Genesis of myocardial repair with cardiac progenitor cells and tissue engineering

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ABSTRACT

Background There is mounting evidence to suggest that the heart has regenerative potential in the event of myocardial injury. Recent studies have shown that a resident population of cardiac progenitor cells (CPCs) in the heart contains both vasculogenic and myogenic lineages. CPCs are able to migrate to the site of injury in the heart for participation in the healing process. The resident CPCs in the heart may also be activated through outside pharmacological intervention to promote their participation in the intrinsic repair process. In the light of these characteristics, CPCs provide a logical source for the heart cell therapy. During the regenerative cardiac process, stem cell niches (a specialised environment surrounding stem cells) provide crucial support needed for their maintenance.

Discussion Compromised niche function may lead to the selection of stem cells that no longer depend on self-renewal factors produced by its environment. The objective of stem cell transplantation associated with tissue-engineered approaches is to create a new modality in the treatment of heart failure. The use of efficient scaffolds will aid to re-establish a favourable microenvironment for stem cell survival, multiplication, differentiation and function. Cardiac tissue engineering using natural and/or synthetic materials in this regard provides a novel possibility in cardiovascular therapeutics.

CARDIAC PROGENITOR CELLS

The adoption of cardiac phenotype by stem cells is critical for any stem-cell-based treatment for myocardial repair. Studies in the experimental animal models[1 2] and clinical trials in humans[3–6] have yielded encouraging results. However, with the emerging controversies regarding bone marrow stem cells to adopt cardiac phenotype[7 8] and arrhythmogenic nature of skeletal myoblasts,[9 10] the field of regenerative medicine continues to be without an ideal donor cell type for cardiac repair. The existence of resident CPCs in the heart has challenged the longstanding dogma that the heart is a terminally differentiated organ.[11] There is mounting evidence to suggest that the heart has regenerative potential in the event of myocardial injury. More recent studies have shown that a resident population of CPCs in the heart has vasculogenic and myogenic lineages.[12] The resident CPCs in the heart can also be activated through outside pharmacological intervention to promote their participation in the intrinsic repair process.[13] The presence of CPCS has been reported in different species including murine, feline, canine, porcine and human hearts.[14–17] Even though the identification of resident population of CPCs is an enormous breakthrough, their origin in the heart remains unknown. CPCs have been identified and isolated on the basis of their markers: c-Kit, Sca-1, stage-specific embryonic antigen-1 (SSEA-1), Oct3/4 and as a side population (SP). These cells are capable of self-renewal, clonogenicity, and migration and homing in to the site of injury in the heart to participate in the repair process.[18] In the light of these characteristics, CPCs provide a logical source of cells for the heart cell therapy. A remarkable advancement in the field of CPCs is the identification and isolation of multipotent adult progenitor cells (MAPCs) in the human heart.[19] MAPCs express the pluripotent-state markers, including Oct-3/4 and Nanog, displayed telomerase activity and exhibited a multilineage differentiation potential.

CARDIOSPHERES

Our experience with porcine CPCs suggested that these cells could be obtained from both left atrium and ventricle samples of an adult pig. The culture of the tissue fragments from atria and ventricle generated migrating cells at 1–2 weeks in the culture. The migrating cells, harvested at 3 weeks, most efficiently formed cardiospheres. Surface marker analysis showed that the cardiospheres included a mixed cell population containing CD15 (SSEA-1), CD31, CD34 and c-Kit positive cells. The c-Kit CPCs may be purified by magnetic activated cell sorting and expanded in c-Kit medium.[20]

Notwithstanding the multipotent nature and high proliferative potential of CPCs, there are several issues which need to be resolved before realisation of their clinical applicability. First, culture conditions should be optimised for their undifferentiated in vitro propagation without compromising their stemness. Second, the choice of cell type requires detailed investigation for maximal benefit in patients: purified CPCs, or differentiated cells (cardiomyocyte, endothelial cell and smooth muscle cell) or a mixture of different cell types. More importantly, strategies aimed at improving cell survival in early stage after transplantation should be developed which otherwise greatly hampers the effectiveness of heart cell therapy.[21]

GENERATION OF PLURIPOTENT STEM CELLS

A more recent advancement in the field of regenerative medicine is the generation of induced pluripotent stem (iPS) cells which are bracketed with embryonic stem cells (ESCs) due to their pluripotent nature and effectiveness in myocardial repair.[22] Such a comparison, however, may be premature unless

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the pros and cons of iPS cell-based heart cell therapy have been realised in proof-of-concept studies in experimental in vitro/animal models. CPCs have a clear advantage, which is unrivalled by both iPS cells and ESCs. Being of cardiac origin, CPCs are reckoned as naturally programmed to develop into all the cell types needed for myocardial regeneration. Moreover, isolation of human endogenous CPCs from endomyocardial biopsy specimen obtained through percutaneous intervention is a leap forward for getting autologous CPCs for clinical use.[23] Hence, unless a well-defined and more refined approach is developed for iPS cells, ease of availability, efficiency of isolation and purification, and the logistic considerations favour CPCs for their clinical use.

STEM CELL NICHE AND CELL HOMING

Migration of stem cells through the blood, across the endothelial vasculature to different organs and to bone marrow niches, requires active navigation, a process termed 'homing.' Homing is required for seeding of the fetal bone marrow by haematopoietic progenitors during development. The efficiency of cell therapy to augment recovery after myocardial ischaemia depends on the sufficient recruitment of applied cells to the target tissue. Homing to sites of active neovascularisation is a complex process depending on a timely and spatially orchestrated interplay between chemokines (eg, SDF-1), chemokine receptors, intracellular signalling, adhesion molecules (selectins and integrins) and proteases.[24] Until now, cell transplantation for cardiac regeneration was limited by poor effects in ventricular function.[25 26] This may be due to the lack of gap junctions between the native myocardium and the grafted cells. Also, cell transplantation seems to be limited by acute death after transplantation. Most cell death occurs during the acute phase after engraftment due to multiple factors. Apoptosis can be induced by anchorage-dependent cells detaching from the surrounding extracellular matrix. Interventions known to enhance transplanted cell survival include heat shock, overexpressing anti-apoptotic proteins, free radical scavengers, anti-inflammatory therapy and codelivery of extracellular matrix molecules.[22] Combined use of such interventions may enhance cell graft survival significantly. Despite these possibilities, cell transplantation constitutes a passive therapeutic approach; the only effect seems to be related to the reduction in the myocardial fibrosis and the limitation of the adverse ventricular remodelling. Development of strategies to improve cell survival and differentiation should be encouraged, for example by using tissue engineering, prevascularisation, preconditioning procedures such as in vitro cell electrostimulation, cell cultures under hypoxia, and combination of angiogenic and myogenic stem cells.[27–31]

The stem cell niche, a specialised environment surrounding stem cells, provides crucial support needed for stem cell maintenance. Compromised niche function may lead to the selection of stem cells that no longer depend on self-renewal factors produced by its environment. An ageing stem cell niche may not support the transplanted stem cells.[32 33]

CARDIAC TISSUE ENGINEERING

Tissue engineering uses a combination of cells, engineering materials, and suitable biochemical factors to improve or replace biological functions. Probably the first definition of tissue engineering was by Langer and Vacanti,[34] who stated it as 'an interdisciplinary field that applies the principles of engineering and life sciences towards the development of biological substitutes that restore, maintain or improve tissue function or a whole organ.' Engineering materials can be implanted or 'seeded' into a natural or synthetic structure capable of supporting three-dimensional tissue formation. These scaffolds are often critical, both ex vivo and in vivo, to recapitulating the in vivo milieu and allowing cells to influence their own microenvironments. Such scaffolds serve at least one of the following purposes: allow cell attachment and migration, deliver and retain cells and biochemical factors, enable diffusion of vital cell nutrients, and exert certain mechanical and biological influences to modify the behaviour of the cells.[35–41] Specially designed bioreactor systems are used to manufacture tissue in vitro which will replace the diseased one in vivo.[42–44]

To achieve the goal of tissue reconstruction, scaffolds should meet some specific requirements. A high porosity and an adequate pore size are necessary to facilitate cell seeding and diffusion throughout the whole structure of both cells and nutrients. Biodegradability is essential, since scaffolds need to be absorbed by the surrounding tissues without surgical removal. The rate at which degradation occurs has to coincide with the rate of tissue formation to ensure that the scaffold is able to provide structural integrity within the body, and eventually it will break down, leaving the newly formed tissue to take over the mechanical load.

TRANSLATIONAL RESEARCH

The clinical application of current tissue engineering includes specialities such as orthopaedics, urology, dermatology, plastic surgery, neurosurgery, dentistry, tracheal replacement and cardiovascular surgery (heart valves and small-calibre vessels).[38 45–47] More than a decade since the first report on successful 3D cardiac cell culture for experimental and potential clinical application, the scientific investigations in the field of myocardial tissue engineering continue to grow. Tissue engineering may contribute to improve the efficiency of cellular therapy for organ regeneration. The cardiac connective tissue is mainly composed of collagen, with smaller amounts of elastin, laminin and fibronectin. There are two types of collagen fibres in the normal adult heart: types I and III which are produced by fibroblasts and myofibroblasts. Type I represents 80% of collagen protein in the heart, and type III is nearly 10%. These fibres provide structural support and give the heart properties that include stiffness and resistance to deformation; they have also been shown as an important role as a link between contractile elements of adjacent myocytes and carrying some information useful for cell function.

Cell transplantation associated with tissue engineering procedures is becoming recognised as a viable strategy to improve myocardial viability and limit infarct growth. Numerous studies are evaluating the benefits of stem cell delivery to injured myocardium, and more recent reports have also described bioartificial matrices that may provide mechanical support and enhance myocardial regeneration. It is important to note that current indications for cell-based regenerative therapy are small ischaemic scars and not large ischaemic lesions responsible for end-stage ventricular failure. Preliminary results of the MAGNUM clinical trial demonstrated that cellular CMP associated with a cell seeded collagen matrix increase the thickness of the infarct scar with viable tissues and help to normalise cardiac wall stress in injured regions, thus limiting ventricular remodelling and improving diastolic function.[48]

FUTURE PROSPECTS

Future developments include bioengineered platforms where stem cells are preconditioned to resist their implantation into a highly stressed myocardial tissue. Basically this approach consists in the development of bioactive membranes made of two integrated materials: (1) one nanofibre matrix made out of self-assembling peptides with molecule-release capacity (for

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growth factors such as VEGF and FGF); and (2) contained in a microscale elastomeric scaffold that provides the mechanical framework (elastic, loading) that will match the cardiac tissue mechanics. Both are essential to promote local angiogenesis in a necrotic affected tissue as well as its regeneration.[49–51] Proteins such as laminins, collagens and integrins, among others, support the needs of cells limited by distance, and this restriction allows for the formation of a basement membrane that provides support for cellular proliferation and supports adhesion-based properties.

Competing interests None.

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