Abstract

Vascular biology plays a critical role in many aspects of normal and disease-relevant physiology, including barrier function, inflammation, cell migration (wound healing and metastasis), thrombosis and hemorrhage, atherosclerosis, as well as angiogenesis. The development of reliable vascular biology tools, such as endothelial cells (ECs), is a critical need for the generation of relevant in vitro human disease models for drug and therapeutic research, as well as enabling regenerative medicine. To that end, we have developed human induced pluripotent stem cell (iPSC)-derived endothelial cells referred to as iCell ECs that exhibit vascular endothelial markers and characteristics, and offer a physiologically relevant human in vitro system to study complex cellular processes of vasculogenesis.

Here, we describe the use of Cell ECs in a real-time electronic cell sensor impedance array technology to monitor endothelial function. We measured the impact of serum, growth factors (e.g. VEGF, EGF, FGF-2), and small molecule angiogenesis modifiers (e.g. SU5402, SU1498) on the proliferation, migration, and invasion of iPSC-derived ECs compared to primary cells, such as HUVECs. We observed that the combination of iCell ECs and a real-time monitoring platform provides a biologically relevant human model system for measuring and quantifying modulation of endothelial cell morphogenesis. The data presented here demonstrate that the use of ECs derived from iPSCs provide a stable, well-defined source of human cells that are well-suited for investigating vasculogenesis and angiogenesis studies and particularly useful for addressing current challenges facing vascular therapeutic development and tissue engineering applications.

Analysis of Proliferation in Real-Time

Cell ECs treated with growth factors (GFs) and inhibitor compounds in a novel, physiological-relevant spraying assay reports on cell morphology relevant to angiogenic signaling dependent Sprouting. Sprouting was assayed by seeding cells for 24 h at 3.0 × 10^4 cells/cm^2 in non confluent culture dishes to allow aggregate formation and then overlaid with mural. Cells were treated with VEGF and images analyzed to generate a measure of sprouting behavior. VEGF treatment in the presence of SU5402 or Nocodazole was then applied to measure the ability of these compounds to inhibit spraying. The representative images of endothelial cell sprouts in full supplement and without inhibitors are shown to illustrate the effect of inhibitors on the spraying behavior. The chart displays a full dose response for the inhibitors and was fitted to generate a mathematical model of the impact of endothelial cell spraying behavior also enabled distinguishing morphogenetic effects of the diffusion independent/independent effects of different mechanistic inhibitor treatments. As can be observed the kinase inhibitor SU5402 induced coordinated migration, whereas the microtubule inhibitor Nocodazole had a more dramatic impact on the overall ability of the cells to adopt a normal spindle-shaped morphology. Images were processed using ImageJ software.

Modulation of Morphogenesis

Inhibition of VEGF-mediated Proliferation: A panel of mechanistically distinct inhibitors of proliferation were assessed at a single concentration for their ability to inhibit the response of iCell ECs to VEGF in a triplicate (left). The IC50 of the kinase inhibitor SU5402 was determined over a full dose response in iCell ECs and HUVECs and illustrated). Differential sensitivity by the two cell types.

Real-Time Assays for Chemotaxis

Migration (left) and invasion (right) of iCell ECs toward VEGF as measured on the ACEA RTCA DP Analyzer. Media preparation and treatment conditions were prepared in bottom chamber of CM plates, and cells seeded at 10,000 cells/well in the top chamber. The electrodes in all wells were coated with E-PC3 matrix. Cells were assayed for 24 h after which time the final cell readout was calculated for comparison. In the cases of inhibition, inhibitor was added to the lower chamber at the concentration shown.

Shown on the left is the migratory potential of the iCell ECs on HUVECs. The real-time impedance data at the top panel demonstrates the migration toward the VEGF signal in the bottom chamber. This growth factor chemotaxis is abrogated by the addition of the receptor kinase inhibitor SU5402 as well as the microtubule inhibitor Nocodazole. A comparison of the migratory potential and sensitivity for a number of these inhibitors between the 2 cell types is shown in the bar chart with Cell EC showing significant chemotaxis range than HUVECs.

iCell EC invasion through a matrigel plug is shown on the right along with inhibition by SU5402 and Nocodazole again. HUVECs were unable to invade through the matrigel plug under the conditions tested. Inhibition of invasion by SU5402 is shown in the bar chart.

Summary

Angiogenesis is a complex process involving an interplay between blood vessel endothelial cells, support cells, and the extracellular matrix (ECM). Traditionally, EC morphogenesis associated with angiogenesis has been modeled in vitro with primary ECs, such as HUVECs, iPSCs, and arterial ECs. However, terminal blood vessel specification and primary EC heterogeneity can limit the use of primary ECs for emerging applications such as drug screening and tissue engineering. Induced pluripotent stem cell-derived endothelial cells (iPS-ECs) present potential benefits over traditional cell function related to angiogenesis. iCell ECs are a robust, reproducible and well-characterized cell type that enables rapid drug screening and analysis of pro-angiogenic function. Here, we have shown that iCell ECs respond to the pro-angiogenic growth factors VEGF and BFGF and may have different signaling mechanisms when compared to HUVECs. We have demonstrated that iCell ECs function in invasion and invasion assays that could be correlated to a physiological model of EC sprouting. Thus, the analysis of iPS-ECs with real-time and end-point monitoring offers a biologically relevant human model of angiogenesis.