Molecular Mechanisms of Vessel Morphogenesis

Elena Rakovoliou [#1, 2], Maria Markou [#1], Eleni Bagli[1], Sofia Bellou [1], Theodore Fotsis[1*] and Carol Murphy[1]

[1] Biomedical Research Institute - FORTH, 45110 Ioannina, Greece

[2] Laboratory of Biological Chemistry, Medical School, University of Ioannina, 45110 Ioannina, Greece

#Contributed equally

* Presenting author: Elena Rakovoliou, elenarakovoliou@gmail.com

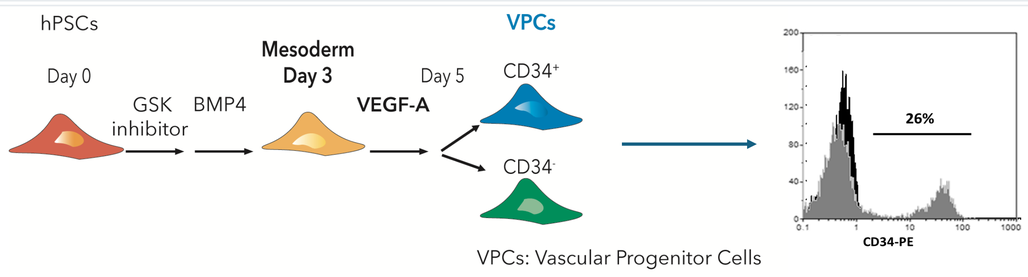
* Corresponding author: Carol Murphy, carol_murphy@bri.forth.gr

Abstract

Regenerative Medicine (RM), based on the immense regenerative capacity of stem cells, is considered the therapy of the future, relies on rapid vascularization for successful cell transplantation. To enhance vascularization, we differentiate human induced pluripotent stem cells (hiPSCs) and human embryonic stem cells (hESCs) via mesodermal intermediates into vascular progenitor cells (VPCs) and endothelial cells (ECs). VEGF-A is crucial for endothelial cell commitment during vasculogenesis and angiogenesis, yet its signaling pathways are not fully defined. Currently there is a lack of data concerning the VEGF-induced signalling cascades that differentiate/commit mesodermal intermediates to VPCs (vasculogenesis) and then to ECs. In addition to VEGF, several other parameters are known to affect vasculogenesis *in vivo*, the most important of which are the presence of Mural Cells (MCs) and flow.

We identified a subset of Day 3 (D3) mesodermal cells, with high VEGFR-2 expression, that respond to VEGF-A, showing elevated ETV2 levels and giving rise to VPCs on Day 5. In vivo labeling of Day 3 (D3) mesodermal cells with a non-functional anti-VEGFR-2-Alexa488 antibody, followed by VEGF-A induction, confirmed that Day 5 (D5) vascular progenitor cells (VPCs) were strongly labeled with internalized anti-VEGFR-2-Alexa488. This demonstrates that a subset of D3 mesodermal cells with high VEGFR-2 expression gives rise to VPCs after VEGF stimulation. Ongoing scATACseq, scRNAseq, and phosphoproteomics aim to clarify the molecular identity of these cells and the signalling cascades of VEGF responsible for vasculogenesis. We are also investigating the impact of flow and mural cells on VPC differentiation using microfluidic models of vasculogenesis.

1. Differentiation of hPSCs to VPCs

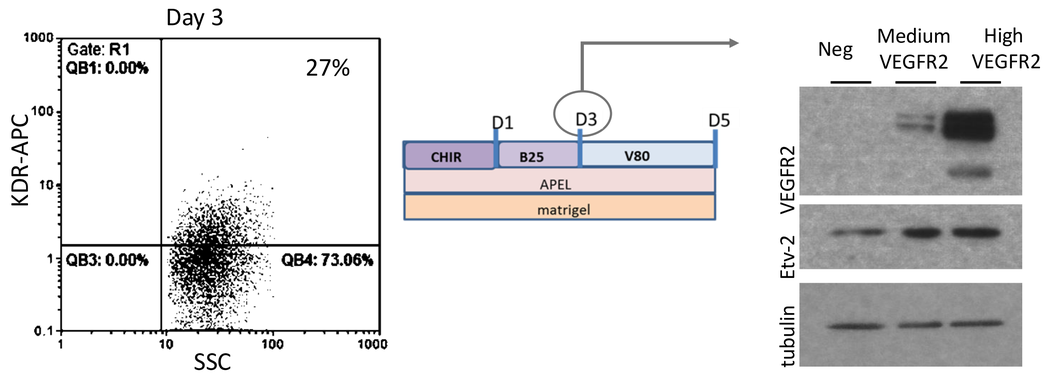


VPCs: Vascular Progenitor Cells

On the 5th day of the differentiation procedure up to 35% of the cells express CD34, a marker of VPCs and could be clearly segregated from CD34- cells

2. Identification of Mesodermal Cells responsive to VEGF-A on Day 3 of Differentiation, giving rise to VPCs

Day 3 cells sorted according to VEGFR2 surface expression

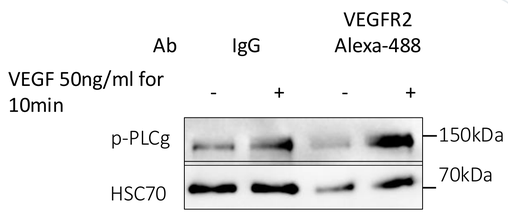


VEGFR2-expressing cells also express the transcription factor ETV2, a marker of mesodermal cell commitment to the endothelial lineage

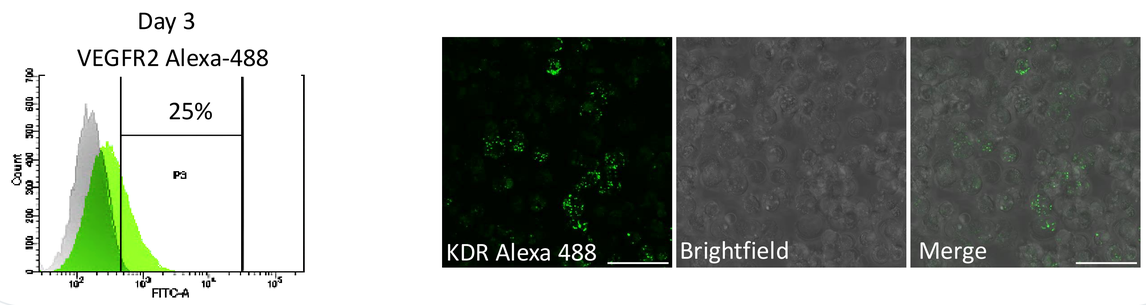
3. Selection of mesodermal cells poised to become VPCs

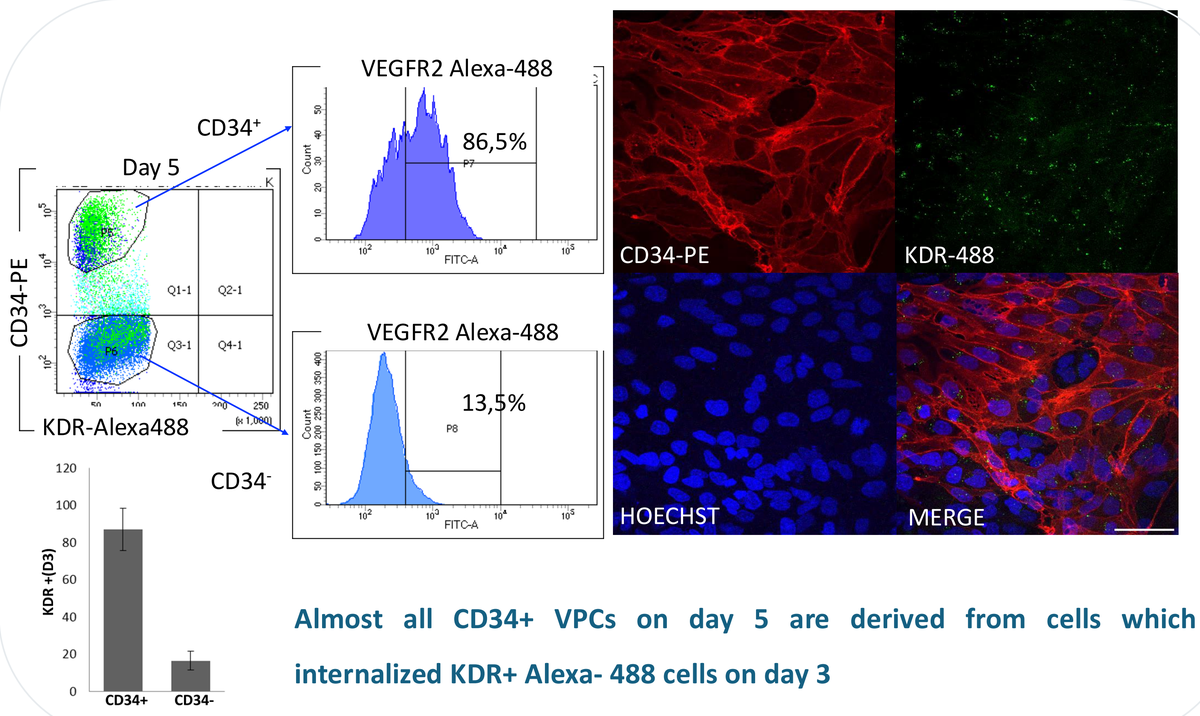
Non-Functional Blocking Anti-VEGFR2 Antibody:

- No KDR Activation in Absence of VEGF
- No Alteration of VEGF Induction



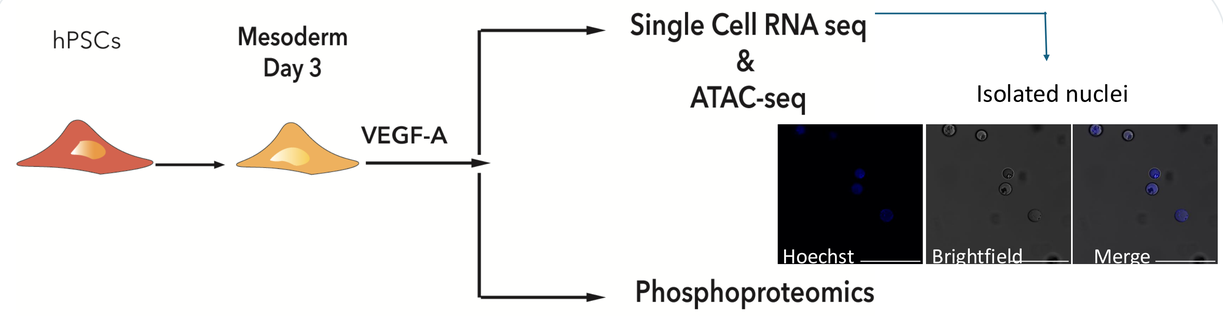
In Vivo Labeling of Day 3 Mesodermal Target Population with a non-functional anti-VEFR2-Alexa 488 antibody





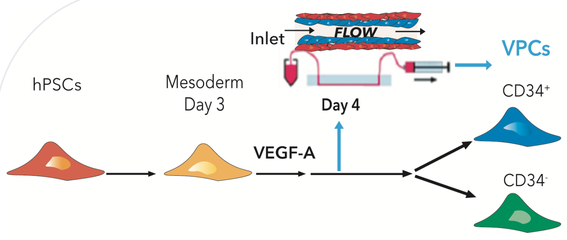
Almost all CD34+ VPCs on day 5 are derived from cells which internalized KDR+ Alexa- 488 cells on day 3

4. Determination of the VEGF-induced transcriptome/ phosphoproteome during vasculogenesis



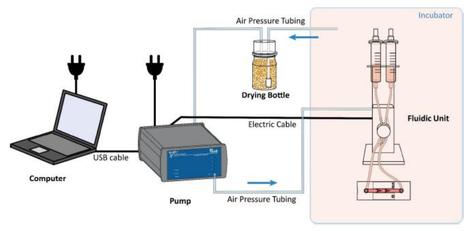
Combined network analysis of RNA-Seq and phosphoproteomic analysis for identification of regulatory networks of vasculogenesis

5. Effect of flow on the differentiation of mesodermal cells to VPCs

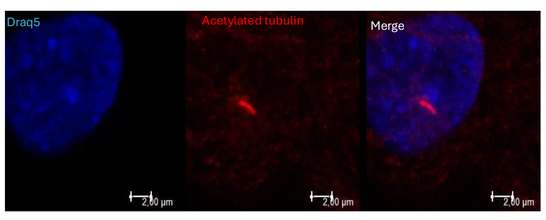


VPCs: Vascular Progenitor Cells

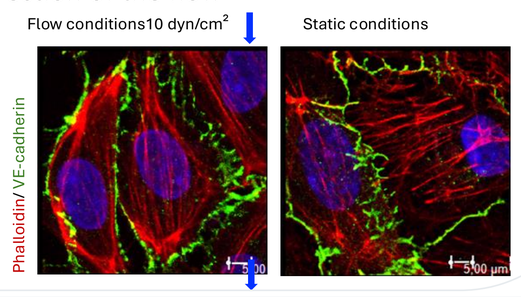
A microfluidic system has been installed to mimic flow conditions



hPSC-derived ECs have primary cilia which allow them to respond to flow, as they act as calcium-dependent mechanosensors that sense blood flow



Shear Stress Influences Cell Morphology, Cell Structure, and Organization. In contrast to static cell culture, cells orient in the direction of the flow, and a rearrangement of the cytoskeleton takes place with actin fibers aligning in the direction of the flow



6. Conclusions

1. We have established an in vitro model of vasculogenesis using human pluripotent stem cells
2. Additionally, we have identified a population of high KDR mesodermal cells which give rise to VPCs
3. We have optimized methods to isolate and characterize these cells by scMultiome and phosphoproteomics - analyses are ongoing to identify the signalling cascades governing vasculogenesis
4. The role of shear stress and flow on vasculogenesis are being dissected using a microfluidic platform incorporating mural cells and the high KDR mesodermal population

REFERENCES

[1] Tsolis K, Bagli E, Kanaki K,Zografou S, Carpentier S, Bei E, Christoforidis S, Zervakis M, Murphy C, Fotsis T, Economou A, J. Proteome Res. 2016, 15, 1995−2007

[2] Markou M, Kouroupis D, Badounas F, Katsouras A, Kyrkou A, Fotsis T, Murphy C, Bagli E, 2020, Front Bioeng Biotechnol;8:278.

The research project is implemented in the framework of H.F.R.I call "Basic research Financing (Horizontal support of all Sciences)" under the National Recovery and Resilience Plan "Greece 2.0" funded by the European Union-Next Generation EU
(H.F.R.I. Project Number: 15009).