ATVB in Focus:
iPSC-Derived Cell Types Relevant to Atherothrombosis

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Induced Pluripotent Stem Cell–Derived Endothelial Cells in Insulin Resistance and Metabolic Syndrome

Ivan Carcamo-Orive, Ngan F. Huang, Thomas Quertermous, Joshua W. Knowles

Abstract—Insulin resistance leads to a number of metabolic and cellular abnormalities including endothelial dysfunction that increase the risk of vascular disease. Although it has been particularly challenging to study the genetic determinants that predispose to abnormal function of the endothelium in insulin-resistant states, the possibility of deriving endothelial cells from induced pluripotent stem cells generated from individuals with detailed clinical phenotyping, including accurate measurements of insulin resistance accompanied by multilevel omic data (eg, genetic and genomic characterization), has opened new avenues to study this relationship. Unfortunately, several technical barriers have hampered these efforts. In the present review, we summarize the current status of induced pluripotent stem cell–derived endothelial cells for modeling endothelial dysfunction associated with insulin resistance and discuss the challenges to overcoming these limitations.

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Key Words: cell differentiation ■ diabetes mellitus, type 2 ■ endothelial cells ■ induced pluripotent stem cells ■ insulin resistance ■ metabolic syndrome X

The endothelium has a wide variety of functions that include maintaining vascular homeostasis, regulation of vasomotor tone, thrombosis, regulation of inflammatory responses, control of vascular permeability, and insulin delivery to different tissues.

Insulin resistance causes many metabolic and cellular abnormalities and adverse clinical outcomes, some of which have been subsumed under the diagnostic category referred to as the metabolic syndrome. The cluster of abnormalities associated with insulin resistance and compensatory hyperinsulinemia include hypertension, increased plasma triglyceride concentrations, and low concentrations of high-density lipoprotein.[1,2] Individuals with insulin resistance are at increased risk of developing type 2 diabetes mellitus (T2D), coronary heart disease, and other vascular abnormalities like retinopathy, nephropathy, and neuropathy.[3] It is now established that endothelial dysfunction is one of the initial steps in the development of vascular complications associated with insulin resistance and the metabolic syndrome. Endothelial dysfunction is characterized by a shift in the homeostatic functions of the endothelium toward a vasoconstrictor, prothrombotic, and proinflammatory state.

Although a small number of endothelial cell lines and primary cultured cells have significantly helped in studies of vascular biology, the difficulty accessing vascular tissues has greatly impacted the ability to select for specific donors with informative genetic backgrounds to study specific pathologies. Now, with the advent of induced pluripotent stem cell (iPSC) technology, the recruitment and generation of endothelial cells from patients with a wide variety of conditions is potentially unlimited.

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Characteristics of Insulin Resistance/Metabolic Syndrome–Associated Endothelial Dysfunction

Insulin resistance and the associated metabolic syndrome are complex traits with both genetic and environmental determinants. Decreases in insulin sensitivity in fat and skeletal muscle are primarily responsible for whole-body insulin resistance and are a necessary precursor to T2D and the metabolic syndrome. Once this pathological condition is initiated, whole-body homeostasis is affected, rendering a wide group of autocrine and paracrine abnormal processes.

Diverse studies have associated the metabolic syndrome as a whole, as well as the composite risk factors such as hypertension and T2D, with endothelial dysfunction. The reactive sustained hyperinsulinemia aimed at maintaining euglycemia in the metabolic syndrome has deleterious consequences. In the vasculature, hyperinsulinemia favors *EDN1* (endothelin-1) secretion, which promotes

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From the Department of Medicine and Cardiovascular Institute (I.C.-O., T.Q., J.W.K.) and Department of Cardiothoracic Surgery and Cardiovascular Institute (N.F.H.), Stanford University School of Medicine, CA; and Veterans Affairs Palo Alto Health Care System, CA (N.F.H.).

Correspondence to Carcamo-Orive, PhD, Department of Cardiovascular Medicine, Stanford University School of Medicine, Falk Cardiovascular Research Bldg, 300 Pasteur Dr, Stanford, CA 94305. E-mail ivantxo@stanford.edu

Nonstandard Abbreviations and Acronyms	
iPSC	induced pluripotent stem cell.
iPSC-EC	induced pluripotent stem cell–derived endothelial cell.
T2D	type 2 diabetes mellitus.

vasoconstriction and contributes to endothelial dysfunction. Moreover, high insulin levels contribute to an increased expression of VCAM-1 (vascular cell adhesion molecule 1, *VCAM1*) and *SELE* (E-selectin) that promote monocyte/lymphocyte recruitment and a proinflammatory state of the endothelium, which feeds back into increased endothelial dysfunction.[4] Furthermore, both prolonged and transient hyperglycemia promotes micro- and macrovascular endothelial dysfunction in animal models.[5,6] Hyperglycemia increases reactive oxygen species, which in turn contribute to a decreased NO bioavailability. Decreased NO synthesis and release is a central hallmark of endothelial dysfunction, while insulin favors vasodilatation stimulating the production of NO by endothelial NO synthase.[7,8] Additionally, hyperglycemia leads to an accumulation of advanced glycation end products that contribute to the decrease of NO bioavailability. Comparable to hyperglycemia, increased circulating free fatty acids lead to lipotoxicity in endothelial cells, rendering an endothelial dysfunction phenotype, at least in part mediated by a decrease in endothelial NO synthase activity and NO production.[4]

These and other studies have offered strong evidence that endothelial cells are direct targets for insulin action and that insulin plays a critical role in the regulation of endothelial cell function even though endothelial cells rely on insulin-independent mechanism for glucose uptake. The critical role of insulin in endothelial cells beyond glucose metabolism is not surprising because the molecular pathways that govern various important endothelial functions, including NO production, are strikingly similar to those associated with glucose and lipid metabolism in metabolically active cells like adipocytes and skeletal muscle cells.[9]

Endothelial dysfunction may also contribute to the disease development by leading to impaired insulin action because of altered transcapillary passage of insulin to target tissues.[10] In addition, reduced expansion of capillary networks decreases blood flow to metabolically active tissues and contributes to abnormal insulin action in glucose and lipid metabolism,[10] which in turn contributes to insulin resistance.

It is believed that metabolic syndrome–associated endothelial dysfunction is a universal consequence of the whole-body metabolic dysregulation. However, the extent to which endothelial dysfunction is associated with the metabolic syndrome may be conditioned by the same pre-existing genetic risk variants that affect metabolically active cell types in the context of insulin resistance or a result of a different set of genetic risk variants (eg, vascular specific alleles that are influenced under conditions of insulin resistance).

Current Advances Modeling Insulin Resistance/Metabolic Syndrome–Associated Endothelial Dysfunction Through iPSC Technology

The multifactorial nature of insulin resistance has added an extra layer of complexity for the modeling of metabolic syndrome–associated endothelial function. Although iPSC technology has demonstrated the capacity to model both Mendelian diseases and more complex traits,[11,12] it has been challenging to generate an iPSC-based human model system to study endothelial function in the context of the insulin resistance.

Among the monogenic forms of diabetes mellitus, iPSCs have been successfully generated from individuals with maturity-onset diabetes mellitus of the young and mitochondrial diabetes mellitus.[13–15] iPSC lines have also been successfully generated from type 1 diabetes mellitus[16–19] and T2D patients,[16,20] although in limited numbers. More recently, iPSC lines derived from patients with mutations in the insulin receptor[21] have proven useful in unraveling metabolic defects in mesenchymal progenitor cells derived from those iPSC lines.[22] In a different study, iPSC-derived cardiomyocytes from patients with T2D reproduced the cardiomyopathic phenotype when exposed to diabetic mediators like high glucose, endothelin-1, and cortisol.[23] None of these studies have analyzed the possible effects of the disease in iPSC-derived endothelial cells (iPSC-ECs).

There are also some examples of animal models of metabolic syndrome from which iPSCs have been derived. For example, iPSCs derived from DahlS.Z-Leprfa/Leprfa (DS/obese) rats offer a new model of metabolic syndrome.[24] More importantly, a recent study derived iPSC-ECs from diet-induced obesity mice that exhibit signs of endothelial dysfunction.[25] Diet-induced obesity iPSC-ECs demonstrated a reduced capacity to form cord-like structures, proliferation, migration, and increased apoptosis in vitro while performing poorly in a hindlimb ischemia model in vivo. Treatment with pravastatin was able to restore cell migration and proliferation while inhibiting apoptosis. Increased NO levels in iPSC-ECs treated with statin was demonstrated to mediate such effects.

Over the past decade, genome-wide association studies have identified at least 100 candidate genes and genomic loci associated with T2D-related and insulin resistance–related traits.[26,27] Although there have been some mechanistic successes,[28,29] efforts to find the causal variants or genes and the molecular mechanisms contributing to the onset and development of disease have not been as successful, in part because of the lack of appropriate human cellular systems where fine-tuned genomic modifications can be performed. With the advent of genome-editing technology combined with iPSC methodology, it is now possible to interrogate disease-associated loci in a specific cellular context.[30] Such an approach is based on the generation of isogenic iPSC lines that share identical genetic background other than the modified disease-associated locus (genome editing–based metabolic disease modeling with iPSCs is reviewed by Yu et al[30]). More recently, Zeng et al[31] generated isogenic human embryonic stem cells with genome-wide association study–identified susceptibility genes for T2D and demonstrated impaired glucose secretion in iPSC-derived pancreatic β-like cells. These seminal studies pinpoint the possibility of using genome editing to study candidate loci to participate in insulin resistance and the metabolic syndrome and, in particular, to interrogate specific variants in the context of endothelial dysfunction.

However, a well-described human iPSC library built from individuals with different degrees of insulin sensitivity, including many insulin-resistant patients with metabolic syndrome has been lacking. To fill this gap, our group has generated a large-scale iPSC library derived from individuals with accurate measurements of insulin sensitivity, the insulin suppression test (steady-state plasma glucose measurements), that represent the full spectrum of insulin response in human populations.[32] This iPSC library is now publicly available at the WiCell Stem Cell Bank. We have begun to use these lines to create iPSC-ECs to investigate several aspects of the metabolic syndrome–associated endothelial dysfunction: (1) interrogate for new loci participating in the disease, (2) validate candidate genes through gene-editing technology and generation of isogenic lines, and (3) study new mechanisms participating in endothelial dysfunction.

Overall, there are encouraging reports suggesting that insulin resistance/T2D/metabolic syndrome can be effectively modeled through iPSC technology. However, there is a current lack of information if these in vitro models can fully reproduce the dysfunctional phenotype in iPSC-ECs. Moreover, there are still some technical challenges, as described below, to overcome in order to accurately model the metabolic syndrome–derived endothelial dysfunction.

Challenges for iPSC-Mediated Modeling of Insulin Resistance/Metabolic Syndrome–Associated Endothelial Dysfunction

After the discovery of iPSCs, several groups demonstrated the possibility of deriving functional endothelial cells through cocultivation with OP9 cells.[33,34] Posteriorly, generation of embryoid body intermediates was also demonstrated to induce endothelial determination of iPSC cells.[35] Currently, there are several improved protocols that enable the generation of endothelial cells in 2-dimensional systems with defined factors and without the need of generating embryoid bodies or cocultivation.[36,37] There are even incipient methods that seek to differentiate endothelial cells in a 3-dimensional system–mimicking vascular cues.[38] The current most relevant protocols for endothelial differentiation have been reviewed elsewhere; however, 2 recent reports deserve special attention. First Patsch et al[39] described a highly efficient protocol for the generation of both endothelial cells and smooth muscle cells in a defined medium. However, one of the critical components (CP21R7) in the differentiation medium is still not currently commercially available. Second, Wu et al[40] described a differentiation protocol that is able to render highly pure endothelial populations without the need for an additional purification step through the use of antiadsorptive agents that inhibit cell attachment of nonendothelial cells.

Although much progress has been achieved in recent years, there is yet not a standardized protocol that yields a pure population of endothelial cells or that maintains the in vitro expansion of endothelial cells without loss of purity or phenotype. Moreover, we have shown high variability on the differentiation efficiency of different iPSC lines from different individuals and even from different iPSC clones derived from the same individual.[32] Our analysis proposed *HOX* genes as a key driver determinant of the differentiation variability. Other works have shown that extracellular matrix coating, serum, and seeding density can also affect differentiation of pluripotent cells to the endothelial lineage.[41,42]

Another layer of complexity for endothelial dysfunction modeling is represented by endothelial cell heterogeneity depending on their particular identity and anatomic location.[43,44] Endothelial cell subtypes can be grossly defined as arterial, venous, lymphatic, or microvascular. In addition, there is also organ-specialized endothelium such as in brain, kidney, or liver, for example.[43,44] This fact is especially relevant for the modeling of insulin resistance–associated endothelial dysfunction that encompasses pleiotropic effects in various vascular beds across the body. Currently, the possibility of generating particular subtypes of endothelial cells is the subject of intensive investigation. We have previously demonstrated the heterogeneous nature of iPSC-ECs and the ability to enrich for subtypes using soluble factors.[45] Other works have also shown the possibility of deriving microvascular or arterial endothelial cells from pluripotent stem cells.[46,47]

One of the main concerns in the iPSC field relates to the extent to which mature cells derived from iPSCs truly resemble the genetic program and functional characteristic of their primary counterparts. Some studies have proposed that there are limited differences between primary endothelial cells and iPSC-ECs,[39,48] whereas others have found larger differences that may affect functionality and the long-term stability of the endothelial identity in iPSC-EC. In particular, a phenotypic shift has been observed in long-term culture of iPSC-ECs, resembling the endothelial-to-mesenchymal transition that happens during normal embryonic development.[49,50] However, it is also possible that a residual fibroblastic population overtakes iPSC-ECs in culture. These observations emphasize the need for additional studies to assess the extent to which iPSC-ECs truly resemble the gene expression program found in their primary counterparts and develop new strategies to maintain a stable endothelial cell phenotype in vitro.

We have summarized the current hurdles for iPSC-EC modeling of endothelial dysfunction in Table 1, and a general view of the future goals to advance and improve endothelial dysfunction modeling through iPSCs is condensed in Table 2. We think that as our control over this experimental system grows, our knowledge about the genes and processes driving insulin resistance–associated endothelial dysfunction will exponentially expand.

Table 1. iPSC to Endothelial Cell Differentiation Barriers

Efficiency: efficacy to generate endothelial cells from iPSC needs improvement.
Variability: iPSC clonal differences of endothelial differentiation efficiency.
Identity: derivation of specific subtypes of endothelial cells.
Fidelity: replication of primary endothelial cell gene expression pattern and functional properties.
Stability: ability to maintain endothelial identity during in vitro expansion.

iPSC indicates induced pluripotent stem cell.

Table 2. Future Goals to Improve iPSC-Derived Models of Endothelial Dysfunction

Improve differentiation efficiency and consistency across different iPSC lines.
Improve the functional and gene expression fidelity of iPSC-ECs compared with their primary counterparts.
Derive specific endothelial cell subtypes.
Maintain endothelial identity during in vitro expansion.
Mimic the environmental cues endothelial cells are exposed in vivo in normal homeostasis and in the context of insulin resistance/metabolic syndrome
Build blood-vessel 3D surrogates comprising the different cell types present in the blood vessels in vivo.

EC indicates endothelial cell; iPSC, induced pluripotent stem cell; and 3D, 3 dimensional.

Concluding Remarks

iPSC-ECs offer a new, unprecedented opportunity to develop model systems to study dysfunctional vasculature in the context of insulin resistance and the metabolic syndrome. Although there are various examples of successful modeling of a complex genetic trait, an appropriate iPSC resource for the study of insulin resistance/metabolic syndrome has been lacking. Additionally, there are few examples of in vitro modeling of endothelial dysfunction through iPSC-ECs. The combination of large-scale iPSC libraries generated by our group and other groups and improvement of the current endothelial differentiation protocols will help to overcome current hurdles and improve our ability to model endothelial dysfunction. Finally, the combination of iPSC and gene-editing technologies is opening new venues to interrogate the gene identity and functional relevance of the multiple loci found to be associated with insulin resistance and the metabolic syndrome by genome-wide association studies.

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Disclosures

None.

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Highlights

- Insulin resistance and metabolic syndrome promote endothelial dysfunction.
- Induced pluripotent stem cell–derived endothelial cells offer a novel framework to model endothelial dysfunction.
- Large-scale induced pluripotent stem cell libraries accompanied with accurate measurements of insulin sensitivity have now been generated.
- Experimental challenges do still hamper the ability to properly model endothelial dysfunction through induced pluripotent stem cell technology.

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