Pathology, protein expression and signaling in myxomatous mitral valve degeneration: Comparison of dogs and humans

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Serotonin;
Valve interstitial cells

Abstract  Myxomatous degenerative mitral valve disease (MMVD) is a common heart disease in dogs. Although several morphological similarities occur between human and canine MMVD differences exist. However, in advanced stages the accumulation of proteoglycans is the main finding in both species.

The extracellular matrix (ECM) in normal canine and human mitral valves is similar. In MMVD of both species proteoglycans is the major alteration, although specific changes in collagen distribution exists.

The valvular expression pattern of matrix metalloproteinases (MMPs) and of their inhibitors (TIMPs) differs, in part, between dogs and humans. The MMPs and TIMPs expression patterns are similar in normal canine and human mitral valves, but they are quite different during degenerative progression.

Valve endothelial cells (VEC) and interstitial cells (VIC) are phenotypically transformed in canine and human MMVD. Inflammation is an unlikely cause of valve degeneration in humans and dogs. There are several lines of evidence suggesting that transforming growth factor β1 (TGF β1) and serotonin signaling may mediate valve degeneration in humans and dogs.

Although human and canine MMVD share structural similarities, there are some differences in ECM changes, enzyme expression and cell transformation, which may reflect a varied pathogenesis of these diseases.

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Introduction

Myxomatous mitral valve disease (MMVD) is a common acquired cardiac disease in older small and medium-sized dogs.\(^1,2\) The mitral valve is comprised of two leaflets (anterior or septal and posterior or mural leaflets). Normal valve leaflets are grossly thin and transparent (Fig. 1A).\(^1\) The myxomatous valves are macroscopically characterized by nodular thickening of the valve leaflets (Fig. 1B).\(^3\) The chordae tendineae are elongated.\(^4–6\) Myxomatous mitral valve disease develops mainly in dogs older than seven years of age.\(^1\) Males are more susceptible to develop the disease than females.\(^7\) The etiology of canine MMVD is still unclear. Hemodynamic, endogenous and genetic factors have been discussed.\(^7,7,8\) In dogs, breeds predisposed to connective tissue disorders including intervertebral disc disease, tracheal collapse and cruciate ligament rupture are also affected with MMVD.\(^9\) Such breeds include Dachshund, Cocker spaniel, Poodle, Schnauzer, Chihuahua and Terriers.\(^1\) This disease is considered to be inherited in some breeds including Cavalier King Charles Spaniel (CKCS) and Dachshund.\(^10–13\)

Myxomatous mitral valve disease in CKCS occurs at an early age and may be caused by a polygenic inheritance.\(^12\) Clinically, the degeneration of the valves and corresponding chordae tendineae causes an inappropriate coaptation of the leaflets leading to valve regurgitation and finally resulting in left sided congestive heart failure.\(^1,2,14–17\)

In man, a morphologically similar disease (Fig. 1C) has been described.\(^18\) Human MMVD causes prolapse or billowing of mitral valve leaflets into the left atrium during systole. The prevalence of MMVD in the human population is associated with age.\(^19\) Mitral valve prolapse, a risk factor for development of MMVD later in life, is more prevalent in females than males.\(^20\) Patients with mitral valve prolapse tend to have low body mass index, but the reason for this phenomenon is uncertain.\(^21\) The etiology of human MMVD is unknown. The risk factor of mitral valve prolapse may be inherited as an autosomal dominant or a polygenic abnormality.\(^22,23\) It often displays familial transmission.\(^24\) Connective tissue disorders

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**Figure 1** Gross findings in mitral valves (A) Normal mitral valve from a 3-year-old dog. The leaflets are thin and regular and the chordae tendineae are uniform. (B) Marked chronic valve disease from 8-year-old dog: The leaflets of the mitral valve are thickened, white in colour and deformed with the free edges rolled upward. There is evidence of chordal rupture (C) Human surgically removed diseased valve from 59-year-old male. The valve is thickened with irregular surface and hemorrhage.

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**Abbreviations**

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<tr>
<td>CKCS</td>
<td>Cavalier King Charles Spaniel</td>
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<td>MMVD</td>
<td>Myxomatous mitral valve disease</td>
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<td>MMP</td>
<td>Matrix metalloproteinase</td>
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<td>MVP</td>
<td>Mitral valve prolapse</td>
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<td>TGF</td>
<td>Transforming growth factor</td>
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<td>TIMP</td>
<td>Tissue inhibitor of metalloproteinase</td>
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<td>VEC</td>
<td>Valve endothelial cell</td>
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<td>VIC</td>
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including Ehlers-Danlos-Syndrome, Osteogenesis imperfecta, Marfan syndrome and Stickler-Syndrome are strongly associated with MVD in humans.  

Normal canine and human mitral valves have three well-defined layers (Fig. 2A): (1) The atrialis layer is mainly composed of elastic fibers. (2) The spongiosa layer is rich in glycosaminoglycans. (3) The fibrosa layer predominantly consists of densely-packed collagen bundles.

The pathology of myxomatous valves in humans and in dogs includes increased cellularity, valve structure disorganization as well as transdifferentiation of valve endothelial (VEC) and valve interstitial cells (VIC). 

In canine degenerative valves, the changes occur primarily in the atrialis layer by an accumulation of transformed VIC. In advanced stages the major structural change, glycosaminoglycan infiltration, appears in the spongiosa layer in both human and canine MMVD (Fig. 2B and C). The degeneration is most pronounced at the free margins of the leaflets. When disease progresses, the amount of glycosaminoglycans increases and invades into the other layers. The elastic fibers become fragmented and split. The collagen bundles are disorientated by glycosaminoglycan infiltration. The collagen fibrils also appear disrupted. Similar changes occur in the chordae tendineae.

In humans, the posterior leaflet is more often and more severely affected than the anterior leaflet. In contrast, the anterior leaflet is more frequently involved in canine MMVD (Table 1). In human myxomatous valves, linear thrombus may be present at the base of the leaflet next to the left atrial wall. In dogs, platelets adhering to the subendothelial collagen have been detected in endothelial lesions, but marked thrombus formation has not been reported. It remains to be determined whether abnormalities of platelet function and morphology or abnormalities in coagulation factors such as plasminogen, are responsible for this finding. The occurrence of stroke secondary to emboli has been reported in humans affected with MMVD. However, some studies suggested that this phenomenon might be
a coincidence. The occurrence of thromboembolism has not been reported in canine MMVD.

### Extracellular matrix

In heart valves, the extracellular matrix (ECM) is produced by endothelial and valve interstitial cells.\(^45,46\) ECM is a complex dynamic physical and chemical network consisting of several components including collagen, elastin, fibronectin, laminin, proteoglycans and glucosamines.\(^47\) ECM is not a static structure, but is a dynamic network constantly being remodelled. The balance between synthesis and degradation maintains a normal structure and homestasis of the valves.\(^48,49\)

About 14 types of collagen have been described.\(^50\) In heart valves of several species, collagen types I, III, IV, V and VI have been identified.\(^31,46\) Collagen I and III are mainly produced by fibroblasts. Collagen IV and VI are synthesized by endothelium, fibroblasts and cardiomyocytes\(^51,52\) and are part of basement membranes.\(^53–55\) Collagen VI is secreted as a tetramer and aggregates extracellularly.\(^56\) It is very resistant against proteolytic digestion and is not detectable by most analytical methods.\(^54,57\) Aupperle et al.\(^31\) described collagen VI for the first time in normal adult canine heart valves and valvular degeneration. Comparable data in humans is not available. Biochemical analyses of collagen VI in canine mitral valves quote \(472 \pm 49\) mg/g collagen content as normal.\(^8\) More detailed biochemical analyses of human mitral valves are available: water content in normal mitral valves is 83%. The collagen, elastin and proteoglycan contents of normal human valves are approximately 60%, 10% and 30% of the dry weight, respectively.\(^33,45,54\)

Elastin and fibronectin are produced from smooth muscle cells, fibroblasts and endothelium.\(^58–60\) Laminin is part of the basement membrane of endothelium and cardiomyocytes.\(^61,62\) The primary role of laminin is the mediation of interactions between cells and the ECM, but laminin can also bind to glycosaminoglycans.\(^62\) Proteoglycans (also known as mucopolysaccharides) are the main component of the ECM\(^63\) and are involved in degenerating processes of elastic fibers.\(^33\)

In general, the specific distribution pattern of ECM components in the mitral valve reflects the mechanical forces during systole and diastole: elasticity of the atrialis layer, stability of the fibrosa layer and flexibility of the spongiosa layer.\(^64\)

Immunohistochemical analyses of the normal canine mitral valve showed that the subendothelial basement membrane consists of moderate amounts of laminin and fibronectin, and small amounts of collagen I, III, IV, VI and heparan sulphate. The atrialis layer is mainly composed of elastic fibers, collagen I, III and VI. The spongiosa layer contains proteoglycans and collagen VI and the fibrosa layer consists of collagen I, III, and collagen VI (Fig. 3A).\(^31\) These findings are in accordance to published data in normal human mitral valves.\(^46,65\)

In human MMVD, the amount of collagen I is mildly decreased and collagen III increased (normal: 74% collagen I, 24% collagen III; MMVD: 67% collagen I, 31% collagen III).\(^45\)

The study from Hadian et al.\(^66\) showed a 10% reduction in total collagen and a 20% reduction in fibrillar collagen content in the myxomatous areas of canine valves with mild to moderate MMVD. Unstable, immature and abnormally cross-linked collagen molecules were identified.\(^66,67\) Furthermore, an accumulation of proteoglycans, lipids and hexosamines was detected in canine MMVD.\(^5,68,69\)

Immunohistochemical studies in mild canine MMVD showed an irregularly split basement membrane. The ECM components, collagen VI and laminin were mildly increased and spread into the atrialis layer. Collagen I and III were no longer arranged in their typical layers, but formed a loose

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and fine fibrillary network in the atrialis and spongiosa layer, resulting in a less intense staining reaction. It may be speculated that the atrial deposition of laminin, fibronectin and collagen IV, act as an intensified diffusion barrier, presumably causing malnutrition of underlying tissues. In advanced canine MMVD, nodular lesions were characterized by peripheral fibronectin accumulations. The intense immunohistochemical expression of fibronectin is in accordance to microarray gene expression analyses performed by Oyama and Chittur, who reported a 3.7 fold increase of fibronectin expression in canine MMVD in comparison to healthy controls. Collagen I was diffusely present throughout the nodular lesions, while collagen III was moderately positive mainly in the center of the nodules. Collagen VI was markedly increased (Fig. 3B). Laminin was not only associated with the basement membranes, but also mildly deposited multifocally on the periphery of the myxomatous nodules.

To the best of the authors’ knowledge, comparable investigations in human MMVD have not been published. However, various congenital connective tissue disorders (Ehlers-Danlos-Syndrome, Osteogenesis imperfecta, Stickler-Syndrome, Marfan-Syndrome) have been reported to correlate to MMVD in humans.

In summary, the ECM in normal canine and human mitral valves is similar. In dogs the early lesions appear in the atrialis layer. In man there are no studies of early alterations. However, in advanced stages, the accumulation of proteoglycans is the main finding in both species (Table 1).

**MMPs/TIMPs**

The ECM is not static, as it is constantly being remodelled and in equilibrium by the cellular synthesis of ECM components and their breakdown by different enzymes. Matrix metalloproteinases (MMPs) appear to play an important role in physiological processes and in the pathogenesis of various diseases affecting the ECM.

MMPs are divided into six groups: (1) collagenase (MMP-1, MMP-8, MMP-13, MMP-18), (2) gelatinase A and B (MMP-2, MMP-9), (3) stromelysin 1 and 2 (MMP-3, MMP-10), (4) stromelysin 3 (MMP-11), (5) metalloelastase (MMP-12), (6) membrane-bound MMPs (MMP-14, MMP-15, MMP-16, MMP-18). In cardiovascular tissues, predominantly MMP-1, -2, -3, -9, -13 and -14 play a role.

MMPs are Ca\(^{2+}\) and Zn\(^{2+}\) dependent proteases that are produced and secreted as inactive zymogens pro-MMPs. Several mediators may induce transcription, translation, secretion or activation of MMPs, for example, angiotensin II, endothelin-1, catecholamines, norepinephrine, TNF-α and interleukine-1β. Oxidative stress or mechanical stress may also alter MMPs and tissue inhibitor of metalloproteinase (TIMPs) expression. Serine proteases as trypsin, plasmin and urokinase may also activate MMPs.

MMPs are inhibited by specific tissue inhibitors TIMPs. Four types of TIMPs, TIMP-1 to TIMP-4, have been described which are all expressed in the myocardium. They are mostly expressed by the same cells which also produce the MMPs. The balance of MMPs and TIMPs appears to be vital for the regulation and homeostasis of the ECM.

The expression of MMPs and TIMPs has been investigated in normal human embryonal, adult,
degenerative canine mitral valves as well as in normal and altered human heart valves.

Immunohistochemical studies in normal canine mitral valves showed: MMP-1, MMP-2, MMP-14 (Fig. 4A) and TIMP-2 were mildly expressed in individual interstitial cells and TIMP-3 (Fig. 5A) expression was intense in numerous interstitial cells in all layers. In contrast, MMP-2, was not seen in normal valves in the study from Obayashi et al. (2011). MMP-9 was not identified in VIC, but was extensively expressed in cardiomyocytes. However, mRNA of MMP-9 was detectable in normal canine mitral valves.

The normal findings in dogs partially contrast to the immunohistochemical studies in normal mitral valves in humans (Table 2), which showed an intense expression of MMP-1, TIMP-1, and TIMP-2, but no or little expression of MMP-2, MMP-3, MMP-9 and TIMP-3 in VIC. MMP-14 expression was not detected in normal or diseased human mitral valves.

With increasing severity of canine MMVD (Table 3) the immunohistochemical expression of MMP-2, and MMP-9 decreased, but MMP-14 (Fig. 4B), TIMP-2 and TIMP-3 (Fig. 5B) expression increased markedly. In contrast, Obayachi et al. found increasing expression of MMP-1 and MMP-3 but negative labeling for MMP-2 and MMP-9 in MMVD. There was a correlation between the elevation in mRNA encoding MMP-14, TIMP-2 and -3 in MMVD, and the expression of these proteins within similarly affected mitral valves. The expression of MMP-1 and MMP-13 progressively increased with advancing disease severity. Furthermore, the concentration of mRNA encoding MMP-14 and TIMP-3 was significantly elevated, and there was additional transcription of genes encoding MMP-1, TIMP-2 and -4 which were not transcribed in normal mitral valves.
The results of the studies in canine MMVD contrast with findings in myxomatous human mitral valves in which there is increased expression of genes encoding MMP-1, -2, -9 and -13 compared with normal valves. Furthermore, the elevated MMPs expression was identified immuno-histochemically in the VIC. The expression of TIMPs was not investigated in these studies. Interestingly, a significant promoter polymorphism of the MMP-3 gene was identified correlating to the severity of MMVD in humans. Elevated expression of MMP-2, TIMP-1 and TIMP-2 was seen in endocarditis, and in some cases of human degenerative valve disease.

In summary, the MMPs and TIMPs expression patterns are similar in normal canine and human mitral valves, but they are quite different during degenerative progression. In canine MMVD, the

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expression of TIMPs is the main finding. In contrast, elevated MMP expression is characteristic in human MMVD. These differing results in normal and myxomatous heart valves in dogs and humans probably reflect different pathogenic pathways of degenerative valve disease, although a progressive course of the disease is seen in both species.

Cells

Two major cell types, VIC and VEC, exist within the valve. These cells have a major function to maintain valve homeostasis in normal conditions and participate in valve repair as well as remodeling in diseased valves. Both VIC and VEC have the ability to produce ECM, chemokines and catabolic enzymes such as MMPs and their tissue inhibitors (TIMPs). These cells have unique characteristics which are different from those described in interstitial and endothelial cells from other tissue types. Furthermore, it has been suggested that VIC isolated from aortic, pulmonary, mitral and tricuspid valves express some molecular components that are different from each other indicating that VIC in different types of valves may have a specific functional role.

Valve endothelial cells

The VEC layer comprises of a single cell lining on the surface of both leaflet sides. Vascular endothelial cells function as a barrier between the leaflets and the blood. Damaged or denuded areas of the endothelial layer have been identified in dogs and humans with MMVD. The damaged area was mostly seen in the distal part of the leaflets. The endothelial cells in affected valves were swollen, separated or had loose contact to each other. Calcium crystals were occasionally found in the ulcerated areas of the endothelial layer of affected valves in humans, but not in dogs. In canine myxomatous valve tissues, VEC appeared with prominent nuclear bulges and became activated by transdifferentiating into α-actin positive cells or myofibroblasts. The endothelial lining looked irregular and not smooth like in normal valves (Fig. 6A and B). An increased NADPH-diaphorase activity and subsequent augmented nitric oxide synthase expression has been found on endothelial cells in areas of myxomatous changes of canine mitral valves. A higher endothelin receptor presentation was also seen in endothelial layers of myxomatous valves in dogs.

Valve interstitial cells

Valve interstitial cell is a major cell type in the heart valve and scatters throughout in all three valve layers. Valve interstitial cells are thought to have a role in maintaining normal valve structure.
and function. An increased number of VIC was seen in human and canine degenerative valves.\textsuperscript{30,32,95} Most VIC accumulated in the atrialis layer.\textsuperscript{30,96} Several researchers suggested that an increased cellularity may be due to the proliferative process. However, a proliferative marker, Ki-67 antigen was not elevated in canine myxomatous valves.\textsuperscript{30} In addition, by using a computerized imaging tool, the number of cells in myxomatous areas was not different in various degrees of the disease suggesting a constant number of cells within the canine diseased valves during the disease progression.\textsuperscript{97}

A similar image was seen in human myxomatous valves. However, image analysis has not been studied yet. In diseased valves, VIC undergo phenotypic transformation. Some VIC turn into an \(\alpha\)-actin positive phenotype.\textsuperscript{30,32} In both human and canine degenerative valves, these \(\alpha\)-actin positive cells or myofibroblasts mostly accumulated in the area underneath the endothelium particularly on the atrial side of the leaflets (Fig. 7A and B).\textsuperscript{98} Several researchers believe that myofibroblasts or \(\alpha\)-actin positive VIC may be a major phenotype that mediates degeneration. A study on human myxomatous valve disease suggested that \(\alpha\)-actin positive VIC might be an activated VIC phenotype which play a role in producing catabolic enzymes and ECM.\textsuperscript{32} However, a co-localization study in dog tissues showed that the expression of MMP-1 and MMP-13 was not restricted to \(\alpha\)-actin positive cells.\textsuperscript{30} In agreement with an immunohistochemical study, an electron microscope study in canine valve tissues suggested that myofibroblast VIC may not have a primary function in production of ECM.\textsuperscript{29}

Secondary to smooth muscle-like structure within these cells, it has been suggested that myofibroblasts may have contractile properties and act as a supporter to maintain valve tone in diseased canine valves. This considers that \(\alpha\)-actin VIC or myofibroblasts in human and canine diseased valves may have different functions in the degenerative process. Several studies performed in human tissues suggested that the myofibroblast might be a precursor that implicates the alteration in valve structure. However, several lines of evidence in canine studies implied that transformation of VIC into myofibroblast phenotype might be a later consequence of the diseased process. The non-muscle embryonic heavy chain myosin, a marker of mesenchymal cell activation was expressed in human and canine diseased valves in a similar pattern of distribution.\textsuperscript{30,32} This VIC phenotype was suggested to be a marker of cell activation in close correlation with pathologic changes within the valves and displayed a similar pattern of distribution with MMP.\textsuperscript{30} An increased expression of CD34, a hematopoietic stromal cell marker involved in stromal cells repair was reported in human mitral valve disease.\textsuperscript{99} This marker has not been studied yet in canine valves. In humans, another phenotype of VIC called osteoblastic VIC has been suggested.\textsuperscript{100} This phenotype has been thought to be involved in forming calcific nodules and cartilaginous structures in human degenerative valves.\textsuperscript{98,100–102} The expression of osteoblastic VIC has not been reported in canine myxomatous valves. However, a chondroblastic activity has recently been described.\textsuperscript{103}

In conclusion, the phenotypic transformation of VIC in humans tends to present itself in a similar manner as in canine MMVD. However, the activity of these transformed VIC may be different in humans and dogs.

### Inflammatory cells

It has been suggested that inflammation is not involved in the degenerative process of human MMVD since the inflammatory cells including macrophages and lymphocytes were negligible in

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**Figure 7** \(\alpha\)-actin immunohistochemistry Positive cells are accumulated mainly in the atrialis layer of canine (A) (magnification 10x) and human MMVD (B) (magnification 10x).
myxomatous valves.\textsuperscript{32,91,98} In canine valves, mast cells were slightly increased in the perimeter of pathologic areas of diseased valves suggesting a potential role of mast cells in this disease. Interestingly, the human myxomatous valve is prone to develop bacterial endocarditis.\textsuperscript{104,105} However, this condition is rare in dogs.\textsuperscript{105} An appearance of mast cells may have a protective role in preventing secondary infection in canine myxomatous valves. Macrophage numbers also increase in canine diseased valves. However, evidence of direct involvement does not exist since the macrophage population mostly accumulated in the disease-free basal zone.\textsuperscript{69} The infiltration of other inflammatory cells was not detected in diseased valves.\textsuperscript{1,16,30}

However, several inflammatory cytokine genes were up-regulated in affected canine valves. It has been suggested that valve endothelium might be a major source of these inflammatory mediators.\textsuperscript{16}

In human valves, chronic inflammation and neovascularization may be seen as post-inflammatory changes.\textsuperscript{106,107} The histologic appearance of these valves showed layer structure destruction with diffuse thick-walled vessels and inflammatory round cell infiltration. However, the vascularization was hardly seen in canine myxomatous valves.

In summary, an inflammatory process is unlikely to be a cause of degeneration in human and canine valves. A post-inflammation may occur in humans\textsuperscript{101} but not in canine MMVD.\textsuperscript{30} Small amounts of mast cells were seen in canine myxomatous valves, but it is unclear if they play a role in the disease process.

The expression of signaling proteins

In affected canine valves, the \(\alpha\)-actin positive VIC and VEC strongly expressed transforming growth factor (TGF) \(\beta1\) and \(\beta3\).\textsuperscript{33,83} The results of the Aupperle et al\textsuperscript{13} indicated an increased expression of TGF-\(\beta1\) and TGF-\(\beta3\) and weak expression of TGF-\(\beta2\) in canine diseased valves. In contrast, Obayashi et al.\textsuperscript{83} described an expression of TGF-\(\beta3\) and of \(\alpha\)\textsuperscript{1}R-II in degenerated mitral valves and unchanged expression of TGF-\(\beta1\) and TGF-\(\beta2\) in clinically normal dogs and dogs with MMVD. These differences may have been attributable to the use of antibodies obtained from different manufacturers, different fixation times, or the immunohistochemistry methods that were used.

An increased expression of TGF-\(\beta1\) was seen in human myxomatous valves in a similar manner to those described in canine valves.\textsuperscript{108} Not only TGF-\(\beta\), but also their receptors are up-regulated in affected canine valves.\textsuperscript{109} In addition, localized production of TGF-\(\beta1\) has been reported in canine myxomatous valves.\textsuperscript{33,109}

The up-regulation of serotonin 5HT2B receptor mRNA and proteins has been demonstrated in canine degenerative valves.\textsuperscript{16,109} Tryptophan hydroxylase 1 (TPH1), a rate limiting enzyme for serotonin production was found up-regulated in diseased human and canine valves.\textsuperscript{109} Interestingly, the expression pattern of TPH 1 is similar to non-muscle embryonic heavy chain myosin VIC phenotype in canine and human myxomatous valves.\textsuperscript{96} The expression of the serotonin transporter, a major structure involved in cellular serotonin uptake, was decreased in late stage canine MMVD.\textsuperscript{110} Thus, there is evidence suggesting that TGF-\(\beta1\) and serotonin signaling may be related to the pathogenesis of human and canine MMVD. The roles of signaling pathways involved in myxomatous degeneration are the subject of ongoing studies.

Conclusion

Human and canine MMVD share several similarities. The structure of the degenerative valves is almost identical in both species. However, there are some differences in details such as the distribution, the type and the amount of ECM. The pathogenesis of MMVD is unclear. In human valves, ECM abnormalities secondary to inherited diseases may be the cause. The alteration of ECM homeostasis i.e. increased production and/or increased degradation by MMPs has been proposed as the cause of canine and human MMVD. The transformation of cells within the canine and human diseased valves has been found, but it is not clear whether this is the cause or result of the degeneration. Inflammation is unlikely to be the cause of MMVD in both species. Finally, changes in serotonin and TGF-\(\beta1\) signaling proteins have been discussed. It is uncertain what the exact roles of these proteins are in the pathogenesis of MMVD. Further studies are needed to clarify in more details about the similarity and difference between human and canine MMVD.

Conflicts of interest

The authors have no conflict of interest.

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Mitral valve disease protein expression and signaling


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