

Integrative Multi-Omics Analysis of VEGF-Driven Vasculogenesis from Human Pluripotent Stem Cells

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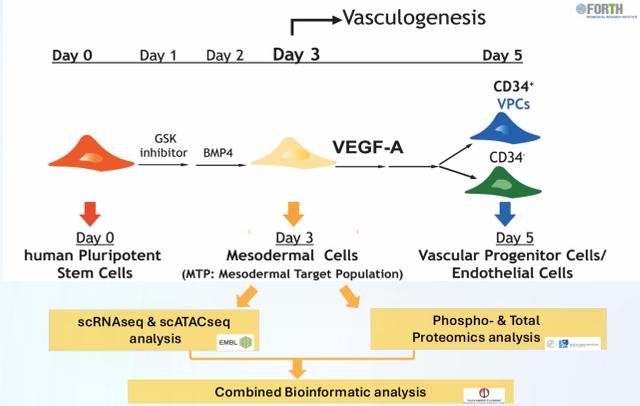
Abstract: Rapid vascularization remains a major bottleneck in regenerative medicine. Vascular Endothelial Growth Factor (VEGF-A) is essential for endothelial commitment during vasculogenesis, yet the exact precise cell population and underlying regulatory networks in humans are unknown. We identified a rare Day-3 mesodermal subset (Mesodermal Target Population, MTP) expressing high VEGFR-2 levels that exclusively gives rise to vascular progenitor cells (CD34⁺ VPCs) upon VEGF-A stimulation. Lineage tracing using a non-functional anti-VEGFR-2-Alexa488 antibody confirmed that only this subset internalizes the receptor and commits to the vascular fate. Using single-cell RNA-seq, scATAC-seq and ultra-deep phosphoproteomics (total mesoderm & purified MTP_5' and 15' post-VEGF), we are mapping the complete transcriptional, epigenomic, and early signalling landscape of human vasculogenesis. Ongoing multi-omics integration will deliver the first molecular blueprint of this process, enabling high-efficiency generation of vascular networks for tissue engineering and regenerative therapies.

1 Regenerative Medicine Challenge

Problem: Transplanted tissues fail without rapid vascularization

GOAL: Identification of regulatory networks of vasculogenesis
Understand VEGF-driven vessel formation to engineer pre-vascularised tissue constructs

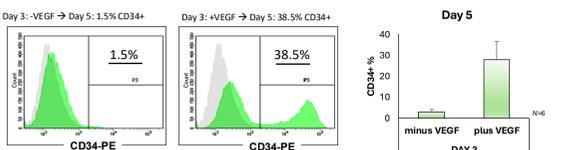
Differentiation of hPSCs to VPCs



2 Identification of Mesodermal Target Population

Day 3 mesodermal population is Heterogenous
Only High VEGFR2 cells → CD34+ VPCs

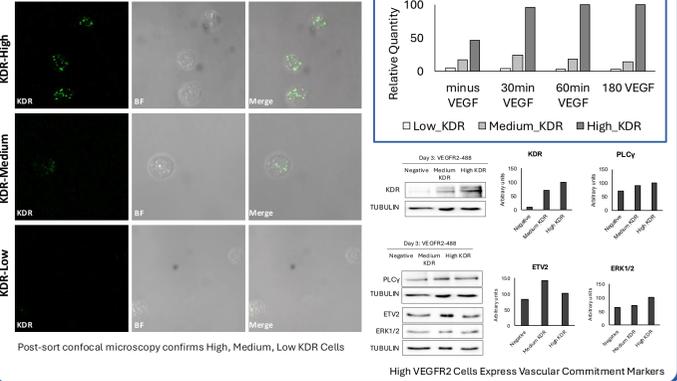
VEGF induces robust VPC generation from Day 3 mesoderm



Upon VEGF induction on day 3, 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

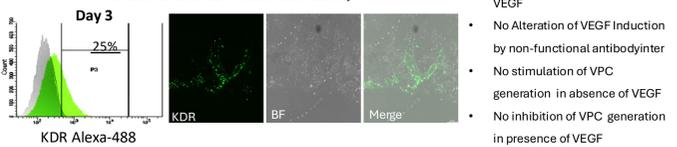
KDR Expression Heterogeneity

Three distinct KDR populations (High, Medium, Low) with corresponding mRNA levels. VEGFR2-expressing cells also express the transcription factor ETV2, a marker of mesodermal cell commitment to the endothelial lineage.

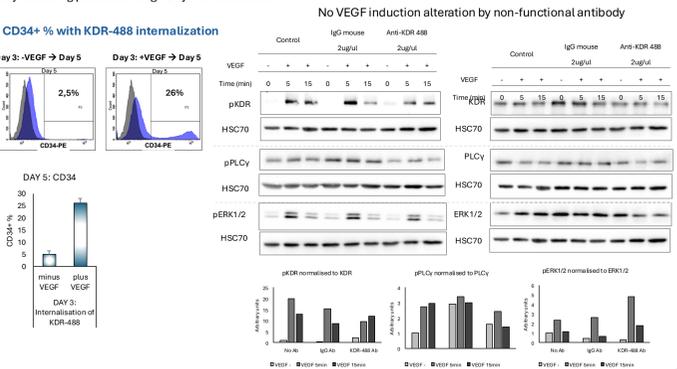
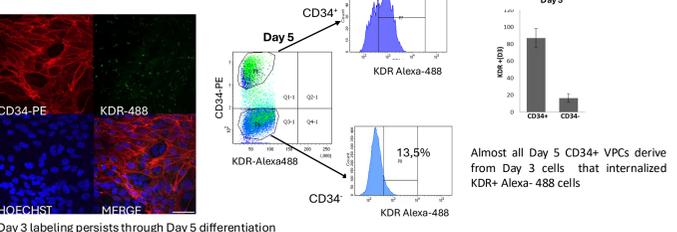


3 Lineage tracing: VEGFR2+ Cells Generate VPCs

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



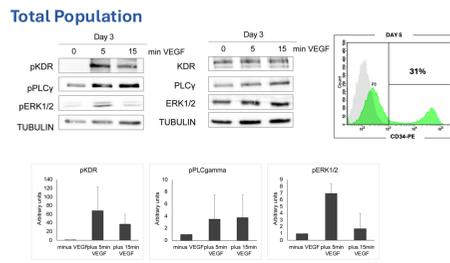
VEGFR2+ Cells Give Rise to VPC



4 High-Throughput Phosphoproteomics: Early VEGF Signalling

Experimental design: MTP day 3 total, split &, KDR-488 sorted cells (High/Medium/Low) at 0, 5, 15min post-VEGF

VEGF Signalling - Activation

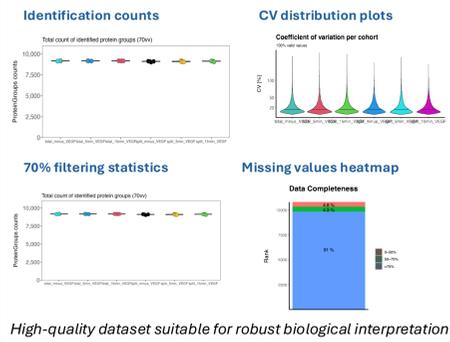


Rapid VEGFR2 phosphorylation triggers PLCγ and ERK1/2 MAPK cascades within 5-15 minutes

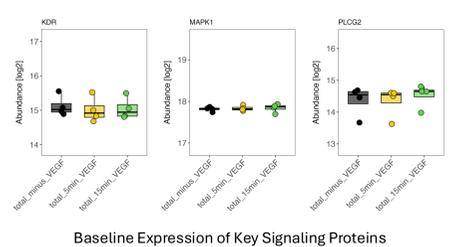
Dataset Quality:

- ~30,000 phosphopeptides quantified → excellent enrichment efficiency
- ~10,000 total proteins detected → High sample quality, efficient digestion and deep coverage
- CV < 20% (excellent reproducibility)
- 70% data completeness across replicates

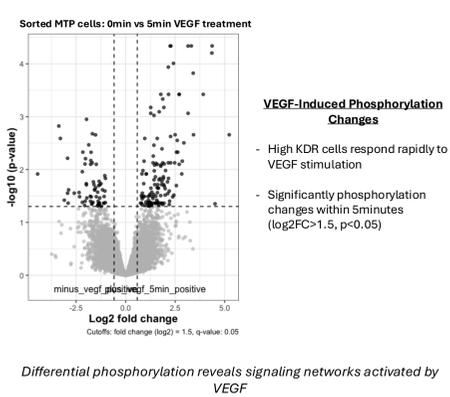
Quality Control Metrics



Total Proteomics: VEGF Pathway Components



Phospho Proteomics: Early VEGF Signalling

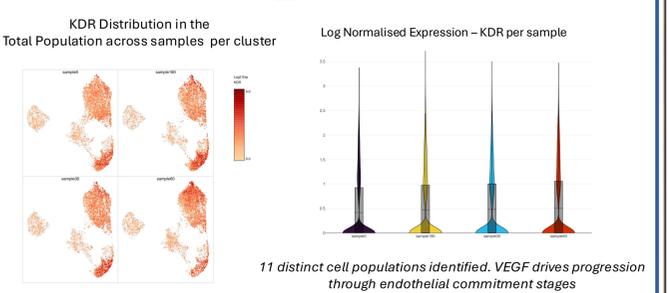
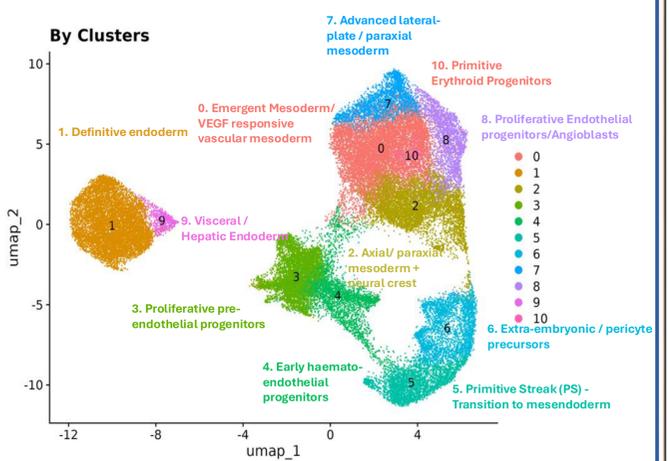


5 Single-Cell Transcriptome & Epigenome

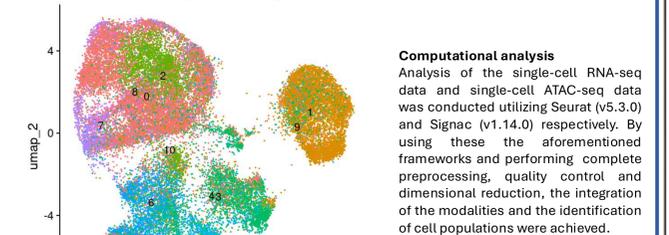
scRNA-seq, scATAC-seq, Integrated Analysis

Samples: Day 3 mesoderm at 0, 30, 60, 180 min post-VEGF

scRNA-seq: UMAP plot showing 11 clusters:



ATAC (scATAC-seq)



KDR distribution analysis: scRNA-seq confirms heterogenous VEGFR2 expression in total Day 3 population, validating FACS sorting strategy

6 Translational Impact

1. Optimized VPC Generation

Target high VEGFR2 cells with precision → higher efficiency, lower cost for regenerative therapies

2. Pre-Vascularized Constructs

Engineer functional vessel networks before transplantation → improved tissue survival and integration

3. Vascularized Organoids

Apply molecular blueprint to create organoids with integrated vasculature → better disease models and drug testing

7 Conclusions/ Next Steps

- Identified rare high-VEGFR2 mesodermal population that exclusively generates CD34+ VPCs upon VEGF stimulation
- Generated ultra-deep phosphoproteomics dataset revealing novel regulators activated within minutes
- Mapped 11 distinct cell populations and transcriptional programs during VEGF-induced differentiation
- Ongoing: Integrating phosphoproteomics and scMultiome data to identify master regulatory networks
- Future: Apply knowledge to generate pre-vascularized organoids for regenerative medicine

MorphoVess Web-page



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