Vascularised Bioartificial Constructs in Regenerative Medicine: Differentiation of human induced pluripotent stem cells to vascular progenitors

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Amphitheater "G. Lianis ", FORTH, Heraklion, Crete

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University of Ioannina

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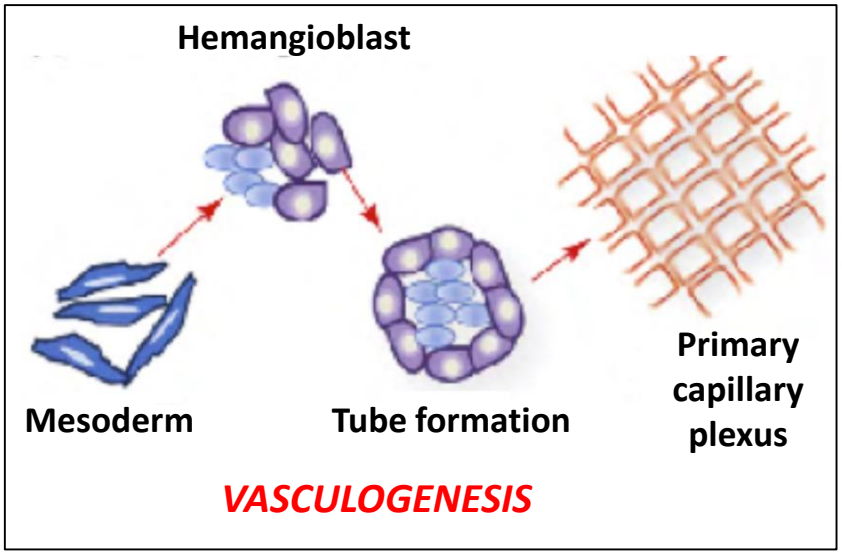

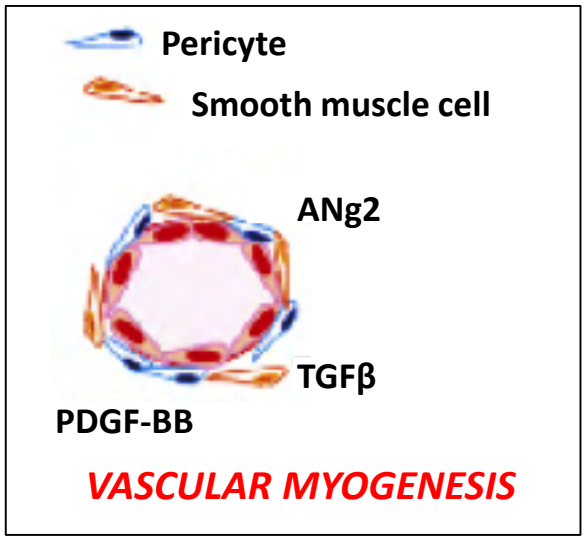

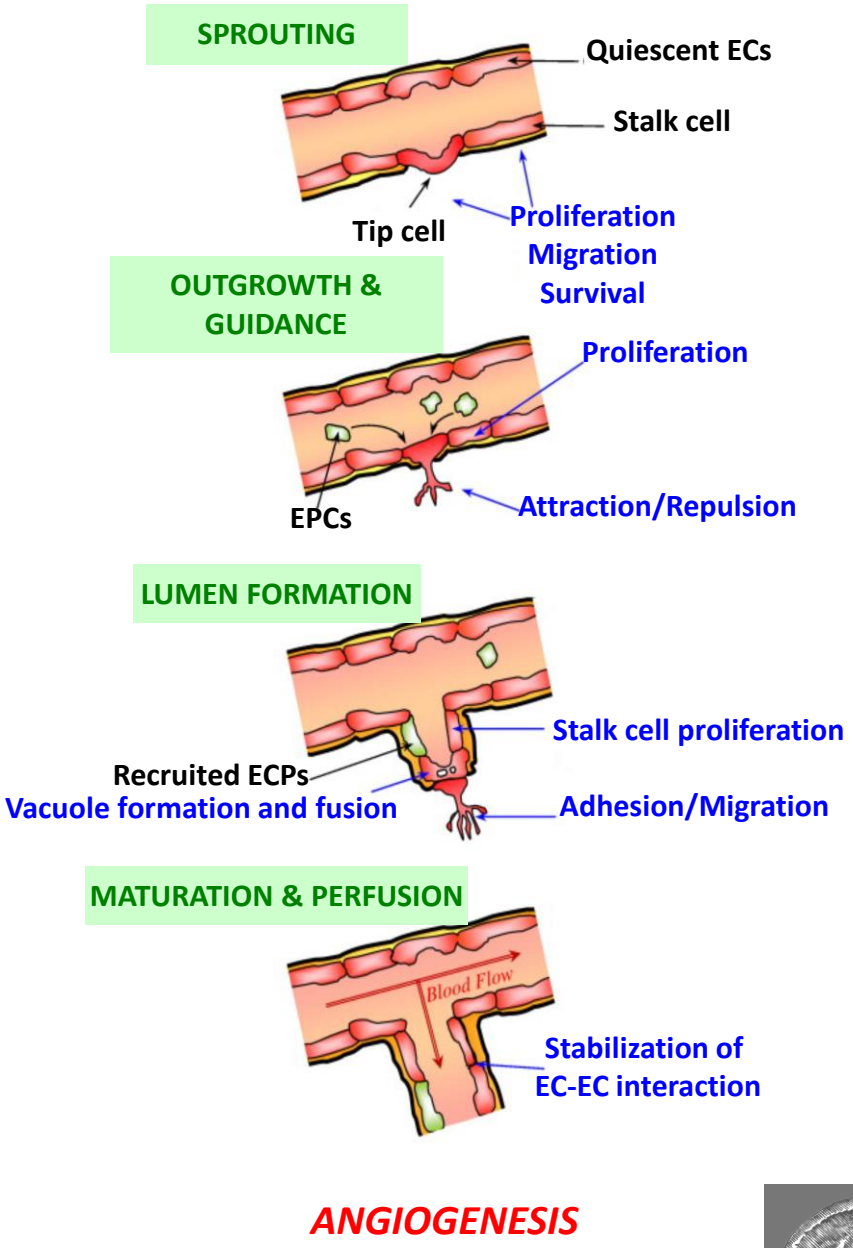

Pardali et al., Trends Cell Biol 2010

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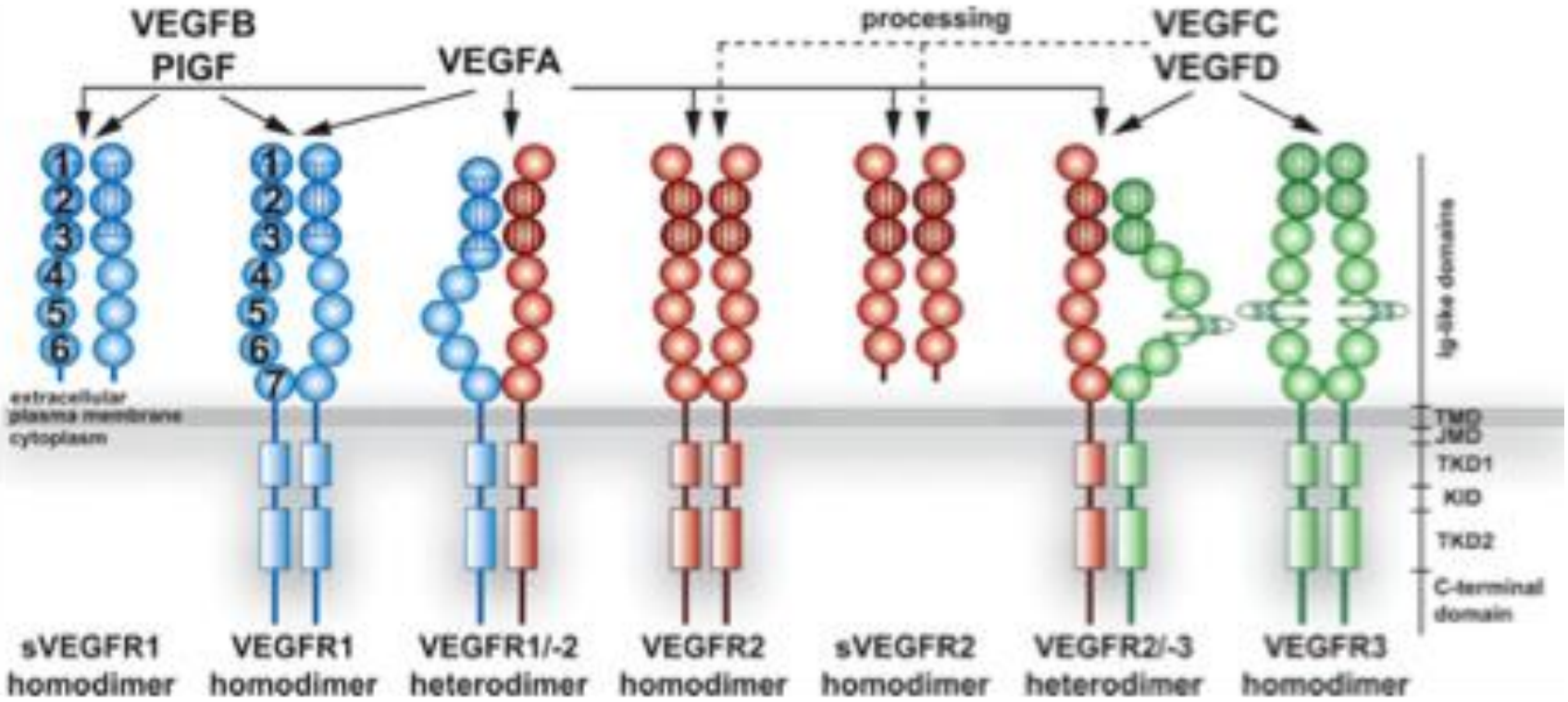
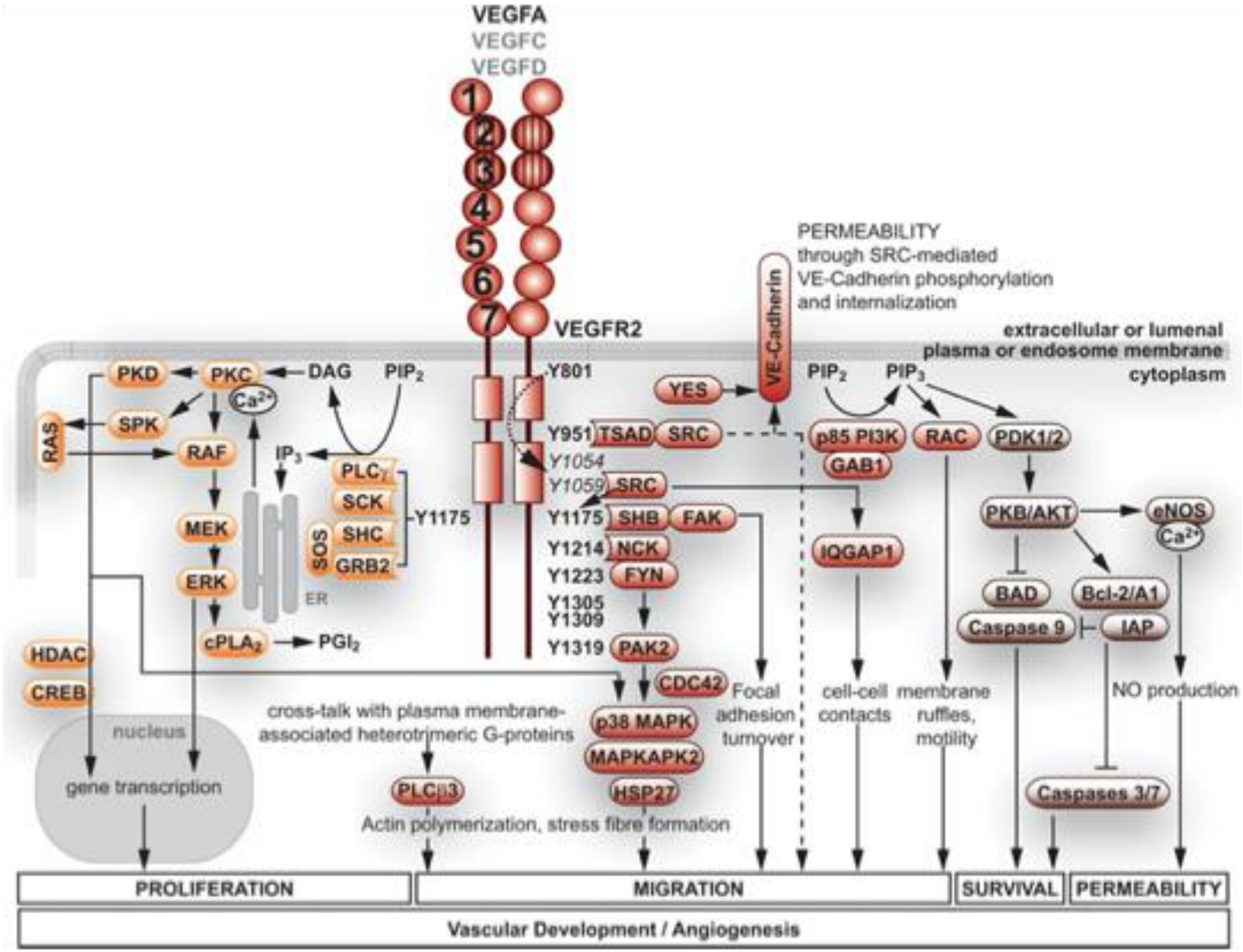

VEGFB
PIGF
VEGFA
processing
VEGFC
VEGFD
1
2
3
4
5
6
7
extracellular
plasma membrane
cytoplasm
Ig-like domains
TMD
JMD
TKD1
KID
TKD2
C-terminal domain
sVEGFR1 homodimer
VEGFR1 homodimer
VEGFR1/-2 heterodimer
VEGFR2 homodimer
sVEGFR2 homodimer
VEGFR2/-3 heterodimer
VEGFR3 homodimer



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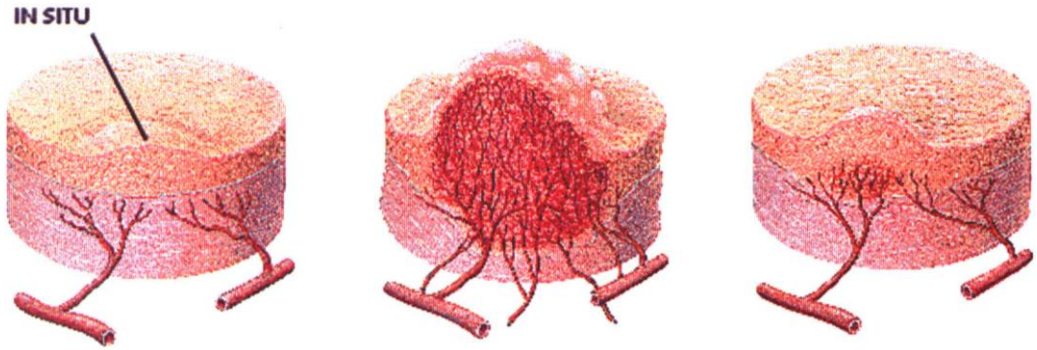
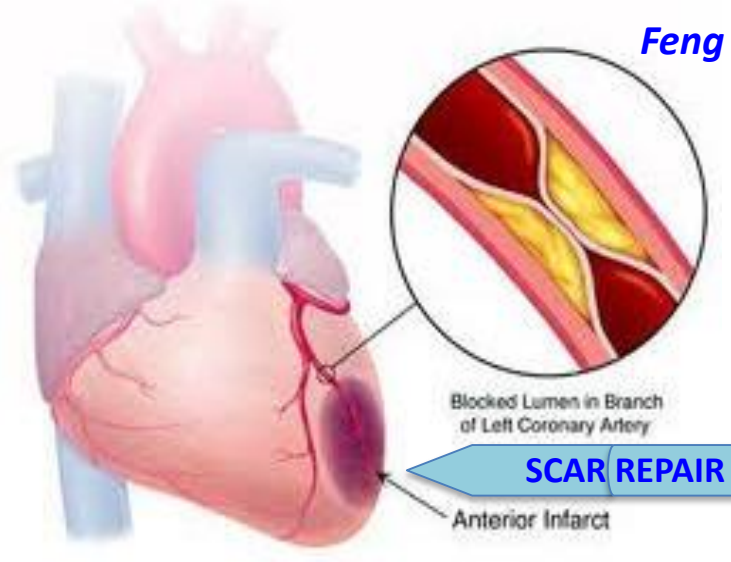
Anti-angiogenesis vs Therapeutic Angiogenesis



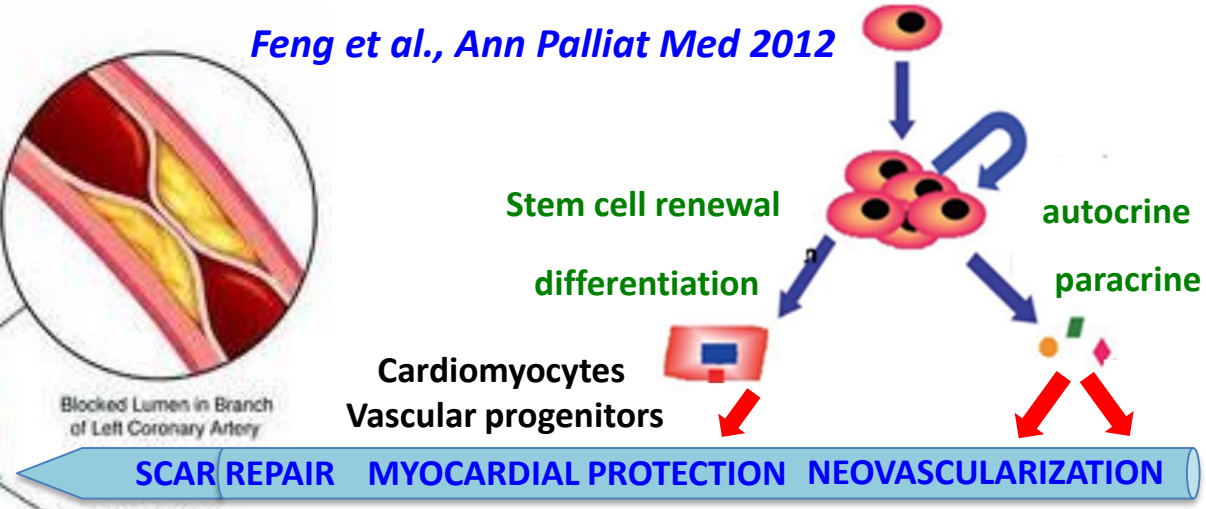
Genetic changes
- Hypoxia

New vessels:
tumor growth

Anti-angiogenic treatment:
tumor regression

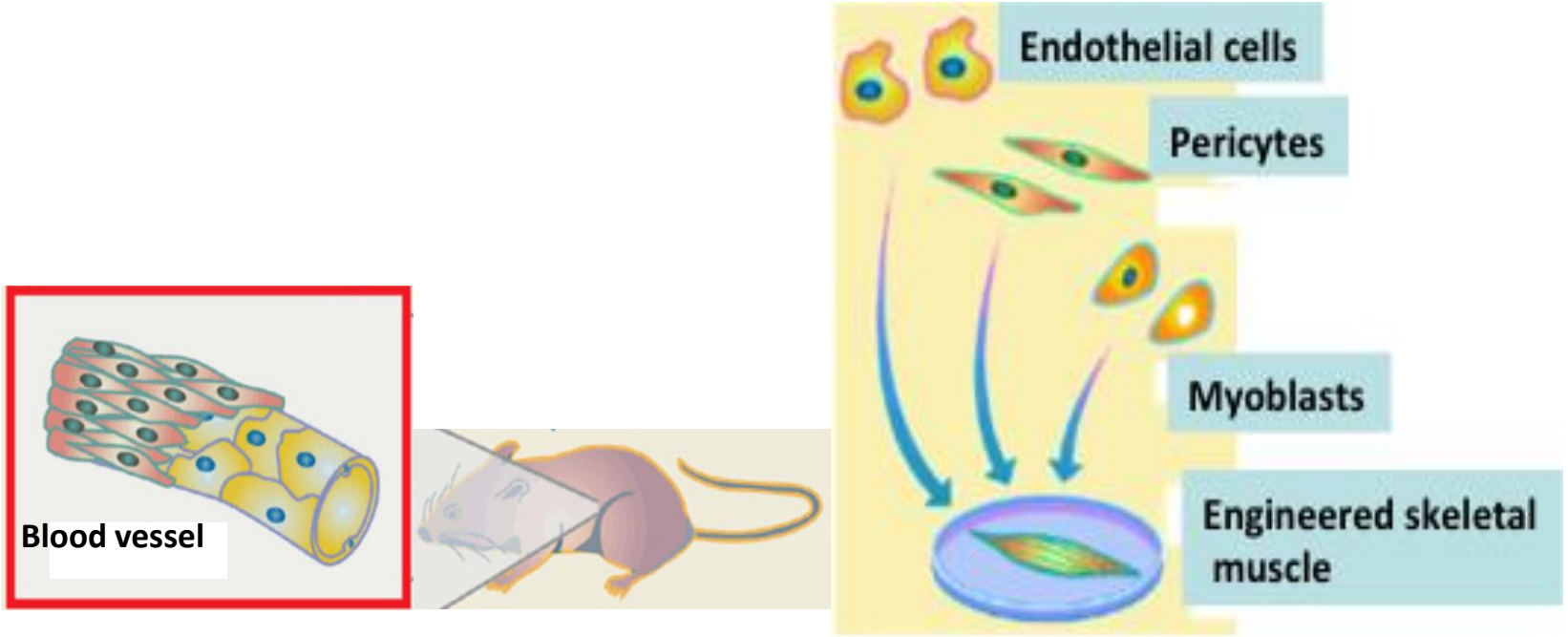


Feng et al., Ann Palliat Med 2012

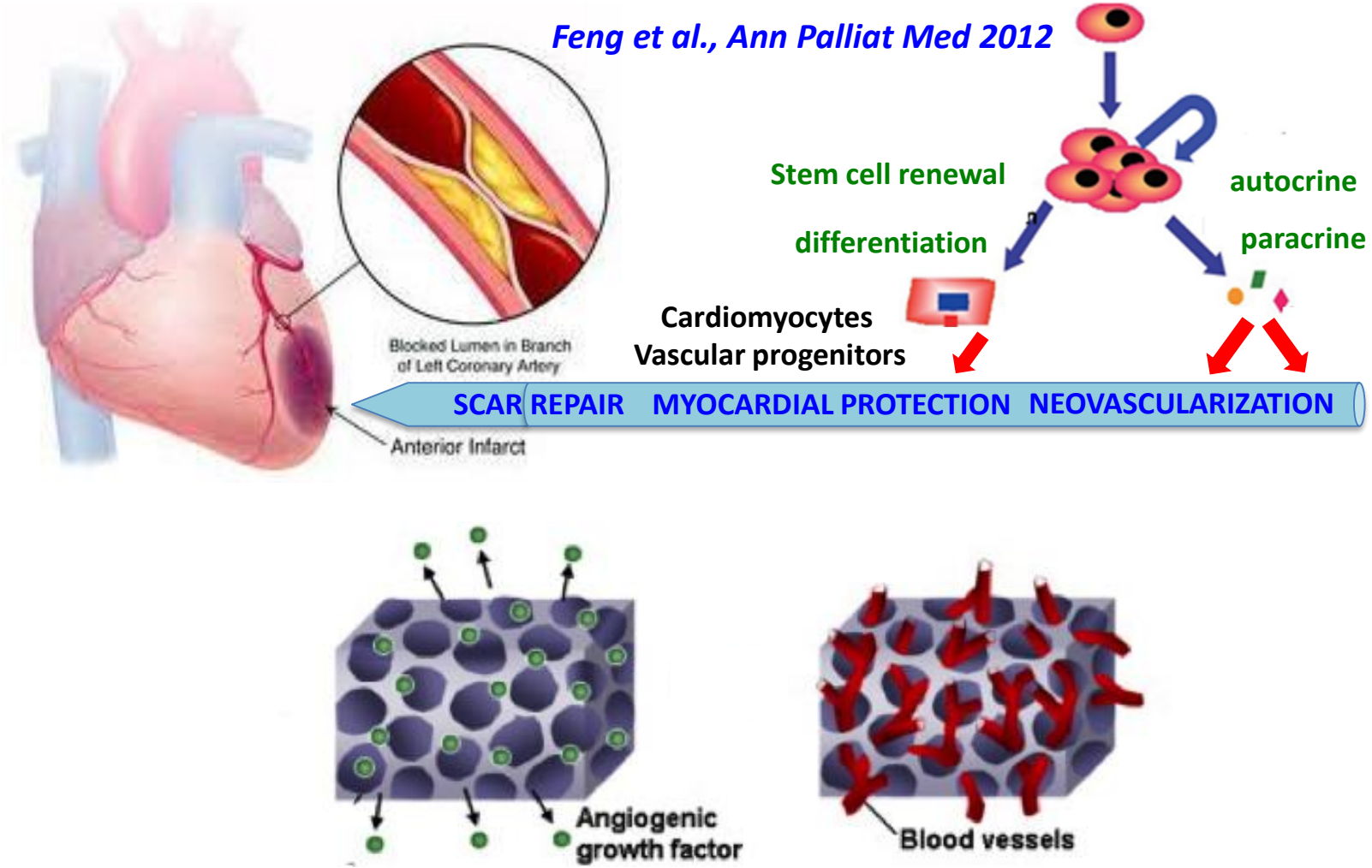


Vascularised tissue engineered constructs for Regenerative Medicine

Jain et al., Nature Biotechnology 2005
Levenberg et al., Nature Biotechnology 2005

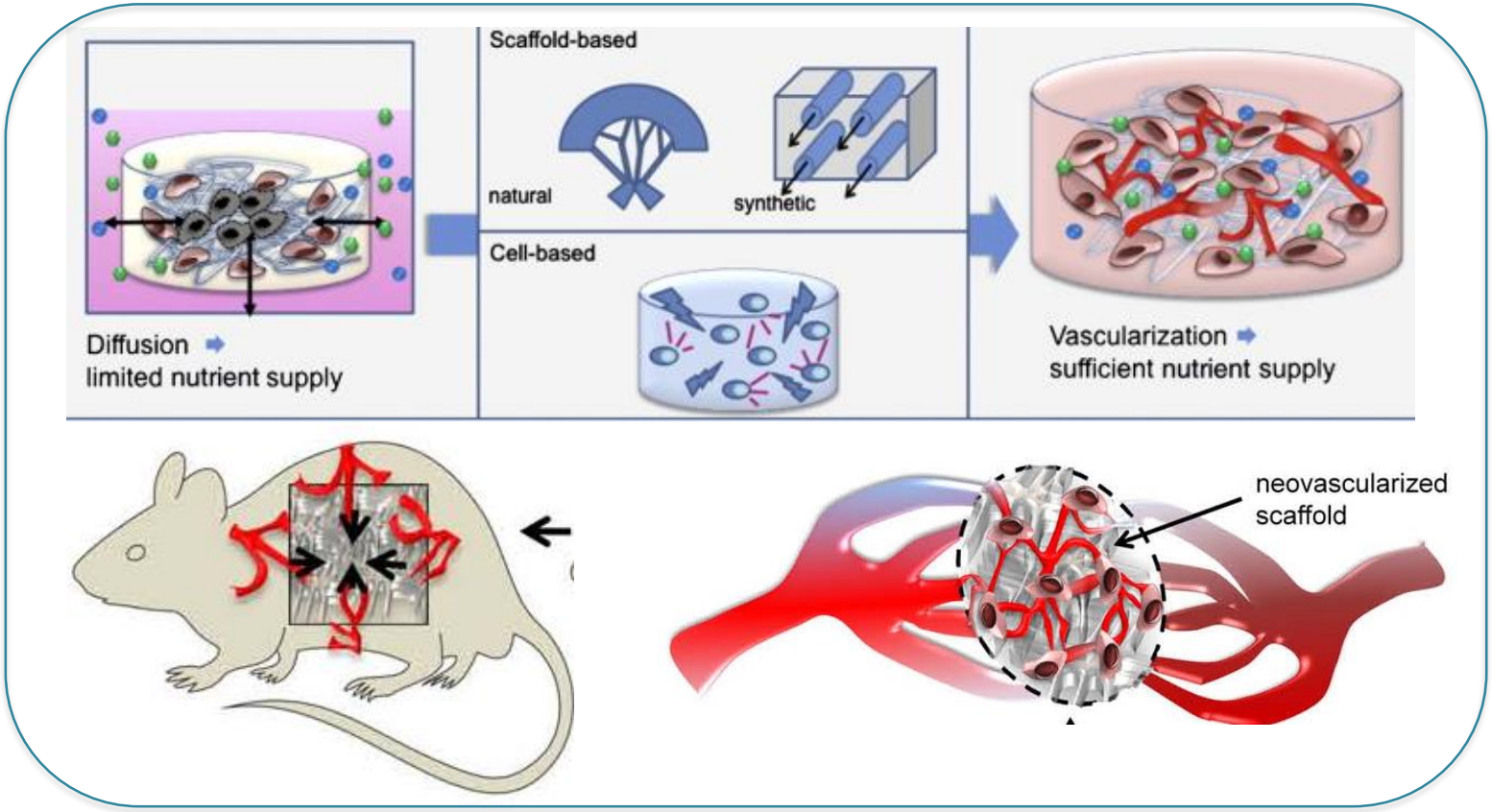


Myocardial Infarct Repair (MIR)



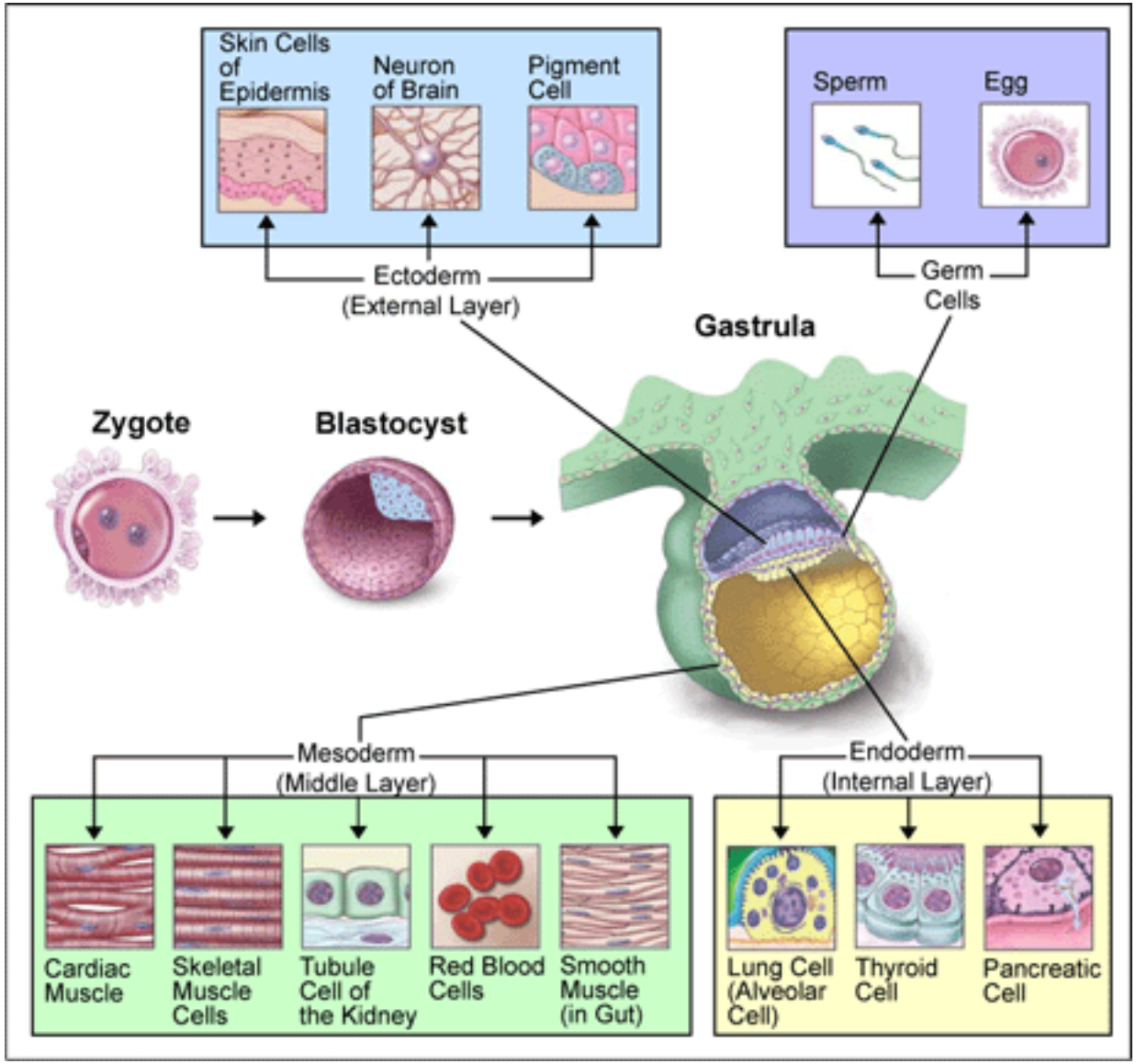
Vascularised Tissue Engineered Constructs in Regenerative Medicine

VEGF signalling in VASCULOGENESIS

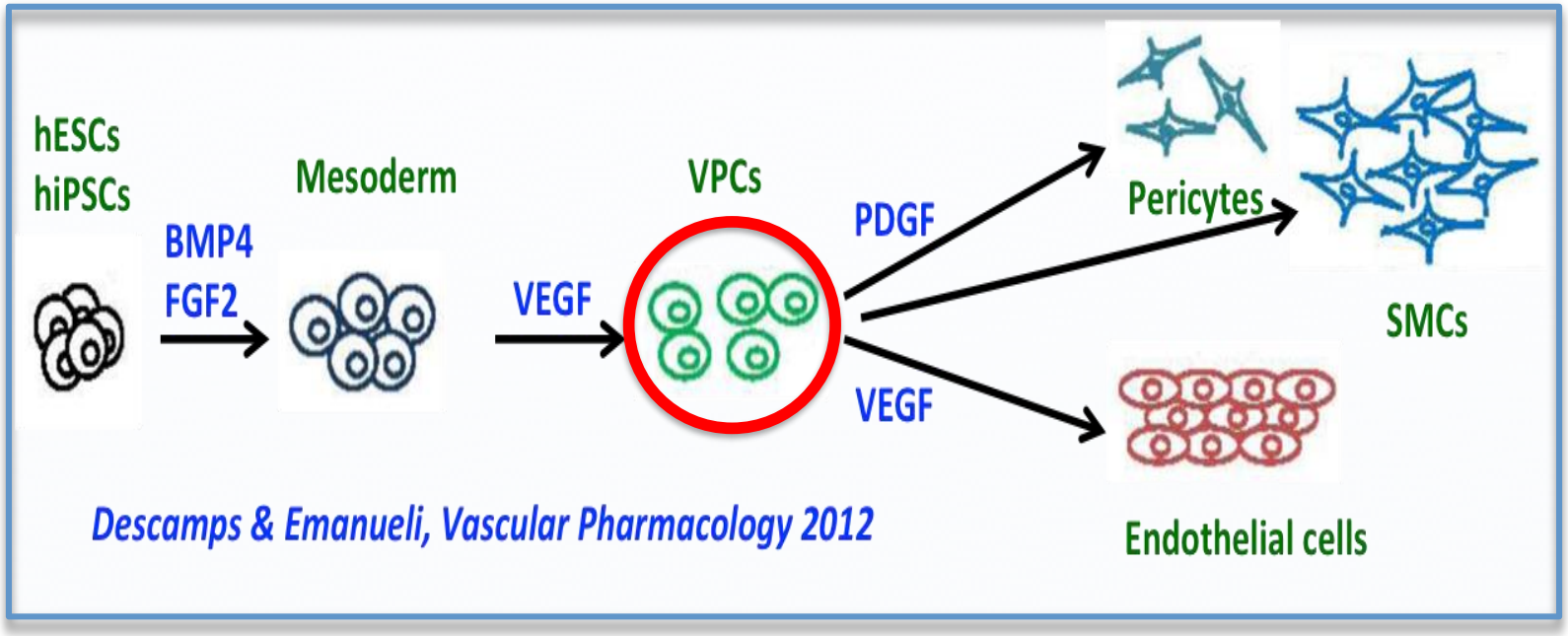


Hurdles: **The signalling cascades of VEGF that differentiate hESCs to *Vascular Progenitor Cells (VPCs)* and subsequently to endothelial cells are not yet characterised.**

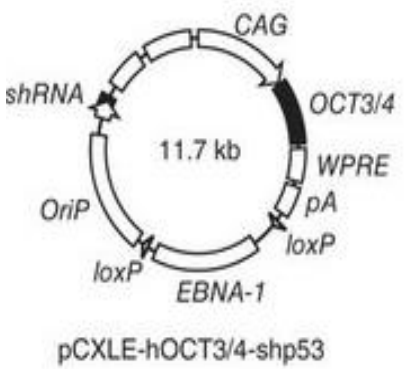
Tissues derived from the three germ layers



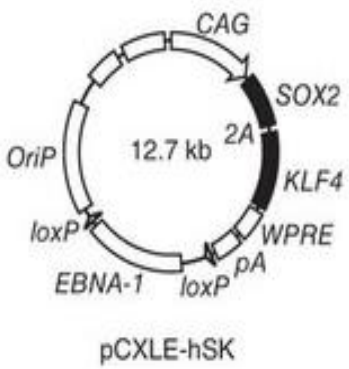
Vascular Progenitor Cells (VPCs)



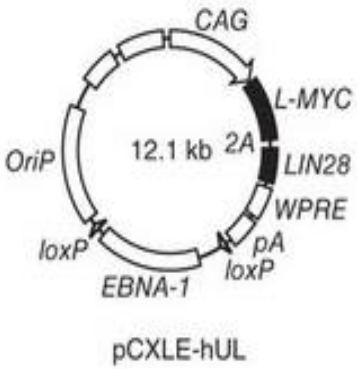
Generation of hiPSCs



pCXLE-hOCT3/4-shp53



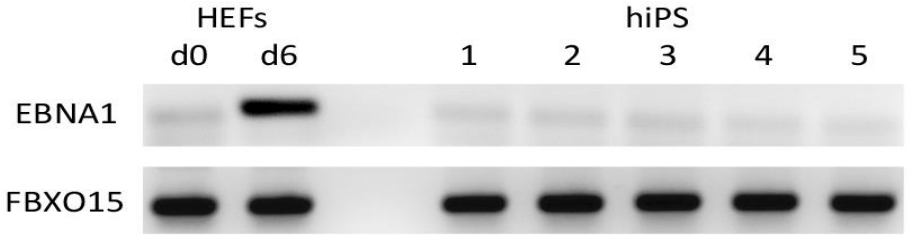
pCXLE-hSK



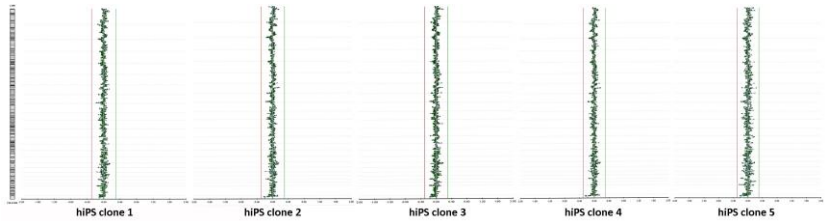
pCXLE-hUL

- **Episomal based approach (Okita et al, 2011)**

Integration free



Genome-wide array based Karyotyping



GeneChip Gene 1.0ST array system
28,869 genes with 764,885 probes
EMBL Genecore facility

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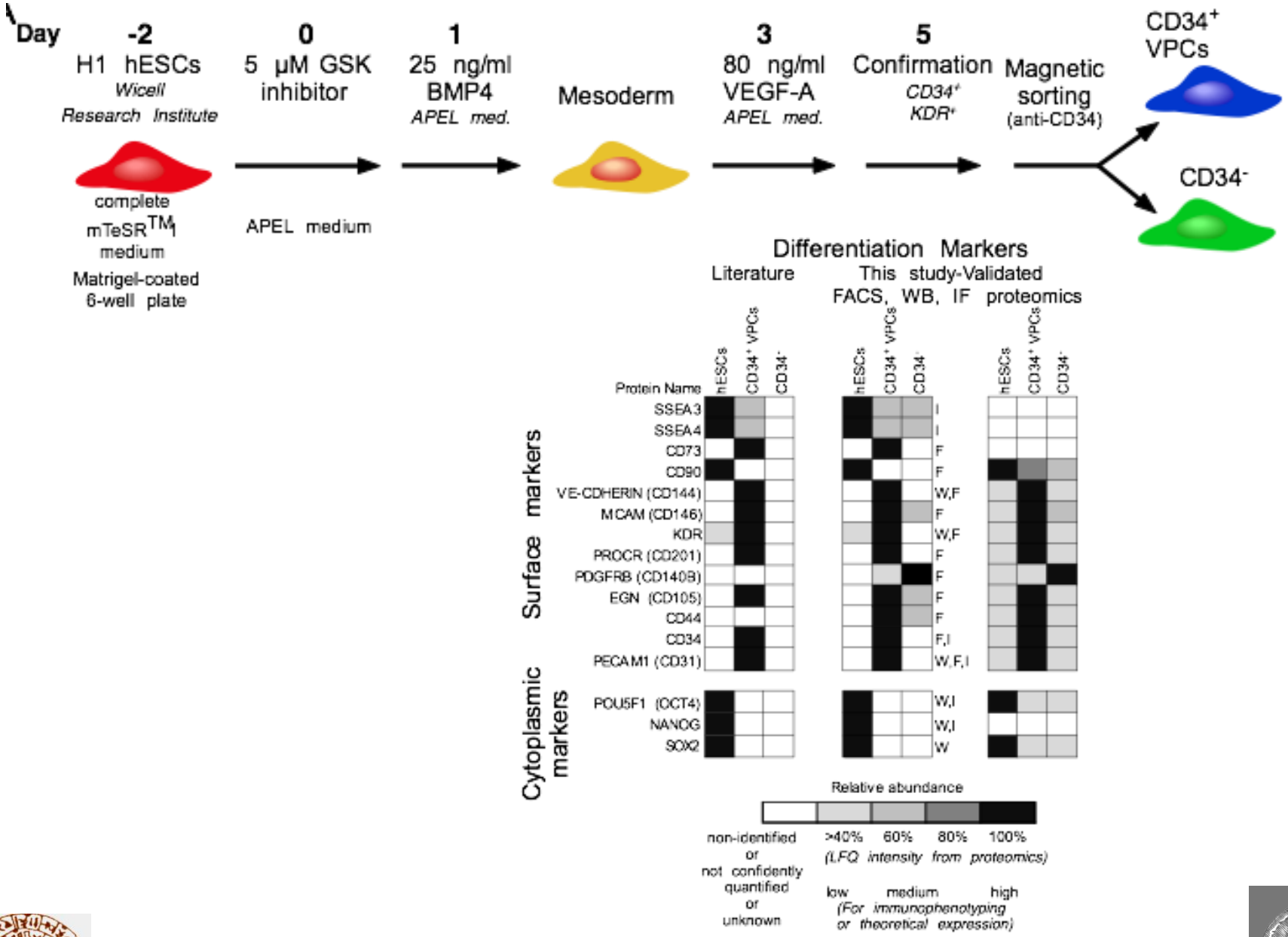

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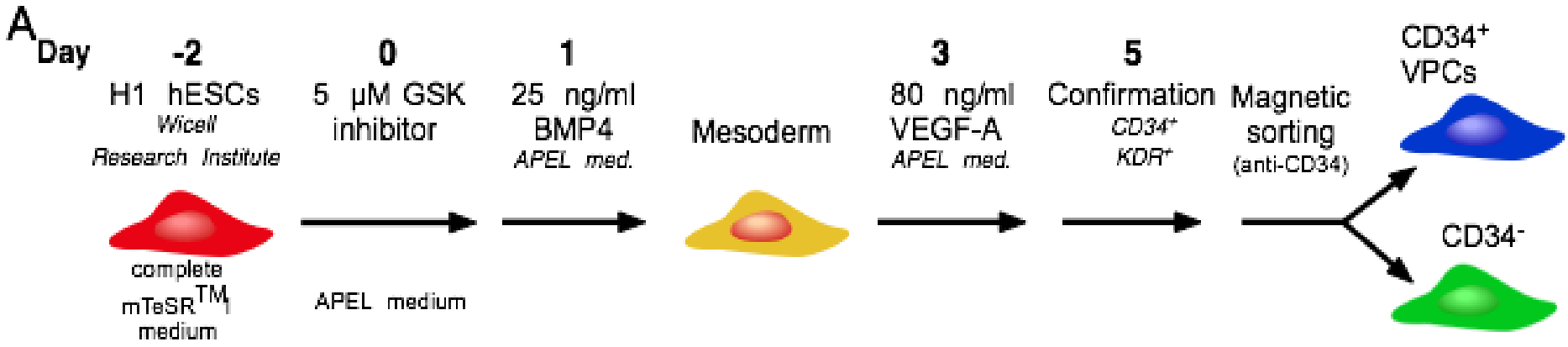
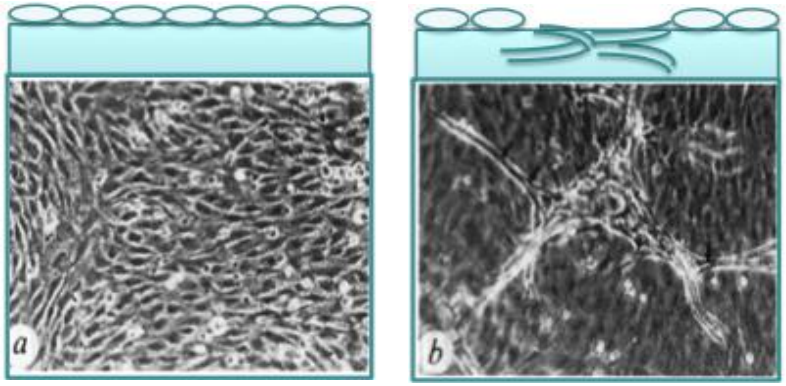
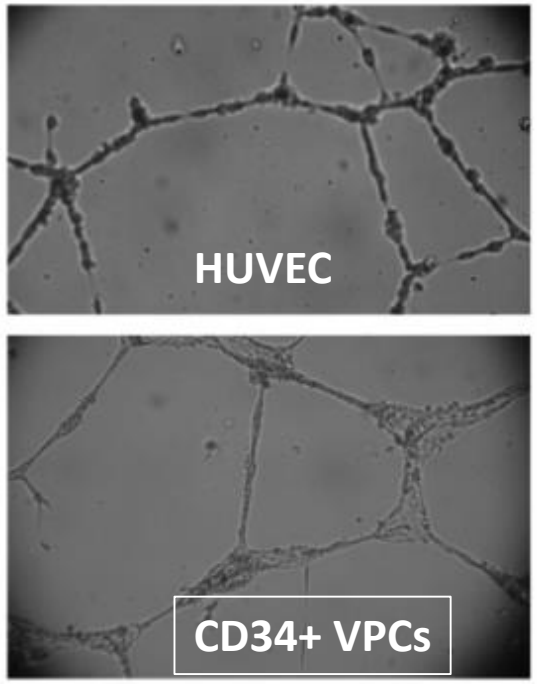
Differentiation of hESCs (H1) to VPCs



In vitro 3D angiogenesis assays

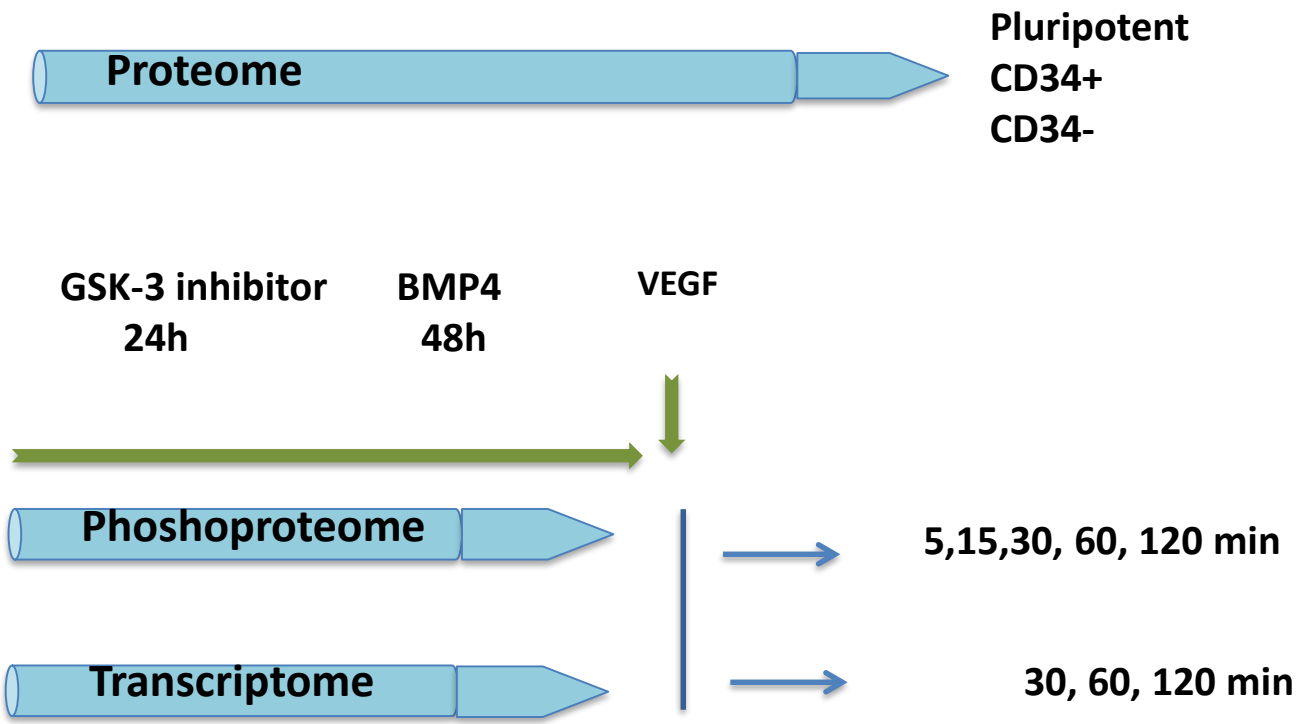


Matrigel Assay

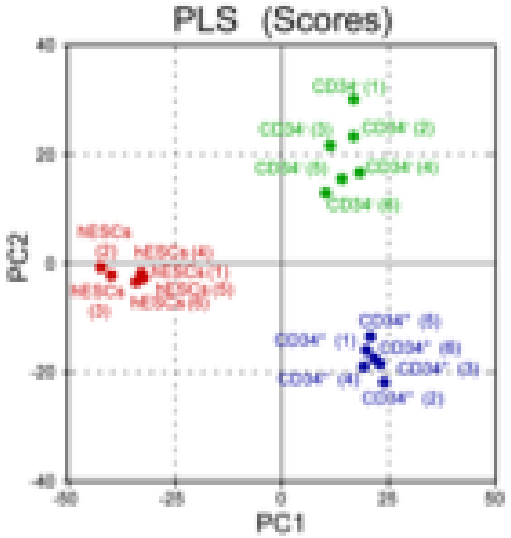


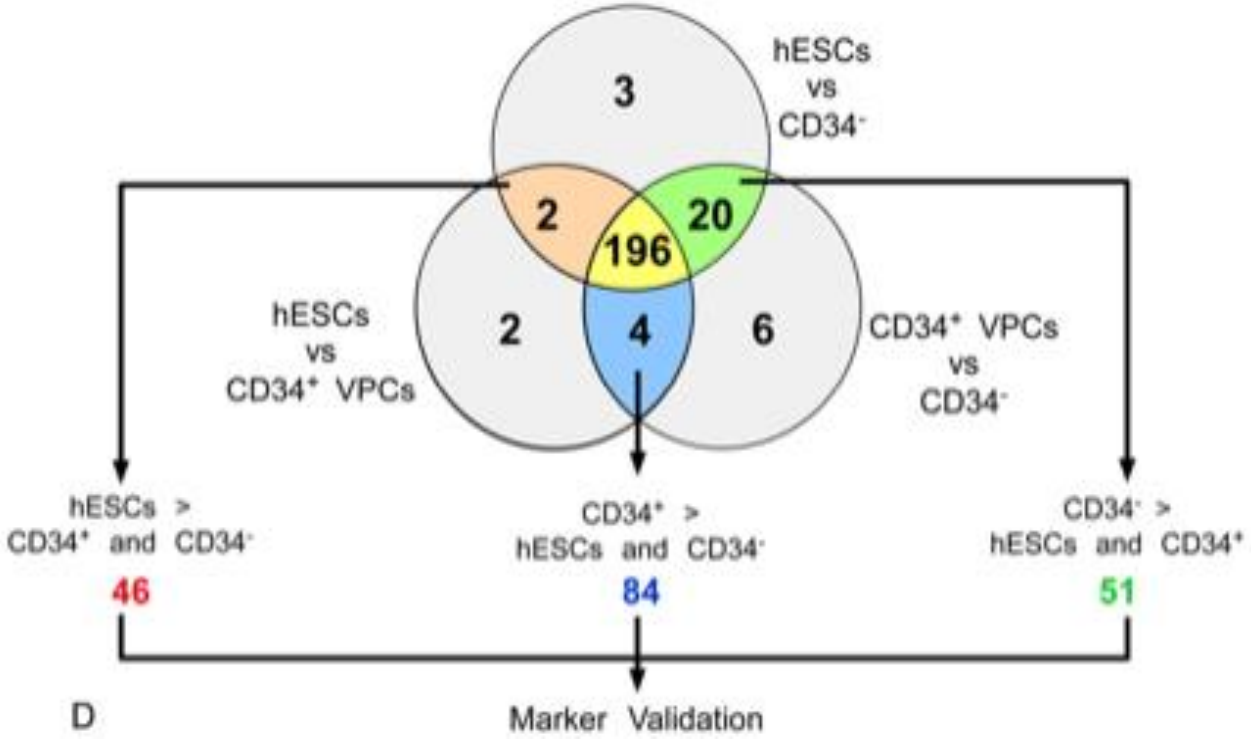
Tsolis, Bagli, Kanaki et al J Prot Res 2016

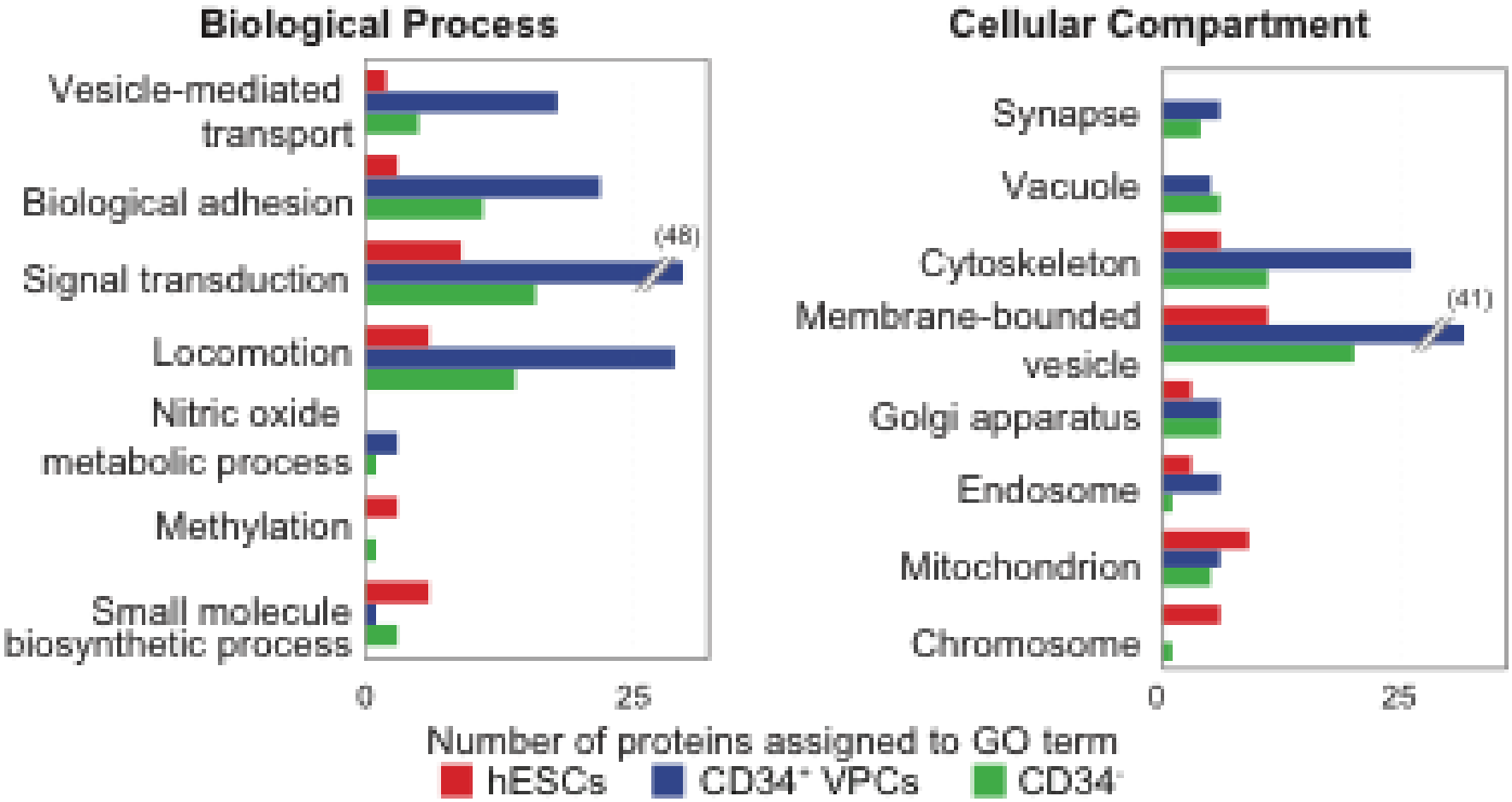
Tan et al., Stem Cells and Development 2013


Proteome
Pluripotent
CD34+
CD34-
GSK-3 inhibitor
24h
BMP4
48h
VEGF
Phoshoproteome
5,15,30, 60, 120 min
Transcriptome
30, 60, 120 min

3 Conditions hiPSCs, CD34^{+}, and CD34^{-}
6 biological repeats
4.491 proteins
Selection of differential protein by the VIP method
Statistcs using the nonparametric Kruskal-Wallis test



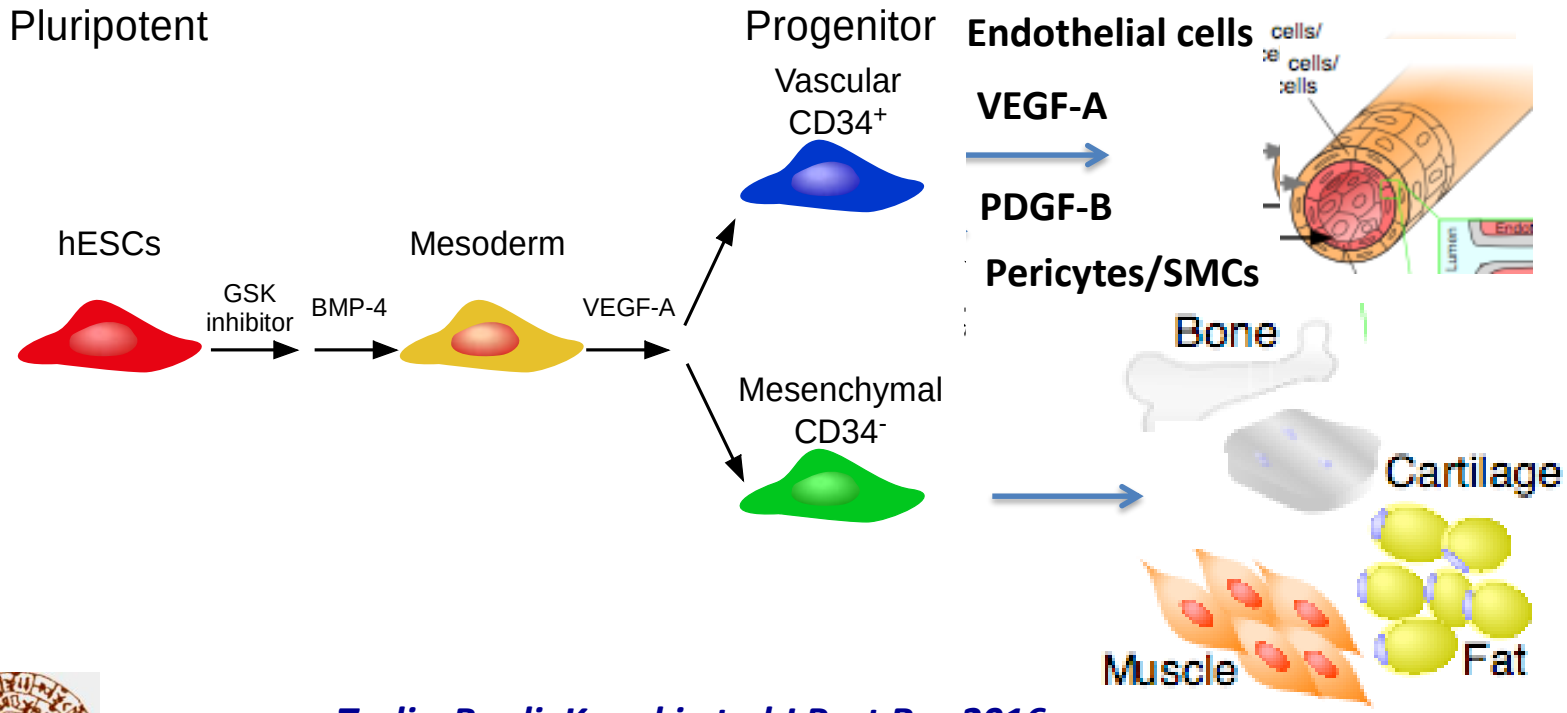



Biological Process
Vesicle-mediated transport
Biological adhesion
Signal transduction
(48)
Locomotion
Nitric oxide metabolic process
Methylation
Small molecule biosynthetic process
0
25
Cellular Compartment
Synapse
Vacuole
Cytoskeleton
Membrane-bounded vesicle
(41)
Golgi apparatus
Endosome
Mitochondrion
Chromosome
0
25
Number of proteins assigned to GO term
hESCs
CD34+ VPCs
CD34-

The isolated CD34+ cells are vascular progenitors

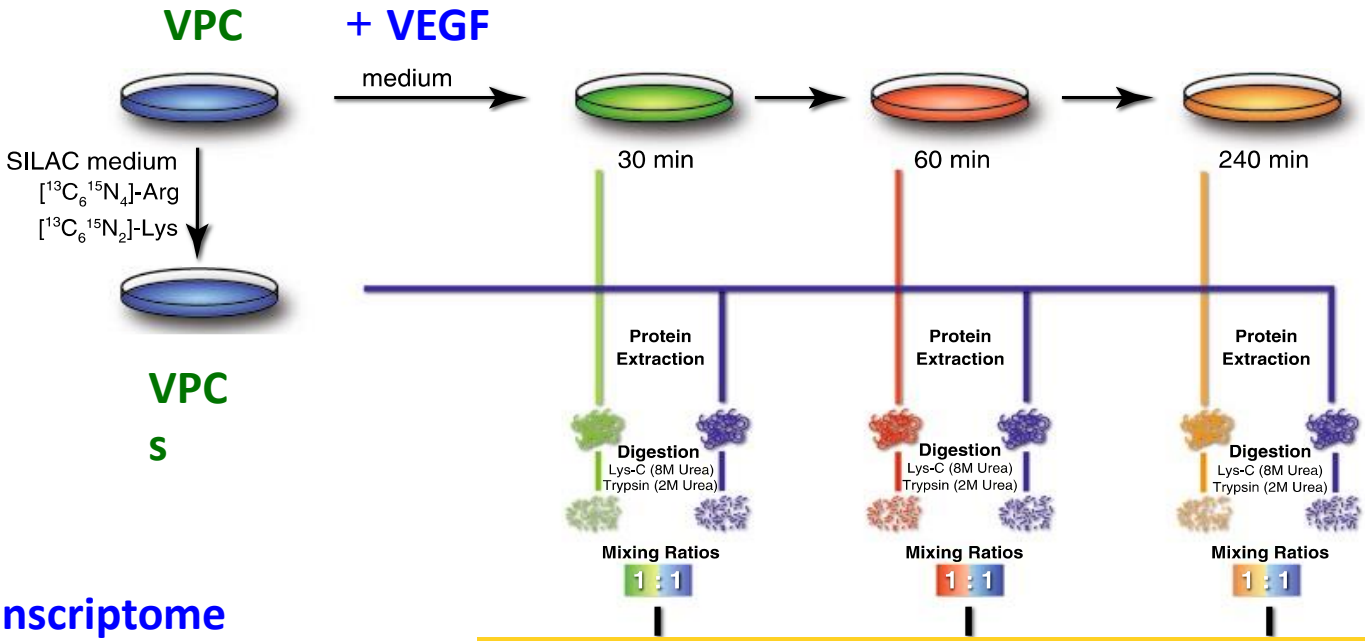
KDR, VE- Cadherin (CDH5), CD31, PROCR (CD201), ERG, CD73, CD44, CD105, CD146 (MCAM), and CD140B (PDGFRB)

From the **86** oversynthesised proteins **33** were related to angiogenesis/vaculogenesis and vascular homeostasis and 7 to mural cell differentiation



Tsolis, Bagli, Kanaki et al J Prot Res 2016

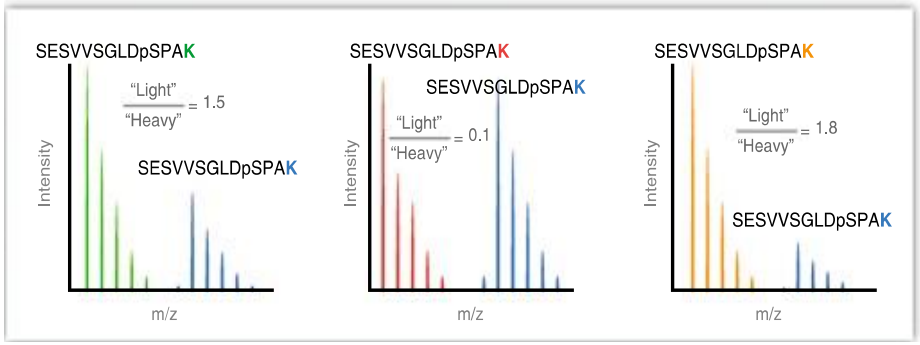
Identification of the VEGF pathways responsible for differentiation of VPCs
The VEGF phosphoproteome



VEGF transcriptome
Bioinformatics
Experimental validation

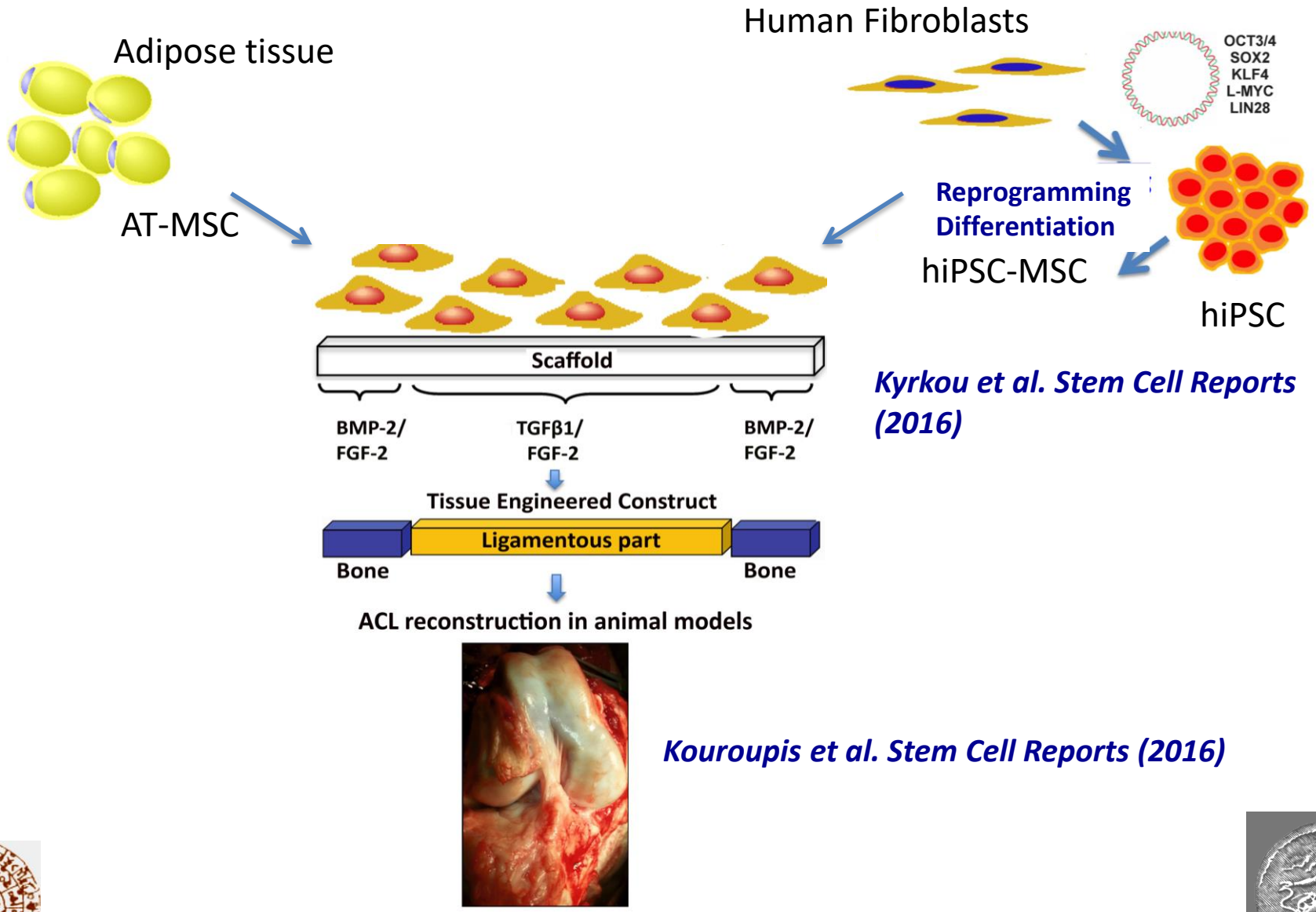
PROFI & Gene expression Facilities - IMBB

Titanium dioxide phospho-enrichment/ LC-MS/MS



Muñoz and Heck, Methods in Molecular Biology 2011

Bioartificial anterior cruciate ligament from Mesenchymal stem cells



Kyrkou et al. Stem Cell Reports (2016)

Kouroupis et al. Stem Cell Reports (2016)

Acknowledgments

CAROL MURPHY (Co-Group Leader)

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MSc STUDENT

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Topalis Pantelis

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Kouroupis Dimitris

Karali Evi

Kanaki Katerina

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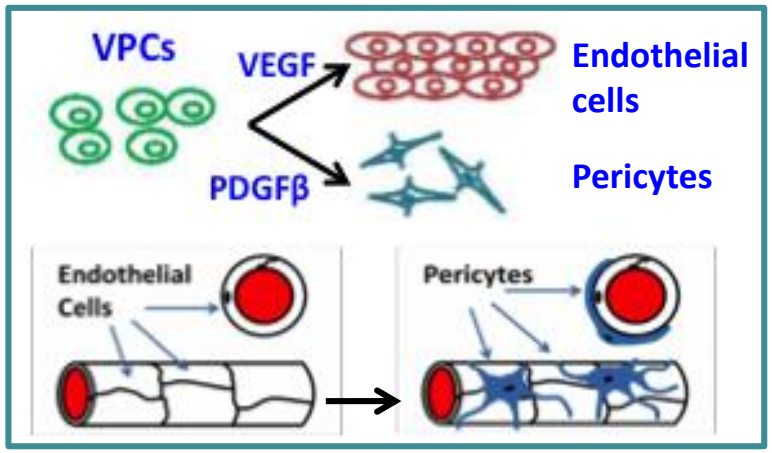
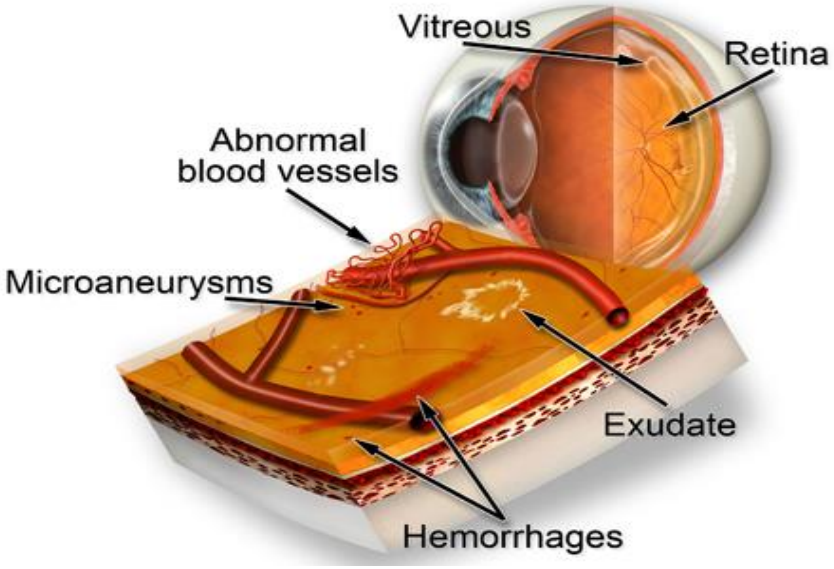
Proliferative Diabetic Retinopathy





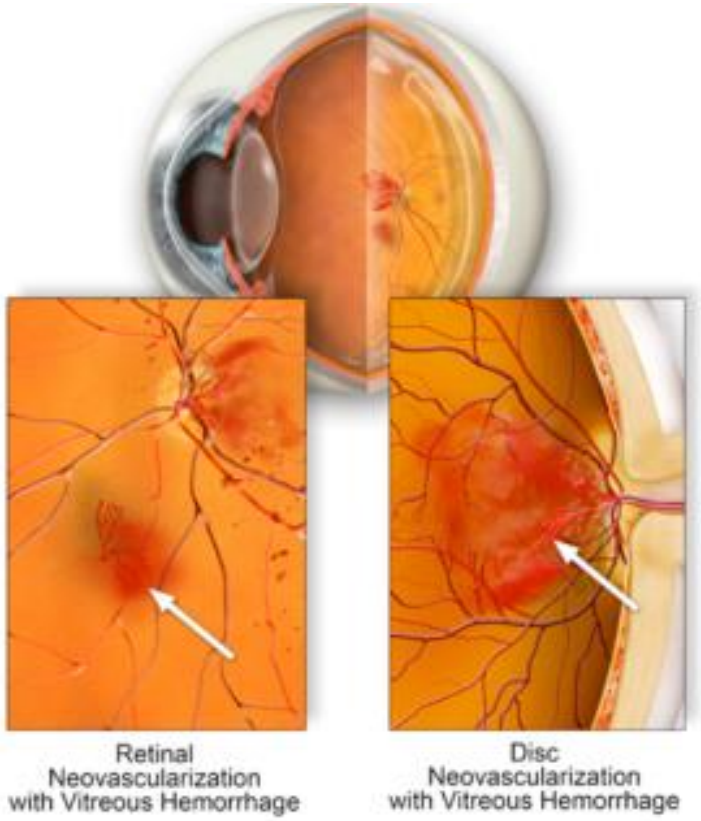
CELL TYPES:
hESCs, hiPSCs, hST-MSCs

ANIMAL MODELS:
- **Diabetic ischemic retinopathy model**
- **Advanced diabetic retinopathy model**
- **Oxygen induced retinopathy model**

COLLABORATORS:
Prof. Antonia Joussen,
Charite Universitatsmedizin Berlin,
Animal facilities and expertise

Prof. Andrew Hunter, Lincoln University, UK
REVAMMAD Marie Curie ITN
Validation of the effect, modeling

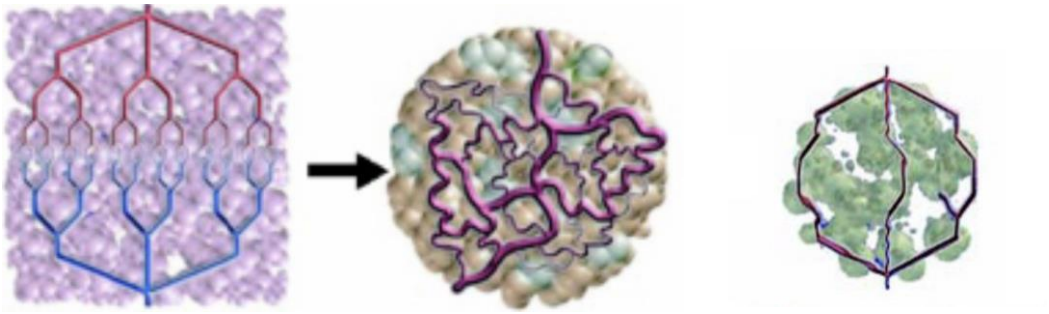
Proliferative Diabetic Retinopathy and tumor angiogenesis

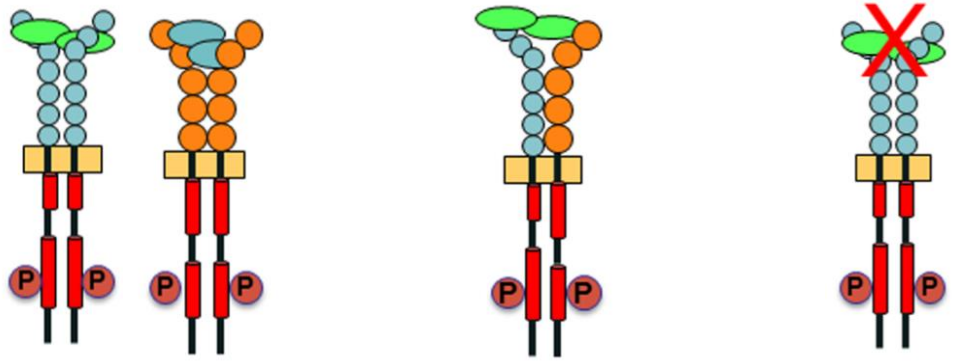


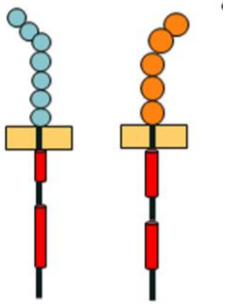
Normal VEGF
Normal vessels

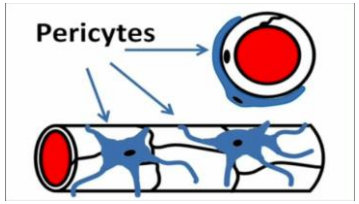
Excessive VEGF
Abnormal vessels

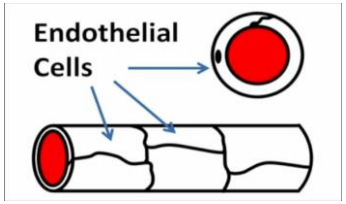
Anti-VEGF agents
No vessels



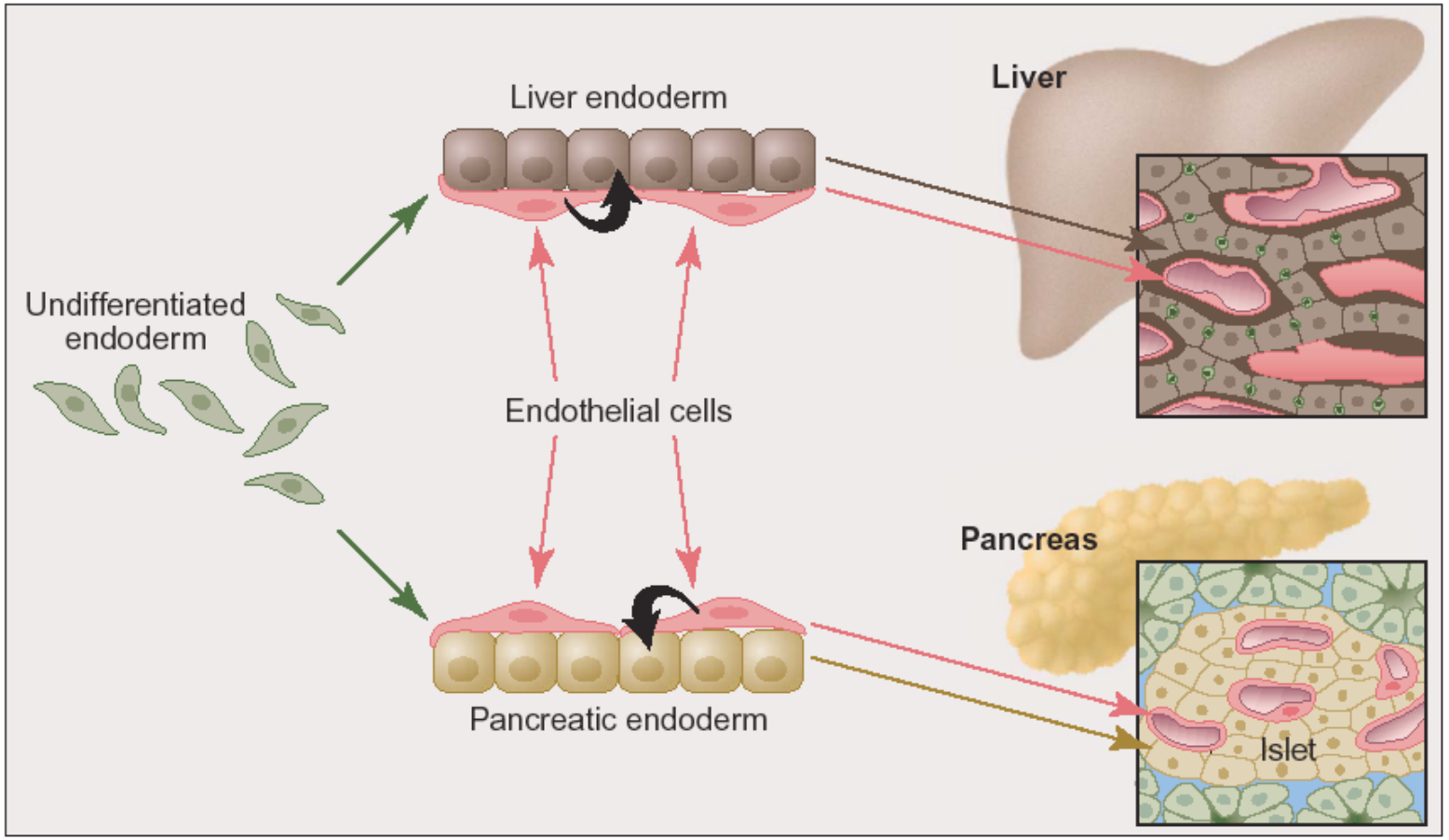








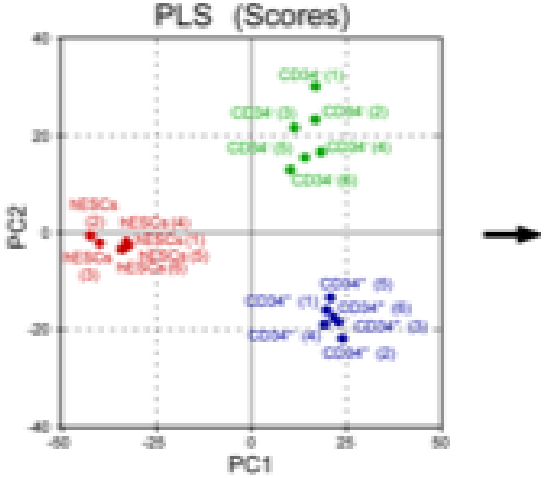
Endothelial cells are crucial players in organ development

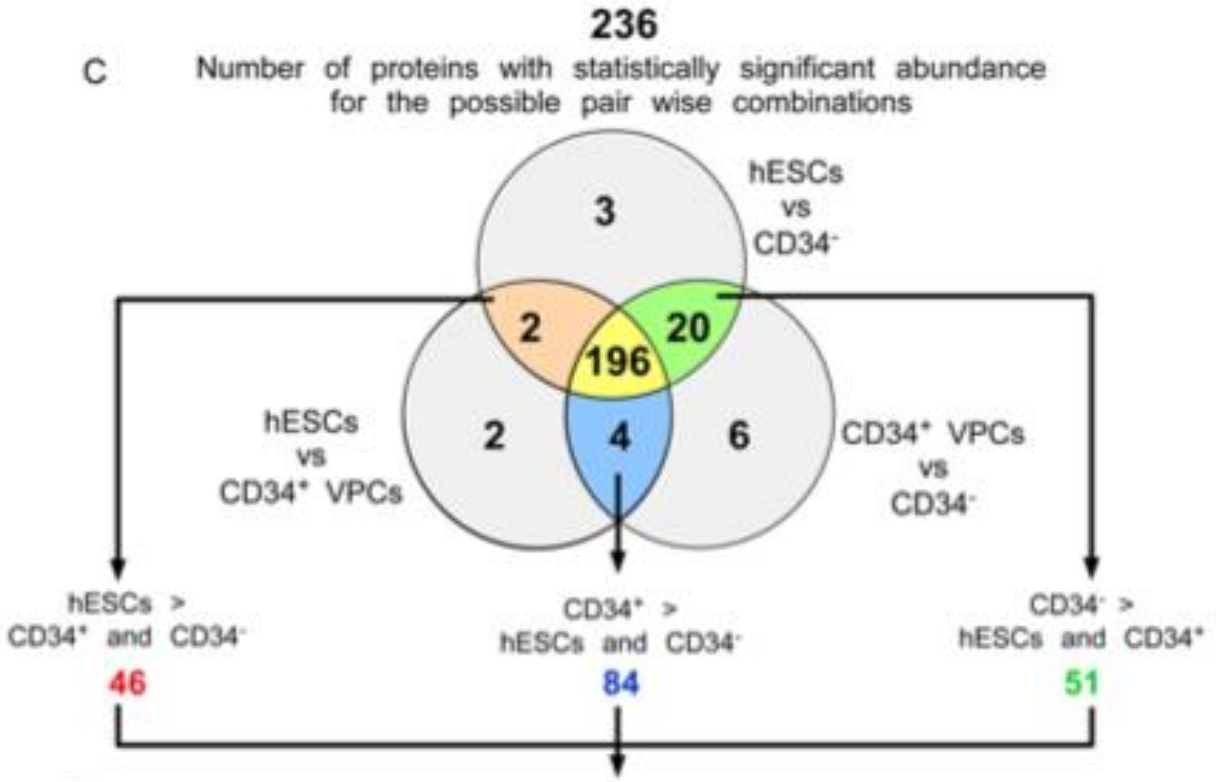


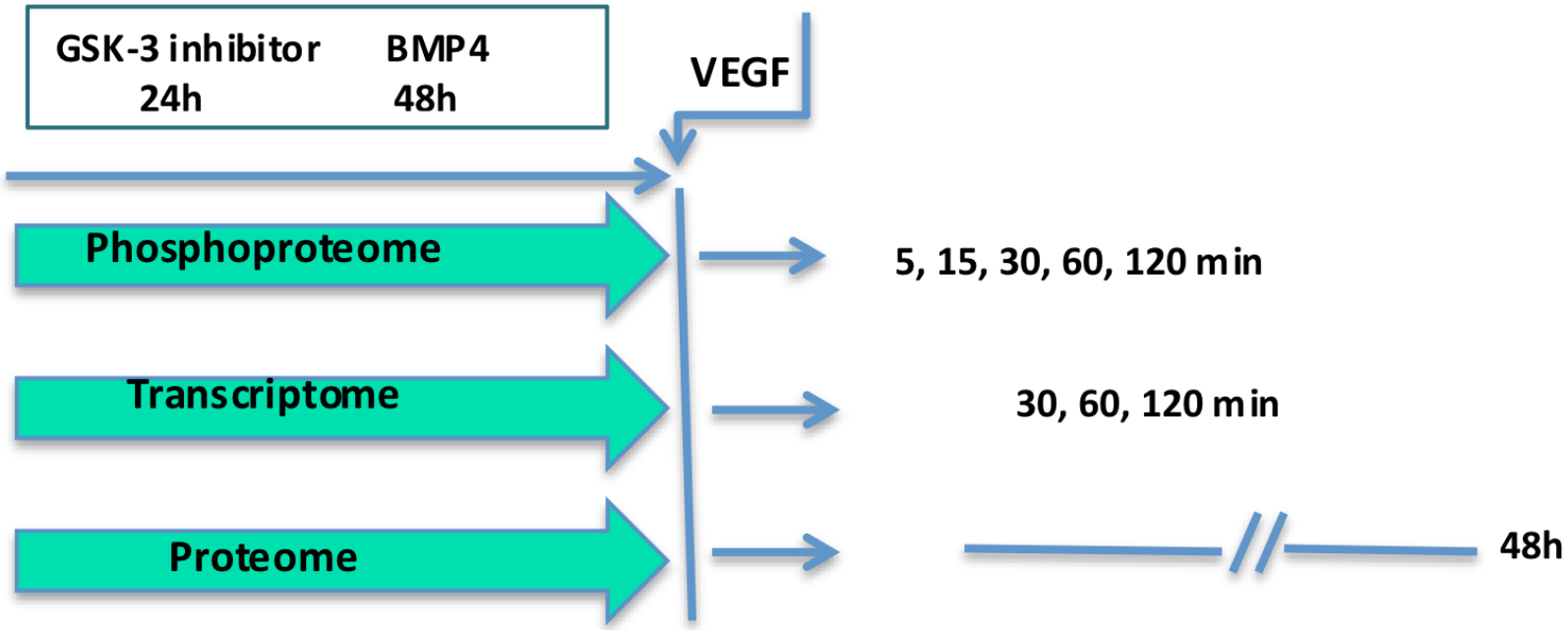
Bahary & Zon, Science 2001
Matsumoto et al., Science 2001
Lammert et al., Science 2001

Proteomics of the hESCs, the CD34+ (VPCs) and the CD34- cells

3 conditions (hESCs, CD34⁺, CD34⁻)
6 biological repeats for each group
4,491 proteins experimentally identified & quantified (min 2 peptides)






GSK-3 inhibitor
24h
BMP4
48h
VEGF
Phosphoproteome
5, 15, 30, 60, 120 min
Transcriptome
30, 60, 120 min
Proteome
48h

Team Members

CAROL MURPHY *(Co-Group Leader)*

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Kouroupis Dimitrios

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Acknowledgments

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Stellas Dimitrios

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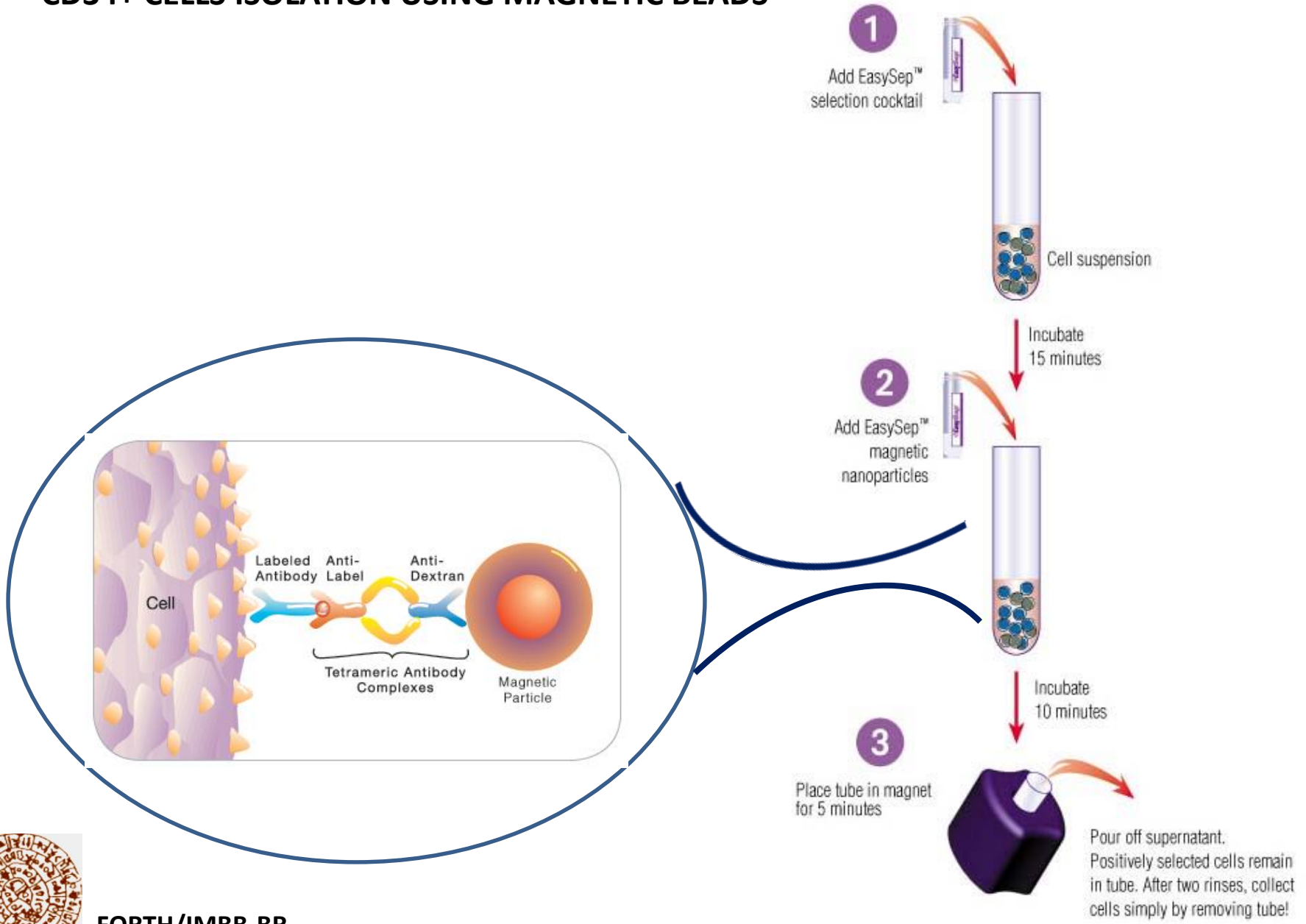
Karali et al. Molecular Cell (in Press)

17.04.2014 → On line

22.05.2014 → In print

Karali Evdoxia

CD34+ CELLS ISOLATION USING MAGNETIC BEADS



GENE NAME	ABBREV.	Fold ↑or↓
0,5hr		
FBJ Murine Osteosarcoma Viral Oncogene	FOS	2.93381693
Early Growth Response 1	EGR1	3.191280212
FBJ Murine Osteosarcoma Viral Oncogene	FOSB	1.280460829
Early Growth Response 1	EGR2	1.247055944
		-1.0929
WT1 antisense RNA		1.00023
1hr		
Early Growth Response 1	EGR1	3.498219969
FBJ Murine Osteosarcoma Viral Oncogene	FOS	2.686333666
FBJ Murine Osteosarcoma Viral Oncogene	FOSB	1.964147637
Early Growth Response 2	EGR2	2.0098222
Early Growth Response 4	EGR4	1.631138464
Nuclear Receptor Subfamily 4, Group A, Member	NR4A3	1.252448781
Early Growth Response 3	EGR3	1.231843601
(Ets Variant 2)	ETV2	1.499910859
Nuclear Receptor Subfamily 4, Group A, Member	NR4A1	1.370193165
Lectin, galactoside-binding, soluble, 8	LGALS8	1.025375687
Jun Proto-Oncogene	JUN	1.074031089
Nicotinamide N-methyltransferase	NNMT	-1.226535577
(Ets Variant 2)	ETV2	1.237468366
Phospholipid scramblase family, member 5	PLSCR5	-1.370018206
Dual Specificity Phosphatase 2	DUSP2	1.069818097
Methyltransferase like 7A	METTL7A	-1.086856357
Lumican	LUM	-1.400412082
2hr		
(Ets Variant 2)	ETV2	3.300306553
ETV2 (Ets Variant 2)	ETV2	3.286132621
Homo sapiens immediate early response 3 (IER3)	IER3	1.321028058
Homo sapiens immediate early response 3 (IER3)	IER3	1.210983167
Homo sapiens nuclear receptor subfamily 4 group	NR4A3	1.015714774
DUSP2 (Dual Specificity Phosphatase 2)	DUSP2	1.731873738
Homo sapiens nuclear receptor subfamily 4,	NR4A1	1.289972478
Homo sapiens small nucleolar RNA, H/ACA box	SNORA11D	-1.096551039
Homo sapiens RNA, 5.8S ribosomal 5 (RNA5-8S5)	RNA5-8S5	-1.22018063

Enriched Pathways

Differential Proteins Were Subjected in Pathway Enrichment Analysis against Wikipathways Database Using WebGestalt Web Tool

	Status	Gene ID	Adjusted p-value
ADHESION - MECHANOTRANSDUCTION			
Integrin-mediated cell adhesion	Activated in $CD34^+$ cells.	VAV3 SRBS1 PAXI TLN1 ITA6 VASP CRK	7.71×10^{-07}
Regulation of Actin Cytoskeleton	Activated in $CD34^+$ cells	PAXI GIT1 GELS CRK RADI MOES PDFRB	0,0001
Focal Adhesion	Activated in $CD34^+$ cells	CO6A2 PAXI LAMC1 TLN1 VEGFR2 ITA6 LAMA1 VASP CRK LAMB2 COIA2 ITA9 PDGFRB	$4,56 \times 10^{-10}$
SIGNAL TRANSDUCTION			
FAS pathway and Stress induction of HSP regulation	Stress induction of HSPs is induced in $CD34^+$ cells.	LMNA HSPB1 SPTAN1	0,008
Insulin Signaling	Increased receptor recycling &	SORBS1 MYO1C KIF5B CRK EHD2	0,0062
AGE-RAGE pathway	Altered between the two cell types.	ALPL MSN NOS3	0.0177
MAPK signaling pathway	Altered between the two cell types.	CRK PPP5C HSPB1 PDGFRB	0.0226
Hepatocyte Growth Factor Receptor	Activated in $CD34^+$ cells	PXN CRK	0.0276
REGULATORY AND METABOLIC PATHWAYS			
Glycolysis and Gluconeogenesis	Increased in $CD34^+$ cells.	PGK1 ENO3 HK2 HK1	0.0008
Urea cycle and metabolism of amino		OAT CKB GATM	0,0008
Angiogenesis	Activated in $CD34^+$ cells, mainly towards migration phenotype.	KDR NOS3	0,0177
mRNA regulation of DNA Damage		CCNB1 PML CDK6	0,0177
Glucuronidation		UGP2 HK1	0,0177
Prostaglandin Synthesis and Regulation	Blocked in $CD34^+$ cells, suggesting reduced synthesis of prostaglandins	ANXA3 ANXA2	0,0225
One Carbon Metabolism	Less active in $CD34^+$ cells, suggesting less DNA methylation compared to the hESCs.	MTR DNMT3B	0,0276

Αγγειωμένες Βιοτεχνητές Κατασκευές στην Αναγεννητική Ιατρική: Διαφοροποίηση Αγγειακών Προγονικών Βλαστικών Κυττάρων από Πολυδύναμα

11η Επιστημονική Διημερίδα ΙΤΕ,
13-14 Οκτωβρίου 2017
Αμφιθέατρο «Γ. Λιάνης», ΙΤΕ, Ηράκλειο Κρήτης

ΘΕΟΔΩΡΟΣ ΦΩΤΣΗΣ
Τμήμα Βιοϊατρικών Ερευνών
Ινστιτούτο Μοριακής Βιολογίας και Βιοτεχνολογίας
Ίδρυμα Τεχνολογίας και Ερευνας (ΙΤΕ/IMBB-BE)
&
Εργαστήριο Βιολογικής Χημείας, Ιατρικό Τμήμα
Πανεπιστήμιο Ιωαννίνων

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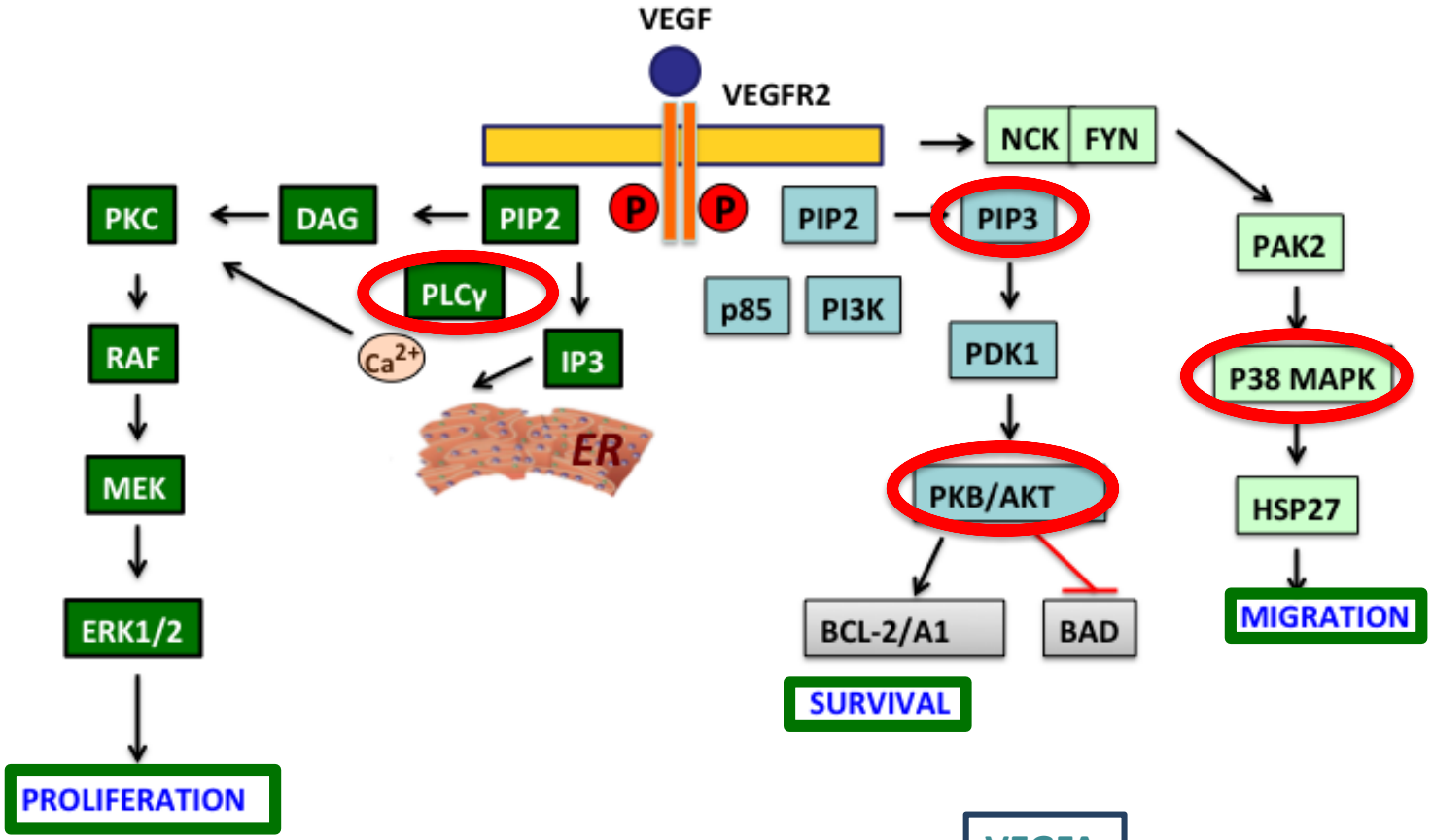
VEGF/VEGFR2 Signal Transduction and Endothelial Cell Responses



VEGFA
VEGFB
VEGFC
VEGFD
PIGF

VEGFR1
VEGFR2
VEGFR3

Modified from
Koch et al., Biochem J 2011

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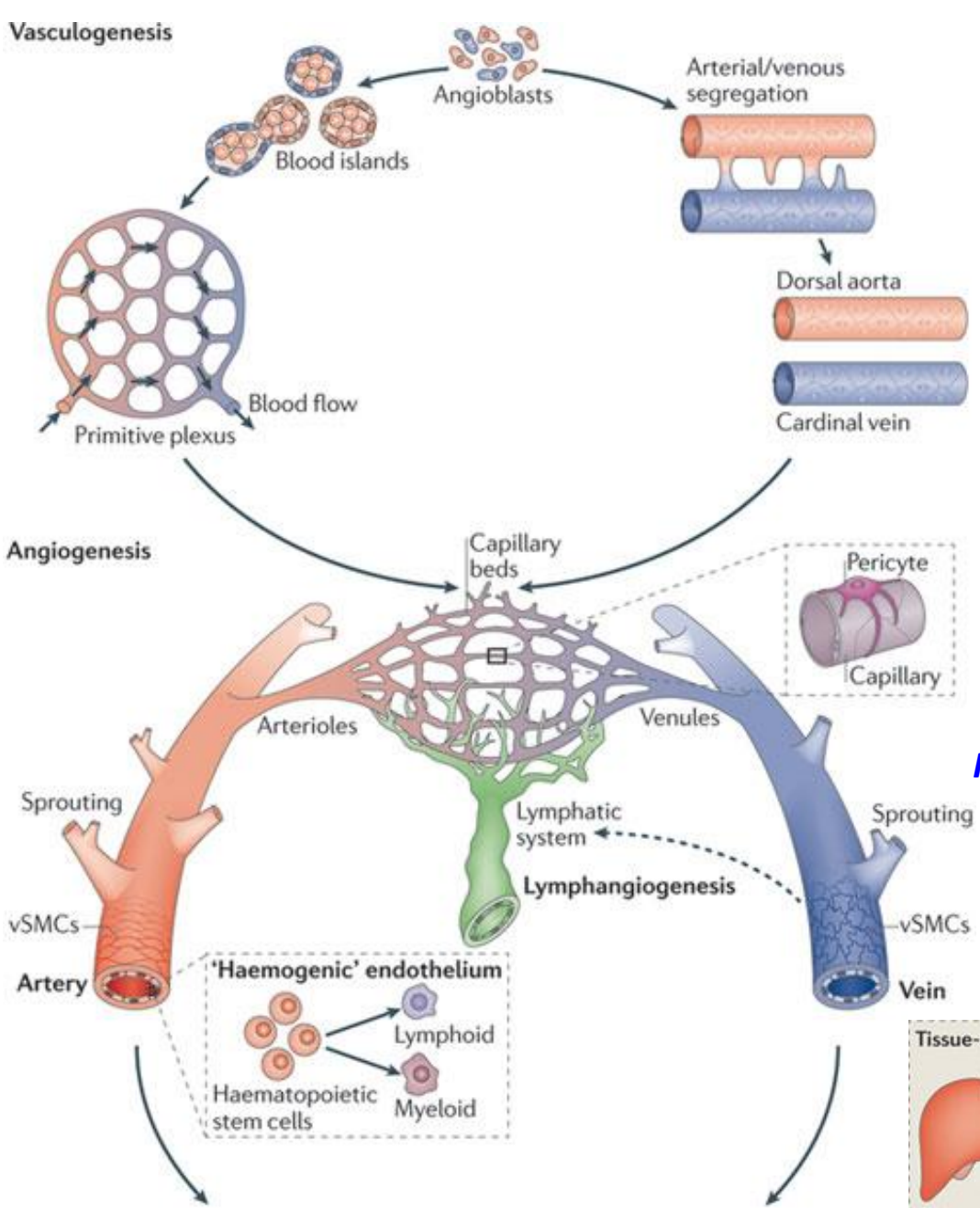
Development of vasculature from progenitor endothelial cells



Herbert & Stainer Nat Rev Mol Cell Biol 2011