tissue engineering. Cells Tissues Organs. 2006;183(3):112-122.
[73] Casiraghi F, Remuzzi G, Abbate M, et al. Multipotent mesenchymal stromal cell therapy and risk of malignancies. Stem Cell Rev. 2013;9(1):65-79.

间充质干细胞的生物学特性及临床应用

殷海斌[1,2]，田　新[2]，杨春会[2,3]，高晓慧[2,3]([1]昆明医科大学，云南省昆明市　650500；[2]昆明医科大学附属儿童医院血液肿瘤科，云南省昆明市　650000；[3]大理学院，云南省大理市　671003)

殷海斌，昆明医科大学在读硕士，主要从事小儿白血病临床治疗方面的研究。
通讯作者：田新，博士，主任医师，硕士生导师，昆明医科大学附属儿童医院血液肿瘤科，云南省昆明市　650000

文章亮点：

文章特点在于回顾总结近年来对于间充质干细胞的生物学特性及临床应用前景的研究。由于间充质干细胞多向分化潜力，且能分泌多种细胞因子，还具有免疫调节能力，已成为再生医学、血液学和免疫学方面细胞疗法的候选细胞，但仍存在利于肿瘤生长和转移、增加侵袭性真菌感染等方面问题。国内外均进行了广泛的应用，并有大的临床多中心对照研究结果。间充质干细胞临床应用中虽有问题，但也是间充质干细胞研究最活跃的领域。

关键词：

干细胞；培养；诱导；移植；间充质干细胞；临床应用；移植物抗宿主病；干细胞移植

主题词：

干细胞；间充质干细胞；间充质干细胞移植；诱导多功能干细胞；细胞培养技术

摘要

背景：间充质干细胞的临床应用是干细胞移植研究的热点，但其安全性和有效性仍存在争议。

目的：回顾总结近年来对于间充质干细胞的生物学特性及临床应用前景的研究。

方法：计算机检索 Pubmed(1990 年 1 月至 2013 年 11 月)和中国知网数据库(2011 年至 2013 年)，检索关键词为“间充质干细胞；分离培养；表面抗体；诱导分化；临床应用”。

结果与结论：间充质干细胞可表达多种表面抗原，但没有特异性，同时其具有自我更新和向骨细胞、脂肪细胞、软骨细胞、骨骼肌细胞和神经细胞等的多向分化能力。由于其多向分化潜力，且能分泌多种细胞因子，还具有免疫调节能力，已成为再生医学、血液学和免疫学方面细胞疗法的候选细胞。间充质干细胞临床试验已处于第三期，尽管数据还不多，已发现存在利于肿瘤生长和转移、增加侵袭性真菌感染等方面问题。即使如此，其在血液系统恶性肿瘤等疾病面方面仍存在良好的应用前景。所以，找出间充质干细胞在体内的作用原理及修复其产生的不良影响是目前干细胞移植研究的重点。

作者贡献：所有作者共同进行实验设计、分析及撰写文章。

利益冲突：文章及内容不涉及相关利益冲突。

伦理要求：二次研究未涉及伦理问题。

学术术语：免疫抑制-是指对于免疫应答的抑制作用，可由天然或人为因素导致。

作者声明：文章为原创作品，无抄袭剽窃，无泄密及署名和专利争议，内容及数据真实，文责自负。

中图分类号: R318　文献标识码: A
文章编号: 2095-4344(2014)14-02282-08

殷海斌，田新，杨春会，高晓慧. 间充质干细胞的生物学特性及临床应用[J].中国组织工程研究，2014，18(14):2282-2289.

(Edited by Bai H/Wang L)