Cell Therapy for Peripheral Arterial Disease: Cellular and Molecular Aspect

Periferik Arter Hastalıkları İçin Hücresel Tedavi: Hücresel ve Moleküler Bakış

Günseli ÇUBUKÇUOĞLU DENİZ,[a]
Serkan DURDU, MD,[a,b]
Aynur KARADAĞ,[a]
Çağın ZAİM, MD,[b]
Arın DOĞAN,[a]
Reza DATOURİ,[a]
Ahmet Rüçhan AKAR, MD[a,b]

[a]Biotechnology Institute,
Ankara University and Stem Cell Institute,
[b]Department of Cardiovascular Surgery,
Heart Center,
Ankara University Faculty of Medicine,
Ankara

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Yazışma Adresi/*Correspondence:*
Serkan DURDU, MD
Ankara University Faculty of Medicine,
Department of Cardiovascular Surgery,
Heart Center, Ankara,
TÜRKİYE/TURKEY
serkandurdu@gmail.com

ABSTRACT The aim of this review is to define the cell based therapy applications, together with revision of up to date literature in peripheral artery diseases, predominantly, the possible mechanisms of the theraupetic effect regarding the experimental studies. Clinical studies about this issue will be mentioned limitedly. The issue titles which must be answered by the angiogenesis studies which are targeted by the cell based therapies will also be mentioned throughout this paper. The term regenerative medicine refers to the strategies and methodologies used in curing conditions as opposed to treating them. Despite more than 10 years of worldwide activity on adult stem cell developmental plasticity (metamoirosis), current results of fundamental research are still in the embryonic stage. Cardiovascular disorders are the leading causes of mortality and morbidity in the developed world. Cell-based modalities have received considerable scientific attention over the last decade for their potential use in this clinical arena. The question, whether or not future improvements on cell-based therapies will provide remarkable improvement in survival and quality of life for millions of patients with cardiovascular disorders still remains unsolved.

Key Words: Stem cell; peripheral vascular disease; angiogenesis; molecular mechanism

ÖZET Bu derlemenin amacı, perifer arter hastalığı ile ilgili güncel yayınları inceleyerek, terapötik etkinin olası mekanizmalarının incelendiği araştırmalar ışığında, hücre tabanlı tedavi uygulamalarını tanımlamaktır. Bu konuyla ilgili klinik çalışmalardan kısıtlı olarak bahsedilecektir. Anjiogenez konusunda yapılan çalışmalar ile incelenen konu başlıklarına da bu derleme içinde yer verilmektedir. Metin içerisinde onarımsal tıp terimiyle kast edilen tedavi yerine tamamen iyileşme yolunda izlenen tıbbi strateji ve metodolojilerdir. Erişkin kök hücrelerinin gelişimsel plastisitesi (metamoirosis) üzerinde yapılan çalışmalar 10 yılı aşkın süredir devam etmekte olsa da, temel bilimsel sonuçlar halen embriyonik aşamadadır. Kardiyovasküler hastalıklar, gelişmiş ülkelerde mortalite ve morbiditenin başlıca sebebidir. Hücre tabanlı tedavi yaklaşımları, geçtiğimiz on yıl içinde klinik alandaki potansiyel kullanım alanları sebebiyle yoğun ilgi görmektedir. İleriki yıllarda hücre-tabanlı tedavi yaklaşımlarındaki gelişmelerin, kardiyovasküler hastalıklardan etkilenen milyonlarca insanın yaşam kalitesine ve hayatta kalımına anlamlı bir fayda sağlayıp sağlayamayacağı halen belirsizliğini korumaktadır.

Anahtar Kelimeler: Kök hücre; periferal vasküler hastalık; anjiogenez; moleküler mekanizma

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THE BRIEF HISTORY OF THE ANGIONESIS STUDIES IN PERIPHERAL ARTERIAL DISEASES

The study reported by Asahara et al.[1] is the first invivo study which assessed the efficacy of the utilization of the endothelial progenitor cells (EPCs) in an experimental ischemia model following the isola-

tion of these cells via magnetic activated cell sorting principle. They reported that the EPCs formed capillary web formation on the 7th day. In 1-6 weeks the nucleus-marked EPCs appeared in the new vessel formation sites and almost all the marked cells were in correlation with the capillary vessel walls. In the detailed immunohistochemical study of these cells, it was shown that the marked EPCs settled in the ischemic site around the vessel web of the new vessel formation site. This study is the innovator of the cell-based therapies.

Kalka et al.[2] has an important implication about peripheral ischemia models. In their experimental study, EPCs which are cultivated by cellular culture environment are used. In the experimental peripheral extremity ischemia model of the athymic rats the efficacy of the human EPCs are compared with the control group. In the isolation of the EPCs, fluorescence-activated cell sorting (FACS) method was used. After the ischemia and cell based therapy process, observations were made in different periods. It was seen that in the cell implantation group autoamputation rate was lowered by 50%, capillary density rate and regional extremity blood flow increased significantly in early periods. This study has an historical importance by means of using the EPCs in peripheral extremity ischemia.

POTENTIAL MOLECULAR MECHANISMS IN ANGIOGENIC PROCESS

For defining the stem cell behaviour and their differantiation process and enlightening the potential molecular mechanisms in these processes, many informations are added to the literature. In defining the cell cycle, "niche" concept is used recently. "Niche" was defined by Schofield in 1978 as spesific anatomic sites which manage the role of stem cells for tissue renewing, protecting and repairing.[3] Niche is mostly in contact with related stem cells and controls the proliferation while blocking the reduction in stem cell number. However, it provides the stem cells' survival without any differantiation. It was reported that there is a failure between stem cells and the niche in tumoral development.[4] Niche is one of the important target sites in stem cell therapy and also it is an important component for tissue hemostasis.

According to the recent data, stem cell niches are the most important mechanisms in stem cell biology and behaviour regulation.[5] Humoral, metabolic, physical, neural and paracrine effects which affect stem cell niche continuously affect the niche and can affect the stem cell behaviour via the organisation of the cells neighbouring it.[6] As a result of the sent messages over the niche, some certain pathways and molecular mechanisms step in and then every stem cell symmetrically can produce two similar young cells or two differantiated cells (symmetric cell division) or every stem cell tends to produce a similar young cell and another differentiated young cell in the same division (asymmetric cell division).[6-8] The Notch receptor which is common in vertebrated and non-vertebrated animals includes one pathway transmembran protein and is modulated by Delta and Serrate ligands; it has very important functions in stem cell behaviour regulation.[9] For example, the protection of differantiation inhibition and immature phenotype for heamopoietic stem cells (HSCs) is provided by the Notch signal; differantiation stimulation is provided by the Wnt signal.[10] Uncontrolled regulation in the Notch and Wnt signal mechanisms and their continuous pathways can cause displastic cell proliferation (cancer formation hypothesis) or insufficient repair in older ages.

HSCs have the ability to renew themselves and to give rise to all lineages of the blood; however, the signals that regulate HSC self-renewal still remain unclear. Wnt signalling pathway has an important role in this process. Overexpression of activated b-catenin expands the pool of HSCs in long-term cultures by both phenotype and function.[11] The researchers conclude that the Wnt signalling pathway is critical for normal HSC homeostasis in vitro and in vivo, and provides an insight into the potential molecular hierarchy of regulation of HSC development.

Some signal ways in the bone marrow (BM) niche have a role in the endothelial differentiation. Osteoblasts in the endosteal niche expressing

Jagged-1 (Jag1) and N-cadherin contact and maintain HSCs by activation of Notch, and might further regulate HSC activity through N-cadherin and β-catenin signalling. The signal interactions between endothelial cell (EC) in vascular niche and HSCs is not well known. HSCs might be transported between niches and subjected to differential regulation in each niche. ECs expressing vascular cell-adhesion molecule 1 (VCAM-1) also associate closely with megakaryocytes and their progenitors through very late activation antigen 4 (VLA4) in response to chemotactic factors, stromal cell-derived factor-1 (SDF1) and fibroblast growth factor-4 (FGF4), and provide a niche for megakaryocyte maturation and platelet production. The immediate juxtaposition of HSCs to ECs also facilitates their rapid mobilization and entry into circulation in response to stress and, in the presence of megakaryocytes, release of platelets directly into the blood. HSCs and haematopoietic progenitor cells as well as megakaryocytes produce vascular endothelial growth factor (VEGF) and other angiogenic factors, which might act in a feedback loop to support ECs in the BM and in the periphery at sites of normal and pathologic angiogenesis.[12] VEGF-A plays a regulator role for the ECs. However which cell group would be chosen for vascular web formation in angiogenic process is not defined. In recent studies it is observed that Delta-like 4 (Dll4)-Notch 1 signal pathway plays a role in the formation of vascular web. Hellstrom et al. showed that inhibition of Notch signalling using c-secretase inhibitors, genetic inactivation of one allele of the endothelial Notch ligand Dll4 or endothelial-specific genetic deletion of Notch1, all promote increased numbers of tip cells. Conversely, activation of Notch by a soluble Jag1 peptide leads to fewer tip cells and vessel branches. Dll4 and reporters of Notch signalling are distributed in a mosaic pattern among ECs of actively sprouting retinal vessels. At this location, Notch1-deleted ECs preferentially assume tip cell characteristics.[12,13]

When detached from their niches or cultured in vitro, media stem cells die rapidly or differentiate to a cell type. This situation displays the importance of self homeostasis of the stem cells. If we understand the niche structure and cell-to-cell, cell-to-matrix relations properly, there will be a chance to create a virtual "wonderland" for the stem cells and the cells that differentiate from them. Thus, the number of the unknowns of regenerative medicine whose importance increases gradually decreases, resulting in an increased probability to treat more effectively. The complex structure and increase in the niche number and function brings up the question whether this kind of relations also arise during the unhealthy conditions. The increase in the knowledge about this issue lets not only the treatment purposed applications but also lets to explain the pathogenesis of the diseases as well. The regulations in the niche for a succesfull the treatment is another important issue to be worked on.

ARTERIAL, VENOUS AND LYMPHATIC ENDOTHELIAL CELLS IN ANGIOGENIC PROCESS

The explanation of the angiogenic process in the organism through the datas gained from embriological studies gives important datas in regenerative medicine applications. Blood vessels contain two types of cells: 1) ECs 2) mural cells (vascular smooth muscle cells and pericytes).[14] These cells which form the vessels show important differences at molecular level in different vessel sites. ECs and signal pathways which affect these cells provide these differences. For example transmembrane ligand, Ephrin (Eph) B2, and its receptor trosyne kinase EphB4 are shown as arterial and venous EC markers, respectively.[15] EphB2 which is assumed as an arterial marker is induced by vascular endothelial growth factor A, triggers Notch signal through VEGF receptor-2 (VEGFR2) or neurophillin-1(NRP1) and is formed with FoxC1 and FoxC2 transcription factors.[13] In the formation of venous system Notch signal is blocked via the COUP-TFII nuclear orphan receptor and a venous marker, EphB4 forms.[13] Serious vascular morphologic defects occurs by the blockage in the formation of EphB2 or EphB4.[15] Angiogenic formation in embrionic or postnatal period is managed via the balance between proangiogenic signals as VEGF and

inhibitors. Chosen ECs are induced by positive signals. By the fusion of EC,EC channels are formed and angiogenic signal regresses by the feedback mechanism when the ischemia reduces.[13]

It was shown in adults that circulating EPCs incorporate the active angiogenesis sites of the BM derived or peripheral blood isolated angioblasts and are the key factors for the re-endothelization.[1] EPCs are determined by the expression of the endothelial marker proteins such as vascular endothelial cadherine, von Willebrand factor, KDR and endothelial nitric oxide synthetase.[16] Ex-vivo cell therapy which is formed by cultivated EPCs transplantation is used for increasing the neovascularization of ischemic tissues succcessfully. The concept accepted recently is that EPCs can derive from BM and CD133/VEGFR2 cells have the endothelial premising capacity. However, it was shown that other BM derived cell populations (e.g. myeloid cells, "side population" cells and mesenchymal cells) and non-BM derived cells which can differ to EPCs also have the same capacity.[17] EPCs during both embrionic and postnatal physiologic terms contribute to the tissue vascularization. In this context, in the adults new vessel formation is not limited to the angiogenesis, includes "vasculogenesis and arteriogenesis".[18] In addition to these spesifications, EPCs can be responsible from endothelization and constitution of non-thrombogenic surfaces in prostethic vascular grafts.[19] However, regenerative or pathologic neovascularization can also occur by the vasculogenic incorporation of circulating EPCs.

CELL SOURCES IN ANGIOGENESIS STUDIES; ONLY ONE CELL SUBGROUP? CELL COMBINATIONS? POTENTIAL USE OF CYTOKINES

Endotelial cell levels in autogen peripheral circulation is to be researched for the assesment of the efficacy of the cell-based therapies in peripheral arterial diseases . In several studies the correlation of the circulating EC levels and peripheral arterial disease (PAD) pathogenesis is researched. In comparative studies circulating EPC spesific m-RNA expressions were observed to be low in the presence of the ischemic PAD.[20] Enhancing the number of the EPCs in circulation became an important target for the clinical studies. In a randomized study including three different PAD groups it was shown that the number of progenitor cells in circulation could be increased after the 4 weeks-exercise educational programme.[21] In another study of diabetic patients, it was detected that there was a direct relationship between EPC rates in circulation and ankle-brachial index and also there was a significant reduction of progenitor cells in diabetic patients with PAD.[22] Shi et al.[23] showed that EPCs in circulation plays a role in vascular repair in dogs. In this study, progenitor cells which are mobilized via a granulocyte colony stimulating factor in prosthetic vascular bypass graft has shown a significant increase in endothelization when compared with the control group. After understanding the important role of EPCs in vascular repair process, experimental studies focused to enhance the efficacy of these progenitor cells. In these studies the statins[24] and the estrogen[25] were found to have a role in enhancement of both EC count and capacity of repair. In addition, it was shown that eritropoietin applications cause an increase in functional EPC count however the mechanism is not clearly understood yet.[26]

In almost all of the experimental models following the studies mentioned above, the efficacy of direct cellular implantation are assessed in peripheral limb ischemia models.

WHICH CELL?

Consistent with the developments in cell based therapies, controlled studies made with different BM subgroups are also subsequently reported. "Mesenchymal cells" which are frequently mentioned in myocardial regenaration studies, may lead to another topic for the question "which cell". An important issue that is disscussed recently is evaluation of the effectiveness of the treatment whether the treatment is achieved by mesenchymal cells or mononuclear cells in peripheral limb ischemia. Iwase et al.[27] compared the efficacy between mononuclear cells and mesenchymal cells in

an experimental leg ischemia created after femoral artery ligation in rats. In this experimental study, it was found that mononuclear and mesenchymal cells stimulated the angiogenesis. Neovascularisation via mesenchymal cell implantation was more significant than neovascularisation via mononuclear cell implantation. Mesenchymal cells differentiated to the ECs more significantly than mononuclear cells, in addition, only the mesenchymal cells differantiated to vascular smooth muscle cells and mesenchymal cells were more tolerable to the ischemic stimuli than mononuclear cells.

PROTEINS RELATED WITH ANGIOGENESIS

In the studies concerning therapy based angiogenesis, another important issue is determination of the proteins related with angiogenesis and transport of the proteins to the ischemic target tissue. Platelet derived growth factors (PDGF) are predominantly researched proteins in angiogenesis studies. PDGFs are the first growth factors that are shown to have a role in vessel maturation. Their sources are the platelets in circulation.[28] There are four factors in PDGF family; PDGF-A, PDGF-B, PDGF-C, and PDGF-D. These factos should be in their dimer position (such as AA, BB, CC, DD) for being physiologically active.[29] Subtype PDGF-BB provides the maturation of the newly forming vessels by interacting the circulating progenitor cells of the vascular pericytes and smooth muscle and and is shown to be responsible for the tumoral lymphangiogenesis.[30] Nevertheless, it was detected that new vessel formation was improved via applying PDGF-BB alone as well as applying together with other angiogenic proteins such as fibroblast growth factor-2 (FGF-2).[31] In addition, PDGF-CC infusion alone also improves the new vessel formation in the ischemia models.[32]

TISSUE ENGINEERING APPLICATIONS

Researchs using tissue engineering methods in peripheral ischemic limb models are other issues discussed in the literature recently. It was found in past studies that collagen matrix supports the angiogenic process.There are experimental studies that showed improvement in new vessel formation by combining the progenitor cells and collagen matrix together and transporting them to the ischemic tissue.[33]

TISSUE ENGINEERED VASCULAR GRAFTS IN CLINICAL USE

Consistent with the developments in polymer chemistry, vascular grafting applications has been extensively researced by means of tissue engineering. Vascular grafts formed after cellular coating by the biodegradable polymers such as polyglycolic acid and polyhydroxyalkanoate were implanted to the lamb carotid artery and aorta. These tissue engineered grafts were still patent 5 months after the implantation.[34] During these studies it was observed that all the biodegradable polymers can not be absorbed. Thus, use of hyaluronic acide esters in biodegradable polymers was argued. It is reported that aproximately total absorbtion occured via the endogen hyaluronidase activity and these polymers stimulates the angiogenesis successfully.[35] Today, tissue engineered grafts are used as extracardiac conduits in congenital cardiac surgery after a short period of cultivation with autologous BM mononuclear cells.[36] These grafts are applied to the right heart portions where the pressure is low. The arterial tissue engineered grafts which work under high blood pressure are still thesubjects of experimental studies and there is still no clinical applications yet.

CLINICAL STEM CELL APPLICATIONS IN CRITICAL LIMB ISCHEMIA

After promising results of the cell based therapies in experimental peripheral ischemia models, the clinical applications are in use today. Adult stem cells derived from the BM continue to be under intense investigation by the researchers while the proportional contributions of angiogenesis and vasculogenesis to postnatal neovascularization remain to be clarified.[37] In a clinical setting, Tateishi-Yuyama et al (TACT study)[38] have recently established that implantation of autologous bone marrow-mononuclear cells (ABMMNC), including EPCs, into ischemic limbs increases collateral vessel formation. Considering the complexity of the angi-

ogenic process, cellular therapeutic strategies may overcome most of the shortcomings related to single growth factor applications. Tateishi-Yuyama et al recently demonstrated improvement in transcutaneous oxygen pressure, rest pain, and pain-free walking time after ABMMNC intramuscular injection in 25 patients with critical limb ischemia (CLI).

Recently, the widest series of patients with isolated thromboangiitis obliterans is reported by our study group.[39] In our study, ABMMNC implantation was applied to 28 patients with Rutherford Grade II-III ischemia. The result of the study suggests that ABMMNC implantation into ischemic limbs of patients with TAO is associated with improved exercise performance, quality of life, ankle brachial pressure index, pain control and augmentation of collateral formation in 24 weeks in 78% of patients. Ischemic ulcers healed completely in 15 patients (83%) and improved markedly in three (17%). The long term follow-ups of the patients are still continuing.

The pilot studies concerning cell based therapies in PAD have positive results. Corresponding to the developments in cell based therapies, there is a need for studies made by more selective cell subtypes. There are no certain evidences for comparison of the efficiencies of cell subtypes. Further prospective randomised studies are needed to compare ABMMNC, EPC and mesenchymal cells.

LEVELS OF EVIDENCE IN CLINICAL STEM CELL APPLICATIONS: IN-VIVO CELL TRACKING

The description of the stem cells is an important milestone in the development of the cell-based therapy options. In determining the BM derived stem cells, the phenotype and morphological specifications are identifying. Mesenchymal cells which were identified as star-like shape fibroblasts in direct microscobic examination gets more attention in regenerative medicine.These cells have a potential for transforming to all three germ layer cells although they are originated from mesoderm.[16] However, because of the similarities in cell morphologies, it is inevitable to have some infirmity in identifying the stem cells. Although there are still some undefined points, stem cells are classified according to their surface antigens. For example, surface markers such as CD105, CD166, CD44 and CD90 are uniformly expressed by mesenchymal cells.[40] The identification of the cell surface antigens may help to some developments in cell isolation. The primary antibody which is specific to the isolated cell surface antigen and the secondary antibodies which are binded to the primary antibody spesific magnetic bead can be evaluated for the magnetic seperation process (magnetic activated cell sorting). Thus, positive and negative cell selection can be achieved. With this method more spesific cell isolatation such as younger progenitor cells which have supreme differentiation capacity to the other tissues (osteogenic, adipogenic etc.) is provided.[41]

Molecular methods are frequently used in studies for identifying the differantiation capacity of the progenitor cells. Progenitor cells which are exposed to the green fleuroscene protein (GFP) transduction are applied to both in-vivo and in-vitro differantiation process. In this context, cardiac and vascular marker expression of the GFP or nuclear Dil paint activity showing cells are shown via the immunohistochemistry methods.[1,42] These findings are the high evidence level findings which show the ex-vivo differantiation ability of the progenitor cells.

VISUALISATION OF NEOVASCULARISATION

Corresponding to the increase in both experimental and clinical stem cell applications in peripheral artery diseases, there is a need for visualization of the achieved neovascularization via the improved techniques. Zhuang et al.[43] found that collateral vessel formations enhanced by cell based therapies are frequently in a diameter of 10-45 μm. It was reported that vessel formations over 200μm of diameter can be visualized by the conventional angiographic display methods. In the same study it was determined that collateral vessel formations under 200 μm of diameter were visualized via the 3D multidetector computed tomography angiography. In future,it will be possible to display the efficacy of the therapy via the cell based and virus mediated angiogenic protein applications for neovascularization.

SUPERMAGNETIC IRON OXIDE (SPIO) MARKED STEM CELLS

The supermagnetic iron oxide (SPIO) nanoparticle-marked stem cells may be detected in vivo. Kraitchman et al.[44] implanted SPIO marked-cells to the experimental myocard infarction sites. SPIO -marked mesenchymal cells were detected by magnetic resonans imaging (MRI) at implantation sites after 14th day of implantation. SPIO marked cells can be detected in the implantation sites via MRI however, this method doesn't give any information about cell viability. There is a risk for false positive detection of macrophage-phagocyted, inviable stem cells. There is not enough information about the toxic effects of the SPIO nanoparticles to the tissues. In addition, there is no study made about the potential nagative effects of SPIO in vasculogenic differantiation of the stem cells. There should be a number of at least 10^8 colonizations of the marked cells for being detected via MRI. Below this cell count density any MRI visualization can not be achieved. Because of these reasons, although this method has gained great attention at the beginning, later it has lost its importance. Prerequisities for clinical applicability of contrast agents for cell labeling are biocompatibility, safety, and nontoxicity for tissues.

Detailed studies are needed for displaying the clinical efficacy of the cell based therapies in ischemia models. One part of these studies are about the nuclear visiualization methods.

USE OF RADIONUCLIDES

Direct labeling of cells with radionuclides provides the advantage of a lower background signal as compared with MRI. However, higher sensitivity is achieved at the cost of lower spatial resolution. Various clinically applicable radionuclides have been used, based on previously established protocols for leukocyte or thrombocyte scintigraphy. Direct labeling with radionuclides appears highly informative for clinical studies addressing homing and biodistribution after cell injection. Hua et al.[45] have detected that the angiogenesis sites were visualized as "hot spot" in perfusion scintigraphy via peptide binded TC99m targeting the αvß3 integrin sites of the endothelial cells in experimental peripheral limb ischemia. The "hot spots" which were the markers of angiogenesis in perfusion scintigraphy were confirmed by immunohistochemical methods. In other words, it was shown that the sites which were the " hot spots" of scintigraphy were consistent with the neovascularization sites immunohistochemically. We think that there will be clinical reflections of the peptide marked perfusion scintigraphy spesific to the endothelial cells for visualizing the theraupetic cell based therapies ahead.

There are studies performed about detection of the radioisotope marked stem cells after communicating to the target site via the single photon emission computed tomography (SPECT) and positron emission tomography (PET) in the tissues. Radioactive isotopes such as [In-111]oxyquinoline (oxine) and [T-99m] hexamethylprophylene amine oxime have been clinically used in the nuclear medicine for labeling autologous white blood cells, which are subsequently infused back to the patients for localization of inflammatory sites. In general, radioisotopes with a relatively long decay half-life are used to track cells during a period of several hours or even days, for instance In-111 (T1/2= 2.8 days) for SPECT and (T1/2= 12.7 h) for PET.[46] Radioactive marked cells can be detected via this method in vivo. However, it is not a properly as there is potential toxic effects of the radioisotope agents on stem cells whatsmore, there can't be any visualization after 7 days because of the radioactivity loss and inviable cells can give radioactivity. And also as the signals taken from marked cells can not give any information about differentiation process, the method was shown in limited number of studies.

MOLECULAR BIOLOGIC METHODS: "REPORTER GENE APPROACHES FOR CELL TRACKING"

Researches for determining the tissue-targeted stem cells as viabl status are still continuing. For direct visualization of the efficacy of the therapy, molecular biologic methods are used. "Tracer Trapping Methods" are recently used to visualize the viabl cells in cell applicated target sites. This method mainly consists of gene transduction that produce pro-

teins which can be detected by SPECT or PET via viral reporters. For this purpose, timidin kinase (*tk*) reporter gene[47] and triple fusion (*TF*) gene[48] transductions are used frequently. As a reporter gene, *tk*, entegrates to the stem cell genomic DNA after introducing to the cellular nuclei. After the transcription and translation steps, primidine analouges and acycloguanosine derivatives, which can be detected by SPECT and PET, deposite in the cell. When the *TF* transduction is used as the method, the products such as monomeric red fleurosans protein (mrfp), firefly luciferase(fluc), truncated timidine kinase (ttk) which can be detected via visiualizing methods are gained. Viabl stem cell follow-up could not be achieved by in vivo cell follow methods untill these defined methods were used recently. A major disadvantage of reporter gene approaches for cell tracking is the requirement for molecular manipulations of the cells under study. Other potential concerns regarding long-term efficacy of the method include reporter gene silencing, in which reporter gene expression decreases over time with progressive cell differentiation. Another important issue is that teratoma formations were observed in experimental studies, especially herpes simplex virus was used as a gene transporter.[48]

HOW SHOULD AN IDEAL NON-INVASIVE IN VIVO IMAGING TECHNIQUE BE?

Ideal, noninvasive in vivo imaging technologies should have capabilities of (1) bioavailibility, safety, and non-toxicity; (2) no genetic modification or perturbation to the stem cell (3) real-time visualization of injected cells either in the target area or throughout the body; (4) single-cell detection at any anatomic location; (5) minimal or no transfer of contrast agent to non-stem cells; (6) noninvasive imaging in the living subject over months to years; (7) no requirement for injectable contrast agent; (8) stem cell quantification; (9) long-term, serial traceability; (10) single cell sensitivity in any location; and (11) reduced falsepositive imaging.[49]

CONTRAST AGENTS

A number of contrast agents and detectors for noninvasive, repeatable visualization of therapeutic cells in vivo have been pursued. Regardless of the level of sensitivity finally achieved, quantification of cell number may be especially difficult when we consider the effects of contrast agent dilution during cell division, the propensity of some contrast agents to transfer to non-stem cells, and certain technical limitations. Such imaging approaches may not only refine the understanding of therapeutic mechanisms in preclinical studies but may also have direct clinical applications. Most of the available cellular molecular imaging techniques are also applicable in humans, and therefore may facilitate rapid translation of cell-based therapies into clinical practice. The ideal imaging technology should permit tracking of injected stem cells for months to years as clinical trials will require long-term follow-up of tissue function or host survival. Finally, injectable contrast agents, such as enzyme substrates, add complexity and cost to stem cell–tracking procedures.

Cell based therapies are improving progressively in cardivascular diseases. In spite of the important results in laboratory studies, better results in in vivo methods which will directly show the clinical efficacy of the cell based therapies are yet to be reached. Displaying of the in vivo, viabl, and long term homing/differantiation processes of the cells is one of the important points in objective display of the therapy efficacy. Combined usage of the molecular and visiualizing methods together will probably help the solution of this problem.

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