МАТЕРИАЛЫ КОНФЕРЕНЦИИ

PRECLINICAL STUDIES OF CELL-GENE THERAPY EFFICACY FOR TREATMENT OF HEART ISCHEMIA

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It was shown the promising results using adipose derivate MMSC transfected with pWZ Blast-VEGF for the treatment of ischemic heart disease. In the group with the introduction of pWZLBlast-VEGF transfected cells compared to control in the area of simulated ischemia, revealed a significant increase in the number of functioning capillaries (by 224,2%, p <0,05), density (by 350,1% p <0,01) and the exchange surface (245, 4%, p <0,01) and pO2 (by 282% p <0,01). Moreover, compared with controls, the introduction of pWZLBlast-VEGF transfected cells led to markedly increased blood vessel migration into the ischemia zone from adjacent tissues.

Introduction

Cardiac cell and gene therapy has already been investigated in experimental and clinical studies in recent years [12, 27, 31]. Different vector systems for gene therapy have been developed. Non-viral vectors are remarkable with regard to safety, but are limited by low transfection efficiency and transient gene expression. In contrast, virus-based vectors transfer the transgene more efficiently, but low organ specificity and immunogenic properties can limit their applicability. Combination with specific application techniques, such as intramyocardial injection, catheter-based perfusion, ultrasound targeted microbubble destruction, or retroinfusion unable to enhance vector efficiency [12, 30, 31]. Low efficacy of myocardial gene transfer and limited duration of transgene expression have been held responsible for the lack of significant clinical success [18].

On the other hand, adult progenitor cells are used in clinical trials to treat patients who have sustained acute myocardial infarction or who have chronic ischemic heart disease. Many of these studies have demonstrated clinical benefits [1, 19, 20, 23, 24, 29]. Mesenchymal stem cells (MSCs) are a subset of stem cells that inhabit the stroma and can be induced to differentiate in vitro into cardiomyocytes, and was shown in a large number of animal and clinical studies of the MSCs efficacy for cardiac repair and regeneration [2, 3, 16, 17, 21, 25, 28] Preclinical studies using transplantation of MSCs demonstrated improved post-infarct left ventricular function and reduction in infarct size, and a decrease in mortality. These improvements were seen despite small numbers of cells undergoing differentiation to cardiomyocytes [10]. A clinical study of MSCs in 69 postinfarct patients also demonstrated improved left ventricular function [22]. However, there remains significant heterogeneity among MSC populations and thus they are less predictable when implanted. Most notably, some studies found that implanted MSCs had differentiated into osteoblasts inside ventricular tissue [13, 15]. This is an obvious cause for concern and needs to be addressed prior to fullscale therapy.

Controlled angiogenesis is an important component of successful tissue regeneration [9] as well as the treatment of ischemic diseases.

Many strategies have been developed to promote vascular growth, including growth factor delivery [7, 26] but is often associated with an initial burst of growth factors and a short half-life in vivo [4]. The uncontrolled diffusion of angiogenic factors may also cause undesirable side effects.

Stem cell therapy holds potential as an alternative approach that may offer advantages by promoting therapeutic angiogenesis through paracrine factor signaling [6, 11] as well as their ability to migrate toward the ischemic tissues [5]. However, the efficacy of using stem cells alone to promote angiogenesis remains limited [30].

Genetic modification of stem cells to express angiogenic factors is a promising approach to further enhance the efficacy of stem cells for therapeutic angiogenesis. Virally modified, VEGF-overexpressing mesenchymal stem cells (MSCs) were reported to enhance angiogenesis [9] in vivo and improve myocardial function [14]. But vectors that integrate into the genome can create mutations and limit the utility of the cells in both clinical and research applications.

We modified rabbit stem cells to express an angiogenic gene encoding VEGF using non viral vector. Transplantation of PBAE/VEGF-modified stem cells significantly enhanced angiogenesis in rabbit of left anterior descending artery of the heart incomplete.

Gene construction

The constructs expressing VEGF were created by digesting the vector VEGF164 (a gift from Bruce Spiegelman, Dana Farber Cancer Institute) with BamH1 and EcoR1, and ligating it to similarly digested pWZLBlast to pWZLBlast-VEGF.

Transfection. Adipose-derived rabbit MSCs were obtained and cultured as previously described [8]. Cells were transfected with VEGF plasmid or control plasmid (EGFP, Addgene plasmid 13031) by using optimized transfection conditions with Lipofectamine 2000 (Invitrogen).

Implantation of Stem Cell-Seeded Scaffolds

Results

Experiments were performed on male Chinchilla rabbits (weight 2,8-3,2 kg). Modeling of left anterior descending artery of the heart incomplete (80%) occlusion was performed by its ligation on the stylet. Immediately after arterial ligation, cells (1.0×10^6 cells per injection) were suspended in rMSC growth medium and injected intramuscularly cardial muscle in place of ischemia. Experimental group of animals (n = 5) was injected with pWZLBlast-VEGF transfected cells, while control group (n = 5) – with EGFP transfected cells

The level of angiogenesis was assessed on day 30 after surgery on microscopic sections of the myocardium, on the basis of determining the number of capillaries, the average diameter of capillaries (d), measured using the eyepiece micrometer, calculating the density (n) (n/mm^2), the exchange surface of capillaries (ESC) and the capacitance of the capillary bed (CCB) on 1 mm^3 myocardial tissue.

Study of partial pressure of oxygen (pO_2) in the area of damage on open-heart surgery was performed by the polarographic method.

The results demonstrate that at the 30 th day after the occlusion in the area of simulated ischemia in control animals, the density of vascular elements was lower than in surrounding areas with initially adequate blood supply.

At the same time in the group with the introduction of pWZLBlast-VEGF transfected cells revealed more dense arrangement of capillaries anastomosing extensively with each other and forming a network.

When morphometric evaluation of capillary zone ischemia, average diameter of capillaries not statistically different in both groups of animals (difference 7.2%, $p > 0.1$).

In the group with the introduction of pWZLBlast-VEGF transfected cells compared to control in the area of simulated ischemia, revealed a significant increase in the number of functioning capillaries (by 224,2%, $p < 0,05$), density (by 350,1% $p < 0,01$) and the exchange surface (245, 4%, $p < 0,01$) and pO_2 (by 282% $p < 0,01$).

Moreover, compared with controls, scaffolds seeded the introduction of pWZLBlast-VEGF transfected cells led to markedly increased blood vessel migration into the into the ischemia zone from adjacent tissues.

Thus the results confirm the previously suggested idea about the perspective of genetically modified multipotent mesenchymal stromal cells for a therapy for ischemic heart damage, and moreover, any defect of any organs and tissues.

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ДОКЛИНИЧЕСКОЕ ИССЛЕДОВАНИЯ ЭФФЕКТИВНОСТИ ГЕННО-КЛЕТОЧНОЙ ТЕРАПИИ ИШЕМИИ СЕРДЦА

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Продемонстрированы многообещающие результаты использования мультипотентных мезенхимальных стромальных клеток жировой ткани, трансфицированных pWZ Blast-VEGF для лечения ишемической болезни сердца. В группе с введением pWZLBlast-VEGF трансфицированных клеток, по сравнению с контролем, в области моделирования ишемии выявлено значительное увеличение числа функционирующих капилляров (на 224,2%, $p<0,05$), плотности капиллярного русла (на 350 , 1% $p<0,01$) и обмена поверхности капиллярного русла(245,4%, $p<0,01$) и pO_2 (на 282% $p<0,01$). Кроме того, по сравнению с контролем, введение pWZLBlast-VEGF трансфицированных клеток сопровождалось значимым увеличением числа анастамозов между сосудистой сетью непораженной области миокарда и новообразующимися сосудоми в зоне ишемии.

GENE-CELL THERAPY OF LIVER FAILURE. EXPERIMENTAL PART I

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We report a method for systemic derivation of functional hepatocyte-like cells from human MSCs, which are readily accessible from adipose tissue.

In summary, our findings indicate that AT MSCs derived from adipose tissue can differentiate into functional hepatocyte-like cells in vitro, in addition to mesodermal and ectodermal lineages. The results of this study shows the perspective of the restricted differentiation potential of adult-derived stem cells. Most important of all, MSCs may serve as a cell source for tissue engineering or cell therapy of liver.

Introduction

One of a pathology for which organ transplantation is the only definitive therapy is a liver failure [9]. Developments of new methods to treat patients with hepatic diseases show the way to eliminate the need for liver transplantation. Identification of specific growth factors that promote liver regeneration has allowed the development and the use of recombinant growth factors, but the success of this strategy was hampered by the short half-life of these proteins and the need to administered them continuously [4, 10]. To overcome this problem, investigators successfully used gene technology to transfer the genes that encode these factors to liver to enhances its proliferation [11].

But the efficicncy and selectivity of in vivo transfection with the introduction of the vector into the main blood stream is very small. Vectors based on viruses show better results but they integrate into the genome, which create limitation of the cells application in clinic. Furthermore, they cause the immune response that severely limits of applicability in clinical practice

On the other hand it was shown that the introduction of mesenchymal stem cells (MSCs) accompanied with a good clinical effect. Moreover, human MSCs have been described as an attractive cellular vehicle for gene.

Secondly it has long been thought that the differentiation potential of adult stem cells is limited to their germ layer of origin, but recent studies have demonstrated that adult stem cells are more plastic than once believed [5].

However, subsequent work by several independent groups has clearly shown that hepatocyte replacement levels after MSCs transplantation are low (<0.01%), unless the MSCs have a selective growth advantage [3, 15]. Furthermore, in most of the cases, fusion with host hepatocytes, but not transdifferentiation of extrahepatic cells, has been described as the underlying principle of the therapeutic effect [1, 12, 14, 17, 18]. More over the failure of other research to show an impact of bone-marrow-derived MSCs on liver regeneration [3, 6, 15], clearly show that the use of bone-marrow-derived cells for the treatment of liver diseases is far from clinical application and that additional basic research on reprogramming is needed.

Adipose tissue MSC (AT-MSCs), like bone marrow, is a mesodermally derived organ that contains stem cells [19].

AT-MSCs share many of the characteristics of their bone marrow counterpart, including cell morphology, extensive proliferation potential, tumor tropism and the ability to undergo multilineage differentiation [2, 8, 15]. But, unlike bone marrow MSCs, AT-MSCs can be obtained by a less invasive method and in larger quantities (40-fold higher compared with that of bone marrow than bone marrow [7] and did not show any detectable chromosomal abnormalities or formation of tumors in the host's tissues.)

Thus the purpose of this study is to investigate the possibility of MSCs differentiation into hepatocytes as a result of cells transfection by using a vector that encodes a hepatocyte growth factor (HGF).