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Vivo study of MSCs on EPCs cells in ischemic heart disease

Jianming Wang[1], Gengbiao Chen[2], Youfeng Guo[1], Xiaohong Yin[1], Xiaodong Wang[3*]

[1]Guangdong Food and Drug Vocational –Technical School, 510663, China

[2]The Second Affiliated Hospital of Sun Yat-Sen University, 510665, China

[3]The First Affiliated Hospital of Guangdong Pharmaceutical University, 510080, China

Abstract: **The treatment of ischemic heart disease mainly depends on the formation of blood vessels and the survival of cardiac myocytes. This paper will research the enhancement of EPC cells by mesenchymal stem cells in the treatment of ischemic heart disease. Mouse bone marrow was collected, MSCs cells were isolated and cultured. Flow cytometry was used to test the expression of CD34 and CD44. Then MSCs cells and endothelial progenitor cells co-culture 48 h, and 24 Wistar rats were randomly divided into EPCs group, EPCs MSCs + group and the control group. MSCs 2×106, EPCs 2×106, EPCs 2×106, and PBS buffer were injected into five different regions around ischemic myocardium. The changes in cardiac function were assessed by echocardiography after 4 weeks. MSCs 2×106, EPCs 2×106, EPCs 2×106, and PBS buffer were injected into five different regions around ischemic myocardium. The changes in cardiac function were assessed by echocardiography after 4 weeks. The rats were sacrificed, and the expression of VEGF was detected by immunohistochemistry and immunofluorescence. The surface markers CD34 and CD44 of MSCs and EPCs were differentially expressed. We successfully isolated MSCs and EPCs cells. Meanwhile, the proliferation index Ki67 was highly expressed, suggesting that MSCs could effectively promote the proliferation activity of EPCs. Echocardiography showed that after MSCs+EPCs treatment, the left ventricular ejection fraction, end-diastolic volume and cardiac volume of the rats were significantly improved, with statistically significant differences from the control group and the EPCs group (P<0.05), Meanwhile, the expression of VEGF in MSCs+EPCs group was also significantly increased (P<0.05). Mesenchymal stem cells can enhance the therapeutic effect of EPC cells in ischemic heart disease, which can promote myocardial angiogenesis, improve ventricular remodeling and cardiac function.**

Keywords: MSCs; EPCs. Ischemic heart disease; VEGF

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*Corresponding Author: Xiaodong Wang, 279407056@qq.com

1. Introduction

In recent years, the application of stem cells in ischemic heart disease has made great progress. When endothelial progenitor cells are mobilized in the peripheral blood, they can be converted into endothelial cells and participate in the regeneration and repair of local blood vessels in tissues. EPCs have strong multidirectional differentiation potential and renewal capacity [1]. Mesenchymal stem cells isolated from bone marrow tissue cells, it can promote proliferation and repair of ischemic tissues cells [2]. Studies have found that planting MSCs in the infarction area can effectively promote myocardial regeneration and peripheral angiogenesis, so as to maintain a good blood supply of ischemic tissues [3]. The application of MSCs in the treatment of ischemic myocardium has become a new therapeutic direction. However, most domestic scholars choose single MSCs EPCs to planting treatment of ischemic myocardium. At present, in China there is no precedent of two kinds of stem cells transplantation at the same time. It may be the reason that the two kinds of cells differentiation under the influence of various factors, a kind of stem cell differentiation, or can be affected by another kind of stem cell biology behavior [4]. the mechanism of its action is not clear, therefore, this article will explore whether mesenchymal stem cells enhance EPC cells in the treatment of ischemic heart disease.

2. Materials and Methods

2.1. Isolation, co-culture and identification of MSCs and EPCs

Mice to death after anesthesia, in a sterile environment, the mice's bilateral tibia and the femur were taken, extracted 5 ml bone marrow tissue. The bone marrow cavity was washed repeatedly, the washed bone marrow liquid was mixed and inoculated in the culture medium, and the culture was conducted in the cell incubator for 48 hours. After the number of cell adherents reached 90%, the cells were digested and subcultured for 3 times, and the cells were collected for follow-up research. EPCs: bone marrow extraction was performed in the same way as MSCs. After 48 hours of cell culture, unattached cells were collected, centrifuged and re-suspended, and uniformly inoculated in culture dishes for continued culture and passage. Co-culture: 5×10^5 MSCs cells, which had been passed for 3 generations, were inoculated in the medium containing.

5×10^5 EPCs were inoculated in the same culture medium and placed in an incubator with 5% CO_2 at

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37℃ for 48h, followed by digestion and separation. The digested cells were mixed into PBS, and the expressions of CD34 and CD44 were detected by flow cytometry.

2.2. Preparation of rat model of chronic ischemic heart disease and transplantation of MSCs and EPCs

Take 40 Wistar rats, preparation of myocardial ischemia model. During the operation, continuous electrocardiogram monitoring was conducted to maintain stable respiration and heart rate. The chest was cut open and coronary arteries were dissociated. Ligation lines were placed from the lower edge of the left auricular appendage to ligate coronary arteries. The model of myocardial infarction was successfully established when the coronary artery was ligated and the myocardial movement in the local area was weakened and the infarct area reached 30%. The model of myocardial infarction was successfully made. 24 successfully model rats were randomly divided into MSCs+EPCs group, EPCs group and control group. MSCs 2×10^6 +EPCs 2×10^6, EPCs 2×10^6 and PBS buffer 200uL were injected into five different areas around ischemic myocardium.

2.3. Echocardiography to evaluate cardiac function

After 4 weeks, the rats were anesthetized again, and the changes of cardiac function in each group were detected by echocardiography, which included left ventricular ejection fraction, end-diastolic volume and cardiac volume.

2.4. Expressions of KI67 and VEGF

After the detection of cardiac function, the rats were killed, and the myocardial ischemic regional tissues were taken, and the expressions of KI67 and VEGF were detected by immunohistochemistry and immunohistochemistry.

2.5. Statistical method adoption

SPSS20.0 statistical software was used, and quantitative data were expressed as mean ± standard deviation. Analysis of variance was used for comparison among multiple groups, and q test was used for further pairwise comparison of results with differences. $P<0.05$ was considered as statistically significant difference.

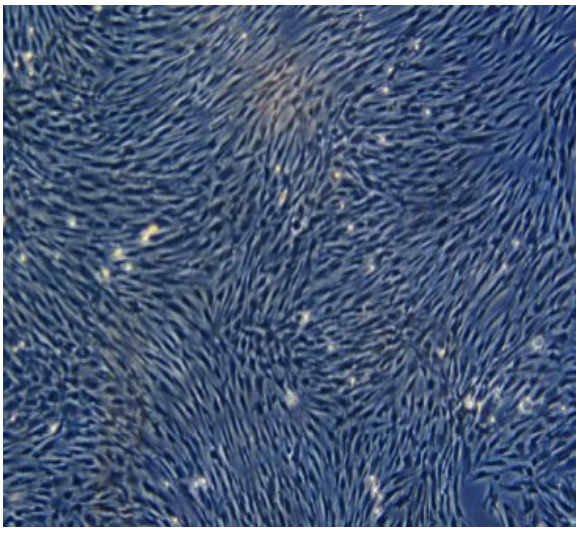

Figure 1. Isolation and culture of MSCs 100X, bar=250um.

3. Results

3.1. Isolation and culture of MSCs

After inoculation, the survival rate of MSCs cells reached 94%, and the cells were polygonal or dense and small round. With the extension of culture time, the cells began to stick to the wall and grew, and the volume gradually increased, and the cells were fibrocell-like (Figure 1).

3.2. MSCs and EPCs markers

After 10 days of adherent culture, flow cytometry showed that MSCs marker CD44 was negative, while CD34 was negative (Figure 2).

3.3. Echocardiography to evaluate the changes of cardiac function in each group

After treatment with MSCs+EPCs, the left ventricular ejection fraction, end-diastolic volume and cardiac volume of the rats were significantly improved ($P<0.05$), and the improvement degree of the EPCs group was significantly better than that of the control group ($P<0.05$), as shown in Table 1.

3.4 Expressions of Ki67 and VEGF

The expression of VEGF and Ki67 in myocardial tissue of MSCs+EPCs group was significantly increased, suggesting that MSCs can effectively promote the proliferation activity of EPCs and the angiogenesis of ischemic areas, shown in Figure 3 and Figure 4.

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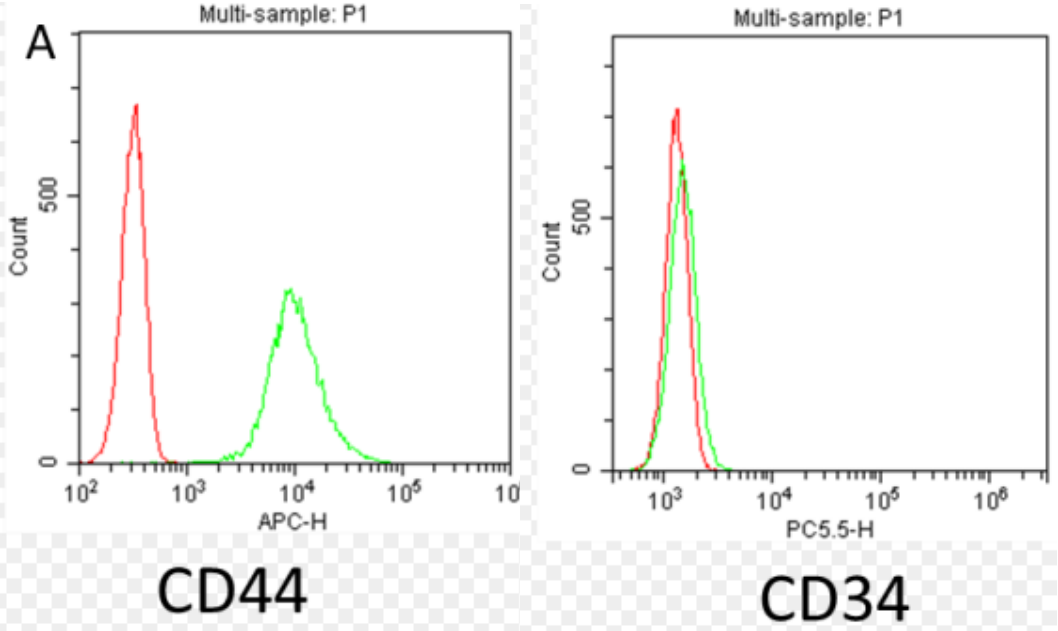

Figure 2. MSCs and EPCs markers of CD44 and CD34.

Table 1. Echocardiography to evaluate the changes of cardiac function in each group

Group	Case	left ventricular ejection fraction	end-diastolic volume	cardiac volume
MSCs+EPCs Group	8	0.288±0.030	299.63±30.25	80.22±12.36
EPCs Group	8	0.195±0.041ab	285.21±28.47ab	63.25±8.95ab
Control Group	8	0.168±0.022	212.58±29.13	55.69±7.14
F		22.361	15.63	8.142
P		0.001	0.001	0.001

Compare with MSCs+EPCs group, aP＜0.05, Compare with control group, bP＜0.05

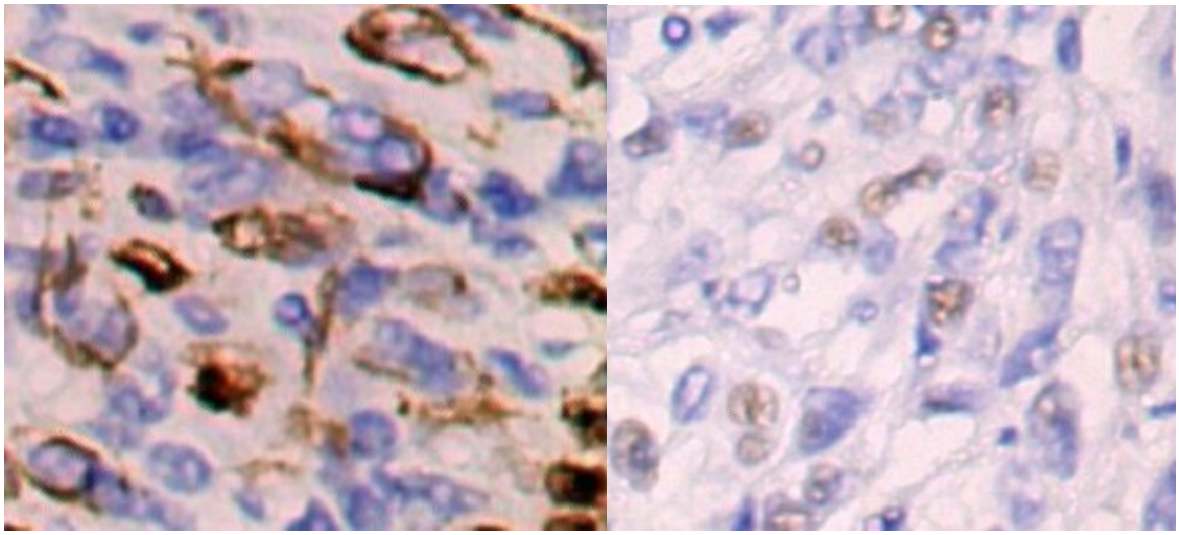

Figure 3. The Expressions of Ki67 (right) and VEGF (Left).

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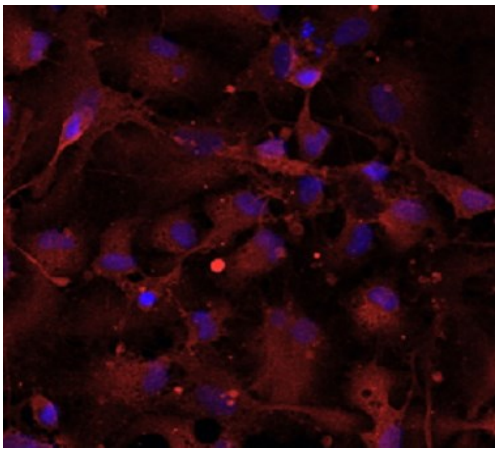

Figure 4. VEGF Expression 400X.

4. Discussion

Ischemic cardiomyopathy is a clinical common disease, the death toll to 1 million people a year, and the increase rate of 20% a year, a serious threat to human life safety. After the occurrence of myocardial infarction, myocardial contraction ability to drop, ventricular remodeling, and heart failure, is the leading cause of death in patients. At present the treatment of myocardial infarction (mi) has to dilate blood vessels, anticoagulation, melting, and stent and vascular bypass, etc. [5]. However, with the progress of vascular reconstruction technology in recent decades, the existing technology is still unable to repair or promote the proliferation of cardiac cells and blood vessels, so the probability of ventricular remodeling and heart failure in ischemic myocardium is still high, and the therapeutic effect is still not ideal. In this clinical context, how to effectively open up the new situation of ischemic heart disease treatment is an urgent problem for clinical medicine. A number of studies have found that MSCs inhibit after myocardial infarction area, can induce the expression of multiple factors and promote the local formation of new blood vessels [6], but there are also other studies have found that pure gene therapy promotes cell proliferation is not obvious on the improvement of the myocardial function. The possible reasons is the heart's own stem cells number is less, by means of gene does not effectively supplement the necrosis of myocardial cells [7]. Therefore, added by exogenous stem cells, the cells grow and myocardial, at the same time through paracrine way to promote the secretion of cytokines, to promote blood vessel growth and improve the effect of blood perfusion [8]. EPCs is early and widely research and application in the repair of ischemic tissues and new blood vessels form a kind of stem cells, which can be directly involved in the repair of ischemic tissues, but stem cell biology is vulnerable to a variety of internal and external factors, the change of the growing environment for stem cell biology also influence [9]. Therefore, how to effective intervention in this phenomenon, is the scientific research need to solve the problem. Some scholars applying MSCs and EPCs to radiotherapy in patients with malignant tumor after treatment, to see if you can reduce the radiation caused by bone damage. It was found that EPCs patients after bone marrow MSCs or simple injection has no obvious new blood vessels, and EPCs joint after injection of MSCs and the formation of new blood vessels of clearly visible at that both through the common application is likely to have positive synergy [10]. This study tried to combined use of MSCs and EPCs trained after transplantation in ischemic myocardial tissue, to observe whether MSCs can enhance the EPC cells in the treatment of ischemic heart disease.

In this study, flow cytometry showed that the expression of CD44, CD29 and CD105 in MSCs was positive, while CD34 was negative, and the isolated cells were consistent with the morphology and surface markers of MSCs. CD34 was positive and CD90 was negative, indicating that the isolated cells were consistent with EPCs morphology and surface markers. The two kinds of cells were further transplanted into ischemic myocardial rats after co-culture, and the results showed that after MSCs+EPCs treatment, the left ventricular ejection fraction, end-diastolic volume and cardiac volume of the rats were significantly improved. It was statistically significant different from the control group and the EPCs group, and the improvement degree of the EPCs group was significantly better than that of the control group. The results showed that the combined application of the two drugs plays an important role in promoting cardiac angiogenesis and improving myocardial blood supply. In the process of using stem cells to treat myocardial infarction, the microenvironment of cells plays an important role. An important problem for stem cell transplantation is that the survival rate of cells in the recipient body is low, and many stem cells will die within hours after transplantation [11]. Studies have found that this process is the result of multiple factors, and cytokines, immune status and ischemia status are important factors for its occurrence [12]. Therefore, how to improve the tolerance of stem cells in transplantation and further exert a series of biological functions such as targeted differentiation, migration and vascular regeneration of endothelial progenitor cells and mesenchymal stem cells are the key to solve the effect of stem cells in treating ischemic cardiomyopathy [13]. In order to further evaluate the positive synergistic effect of the combined transplantation of two kinds of stem cells in improving the success rate of treatment, a rat model of ischemic cardiomyopathy was selected in this study. Pathological results of this study showed

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that EPCs group MSCs + myocardial tissue VEGF expression is significantly increased, and the rest is similar between the two groups have statistical significance. VEGF plays an important role in cell differentiation and proliferation, domestic guo [14] the study found that MSCs under the induction of VEGF can be in the direction of the endothelial cells differentiation, and promote endothelial progenitor cells and the formation of new blood vessels. Abroad Lopez [15] found that MSCs and EPCs can co-culture to form vascular microstructures. The key molecule mediating the mutual interaction between the two is VEGF. So the expression of VEGF can be an important indicator of the therapeutic effect of stem cell transplantation. The results of this study preliminarily confirmed that MSCs and EPCs have a positive synergistic effect in promoting the neovascularization of ischemic myocardial tissue. VEGF may be an important factor for MSCs to promote the differentiation of EPCs. It may be involved in the repair and regeneration of damaged blood vessels, so as to achieve the purpose of treatment.

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