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Regenerative medicine in cardiovascular diseases – an update

Y. CAO[1], P.-Y. ZHANG[2]

[1]Department of Cardiology, Xuzhou Hospital of Traditional Chinese Medicine, Xuzhou, Jiangsu, P.R. China
[2]Department of Cardiology, Xuzhou Central Hospital, The Affiliated Xuzhou Hospital of Medical College of Southeast University, Xuzhou, Jiangsu, P.R. China

Abstract. – The unique ability of regenerative medicine to differentiate into any cell of the three germ layers (endoderm, ectoderm, and mesoderm) is of immense clinical importance. They have an unique capacity for unlimited self-renewal. Furthermore, pluripotent stem cells (PSC), including human embryonic (hESC) and induced pluripotent stem cells (hiPSCs), hold great potential as an unlimited source of functional as well as transplantable cells applications. Specifically, in the context of cardiovascular and ischemic diseases, it is believed that hESC-derived endothelial cells (hESC- ECs) could be used to stimulate angiogenesis or vasculogenesis in ischemic tissues, thereby restoring blood supply to the affected area. The present review article is focused on the current aspects of the regenerative medicine during cardiac disorders.

Key Words
Regenerative medicine, Cardiac disorders, Stem cells.

Introduction

Regenerative medicine incorporates all strategies for the repair, replacement or regrowth of damaged/destroyed tissues and organs. It involves the use of biomaterials, human genes, proteins or cells. In peripheral arterial disease (PAD) and critical limb ischemia (CLI), early regenerative medicine strategies focused on the administration of angiogenesis-inducing growth factors, such as vascular endothelial growth factor (VEGF) or fibroblast growth factor (FGF)[1,2]. Angiogenesis is defined as the migration and proliferation of differentiated endothelial cells (ECs) to allow sprouting of new capillary branches from existing vessels to stimulate the development of new blood vessels in the affected tissue. Although early results from these trials were promising, and the treatment using these factors were found to be safe but there were no lasting clinical affects[3]. This might be due to insufficient doses or a very large amount of protein might be needed to have a significant effect, something that is unrealistic in a clinical setting.

Gene Therapy

Gene therapy has a unique ability to administer some different angiogenic factors. Adenoviral vectors are used on a large scale during gene therapy studies; however, some high profile serious adverse events during clinical trials have led to major questions about the safety[4]. Another strategy, which has been investigated in the setting of PAD and CLI, is the administration of naked plasmid DNA encoding angiogenic factors. These therapies primarily aimed to increase the duration of transgene expression. Despite this, expression of the transgene remained low, as plasmids have low transfection efficiency[5].

The first case of administration of naked plasmid DNA, encoding for VEGF, was reported almost 20 years ago[6], but without any definitive conclusions. Since then, numerous clinical and pre-clinical studies have been performed, using a variety of VEGF family members and viral vectors. A phase I clinical trial confirmed the safety of adenoviral-mediated delivery of VEGF cDNA to patients with PAD, although no conclusions could be drawn about efficacy[7]. In 2012, a 10-year safety follow-up on patients treated with an injection of either an adenoviral vector containing a VEGF-encoding plasmid, or a plasmid/liposome combination, directly into an ischemic lower limb, confirmed the long-term safety of this approach[8]. Currently, there are 22 known FGF

Corresponding Author: Peiying Zhang, MD; e-mail: zpying58@126.com

ligands involved in angiogenesis[9], and cardiovascular gene therapy trials have been performed using some these isoforms. Trials with Sendai virus expressing human FGF-2, showed a significant improvements in limb function[10]. Some gene therapy clinical trials, using other angiogenic factors, (such as hypoxia-inducible factor-1α (HIF-1α), hepatocyte growth factor (HGF) and developmentally regulated endothelial locus-1), have been performed in patients suffering from IC and CLI[11,12]. Although, no significant safety issues were reported in any of these trials, and the results were mixed. Overall, strategies using angiogenic growth factors to treat PAD and CLI have been largely unsuccessful.

Cell Therapies

Currently, there are many clinical trials involving stem cell therapies in the treatment of CLI but the results have been varied. The majority of these studies involved the transplantation or injection of autologous cell populations broadly called as adult stem cells, including mesenchymal stem cells (MSC), bone marrow mononuclear cells (BM-MNC) and endothelial progenitor cells (EPC). Autologous cell types have an advantage of the decline in the risk of immune rejection.

MSCs originate in the stromal compartment of the bone marrow, where they make up only a small fraction of the total nucleated cells. Other sources of cells with mesenchymal potential have also been reported, for example, adipose tissue and skeletal muscle[13]. These cells are described as multipotent, non-hematopoietic and fibroblast-like, possessing the ability to differentiate into some cell types, including bone, fat, and cartilage[14]. Although their cell surface marker profile has been debated[15], their broad potential in the field of regenerative medicine is widely accepted[16]. In preclinical models of PAD and CLI, treatment of animals with ECs from MSCs derived from numerous sources, perfusion rates of ischemic limbs were significantly higher in in treated animals as compared to control animals[17]. However, in clinical trials, the results have been varied, and many trials have shown no significant improvement in secondary outcomes[18].

Pluripotent Stem Cells

These cells have unique abilities as they could be differentiated into any of the three viz. endoderm, ectoderm, and mesoderm. Further, they have the capacity for unlimited self- renewal. HPSCs hold great potential for some diverse scientific and clinical applications, including pharmacology, toxicology, cellular-based therapies and regenerative medicine. Currently, there are 2 different types of pluripotent stem cells, which have been described – embryonic stem cells (ESCs) and induced pluripotent stem cells (iPSCs) (Table I).

Table I. Regenerative medicine prospects.

Regenerative medicine Human Pluripotent Stem Cells (HPSCs) hold great potential for some diverse scientific and clinical applications, including regenerative medicine	
	Types:
1. Human embryonic stem cells (HESCs)	**2. Induced pluripotent stem cells (iPSCs)**
Pros • The cells were obtained from the inner cell mass (ICM) of the blastocyst stage embryo • Regarded as the 'gold standard'	***Pros*** • It involves the use of autologous programmed cells. • It has potential to provide patient-and disease-specific autologous cells for transplantation • The method of iPSC derivation does not require the use of human embryos, so ethically more appropriate
Cons • Heterogeneity between these cell lines • Ethical concerns, like fertilized embryos destruction • The potential of teratoma formation is high • Chances of rejection by the host are high	***Cons*** • The mechanisms governing pluripotency is complex and is poorly understood • Highly efficient, 3D EB-based culture systems are difficult to scale up to produce clinically relevant numbers of cells • Expensive

Human Embryonic Stem Cells

The human embryonic stem cells (hESCs) are important tools in the fields of developmental biology and regenerative medicine[19]. In 1998 Thomson[20] used human embryos, produced by *in vitro* fertilization (IVF), to isolate and culture the first human-derived ESCs. The cells were obtained from the inner cell mass (ICM) of the blastocyst stage embryo, isolated by immune-surgery and cultured on irradiated mouse embryonic fibroblasts (MEF). Cells were grown as colonies and individual undifferentiated colonies were manually selected and dissociated into clumps until the cell line was established. These cells, generated in 1998, are still used in research today and are widely regarded as the 'gold standard' hESC lines. Moreover, efforts are made to advance the culture conditions to a clinically acceptable standard. In addition to the original 5 lines, a large number of other hESC lines have now been generated. However, there is considerable heterogeneity between these cell lines[21]. Further, there are numerous caveats that must be addressed before such strategies could be considered for routine use clinically. Firstly, there are some ethical concerns, like fertilized embryos destruction[22]. Secondly, the presence of undifferentiated cells increases the potential of teratoma formation. Another caveat is their immunogenicity. In a similar way to tissue and organs, transplantation of hESCs is an allogeneic process, and rejection by the host is a very real prospect. Several studies[23] have shown varying degrees of immune response elicited by these cells, with some reports even suggesting that hESCs are immune-privileged. Circumvention of rejection might be possible via the generation of hESC banks containing immune-phenotyped lines, although this requires a large time and economic investment[24]. A recently published trial, evaluated hESC-derived retinal pigment epithelium in the treatment of patients with age-related macular degeneration and Stargardt's macular dystrophy[25]. This study provided evidence of their safety, graft survival and possible biological activity.

Induced Pluripotent Stem Cells

One way to overcome the issue of hESC immune-rejection is via the use of autologous cells. In 2007 Takahashi et al[26] reprogrammed both mouse and human adult fibroblasts to pluripotency by transduction of four defined factors: OCT4, SOX2, KLF4, and c-MYC. The pluripotent cells generated, known as induced pluripotent stem cells (iPSCs) are similar to hESCs in morphology, proliferation, surface antigens (SSEA3, SSEA4, TRA1-60, and TRA1-81) and gene expression (OCT4, NANOG, SOX2). Further, they were able to produce cells from all 3-germ layers both *in vitro* and *in vivo* teratoma formation assays. Although hESCs are still considered the 'gold standard' regarding developmental biology, these cells have the potential to provide patient-and disease-specific autologous cells for transplantation. Additionally, iPSCs could be used as excellent *in vitro* models of disease[27]. Moreover, as the method of iPSC derivation does not require the use of human embryos, using these cells circumvents the ethical issues faced with the use of hESCs.

Regulation of Pluripotency

The mechanisms governing pluripotency in these cells are still relatively poorly understood. Thus far, research has identified a group of key transcription factors, playing essential roles in maintenance and control of pluripotency – OCT4, SOX2, and NANOG. Indeed, these factors are used in the reprogramming of somatic cells to a pluripotent state. Much of the original work was performed in mESCs, before the derivation of hESCs in 1998, with many of the mechanisms conserved between the two systems.

OCT4 has a key role in the regulation as well as in the establishment of ICM pluripotency[28]. In hESCs *in vitro*, knockdown of OCT4 results in rapid changes in morphology, a marked reduction in growth rate and cell surface marker expression, including down-regulation of SSEA3, SSEA4, and TRA1-60[29]. Cells deficient in OCT4 also showed a clear up-regulation of differentiation-associated markers, particularly genes associated with differentiation to trophectoderm, endoderm, and mesoderm[30]. Up-regulation of OCT4 showed association with the changes in genes associated with mesodermal and endodermal differentiation[31]. Additionally, RNAi-induced silencing of OCT4 induced a change in >1000 genes, with both positive (e.g. pluripotency-associated TFs) and negative (e.g. mesoderm, endoderm and ectoderm-associated genes) regulation of different gene sets[32]. OCT4 also interacts with SOX2, a member of the SRY-related HMG-box (Sox) family, for the purpose of regulation of pluripotency[33]. Further, it was also shown that NANOG was expressed in the ICM of blastocyst stage pre-implantation human embryos, but not in some of the earlier-stage embryos, demonstrating a role for NANOG in the maintenance of pluripotency.

OCT4, SOX2, and NANOG were found to co-occupy the promoter region of 353 different genes, with binding sites occurring nearby. The three factors were found to regulate pluripotency by binding and transcriptionally activating genes. Moreover, they bind and transcriptionally inactivate genes that promote development, such as HOXB1and PAX6[34]. Indeed, targeted down-regulation of any one of these three factors results in a decrease in the expression of the other two. Thus, a synergy exists between these three factors, forming an auto-regulatory loop, and working to regulate a large number of differentiation and pluripotency associated genes.

Endothelial Differentiation of Pluripotent Stem Cells

Regarding vascular regeneration and stimulation of angiogenesis, hPSC-derived endothelial cells (hPSC-ECs) are thought to have the greatest potential, although methods of derivation remain suboptimal. Thus far, there have been a large number of publications describing protocols for the derivation of ECs from hPSCs[35]. Further, there are two main approaches, which have been taken when generating hESC-ECs; 3D embryoid body (EB)-based culture systems and 2D monolayer culture systems.

Endothelial-associated genes, including Pecam-1 (CD31), VE-Cadherin (CD144) and CD34, are often elevated during spontaneous EB-based differentiation of hESCs[36]. However, the efficiency of these differentiations is low. Other studies[37] showed that addition of VEGF into the system could increase the numbers of cells expressing CD31 and CD144. Further, these cells could be isolated and cultured to obtain higher percentages of CD31+ cells[38].

3D Embryoid Body (EB)-based Culture Systems

3D EB-based direct differentiation protocols are efficient in generating hESC-EC or hiPSC-ECs[39]. Rufaihah et al[40] used hiPSCs to generate cells, which were positive for CD31, CD144, endothelial nitric oxide synthase (eNOS) and von Willibrand Factor (vWF). Moreover, these cells demonstrated a high degree of transcriptional similarity between hESC-ECs and post-natal ECs[41]. Although highly efficient, 3D EB-based culture systems are difficult to scale up to produce clinically relevant numbers of cells due to methods of EB formation.

2D Monolayer Culture Systems

This protocol resulted in up-regulation of endothelial-associated CD31 and down-regulation of pluripotency-associated genes such as OCT4. These cells expressed high levels of endothelial-associated genes, and performed functionally in both *in vivo* and *in vitro* models[42]. Recently, a method for simultaneous derivation of ECs and pericytes from hiPSCs has been published[43]. Furthermore, Patsch et al[44] also described a highly efficient monolayer-based system for the derivation of ECs from hPSCs.

Conclusions

It could be concluded from above discussion, that regenerative medicine holds a strong potential for revascularization and angiogenesis. These data are promising for the potential translation of these technologies into the clinic, as a possible therapeutic approach.

Conflict of Interests

The Authors declare that they have no conflict of interests.

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