International Journal of Cardiology 197 (2015) 116–122

Contents lists available at ScienceDirect

International Journal of Cardiology

journal homepage: www.elsevier.com/locate/ijcard

Review

Generating induced pluripotent stem cell derived endothelial cells and induced endothelial cells for cardiovascular disease modelling and therapeutic angiogenesis

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ARTICLE INFO

Article history:
Received 21 January 2015
Received in revised form 23 May 2015
Accepted 17 June 2015
Available online 18 June 2015

Keywords:
Induced pluripotent stem cells
Endothelial cells
Angiogenesis
Cell therapies
Peripheral vascular disease
Reprogramming

ABSTRACT

Standard therapy for atherosclerotic coronary and peripheral arterial disease is insufficient in a significant number of patients because extensive disease often precludes effective revascularization. Stem cell therapy holds promise as a supplementary treatment for these patients, as pre-clinical and clinical research has shown transplanted cells can promote angiogenesis via direct and paracrine mechanisms. Induced pluripotent stem cells (iPSCs) are a novel cell type obtained by reprogramming somatic cells using exogenous transcription factor cocktails, which have been introduced to somatic cells via viral or plasmid constructs, modified mRNA or small molecules. IPSCs are now being used in disease modelling and drug testing and are undergoing their first clinical trial, but despite recent advances, the inefficiency of the reprogramming process remains a major limitation, as does the lack of consensus regarding the optimum transcription factor combination and delivery method and the uncertainty surrounding the genetic and epigenetic stability of iPSCs. IPSCs have been successfully differentiated into vascular endothelial cells (iPSC-ECs) and, more recently, induced endothelial cells (iECs) have also been generated by direct differentiation, which bypasses the pluripotent intermediate. IPSC-ECs and iECs demonstrate endothelial functionality in vitro and have been shown to promote neovessel growth and enhance blood flow recovery in animal models of myocardial infarction and peripheral arterial disease. Challenges remain in optimising the efficiency, safety and fidelity of the reprogramming and endothelial differentiation processes and establishing protocols for large-scale production of clinical-grade, patient-derived cells.

1. Introduction

Standard therapy for atherosclerotic coronary and peripheral arterial disease includes medical therapy targeting modifiable risk factors, anti-platelet agents and interventions to restore blood flow to the ischaemic myocardium or limb [1]. However, up to 30% of patients are unsuitable for these procedures as diffuse long segments of disease preclude effective revascularisation [2]. Recently, therapeutic angiogenesis has emerged as a potential strategy for treating limb ischaemia in these patients to supplement current interventions. Pre-clinical research has shown that treatment with pro-angiogenic growth factors and cytokines stimulates new vessel growth and improves blood perfusion recovery in animal models of disease, but clinical trials using pro-angiogenic proteins and gene therapy have so far failed to demonstrate more than minor, short-term improvements in patient outcomes [3].

Stem cell therapies are a promising alternative because stem cells can be differentiated to be functionally equivalent to the endogenous cell types they are intended to support. Induced pluripotent stem cells (iPSCs) are a novel cell type, derived from somatic cell reprogramming via overexpression of exogenous transcription factors [3]. These reprogrammed pluripotent cells are then capable of being differentiated into many different mature cell types, including vascular endothelial cells (iPSC-ECs) [4–6]. Similarly, induced endothelial cells (iECs) can be produced by transdifferentiating adult fibroblasts directly to endothelial cells, bypassing the pluripotent stem cell intermediate [7,8]. IPSC-ECs and iECs have several potential advantages over other cell types in that they do not require the use of embryonic cells and have minimal immunogenicity due to their autologous origins. However, the optimum reprogramming and differentiation method remains unclear and more research is required to better understand the underlying processes, the phenotype and in vitro behaviour of the resultant endothelial-like cells and their in vivo functionality before they can be trialled in a clinical setting. This review summarises recent progress in the field of cellular reprogramming and evaluates current evidence

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http://dx.doi.org/10.1016/j.ijcard.2015.06.038
0167-5273/

supporting the use of pluripotent stem cell derived endothelial cells for basic science research and therapeutic neovascularisation.

2. Therapeutic angiogenesis for vascular regeneration

Vascular endothelial growth factor (VEGF) is a key pro-angiogenic protein, and accordingly, VEGF-antibodies or VEGF receptor inhibitors have proven successful in treating pathological angiogenesis in cancers, diabetic retinopathy and age-related macular degeneration. However, the use of VEGF and other pro-angiogenic cytokines to enhance ischaemia-mediated angiogenesis in humans has thus far been almost entirely unsuccessful, despite promising pre-clinical data from animal models [9–12]. Phases I and II clinical trials using recombinant VEGF, fibroblast growth factors 1 and 2 (FGF1, bFGF) and granulocyte/macrophage colony stimulating factor (GM-CSF) proteins to treat patients with angina demonstrated that pro-angiogenic factors could be safely delivered via intracoronary, intravenous and epicardial routes and that the treatments themselves were well-tolerated, although high doses of VEGF induced hypotension [3,13–15]. Protein therapy was associated with modest improvements in some end points, such as exercise treadmill time and angina frequency, but no significant improvements in myocardial perfusion were found. Similarly, delivery of VEGF via gene therapy again yielded modest improvements in some measures of blood flow, but later, larger trials found no significant, long-term differences in primary endpoints such as myocardial perfusion or death [16,17].

VEGF, FGF1 and hepatocyte growth factor (HGF) plasmid gene therapy has also been trialled in patients with peripheral arterial disease. Small, uncontrolled studies have reported intramuscular and intra-arterial delivery of phVEGF$_{165}$, a VEGF gene-carrying plasmid, promotes collateral vessel growth, increases ankle-brachial index and improves ischaemic ulcer healing [18–20]. A double-blind, placebo controlled trial by Kusumanto et al. found similar improvements, but no significant change in rate of amputation at 100 days, the primary endpoint of the study [21]. Placebo controlled trials of FGF1 and HGF gene transfer have produced encouraging results, but a larger, phase III trial of FGF1 found no significant improvements in the primary end points of wound healing, time to major amputation or survival [22–26].

The failure of clinical studies to translate therapeutic benefits seen in animal models to substantial improvements in quality of life for patients suggests the problem may be with delivery of pro-angiogenic factors rather than with the biological activity of the treatments themselves. It may be that simultaneous administration of multiple factors or additional supporting cells or structures is necessary to optimise the local environment for supporting neo-vessel growth. Cell-based therapies may circumvent some of the problems (e.g. short half-life, dose limiting effects) that have plagued trials using proteins. The ability of cells to secrete multiple pro-angiogenic factors as well as potentially incorporating into the vasculature themselves means that cells may also have greater scope for enhancing revascularisation. Various stem cell-based therapies have already shown pro-angiogenic capabilities in pre-clinical and clinical studies [2,27–29]. It is therefore imperative to determine the best source of stem cells and how stem cell-derived endothelial populations can be generated and delivered to maximise efficacy and safety.

3. Stem and progenitor cell therapies for therapeutic angiogenesis

The general term 'stem cell' describes any undifferentiated cell that has the ability to differentiate into multiple specialised cell types. Pluripotency refers to the ability of a cell to differentiate into any and all cell types of the body, excluding extraembryonic tissue. Embryonic stem cells (ESCs) and iPSCs are two examples of pluripotent cell types. Stem cells that have differentiated further, committed to a particular lineage and are only capable of becoming cell types from that lineage are referred to as multipotent cells. Adult stem and progenitor cells are usually multipotent and can be isolated from three postnatal tissues: bone marrow, adipose tissue and blood (including umbilical cord blood). Multipotent cells have decreased capacity for self-renewal compared to pluripotent cells and can only form a limited number of different cell types. Previous reviews have highlighted the advantages and limitations of each of the three major stem cell types as potential cardiovascular regenerative therapies [30–32].

4. IPSCs and iPSC-ECs

Induced pluripotent stem cells (iPSCs) are a novel cell type, derived by reprogramming somatic cells via overexpression of exogenous transcription factors. These cells were first generated by Takahashi and Yamanaka, who published their groundbreaking development in 2006 [33]. They assayed selected candidate genes as factors to induce pluripotency and introduced the genes to mouse embryonic fibroblasts (MEFs) by retroviral transduction, methodically testing combinations of genes until they established four key factors, which appeared to be necessary for iPSC colony formation [33]. Since 2006, multiple studies have replicated these results in human cells and cells from other species, modifying and refining the reprogramming process and differentiating iPSCs to mature cell types for translational research.

Induced pluripotent stem cells can theoretically be differentiated to any mature cell type derived from the three primary germ layers. To date, iPSCs have been successfully differentiated to many different cell types, including neural cells, lung and airway epithelial cells, cardiomyocytes and endothelial cells [34]. This, coupled with their autologous, non-controversial origins, means that iPSCs are now being used to study a wide range of conditions. Recently, the first clinical study using iPSCs commenced in Japan. The study will test the effectiveness of retinal pigment epithelium derived from iPSCs as a treatment for age-related macular degeneration and, if successful, will pave the way for further clinical studies using iPSCs [35].

4.1. Generating iPSCs and iPSC-ECs

Induced pluripotent cells are most commonly derived from dermal fibroblasts, although iPSCs have also been generated from many other cell types, including adipose stromal cells, hepatocytes, gastric epithelial cells, dental pulp stem cells, cord blood cells and peripheral blood mononuclear cells [36,37]. Takahashi and Yamanaka generated iPSCs by introducing retroviral vectors into fibroblasts in order to induce overexpression of four transcription factors, Oct3/4, Klf4, Sox2 and c-Myc (OKSM) [33]. Oct3/4 and Sox2 are essential factors for stem cell self-renewal and Klf4 has been shown to repress p53, an important cell-cycle regulator and tumour suppressor gene, which suggests Klf4 may activate stem cell specific genes via repression of p53 [33]. C-Myc is an oncogene and master regulatory gene, which appears to be useful for iPSC proliferation and activation of pluripotency genes via histone acetylation [33]. However, it is not likely to be suitable for clinical applications and was subsequently found to be dispensable for reprogramming somatic cells, although its absence significantly reduced the efficiency [38–40]. IPS cell lines have also been generated using a four-factor combination comprised of Oct3/4, Sox2, Nanog and Lin28, a microRNA binding protein, which appears to increase reprogramming frequency without being vital to the process [41]. Buganim et al. recently reported generating high quality iPSCs using Sall4, Nanog, Esrrb and Lin28. Although the absence of c-Myc significantly reduced the number of colonies formed, the "SNEL" iPSCs were capable of generating chimaeric mice and demonstrated greater developmental potential than OKSM iPSCs, as evidenced by greater numbers of viable mouse pups birthed after tetraploid complementation and lower frequencies of genetic or chromosomal abnormalities, such as Trisomy 8 [39].

Initially, iPSCs were generated by retroviral or lentiviral transduction of the reprogramming factors. The concern with this method is that