Mechano-regulation of protein CDK8 in Vasculature

Mimi Jüng[1,3], Nazli Gharraee[2,3], Susan Lessner[2,3]

[1] *University of Rochester,* Department of Biomedical Engineering,
[2] *University of South Carolina,* Department of Biomedical Engineering, [3] *University of South Carolina School of Medicine,* Department of Cell Biology & Anatomy

UNIVERSITY OF South Carolina
School of Medicine Columbia

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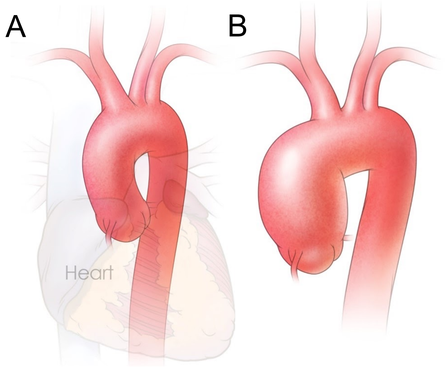
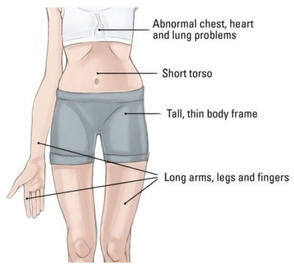
College of Engineering and Computing

Introduction





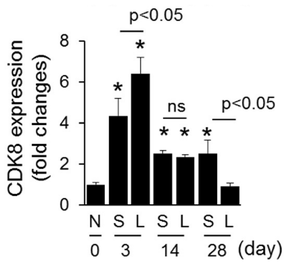


Figure 1:
a) Healthy aortic arch
b) Thoracic Aortic Aneurysm (TAA), characterized by arterial dilation due to weakening of the aortic walls. Rupture may cause massive hemorrhaging and premature mortality and morbidity.

Figure 2: Marfan Syndrome: A genetic disorder of the connective tissue that commonly manifests in aortic enlargement that leads to TAA. The most common genetic defect is a mutation in fibrillin-1, which causes defects in the elastic fibers of the aortic media.

Figure 3: Cyclin-dependent kinase 8 (CDK8), a transcription-regulating kinase, expression is upregulated after carotid ligation injury in mice. Graph compares normal (N), sham (S), and ligation (L).

Mature vascular smooth muscle cells (SMCs) undergo dedifferentiation in response to injury, for example during TAA. CDK8 has been found to be a critical mediator of vascular SMC dedifferentiation. It is a possible target for treatment of vascular diseases caused by abnormal phenotypic switching of vascular SMCs.

Aim: To look for evidence of mechano-regulation of CDK8 in vasculature.

Methods

Animal Model:

Marfan Syndrome Model: Fibrillin-1 (Fbn1 C1041G/+) Heterozygotes (Het)
Control Group: Black 6 (C57Bl/6) Wildtype (WT) +/+
6-month Male + Female mice (n=4-6 for each group)

Fluorescent Immunohistochemistry (IHC)

Primary Antibody: Anti-CDK8 in 10% Goat Serum
Secondary Antibody: Biotinylated Goat Anti-Rabbit IgG in 10% Goat Serum
Tertiary Reagent: Rhodamine Red X-Streptavidin in 50 mM Tris HCl

Fluorescent Microscopy

Image of aorta section on Zeiss Axioskop 2, under 100x magnification

Analysis

Fractional CDK8 content =
Total CDK8 area (pixels) / Total aortic wall cross sectional area (pixels) x 100%

Two Way ANOVA performed to determine statistical significance

Results

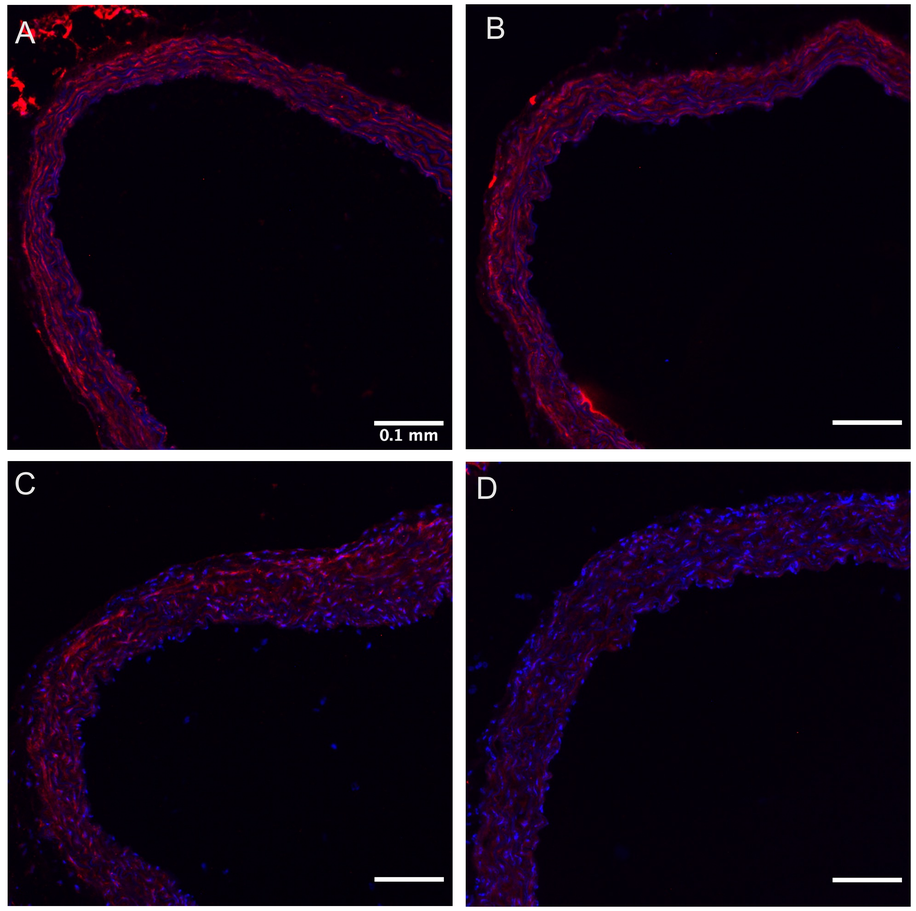


Figure 4: Representative IHC images of 6-month WT (male in a and female in b) and Het (male in c and female in d) mice. Blue shows nuclear staining, red shows CDK expression, and magenta indicates CDK8 positive nuclei. Images were taken at 100x magnification.

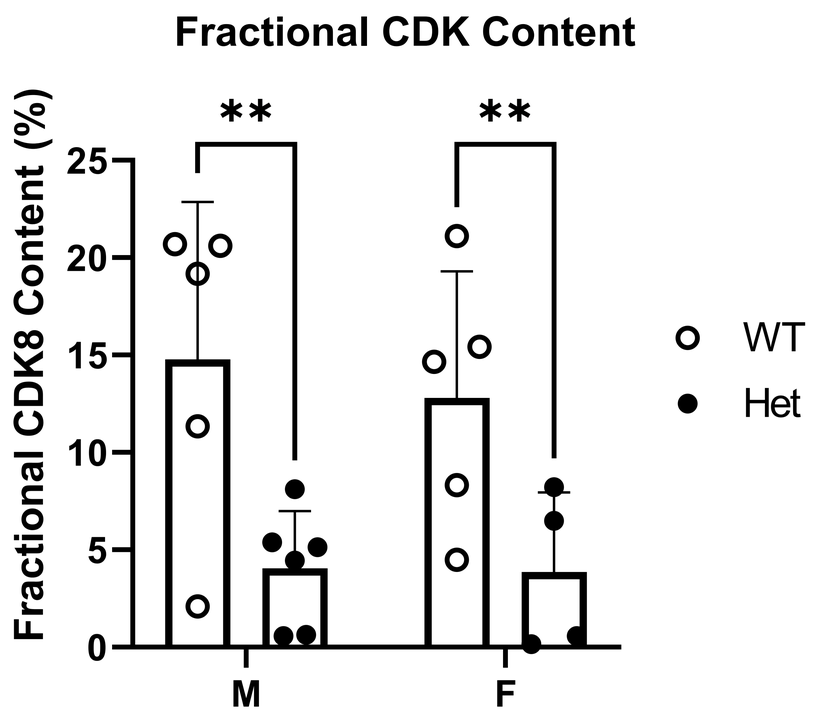


Figure 5: Fractional CDK8 content in 6-month WT and Het mice, male (M) and female (F). ** represents a p-value ≤ 0.01.

Conclusion

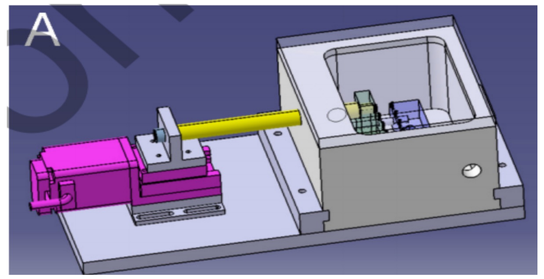
- Significantly lower fractional CDK8 content in Fbn1 heterozygous mice at 6 months than wild type (p-value ≤ 0.01.)
- Found no effect of sex on fractional CDK8 content in both genotypes (p-value > 0.05)
- An association between CDK8 expression and genotype has been identified at 6 months

Discussion

- These are surprising results since previous work demonstrates a loss of SMC contractile phenotype at 6 months during vascular SMC dedifferentiation.
- The 6-month time point is prior to the time point when significant changes in aortic diameter occur. Therefore, more work is needed to further investigate the role of CDK8 in vasculature.

Future Directions

In *vitro* Bioreactor Culture of Vascular Tissue: induce uniaxial cyclic stretch on rings cut from WT mouse ascending aortas. Different time cycles, stretch ratios, and frequencies will be tested on arterial samples to determine CDK8 expression.



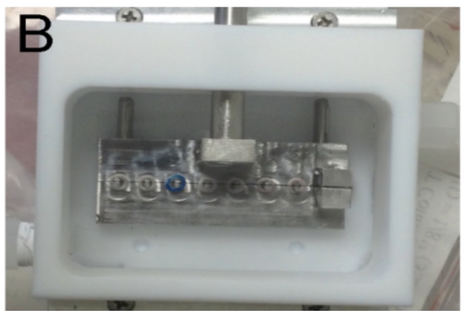


Figure 6: Miniature bioreactor schematic (a) and top view of device (b)

Time course study: mice of different time points (3, 9, and 12 month) will be used to track CDK8 expression with the development of the aneurysm. Samples of corresponding age and sex from a previous study are readily accessible.

3 month → 6 month → 9 month → 12 month

Acknowledgments

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References

Leach, D.(2017). *The Role Of Cyclin-Dependent Kinase 8 In Vascular Disease.* (Doctoral dissertation). Retrieved from https://scholarcommons.sc.edu/etd/4523
Shazly, T., Rachev, A., Lessner, S. *et al.* On the Uniaxial Ring Test of Tissue Engineered Constructs. *Exp Mech* **55,** 41–51 (2015). https://doi.org/10.1007/s11340-014-9910-2