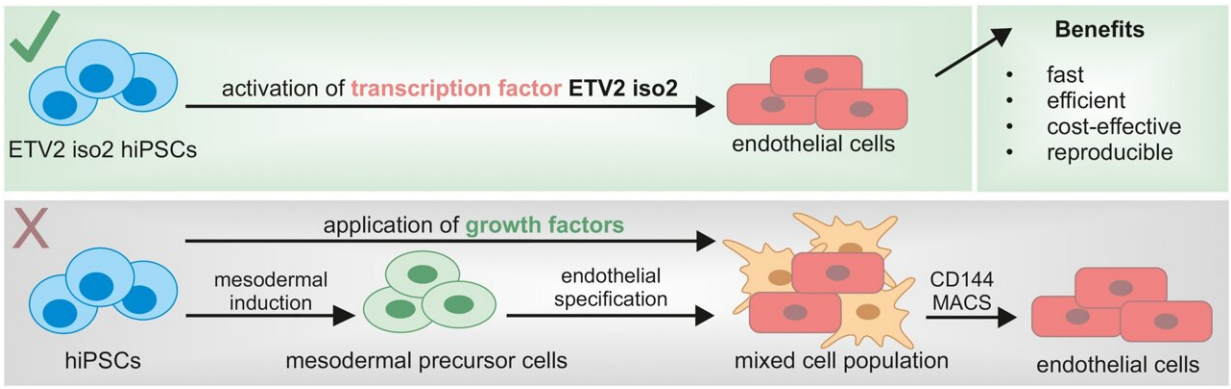
Researchers establish efficient, cost-effective method for generating endothelial cells from stem cells

July 31 2024



Credit: *Cardiovascular Research* (2024). DOI: 10.1093/cvr/cvae129

The innermost layer of blood vessels is formed by endothelial cells, which in turn play a role in the development of diseases of the cardiovascular system. Human endothelial cells are therefore required for the "in vitro" investigation of the causes of these diseases.

Researchers at the University Hospital Bonn (UKB) and the University of Bonn have now established a highly efficient, cost-effective and reproducible way to generate functional endothelial cells from human induced pluripotent stem cells (hiPSCs) for tests in cell culture dishes.

The results of the study have now been published in the journal *Cardiovascular Research* .

Endothelial cells that line the inside of blood vessels perform a number of tasks in the human body, such as regulating blood pressure and blood clotting. They also play a role in the development of diseases of the cardiovascular system. Human endothelial cells are needed to study the basis of these diseases outside the human body.

"Human induced pluripotent stem cells (hiPSCs) are a promising approach for this. Since they are not yet committed to a specific tissue type, they have the potential to differentiate into many different cell types—including endothelial cells," says co-corresponding and senior author Prof. Bernd K. Fleischmann , Director of the Institute of Physiology I at the UKB and member of the Cluster of Excellence ImmunoSensation2 and the Transdisciplinary Research Area (TRA) "Life & Health" at the University of Bonn.

Various differentiation strategies for hiPSCs into endothelial cells have already been developed in the past. One of the most efficient approaches to date is based on the use of different growth factors in combination with a purification step to enrich the successfully generated endothelial cells. In another approach, so-called transcription factors are specifically activated to control the conversion of hiPSCs into endothelial cells.

Recently, an international research team led by George Church from Harvard Medical School in Boston (U.S.) and Volker Busskamp from the UKB identified the transcription factor "ETS variant transcription factor 2," abbreviated ETV2, as an important driver in this process.

Furthermore, the team has developed a hiPSC line in which the transcription factor ETV2 can be specifically activated by the addition of the antibiotic doxycycline. This particular stem cell type is called

"PGP1 ETV2 iso2."

Fast, cost-effective and reproducible route to human endothelial cells

Dr. Sarah Rieck's research group from the Institute of Physiology I, together with Kritika Sharma from Prof. Busskamp's team at the UKB Ophthalmology clinic has improved the differentiation protocol for the PGP1-ETV2-iso2 line (ETV2 protocol) and compared it with the strategy using growth factors.

"We were able to show that the ETV2 protocol we improved is more efficient and cost-effective than the protocol with growth factors," says co-corresponding and first author Dr. Rieck, who also conducts research at the University of Bonn.

This is because it delivers endothelial cells more quickly, requires fewer additives for the culture medium and does not require an additional purification step. Furthermore, the process is highly reproducible and can be easily transferred to other hiPSC lines.

The resulting cells are not contaminated with other cell types and are also stable over longer cultivation periods. They produce proteins characteristic of endothelial cells and also show typical functional properties of endothelial cells. By modifying the differentiation protocol, it is also possible to preferentially obtain endothelial cells with arterial or venous characteristics.

Although they are similar to the endothelial cells differentiated with the growth factor protocol, there is evidence that the endothelial cells of the ETV2 protocol have a slightly higher degree of maturity.

"Compared to human endothelial cells from the umbilical vein, however, both types of hiPSC-derived endothelial cells are not fully developed, which is probably due to a lack of external influences such as the absence of blood flow," says co-author Prof. Busskamp, head of the "Neurodegenerative Retinal Diseases" research group at the UKB and member of the Cluster of Excellence ImmunoSensation 2 and the Transdisciplinary Research Area (TRA) Life & Health at the University of Bonn.

For the future, the Bonn researchers assume that the PGP1 ETV2 iso2 line and the endothelial cells generated from it will be used to model and study diseases of the human vasculature in which the endothelium is involved in the cell culture dish.

This scientific question is being researched by Dr. Rieck and Prof. Fleischmann in project C01 in the DFG Collaborative Research Center Transregio (TRR) 259 Aortic Diseases. The endothelial cells can also be used in organoid research to develop organoids with a vascular system.

"Apart from this, we are also interested in which cultivation methods increase the 'degree of maturity' of the endothelial cells following differentiation, so that their profile corresponds more closely to that of adult endothelial cells," says Dr. Rieck.

More information: Sarah Rieck et al, Forward programming of human induced pluripotent stem cells via the ETS variant transcription factor 2: rapid, reproducible, and cost-effective generation of highly enriched, functional endothelial cells, *Cardiovascular Research* (2024). DOI: 10.1093/cvr/cvae129

Provided by University Hospital of Bonn