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Research advances in mechanisms by which bone marrow stem cells improve myocardial function☆

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Abstract

BACKGROUND: Bone marrow mesenchymal stem cells transplantation can significantly improve the cardiac structure reconfiguration and cardiac function through affecting the myocardial cell formation and myocardial angiogenesis.

OBJECTIVE: To summarize the theoretical basis of bone marrow mesenchymal stem cells transplantation for the treatment of ischemic cardomyopathy through researching the effect of bone marrow mesenchymal stem cells transplantation on myocardial function.

METHODS: The Chinese Biomedical Literature database and Medline database from 1994 to 2011 were used to search the reviews and reports that relate to the application of bone marrow mesenchymal stem cells transplantation for the treatment of ischemic cardomyopathy, and the research progress was analyzed.

RESULTS AND CONCLUSION: A total of 37 articles were included for the review. Autologous application of bone marrow mesenchmal stem cells transplantation can be amplified *in vitro* without immune rejection and can avoid the ethical controversy. Research of bone marrow stem cells for the treatment of acute myocardial infarction is still in the early stage. With the development of the research, bone marrow mesenchymal stem cells become a new method for the treatment of ischemic cardiomyopathy if we can find an effective and safe dosage.

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INTRODUCTION

Coronary heart disease (CHD) has become a common and frequently encountered disease in China. At present, interventional therapeutic technique and thrombolytic therapy cannot reverse the myocardium which is necrosis[1].The advantages include relatively easy to obtain, no rejection for autologous cells transplantation and able to differentiate into cardiocytes[2]. This paper primarily expounds the theory of bone marrow stem cells transplantation clinically for the treatment of ischemic cardomyopathy.

MATETIALS AND METHODS

Data source

Retrieval-related contents: Yu Gui-ping.
Retieval period: 1996–2011.
Keywords: myocardial infarction, heart failure, mesenchymal stem cells.
Retrieval database: http://www.ncbi.nlm.nih.gov/Pubmed

Inclusion criteria

Retrieval methods: Inclusion criteria: articles on bone marrow stem cells that used for the treatment of ischemic cardiomyopathy, and studies that published recently or in high impact journals.
Exclusion criteria: repetitive articles were excluded.

Data extraction

A total of 66 English articles were retrieved electronically. The titles and abstracts were screened. Among the 66 articles, 10 were rejected for objective independence and 18 for repetitive contents; finally, 37 articles were retained for further analysis.

RESULTS

Bone marrow mesenchymal stem cells

Origin of bone marrow mesenchymal stem cells

Stem cell was a kind of undifferentiated cell which has self-replication ability and could be differentiated into at least one kind of cell function. With certain conditions, bone marrow mesenchymal stem cells belonged to adult stem cells, mainly including mesenchymal stem cells, hematopoietic stem cells, and endothelial progenitor cells [3-4]. Moreover, it had plasticity(lateral differentiation) and proficiency

development crossing germ layer[5-8].

Character of bone marrow mesenchymal stem cells

In vitro study showed that bone marrow mesenchymal stem cells displayed different surface antigen characteristics in different separation and culture environments. After multiple passages, its potency of differentiation was weakened with the increasing of the passage[9]. Its basic features were: (1) Source of bone marrow occupied one of 10 000 to 100 000 mononuclear cells. (2) Culturing *in vitro* showed the adherent growth and the formation of fiber colony forming unit. (3) It could be differentiated into more than three kinds of mesenchymal clones[10]. (4) Its genetic background was stable. After 20 passages, it would not lose its feature of differentiation. (5) Bone marrow mesenchymal stem cells had unique phenotype, showing as SH2, SH3, CD29, CD44, CD71, CD90, CD106, CD120a, CD124 and CD166 were positive, but CD14, CD34 and CD45 were negative[11-12]. (6) Even continuous generation cells could maintain normal chromatin and telomere activity. Its proliferation was prosperous and the multiplication time *in vitro* was around 30 hours. 10% cells were in S period[13]. Cells could expand into 15−70 multiplication periods[14-15]; (7) After excessive proliferation, cells would lose the pluripotent differentiation potential and tend to caducity or death[14].

Cardiomyocytes differentiation of bone marrow mesenchymal stem cells

In vivo experiment

The bone marrow mesenchymal stem cells were injected into cardiac muscle after separation, purification and induction and differentiation. Injection through coronary artery or directly injected in myocardium. In 1999, Tomita *et al* [16] made differentiation of adult cardiocytes of bone marrow cell in rat.

In 2000, Wang *et al* [17] used the adult homogenic rat as donor and receptor to imitate auto-transplantation. Stem cells from marrow of donor leg were cultured and proliferated before 4',6-diamidino-2'-phenylindole (DAPI) mark was injected into the receptor rat's myocardium, and then it was to respectively take heart transplantation place to test at the 4th day and the 12th week and the donor cells could be seen at any time point. The cells could be transformed to myocardial cells and replaced the necrotic and died mal cells with the function of part microenvironment.

Toma *et al* [18] has successfully made the adenovirus of LacZ gene to transfect into human bone marrow stem cells (hBMSCs) with the regulation and control of Rous sarcoma virus-long terminal repeat (RSV-LTR) and cytomegalovirus (CMV), and the marked hBMSCs were directly injected into the ventriculus sinister of rat with severe combined immunodeficiency disease (SCID). Moreover, they successfully differentiate and show specificity albumen of myocardium in cardio microenvironment: myocardium troponin T and phospholamban on Ca-ATP was positive.

In the *in vitro* control group, the MyoD-hBMSCs were differentiated into skeletal muscle controlled by myoD gene, and they also found that the efficiency of RSV regulatory sequence was far higher than that of CMV; the highest transfect efficiency appeared when the concentration of the virus was 1 000−1 500 mol/L. Rangappa *et al* [19] put mice leukemia virus (MoMuLV) with LacZ gene into the packing cells to transfect hBMSCs. The hBMSCs of the combined LacZ gene production-gal were considered as markers. Fluorescence X-gal staining showed that conversion rate reached 65%. After transplantation, hBMSCs were parallel arranged with cardiocytes of donor. The test of vWF factor showed that hBMSCs could promote the formation of micro artery and it continued to compose β-gal for 2 months in host, showing that hBMSCs could be regarded as carrier to lead the target gene into cell of host. Transplanted through cyclic system, hBMSCs could live in myocardium of host and differentiate into cardiac-like cells[20]. In addition, Bayes Genis and others treated the patient that receiving cardiac transplantation with bone marrow stem cells transplantation and proved that bone marrow stem cells could differentiate into cardiac-like cells[21]. Unite Transplantation: Min *et al* [22] transplanted the hBMSCs marked by green fluorescent protein and human fetus cardiocytes (hFCs) into infarct myocardium of pig model and found that the improvement of unite transplantation group was obviously higher than that of hBMSCs transplantation group, which illustrates that the mutual function between hBMSCs and hFCs has substantial positive function for cell differentiation and promoting the formation of vessel. Recently, some people think combining myoblast of skeletal muscle plus bone marrow stem cells transplantation is better than singly using any one cell[23].

In vitro experiment

Building cardiomyogenic cells (CMG): the research of Tomita *et al* [16] found that the MSCs induced by 5-azacytiding (5-aza) *in vitro* could mutually connect and form polykaryocyte and myotube after cultured for 10 days, and after 21 days, the expressions of cardiac troponin Ⅰ (cTnⅠ) and myosin heavy chain were positive. This shows that the MSCs could differentiate into cardiocytes after *in vitro* induction. Through methylation, some static genes of 5-aza made MSCs had the possibility to differentiate into cardiocyte. However, Liu *et al* [24] think that such activation is invalid for non-immortalized MSCs.

In 1999, Makino *et al* [25] *in vitro* induced MSCs to differentiate into myocardium successfully for the first

time. The author made primary culture and subculture for cells from marrow. Continuous passage of adherence MSCs for 4 months became immortalization cells; limitedly dilute monoclonal subculture, induced by 3 μmol/L 5-aza, observed with microscope after 24 hours showed the mark spontaneously pulse cell line could be seen, and then utilized clone technology to separate and culture and then induced with 5-aza for another 24 hours, repeated the above operation and obtained cell line for the second time, which was the undifferentiated CMG. At the beginning, cells showed as fibrocyte. After 1 week induction, 30% of cells became greater, showing globular or extended as virgate to the same direction. After 2 weeks, it connected with cells around it. After 3 weeks, cells were connected with intercalated disc and formed myotube. There was phenotype cardiocyte and actively and spontaneously pulse, which called as differentiated CMG[26].

Clinical research on stem cells transplantation for the treatment of heart disease: Based on the experiment and research, in 2001, stem cells transplantation was used to treat the heart disease Ⅰ period clinical research. The report of the first example was from the experiment made by Hakuno *et al* [27]. They selected 20 patients who suffered acute myocardial infarction and treated by regular coronary artery recanalization and support implantation. Patients were divided into control group with 10 cases and cell treatment group with 10 cases. The treatment group input autologous marrow stem cells through coronary artery.

We think the transplantation of MSCs is related to contract function of ventriculus sinister of AMI patients. Mann[28] adopted the method to inject the MSCs into coronary artery and CPS to treat old MI. Selected 75 patients who suffered MI within at least 3 months and had stable ischemic heart disease. The patients were randomly divided into three groups, including control group with 23 patients, CPC treatment group with 28 patients; after 3 months, the original control group was randomly divided into CPC treatment group with 10 patients and MSCs treatment group with 11 persons. After 3 months, we made a follow-up. Results showed that only the EF of patients in MSCs group was obviously increased (+2.9%) compared with that before surgery. However, there was no obvious changes in CPC group (−0.4%) and control group (−1.2%); moreover, the improvement of heart function was related to the regularly inputting of MSCs into infarct part and improvement of contract function. Cross experiment revealed that injection of MSCs into coronary, which was related to the improvement of contract function of heart. We think that after injecting MSCs for 3 months, EF will obviously increased to a certain degree, which is available and secure to use cell treatment to treat MI patients.

Morimoto *et al* [29] used RT-PCR to test mRNA in order to research surface receptor of CMG and find undifferentiated CMG express a AI, a B1 and a D1 and adrenergic receptor. Induced by 5-aza, it could expressed β1, β2, M1 and M2 receptor. After giving phenylephrine, lsoproterend, prazosin, propranolo, atropine and M2 AFD116, we tested ERK1/2, CAMP, relevant signal path of TP3, contract rate of cardiocytes and others and found that these receptors have functional conduction access and could effectively regulate and control the function of cells.

Waksman *et al* [30] made detailed research on the morphology, electric physiological property and others of differentiated CMG, and the results indicated: (1) ultrastructure was similar with myocardium but not skeletal muscle; (2) had fetus ventricle muscle cell phenotype; (3) expression of transcription factor; undifferentiated CMG could express GATA-4, TER-1, NKX2.5, HAND and MEF2-C and the differentiated CMG could express MEF2-A and MER2-D; (4) action potential was similar with myocyte of embryo and ventricle: differentiated CMG appeared in at least two different kinds of action potentials after 28 days, atrionector potential and ventricular muscle potential. Atrionector potential showed relatively high resting membrane potential. The slow depolarization of late stage of diastole was just like pace-making potential; ventricular muscle action potential was little sharp and bended top. These ventricular muscle action potentials recorded by spontaneous beat cell had several features: (1) relatively long interphase or platform of action potential; (2) relatively high resting membrane potential; (3) depolarization of late stage of diastole like pacemaker. After processed by 5-aza for 3 weeks, it just recorded the atrionector potential and could record the ventricular muscle potential after 4 days and gradually took the main position. Most of action potential of myotube cells was myocyte potential. 5-aza induced MSCs to differentiate into myoblast, whose accurate mechanism was unclear. In addition, the separation, purification, identification and culture method of MSCs and how to improve the inducing rate of CMG and other difficulties are waiting to solve. Fuchs *et al* [31] researched on 5 heart pseudomyxoma peritonei and one undifferentiated sarcoma, which showed that tumor could express the specificity factor which was not expressed in the differentiated process of myocardium: NKX2.5/CSX, CATA-4, MEF-2, eHAND, corresponding mRNA and protein production. The ultrastructure of tumor showed the differentiation of immature interstitial cells. This discovery supported that pseudomyxoma peritonei may roots in mesenchymal adult myocardium progenitors cells, indicating transplanting undifferentiated cardiocytes into heart have potential formation of tumor. At present, whether bone marrow stem cells may differentiate into tumor *in vivo* is still unclear.

Homing of bone marrow stem cells

Ciulla *et al* [32] made experiment on MSCs directionally home to myocardium by peripheral injection, which indicated that peripheral stem cells could reach the damaged cardiac muscular tissue *via* blood circulation. Bittira *et al* [33] injected the stem cells marked by lacZ receptor gene into the penis vein of mouse. It proved the capacity of stem cells for settling in myocardium. Song *et al* [34] studied that autologous MSCs transplantation repairing damaged myocardium possessed its inherent limitation for the viability of transplanted stem cells. Adhesive force is the premise of cells survival as well as the key factor of the MSCs differentiation.

Stem cells in blood circulation can directionally reach the infracted myocardium and participate in tissue regeneration. But the exact motivation that promotes its position and cell proliferation has not been clear yet. It has known that the cell membrane of candidate stem cells expresses C-kit antigen and stem cells factor is the ligand. The experiment indicated that SCF rapidly generate after many tissues (including myocardial injury) were injured. Therefore, the way of C-kit/SCF may be one of the mechanisms promoting stem cells to migrate to myocardial infarction region. Besides, VEGE may also played an important role in migration of stem cells. $CD34^+$ cells and candidate stem cells expressed VEGF receptor Ⅰ and Ⅱ respectively, and could migrated to myocardial ischemia region by the interaction with VEGF in damaged region[35]. Rafii *et al* [36] tested that MSCs could home to nearby infracted myocardium. At 4 weeks after myocardial infarction, monocyte chemotactic protein 3 (MCP-3) could make MSCs home to heart. After injecting stem cells, the cardiac function of MCP-3 expressive group was improved (88.7%, $P < 0.001$). But the improvement of cardiac function that constrains the expression of MCP-3 was not undesirable (8.6%, P=0.47). Someone holds it is the inflammatory reaction after myocardial necrosis releasing various inflammatory mediators which arouses the directional migration of stem cells. The regulation mechanism of internal stem cells transforming to myocardial cells has not been known. Most of the views consider that it is related to microenvironment. The possible mechanism is that after stem cells enter the microenvironment, the around myocardial microenvironment can provide cardiac growth factors needed by specific differentiation and the molecule needed by other differentiations, and can promote stem cells differentiate into myocardial cells in myocardial microenvironment, which is so-called "environment induces differentiation" theory[37].

To sum up, it is a long way to make clear the mechanism of MSCs in perfecting myocardial function, achieve the best therapeutic effect and reduce complication as far as possible in order to formulate a set of perfect and systematic therapeutic schedule, so the mass, strict and normalized foundation and clinical experimental studies are need in future.

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骨髓间充质干细胞移植改善心肌功能机制的研究与进展☆

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文章亮点：自体骨髓干细胞移植无免疫排斥反应，并可在体外大量扩增，但其在治疗心肌梗死的作用机制研究仍处于初期阶段。

关键词：缺血性心脏病；心力衰竭；骨髓间充质干细胞；血管再生；组织工程

摘要

背景：骨髓间充质干细胞移植可以通过影响心肌细胞形成和心肌血管再生，改善心脏结构的重构和心功能。

目的：通过对骨髓间充质干细胞移植改善心肌功能机制的研究来初步阐述骨髓间充质干细胞移植临床治疗缺血性心脏病理论依据。

方法：电子检索中国生物医学文献数据库和计算机 Medline 数据库 1994 年至 2011 年收录的关于干细胞在缺血性心脏疾病中应用的相关综述和论文报告，并分析其研究进展。

结果与结论：共纳入相关文献 37 篇。骨髓干细胞移植自体应用时无免疫排斥反应，可在体外大量扩增，避免伦理争论。骨髓干细胞治疗心肌梗死的研究仍处于初期阶段，随着研究深入，如果找到临床应用有效、安全的剂量，其必将为防治缺血性心脏病提供全新的治疗手段。

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