

Research Proposal: The Role of Cardiac Fibroblasts in Calcific Aortic Stenosis

Background:

Aortic valve stenosis is characterized by a narrowing of the aortic valve located in the heart between the left ventricle (LV) and the aorta. The aortic valve regulates the unidirectional flow of blood out of the LV. During isovolumetric contraction, the LV contracts without a change in LV volume. This continues until the left ventricular pressure becomes greater than that in the aorta which subsequently causes the aortic valve to open and blood to be forcefully ejected from the heart.¹ Stenosis of the aortic valve causes higher systolic pressures to build in the LV inducing an increase of afterload in the LV. Constant increased afterload will lead to myocardial thickening as the myocytes hypertrophy in response to pressure overload. Cardiac hypertrophy can lead to eventual congestive heart failure (CHF) which is characterized by reduced cardiac output and decreased ejection fraction and can lead to serious complications.²

Calcific aortic stenosis (CAS) is the most common cause of valve replacement in the United States and affects 2-3% of the population over 75.³ CAS is caused by deposition of lipids, minerals and inflammatory cells followed by calcification of the surrounding tissues. The calcified aortic valve is resistant to systolic blood flow and contributes to higher LV pressures as well as regurgitation of blood from the aorta back into the LV. CAS can be diagnosed in patients by listening for systolic murmurs, visualizing the calcified aortic valve via echocardiography and pulse-wave Doppler measurements for increased blood velocity across the aortic valve. The symptoms of severe aortic stenosis include angina, syncope and CHF.

The pathophysiology of CAS resembles that of coronary artery disease (CAD). Observational studies have noted that patients at risk of CAD are also at risk of developing aortic stenosis. Risk factors for CAS include hypercholesterolemia, elevated low-density lipoprotein (LDL), elevated triglycerides and elevated lipoprotein(a).⁴ In a fashion similar to the development of atherosclerotic plaques, aortic stenosis involves the recruitment of inflammatory macrophages and lymphocytes along with lipid accumulation along the basement membrane of the valve leaflets (Fig. 1). However, recent studies have discovered the accumulation of fibroblast and myofibroblast stromal cells within diseased valves. In addition, osteopontin (OPN) and other bone-associated proteins have been found to be expressed in the disease state.⁵

Cardiac fibroblasts (CF) are the most numerically abundant cell type in the heart. CF play an essential role in extracellular matrix (ECM) protein deposition, paracrine signaling and cardiac remodeling. Dysregulation of fibroblast activation can lead to excess fibrosis as seen in individuals with cardiac hypertrophy and CHF. While fibroblasts play an essential role in fibrotic disease progression and have been shown to release a variety of pro-inflammatory cytokines as well as OPN, their role in the pathogenesis of aortic stenosis is unclear.⁶

Open Question

The studies outlined in this proposal will explore the potential role of cardiac fibroblast activation in the disease progression of CAS. While fibroblasts are known to be major contributors to fibrosis and adverse cardiac remodeling during cardiac hypertrophy and CHF, their role in the pathogenesis of CAS has not been fully examined. In this study I propose the **hypothesis** that cardiac fibroblast infiltration and activation plays an important role in the pathogenesis of aortic valve stenosis. CF may play a role in releasing inflammatory cytokines and ECM proteins including osteopontin which could directly contribute to inflammatory cell accumulation and calcification.

Experimental Approaches

1. In the course of this study, I seek to first identify any changes in resident fibroblast content between patients suffering from severe CAS and those without noticeable aortic valve calcification. This would require obtaining tissue from consenting human patients undergoing valve replacement. Furthermore, established mouse and New Zealand white rabbit models of CAS will be used for additional *in vivo* and *ex vivo* experiments. These models are obtained by feeding animals with a high cholesterol diet and using LDL receptor knockout mice, where applicable.⁷ After obtaining samples from human patients and animal models,

immunohistochemistry and quantitative PCR will be used to assess for the amount of cardiac fibroblast and myofibroblast content as well as levels of inflammatory cytokines, ECM proteins, and OPN. Previous observational studies have described an increase in OPN and osteoblast-like cells in calcified aortic valves.⁷ It is not known whether resident cardiac fibroblasts are the predominant cell type expressing OPN and matrix proteins.

Immuno-histochemical staining of valve tissue with discoidin domain receptor 2 (DDR2), a protein specific for CF and not expressed in myocytes, smooth muscle, endothelial or vascular cells will identify CF.⁸ An increase in CF corresponding with severe CAS would suggest that fibroblast recruitment plays a major role in disease progression. Furthermore, CF colocalization with increased matrix proteins such as OPN, collagen and fibronectin (FN) will provide additional support that CF release factors that directly stimulate calcification. Quantitative PCR will be used on isolated cells to directly assay any increases in inflammatory and ECM gene expression in diseased versus normal tissues.

2. According to my hypothesis, I expect to see an increase in CF number in aortic valve tissue isolated from subjects with CAS correlative with increased matrix and OPN deposition. To assess the importance of OPN expressed in CF, cardiac fibroblast-specific OPN knockout mice will be generated. These knockouts will be generated using a Cre/loxP system and the Cre recombinase placed under control of the CF specific DDR2 promoter. The knockout mice will be subjected to a high cholesterol diet and the progression of CAS monitored in conjunction with WT littermates. I would expect mice with CF lacking OPN expression to show less advanced aortic calcification following a high cholesterol diet. Since the knockout should not affect any increases in fibroblast localization to potential lesions in the aorta, one would not expect a decrease in CF recruitment in the hypercholesterolemic state.

The generation of a fibroblast-specific OPN knockout would strongly support the current hypothesis. However, this is not a trivial task, and there is some controversy about the expression efficiency of using a DDR2 promoter. This is a caveat of the current experimental plan. A OPN^{-/-} knockout mouse is available, but this would be less convincing a model in examining the fibroblast specific role of CAS progression.

3. Recent studies have found cAMP-raising agonists to be able to prevent and reverse CF transformation into the activated myofibroblast phenotype.⁹ Since drugs specifically targeting fibroblast activation do not exist, it would be interesting to assess the possibility of raising cAMP levels in preventing CAS. This can be done in animal models via systemic injections of isoproterenol (Iso), a β -adrenergic receptor agonist or forskolin (Fsk), an adenylyl cyclase (AC) agonist concurrent with a high cholesterol diet. A more invasive treatment regimen involves intracoronary gene delivery of adenylyl cyclase 6, the most abundant AC in fibroblasts.⁹ This eliminates many of the potential off-target complications of systemic Iso or Fsk treatment. However, gene expression from adenoviral-mediated delivery is transient and may be as short as one week. Depending on the proposed scope of the project, a cardiac specific AC6 overexpressing mouse may be generated and used for study.

Regardless of delivery method, the effect of raising cAMP on fibroblast activation and population in the aortic valve area will be analyzed. If myofibroblast activation plays an essential role in pathophysiology, preventing CF transformation may prevent or hinder CAS progression. It may be possible that CF to myofibroblast transformation, which may be prevented by raising cAMP levels in the fibroblast, is necessary for aggressive calcification seen in severe CAS.

A clinical parallel that can be studied for this aim involves the long-term tracking of patients at risk for CAS. ACE inhibitors and angiotensin receptor blockers (ARB) are routinely prescribed for patients with hypertension or CHF. It may be interesting to track any correlation with CAS high risk patients who are taking the ACE inhibitor/ARB regimen with decreased fibroblast activation and CAS severity with those that are not prescribed such a regimen. Because it is known that inhibiting the renin-angiotensin pathway can inhibit CF activation, it may also serve to prevent the formation of calcific lesions seen in CAS.

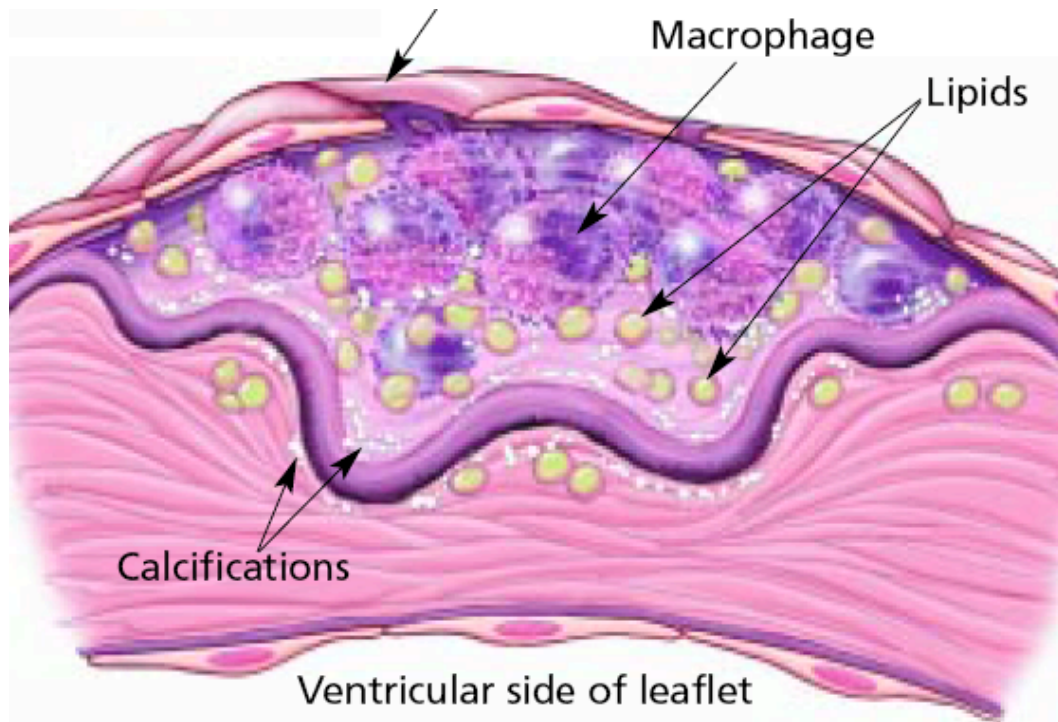


Figure 1. from Novaro G.M., Griffin B.P., Calcific aortic stenosis: Another face of atherosclerosis? *Cleveland Clinic Journal of Medicine*. 2003; 70:471-477

-
- ¹ Wiggers, Carl J., *Physiology in Health and Disease*. Lea & Febiger; 5th ed., 1949
- ² Katz, Arnold M., *Heart Failure: Pathophysiology, Molecular Biology, and Clinical Management*, Lippincott Williams & Wilkins, 2000
- ³ Lindroos M., Kupari M., Heikkila J., Tilvis R., Prevalence of aortic valve abnormalities in the elderly: an echocardiographic study of a random population sample. *J Am Coll Cardiol*. 1993; 21:1220 –1225.
- ⁴ Novaro G.M., Griffin B.P., Calcific aortic stenosis: Another face of atherosclerosis? *Cleveland Clinic Journal of Medicine*. 2003; 70:471-477
- ⁵ Mohler E.R., et al., Detection of osteopontin in calcified human aortic valves. *Arterioscler Thromb Vasc Biol*. 1997; 17:547-552
- ⁶ Lenga Y., et al. Osteopontin expression is required for myofibroblast differentiation. *Circ Res* 2008; 102(3): 319-327
- ⁷ Rajamannan N.M., Calcific aortic stenosis: Lessons learned from experimental and clinical studies. *Arterioscler. Thromb. Vasc. Biol*. 2009; 29:162-168
- ⁸ Goldsmith E.C., et al., Organization of fibroblasts in the heart. *Dev Dyn* 2004; 230(4): 787-794
- ⁹ Swaney J.S., et al., Inhibition of cardiac myofibroblast formation and collagen synthesis by activation and overexpression of adenylyl cyclase. *Proc Natl Acad Sci*. 2005; 102(2): 437-442