Vascular research using human pluripotent stem cells and humoral factors

Masakatsu Sone and Kazuwa Nakao

Department of Medicine and Clinical Science, Kyoto University Graduate School of Medicine, Kyoto 606-8507, Japan

Abstract. Embryonic stem (ES) cells are pluripotent cells collected from the inner cell mass of blastocysts. Induced pluripotent stem cells exhibit characteristics and pluripotency similar to ES cells, even though they were generated from adult somatic cells. We have been investigating the vascular differentiation kinetics of human pluripotent stem cells (PSCs) and their application to human vascular research and clinical medicine. In this review, we present an overview of recent vascular research using human PSCs, focusing on the role of humoral factors and their receptors. We also discuss possible future application of human PSCs to translational research on human vascular disorders.

Key words: Human induced pluripotent stem cell, Human embryonic stem cell, Vascular endothelial cell, Vascular smooth muscle cell, Vascular hormone

Embryonic stem (ES) cells are pluripotent cells collected from the inner cell mass of blastocysts. Mouse ES cells were first established by Evans et al. in 1981 [1]. Subsequent establishment of human ES cells was difficult owing to the many differences between mouse and primate ES cells [2]. After 17 years have passed since the establishment of mouse ES cells, human ES cells were first established by Thomon JA, et al. in 1998 [3]. Recently, induced pluripotent stem (iPS) cells were generated from both mouse and human somatic cells through the introduction of defined factors [4, 5, 6]. Although iPS cells are derived from adult somatic cells, their characteristics and pluripotency are nearly identical to those of ES cells. In this review, we present an overview of ongoing vascular research making use of pluripotent stem cells (PSCs), especially human PSCs, focusing on the roles of hormonal factors and their receptors.

Induction of vascular cells from human PSCs

The differentiation of hemangioblast-like cells from mouse ES cells was first reported in 1998 [7]. Flk1-positive cells derived from mouse ES cells differentiated into sheet-like clusters of VE-cadherin- (CD144), PECAM-1- (CD31) and CD34-positive vascular endothelial cells when co-cultured with OP9 feeder cells [8]. Mural cells (i.e., vascular smooth muscle cells and pericytes) were also differentiated from the same Flk1-positive cells induced from mouse ES cells [9]. In primates, however, Flk1 is expressed even in undifferentiated ES cells, and the vascular differentiation kinetics differ from those of mouse ES cells [10]. In 2003, human ES cells were established in Japan, where we succeeded in inducing and isolating vascular endothelial cells and mural cells from human ES cells [11]. In the meanwhile, Yamanaka et al. established iPS cells from mouse and human fibroblasts [4, 5]. These iPS cells were capable of differentiating into vascular cells in the same manner as mouse and human ES cells [12, 13, 14]. Moreover, a modified serum and feeder-free method for the induction of vascular endothelial cells from human ES/iPS cells was recently reported [15, 16], and we are continuing to modify and refine the method for stable differentiation of various human iPS lines.

Vascular differentiation and humoral factors

Humoral factors and their receptors play key roles in the pathway along which human PSCs differentiate into vascular cells (Fig. 1). Flk1 (also known as
VEGFR-2) is one of the VEGF receptors and is known to be a marker of proximal lateral mesoderm in mouse [6]. Induction of vascular endothelial cells, mural cells and cardiomyocytes from Flk1-positive cells has been confirmed in both mouse and human ES cells [9, 11, 17, 18]. VEGF dose-dependently induces differentiation of Flk1-positive cells into vascular endothelial cells. In mouse ES cells, another VEGF receptor, Flt1 (also known as VEGFR-1), is expressed at a later phase of vascular differentiation, and the delayed expression of VEGFR-1 correlates with an increase in dose sensitivity to VEGF [19]. This VEGF dose sensitivity is also observed in human ES cells. These vascular differentiation processes of ES cells are almost identical in iPS cells [12, 14]. In mouse ES cells, adrenomedul- lin acts via its second messenger, cAMP, to induce arterial differentiation of vascular endothelial cells [20]. Notch and GSK3β-mediated β-catenin signaling is activated downstream of cAMP via phosphatidylinositol-3 kinase and induces differentiation to arterial endothelial cells [21]. Inhibition of GSK3β also induces arterial differentiation in human ES/iPS cells [16].

Vascular smooth muscle cells are believed to derive from mesoderm, neural crest or epicardial cells and to then migrate to form the vessel wall; however, difficulty in preparing pure populations of these lineages has hampered dissection of the mechanisms underlying vessel formation. It is reported that Flk1-positive cells derived from mouse ES cells can differentiate into both endothelial and vascular smooth muscle cells, and so they referred to these cells “vascular progenitor cells” [9]. In humans, some TRA1-60-negative cells derived from human ES cells express Flk1 and PDGF receptor β. Stimulation of these cells with VEGF induces vascular endothelial cells, while stimulation with PDGF-BB induces vascular smooth muscle-like cells [11]. In another recent study, human PSCs were induced to differentiate into the synthetic vascular smooth muscle cell phenotype in medium containing high serum with PDGF-BB and TGF-β1, after which serum starvation and PDGF-BB deprivation caused maturation towards the contractile vascular smooth muscle cell phenotype [22]. On the other hand, heterogeneity of embryological origins is a hallmark of vascular smooth muscle cells in vivo. In one study, for example, human PSCs were initially induced to form neuroectoderm, lateral plate mesoderm or paraxial mesoderm, and each of these intermediate populations was then further differentiated towards vascular smooth muscle cells. Notably, the derived vascular smooth muscle cell subtypes recapitulated the unique proliferative and secretory responses to cytokines previously documented in studies using aortic SMCs of distinct origins [23]. This result suggests heterogeneous origins in the development of vascular smooth muscle cells.

![Diagram](image-url)
**Future application of human iPS cells for vascular research**

Vascular differentiation from PSCs is a productive area of basic research; however, clinical application of PSCs has not yet been achieved. Applications of a PSC-based vascular differentiation system can be separated to three categories (Fig. 2). The first is application for vascular regeneration therapy. As for somatic progenitor cells, after reports that bone marrow-derived CD34-positive mononuclear blood cells have the potential to differentiate into vascular endothelial cells and were referred to as “endothelial progenitor cells” (EPCs) [24], several clinical studies entailing transplantation of bone marrow stem cells as vascular regeneration therapy for ischemic diseases were performed [25-28]. In those studies, transplantation of bone marrow stem cells induced angiogenesis and relief of ischemic symptoms. Notably, however, the transplanted cells rarely survived as vascular endothelial cells, and the same effect was observed after transplantation of peripheral mononuclear blood cells [29]. Consequently, the clinical effect is not thought to be due to vessel formation by the transplanted cells, but to production of angiogenic factors. A report also showed that the implanted cells do not secrete angiogenic factors at levels sufficient to induce neovascularization; they instead stimulate muscle cells to produce angiogenic factors, thereby promoting neovascularization of ischemic tissues [30]. Vascular cells derived from human PSCs have been transplanted into animal models of ischemia in several studies [31-35], but no studies have yet been carried out in humans due to both ethical considerations and technical problems. So far, for example, transplanted vascular cells form only capillaries. Perhaps combining these cells with tissue engineering might lead to a breakthrough in their application [36-38].

The second application category is research into cell biology and drug discovery. Human PSC-based vascular differentiation systems enable one to observe and investigate the cellular and molecular mechanisms underlying vascular development in vitro. In addition, with these cells one can investigate the characteristics of vascular cells at early stages during differentiation. For example, we reported that Sirt1 expression is higher early during differentiation of vascular endothelial cells from human PSCs than it is in human adult endothelial cells, and that Sirt1 plays a key role in endothelial cell functions [16]. These systems might also enable identification of novel molecules responsible for embryonic vasculogenesis and support the discovery of new drugs for vascular regeneration therapy.

The third application category is research into the use of patient-specific iPS cells. iPS cells can be established from any human being, irrespective of their genetic background. The establishment of iPS cell lines from patients with inherited diseases presenting vascular abnormality should enable clarification of their pathogenesis. Patient-specific iPS cells are also useful for constructing in vitro models that can facilitate understanding of disease mechanisms and screening for more effective and safer drugs. For research
using patient-specific IPS cells, refined methods of differentiating and isolating cells for target tissue is indispensable. Although recently there has been a great deal of research into patient-specific IPS cells [39-52], research into their use in the vasculature and endocrine organs is still rare [53]. We are now investigating the pathogenesis of several inherited vascular disorders with collaborators.

Acknowledgement

We thank our laboratory members and collaborators for their helpful support and thoughtful discussion. Work carried out in our laboratories is supported by Grants-in-Aid for Scientific Research from the Japan Society for the Promotion of Science and grants from the Japanese Ministry of Health, Labour and Welfare.

References


