Expression of smooth muscle cell markers and co-activators in calcified aortic valves

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Aims

Similar risk factors and mediators are involved in calcific aortic stenosis (CAS) and atherosclerosis. Since normal valves harbour a low percentage of smooth muscle cells (SMCs), we hypothesize that the SMC phenotype participates in the pathogenesis of CAS.

Method and results

We analysed 12 normal and 22 calcified aortic valves for SMC markers and the expression of co-activators of SMC gene expression, myocardin and myocardin-related transcription factors (MRTF-A/B). Transforming growth factor β (TGFβ1) was used to upregulate SMC markers and co-activators in valve interstitial cells (VICs) and transmission electron microscopy (TEM) was used to detect the presence of SMC in atypical regions of the valve leaflets. Smooth muscle cell markers and co-activators, myocardin, MRTF-A, and MRTF-B, demonstrated an increased incidence and aberrant expression around calcified nodules in all 22 calcified valves as well as in surface and microvessel endothelial cells. Smooth muscle cell markers and MRTF-A were significantly increased in calcified valves. Transforming growth factor β1 (TGFβ1) (10 ng/mL) was able to significantly upregulate the expression of some SMC markers and MRTF-A in VICs. Transmission electron microscopy of the fibrosa layer of calcified valves demonstrated the presence of bundles of SMCs and smooth muscle-derived foam cells.

Conclusion

Smooth muscle cell markers and co-activators, myocardin and MRTFs, were aberrantly expressed in calcified valves. Transforming growth factor β1 was able to significantly upregulate SMC markers and MRTF-A in VICs. Transmission electron microscopy unequivocally identified the presence of SMCs in calcified regions of valve leaflets. These findings provide evidence that the SMC phenotype plays a role in the development of CAS.

Keywords

Human • Valve • Cardiac • Calcification • Smooth muscle cells

Introduction

Calcific aortic stenosis (CAS) is the most common valvular condition in the developed world with an incidence of 2–7% in individuals aged over 65 years, and aortic valve sclerosis is present in more than 25% of patients older than 65 years. Atherosclerosis is the most common vascular disease with clinical manifestations being present in 66% of men and 50% of women over the age of 40 years. Calcific aortic stenosis and atherosclerosis have many common features in relation to risk factors and histopathological lesions with similar pathogenic pathways and mediators being proposed in both diseases. The initiating factors include inflammation, damage to the endothelium, infiltration by inflammatory cells, lipids, low shear stress, cytokines, and reactive oxygen species. In both diseases, the tissues are tri-layered with the aortic valve leaflet being divided into the fibrosa, spongiosa, and ventricularis and the artery into the intima, media, and adventitia.

Valve interstitial cells (VICs) are the predominant cell types residing in the valve leaflets and smooth muscle cells (SMCs) form the bulk of the vessels.

The VIC population consists of a number of different phenotypic and functional types of cells. By far, the majority of the population consists of pleomorphic fibroblastic VICs with some SMCs. These VICs are derived through endothelial-to-mesenchymal transition (EMT) and maintained by a contribution of haematopoietic stem cells. Smooth muscle cells have diverse embryological origins and there are only a few reports describing the presence of SMCs in small studies of normal human aortic valves.

Fibroblasts are cells of mesenchymal origin producing a wide variety of extracellular matrix proteins, growth factors, and proteases and their characterization relies on morphological, proliferative, and
Myofibroblasts are identified by the neo-expression of \( \alpha \)-smooth muscle actin (\( \alpha \)-SMA) and the absence of desmin, smooth muscle myosin heavy chain (SM-MHC), h-caldesmon, and smoothelin distinguishing them from SMCs. They combine ultrastructural and functional features of SMCs and fibroblasts.\(^{15}\)

The term ‘smooth muscle cell’ is used to include any connective tissue cell that forms a coating around the endothelial tubes. These cells exhibit a wide range of different phenotypes at different stages of development and in different tissues.\(^{16}\) During early stages of vasculogenesis, SMCs are highly migratory and proliferative exhibiting very high rates of synthesis of ECM components. Conversely, in adult blood vessels, SMCs are non-migratory, non-proliferative, and low rates of synthesis of ECM components. The mature, fully differentiated SMC expresses a repertoire of appropriate receptors, ion channels, signal transduction molecules, calcium regulatory proteins, and contractile proteins necessary for the unique contractile properties of the SMC.\(^{17}\)

Smooth muscle cells can be identified by several markers of their differentiation/maturation, including \( \alpha \)-SMA, calponin, SM22, and SM-MHC isoforms (SM1 and SM2). Expression of SM-MHC isoforms is strictly limited to fully differentiated SMCs.\(^{18}\) Ultrastructurally, SMCs have a complete external lamina, many pinocytotic vesicles, perinuclear mitochondria, and varying amounts of myofilaments and focal densities depending on their phenotypic and functional (contractile or synthetic) state. Smooth muscle cells exhibit extensive phenotypic modulation and plasticity.\(^{19,20}\)

We hypothesized that similar to atherosclerosis, SMCs play an important role in the development of CAS and hence this study was designed to assess their expression, regulation, and unequivocal identification in normal and calcified valves.

\section*{Methods}

\subsection*{Tissue}

Twelve normal valves, free from calcification (mean age 48 years, 9 males: 3 females; age range 6–58 years; SD = 13.16) and 22 human calcified aortic valves (mean age 56.8 years, 17 males: 5 females; age range 17–61 years; SD = 23.92) were used in this study. The normal valves were obtained from patients free from cardiovascular and valvular complications based on history, macroscopic, and microscopic evaluation. These were unused valves from healthy, heart donors, and most of them died from a cerebral hemorrhage due to head trauma with no underlying diseases. The ischaemic time for fresh tissue harvest did not exceed 24 h and was comparable in control and calcified groups. None of the patients had pure insufficiency; all had predominant stenosis with varying degrees of insufficiency. Left ventricular dysfunction was not considered a contraindication; however, patients with advanced cancer or other debilitating diseases with a life expectancy of less than 6 months were excluded. None of the calcified valves was from bicuspid valves.

Normal VICs were used for the cell culture analyses. The calcified valves used for the EM analysis were from patients without coronary artery disease. Sections of tissue from each valve were fixed in 10% formalin and sectioned for immunostaining, fixed in 3% glutaraldehyde for electron microscopy, and the remainder used for cell culture to isolate VICs. This study was approved by the Royal Brompton hospital ethics review board and informed consent was obtained from the subjects.

\subsection*{Statistics}

A Mann–Whitney U test and a Kruskal–Wallis test followed by a Dunn’s test were used to test for significance where appropriate and tests were two-sided. GraphPad Prism 5 was used for the statistical analyses. A P-value of \(< 0.05\) was considered significant.

\subsection*{Results}

\section*{Smooth muscle markers are aberrantly expressed in calcified valves}

Normal human aortic valves harbour a very small percentage of SMCs which reside in the base of the ventricularis (Figure 1). This basal region of the ventricularis stains positive for early markers of SMC expression such as \( \alpha \)-SMA and SM22, and late markers of differentiation such as desmin and SM-MHC. Occasionally, isolated or small groups of 2–6 SMC marker-positive cells were observed in the ventricularis from the base to the belly region, but no SMC marker-positive cells were observed from the belly to the co-apting edge. SMC marker-positive cells were not observed in the spongiosa or the fibrosa of normal aortic valves.

Early and late SMC marker-positive cells were also observed in their usual location within the base of the ventricularis in calcified valves. However, calcified valves demonstrated an increased prevalence of SMC marker-positive cells with an aberrant and uncharacteristic localization to the spongiosa and fibrosa layers. Numerous SMC marker-positive cells were observed within the calcified regions and notably SMCs were observed along most of the calcified front towards the ventricularis (Figure 2). Early and late markers of SMCs were detected in all three layers of the calcified valve, predominantly adjacent to the calcified regions (Figure 2B–E). Distal from the calcified regions, SMC marker positivity was not often observed. Smooth muscle cell markers and co-activators were assessed in control and calcified valves and were found to demonstrate significantly increased expression of \( \alpha \)-smooth muscle actin (\( P = 0.03\)), calponin (\( P = 0.02\)), SM22 (\( P = 0.03\)), caldesmon (\( P = 0.03\)), smooth muscle myosin (\( P = 0.03\)), and MRTF-A (\( P = 0.03\)) (Supplementary material online, Table S1). Levels of myocardin and desmin were not significantly increased (Figure 2F and G) and the MRTF-B antibody was not suitable for the western analysis (Supplementary material online, Table S1).

Calcified valves contained both myofibroblasts (\( \alpha \)-SMA-positive, SM-MHC-negative) and SMCs (\( \alpha \)-SMA-positive, SM-MHC-positive); however, in this cohort of end-stage calcified valves, SMCs were more abundant than myofibroblasts. A greater degree of SMC marker and co-activator expression was observed in cusps which were severely calcified compared with those which were initially calcified and appeared normal macroscopically, indicating that the extent of SMC expression was related to the degree of calcification; however, this requires validation in a greater number of samples.

Some of the aortic and ventricular facing surface endothelial cells (ECs), some ECs lining microvessels, and the vasculature of larger vessels resulting from neangiogenesis were also positive for SMC markers (Figures 2D and E, 3). Usually isolated surface ECs were...
not observed expressing SMC markers but groups of adjacent ECs were observed to be positive. Using double labelling, some VICs and sub-ECs expressing SMC markers were also found to co-express bone markers such as osteopontin within the fibrosa (Figure 3G).

Smooth muscle cells are not proliferative in calcified valves
No positivity for either Ki67 or PCNA with SMC markers was observed (data not shown).

Smooth muscle gene co-activators are aberrantly expressed in calcified valves
Normal valves showed expression of MRTF-A, MRTF-B, and myocardin localized to the base of the ventricularis, similar to the expression of SMC markers (Figure 4D). There was no expression of these co-activators in the spongiosa or fibrosa layers or in the belly region and free edge of the leaflets (Figure 4A–C).

Numerous cells with increased expression of all three co-activators were observed with an uncharacteristic localization to the spongiosa and fibrosa layers in calcified valves. Abundant cells demonstrated positivity for MRTF-A, MRTF-B, and myocardin adjacent to and within the calcified regions as well as in both aortic and ventricular facing surface ECs, the ECs of the microvessels, and in bundles of cells reminiscent of SMC bundles (Figure 4E–J).

All the calcified valves demonstrated an increased and aberrant expression of calponin, SM22, SM1, and SM2. An aberrant expression for desmin, smooth muscle myosin, α-SMA, and caldesmon was observed in 58–92% of the calcified valves (Figure 5A) with the expression of desmin being the least aberrantly expressed.

An uncharacteristic expression of SMC markers in ECs was observed in 41% of the calcified valves. An uncharacteristic expression of co-activators of smooth muscle gene expression was observed in 53–71% of the calcified valves (Figure 5B).

Transforming growth factor β1 is able to upregulate the expression of myocardin-related transcription factor-A and smooth muscle cell markers in human valve interstitial cells
Valve interstitial cells were treated with concentrations of transforming growth factor β1 (TGFβ1) (0.25, 1, 5, and 10 ng/mL) for 4 h and the expression of MRTF-A, MRTF-B, and myocardin was assessed. Normal untreated VICs, isolated from normal valves, showed very low numbers of MRTF-A, MRTF-B, and myocardin-positive cells. Less than 1% of normal VICs showed nuclear expression of MRTF-A and MRTF-B with no nuclear expression of myocardin. Upon treatment with TGFβ1 (10 ng/mL) for 4 h, an increased incidence of nuclear expression of MRTF-A (control: median = 2.0, IQR = 1–3.5; TGFβ1: median 6.5, IQR = 4.5–10) was observed in aortic VICs, P = 0.01 (Figure 6B–D). Transforming growth factor β1 was not able to induce the nuclear expression of MRTF-B or myocardin. Smooth muscle cell markers and MRTF-A were upregulated after treatment with TGFβ1 (10 ng/mL) for 96 h as assessed by western blotting (Figure 6E) with significant increases in α-SMA (P = 0.01), calponin (P = 0.02), SM22 (P = 0.02), caldesmon (P = 0.04), SM-MHC (P = 0.01), and MRTF-A (P = 0.02) (Supplementary material online, Table S2).
Figure 2. A haematoxylin and eosin stained section of a calcified valve (A). The calcification is on the fibrosal side of the valve. Labelled insets (B–E) are magnified on the right. Increased cellularity is seen as intense pink staining along the calcified front from the top of (C) to (E). Staining of sequential sections with omission of primary antibody (B-VE), α-smooth muscle actin (B α-SMA), and SM22 staining around a nodule (B SM22), calponin staining in abundant cells within the spongiosa (C CALP), smooth muscle myosin staining in the endothelial cells and VICs (D SMM) and caldesmon staining in VICs and smooth muscle of the vasculature (E CALD). Normalized quantitative data on the median expression levels of SMC markers and co-activators in control (CONT, n = 3) and calcified (CALC, n = 3) valves (F) and representative blots (G). N: nodule, *p < 0.05.
was not able to significantly upregulate desmin, or myocardin and the antibody for MRTF-B was unsuitable. Upregulation of SMC markers and co-activators by immunofluorescence is not shown as normal VICs are a heterogeneous population and varying numbers of untreated cells express some of these markers to varying degrees thus the images can be selective.

The fibrosal layer of calcified valves harbours smooth muscle cells and smooth muscle-derived foam cells

We analysed the fibrosal layer and found distinct differences in morphology and ultrastructural features of cells in normal and calcified valves.
calciﬁed valves. Normal valves showed typical features of ﬁbroblasts; isolated and spindly with long cytoplasmic extensions, having a prominent rough endoplasmic reticulum, an absence of myoﬁlaments and basal lamina, and the presence of some focal adhesions (Figure 7A).

In calciﬁed valves, myoﬁbroblastic features were identiﬁed; a well-developed rough endoplasmic reticulum and Golgi apparatus, partial basal lamina with points of plasmalemmal attachment, some peripheral myoﬁlaments, a few dense bodies running parallel to the long axis, a notched nucleus, and pinocytotic vesicles (Figure 7B).

The ﬁbrosal layer of calciﬁed valves showed bundles of elongated cells in groups (Figure 7C). These cells had a complete basal lamina and the cells were packed with longitudinal myoﬁlaments and dense plasmalemmal plaques (Figure 7D). These cells displayed the typical characteristics of SMCs including dense focal adhesions anchoring the myoﬁlaments, perinuclear mitochondria, few cytoplasmic organelles, and classical pinocytotic vesicles in clusters in the submembrane (Figure 7E). Predominantly, contractile SMCs were observed packed with myoﬁlaments and very little rough endoplasmic reticulum.

Having identiﬁed SMCs unequivocally in the ﬁbrosal layer, we sought to identify SMC-derived foam cells in the ﬁbrosa. Foam cells were easily identiﬁed based on their inclusion of lipid vacuoles. Foam cells derived from SMCs were identiﬁed in the ﬁbrosa of calciﬁed valves based on the abundant presence of longitudinal myoﬁlaments together with lipid vacuoles (Figure 7F).

**Discussion**

This study has documented for the ﬁrst time the pattern of SMC expression in normal and calciﬁed valves. Normal aortic valves harbour a small population of SMCs in the base of the ventricularis as shown by the expression of early and late speciﬁc markers of SMCs. The contractile apparatus of SMCs is characterized by high expression levels of α-SMA, caldesmon, calponin, and SM-MHC. These markers co-localized within the same cells of the aortic valve ventricularis. We could not identify myoﬁbroblasts in this region of the valve as all the cells positive for expression of α-SMA, calponin, and SM22 also expressed SM-MHC. These data, which represent a larger cohort of normal, human aortic valves, conﬁrms the previous smaller studies describing SMCs in the base of the ventricularis and in porcine aortic valves but is in contradiction to one documenting the presence of SMCs in the outer edge of the ﬁbrosa using anti-smooth muscle myosin antibodies. Electron microscopy was able to clearly identify the classical features of SMCs in the aortic valves and modelled the location and spatial arrangement of the SMC bundles to the ventricularis, running circumferentially. Single SMCs were not observed in the coapting regions or along the ﬁbrosa.

Using immunocytochemistry, Cimini et al. also demonstrated bundles of smoothelin stained cells localized to the ventricularis of the aortic valves. The use of α-SMA cannot distinguish between myoﬁbroblasts and SMCs as this is shared by both types of cells; however, in another study, α-SMA was shown to be prominently expressed in the region subjacent to the ventricular endocardium in porcine valves and alluded to as SMCs.

Calciﬁed valves demonstrated abundant and strong positivity for early and late markers of SMCs, α-SMA, smooth muscle actin, SM22, and calponin are early markers of SMC contractile proteins which are shared by myoﬁbroblasts and SMCs; however, SM-MHC is considered to be a speciﬁc marker of SMCs. Smooth muscle cell markers, including SM-MHC, were observed adjacent to and within the calciﬁed regions and often seen as a ridge of staining along the calciﬁed front. Endothelial cells were also positive for early and late SMC markers, both on the aortic and ventricular side of the valves as well as in capillary endothelium and the musculature of the larger neo-vessels. Neo-angiogenesis in calciﬁed valves has been reported with the expression of α-SMA within the vasculature; however, the expression of SM-MHC has not been reported in the endothelium of these neo-vessels. Increased expression of SMCs, distal and separate to the neo-vessels, has not been previously documented in human calciﬁed valves, and this ﬁnding is another factor in support of the similarities between CAS and atherosclerosis, though in atherosclerosis, the resident SMC population de-differentiates from a contractile to a synthetic, proliferative, migratory type. Smooth muscle cells have been shown to give rise to osteochondrogenic cells within calciﬁed arterial media as well as atherosclerotic lesions and this may also be the case for CAS. The abundance of
cells expressing SMC markers, specifically SM-MHC, seen in calcified valves may have originated through proliferation of a small number of resident SMCs or differentiation of resident/infiltrating stem cells; however, transdifferentiation of VICs, directly or indirectly via the myofibroblast phenotype, and valvar ECs into SMC-marker expressing cells may be more likely due to the number and localization of these SMC markers in the fibrosa, spongiosa, and endothelium, distal to the normal localization of SMCs in the aortic valve.

The SMCs identified here were not of a proliferative type due to the absence of Ki67 and PCNA. We believe that proliferation has occurred during the initial calcification process of the resident fibroblasts; however, in these end-stage calcified valves, the SMCs are not proliferating. The different phenotypes of SMCs, namely contractile and synthetic, differ in their proliferative capacities and contractile SMCs, as shown in our cohort of end-stage calcified valves by the presence of abundant myofilaments, are not usually proliferative but quiescent whereas synthetic SMCs are highly proliferative. Additionally myocardin has been shown to induce the expression of micro-RNA-1 which has a significant anti-proliferative effect.

We found abundant expression of myocardin, MRTF-A and -B in calcified human valves which has not been previously reported. Myocardin is a smooth and cardiac muscle-specific transcriptional co-factor for a variety of smooth muscle-specific genes and MRTFs are broadly expressed in the embryo and adult. These co-activators bind to the globally expressed transcription factor serum response factor (SRF). This co-activator–SRF complex regulates gene expression via binding to the serum response element or CArG box to drive SMC gene expression and nearly every smooth muscle contractile gene identified to date contains a functional CArG box within its promoter region. The increased expression of these co-activators

**Figure 6** The expression of MRTF-A in normal VICs (A), expression of MRTF-A in VICs treated with TGF-β1 (B), and co-localization of MRTF-A with DAPI (C) demonstrating nuclear expression. Graph showing the significant increase in the number of cells expressing MRTF-A after treatment with TGF-β1. Representative blots (E) and graphs showing median quantitative data (F) on the expression of smooth muscle markers and co-activators before and after treatment with TGFβ1 at time points of 0, 24, and 96 h (n = 6). *: P < 0.05 compared with time point 0.
would support the increased and aberrant expression of the SMC markers in calcified valves and provides further support that these are true SMCs.

The expression of SMC markers and co-activators of SMC gene expression is a novel finding in calcified valve ECs. During development of the valve, valvular endothelium undergoes EMT7–9 and this process continues in adult valves with a lower incidence9,29; however, a more advanced differentiation into SMCs defined by the expression of SM-MHC rather than α-SMA has, to our knowledge, not been described in human valves. Human ECs (HUVECs) have been shown to transdifferentiate into SMCs when treated with TGF-β and PDGF-BB30; and mature endothelium has been shown to undergo EMT and differentiate into SMCs expressing α-SMA, SM22α, calponin, and smooth muscle myosin.31 Cyclic strain has also been shown to induce endothelial to SMC/mesenchymal transdifferentiation.32–34 The result of increased EMT is not only increased fibrosis and excessive extracellular matrix deposition due to the generation of fibroblasts, myofibroblasts, and potentially SMCs but also a loss of endothelium is also a consequence and exacerbates tissue damage.

Transforming growth factor β1 was used to upregulate MRTFs as it is a proinflammatory cytokine and a mediator of the myofibroblast phenotype in human VICs35,36; and increased TGFβ1 signalling has been reported in patients with rheumatic heart disease including...
valvular fibrosis and calcified human aortic valves. Transforming growth factor β1 has been shown to increase the expression of α-SMA in VICs; however, its effects on other myofibroblastic and SMC markers in VICs has not been assessed. Transforming growth factor β1 has been shown to promote the differentiation of valvular ECs into a mesenchymal phenotype with the expression of α-SMA, but the expression of MRTFs has not been previously shown. We demonstrated that TGFβ1 was able to upregulate the nuclear expression of MRTF-A, but not of MRTF-B or myocardin, in VICs. Transforming growth factor β1 was unable to upregulate the nuclear expression of MRTF-B and this finding is similar to that found by Tomasek and co-authors using rat fibroblasts. Actin dynamics control the subcellular localization of MRTFs which themselves are responsible for SRF activity and SMC-specific gene expression. G-actin sequesters MRTFs in the cytoplasm by direct binding to the N-terminal RPEL motifs. The increase in the F-actin/G-actin ratio by the Rho family or the downstream kinase ROCK triggers a release of MRTFs from the cytoplasm allowing nuclear translocation and activation of SM promoters (Figure 8). This further supports the role of TGFβ in valve calcification.

We were able to identify unequivocally SMCs ultrastructurally by transmission electron microscopy (TEM) and documented the classical features of SMCs in the spongiosa and fibrosa of calcified valves. Smooth muscle cells have been identified using TEM in normal human aortic valves where they were located to the ventricularis but have not been previously reported in calcified valves. Additionally, we identified SMC-derived foam cells in the fibrosa of calcified valves by the presence of lipid vesicles in conjunction with longitudinal myofilaments. Foam cells have been identified in bioprosthetic valves, calcified valves, and specifically monocyte-derived foam cells were demonstrated in valvular diseases; however, the presence of SMC-derived foam cells have not been previously reported.

It has been suggested that fibroblasts, myofibroblasts, and vascular SMCs derive from a common stem-like cell and it is accepted that a myofibroblast precursor exists in the valve which subsequently differentiates into a SMC/osteoblast phenotype. The diverse embryological origins of vascular SMCs are well recognized; however, it remains unclear whether the site-specific presentation of some acquired vascular diseases, including regional susceptibility to

**Figure 8** TGFβ signalling, mechanical force, and stress signals result in activation of Rho-dependent signalling with the dissociation of MRTFA/B from monomeric G-actin. G-actin is then able to polymerize into F-actin and MRTFA/B is free to translocate to the nucleus and potentiates the activity of SRF (serum response factor). This in turn activates transcription of genes encoding a subset of contractile SMC proteins. TGFβ signalling induces phosphorylation of receptor-associated SMADs which form a complex with the common SMAD4 and translocate to the nucleus to bind the SMAD binding element (SBE) resulting in transcription of myocardin. Inhibitory proteins such as KLF4, HRT2, Foxo4, and GATA6 associate with myocardin repressing transcription of SMC contractile proteins. The pathways depicted have been proven. CARG - CCAAT element, CC(A/T)6GG; F-actin, filamentous actin; Foxo4, forkhead box 04; G-actin, globular actin; GATA6, GATA binding factor 6; HRT2, hairy related transcription factor 2; KLF4, Krupple-like factor 4; MRTF-A/B, myocardin-related transcription factor A/B; Rho, GTP-binding protein; ROCK, Rho-associated protein kinase; SBE, SMAD binding DNA element; SMAD, mothers against decapentaplegic (MAD) homolog and C. elegans Sma; SRF, serum response factor; TGFβ, transforming growth factor β; TGFB1, transforming growth factor β receptor 1; TGFB2, transforming growth factor β receptor 2.
atherosclerosis, vascular calcification, and aortic aneurysm distribution is attributable to SMCs’ intrinsic developmental origins. The most deleterious atherosclerotic lesions occur frequently in the coronary arteries which arise from the lateral plate mesoderm and the basal aortic root is also derived from the lateral plate mesoderm, and these cells may be equally susceptible to calcification and disease and this process may be mutually targeted by therapeutic drugs.

Common mechanisms of calcification have been proposed in CAVD and arteriosclerosis which are supported by the recent findings of microparticles of the same architecture, composition, and crystallinity, suggesting that spherical particles are not only the first mineralized structure formed in cardiovascular tissues, but also that the formation of calcific lesions among apparently disparate diseases may follow a common process. However, clinical studies suggest that calcification is patient specific and multifactorial in origin.

This report documenting the increased presence and significant up-regulation of early and late SMC markers and co-activators of smooth muscle gene expression together with upregulation of MRTF-A by TGF β1 and the identification of SMCs ultrastructurally is novel. The process of calcification drives the VICs to a myofibro-mineralized structure formed in cardiovascular tissues, but also crystallinity, suggesting that spherical particles are not only the first mineralized structure formed in cardiovascular tissues, but also that the formation of calcific lesions among apparently disparate diseases may follow a common process. However, clinical studies suggest that calcification is patient specific and multifactorial in origin.

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