In Vitro and In Vivo investigation into the properties of VEGFB

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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

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 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

Growth Factors

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

Cell Migration/scratch area

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<th>50ng/ml VEGFA</th>
<th>250 ng/mL VEGFB</th>
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<tbody>
<tr>
<td>Cell Migration/scratch area</td>
<td>30</td>
<td>50</td>
<td>40</td>
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VEGF

Ctrl 50ng/ml

250 ng/mL VEGFB

Cell migration

Cell proliferation

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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