cases of isolated MVP. In a family suffering a mild form of MVP, we found a non-synonymous rare variant in the Rac1 specific GTPase Activating Protein *ARHGAP24* gene encoding ARHGAP24-D92N mutant protein. Interestingly, ARHGAP24 protein was previously shown to interact with FlnA and was thus named FilGAP. Also, our previous studies established the potential involvement of FilGAP and small GTPases (RhoA, Rac1) in FlnA-associated MVP. Complete exon sequencing of *ARHGAP24* in 95 additional MVP patients identified, according to genetic databases, 3 other non-synonymous rare sequence variations encoding FilGAP-R95Q; P417H and T481M mutants. Based on this genetic analysis, HEK293 cells were used to analyze the effects of theses mutations. The role of FilGAP is to decrease Rac1 activity and we showed that all the mutations identified are loss of function mutations mutated leading to increase Rac1 activity. Increased spreading of mutant FilGAP expressing cells corroborates this increase in Rac activity. In addition, we showed FlnA poorly co-immunoprecipitates FilGAP-P417H and T481M suggesting reduced FlnA/FilGAP interaction might be the molecular mechanism responsible for the loss of function of these two mutations. The molecular mechanism involved for the two other mutations (R92Q and D92N) is under investigations. Together, we identified a new gene involved, like FlnA, in cellular mechanical stress response pathway suggesting common physiopathological mechanisms are at work in FilGAP and FlnA associated valvulopathy.

0122

The serotonergic system in pathological human cardiac valves. What is the role of progenitors cells expressing the 5-HT2B receptor?

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Many compounds (pergolide, cabergoline, fenfluramine, ectasy) were described as inducers of fibrotic valvular lesions, a rare but severe drug reaction. All these drugs share in common the pharmacological property to activate a serotonergic receptor subtype, the 5HT2B. Together with the well known “carcinoid heart” that is a valvulopathy due to high amounts of circulating serotonin, these observations lead to the hypothesis that cardiac valves express a "serotonergic system" that could be activated by 5-HT or 5-HTR agonists. The aim of this work was to characterize the pattern of expression of 5-HT2A,2B,4 receptors, the serotonin transporter (SERT) and the biosynthesis peripheral enzyme (Tph1) in various valvulopathies. Thirty degenerated human valves were collected: 11 calcified aortic valves (CAV), 5 sclerotic aortic valves (SAV), 11 dystrophic mitral valves (DMV). They were analyzed by RT-qPCR and immunohistochemistery. All samples express 5HT2A,2B,4 receptors, SERT and Tph1. In these valve tissues, the amount of 5HT2B receptor (5HT2B R) mRNA is higher than the 5HT2A one (5HT2A R) : Δ Ct (5HT2B R -18S) = 12,53±1,12 vs Δ Ct (5HT2A R -18S) = 15,95±2,37 for CAV, Δ Ct (5HT2B R -18S) = 13,04±2,62 vs Δ Ct (5HT2A R -18S)=16,00±1,46 for SAV, Δ Ct (5HT2B R -18S) = 12,34±0,77 vs Δ Ct (5HT2A R -18S) = 16,14±0,86 for DMV. The amounts of SERT, Tph1 and 5HT4 receptor mRNA are negligible whatever valve and etiology. At a topographical point of view, 5HT2BR expression is found in endothelial cells (at the valve surface) but also inside valve lesions, by interstitial cells (smooth muscle α-actin and vimentin positive cells) located in an abundant glycosaminoglycan matrix. Characterization of these cells is in progress. In particular, we characterize the high amount CD34+ hematopoietic progenitors that are highly present in fibromyxoid lesions. To summarize, 5HT2A,2B,4 receptors, SERT and Tph1 are expressed in aortic and mitral diseased valves. The amounts of 5HT2A,2B R mRNA are equal between mitral and aortic valves. The contribution of the two 5-HT2 receptors in valve degeneration is now under investigation whatever the pathological process considered.

0312

Characterization of human valvular interstitial cells isolated from normal and fibrocalcified aortic valves

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Purpose: Aortic Valve Stenosis (AVS) affects 2% to 6% of population over 65 years in industrialized countries. This atherosclerosis-like pathology involves Valve Interstitial Cell (VIC) proliferation and commitment to osteoblast-like cells. This prevalent cell type of aortic valve presents five identifiable phenotypes: embryonic progenitor endothelial/mesenchymal cells, progenitor, quiescent, activated and osteoblastic VICs. To study the pathophysiology of AVS, their ***in vitro*** cultures are frequently used. Our purpose is to characterize VICs isolated from normal and fibrocalcified human aortic valves and analyze their ***in vitro*** behavior.

Methods: We collected 5 normal and 5 fibrocalcified human aortic valves. VICs were isolated by collagenase digestion. Characterization is assessed at different passages (2 to 5) by immunofluorescence. Analyzed markers consist of progenitor cell markers (SSEA4, ABCG2, CD90, NG2 and OsteoBlast CaDHerin (OB-CDH)), fibroblast markers (vimentin and HSP47) and smooth muscle cell (SMC) marker (α-actin). By blue trypan and MTS, we compared the viability and proliferation of VICs in standard and starvation medium at 48 hours.

Results: Independently of their origin, VICs express all progenitor cell markers. Fibroblasts markers are expressed twice more by pathological VICs and four times more for SMC marker. In standard medium, VICs viability is similar (96,7±2,4 % ***vs*** 96,4±2,3 % ; normal ***vs*** pathological ± SEM). Pathological VICs proliferate more than normal VICs (2,2±0,7 ***vs*** 1,6±0,4 ; OD/OD control). In starvation medium, viability is significantly reduced for pathological VICs (89,6±7,9 % ***vs*** 76,5±5,3 %) but still proliferate in opposition with normal VICs (1,7±0,6 vs 1,2±0,3).

Conclusion: All VICs phenotypes are found ***in vitro*** with no culture selection but in different ratios according to their origin. These new data in VICs isolated from normal or pathological human aortic valves allow us to approve their use ***in vitro***.