Vascular Smooth Muscle Cell Phenotype Switching in Carotid Atherosclerosis

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INTRODUCTION

- >100,000 CEAs annually
- ~↓50% asymptomatic
- ~↑50% with stroke/TIA
BACKGROUND: Carotid atherosclerosis
BACKGROUND: VSMCs

BACKGROUND: HDAC9

- “Healthy” VSMCs, express contractile genes
  - ACTA2, SM22, MYH11
- Disease triggers binding of complex and silences expression of contractile genes
  - Explore role of VSMC phenotype switching in carotid atherosclerosis

Lino Cardenas et al. *Nature Communications*. 2018
DESIGN:

Cell model
Primary human VSMCs treated with cholesterol and phospholipids

Animal models
Mice Hdac9⁻/⁻:Tagln-cre LDLR⁻/⁻ on high fat diet

Surgical Specimen
Molecular expression patterns and genetic data (control, asymptomatic, symptomatic)
RESULTS: Cell Model
## RESULTS: Cell Model

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Chol:MβCD</th>
<th>OxPAPC</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>MC</strong></td>
<td><img src="MC.png" alt="Image" /></td>
<td><img src="MC.png" alt="Image" /></td>
<td><img src="MC.png" alt="Image" /></td>
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<tr>
<td>VSMC</td>
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<td><img src="VSMC.png" alt="Image" /></td>
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<td>Lipid</td>
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<td>SiCTRL</td>
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<td>SiHDAC9</td>
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<td><img src="SiHDAC9.png" alt="Image" /></td>
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</tbody>
</table>

### # of migrated monocytes per frame

- **Control**
- **Control siHDAC9**
- **CH**
- **CH siHDAC9**

**p < 0.05**

<table>
<thead>
<tr>
<th>Incubation time</th>
<th># of migrated monocytes per frame</th>
</tr>
</thead>
<tbody>
<tr>
<td>4 hr</td>
<td><img src="Graph.png" alt="Graph" /></td>
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<tr>
<td>12 hr</td>
<td><img src="Graph.png" alt="Graph" /></td>
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</tbody>
</table>

**Graph:**

- **Control**
- **Control siHDAC9**
- **CH**
- **CH siHDAC9**

**p < 0.05**
RESULTS: Cell Model

What is PN-1?

- PN-1 relative expression
- Fold change (vs Normal media)
  - SERPINE2
  - ACTN1
  - ANXA1
  - SPARC
  - PLOD1
  - ANXA2
  - LGALS3
  - PGK1
  - PRDX1
  - MMP2

0 2 4 6

Normalized mRNA expression

*** p<0.005

RESULTS: Surgical Specimen

**Control**
- Asymptomatic
  - n=35
- Symptomatic
  - n=24

**Control**
- n=13

**Asymptomatic**
- n=35

**Symptomatic**
- n=24
RESULTS: Surgical Specimen

Control n=13
Asymptomatic n=35
Symptomatic n=24
RESULTS: Surgical Specimen
NEXT: On the horizon

• What’s causing the change? How to stabilize the contractile phenotype to mitigate or prevent disease progression
  • What is PN-1
  • HDAC9 associated proteins and pathways

• Further delineate VSMC – macrophage relationship

• Unbiased discovery complex tissues – plaque, vasculature

• Exploring the potential of patient tissues
  • Similar models to expand our understanding of other vascular pathologies
NEXT: Banking for the future/now

- MGH Vascular Tissue Bank
  - Control tissues
    - carotid, segmental aortic arch, descending thoracic aortic, abdominal aortic tissue, lower extremity vasculature
  - Disease specific tissues
    - Marfan, vEDS, LDS, sporadic TAA, AAA, Type A and B dissection, carotid tissue, pulmonary veins with AF
  - Total of over 200 unique patients
Why use single nuclei sequencing

- Vascular tissues are composed of many different cell types which change in phenotype throughout health and disease
  - Agnostic approach to evaluate vascular tissue and discover its diversity

- Comprehensively assess the expression status of different cell types and changes in gene expression in health and disease
  - Identify rare cell populations that are specific to disease
  - Identify targets for treatment

- Why hasn’t this been done already?
TISSUE USE(S): Single nuclei analysis

- Tissue isolation
- Section, digest, homogenize, filter
- Filtered nuclei in suspension

- Nuclei captured with barcoded beads + reagents
- Nuclei are lysed and undergo reverse transcription
- Barcoded cDNA sequenced for analysis
CONCLUSION

• Preserving the VSMCs contractile phenotype may have a role in atherosclerosis and vascular tissue degeneration

• Vascular tissue is valuable
  • Patient’s role in research and discovery

• Surgical outcome improvement, quality of life, morbidity and mortality
  • Biology is inherently associated with outcomes
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Thank you!
Questions/Comments/Suggestions

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