Aortic Valve Knockout of pRb Increases Calcific Aortic Valve Disease Characteristics

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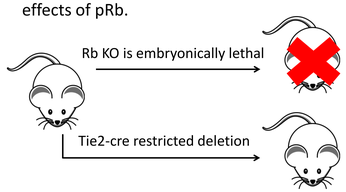
Introduction

- Calcific aortic valve disease (CAVD) affects over 8 million people in the US [1]; currently, the only treatment for severe disease is total valve replacement surgery.
- The lack of basic disease mechanisms underlying CAVD has limited the number of pharmaceutical targets available for treatment; better understanding may lead to better therapies.
- Changes in valve tissue mechanics, composition, and fiber orientation have all been measured in the end stages of the disease, but it is unclear how these properties are altered during disease progression.
- *In vitro* models that allow for controlled experimental manipulation of environments relevant to the aortic valve may help parse out specific disease mechanisms.

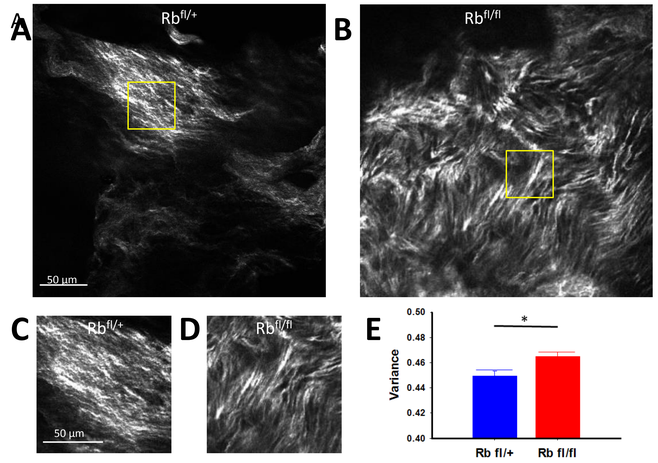
Retinoblastoma Protein Model

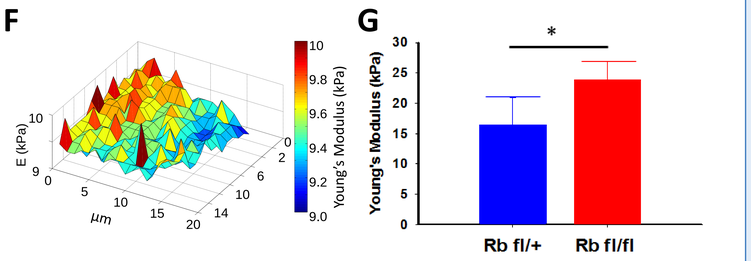
- The loss of retinoblastoma protein (pRb) has been shown to produce cells that mineralized more heavily *in vitro* [2].
- pRb also regulates bone formation through interaction with runx2 [3].
- We created a knockout mouse model to study the effects of pRb.



- Porcine aortic valve interstitial cells (aVICs) were also used to create an *in vitro* model using viral transfection to knockdown Rb.
- aVICs were seeded on 2D, polyacrylamide (PAAM) gels at physiology stiffnesses and dosed for 24 hours with TGF-β_1 to induce activation.

Results: Changes in Valve Leaflet ECM



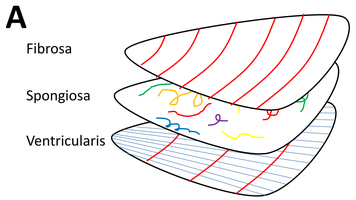


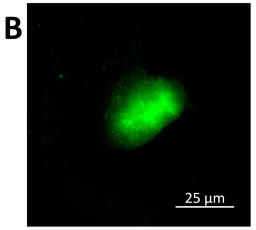
- Second harmonic generation (SHG) images of collagen showed an increase in directional variance (E) in the Rb$^{fl/fl}$ mice compared to the Rb$^{fl/-}$ leaflets. Figures (A) and (B) are representative SHG images while (C) and (D) show zoomed in sections of collagen fibers.
- Atomic force microscopy (AFM) was used to measure the Young's Modulus of the valve leaflets. A representative heat map of leaflet tissue is shown in (F). There was a significant increase in leaflet stiffness in the Rb$^{fl/fl}$ mice (G).

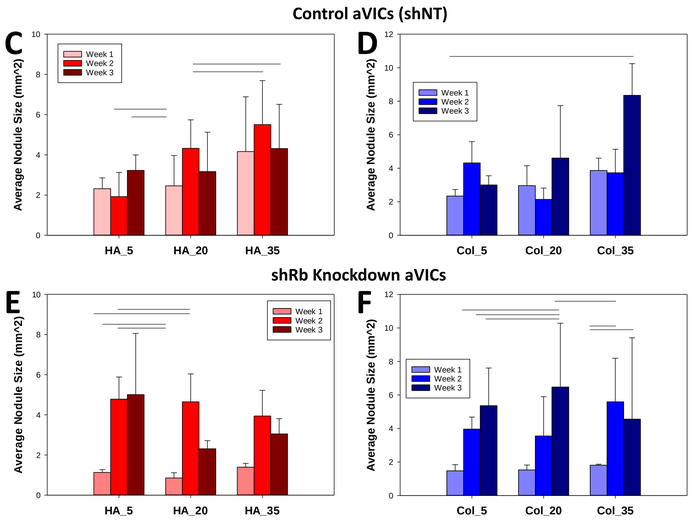
*=p<0.05

Results: *In Vitro* aVIC Modeling

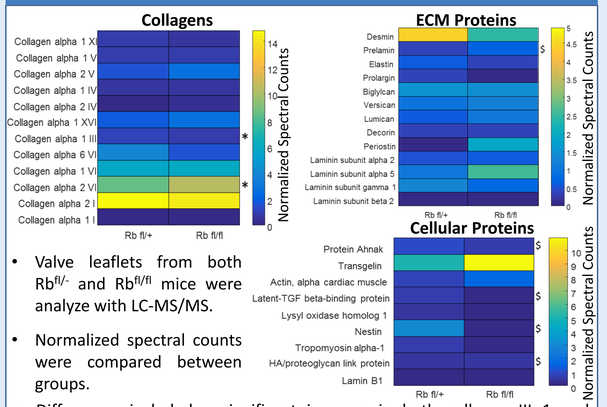
- PAAM gels were created to model changing tissue stiffness associated with CAVD and designed to incorporate different binding proteins to assess the effect of specific ECM components in the fibrosa and spongiosa (A).
- 5 kpa (healthy valve tissue), 20 kPa (beginning of disease), and 35 kPa (diseased valve tissue) [4] stiffnesses were tested with either collagen or hyaluronic acid (HA) as a binding motif. Stiffness was confirm using AFM. Calcific nodules were stained with fluo-4 AM, a calcium dye (B).
- Over a 3 week culture period, significant changes were seen in the average nodule size of differing conditions. The shRb knockdown aVICs demonstrated increased nodule growth compared to the shNT aVICs after 1 week in culture with HA and collagen gels (C-F).







Results: Proteomics Analysis



- Valve leaflets from both Rb$^{fl/-}$ and Rb$^{fl/fl}$ mice were analyze with LC-MS/MS.
- Normalized spectral counts were compared between groups.
- Differences included a significant increase in both collagen IIIα1 and collagen VIα2 in the Rb$^{fl/fl}$.
- The Rb$^{fl/fl}$ valves also showed an increase in latent TGF-β_1 binding protein.
- The knockout model further demonstrated a decrease in nestin and HA/proteoglycan link protein.

*=p<0.05; $=p<0.1

Conclusions

- Knocking out Rb decreases collagen fiber organization and increases aortic leaflet stiffness.
- Knockdown of Rb in aVICs demonstrates an increased propensity for calcification on lower stiffness PAAM gels.
- Proteomics analysis of Rb$^{fl/fl}$ valves shows KO mice have more, less mature collagen and proteoglycans.

References

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Acknowledgements

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