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Application of induced pluripotent stem cells to model smooth muscle cell function in vascular diseases

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Abstract

Vascular smooth muscle cells (SMC) play an essential role in remodeling the vasculature during disease progression. Induced pluripotent stem cells (iPSC) provide an attractive approach to obtain autologous SMC source for patient-specific disease modeling. Here we discuss the current methods to 1) derive functional SMC from iPSC, 2) model vascular diseases using SMC generated from patient-derived iPSC, and 3) modulate microenvironmental cues to enhance cellular differentiation and functionality and better mimic the physiological environment. We emphasize that continuous exploration of biomaterial technologies to engineer a more SMC-specific microenvironment will provide further insight on complex vascular diseases.

Graphical abstract

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I. Introduction

Vascular smooth muscle cells (SMC) play an important role in remodeling the vasculature [1]. SMC display plasticity in switching between contractile and proliferative phenotypes to regulate vessel tone and blood pressure in response to environmental stimuli [2]. Therefore, development of effective therapeutic strategies for vascular diseases relies on the understanding and modulation of SMC function. However, this is often challenging due to the limited proliferation capacity and quick senescence acquisition of SMC in culture [3].

Induced pluripotent stem cell (iPSC) technology provides an alternative to tissue isolation for obtaining large quantities of autologous cells [4, 5], and opens a new venue for patient-specific disease modeling [6]. The ability to recapitulate human pathophysiological conditions in vitro offers a potential advantage over animal models in addressing the cross-species discrepancy issues [7]. Since in vitro cell-based assays fail to effectively model the complexity of 3D interactions in tissues or organs [8, 9], it is crucial to fabricate 3D tissue constructs with relevant microenvironmental cues to serve as a more biomimetic human cell-based disease model. In this review, we aim to discuss the current methods to model vascular diseases using SMC generated from patient-derived iPSC, and further explore the functional role of SMC at the tissue level through microenvironmental cues and engineered vascular constructs (Figure 1).

II. Current status of iPSC-derived SMC generation and applications

A. Generation of SMC from human iPSC

Several studies have reported the derivation of functional SMC from human iPSC till date. Lee et al. first described the use of iPSC derived from human aortic vascular smooth muscle cells (hAVSMC) to differentiate into SMC through embryoid body (EB) formation followed by smooth muscle differentiation culture medium [10]. The generated SMC closely resembled native SMC at both the transcriptional and functional levels, while the pluripotent characteristics were effectively silenced [10].

While the EB formation method closely mimics the native embryonic development process, it has drawbacks such as uneven exposure of soluble factors to cells in the interior or exterior of the EB [11], heterogeneity between EB in terms of size and shape [12], and in most cases the need to separate SMC from other cell types present within the EB [13]. Thus, more recent studies have used EB modification strategies [14] or alternative methods involving monolayer-directed differentiation [15–17] for a more consistent generation of larger quantities of SMC with higher cell purity.

For instance, Bajpai et al. described a SMC differentiation method that undergoes a mesenchymal stem cell (MSC) intermediate [15]. The resulting SMC both expressed and stained positive for major smooth muscle markers, ACTA2, CNN1, and MYH11. Furthermore, the derived SMC displayed high contractile function in response to receptor- and non-receptor-mediated agonists when fabricated into a small-diameter cylindrical construct using fibrin hydrogels [15].

Alternatively, Cheung et al. took a more comprehensive approach to generate SMC from three distinct intermediate lineages (neuroectoderm, lateral plate mesoderm, and paraxial mesoderm) [16]. iPSC were differentiated into a desired intermediate lineage using a specific set of small molecules and growth factors, followed by a combination of TGF β and PDGF-BB to further differentiate them into functional SMC. This resulted in high differentiation efficiency for all three intermediate lineages, where over 80% of total cell population expressed the mature SMC marker, MYH11. These differentiated SMC also displayed contractile function similar to that of the native aortic SMC [16].

The rationale for exploring different intermediate lineage comes from the observation that SMC in the native vasculature have multiple embryological origins [18] and many vascular diseases are site-specific [19]. Cheung et al. speculated that lineage-specific differentiation of SMC function could be the cause of this spatiotemporal difference in disease susceptibility [16]. However, their SMC from different lineages appeared to be phenotypically and functionally indistinguishable, although origin-specific responses to certain cytokines and extracellular matrix (ECM) remodeling proteins were observed. Nevertheless, their subsequent study demonstrated that SMC derived from iPSC of Marfan syndrome patients showed distinct origin-specific disease phenotype [20]. SMC derived from neuroectoderm lineage displayed a more severe form of fibrillin abnormality and increased TGF β 1 activation than that derived from other lineages. This is consistent with the observation that the aortic root, primary site of thoracic aortic aneurysm (TAA), is predominantly populated by neuroectoderm-derived SMC [18]. These results suggest that while the primary contractile function of the reprogrammed SMC remains uniform, the pathological stimuli may trigger a more specific phenotype switching that is partially governed by its embryological origin [21]. The complexity of molecular regulation involved in SMC plasticity [1] suggests that this could not be the sole reason; further investigation of different disease phenotypes specific to certain SMC subtype would provide the insight [22].

Since endothelial cells (EC) that comprise the vessel lumen are highly proliferative and easily obtainable [23], there is lesser need for an alternative cell source. However, the generation of both EC and SMC from the same cell source would be ideal for patient-

specific disease modeling. A recent study by Patsch et al. used chemically defined conditions and growth factors to differentiate iPSC into lateral mesoderm before being induced into functional SMC or EC [17]. Their protocol largely reflects what has already been established in a number of previous publications that derive EC from human embryonic stem cells (ESC) [24, 25], with PDGF-BB and VEGF as well-known factors to direct the SMC and EC differentiation, respectively [26]. Nevertheless, Patsch et al. adds novelty by achieving high conversion efficiency (95.4% for SMC and between 61.8% and 88.8% for EC) within a short period of time (2 weeks) [17]. Furthermore, this study, together with the study by Cheung et al., validates the reliability and efficacy of using chemically defined conditions for SMC differentiation.

B. iPSC-derived SMC for modeling vascular diseases

Reprogramming patient cells to generate disease models offers many advantages. For instance, having patient-derived iPSC eliminates the need for genetic manipulation of normal iPSC or extensive preparation of transgenic animal models to observe a genetic defect. This is particularly helpful for modeling complex genetic disorders. Furthermore, patient-derived iPSC have the therapeutic potential to be used in precision medicine. Here we describe two iPSC-based vascular disease models that have provided great insight into the disease mechanisms.

1. Hutchison-Gilford Progeria Syndrome (HGPS)—HGPS is a genetic disease caused by a mutation in the lamin A/C gene (LMNA) that generates an abnormally truncated mutant known as progerin. The accumulation of progerin leads to several aging-associated nuclear defects that affect mesenchymal lineages, particularly the vascular SMC [27]. Autologous patient cell source is limited due to the rarity and severity of this disease, and thus iPSC present a valuable alternative for modeling the disease.

Zhang et al. and Liu et al. were the first two groups to generate iPSC derived from HGPS patients [27, 28]. While the HGPS fibroblasts isolated from patients displayed the nuclear deformation and premature senescence due to progerin accumulation, such disease phenotypes were silenced in the reprogrammed HGPS-iPSC. Furthermore, the HGPS-iPSCs did not differ from normal iPSC at both epigenetic and transcriptional levels [28]. However, when HGPS-iPSC were differentiated back into somatic cells such as MSC or vascular SMC, LMNA and progerin expression were restored and the differentiated cells displayed the defective phenotype. This reversible disease phenotype through iPSC reprogramming provides an insight to the critical role of progerin in HGPS disease mechanism. Furthermore, the directed differentiation of HGPS-iPSC allowed for the identification of a novel senescence-related marker [28].

HGPS is one of the more extensively studied diseases when it comes to using iPSC as a disease-modeling tool. Because its symptoms closely resemble that of the normal aging process and reprogrammed HGPS-iPSC silence the age-associated molecular marks, there is also a great interest in using HGPS as a model to understand chronic aging mechanisms [29]. For instance, a recent study by Soria-Valles et al. used iPSC from patients with Néstor-Guillermo progeria syndrome (NGPS), another variant of progeria disease, to identify NF-

κ B hyperactivation as a critical cell reprogramming barrier [30]. Similar transcriptional change was also observed in cells from HGPS patients and advanced-age donors (chronic aging), and NF- κ B inhibition significantly enhanced reprogramming efficiency in all three donors. Furthermore, the expression of histone methyltransferase DOT1L, a major effector of NF- κ B barrier, in progeroid mice model extended the longevity. This proof-of-concept study demonstrates the potential use of progeria as a study model for chronic aging.

2. Marfan syndrome (MFS)—MFS is a genetic disorder caused by a mutation of the FBN1 gene that encodes fibrillin-1, a major constituent of microfibrils in the ECM [31]. Patients with MFS most often face premature death due to the development of TAA. FBN1 plays an important role in the TGF β 1 pathway, and the abnormal fibrillin fiber formation leads to an excessive TGF β activation that is closely associated with the progression of TAA [32]. It was shown that losartan, an angiotensin II receptor type 1 blocker that can reduce TGF β 1 activity, could effectively prevent aortic root dilation in MFS mice [33]. However, recent clinical trials with losartan failed to show any significant improvement in MFS patients [34, 35]. This example of cross-species discrepancy reemphasizes the need for a more predictive pre-clinical model that is based on human cells to identify pathogenesis specific to humans.

Moreover, there is a need for investigating the exact molecular mechanism of TGF β signaling pathway in TAA progression, as studies have generated controversies as to the effect of TGF β activation on aneurysm formation in general, where some reported that the effect appears to be temporally dependent on the degree of TAA progression [20]. Recently, Granata et al. generated iPSC from dermal fibroblasts of patients with MFS [20]. SMC differentiated from these MFS iPSC showed that losartan could restore only certain downstream pathways of TGF β signaling, in particular the matrix metalloproteinase expression level and ECM (fibrillin) organization, to normal conditions. However, the increase in apoptosis caused by the downstream p38 pathway was unaffected. Furthermore, it was speculated that fibrillin degradation leads to vascular stress and abnormal mechanotransduction that is independent of the TGF β pathway. These results suggest for more specific therapeutic targets to reverse the defective phenotype in MFS.

C. Microenvironmental cues for modulating iPSC-derived SMC

1. Engineered microenvironment for modulating cellular reprogramming and iPSC-derived SMC functionality—Modulating the epigenetic state of somatic cells via engineering of the cellular microenvironment is an interesting strategy for cellular reprogramming. In particular, 3D microenvironment enhances cellular reprogramming efficiency and helps maintain pluripotency [36, 37]. Caiazzo et al. found that the physical cell confinement imposed by the 3D cell encapsulation in poly(ethylene glycol) (PEG)-based hydrogel improved cellular reprogramming. The pronounced morphological changes induced chromatin remodeling and accelerated mesenchymal-to-epithelial transition, which are two key events for the initiation of iPSC generation [36]. Li et al. demonstrated that microgrooved polydimethylsiloxane (PDMS) surface or aligned poly (L-lactide-co-caprolactone) nanofibers accelerated reprogramming of murine and human fibroblasts [37]. In particular, microgrooves with a 10 μ m spacing and 3 μ m height promoted the expression of

epithelial and pluripotency markers by triggering a more elongated morphology and inducing histone modifications.

To mimic the physiological environment of native blood vessels, the proliferation and maturation of iPSC-derived SMC (iPSC-SMC) exposed to various topographical and biomechanical stimuli have also been studied. When iPSC-SMC were cultured on 3D macro-porous nanofibrous poly(L-lactide) (PLLA) scaffolds, the expression levels of SMC-specific marker genes were highly upregulated while pluripotent marker gene expression was suppressed [38]. Upon subcutaneous implantation of iPSC-SMC/PLLA scaffold constructs, the implanted cells maintained the SMC phenotype and promoted the formation of vascular structure with robust collagenous matrix deposition. Furthermore, PLLA nanofiber scaffold with a smaller pore size supported SMC growth better and facilitated cell-cell interaction in the pores, resulting in higher ECM deposition than in the scaffold with a larger pore [39].

As SMC in native blood vessels are constantly exposed to mechanical stimulation, biomechanical cues that mimic those in the *in vivo* environment should play a role in phenotypic development of vascular SMC. Various subtypes of tensile strain including uniaxial, equibiaxial, and circumferential tensile strain can alter the mechanotransduction of vascular SMC [40]. When both cyclic uniaxial and circumferential tensile strains were applied to human iPSC-SMC embedded in collagen gels, the cells displayed mechanotransductive cytoskeleton remodeling and ECM gene expression [41]. Long-term circumferential stimulation is more effective than uniaxial strain stimulation in improving the mechanical properties of iPSC-SMC vascular constructs through upregulation of collagen and elastin expression.

2. Assessment of SMC function through engineered vascular tissue

constructs—A physiologically relevant 3D structure to study the function of vascular SMC would be a blood vessel. While extensive efforts have been made to fabricate tissue engineered blood vessels (TEBV) as vascular substitutes for treating coronary artery and peripheral vascular diseases [42], it was only recently that TEBV have been viewed as an attractive platform for disease modeling [43]. The lack of cell number sufficient to build a tissue had been a major hurdle, and much effort was shifted to using decellularized scaffolds for *in vivo* application [44]. As this would not be an option for disease modeling, iPSC offer an attractive alternative.

iPSC-SMC cultured on various scaffolds proliferated efficiently and produced ECM proteins such as collagen to create a vascular microenvironment seen in the native tissue [39, 45]. SMC contractile markers ACTA2, CNN1, and MYH11 were also expressed in TEBV after prolonged *in vitro* culture for up to 9 weeks [45]. In terms of vascular functionality, the contraction force of the tissue construct increased in response to the addition of vasoconstrictor agonists such as carbachol. Furthermore, a tissue construct consisting of iPSC-SMC from supra-aortic stenosis patients showed reduced level of contractility and increased proliferation rate [14], demonstrating the potential for disease modeling at a tissue level.

Recently our group and others have generated TEBV using MSC [46] or fibroblasts [47] for in vitro modeling. Despite lacking the SMC component that would be crucial for replicating the vascular remodeling during disease progression, these TEBV were highly functional in terms of vasoactivity, and contained a functional endothelium that could be activated under inflammatory conditions [46, 47]. The incorporation of iPSC-SMC into these constructs should further improve the TEBV vasoactivity and facilitate the assessment of SMC function as readout for drug screening.

III. Challenges and Perspectives

Challenges remain to apply iPSC for in vitro vascular disease modeling. Individual genetic variants among patients and healthy donors who do not share the genetic background present difficulty in accurately interpreting a disease phenotype [6]. This challenge can be addressed through the usage of genome editing tools such as CRISPR/Cas9 or TALEN that can create site-specific gene edits. This will allow for the generation of isogenic cells that only differ at the disease-relevant mutation site, and thus enable a more accurate phenotype comparison [48].

While studying the molecular mechanism behind several monogenic vascular diseases have been achieved, modeling more complex vascular diseases such as hypertension or atherosclerosis remains a major challenge. Diseases associated with aging or epigenetic influences are hard to be reproduced on cells derived from iPSC [49]. The epigenetic memory of the starting cell source also may not be completely eliminated, which may have an unpredictable behavior on the disease model.

In vitro disease modeling through iPSC greatly enhances our understanding of SMC function under human pathophysiological conditions, and eases the process of identifying novel biomarkers and pathways associated with disease progression. Furthermore, SMC have a highly plastic nature that is greatly dependent on their microenvironmental stimuli. Thus, creating a correct microenvironment setting is crucial for accurately modeling the role of SMC in vascular diseases. So far, many of the conventional methods for controlling cellular reprogramming and differentiation of iPSC into SMC lack the relevant physical and biochemical signals of microenvironment. Engineering the vascular microenvironment using well-defined biomaterials can work synergistically with traditional reprogramming approaches to improve the reprogramming efficiency and SMC function. In addition, emerging technologies such as predictive computational modeling, high-throughput biomaterial arrays, and 3D bioprinting can help select a more SMC-specific microenvironment made up of suitable materials with optimal mechanical properties and biochemical compositions. The cooperation of both cellular engineering and biomaterial technologies will have a synergistic impact on direct cellular reprogramming and provide valuable insights for advancing engineered cell-based clinical approaches to disease modeling, drug screening, and regenerative medicine.

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Abbreviations

SMC	smooth muscle cells
iPSC	induced pluripotent stem cells
hAVSMC	human aortic vascular smooth muscle cells
EB	embryoid body
MSC	mesenchymal stem cells
ACTA2	smooth muscle actin
CNN1	calponin
MYH11	smooth muscle myosin heavy chain
ECM	extracellular matrix
TGFβ	transforming growth factor beta
PDGF-BB	platelet-derived growth factor BB
TAA	thoracic aortic aneurysm
EC	endothelial cells
ESC	embryonic stem cells
VEGF	vascular endothelial growth factor
HGPS	Hutchinson-Gilford Progeria Syndrome
LMNA	lamin A/C gene
NGPS	Néstor-Guillermo Progeria Syndrome
NF-κB	nuclear factor kappa-light-chain-enhancer of activated B cells
MFS	Marfan Syndrome
PLLA	poly(L-lactide)
iPSC-SMC	iPSC-derived SMC
TEBV	tissue engineered blood vessels

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* of special interest

** of outstanding interest

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Highlights

- Functional smooth muscle cells can be derived from induced pluripotent stem cells
- iPSC-SMC models are used to understand and identify vascular disease mechanisms
- Modulating microenvironment cues improves cellular fate and functionality

Figure 1. Schematic overview of approaches to 1) differentiate smooth muscle cells (SMC) from induced pluripotent stem cells (iPSC), 2) use SMC to model vascular diseases, and 3) modulate cellular function through different microenvironmental cues.