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Strong association between activated valvular interstitial cells and histopathological lesions in porcine model of induced mitral regurgitation ☆

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Myxomatous mitral valve disease (MMVD) is characterized by valvular degeneration and weakening of the mitral valve apparatus leading to mitral valve prolapse and mitral regurgitation (MR) [1–3]. These pathological changes result from dysregulation of extra-cellular matrix homeostasis including deposition of glycosaminoglycans, disruption of collagen and elastin, and transformation of valvular interstitial cells (VIC) to active myofibroblasts characterized by the presence of alpha smooth muscle actin (α-SMA) [4–6]. It is generally accepted that activated myofibroblast contribute to myxomatous changes found in MMVD [4,6–8]. However, the degree to which hemodynamic changes associated with MR contribute to myofibroblast transformation and the progression of myxomatous pathology is unclear [9]. A study of surgically-induced MR in sheep suggests that turbulent flow in the vicinity of a mitral leaflet may be sufficient to induce mitral valve remodeling [10]. Chronic MR furthermore leads to left-sided volume overload which over time causes chamber dilation, wall thickening and compromised myocardial function [11]. Ventricular intramyocardial arteriosclerosis and fibrosis have been reported in the left ventricle (including papillary muscles) in canine MMVD [12,13]. As part of the mitral valve apparatus, this could cause or further perpetuate MR.

In the present study, we investigated the effect of MR on mitral valve and myocardial remodeling, including transformation of VIC into active myofibroblasts. Data results from 28 female pigs, 10–12 weeks old. At study start and end (a time-span of 8 weeks), clinical examination, blood sampling and echocardiography were performed. The pigs underwent surgical induction of MR or sham operation as previously described [14] and based on final severity-level of MR (relative to the size of the left atrium [15]), the pigs were divided into 3 groups: mild MR (mMR, $10\% < MR \leq 50\%$, $n = 10$), moderate to severe MR (sMR, $MR > 50\%$, $n = 6$) and control (CON, $MR \leq 10\%$, $n = 12$) (Table 1). The anterior mitral valve (MV) leaflet and samples of left ventricular anterior papillary muscle (AP) and free wall (LV) were collected. MV degeneration was graded macroscopically (veterinary Whitney scale [16]) and histopathologically. Myocardial fibrosis and narrowing of intramyocardial arteries (lumen/area ratio, LAR [12]) were graded histologically. Valvular α-SMA expression was analyzed and graded immunohistochemically. Group-wise analyses were performed using Fisher's exact test and Bonferroni-correction was applied on post-hoc pair-wise comparisons. Table 1 summarizes the results.

Whitney score ($p = 0.0009$) and severity of histopathological lesions ($p = 0.01$) differed significantly among groups, with higher scores in sMR compared to CON ($p = 0.01$ and $p = 0.009$, respectively). The valvular lesions consisted primarily of increased cellular density and/or fibrosis, but without the presence of myxomatous degeneration. More extensive valvular lesions were found only in the mMR and sMR groups and are described in further detail in Table 2. In the induced-MR groups, lesions were located mainly to the atrialis and extended into the spongiosa when more extensive. Interestingly, in the CON group 50% of the lesions were found at the non-flow side of the valve. Fig. 1A–C illustrates valves with none, moderate or extensive histological lesions. For α-SMA, there was an increased amount of positive cells in the mMR ($p = 0.01$) and sMR ($p = 0.03$) compared to CON (overall $p = 0.002$), but with no difference between the mMR and sMR groups. Of great interest was the finding of co-localization between lesions and α-SMA positive cells in 83% of the valves with moderate or severe lesions

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Table 1
Results summary.

	CON (N = 12)	mMR (N = 10)	sMR (N = 6)
Echocardiography			
MR pre (%)	5 (0–5)	5 (1.25–10)	5 (7.5–10)
MR post (%)	5 (3.75–10)^{2,3}	22.5 (20–30)^{1,3}	95 (82.5–100)^{1,2}
Mitral valve grading			
Macroscopically[a] (%) (0/1/1.5/2/2.5)	27/73/0/0/0^{3}	0/44/33/11/11	0/20/0/80/0^{1}
Histopathologically (%) (none/focal/moderate/extensive)	45/55/0/0^{3}	50/12.5/12.5/25	0/20/40/40^{1}
VIC activation/α-SMA (%) (none/mild/moderate/heavy)	0/89/11/0^{2,3}	0/12.5/75/12.5^{1}	0/17/66/17^{1}
Myocardial remodeling			
LV fibrosis[a] (%) (0/0.5/1)	67/33/0^{3}	40/50/10	0/50/50^{1}
AP fibrosis[a] (%) (0/0.5/1)	58/42/0	40/40/20	0/60/40
LV LAR (ratio)	0.422 (0.373–0.454)	0.424 (0.308–0.480)	0.397 (0.372–0.470)
AP LAR (ratio)	0.349 (0.291–0.415)	0.375 (0.317–0.441)	0.408 (0.371–0.464)

The table summarizes results from 28 pigs subjected to induced mitral regurgitation (MR) or sham operation. The groups were: control (CON, MR ≤ 10%), mild MR (mMR, 10% < MR ≤ 50%) and moderate–severe MR (sMR, MR > 50%). Echocardiographic variables were analyzed by non-parametrical Kruskal–Wallis test of Wilcoxon rank sum scores and are listed as median and interquartile ranges. Categorical variables were analyzed using Fisher's exact test and are listed in percentages. All pair-wise comparisons were Bonferroni-corrected. Within each row, superscript numerals indicate that the group is statistically significantly different from CON[1], mMR[2] and sMR[3]. Non-italicized superscripts indicate a p value <0.05 and italicized superscripts indicate a p value < 0.01. α-SMA: alpha smooth muscle actin, AP: anterior papillary muscle, LAR: lumen/area ratio (intramyocardial arteries), LV: left ventricle, post: study end, pre: study start. MR% is previously reported in a parallel study [14].
[a] Original scales range from 0 to 4 in macroscopical Whitney score and fibrosis score [12,16].

(Fig. 1D–F) but in none of the valves with none or mild lesions. For myocardial remodeling, only sMR had a higher LV fibrosis score compared to CON ($p = 0.04$) (Table 1 and Fig. 2).

A hallmark of MMVD is differentiation of the VIC from a quiescent fibroblast phenotype to the activated myofibroblast characterized by the expression of α-SMA [7,17,18]. In the current study, the presence of MR led to upregulation of α-SMA in valvular cells. Only mild VIC activation was found in the control group, whereas presence of either moderate or extensive activation was found in both mMR and sMR groups. Not surprisingly, the valves with the most severe lesions and highest Whitney score also showed the strongest presence of α-SMA, however, not necessarily restricted to the sMR group. This could be explained by local differences in turbulence, shear stress and jet-direction between the intervention groups [19]. A previous study in sheep showed that MR alone was sufficient for valvular remodeling, which was attributed to increased turbulence and shear stress [10]. The study, however, did not separate the intervention group into various levels of MR-severity as done in the current study. Left ventricular dilation was found in the sMR group as reported previously [14]. By affecting the size of the mitral annulus, this could lead to increased valvular tension, which may have contributed to VIC-activation [20,21].

The pathological findings revealed a difference in the severity of histopathologic lesions between CON and the intervention groups. The control group had fewer and milder lesions, which were distributed differently, involving the non-flow side of the valve. This indicates that even innocent MR leads to a certain level of stress. Perhaps this reflects a rather dynamic environment where lesions can be located in multiple locations and possibly resolve and "re-locate" with a changing flow pattern. Mild MMVD prior to study start could be a cause of mild lesions, however, unlikely considering the young age of the pigs.

The histopathologic lesions found in this study involved mainly the atrialis, but extended into the spongiosa when more extensive. Interestingly, subendothelial accumulation of myofibroblasts (activated VIC) is a well-known feature of MMVD [3,7,22–24]. Lesions consisted mainly, though, of increased cellular density and fibrosis, but the findings represent a response caused by abrupt induction of MR. Unlike spontaneous (slowly progressing) disease, the pigs experienced very immediate changes in blood flow. This could explain the lack of ECM components such as glycosaminoglycans and proteoglycans found in spontaneous MMVD. Eight weeks may have been an insufficient time for extensive myxomatous degeneration, which was also reflected in Whitney scores no higher than 2–3. Considering this short duration of MR, significant lesions were induced and the induction of α-SMA is very intriguing. This confirms that hemodynamic changes participate in VIC-activation, which could mediate myxomatous-like changes over time.

Table 2
Additional valvular histopathology.

Histopathological description	N
Subendocardial fibroblasts and macrophages	2
Foci of inflammatory cells	1
Central necrosis	2
Neutrophilic plugging of vessels	2
Extensive cellular proliferation with cartilaginous metaplasia	1
Focal hemorrhage	1
Acute focal endothelial thrombosis	2

The table describes additional histopathological findings in mitral valves with more extensive histopathological lesions. Extensive lesions were found solely among pigs subjected to induced mitral regurgitation (MR) and were present in both the mild and moderate–severe MR groups. Several of the above characteristics could be found within the same lesion/valve but in none of the mildly affected valves or sham-operated control pigs.

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