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Review on the Method of Generating Vascular Cells from iPSCs and Its Application

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Abstract. Induced Pluripotent Stem Cells (iPSCs) embryonic-like stem cells are reprogrammed from skin or adult somatic cells. iPSC-derived cardiovascular cells have been widely used in many cardiovascular disease models and drug discovery. This paper reviews and summarizes the method of generating three different types of iPSC-derived cardiovascular cells, including iPSC-derived Endothelial Cells (ECs), Cardiomyocytes (CMs), and Vascular Smooth Muscle Cells (vSMCs), and the application of these iPSC-derived cardiovascular cells. The diseases discussed in this paper are pulmonary arterial hypertension (PAH) and critical limb ischemia (CLI). Additionally, construction of tissue-engineered blood vessels (TEBVs) for vascular grafting has also been discussed. To conclude, iPSCs, as a promising technique, is used in disease modeling, drug screening, tissue engineering, and the discovery of specific cardiovascular therapies for various patients.

Keywords: induced pluripotent stem cells, cardiovascular disease, differentiation, engineered heart tissue

1. Introduction

Induced Pluripotent Stem Cells (iPSCs) are a type of pluripotent stem cell. They are reprogrammed from skin or adult somatic cells to embryonic-like stem cells. Shinya Yamanaka and his colleagues first reported this discovery in 2006 at Kyoto University. iPSCs are reprogrammed by four transcription factors (Oct4/Sox2/c-Myc/Klf4) [1] and can be differentiated to different types of cells that researchers are interested in. This is a novel method for iPSCs' application in drug screening, disease modeling, and cell therapy. For the drug discovery process, researchers usually need to first understand the mechanism of the disease. Although animal models have been used in various research, animals are different from humans in many ways. For example, the resting heart rate for a mouse is around 500 to 700 bpm, which is 10 times faster than that of humans [2]. Moreover, it is difficult for the primary cell lines, a popular method used in drug discovery, to get some specific cells. For instance, it is complexed and limited for researchers to access endothelial cells (EC) from pulmonary arterial hypertension (PAH) patients [3]. iPSCs provide a chance for generated specific cell for disease modeling and 3D organoid/organ construction. Therefore, the differentiation of iPSCs is a powerful method in future studies [2].

In this paper, the method of differentiating iPSC-ECs, iPSC-CMs, and iPSC-vSMCs and some applications of these iPSC-dervied cardiovascular cells will be summarized. Additionally, the future research directions and the potential of iPSC for drug discovery will be discussed.

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2. The differentiation of iPSC-ECs and vSMCs from iPSC

2.1. The method of differentiating iPSC-ECs from iPSC

Endothelial cells compose the thin membrane inside the heart and blood vessels and take charge of regulating exchanges between vessels and surrounding tissues as well as the vascular relaxation and contraction [4].

To differentiate iPSCs to ECs, human iPSCs will first be maintained on a matrigel-coated plate for three days before the differentiation to maintain an undifferentiated morphology. Then the iPSCs will differentiate to mesodermal fate by GSK3 inhibition and BMP4 treatment and finally induce vascular lineages by treating cells with EC growth factors (VEGF and FGF). Since productivity of EC differentiation from iPSCs is relatively suboptimal, to purify iPSC-EC, magnetic-activated cell sorting (MACS) is used against CD144, which is the mature endothelial surface marker. The cell containing CD144+ will be magnetically labeled with CD144 MicroBead, so the non-iPSC-ECs will be filtered out. A two-week differentiation process produced cells exhibiting acetylated low-density lipoproteins (LDL), nitric oxide production, and surface adhesion molecules similar to primary endothelial cells. The Pearson correlation coefficient between iPSC-ECs and the primary EC is r=0.93, which proves the significance of their similarity. Additionally, as iPSCs differentiate into endothelial cells, the pluripotent markers NANOG, UTF1, and SOX2 are deactivated [3].

Four major subpopulations from iPSCs-ECs have been identified by the scRNA-seq analysis. Genes CLDN5, APLNr, GJA5, and ESM1 are enriched in each of the four subpopulations. The generated iPSC-ECs include seven individual clusters (CC0 to CC6) that include not only bona fide iPSC-ECs, but also cardiomyocytes and other mesodermal cell types. Each cluster is distinguished by the expression of APLNR, GJA5, and ESM1 genes. Remarkably, GJA5 can serve as a reliable marker for human arterial iPSC-ECs as GJA5 + iPSC-ECs express four genes related to arterial EC development [5].

2.2. The method of differentiating iPSC-CMs from iPSC

The cardiomyocytes (CM) of the heart pump blood throughout the human body through their contractile function. CMs produce TNF-α, an inflammatory cytokine that has a diverse range of signaling under conditions of some treatments including lipopolysaccharide (LPS) [6]. To generate iPSC-CMs, the generation of cardiogenic mesoderm is necessary, which is the same as the generation of iPCS-ECs. The use of insulin in a stage-dependent manner is significant during iPSC-CMs differentiation. For the first three days, B-27 Supplement Minus Insulin, ascorbic acid, activin A, CHEER 990-21, and BMP4 will be used in a culture medium. Then the culture medium will be changed to B-29 Supplement Minus Insulin, ascorbic acid, and XAV939 for the next two days. On the day 6, only B-27 Supplement Minus Insulin and ascorbic acid will be used. On the day 7, the B-27 Supplement with B-27 Plus Insulin and ascorbic acid will be used and the first beating areas start to appear [7].

2.3. The method of differentiating iPSC-vSMCs from iPSC

The smooth muscle cells of the arteries lie in the media layer, where they participate in arterial remodeling and atherosclerosis at all stages. To differentiate iPSC-vSMCs from iPSCs, the iPSCs first differentiate into mesoderm, by activating Wnt signaling by GSK3 inhibition with CP21 or CHIR combined BMP4. The factors that promote VSMC formation are ActivinA and PDGF-BB. After ActivinA and PDGF-BB have applied on the cell, the original cell forms almost all CD140b +(PDGFRB) cells and detect no CD144 + cell virtually. According to Patsch, Christoph, et al, the generated cell also presents other VSMC-specific markers, including myosin IIB and αSMA. The efficiency of the generation of iPSC-VSMCs is higher than for the iPSC-ECs. [3] This method could generate an average of 95.4% CD14b+ cell, so iPSC-VSMCs require no purification step depending on the nearly homogeneous. By the research from Cheung, Christine, et al., a chemically defined monolayer system that has high efficiency of generating original iPSC-vSMCs has been created.

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Generated iPSC-vSMCs have contractile function and take part in blood vessel formation. The pure iPSC-vSMCs express early SMC markers, ACTA2, TAGLN, and CNN1, and late SMC markers, SMTN and MYH11, so the cells represent truly origin-specific subtypes [8].

3. The Application of iPSCs in specific diseases

3.1. PAH

Pulmonary arterial hypertension (PAH) is a disease that causes progressive right heart failure due to increased pulmonary vascular resistance. Five years after diagnosis, the majority of PAH patients pass away or require organ transplants. The recent primary therapies for PAH are justified by the need to increase the vasodilatory mediators produced by pulmonary artery endothelial cells. For example, calcium channel blockers are used to prevent calcium from entering and relax heart and blood vessel cells [9]. More research is required to develop long-term treatments because existing treatments have a limited ability to reverse pulmonary vascular remodeling. Because of limited access to the ECs from PAH patients, iPSC-Ecs are used to model the disease. A familial form of PAH accounts for 15% of cases, and 70% have mutations in bone morphogenetic protein receptor 2 (BMPR2) that cause haploinsufficiency. Interestingly, only twenty percent of the BMPR2 mutation carriers in FPAH families have clinical symptoms, which suggests that the genetic variation has alleviated the disease. Gu, Mingxia, et al. found that the FPAH-iPSC-ECs showed disease characteristics including reduced adhesion, migration, survival, and angiogenesis compared to unaffected mutation carriers (UMC) iPSC-ECs and control cells. Moreover, UMC iPSC-ECs show BMPR2 activators increase and inhibitor reduce, which could give the explanation for the differences between the FPAH iPSC-ECs and UMC iPSC-ECs. The FPAH iPSC-ECs with the BMPR2 mutation are unable to respond to BMP4 via noncanonical pP38 signaling pathway. After CRISPR-Cas9 mediated homology directed repair the BMPR3 mutation, FPAH iPSC-ECs show normal levels of normal pP38 signaling pathway and cell adhesion [10].

The study from Sa, Silin, et al. has also shown that a high kisspeptin 1 level (KISS1) has a relationship with a reduced migration rate and a low carboxylesterase 1 level (CES1), which affect the survival of the cells within the patient. FK506 is an activator of BMPR2, signaling as an immunosuppressant. It is a drug approved by FDA. FK506 permits the phosphorylation of BMPR2 and downstream signaling by dissociating FKBP12 from BMPR2 coreceptor. Moreover, Elafin improves angiogenesis in IPAH/HPAH PAEC by inhibiting neutrophil elastase and improving BMPR2 signaling in caveolin-1 dependent manner as another potential therapy for severe PH in rats. FK506 and Elafin are both in connection with reducing an anti-migratory factor, slit guidance ligand 3 (SLIT3) [9].

iPSC-ECs could also be used in drug screening for pulmonary arterial hypertension. Researchers exposed iPSC-ECs from six PAH patients to 4,500 compounds and Cell survival after serum withdrawal was evaluated. The result shows that the compound AG1296 has increased the abundance of bone morphogenetic protein receptors. There is a stronger correlation between AG1296 and the anti-PAH gene signature, as well as improved vascular function and BMPR2 signaling compared to other tyrosine kinase inhibitors (TKIs) [11].

iPSCs can not only be used for 2-dimensional purposes for PAH, but also for 3-dimensional disease modeling. According to Llucià-Valldeperas et al., a pressure-overloaded right ventricle dysfunction model is created using 3-dimensional engineered heart tissue (EHT) and iPSC-CMs. The generation of iPSC-CM from PAH patients has been incorporated into EHT for 28 days. Both iPSC-CMs and EHT from patients show increasing contraction and relaxation times compared to the control group. This shows that further optimization of the model might be used as a platform for disease modeling [7].

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3.2. Critical limb ischemia

Peripheral artery disease (PAD) is normally triggered by the buildup of fatty plaque in blood vessel, which results in narrowed arteries, thus causing the reduction of the blood flowing to peripheral tissue. The affected tissues mainly exist in heads, organs, and limbs. As the PAD developed, it might become critical limb ischemia (CLI), which is related to a higher risk of limb amputation and cardiovascular death. ECs and SMCs compose blood vessels and they form the inner lining of the vessel wall and surface of the vascular tube. Therefore, to regenerate peripheral arteries for ischemic tissues, both ECs and SMCs are needed. In the research from Park et al, iPSC-ECs and iPSC-vSMCs are used together to discover the combination therapy in murine hindlimb ischemia model. In the presence of co-transplantation of ECs and SMCs, capillaries and arteries were formed as a result of angiogenesis. ECs migrating, proliferating, and tubulating in vSMC-conditioned medium were sparked by exosomes released from these cells. iPSC-SMCs can improve the efficiency of iPSC-ECs by exosome-mediated paracrine mechanism [12].

3.3. Tissue engineering

iPSCs are not only used for discovery of mechanisms for many disease and patients' specific drug screening, but also provides a potential source of vascular cells for tissue engineering, particularly for vascular grafting. Vascular grafting is suitable for various cardiovascular diseases, including aortic aneurysm, ischemic heart disease and peripheral vascular diseases. However, current synthetic vascular systems face plenty of challenges, including infection, thrombosis, and lack of growth potential for child patients. Therefore, some scientists started using iPSCs in the field of tissue engineering [13]. A paper by Wang et al. describes a new macroporous and nanofibrous (NF) poly (L-lactic acid) scaffold for the seeding of iPSC-vSMCs for development of tissue-engineered blood vessels (TEBVs) [13]. In nude mice, the vSMC scaffolds interacted with collagenous matrix to form robust collagen deposition, preserving the differentiated vSMC phenotype [13]. Reuslts from the research of Luo et al. [14] show that iPSC-vSMCs mechanical strength can be augmented by inclusion of pulsatile radial stretching. iPSC-vSMC biomechanical properties were significantly enhanced by increasing the ultimate strain to 3% and maintaining the pulse rate at 110-120 bpm to generate hiPSC-TEVGs. Implanting hiPSC-derived TEVGs into a rat aortic model showed perfect patency without luminal dilation, and they were mechanically and contractilely active [14].

4. Conclusion

Due to the limited access to patient cardiovascular cells and deficiencies in animal studies, cardiovascular drug discovery and regenerative therapies face enormous challenges. The promising technology based on the iPSCs has provided a platform for various applications including cardiovascular drug screening, disease modeling, regenerative medicine, and tissue engineering. By generating from human somatic cells, iPSC-derived cardiovascular cells could also help researchers to understand patient-specific disease mechanisms. Many cardiovascular diseases still have no permanent therapies, including pulmonary arterial hypertension (PAH) and peripheral artery disease (PAD) mentioned in this paper. These diseases may cause death after diagnosis, and iPSC could contribute to both the drug screening and mechanism understanding of the diseases. Moreover, iPSC-derived cells combined with other tools like CRISPR-Cas 9 could help researchers to discover more patient-specific therapies and drug discovery. However, using iPSC for disease modeling, tissue engineering and drug screening still has some challenges, including maintaining the differentiated iPSC-derived cell during the disease modeling, and generating tissue-specific-subtypes of iPSC derived cells. Since the human cardiovascular system is involved in multiple different cells, vivo model validation is essential in order to test the results from iPSC-based in vitro studies. Overall, iPSCs is a promising technique to use in therapies discovery for various cardiovascular diseases.

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