|  |
| --- |
| Μolecular Mechanisms of Vessel Morphogenesis  **Rakovoliou Elena1, 2**#**, Markou Maria 1, Eleni Bagli 1, Bellou Sofia 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  # Presenting author: Eleni Rakovoliou, email: [elenarakovoliou@gmail.com](file:///C:\Users\ΕΙΡΗΝΗ\Νέος%20φάκελος\Downloads\elenarakovoliou@gmail.com)  \* Corresponding author: Carol Murphy, email: [carol\_murphy@bri.forth.gr](file:///C:\Users\ΕΙΡΗΝΗ\Νέος%20φάκελος\Downloads\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. However, there are still issues that need to be addressed before translation in the clinic. One important factor in the success of RM is the speed by which the transplanted cells are vascularised by the host. Delayed vascularisation of the implant results in low cell survival rates. Thus, ideally the tissue engineered construct should be already vascularised in vitro prior to implantation in vivo. We have already initiated a programme for vascular engineering based on reprogramming human fibroblasts to generate human induced pluripotent stem cells (hiPSCs) and subsequent differentiation of hiPSCs or human embryonic stem cells (hESCs), via mesodermal intermediates, to vascular projenitor cells (VPCs) and then to Endothelial (ECs)1 and mural cells (MCs)2. VEGF is the main growth factor responsible for the endothelial cell commitment during vasculogenesis and angiogenesis. 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 MCs and flow.

To explore the VEGF-A induced signalling responsible for the transition of mesodermal cells to VPCs, first we sought to identify the mesodermal cell population (Day 3 of the differentiation process), responsive to VEGF-A stimulation, which give rise to VPCs. FACS sorting of D3 mesodermal cells based on the expression of VEGFR-2 (negative, medium, high) revealed increased protein levels of ETV2 (a transcriptional factor known to be crucial for endothelial lineage commitment) in cells expressing high levels of VEGFR-2. Moreover, in vivo labeling of D3 mesodermal cells with a non-functional anti-VEGFR-2-Alexa488 antibody prior to induction with VEGF-A, allowed us to confirm that VPCs on D5 were strongly labelled with the internalized anti-VEGFR-2-Alexa488 antibody. Based on the above evidence, it is clear that a subset of D3 mesodermal cells expressing high levels of VEGFR-2 give rise to VPCs following VEGF induction. These cells have been isolated and single cell ATAC seq, RNAseq and phosphoproteomic analysis are ongoing.

In addition to the role of VEGF, we are addressing the role of flow and mural cell involvement on the differentiation efficiency of mesodermal cells to VPCs, using microfluidic systems to recapitulate the early stages of vasculogenesis.

**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.