

In Vitro and *In Vivo* investigation into the properties of VEGFB

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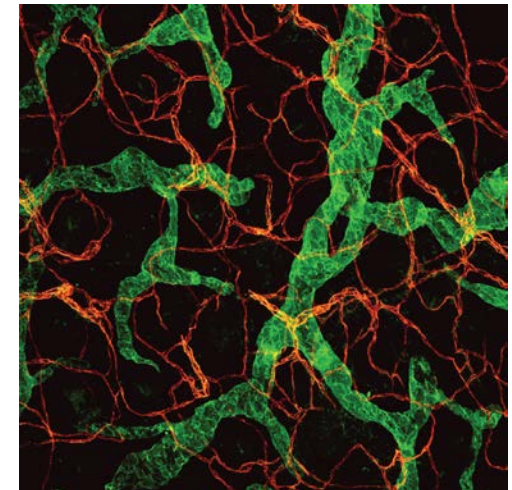
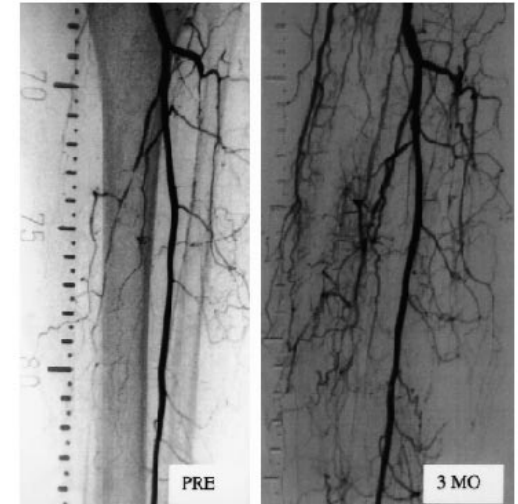
Me



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Angiogenesis

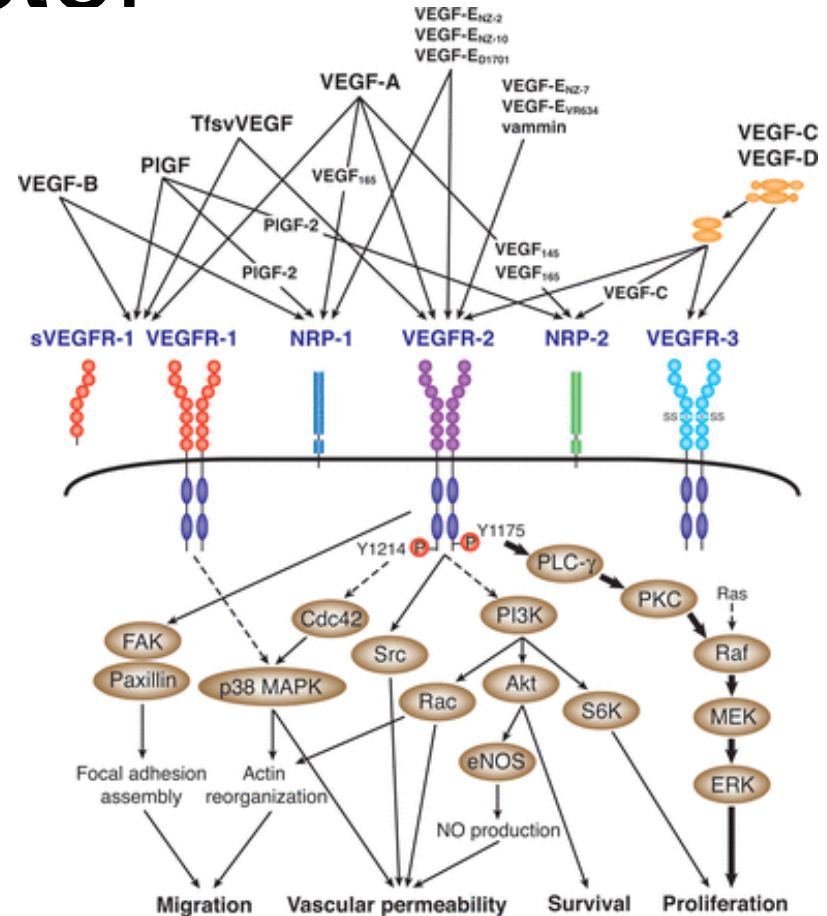
- Angiogenesis- development of blood vessels
 - Begins in embryonic stages; continues in maintenance role throughout adulthood
- Transitional relevance in:
 - Cancer
 - Heart Disease
 - Obesity/Diabetes



Alitalo. *Nature Medicine*.

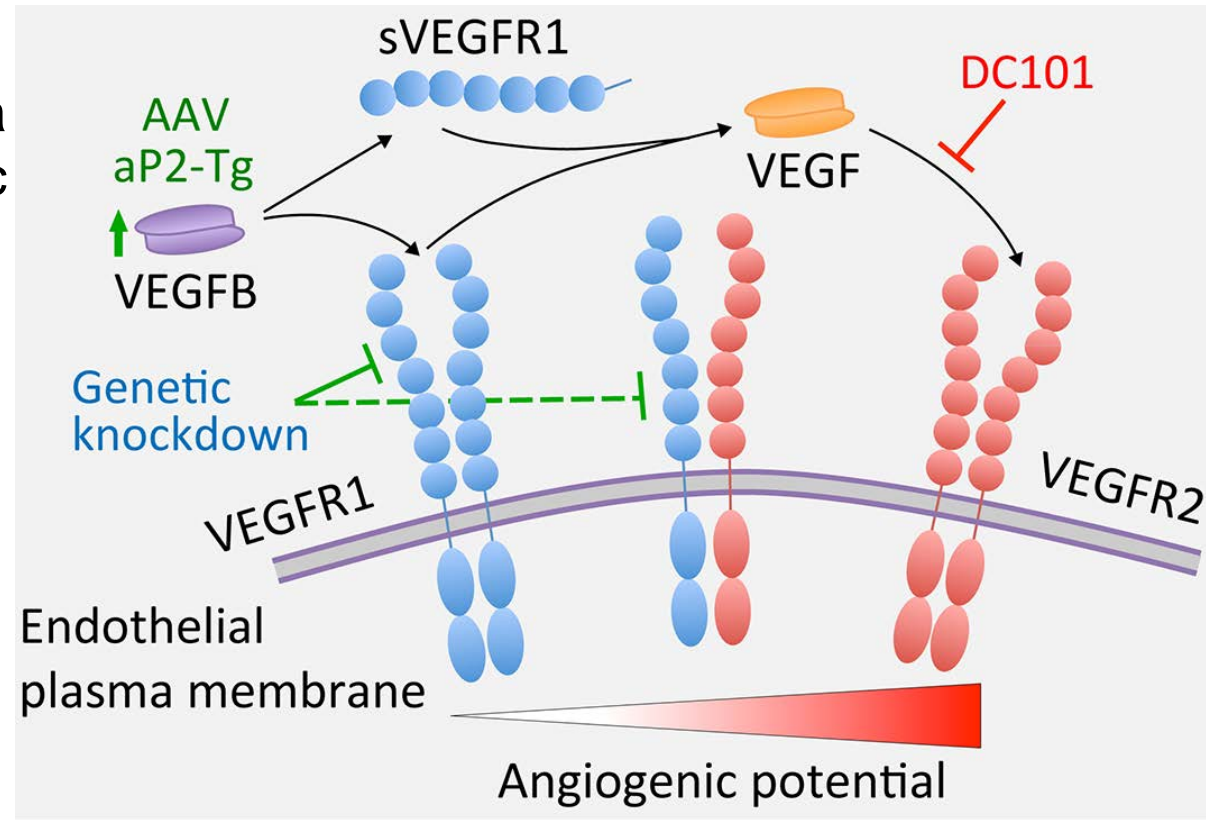
Vascular Endothelial Growth Factor

- VEGF family is a crucial regulator of angiogenesis
 - Growth, direction, and permeability
- VEGF receptor (VEGFR) family
 - Binds VEGF molecules with Immunoglobulin extracellular domain; initiates proliferative/survival response by intercellular tyrosine kinase mechanism



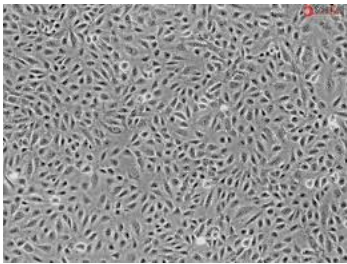
VEGFB

VEGFB has previously been assumed to play a minor role in angiogenic development; however recent developments indicate a nuanced VEGFB mechanism.



Investigative Approach

- How do we study these molecules?
- *In Vitro vs In Vivo*
 - *In Vitro*- mechanistic insight; (ideally) supports *In Vivo* observations
 - *In Vivo*- physiologically relevant, holistic results



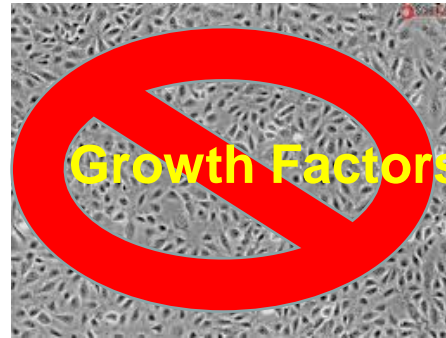
In vitro model: Human Umbilical Vein Endothelial Cells

Starvation/Signaling: What is the cellular signaling response to VEGFA and VEGFB?

Culture HUVEC cells in nutrient poor media

Expose cells to VEGFA and/or VEGFB, lyse cells, and separate cellular proteins by Western Blot

Conditions are compared based on band signal intensity in Western blot



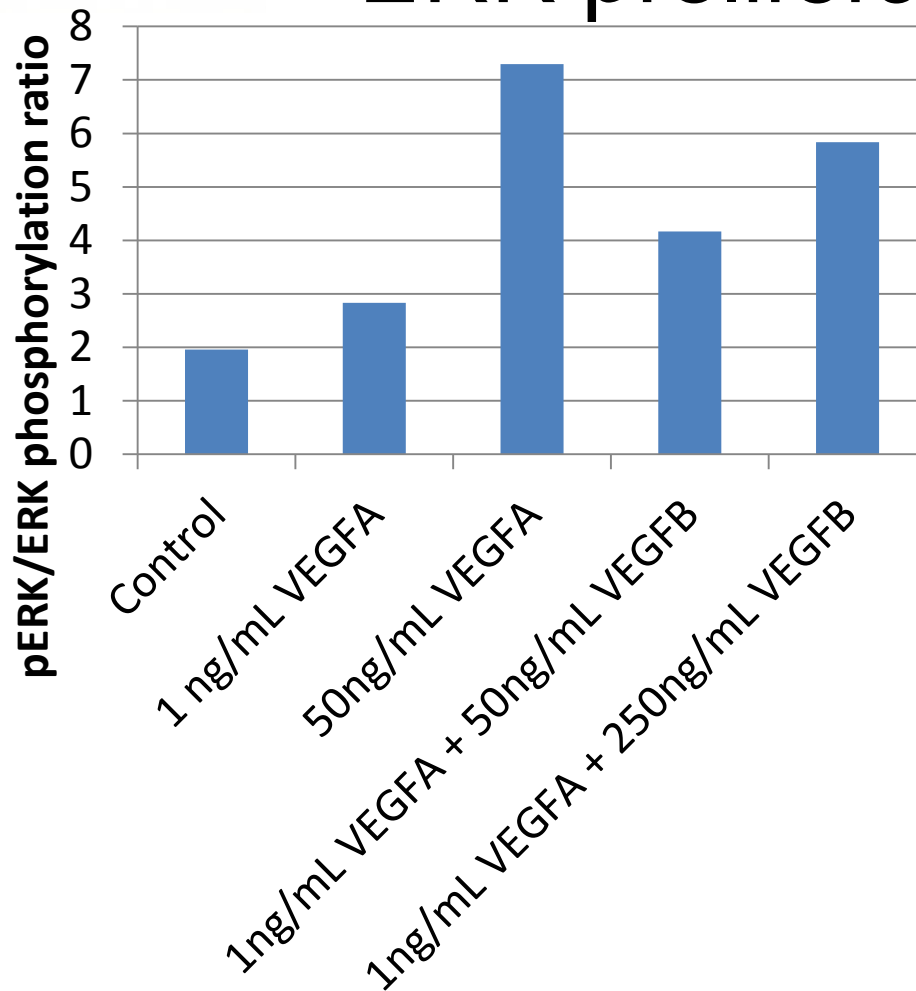
Addition of VEGF's



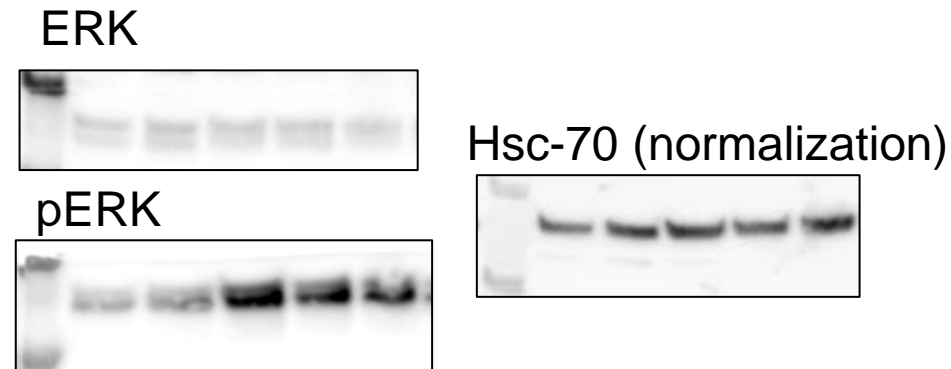
Lyse Cells

Quantify results in Imaging Software

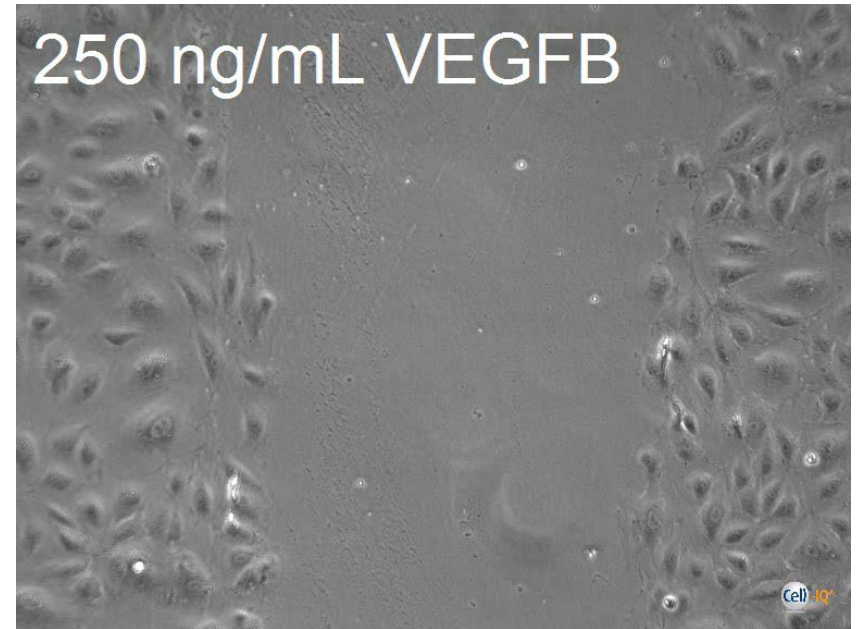
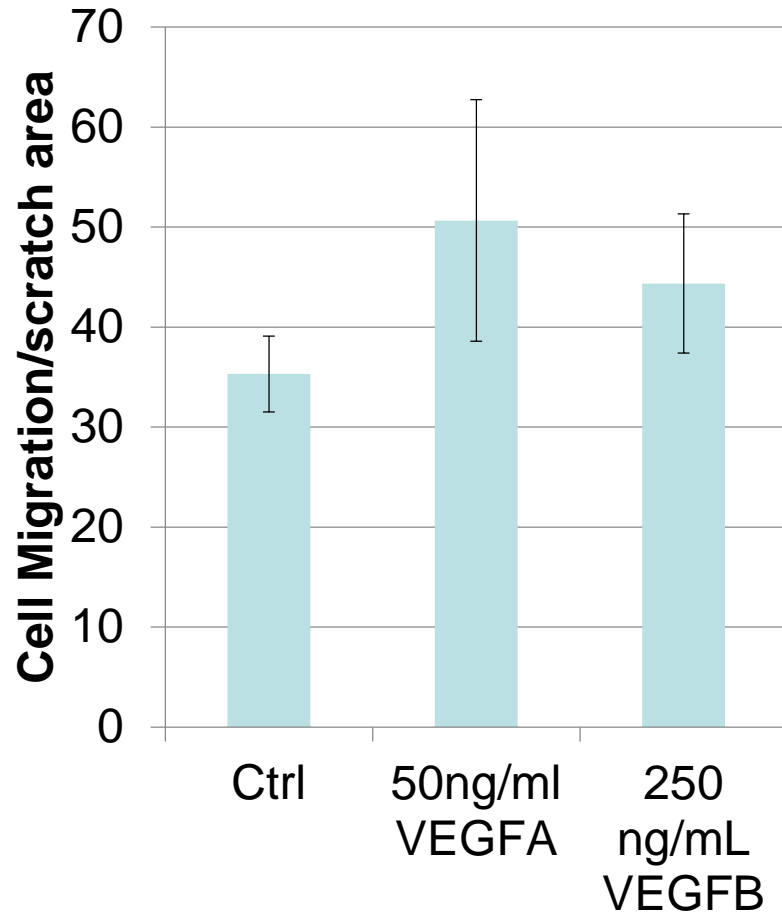
Western Blot indicates activation of ERK proliferation pathway



- ERK protein phosphorylation is the beginning of a proliferative cell program
 - Higher ratio= More cells are growing!
- Results:
 - Low levels of VEGFA can produce an enhanced angiogenic response in the presence of VEGFB



Wound Healing Assay



How does AAV injection of VEGFB affect white adipose tissue vascularity?

Development

- Mice are injected with an AAV for VEGFB
- Induces recombination to produce VEGFB in adipose tissues

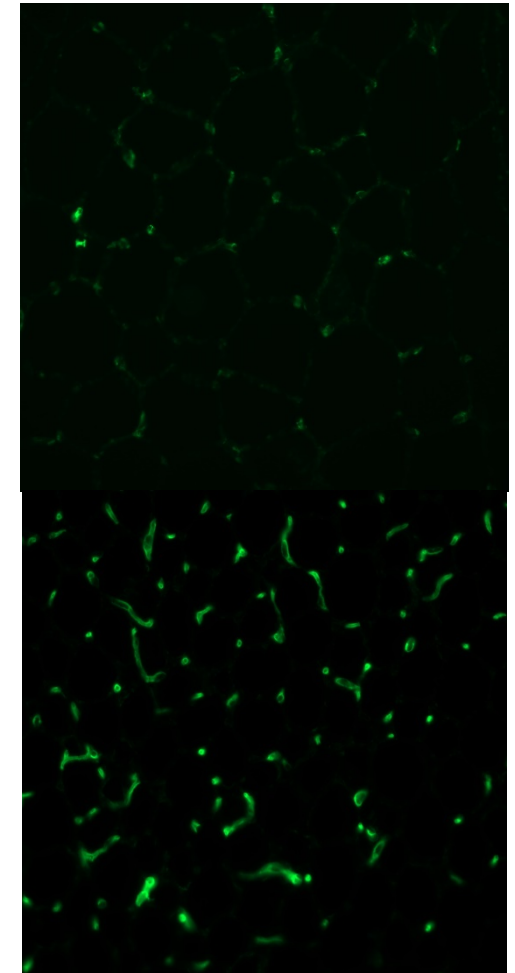
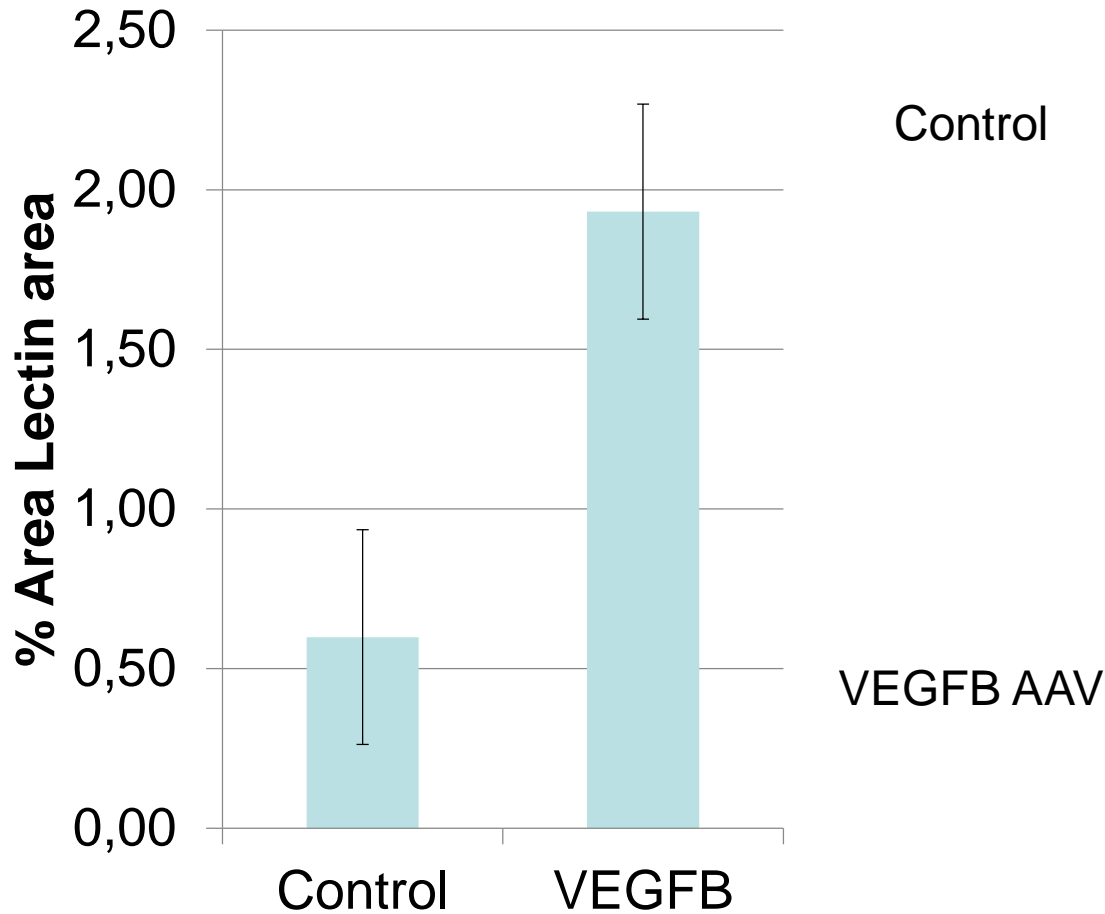
Execution

- Adipose tissues preserved in paraffin sections, stained with Lectin proteins
- Lectin staining- visualization of EC vessels

Compare and Validate

- We believe that VEGF B should incite the development of blood vessels
- Western Blot to confirm results

How does AAV injection of VEGFB affect white adipose tissue vascularity?



In vivo model: Mice

- Western Blot confirms VEGFB AAV was successful, validating results.



What I have learned

- Science requires A LOT of teamwork and time
 - Genetics/cloning- creating mouse lines
 - Proteomics- generating proteins for studying
 - Biochemistry- Executing experiments with precision and control
 - Communication- making results matter