Vascular Smooth Muscle Cell Phenotype Switching in Carotid Atherosclerosis

Elizabeth L. Chou, MD

Massachusetts General Hospital

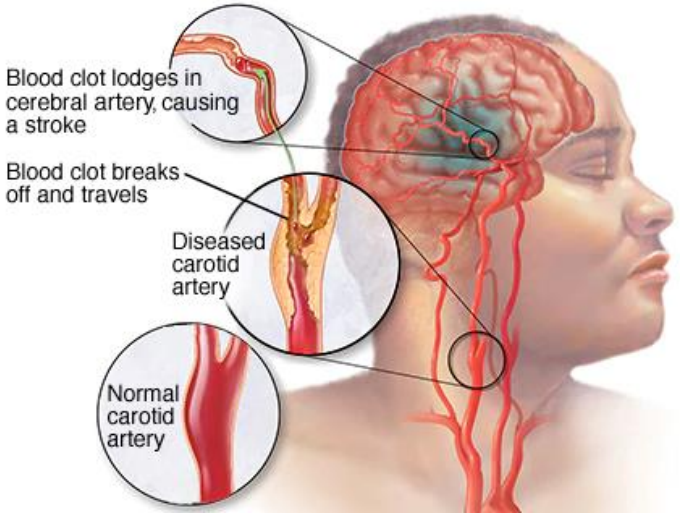
Vascular and Endovascular Surgery, PGY3

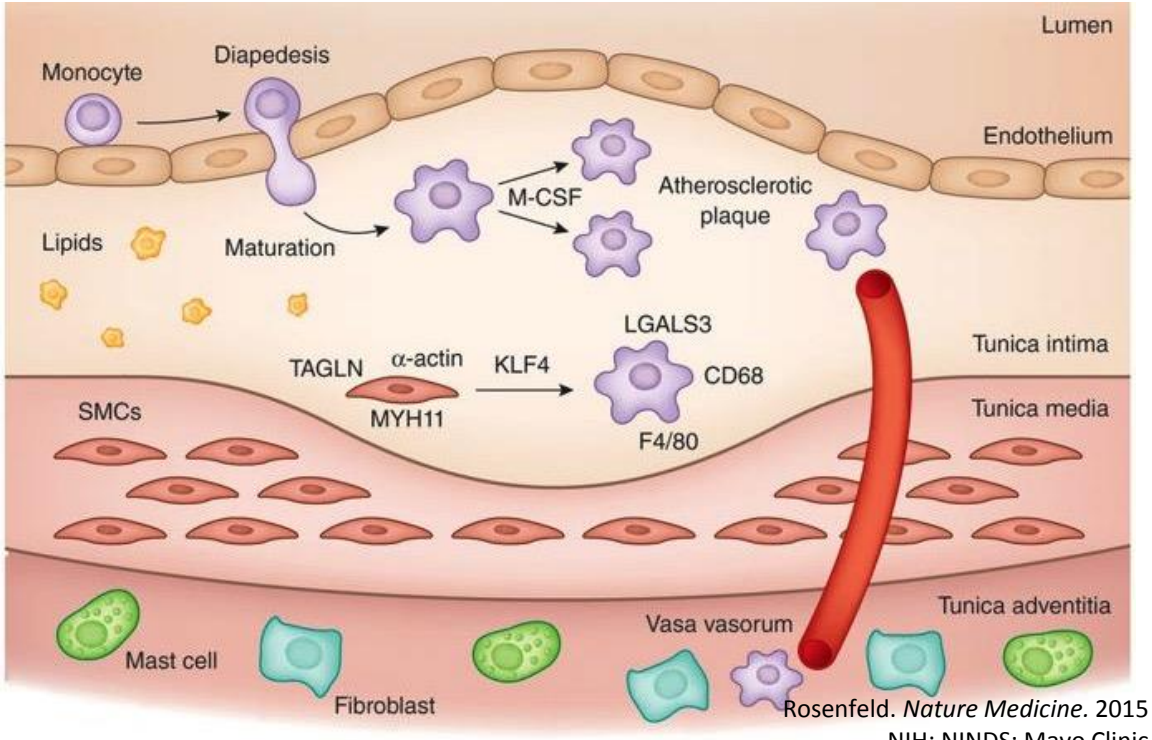
Cardiovascular Research Center

ELCHOU@mgh.harvard.edu

@LizChou

INTRODUCTION

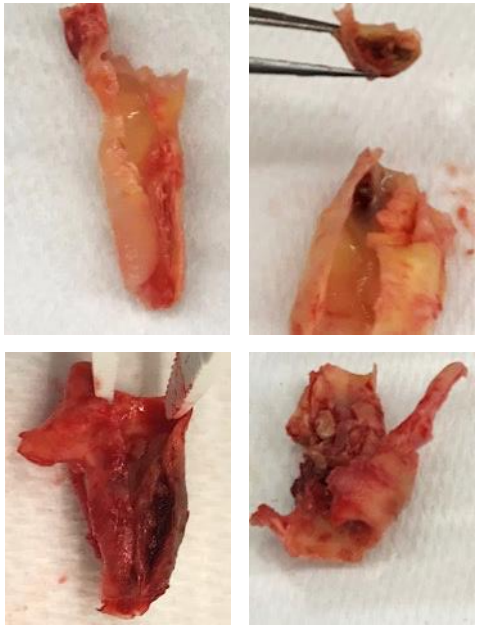


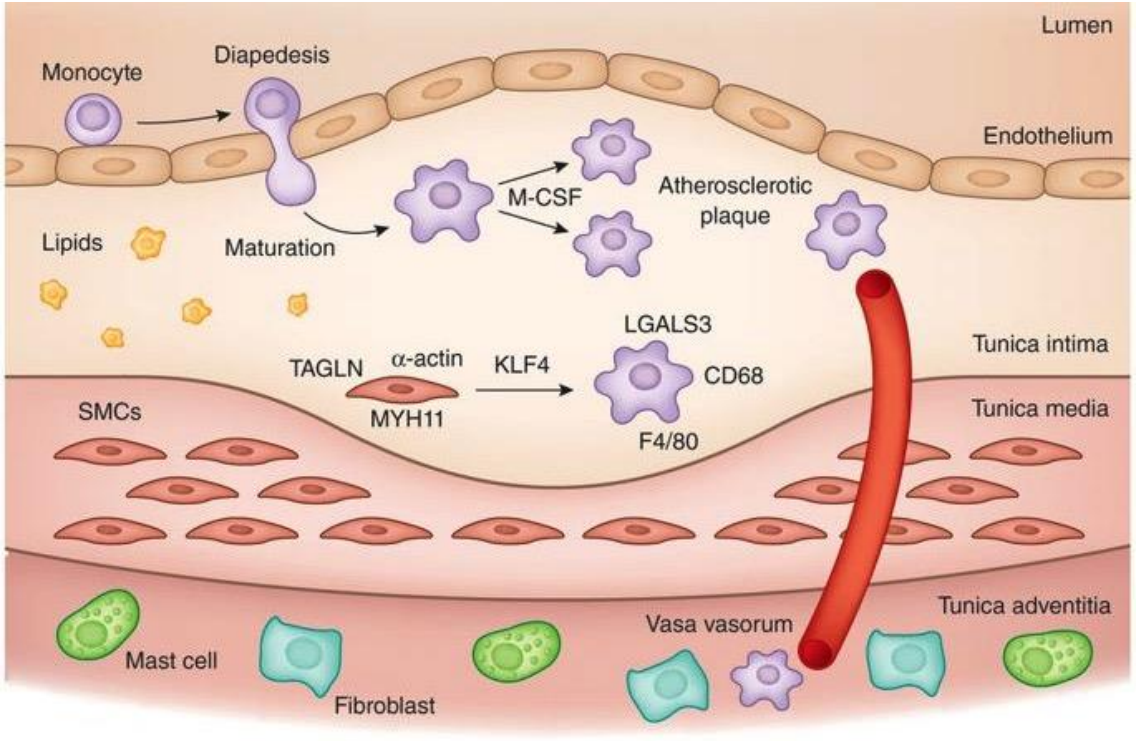


- >100,000 CEAs annually
- ~↓50% asymptomatic
  - ~↑50% with stroke/TIA

Rosenfeld. *Nature Medicine.* 2015
NIH: NINDS; Mayo Clinic
Wallaert. *Stroke*. 2012

BACKGROUND: Carotid atherosclerosis

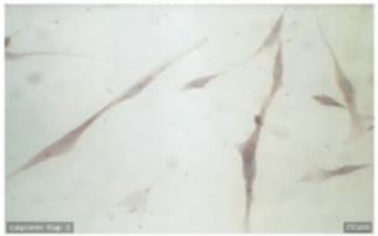




Rosenfeld. *Nature Medicine*. 2015

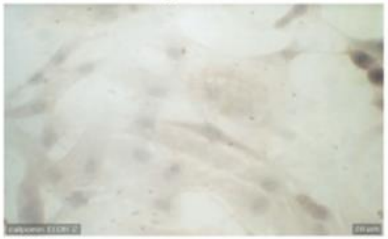
BACKGROUND: VSMCs

Contractile



Arterial injury,
Growth factors
⇄
Arteriogenesis

Synthetic



Marker of differentiation:

Decreased cell size

- Spindle elongated morphology

Decreased ECM production

- Predominance collagen IV, laminin

Increased Contractile protein expression

- SM-MHC2, calponin,actin

Decreased migration

- Decreased MMPs, increased TIMPs

Marker of de-differentiation:

Increased cell size

- Hypertrophic appearance
- "Hill and valley" growth

Increased ECM production

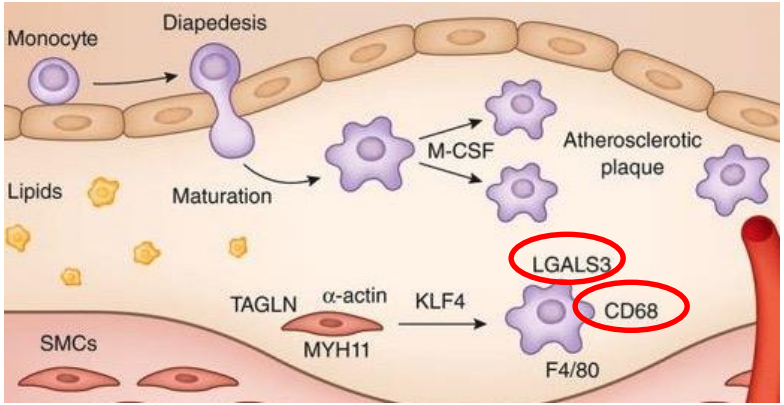
- Predominance collagen III, fibronectin

Decreased Contractile protein expression

- Increase osteopontin

Increase migration

- Increased MMP-1 and MMP-3



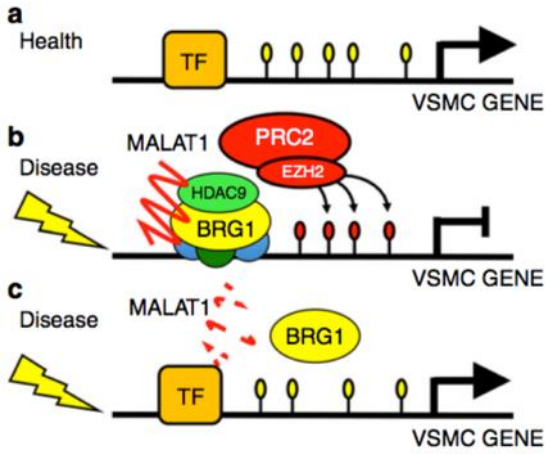
Rzucidlo et al. *J Vasc Surg.* 2007
Rosenfeld. *Nature Medicine.* 2015

BACKGROUND: HDAC9

nature genetics

Genome-wide association study identifies a variant in *HDAC9* associated with large vessel ischemic stroke

The International Stroke Genetics Consortium (ISGC)[1] & the Wellcome Trust Case Control Consortium 2 (WTCCC2)[1]

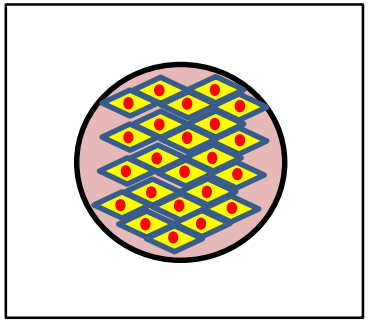


- "Healthy" VSMCs, express contractile genes
  - ACTA2, SM22, MYH11
- Disease triggers binding of complex and silences expression of contractile genes

➢ Explore role of VSMC phenotype switching in carotid atherosclerosis

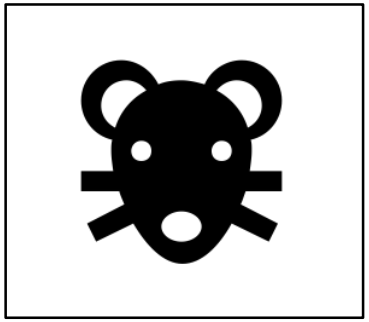
ISGC et al. *Nature Genetics*. 2012
Lino Cardenas et al. *Nature Communications.* 2018

DESIGN:



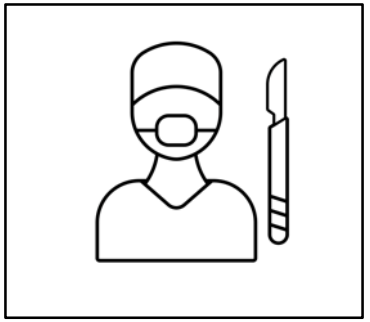
Cell model

Primary human VSMCs treated with cholesterol and phospholipids



Animal models

Mice Hdac9$^{-/-}$:Tagln-cre LDLR$^{-/-}$ on high fat diet

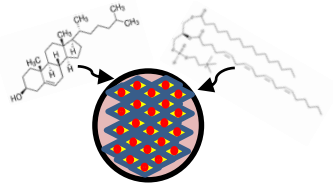


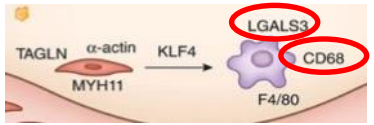
Surgical Specimen

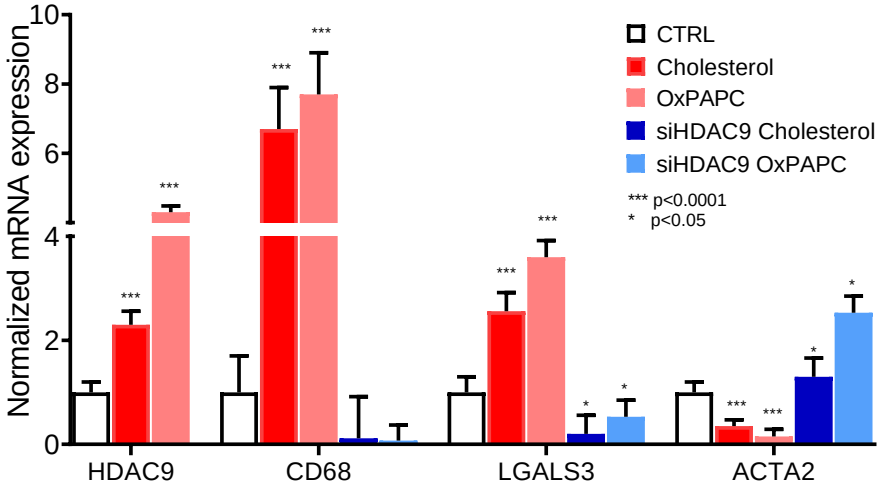
Molecular expression patterns and genetic data

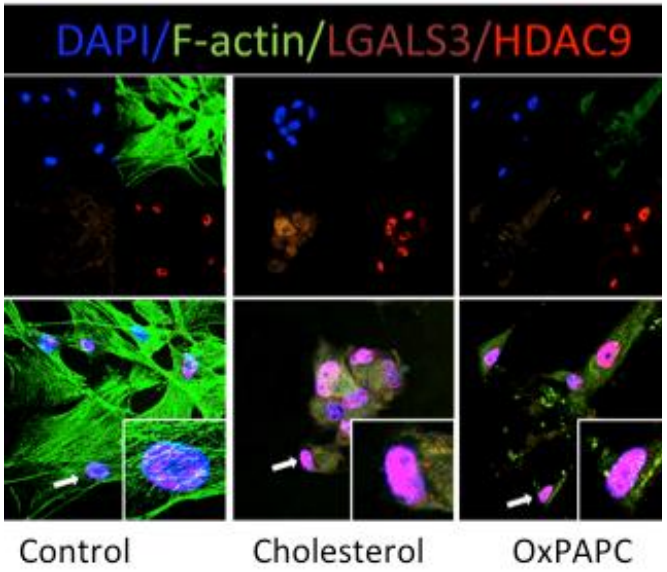
(control, asymptomatic, symptomatic)

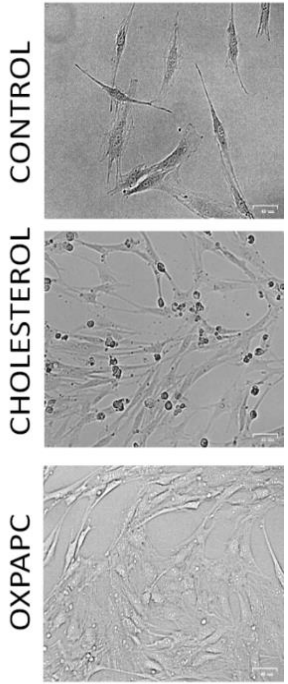
RESULTS: Cell Model



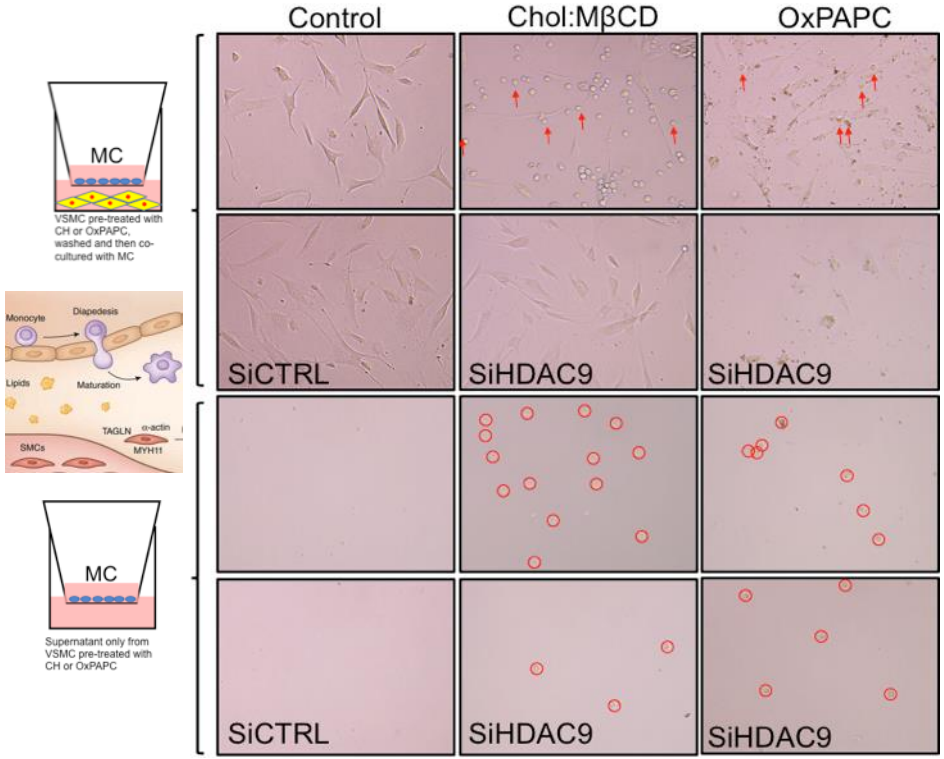


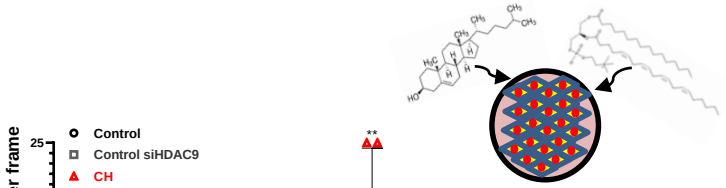


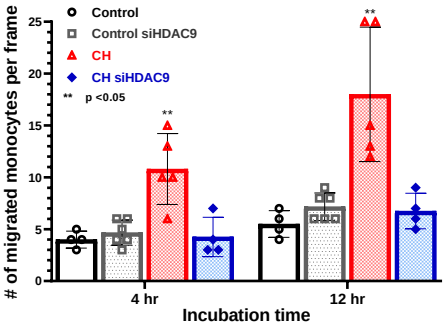


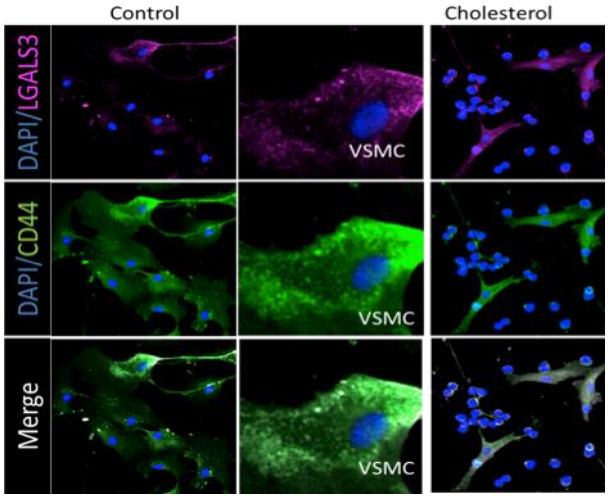


RESULTS: Cell Model

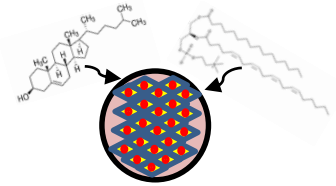


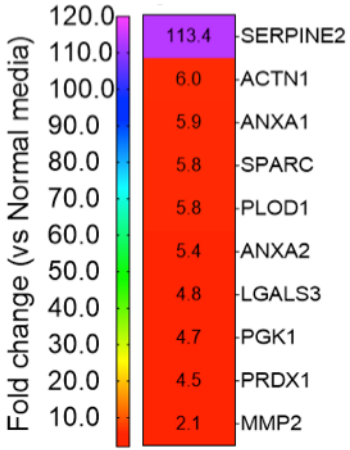


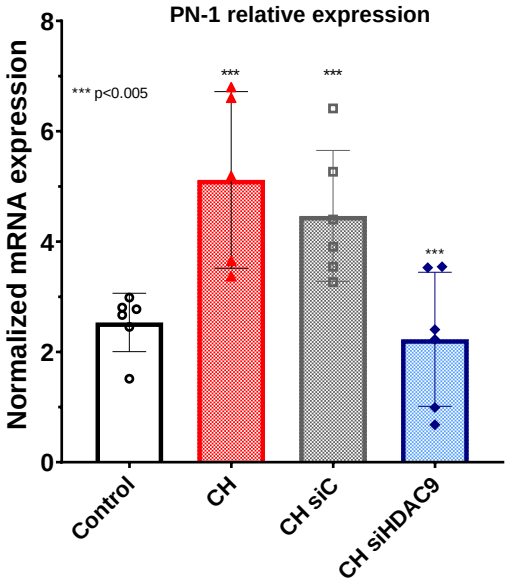




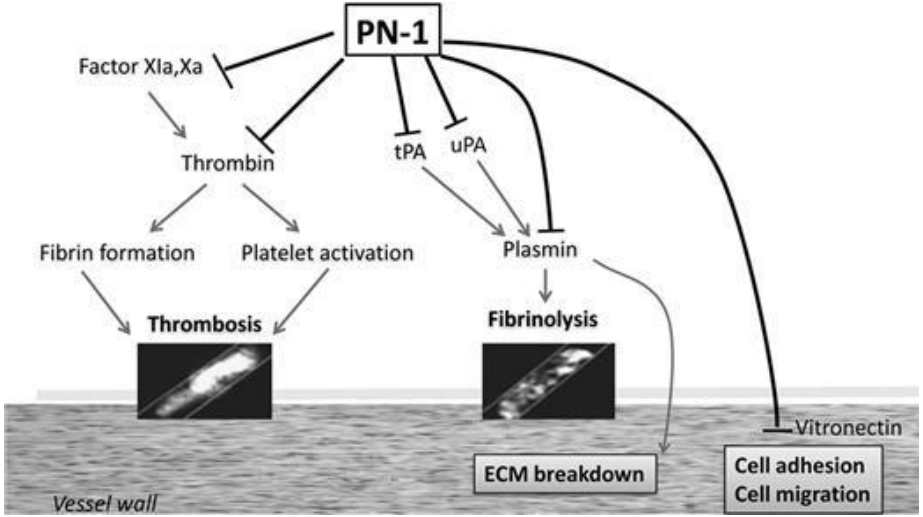
RESULTS: Cell Model





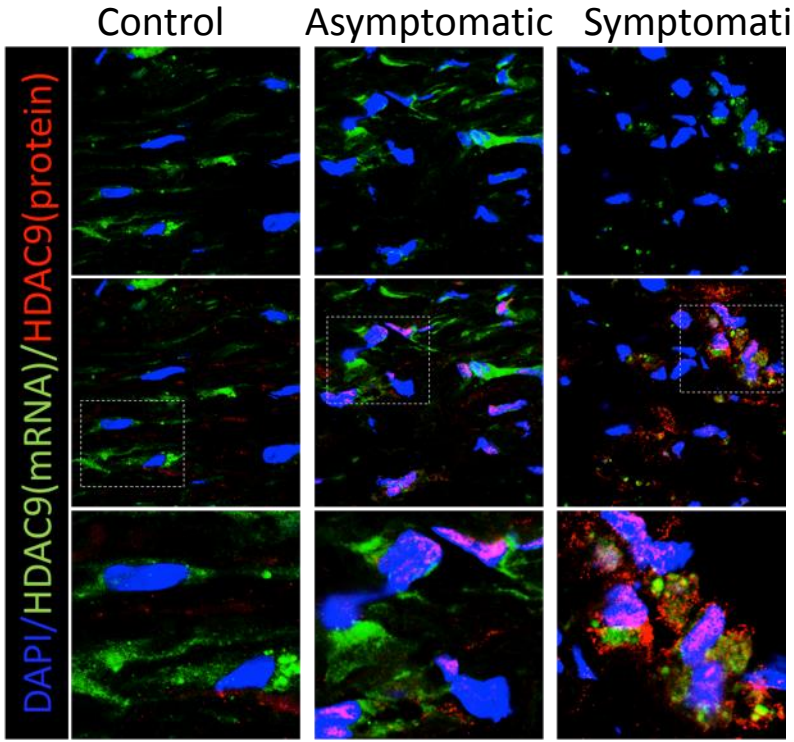


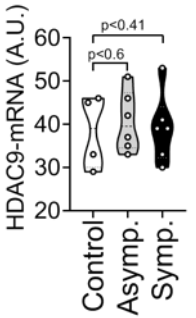
What is PN-1?

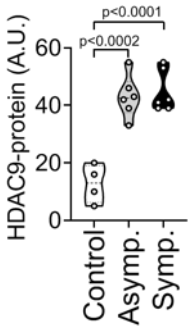


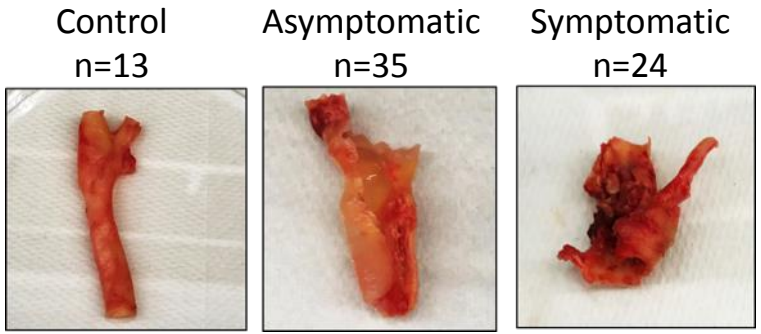
Bouton. *Blood.* 2012

RESULTS: Surgical Specimen



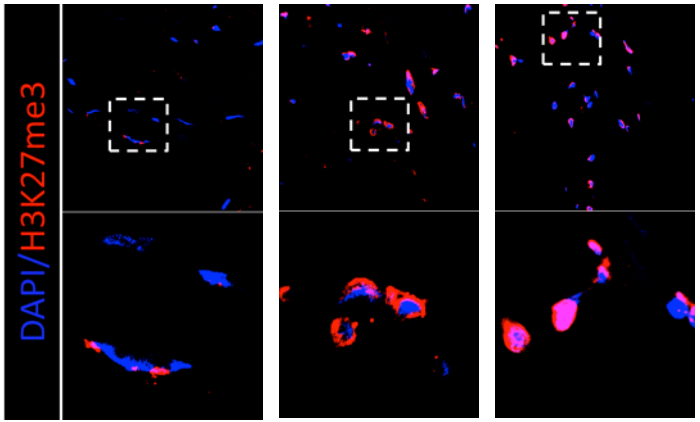


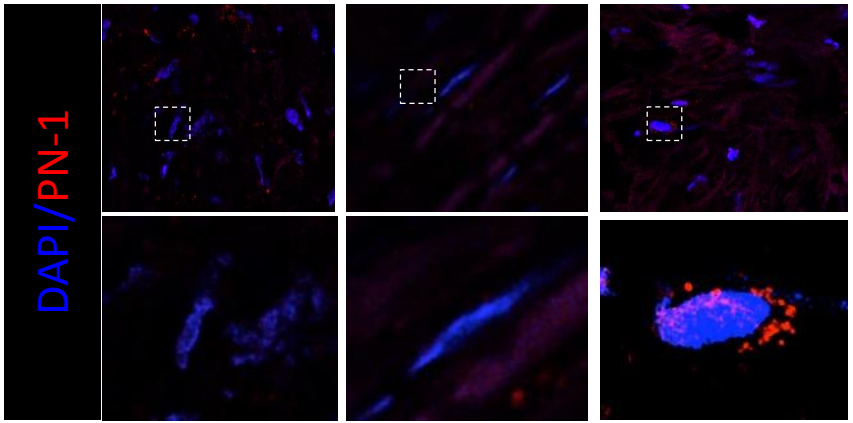




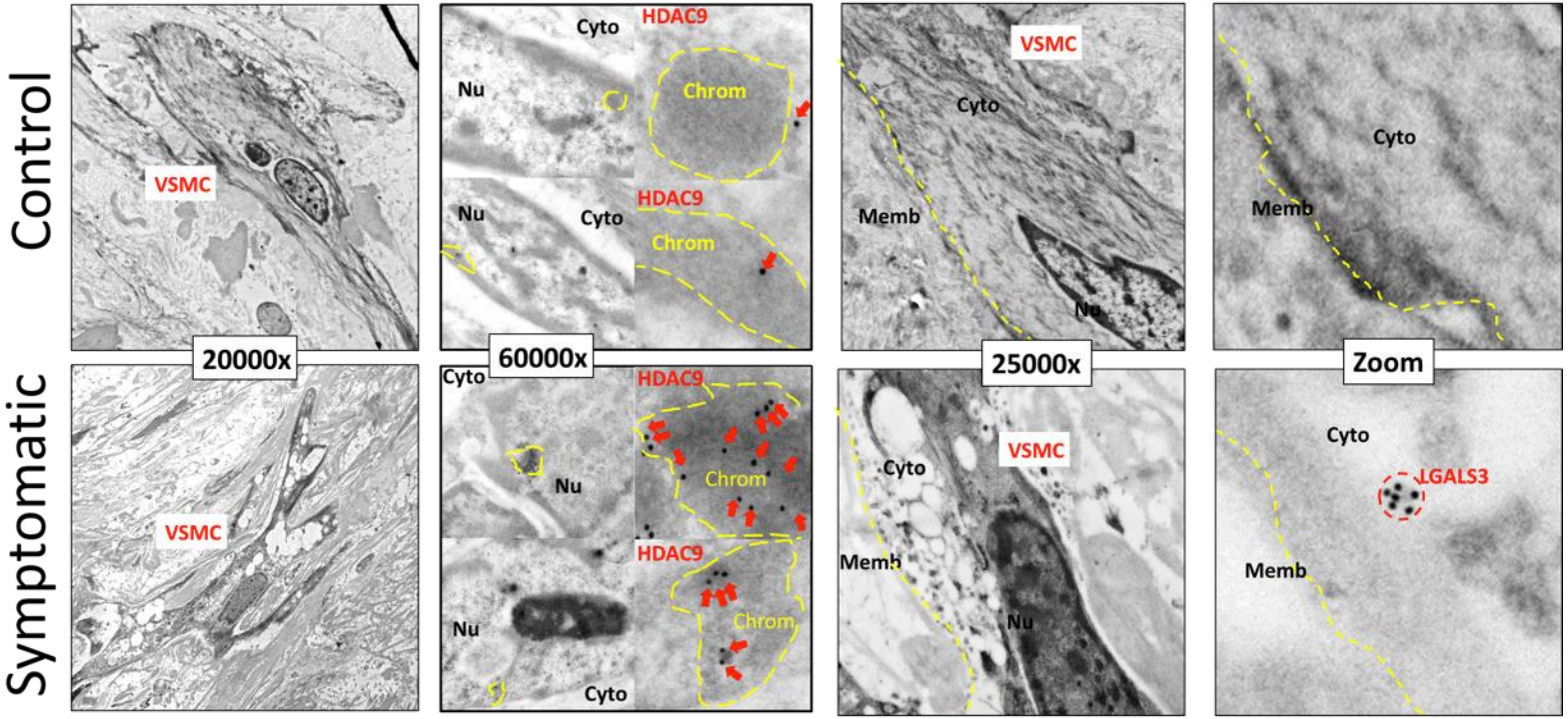
RESULTS: Surgical Specimen

Control	Asymptomatic	Symptomatic
n=13	n=35	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

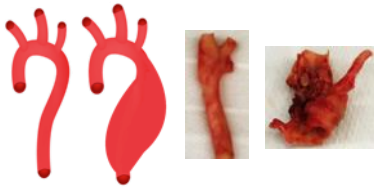
NEXT: Banking for the future/now

- **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**

NEXT: Why use single nuclei sequencing

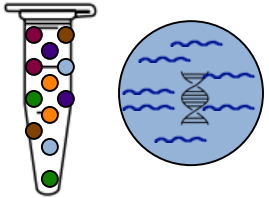
- **Vascular tissues are composed of many different cell types which change in phenotype throughout health and disease**
    - Agnostic approach to evaluate vascular tissue and discover its diversity
- **Comprehensively assess the expression status of different cell types and changes in gene expression 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

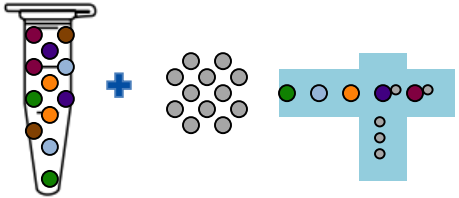


Tissue isolation

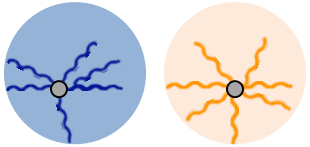
Section, digest, homogenize, filter



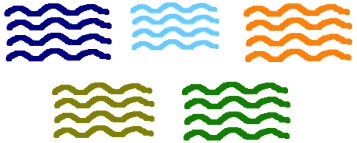
Filtered nuclei in suspension



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

ACKNOWLEDGEMENTS

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Thank you!
Questions/Comments/Suggestions

ELCHOU@mgh.harvard.edu
@LizChou