Amino Acids (2017) 49:1981–1997
DOI 10.1007/s00726-017-2432-3

REVIEW ARTICLE

Human interstitial cellular model in therapeutics of heart valve calcification

Caimei He[1,2] · Hai Tang[3] · Zijian Mei[3] · Nichujie Li[3] · Zhi Zeng[3] · Kwame Oteng Darko[1] · Yulong Yin[2,4] · Chien-An Andy Hu[2,4,5] · Xiaoping Yang[1,2,4]

Received: 28 March 2017 / Accepted: 27 April 2017 / Published online: 23 May 2017

Abstract Calcific aortic valve disease is a common, severe heart condition that is currently with no proven, effective drug treatment and requires a surgical valve replacement or an entire heart explanation. Thus, developing novel, targeted therapeutic approaches becomes a major goal for cardiovascular disease research. To achieve this goal, isolated heart valve interstitial cells could be an advanced model to explore molecular mechanisms and measure drug efficacy. Based on this progress, molecular mechanisms that harbor components of inflammation and fibrosis coupled with proteins, for example, BMP-2, TLRs, RANKL, Osteoprotegerin, have been proposed. Small molecules or antibodies targeting these proteins have shown promising efficacy for either reversing or slowing down calcification development in vitro. In this review, we summarize these potential therapeutics with some highlights of interstitial cellular models.

Keywords Valve calcification · Heart valve interstitial cells · BMP-2 · TLRs · RANKL · Osteoprotegerin

Introduction

Heart valve diseases are the main cause of cardiovascular disease-related deaths (Mozaffarian et al. 2016). Heart valve calcification was recognized as a degenerative disease but a reversibility for this event has been realized recently (Wu et al. 2016; Schoen and Gotlieb 2016). Calcific aortic valve disease (CAVD) starts from the accumulation of mineralized nodules on the valve cusps and results in stenosis of the valve in general (Gohlke-Bärwolf et al. 2013; Iung and Vahanian 2011). Stenosis of the valve has very poor prognosis, the only clinical available therapy for severe disease conditions is surgical valve replacement. Compared to surgery treatment, pharmaceutical intervention is much more durable with low

Handling Editors: C.-A.A. Hu, Y. Yin, Y. Hou, G. Wu, Y. Teng.

✉ Yulong Yin
yinyulong@isa.ac.cn

✉ Chien-An Andy Hu
AHu@salud.unm.edu

✉ Xiaoping Yang
Xiaoping.Yang@hunnu.edu.cn

[1] Department of Pharmacy, School of Medicine, Hunan Normal University, Changsha 410013, Hunan, People's Republic of China

[2] Animal Nutrition and Human Health Laboratory, College of Life Sciences, Hunan Normal University, Changsha 410013, Hunan, People's Republic of China

[3] Department of Clinic Medicine, School of Medicine, Hunan Normal University, Changsha 410013, Hunan, People's Republic of China

[4] Institute of Subtropical Agriculture, Research Center for Healthy Breeding of Livestock and Poultry, Hunan Engineering and Research Center of Animal and Poultry Science and Key Laboratory for Agroecological Processes in Subtropical Region, Scientific Observation and Experimental Station of Animal Nutrition and Feed Science in South-Central, Ministry of Agriculture, Chinese Academy of Science, Changsha 410125, People's Republic of China

[5] Department of Biochemistry and Molecular Biology, University of New Mexico School of Medicine and Health Sciences Center, MSC 08 4670, Fitz 258, 915 Camino de Salud, Albuquerque, NM 87131-0001, USA

risk. Thus, efficient drug treatment for this clinical setting is needed and elaboration of molecular mechanisms will pave the way to develop novel treatment tools.

Stenosis and regurgitation of heart valves can be considered direct consequences of valvular cell and molecular changes, triggered by chemical and mechanical stresses. Mechanical stimuli to valve cells or tissues in culture have demonstrated that valvular degenerative processes can be triggered in short time spans and mimic cell transformation in pathological processes (Balachandran et al. 2011). The morphogenesis of atrioventricular and semilunar valves is a complex process associated with changes in cardiac morphology and hemodynamics. Early embryonic heart is surrounded by primary myocardium of single tube endocardial cells. In the process of circulation, the primary myocardial secretion of hyaluronic acid-rich gel-like matrix, known as the heart of jelly, projects into the room, depending on the levels of the swelling junction and outflow (Zhao and Rivkees 2000).

Thus, cells are useful for investigating the molecular mechanisms as traditional in vitro models. Several efforts have been contributed to develop the cell models since unlike cancer cell in cancer research, heart valve cells are very limited. Two distinct cellular phenotypes including interstitial and endothelial cells are found within the valve. The former covers the surface of the cusps and the latter forms a network within the extracellular matrix within the body of the cusp. Valve interstitial cells have distinct embryonic origins in the endocardium and cardiac neural crest cells. Heart valve interstitial cells play a critical role in the onset and progression of valvular pathological processes and are closely connected to heart valve tissue remodeling and repair (Masjedi et al. 2016). These cells express certain characteristics of both fibroblasts and skeletal myocytes. Interstitial cells from other tissues have been shown to contract and to express sarcomeric (muscle) genes. In addition, these cells express certain regulatory (transcription factor) genes. The unique alignment of these cells may, at least in part if not complete, be controlled by tissue polarity signals transmitted by members of the frizzled family of vertebrate tissue polarity genes. Interstitial cells from all four valves have been successfully isolated and cultured of recipient hearts obtained during transplantation. Interstitial cells express a number of genes which have been confirmed to display profound physiological functions for heart valves. These include members of the contractile elements such as MHC and troponins (Roy et al. 2000). The presence of members of the frizzled family, which specifically orients cell polarization, implies that interstitial cells are arranged in the valve tissue based on its undiscovered regulatory patterns. Therefore, the finding of a number of functionally important genes in isolated interstitial cells from the human heart valves suggests their role in particular physiological functions (Roy et al. 2000).

Valve interstitial cells are known to exist and function within the highly dynamic valve tissue environment, including very high physiological loading rates (Ayoub et al. 2016). The mechanical function of these cells has been investigated recently using computational continuum mechanics model and incorporate realistic stress-fiber geometries, force–length relations via Hill model. This model can force contraction to clarify alpha-smooth muscle actin and F-actin expression levels and strain rate (Sakamoto et al. 2016; Sakamoto et al. 2017). Both F-actin and α-SMA contributed substantially to stress fiber force generation, exerting a great role in valvular interstitial stress fiber function and the potential for strain rate-dependent effects in pathological states (Sakamoto et al. 2017). High levels of α-SMA expression are related to the potential for strain rate-dependent effects in pathological states, indicating that α-SMA has a unique effect on the valvular cellular in vivo microenvironment (Sakamoto et al. 2017). Strain is a newly defined factor associated with heart valve calcification. Strain modifies tension in the monolayer, resulting in cell contacts, migration, apoptosis, and mineralization to influence nodule formation at different points. Interestingly, TGF-β1, one of important growth factors, increases tension in the monolayer in parallel with α-smooth muscle actin (αSMA) expression. Continual exposure to strain accelerates aggregation of calcified mature nodules surrounded by an apoptotic ring with a necrotic core (Fisher et al. 2013).

Moreover, radiation generated by a cesium-137 irradiator for 10 Gy can induce the osteogenesis in human aortic valve interstitial cells with osteogenesis markers for bone morphogenetic protein 2, osteopontin, alkaline phosphatase, and Runx2 (Nadlonek et al. 2012). Unexpectedly, Sun et al. reported that more calcium deposition and higher alkaline phosphatase activity in human mitral valve interstitial cells than human aortic valve interstitial cells after treating both cells with mineralization medium, indicating that human mitral valve interstitial cells are more susceptible to in vitro calcification (Sun et al. 2013).

Valve cusps interstitial cells have cuboidal and spindle-shaped features with swirling pattern (Brand et al. 2006). These cells have been characterized with cardiac muscle and troponin complex with the feature of machinery for contractile responses (Johnson et al. 1987). In another hands, these cells can communicate with each other via the extracellular matrix, and to respond to their environment, allowing heart valves to function in an optimal and efficient way (Taylor et al. 2003). These cells express genes encoding components of the *wnt/β-catenin* signaling pathway, indicating the role of this pathway in the embryonic development of the valves (Butcher and Markwald 2007). These cells make important matrix components including

collagen, elastin, proteoglycans, glycoproteins, growth factors, cytokines and chemokines, as well as matrix remodeling enzymes, the matrix metalloproteinases (MMPs) and their tissue inhibitors. Functionally, VICs have the dual ability to secrete matrix components and to maintain valvular contractile function (Chester and Taylor 2007). Meaningfully, cellular shape regulates valve contractile function independent of phenotype (Tandon et al. 2016).

It has been found that VICs are located inside of heart valves with a heterogeneous cell population. Unexpectedly, greatest amount of the calcific markers has been found in VICs isolated from the noncoronary cusp as compared to these isolated in the coronary cusps. Also, noncoronary VICs have the highest collagen content (Masjedi et al. 2016). Interestingly, there is a significant difference of calcification potential among four valves with a greatest predisposition to calcification in aortic valves (Masjedi et al. 2016).

Valvular interstitial cells can demonstrate a pericyte-like phenotype to regulate valve endothelial cell (VEC) organization. VICs controlled early VEC network organization, and then guided later VEC network contraction through chemoattraction. Invasive spheroids with 3D angiogenic-like sprouts were formed in valve cellular culture systems (Arevalos et al. 2016). The persistence of activation was observed in a high-throughput culture system with cultured collagen-coated polyacrylamide substrates of predefined stiffness, allowing the formation of very low substrate stiffness level to "deactivate" them (Quinlan and Billiar 2012). VICs is highly sensitive to substrate stiffness, demonstrating the importance of the mechanical characteristics of materials used for valve repair or for engineering valve tissue (Quinlan and Billiar 2012).

The cells isolated from both calcified and non-calcified human aortic valve specimens were used to define cellular origin of valve calcification. The results showed that the levels of CD34 expression are key player for this calcification procedure in mesenchymal stem-like conditions (Nomura et al. 2013). Compared with CD34-positive cells, CD34-negative mesenchymal stem-like cells were significantly more sensitive to high inorganic phosphate, calcifying easily in response to these culture conditions (Nomura et al. 2013). Furthermore, the results obtained from immunohistochemical staining showed that mesenchymal stem-like cells with higher population of CD34-negative compared with CD34-positive were localized in calcified aortic valve specimens. These data suggest that CD34-negative mesenchymal stem-like cells are important components for calcification of the aortic valve (Nomura et al. 2013). Importantly, it has been raised that cell density is a robust factor affecting several key proteins, including β-catenin, *N*-cadherin, cofilin, α-smooth muscle actin, transforming growth factor β and nuclear pSmad2/3 (Xu et al. 2012). The roles of sphingosine-1-phosphate and expression of relevant receptors in valve tissue and in valvular interstitial cells have been determined. They serve essential functions in maintenance of valvular structure, associating with the function of contraction and regulating the tissue tension signaling via RhoA and ROCK (Witt et al. 2012).

Mineralization medium generally causes the increase of the expression of osteogenic markers and the decrease of the expression of myofibroblastic markers (Monzack and Masters 2011). VICs displayed distinct difference of expression levels and patterns of these markers than the osteoblastic cell types (Monzack and Masters 2011). Addition of mineralization medium to VICs resulted in the lack of alpha-SMA increases, implying that it is unnecessary for these cells progress through a myofibroblastic stage before reaching the feature of an osteoblast-like gene. Only the osteogenic cell types proved a comprehensible increase in osteocalcin (Monzack and Masters 2011).

Downregulation of the proteins α-SMA, Vimentin, and Calponin has been observed in pathological stretch and pressure which inhibits the contractile (Thayer et al. 2011). Calponin downregulation implies depolymerization of actin filaments and possible conversion to a more synthetic phenotype in aortic valve interstitial cell phenotype (Thayer et al. 2011).

Thus, a gene program of development in aortic valve disease is reactivated, contributing to the molecular mechanisms underlying valve calcification in disease. Collectively, this valve interstitial cell model is a valuable tool to explore the functional and molecular mechanisms of heart valve calcification and to verify novel targets for therapeutic intervention. In summary, the central role of interstitial cell on heart valve calcification is depicted in Fig. 1.

Pathways of heart valve calcification based on interstitial cellular model

During heart valve calcification, valvular interstitial cells from mineralized valves activate molecular and cellular pathways related to valve development and bone differentiation (Rajamannan et al. 2003; Wirrig et al. 2011). Osteoblast and bone markers such as osteopontin, bone sialoprotein, osteocalcin, alkaline phosphatase, and the osteoblast-specific transcription factor Runx2 determined by RT-PCR revealed increased mRNA levels of these genes in the calcified valves. Interestingly, there was no change in alkaline phosphatase mRNA level but an increase in the protein expression in the diseased valves, indicating the presence of post-transcription modification (Rajamannan et al. 2003). Furthermore, VIC activation, ECM disorganization, and markers of valve mesenchymal and skeletal chondrogenic progenitor cells were determined in

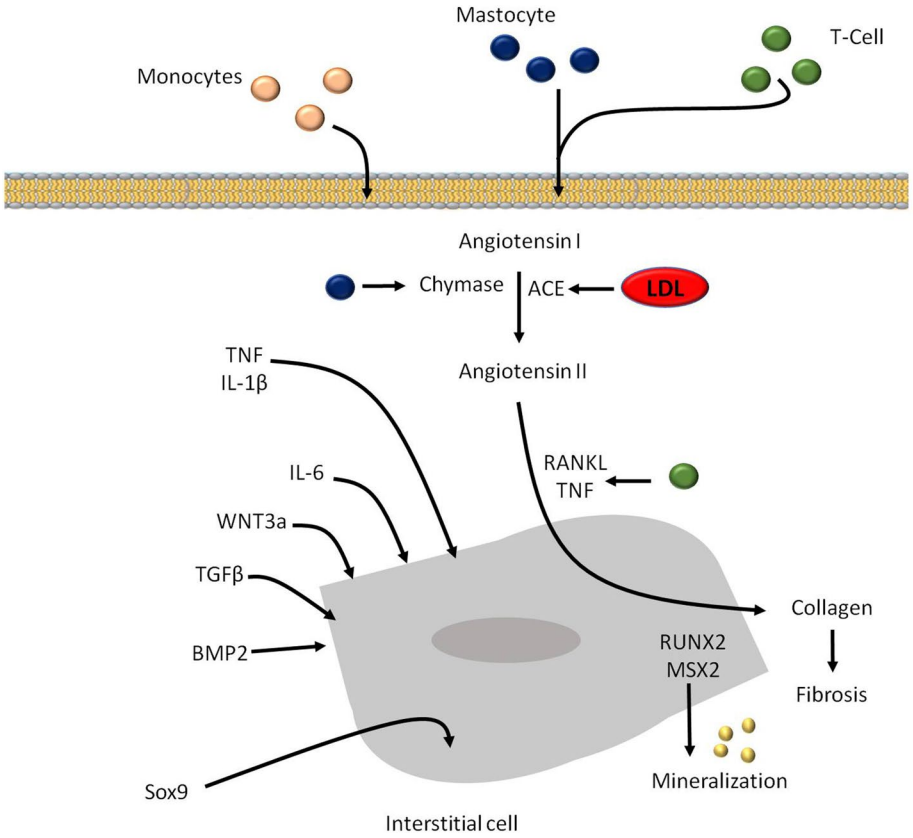

Fig. 1 The central role of interstitial cell on heart valve calcification. Monocytes, mastocyte and T cell are the cells which initiate the early events of calcification-associated inflammation but interstitial cells play a central role on mineralization as inflammation effectors

both pediatric and adult tissues (Wirrig et al. 2011). Activated BMP signaling, increased expression of cartilage and bone-type collagens the osteogenic marker Runx2 were observed in adult diseased aortic heart valve in comparison with control valves (Wirrig et al. 2011). It has been reported that other pathways related to bone and cartilage formation, including bone morphogenetic protein (BMP), Notch, and Wnt signaling, are involved in valve calcification (Garg et al. 2005; Rajamannan 2011; Xu and Gotlieb 2013; Yang et al. 2009a). It has been demonstrated that Notch1 regulates matrix gla protein and calcification gene networks such as atherosclerosis-related gene connexin 40 and matrix gla protein. Sequestering bone morphogenetic proteins (BMPs) can to inhibit soft tissue calcification (White et al. 2015). Notch 1 mediates the pro-osteogenic response to toll-like receptor 4 stimulation in human aortic interstitial cells, leading the calcification via modulation of ERK1/2 and nuclear factor-kB activation (Zeng et al. 2013). Notch 1 may express the inhibitory role in calcific aortic valve disease via direct targets of the transcription factor Sox9 (Acharya et al. 2011). Sox9 is a well-recognized transcription factor, binding the DNA and activating the transcription (Ng et al. 1997). Calcific lesions in heart valve leaflets were observed in Sox9(fl/+);Col2a1-cre mice, correlated with, increased expression of bone-related genes and activation of inflammation and matrix remodeling processes. Specific knockdown of Sox9 in heart valves in vitro caused ectopic calcification, confirming the critical role of Sox9 in valvular calcification (Peacock et al. 2010). Recent evidence demonstrated that valve endothelial cell-derived Tgfβ1 signaling promotes nuclear localization of Sox9, associated with alleviated calcification. Reduced Sox9 expression and nuclear localization precedes the onset of calcification in valve interstitial cells. Interestingly, valve endothelial cells can prevent Sox9 nuclear export and calcific nodule formation i. In contrast, removal of Tgfβ1 in the endothelium leads to decreased Sox9 expression and calcific aortic valve disease (Huk et al. 2015). Sox9 nuclear localization has been observed in cultured chicken embryo aortic valves (Fang et al. 2014). Nodular cells strongly express nuclear Sox9 and its downstream extracellular matrix genes, including Aggrecan, Col2a1 and Col10a1, which is associated with the Wnt/β-catenin signaling pathway (Fang et al. 2014).

Notch 1 mutation leads to valvular calcification through enhanced myofibroblast mechano-transduction, resulting in dystrophic—but not osteogenic—calcification (Chen et al. 2015). A knockdown of LncRNA H19 increased the expression of Notch1 and decreased the levels of Runx1 and BMP2. LncRNA H19 silenced

Notch1 by preventing the recruitment of its promoter. Rescue experiments demonstrated that the transfection of a vector encoding for the active Notch intracellular domain prevented LncRNA H19-induced calcification of valve interstitial cells. Deregulation of DNA methylation in the promoter of H19 during calcific aortic valve disease is associated with a higher expression of LncRNA H19, which promotes an osteogenic procedure by interrupting the expression of NOTCH1, confirming the role of NOTCH1 (Hadji et al. 2016). Similarly, Notch signaling pathway increases bone morphogenetic protein-2 to induce osteogenic differentiation and mineralization in vascular smooth muscle cells (Shimizu et al. 2011). Based on the data collected from transgenic animal model, BMP signaling has shown a crystal clear effect on aortic valve calcification (Gomez-Stallons et al. 2016).

Recently, a novel mechanism regarding the role of non-canonical Wnts signaling pathway has been proposed (Albanese et al. 2016). Significant correlation between WNT5b and WNT11 overall staining and presence of calcification, lipid score, fibrosis, and microvessels has seen in human aortic valve calcification. Abundant immunostaining for WNT5b and WNT11 was observed in fibrosis, inflammatory cells, and activated myofibroblasts in areas of calcified foci, which further confirmed by either Western blot or RT-PCR in stenotic aortic valves, particularly in bicuspid valves. Incubation of HAVICs isolated from non-calcified valves with the non-canonical Wnts significantly enhanced cell apoptosis and calcification. Mitogen-activated protein kinase 38β and GSK3β inhibitors significantly reduced the mineralization of HAVICs, demonstrating the involvement of both MAPK 38 and GSK3β signaling. On the other hand, Raman spectroscopy confirmed the presence of hydroxyapatite, one form of the inorganic phosphate deposits. Furthermore, in response to WNT5a and WNT11, significant increase in hydroxyapatite deposition was detected in HAVICs. These deposits could be observed in both HAVICs treated with Wnts and calcified human aortic valves (Albanese et al. 2016). The condense data collectively convinced the production of hydroxyapatite deposition in heart valve calcification.

Rho signaling pathway is involved in regulating normal smooth muscle and endothelial cell function. Increased Rho A and Rho kinase (ROCK) activity in valvular interstitial cells is correlated to increased nodule formation in vitro (Gu and Masters 2011). Rho/ROCK may be a potential powerful therapeutic target for treating heart valve calcification. Sung et al. revealed that cadherin-11 overexpression increases the expression of RhoA and Sox9, causing calcification and extensive pathogenic extracellular matrix remodeling (Sung et al. 2016). Administration of a Rho-associated protein kinase inhibitor alleviated nodule formation, supporting the role of the small GTPase Rho/Rho-associated protein kinase signaling pathway (Sung et al. 2016).

Contractility of interstitial cells mediates internal stress state and organization of extracellular matrix of aortic heart valve. Osteogenesis disrupts mechanical phenotype of interstitial cells and drives disorganization, nodule formation, and pro-calcific signaling via a RhoA-dependent mechanism (Farrar et al. 2016). A ROCK-dependent manner has been observed during the event of regulation of VICs on the early VEC network. Typical angiogenic-like phenotypes are dependent on ROCK activation. These results suggest that exposure of ectopic sustained angiogenic environment during the early stages of valve disease enhances activities influenced by both VECs and VICs, contributing to neovessel formation and the progression of CAVD (Arevalos et al. 2016).

Furthermore, other mechanisms, including matrix remodeling, lipid accumulation in the valve, and dysfunction of the renin–angiotensin system have been proposed to detail the procedure of heart valve calcification (Rajamannan et al. 2007). Ang II might act on the Ang II receptor on valvular interstitial cells and lead to activation of the Wnt/β-catenin pathway. Thus, angiotensin II induces the activation, differentiation and proliferation of myofibroblasts, that is, heart valve interstitial cells, and finally, osteoblast-like phenotype transformation, leading to calcification of heart valves (Xie et al. 2016). Active investigation in clinical trials has been conducted in angiotensin receptor blockers (ARB)s and angiotensin convertase enzyme inhibitors (ACEI)s to treat heart valve calcification, but has been proven to be unsuccessful in slowing the progression of calcific aortic valve disease (Peltonen et al. 2017).Vasoactive factors are involved in the progression of calcific aortic valve disease. Thus, it has been proposed that endothelin and renin–angiotensin systems seem to be the most prominent targets for therapeutic interventions in the view of valvular pathogenesis. Circulating vasoactive factors may provide targets for diagnostic tools of calcified aortic valve disease. An early observation disclosed that ARBs but not ACEs were found to abolish the increased risk of mortality associated with hypertension (Capoulade et al. 2013). Ang II stimulation significantly induced vascular calcification through activation of RANKL. Furthermore, RANKL activated renin–angiotensin II system, especially angiotensin II-converting enzyme and Ang II type 1 receptor. Cross-talk between renin–angiotensin II system and RANKL system might work as a vicious cycle to accelerate vascular calcification in atherosclerosis (Osako et al. 2013). Fibroblast growth factor 2 formulation can modulate the phenotype and function of human VICs (Latif et al. 2015). Principally, it comes from an early observation that fibroblast growth factor-2 enhances in vitro heart valve interstitial cell repair via Akt1 pathway (Han and Gotlieb 2012).

Mature heart valves have complicated structures consisting of three layers of very well-organized extracellular matrix, mainly composed of collagens, proteoglycans and elastin. In contrast to healthy valves, myxomatous valve disease is the most common cause of mitral valve prolapse in the human population and is characterized by an abnormal abundance of proteoglycans (Barnette et al. 2013). Independently, Osman et al. found that binding of LDL to proteoglycans by transforming growth factor-β1 in aortic valve interstitial cells enhanced the movement to the calcification, serving as an early event associated with Smad2-dependent glycosaminoglycan elongation (Osman et al. 2013).

Scleraxis (Scx) is a basic helix-loop-helix transcription factor, which is required for establishing heart valve structure during remodeling stages of valvulogenesis. Restructuring heart valves from Scx null mice express decreased levels of proteoglycans, particularly chondroitin sulfate proteoglycans, while overexpression of Scx in embryonic avian valve precursor cells and adult porcine valve interstitial cells increases chondroitin sulfate proteoglycans, identifying that Scx is positively regulated by canonical Tgfβ2 signaling during this process and this procedure is attenuated by MAPK activity (Barnette et al. 2013). Scx is increased in myxomatous valves evidenced from both human patients and mouse models, and overexpression of Scx in human mitral valve interstitial cells modestly increases proteoglycan expression consistent with myxomatous mitral valve phenotypes (Barnette et al. 2013). Higher levels of TRAIL were detected in calcific aortic valves and in sera from the same patients compared to controls (Galeone et al. 2013). Figure 2 describes the pathways of Notch, BMP and Angiotension-II that induce increase of calcification under osteogenic conditions.

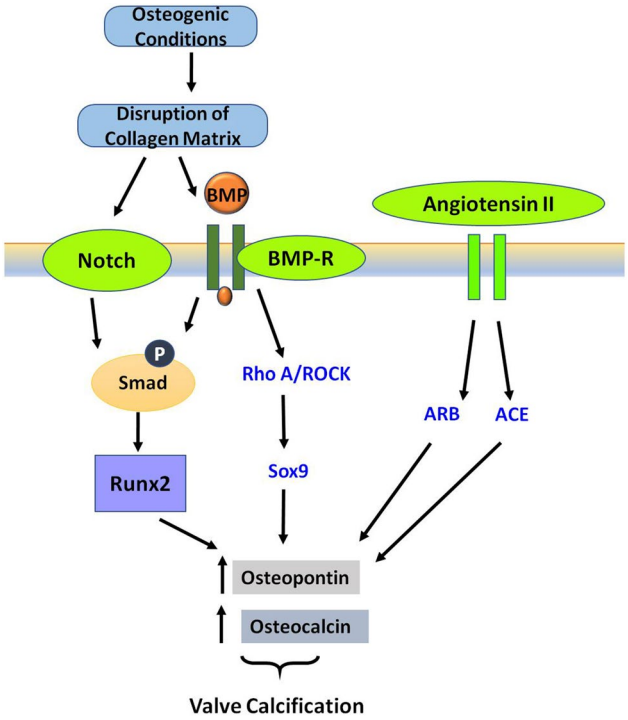

Fig. 2 Osteogenic conditions enhance the signaling pathways of either Notch or BMP which induce the activation of Smad and upregulation of Runx2 or RhoA/ROCK/Sox9, thus resulting in the increase of calcification biofactors including osteopontin and osteocalcin. Angiotensin II-activated ARB and ACE which gives other potential targets for treating heart valve calcification

Inflammatory mechanisms verified via interstitial cell models

Both adaptive and innate immune systems play a critical role on cardiovascular diseases (Mathieu et al. 2015). Several studies have demonstrated the correlation between inflammation and aortic valve calcification (Helske et al. 2007). Inflammation markers, including macrophages, T lymphocytes, and pro-inflammatory mediators such as interleukin-1, transforming growth factor-beta, and tumor necrosis factor-alpha are present in calcified human heart valves (Rajamannan et al. 2007).

We revealed that human AVICs express functional toll-like receptor TLR2 and TLR4, important mediators of the innate immune response and inflammation (Meng et al. 2008). The similar observation has been consistently reported with viral and bacterial patterns (López et al. 2012). TLR4 and TLR2 ligands induced an early activation of NF-κB and p38 MAPK activation in aortic valve interstitial cells (López et al. 2012).

Furthermore, differential TLR expression and differential response to TLR agonists distinguish AVICs from PVICs and are correlated to a pro-osteogenic phenotype. In the meantime, we examined that human aortic and pulmonary valve, both tissue and isolated interstitial cells, express different levels of TLR2 and TLR4 (Yang et al. 2009b). Stimulation of TLR2 and TLR4 signaling pathways results in differential expression of pro-inflammatory and pro-osteogenic factors in AVICs and PVICs. In contrast, silencing TLR2 and TLR4 blocks the pro-osteogenic response in AVICs. We observed that BMP-2 is related to TLR2 and TLR4 to osteogenic phenotypic changes. TLR2, TLR4, and BMP-2 expression differ in AVICs isolated from normal versus stenotic valves (Yang et al. 2009b). In comparison of adult and pediatric patient aortic heart valve interstitial cells, adult AVICs exhibit greater inflammatory and osteogenic responses to TLR4 stimulation than pediatric AVICs (Deng et al. 2015). Inhibition of Stat3 enhanced p-38 MAPK and NF-κβ phosphorylation in pediatric AVICs after TLR4 stimulation. The enhanced responses in adult AVICs are at least partly

due to lower levels of Stat3 activation in response to TLR4 stimulation relative to pediatric cells. Stat3 functions as a negative regulator of the TLR4 responses in human AVICs (Deng et al. 2015). These data described above confirm that these important inflammation-associated markers exist consistently in heart valves both in human tissues and isolated cells.

On the other hand, AVICs stimulated with TLR agonists upregulate BMP-2 and runt-related transcription factor 2 (Runx2) (Meng et al. 2008; Babu et al. 2008), giving crucial functional features of these interstitial cells. Consistently, BMP-2 and Runx-2 have both been detected in calcified valve leaflets (Rajamannan et al. 2003; Mohler et al. 2001). The changes in Runx2 and osteopontin levels were preceded by phosphorylation of Smad1 and extracellular signal-regulated kinase 1/2 but not p38 mitogen-activated protein kinase. Decreasing Smad1 by silencing approach reduced Runx2 and osteopontin levels, whereas inhibition of extracellular signal-regulated kinase 1/2 reduced osteopontin expression without influencing Runx2 expression (Yang et al. 2009a). In addition, AVICs stimulated with BMP-2 upregulate alkaline phosphatase (ALP) (Osman et al. 2006), and formation of calcified nodules in VICs is dependent on ALP activity (Mathieu et al. 2005). Another novel observation is that TLR4-mediated ICAM-1 expression in human aortic valve interstitial cells is related to facilitation of microfilaments (Song et al. 2011). All data highlighted the important role of inflammation and its relevant pathways on heart valve calcification. Interleukin 18 (IL-18), one of the important inflammation factors, is expressed in stenosis aortic valves and is positively related with the clinical severity of aortic stenosis. IL-18 significantly enhanced alpha-smooth muscle actin gene and protein expression. IL-18 treatment also promoted the expression of osteopontin and Runx2 mRNA. IL-18 could induce ALP activity in the presence of conditioning medium. IL-18 markedly enhanced NF-κB p65 phosphorylation in VICs (Zhou et al. 2016). Clinical evidence has shown that coronary artery calcification is associated with rheumatoid arthritis-induced inflammation (Wahlin et al. 2016).

TNF-α accelerated the calcification and ALP activity of HAVICs from calcific aortic valve stenosis. Gene expressions of ALP, BMP2, and distal-less homeobox 5 (Dlx5) were significantly increased as well (Yu et al. 2011).

High-mobility group box-1 protein (HMGB1), another critical biomarker of inflammation, induces production of pro-inflammatory cytokines, accelerates the osteoblastic differentiation and promotes osteogenic phenotype changes including calcification in VICs (Wang et al. 2016). Conversely, osteopontin, one of the important osteogenic phenotypic markers, regulates the HMGB1 expression (Passmore et al. 2015). It has been observed that HMGB1 mediates high-glucose-induced calcification as well (Wang et al. 2016). Clinic-related observation has shown that HMGB1 remained independent predictors of the non-calcified plaque burden with a high positive predictive value for the presence of non-calcified and remodeled plaque (Andrassy et al. 2012).

Autotaxin (ATX) transforms lysophosphatidylcholine into lysophosphatidic acid. Bouchareb et al. observed high levels of ATX in mineralized aortic valves collected from patients. Furthermore, ATX colocalized with oxidized phospholipids and apolipoprotein(a) and valve interstitial cells also expressed ATX. Mechanistically, ATX-lysophosphatidic acid accelerates the mineralization of the aortic valve through a nuclear factor κB/interleukin 6/bone morphogenetic protein pathway. A mouse model has model revealed that when ATX is overexpressed, lysophosphatidic acid increases a strong deposition of hydroxyapatite of calcium in aortic valve leaflets and accelerates the development of CAVD (Bouchareb et al. 2015). Cytokines, including IL-1β, can induce the formation of IL-6 and IL-8, and activate NF-kB pathway (Sun et al. 2015). On the other hand, neutralization of BMP-2 or TGF-β1 attenuates the expression of ALP, osteopontin, and Runx2 in cells (Song et al. 2015). However, neutralization of both BMP-2 and TGF-β1 decreases the expression of these osteogenic biomarkers and calcium deposition (Song et al. 2015). Expression of phosphorylated Smad1 and Smad3 has been found in cells exposed to biglycan, and knockdown of Smad1 or Smad3 attenuates the effect of biglycan on the expression of osteogenic biomarkers. Further evidence confirmed that the Smad 1/3 pathways are involved in the mechanism of AVIC pro-osteogenic reprogramming (Song et al. 2015). In addition, myofibroblasts with enhanced protein expressions of β-catenin, TGF-β1, Wnt3A, and phosphorylated Smad2/3 are found in the fibrosa fed with atherogenic diet, indicating the correlation among β-catenin, TGF-β1, Smad pathways (Chen et al. 2011).

Fernández-Pisonero et al. demonstrated the synergy between bioactive lipid sphingosine 1-phosphate and lipopolysaccharide (Fernandez-Pisonero et al. 2014). This observation is very important since it explains the hallmarks of lipid accumulation and inflammation in the stenosis-related aortic calcification. Co-administration of sphingosine 1-phosphate and LPS induce synergistic pro-inflammatory responses including the upregulation of IL-6, IL-8, COX-2 and ICAM-1. Profoundly, these effects are more dramatic in aortic valve interstitial cells from stenotic than control valves. The authors demonstrated the involvement of sphingosine 1-phosphate receptors 1/3 and Toll-like receptor-4, and downstream signaling such as p38/MAPK, protein kinase C, and NF-κB (Fernandez-Pisonero et al. 2014). Furthermore, sphingosine 1-phosphate caused calcium deposition, increased the expression of tBMP-2 and ALP, and enhanced the effect of LPS, an effect that was partially blocked by

inhibition of sphingosine 1-phosphate receptors 3/2 signaling (Fernandez-Pisonero et al. 2014). Both innate and adaptive immunity influencing aortic valve calcification has been recently proposed due to the facts including production of leukotrienes, existence of oxidized lipid species, activation of innate immune responses and trigger of adaptive immune responses during cardiovascular calcification (Mathieu et al. 2015). Human CAVD tissues are infiltrated by lipids (Meredith et al. 2013). Oxidized LDL upregulated phosphate inorganic transporter 1, a sodium-phosphate ion cotransporter in human aortic valve interstitial cells, may explain the effect of oxidized LD on induction of osteogenic changes (Nadlonek et al. 2013a). Highly expressed lipoprotein-associated phospholipase A2 has been found in calcific aortic valve. In vitro, lysophosphatidylcholine, the product of Lp-PLA2 activation increased the expression of ALP, the ectonucleotide pyrophosphatase/phosphodiesterase 1 enzyme, sodium-dependent phosphate cotransporter 1 (encoded by the SLC20A1 gene), and osteopontin. Lysophosphatidylcholine-induced mineralization involved ectonucleotidase enzyme as well as apoptosis through a protein kinase A-dependent pathway has been demonstrated (Mahmut et al. 2014). Independently, Suzuki et al. discovered that an increased expression of osteogenic molecules is associated with the expression of groups IIE and V phospholipase A2 (Suzuki et al. 2014). Furthermore, the degree of immunoreactivity and gene expression levels of PLA2 s-IIE and -V significantly correlated with those of BMP-2, osteopontin and ALP. In addition, immunoreactivity for cyclooxygenase (COX)-1, COX-2 and 5-lipoxygenase, downstream enzymes of PLA2 in the arachidonic acid cascade, was colocalized with that for PLA2 s-IIE and -V in cells expressing α-smooth muscle actin and macrophages expressing CD68 (Suzuki et al. 2014). Epigenetic regulation of 5-lipoxygenase in the phenotypic plasticity of valvular interstitial cells has been observed in aortic valve stenosis. Calcified tissue exhibited decreased DNA methylation in the promoter of the gene encoding the pro-inflammatory enzyme 5-lipoxygenase, accompanied by increased mRNA levels of 5-lipoxygenase (Nagy and Bäck 2012). The typical mesenchymal-type cell–cell adherens junctions (AJs) connecting VICs appear as normal *N*-cadherin based puncta adhaerentia (Barth et al. 2012). Plakophilin-2, known as a major cytoplasmic plaque component of epithelial desmosomes, is recruited to and integrated in the plaques of VIC adherens junctions as a major component under growth conditions with enhanced proliferation, i.e., in fetal heart valves and in cell cultures (Nagy and Bäck 2012). Plakophilin-2 has recently also been detected in adherens junctions of cardiac myxomata (Rickelt et al. 2010).

Nucleotide P2Y2 receptor (P2Y2R) is a newly defined protein which contributes to vascular inflammation and dysfunction. Direct evidence has shown that P2Y2R is involved in neointimal hyperplasia (Agca et al. 2016). Global deficiency of this receptor slows down the formation of vascular calcification (Qian et al. 2017). Mechanistically, P2Y2R may bind to integrin to promote Wnt3a- and EGF-induced epithelial tubular formation (Ibuka et al. 2015). P2Y2R shows the ability to repress IL-6 expression via Akt. P2Y2R and Akt modulate the NF-κB pathway and its downstream target IL-6, a strong promoter of the mineralization of VICs (El Husseini et al. 2014). MAPK activation can be induced by TGF-beta1, promoting collagen synthesis, nodule formation, redox stress and cellular senescence (Das et al. 2013).

Reactive oxygen species (ROS) generated by hydrogen peroxide induced relocalization of phosphorylated γH2AX, MRE11, and XRCC1 proteins with expression of osteogenic signaling molecule RUNX2 via AKT, implying the accumulation of ROS and remodeling of the microstructure of the cusp characterize aortic valve sclerosis, the early phase of calcific aortic valve disease (Branchetti et al. 2013). Figure 3 highlights the critical role of ERK/Akt on either inflammatory factors-triggered Smad pathway or Integrin-induced Wnt pathway for calcification although latter may go through β-Catenin/alkaline phosphatase.

Fibrosis-related calcification based on interstitial cell results

Calcific aortic stenosis involves fibro-calcific remodeling of the aortic valve that causes restriction of blood flow (Lindman et al. 2016). Accumulation of fibrotic collagen leads to thickened and stiffened valve leaflets and a deterioration in function, which is the hallmark of calcification initiation (Adiguzel et al. 2009). The association between collagen accumulation and calcification in cardiovascular diseases is reminiscent of features of atherosclerosis, in which a collagen-rich fibrotic cap encapsulates an underlying calcification (Adiguzel et al. 2009). Principally, after an atherosclerotic lesion develops, collagen accumulation is desirable to stabilize the plaque and minimize the potential for rupture and thrombosis (Libby 2013). Stability has historically been quantified by the thickness of collagen in the fibrotic cap (Libby and Sasiela 2006). Interestingly, biomechanical analyses indicate that underlying calcifications might further stabilize the plaque, indicating that calcification accelerates the formation of plaque. Surprisingly, clinical evidence showed that statins do not decrease the prevalence of vascular calcification, implying that neither inhibiting HMG-CoA reductase nor lowering cholesterol levels blocks the development of vascular calcification (Saremi et al. 2012; Houslay et al. 2006). Thus, cholesterol may not be a critical factor influencing progress of vascular calcification. In contrast, ACE inhibitors reduce left ventricular hypertrophy beyond their effects on blood pressure

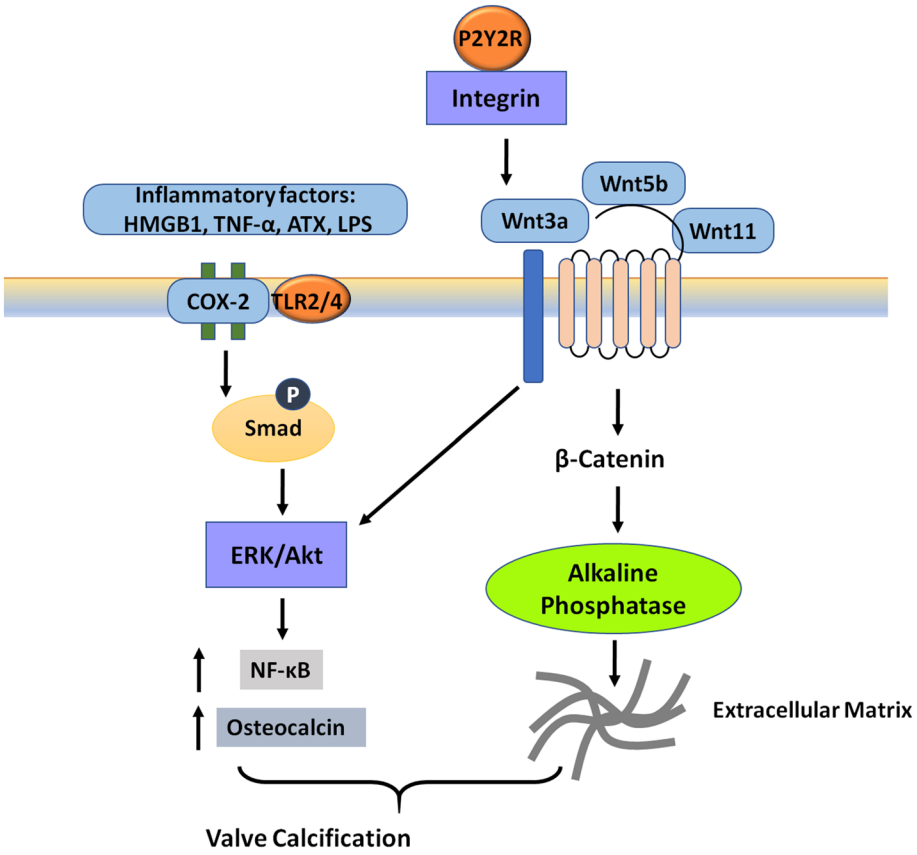

Fig. 3 Inflammation-associated factors including HMGB1, TNF-ATX, LPS or even integrin trigger the pathway of TLR, COX-2 and Wnts, causing the activation of Smad and upregulation of β-catenin. Activation of either ERK or Akt is the critical components of these events

with positive effects on myocardial fibrosis, diastolic function and clinical outcomes (Brilla et al. 2000; López et al. 2001).

The relationship between calcification and OPG/RANKL/TRAIL pathway

Osteoporosis is related to the increase of incidence of aortic valve stenosis and rapid development of disease progression, reserving a close relationship to the cycle of bone lose and formation with the calcification (Persy and D'Haese 2009; Aksoy et al. 2005). Serum levels of osteopontin, osteoprotegerin and RANKL are useful as biomarkers for estimating the degree of calcification in human carotid atheroma (Higgins et al. 2015). Comparably, Runx2 has been found essential to an osteochondrogenic phenotype and subsequent calcification, which may be independent of systemic lipid metabolism, RANKL expression and macrophage infiltration. Runx2 deletion in smooth muscle cells inhibits vascular osteochondrogenesis and calcification but not atherosclerotic lesion formation (Lin et al. 2016). In contrast, twist-related protein 1 negatively regulated osteoblastic transdifferentiation of human aortic valve interstitial cells via directly inhibiting Runx2 expression (Zhang et al. 2014). Also, low level of Twist1 has been observed in calcified aortic valves and overexpression of Twist1 inhibits the trans-differentiation of VICs into osteoblasts and its downstream osteoblastic markers (Zhang et al. 2014).

An early observation has shown that osteoprotegerin deficiency develops early onset osteoporosis and arterial calcification, predicting the role of osteoprotegerin in calcification (Bucay et al. 1998). Overexpression of RANKL during calcific aortic stenosis might have an important role in the pathogenesis, since secreted RANKL activates vascular interstitial cells to produce extracellular matrix (Kaden et al. 2004; Weiss et al. 2013). In contrast, it has been shown that RANKL promotes vascular calcification while osteoprotegerin (OPG) acts as a RANKL decoy receptor to block this effect (Harper et al. 2016). Figure 4 explains the effect of OPG/RANKL/TRAIL pathway on heart valve calcification by comparing the differences of healthy and calcified valve.

Development of therapeutic strategies

Increased COX2 expression in heart valve calcification has been found and pharmacological inhibition of COX2 by celecoxib reduces calcification and blocks osteogenic

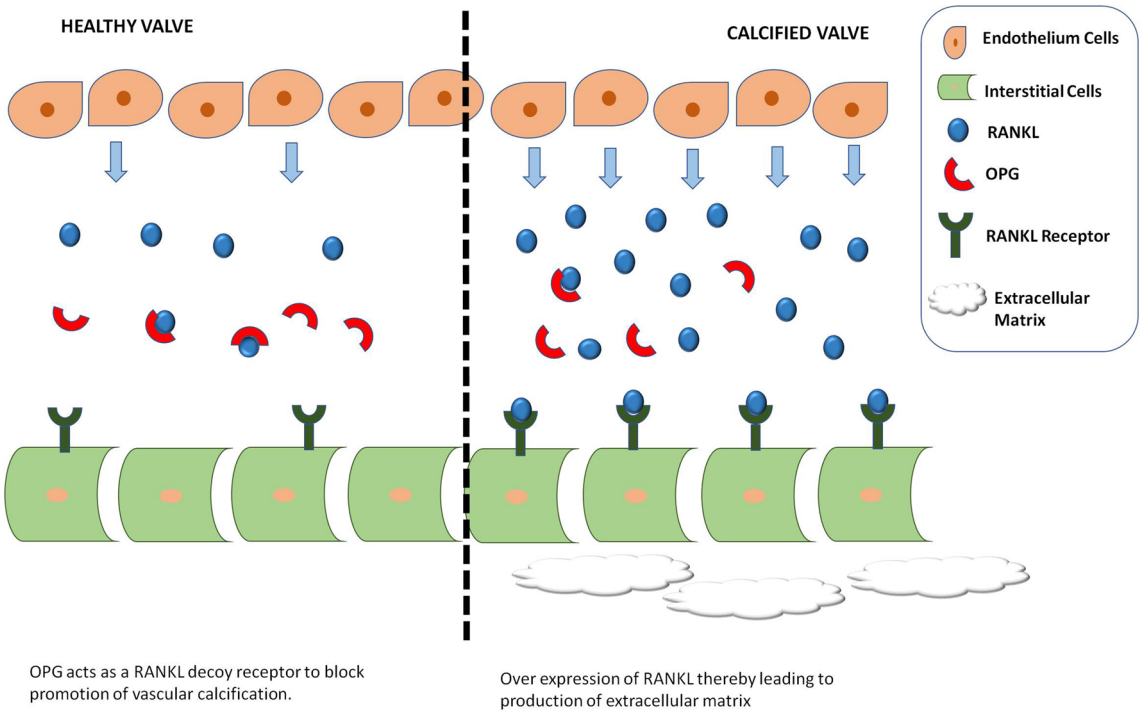

Fig. 4 The role of OPG/RANKL/TRAIL pathway on heart valve calcification. In calcified valve, endothelial cells released higher levels of RANKL which binds to the RANKL receptor and activates the event of extracellular matrix, compared to healthy valve. Activated RANKL pathway released more extracellular matrix, accelerating the process of calcification

gene expression, suggesting that targeting inflammation is an efficient approach to treat heart valve calcification (Wirrig et al. 2015).

The nodule formation could be blocked by specific inhibitors including H1152 and Y27632 (Gu and Masters 2011). Thus, Rho/ROCK is a potential therapeutic target for treating heart valve calcification. Impressively, Witt et al. demonstrated that polyunsaturated fatty acids, including several active components such as docosahexaenoic acid and arachidonic acid can reverse activation of valve interstitial cells into contractile myofibroblasts via decreasing the activity of RhoA and enhanced the ratio of G/F-actin (Witt et al. 2014).

In addition, Fasudil, an inhibitor of the Rho kinase pathway, can also selectively inhibit nodule formation via inhibition of Smad2 phosphorylation, Erk1/2 phosphorylation and p38 MAPK phosphorylation, thus confirming the potential clinical significance of inhibiting the Rho kinase pathway (Das et al. 2013).

Either docosahexaenoic acid or arachidonic acid can decrease G-actin-regulated nuclear translocation of myocardin-related transcription factors, co-activators of serum response factor. C3 transferase can directly block the RhoA as well (Das et al. 2013). Thus, diets with high ratio of polyunsaturated fatty acids may be a useful administration approach to slow down the progress of heart valve calcification.

Dynamically, biological hydrogels could improve the valve replacement situation via the printed trileaflet valve conduits (Duan et al. 2014). 3-D printable formulations of hybrid hydrogels are developed based on methacrylated hyaluronic acid and methacrylated gelatin, and used to bioprint heart valve conduits containing encapsulated human aortic HAVICs. HAVIC encapsulated within bioprinted heart valves maintained high viability, and remodeled the initial matrix by depositing collagen and glycosaminoglycans, implying that HAVIC could be used as the building blocks for preparing the artificial heart valves in near future (Duan et al. 2014). Furthermore, a clickable chemical reaction by reacting an 8-arm poly(ethylene glycol) alkyne with an azide-functionalized photodegradable crosslinker, photodegradable hydrogel substrate allows to precisely control substrate elasticity and topography in situ (Kirschner et al. 2014). Reducing surface modulus could partially reverse VIC activation on stiff surfaces and anisotropic topographies could subsequently re-activate it. These dynamic substrates provide unique opportunities to decipher the complicated role of matrix cues on the plasticity of VIC activation (Kirschner et al. 2014). Preservation of the quiescent fibroblast phenotype of VICs by soft hydrogels displays much better performance than stiff plastic plates (Wang et al. 2013).

The role of stiffness and adhesivity of hydrogels on VIC behavior has been explored using synthesized methacrylated hyaluronic acid and oxidized methacrylated hyaluronic acid (Duan et al. 2013). Adding methacrylated-gel in methacrylated hyaluronic acid enhanced VIC migration, cell spreading and proliferation from encapsulated spheroids and better maintained the VIC fibroblastic phenotype (Duan et al. 2013). Fine tuning the blends of fast-degrading poly(glycerol sebacate) (PGS) and slowly degrading polycaprolactone (PCL) to prepare the hybrid polyester scaffolds which is used to explore the effect of in vitro degradation and ECM secretion on the mechanical properties. Higher ECM protein secretion was observed in VICs seeded on PGS–PCL scaffolds compared with VICs seeded on PCL (Sant et al. 2013). Faster-degrading PGS component of PGS–PCL scaffolds increased the degradation rate. On the other hand, VICs could restructure the synthetic scaffold, depositing novel matrix proteins and maintaining the mechanical properties of the scaffolds (Sant et al. 2013). Furthermore, VICs can be cultivated on anisotropic poly(glycerol sebacate) scaffolds to generate biomimetic in vitro models with clinically relevant cells (Masoumi et al. 2013).

Osteoprotegerin pathways related to RANKL, OPG and RANK are the useful molecular basis for developing the approaches for treating vascular diseases (Dawson and Lawrie 2017). Further, osteopontin protects VICs against in vitro calcification due to its interaction with CD44, demonstrated by proximity ligation assay (Poggio et al. 2014).

Nodule formation was inhibited by various pathway inhibitors. For instance, SB431542, the inhibitor of the Smad pathway, SB203580, the inhibitor of the P38 MAPK pathway, and U0126, the inhibitor of the Mekl/2/Erk1/2 pathway can slow down the calcification (Das et al. 2013). Furthermore, small-interfering RNA for knock-downing cadherin-11 abrogated calcific nodule formation, implying that robust cell–cell connections are necessary in generating tension for calcific nodule morphogenesis, which is consistently associated with TGF-beta1 adminstration (Hutcheson et al. 2013).

We revealed that Noggin, a protein blocking BMP-2, inhibits the calcification in human heart valve interstitial cells (Yang et al. 2009b). This finding is consistently supported by recent evidence that Noggin alleviates the osteogenic activation and osteoblast differentiation has been released as well. Osteoblastic differentiation and cell calcification of adamantinomatous craniopharyngioma are induced by bone morphogenetic protein-2 (Poggio et al. 2013). In meantime, broadly blocking BMP-2 pathway with inhibiting Smad1 and ERK1/2 could reduce the events of heart valve calcification (Yang et al. 2009a).

Clinical evidence demonstrated that metformin administration was independently associated with a lower below-the-knee arterial calcification score, contributing to metformin's recently defined vascular protective effect (Mary et al. 2017). In contrast, retinoic acid treatment in mature heart valves enhanced calcific processes in vitro, which can be attenuated by Sox9 overexpression (Peacock et al. 2010).

One of non-glucocorticoid steroids,21-aminosteroid (U-74389G), could serve as an antioxidant to inhibit peroxidation of lipids via entering cell membranes. This small molecule shows a clinic potential to defend ischemia/reperfusion injury of the central nervous and liver (Alhan et al. 2006; Chang et al. 2011). Inhibition of anti-inflammatory NF-kB may mediate these anti-inflammatory effects of this drug (Okada et al. 2000; Reeves et al. 2013). Recently, the NF-kB inhibitory activity of two 21-aminosteroids, anecortave and VBP15, were found to be dependent on binding to the glucocorticoid receptor, contrary to previous models of the mechanism of action of this class of drugs (Reeves et al. 2013; Baudy et al. 2012; Heier et al. 2013). Sun et al. demonstrated that NF-kB pathway is involved in the suppression of U-74389G on osteoblastic differentiation in AVICs. The negative effects of U-74389G on osteogenic gene expression and mineralization of AVICs was blocked by glucocorticoid receptor antagonist mifepristone and the NF-kB inhibitor Bay 11-7082 (Sun et al. 2015).

Raloxifene belongs to the second generation of selective estrogen receptor modulators, which exhibits estrogen-like effects on cardiovascular and bone tissues and antiestrogen effects on uterine and breast tissues with significant tissue selectivity (Barrett-Connor et al. 2006). Raloxifene induces cell death associated with autophagy; the mechanism was mediated by the activation of AMP-activated protein kinase (AMPK) pathway via decreases in intracellular ATP in cancer cells. The overactivation of autophagy leading to cell death maybe one of the important mechanisms of the therapy effect of Raloxifene (Kim et al. 2015).Thus, the effects of Raloxifene on the proliferation and apoptosis of human aortic valve interstitial cells were determined, showing that Raloxifene reduced the ratio of the S stage and the cell apoptosis rate of AVICs. Furthermore, Raloxifene decreased the mRNA and protein expressions of caspase-3 and caspase-8, indicating the effect of Raloxifene on apoptosis cell signaling pathway (Fu et al. 2016).

Small interfering RNA, c-Jun MAPK and NF-kB inhibitors can slow down the progression of heart valve calcification process (Wang et al. 2016). Inhibition of NF-kB but not ERK1/2 could prevent interleukin-1β-induced inflammatory phenotype in human AVICs, leading to the prevention of the interleukin-1β-amplified osteogenic changes (Nadlonek et al. 2013b). This observation implies that NF-kB may play an important role in the process of interleukin-1β-induced heart valve calcification (Nadlonek et al. 2013b). In contrast, inhibition of either NF-kB or ERK1/2 can reduce lipopolysaccharide-induced

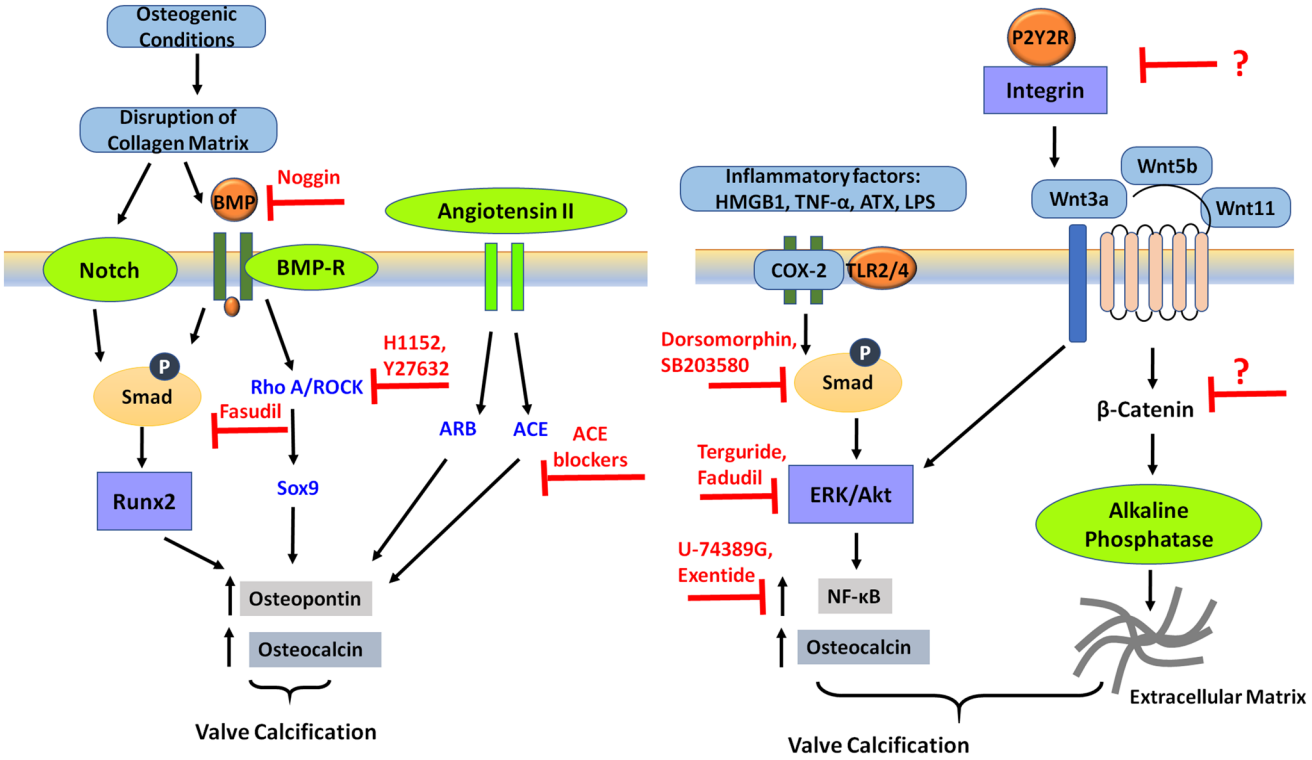

Fig. 5 The effect of various therapeutic candidates targeting BMP, Smad, RhoA, ROCK, ACE, ERK/Akt, NF-κB, etc., on attenuating cellular calcification has been examined. The strategy targeting either P2Y2R/Integrin or β-catenin is under investigation

bone morphogenetic protein-2 and alkaline phosphatase expression (Zeng et al. 2013).

Delivery of antioxidant enzymes may be an efficient approach to reverse the calcification, as antioxidant enzymes block ROS-induced DNA damage (Miller et al. 2011). Rush et al. provided the evidence that surface chemistry modulates VIC phenotype and calcified tissue deposition independent of osteoblastic-inducing media additives (Rush et al. 2015).

Users of angiotensin receptor blockers showed a lower remodeling score of stenotic aortic valves from a clinical observation (Côté et al. 2011). An earlier observation reports the evidence of lower events of progression of coronary artery calcification (Maahs et al. 2007).

Targeting NF-kappa B signaling pathway using Exenatide, an inhibitor of agonist of GLP-1, can inhibit calcification (Zhan et al. 2014). Blocking angiotensin receptor can reduce fibrosis and the IL-6 level in aortic valve calcification (Côté et al. 2013).

5-HT-induced activation of extracellular signal-regulated kinase (ERK) 1/2, an initiator of cellular proliferation and activity, was blocked by terguride in valvular interstitial cells. The stimulatory effect of 5-HT on [^{3}H] proline incorporation, index of extracellular matrix collagen, was blocked by terguride as well (Kekewska et al. 2012).

Dorsomorphin, an inhibitor of Smads1/5/8 phosphorylation, significantly inhibited the enhancement of TNF-α-induced calcification, ALP activity, Smad phosphorylation, and Dlx5 gene expression of HAVICs from calcific aortic valve stenosis. These results indicate that inhibition of BMP2-Dlx5 pathway may slow down the heart valve calcification (Yu et al. 2011). Figure 5 summarizes the findings of several therapeutic candidates that target BMP, Smad, RhoA, ROCK, ACE, ERK/Akt, and NF-κB on calcification.

Future prospects

All together, calcification is a controllable, interventable and targetable degenerative process. Isolated interstitial cells are an ideal in vitro model to explore molecular mechanisms and validate the druggable targets associated with heart valve calcification.

Although we focus on human interstitial cells in this present review, porcine heart valve interstitial cells are becoming an alternative model. The growth of the heart in swine from birth to four months is parallel to that in humans from birth to mid-teens (Swindle et al. 2012) and remodeling caused by atherosclerosis in minipigs closely resembles that in humans (De Smet et al. 1998). Swine can also develop spontaneous

valvular atherosclerotic lesions, a precursor to calcification (Guerraty and Mohler 2007; Simmons et al. 2005). Isolated porcine AVICs appear more homogenous than murine or leporine VICs and have a high recovery rate after being frozen, resulting in a more extensive use in in vitro studies (Johnson et al. 1987).

Since mice are easy to handle, affordable to administrate, and available to genetic mutants, mice have been another popular models of heart valve calcification. Murine AVICs could be harvested from a variety of genetically altered models such as ApoE$^{-/-}$, Notch1$^{+/-}$, and LDLr$^{-/-}$ (Guerraty and Mohler 2007; Awan et al. 2011). However, murine valvular structure is significantly different from that of human: human valve has a trilaminar structure, whereas murine valve is dilaminar, a fibrosa and a spongiosa (Treuting and Dintzis 2011).

In summary, isolated VICs are useful cellular models to explore the molecular mechanism and potential therapeutics to treat heart valve calcification. Although a lot of progress have been made, the most obviously challenges are the physiological difference between the cell culture system and in vivo heart valve calcification. Thus, the approaches recently developed using the synthetic biodegradable polymer to restructure the 3-D model culture in VICs should be a promising future direction. We anticipate that with the concerted efforts of scientists, clinicians, and available drugs an effective treatment of heart valve calcification will emerge soon.

Acknowledgements This project was supported by the National Innovation Student Program (201430191040) to H.T., Z. M., N. L., Z. Z., the National Key Research and Development Program of China (2016YFD0501201) to Y. Y. and X. Y., Open Fund of National Laboratory of Subtropical Agriculture Ecology Process, Chinese Academy of Sciences (ISA2016204), and Xiaoxiang Endowed University Professor Fund of Hunan Normal University (No. 840140-008) to X.Y, Chinese Academy of Sciences Visiting Professorship for Senior International Scientists Grant (no. 2016VBB007) to C. A. H.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical statements This review does not involve any human participants and animal work.

References

Acharya A, Hans CP, Koenig SN, Nichols HA, Galindo CL, Garner HR, Merrill WH, Hinton RB, Garg V (2011) Inhibitory role of Notch1 in calcific aortic valve disease. PLoS One 6(11):e27743

Adiguzel E, Ahmad PJ, Franco C, Bendeck MP (2009) Collagens in the progression and complications of atherosclerosis. Vasc Med 14(1):73–89

Agca Y, Qian S, Agca C, Seye CI (2016) Direct evidence for P2Y2 receptor involvement in vascular response to injury. J Vasc Res 53(3–4):163–171

Aksoy Y, Yagmur C, Tekin GO, Yagmur J, Topal E, Kekilli E, Turhan H, Kosar F, Yetkin E (2005) Aortic valve calcification: association with bone mineral density and cardiovascular risk factors. Coron Artery Dis 16(6):379–383

Albanese I, Yu B, Al-Kindi H, Barratt B, Ott L, Al-Refai M, de Varennes B, Shum-Tim D, Cerruti M, Gourgas O, Rhéaume E (2016) Role of noncanonical wnts signaling pathway in human aortic valve calcification. Arteriosclerosis, thrombosis, and vascular biology. ATVBAHA-116

Alhan E, Türkyılmaz S, Erçin C, Kural BV (2006) Effects of lazaroid U-74389G on acute necrotizing pancreatitis in rats. Eur Surg Res 38(2):70–75

Andrassy M, Volz HC, Schuessler A, Gitsioudis G, Hofmann N, Laohachewin D, Wienbrandt AR, Kaya Z, Bierhaus A, Giannitsis E, Katus HA (2012) HMGB1 is associated with atherosclerotic plaque composition and burden in patients with stable coronary artery disease. PLoS One 7(12):e52081

Arevalos CA, Berg JM, Nguyen JM, Godfrey EL, Iriondo C, Grande-Allen KJ (2016) Valve interstitial cells act in a pericyte manner promoting angiogenesis and invasion by valve endothelial cells. Ann Biomed Eng 44(9):2707–2723

Awan Z, Denis M, Bailey D, Giaid A, Prat A, Goltzman D, Seidah NG, Genest J (2011) The LDLR deficient mouse as a model for aortic calcification and quantification by micro-computed tomography. Atherosclerosis 219(2):455–462

Ayoub S, Ferrari G, Gorman RC, Gorman JH, Schoen FJ, Sacks MS (2016) Heart valve biomechanics and underlying mechanobiology. Compr Physiol 6(4):1743–1780

Babu AN, Meng X, Zou N, Yang X, Wang M, Song Y, Cleveland JC, Weyant M, Banerjee A, Fullerton DA (2008) Lipopolysaccharide stimulation of human aortic valve interstitial cells activates inflammation and osteogenesis. Ann Thorac Surg 86(1):71–76

Balachandran K, Bakay MA, Connolly JM, Zhang X, Yoganathan AP, Levy RJ (2011) Aortic valve cyclic stretch causes increased remodeling activity and enhanced serotonin receptor responsiveness. Ann Thorac Surg 92(1):147–153

Barnette DN, Hulin A, Ahmed AI, Colige AC, Azhar M, Lincoln J (2013) Tgfβ-Smad and MAPK signaling mediate scleraxis and proteoglycan expression in heart valves. J Mol Cell Cardiol 65:137–146

Barrett-Connor E, Mosca L, Collins P, Geiger MJ, Grady D, Kornitzer M, McNabb MA, Wenger NK (2006) Effects of raloxifene on cardiovascular events and breast cancer in postmenopausal women. N Engl J Med 355(2):125–137

Barth M, Rickelt S, Noffz E, Winter-Simanowski S, Niemann H, Akhyari P, Lichtenberg A, Franke WW (2012) The adhering junctions of valvular interstitial cells: molecular composition in fetal and adult hearts and the comings and goings of plakophilin-2 in situ, in cell culture and upon re-association with scaffolds. Cell Tissue Res 348(2):295–307

Baudy AR, Reeves EK, Damsker JM, Heier C, Garvin LM, Dillingham BC, McCall J, Rayavarapu S, Wang Z, Vandermeulen JH, Sali A (2012) Δ-9, 11 modification of glucocorticoids dissociates nuclear factor-κB inhibitory efficacy from glucocorticoid response element-associated side effects. J Pharmacol Exp Ther 343(1):225–232

Bouchareb R, Mahmut A, Nsaibia MJ, Boulanger MC, Dahou A, Lépine JL, Laflamme MH, Hadji F, Couture C, Trahan S, Pagé S (2015) Autotaxin derived from lipoprotein (a) and valve interstitial cells promotes inflammation and mineralization of the aortic valve. Circulation. CIRCULATIONAHA-115

Branchetti E, Sainger R, Poggio P, Grau JB, Patterson-Fortin J, Bavaria JE, Chorny M, Lai E, Gorman RC, Levy RJ, Ferrari

G (2013) Antioxidant enzymes reduce DNA damage and early activation of valvular interstitial cells in aortic valve sclerosis. Arterioscler Thromb Vasc Biol 33(2):e66–e74

Brand NJ, Roy A, Hoare G, Chester A, Yacoub MH (2006) Cultured interstitial cells from human heart valves express both specific skeletal muscle and non-muscle markers. Int J Biochem Cell Biol 38(1):30–42

Brilla CG, Funck RC, Rupp H (2000) Lisinopril-mediated regression of myocardial fibrosis in patients with hypertensive heart disease. Circulation 102(12):1388–1393

Bucay N, Sarosi I, Dunstan CR, Morony S, Tarpley J, Capparelli C, Scully S, Tan HL, Xu W, Lacey DL, Boyle WJ (1998) Osteoprotegerin-deficient mice develop early onset osteoporosis and arterial calcification. Genes Dev 12(9):1260–1268

Butcher JT, Markwald RR (2007) Valvulogenesis: the moving target. Philos Trans R Soc Lond B Biol Sci 362(1484):1489–1503

Capoulade R, Clavel MA, Mathieu P, Côté N, Dumesnil JG, Arsenault M, Bédard É, Pibarot P (2013) Impact of hypertension and renin–angiotensin system inhibitors in aortic stenosis. Eur J Clin Invest 43(12):1262–1272

Chang YT, Wann SR, Hsieh KH, Liu YC, Chang CH, Huang MS, Huang CI, Chang HT (2011) The effects of lazaroid U-74389G in a rat sepsis model. Inflamm Res 60(1):29–35

Chen JH, Chen WL, Sider KL, Yip CY, Simmons CA (2011) β-Catenin mediates mechanically regulated, transforming growth factor-β1-induced myofibroblast differentiation of aortic valve interstitial cells. Arterioscler Thromb Vasc Biol 31(3):590–597

Chen J, Ryzhova LM, Sewell-Loftin MK, Brown CB, Huppert SS, Baldwin HS, Merryman WD (2015) Notch1 mutation leads to valvular calcification through enhanced myofibroblast mechanotransduction significance. Arterioscler Thromb Vasc Biol 35(7):1597–1605

Chester AH, Taylor PM (2007) Molecular and functional characteristics of heart-valve interstitial cells. Philos Trans R Soc Lond B Biol Sci 362(1484):1437–1443

Côté N, Couture C, Pibarot P, Després JP, Mathieu P (2011) Angiotensin receptor blockers are associated with a lower remodelling score of stenotic aortic valves. Eur J Clin Invest 41(11):1172–1179

Côté N, Mahmut A, Fournier D, Boulanger MC, Couture C, Després JP, Trahan S, Bossé Y, Pagé S, Pibarot P, Mathieu P (2013) Angiotensin receptor blockers are associated with reduced fibrosis and interleukin-6 expression in calcific aortic valve disease. Pathobiology 81(1):15–24

Das D, Holmes A, Murphy GA, Mishra K, Rosenkranz AC, Horowitz JD, Kennedy JA (2013) TGF-beta1-Induced MAPK activation promotes collagen synthesis, nodule formation, redox stress and cellular senescence in porcine aortic valve interstitial cells. J Heart Valve Dis 22(5):621–630

Dawson S, Lawrie A (2017) From bones to blood pressure, developing novel biologic approaches targeting the osteoprotegerin pathway for pulmonary vascular disease. Pharmacol Ther 169:78–82

De Smet BJ, Van der Zande J, Van Der Helm YJ, Kuntz RE, Borst C, Post MJ (1998) The atherosclerotic Yucatan animal model to study the arterial response after balloon angioplasty: the natural history of remodeling. Cardiovasc Res 39(1):224–232

Deng XS, Meng X, Zeng Q, Fullerton D, Mitchell M, Jaggers J (2015) Adult aortic valve interstitial cells have greater responses to toll-like receptor 4 stimulation. Ann Thorac Surg 99(1):62–71

Duan B, Hockaday LA, Kapetanovic E, Kang KH, Butcher JT (2013) Stiffness and adhesivity control aortic valve interstitial cell behavior within hyaluronic acid based hydrogels. Acta Biomater 9(8):7640–7650

Duan B, Kapetanovic E, Hockaday LA, Butcher JT (2014) Three-dimensional printed trileaflet valve conduits using biological hydrogels and human valve interstitial cells. Acta Biomater 10(5):1836–1846

El Husseini D, Boulanger MC, Mahmut A, Bouchareb R, Laflamme MH, Fournier D, Pibarot P, Bossé Y, Mathieu P (2014) P2Y2 receptor represses IL-6 expression by valve interstitial cells through Akt: implication for calcific aortic valve disease. J Mol Cell Cardiol 72:146–156

Fang M, Alfieri CM, Hulin A, Conway SJ, Yutzey KE (2014) Loss of β-catenin promotes chondrogenic differentiation of aortic valve interstitial cells. Arteriosclerosis, thrombosis, and vascular biology. ATVBAHA-114

Farrar EJ, Pramil V, Richards JM, Mosher CZ, Butcher JT (2016) Valve interstitial cell tensional homeostasis directs calcification and extracellular matrix remodeling processes via RhoA signaling. Biomaterials 105:25–37

Fernandez-Pisonero I, Lopez J, Onecha E, Duenas AI, Maeso P, Crespo MS, San Roman JA, García-Rodríguez C (2014) Synergy between sphingosine 1-phosphate and lipopolysaccharide signaling promotes an inflammatory, angiogenic and osteogenic response in human aortic valve interstitial cells. PLoS One 9(10):e109081

Fisher CI, Chen J, Merryman WD (2013) Calcific nodule morphogenesis by heart valve interstitial cells is strain dependent. Biomech Model Mechanobiol 12(1):5–17

Fu Z, Luo B, Li M, Peng B, Wang Z (2016) Effects of Raloxifene on the proliferation and apoptosis of human aortic valve interstitial cells. BioMed Res Int. doi:10.1155/2016/5473204

Galeone A, Brunetti G, Oranger A, Greco G, Di Benedetto A, Mori G, Colucci S, Zallone A, Paparella D, Grano M (2013) Aortic valvular interstitial cells apoptosis and calcification are mediated by TNF-related apoptosis-inducing ligand. Int J Cardiol 169(4):296–304

Garg V, Muth AN, Ransom JF, Schluterman MK, Barnes R, King IN, Grossfeld PD, Srivastava D (2005) Mutations in NOTCH1 cause aortic valve disease. Nature 437(7056):270–274

Gohlke-Bärwolf C, Minners J, Jander N, Gerdts E, Wachtell K, Ray S, Pedersen TR (2013) Natural history of mild and of moderate aortic stenosis—new insights from a large prospective European study. Curr Probl Cardiol 38(9):365–409

Gomez-Stallons MV, Wirrig-Schwendeman EE, Hassel KR, Conway SJ, Yutzey KE (2016) Bone morphogenetic protein signaling is required for aortic valve calcification. Arteriosclerosis, thrombosis, and vascular biology. ATVBAHA-116

Gu X, Masters KS (2011) Role of the Rho pathway in regulating valvular interstitial cell phenotype and nodule formation. Am J Physiol Heart Circ Physiol 300(2):H448–H458

Guerraty M, Mohler ER (2007) Models of aortic valve calcification. J Investig Med 55(6):278–283

Hadji F, Boulanger MC, Guay SP, Gaudreault N, Amellah S, Mkannez G, Bouchareb R, Marchand JT, Nsaibia MJ, Guauque-Olarte S, Pibarot P (2016) Altered DNA methylation of long noncoding RNA H19 in calcific aortic valve disease promotes mineralization by silencing NOTCH1 clinical perspective. Circulation 134(23):1848–1862

Han L, Gotlieb AI (2012) Fibroblast growth factor-2 promotes in vitro heart valve interstitial cell repair through the Akt1 pathway. Cardiovasc Pathol 21(5):382–389

Harper E, Forde H, Davenport C, Rochfort KD, Smith D, Cummins PM (2016) Vascular calcification in type-2 diabetes and cardiovascular disease: integrative roles for OPG, RANKL and TRAIL. Vasc Pharmacol 82:30–40

Heier CR, Damsker JM, Yu Q, Dillingham BC, Huynh T, Van der Meulen JH, Sali A, Miller BK, Phadke A, Scheffer L, Quinn J (2013) VBP15, a novel anti-inflammatory and membrane-stabilizer, improves muscular dystrophy without side effects. EMBO Mol Med 5(10):1569–1585

Helske S, Kupari M, Lindstedt KA, Kovanen PT (2007) Aortic valve stenosis: an active atheroinflammatory process. Curr Opin Lipidol 18(5):483–491

Higgins CL, Isbilir S, Basto P, Chen IY, Vaduganathan M, Vaduganathan P, Reardon MJ, Lawrie G, Peterson L, Morrisett JD (2015) Distribution of alkaline phosphatase, osteopontin, RANK ligand and osteoprotegerin in calcified human carotid atheroma. Protein J 34(5):315–328

Houslay ES, Cowell SJ, Prescott RJ, Reid J, Burton J, Northridge DB, Boon NA, Newby DE (2006) Progressive coronary calcification despite intensive lipid-lowering treatment: a randomised controlled trial. Heart 92(9):1207–1212

Huk DJ, Austin BF, Horne TE, Hinton RB, Ray WC, Heistad DD, Lincoln J (2015) Valve endothelial cell–derived Tgfβ1 signaling promotes nuclear localization of Sox9 in interstitial cells associated with attenuated calcification. Arteriosclerosis, thrombosis, and vascular biology. ATVBAHA-115

Hutcheson JD, Chen J, Sewell-Loftin MK, Ryzhova LM, Fisher CI, Su YR, Merryman WD (2013) Cadherin-11 regulates cell–cell tension necessary for calcific nodule formation by valvular myofibroblasts. Arterioscler Thromb Vasc Biol 33(1):114–120

Ibuka S, Matsumoto S, Fujii S, Kikuchi A (2015) The P2Y2 receptor promotes Wnt3a-and EGF-induced epithelial tubular formation by IEC6 cells by binding to integrins. J Cell Sci 128(11):2156–2168

Iung B, Vahanian A (2011) Epidemiology of valvular heart disease in the adult. Nat Rev Cardiol 8(3):162–172

Johnson CM, Hanson MN, Helgeson SC (1987) Porcine cardiac valvular subendothelial cells in culture: cell isolation and growth characteristics. J Mol Cell Cardiol 19(12):1185–1193

Kaden JJ, Bickelhaupt S, Grobholz R, Haase KK, Sarıkoç A, Kılıç R, Brueckmann M, Lang S, Zahn I, Vahl C, Hagl S (2004) Receptor activator of nuclear factor κB ligand and osteoprotegerin regulate aortic valve calcification. J Mol Cell Cardiol 36(1):57–66

Kekewska A, Görnemann T, Jantschak F, Glusa E, Pertz HH (2012) Antiserotonergic properties of terguride in blood vessels, platelets, and valvular interstitial cells. J Pharmacol Exp Ther 340(2):369–376

Kim DE, Kim Y, Cho DH, Jeong SY, Kim SB, Suh N, Lee JS, Choi EK, Koh JY, Hwang JJ, Kim CS (2015) Raloxifene induces autophagy-dependent cell death in breast cancer cells via the activation of AMP-activated protein kinase. Mol Cells 38(2):138

Kirschner CM, Alge DL, Gould ST, Anseth KS (2014) Clickable, photodegradable hydrogels to dynamically modulate valvular interstitial cell phenotype. Adv Healthc Mater 3(5):649–657

Latif N, Quillon A, Sarathchandra P, McCormack A, Lozanoski A, Yacoub MH, Chester AH (2015) Modulation of human valve interstitial cell phenotype and function using a fibroblast growth factor 2 formulation. PLoS One 10(6):e0127844

Libby P (2013) Collagenases and cracks in the plaque. J Clin Investig 123(8):3201–3203

Libby P, Sasiela W (2006) Plaque stabilization: can we turn theory into evidence? Am J Cardiol 98(11):S26–S33

Lin ME, Chen TM, Wallingford MC, Nguyen NB, Yamada S, Sawangmake C, Zhang J, Speer MY, Giachelli CM (2016) Runx2 deletion in smooth muscle cells inhibits vascular osteochondrogenesis and calcification but not atherosclerotic lesion formation. Cardiovasc Res 112(2):606–616

Lindman BR, Clavel MA, Mathieu P, Iung B, Lancellotti P, Otto CM, Pibarot P (2016) Calcific aortic stenosis. Nature reviews. Dis Prim 2:16006

López B, Querejeta R, Varo N, González A, Larman M, Ubago JL, Díez J (2001) Usefulness of serum carboxy-terminal propeptide of procollagen type I in assessment of the cardioreparative ability of antihypertensive treatment in hypertensive patients. Circulation 104(3):286–291

López J, Fernández-Pisonero I, Dueñas AI, Maeso P, San Román JA, Crespo MS, García-Rodríguez C (2012) Viral and bacterial patterns induce TLR-mediated sustained inflammation and calcification in aortic valve interstitial cells. Int J Cardiol 158(1):18–25

Maahs DM, Snell-Bergeon JK, Kinney GL, Wadwa RP, Garg S, Ogden LG, Rewers M (2007) ACE-I/ARB treatment in type 1 diabetes patients with albuminuria is associated with lower odds of progression of coronary artery calcification. J Diabetes Complicat 21(5):273–279

Mahmut A, Boulanger MC, El Husseini D, Fournier D, Bouchareb R, Després JP, Pibarot P, Bossé Y, Mathieu P (2014) Elevated expression of lipoprotein-associated phospholipase A2 in calcific aortic valve disease: implications for valve mineralization. J Am Coll Cardiol 63(5):460–469

Mary A, Hartemann A, Liabeuf S, Aubert CE, Kemel S, Salem JE, Cluzel P, Lenglet A, Massy ZA, Lalau JD, Mentaverri R (2017) Association between metformin use and below-the-knee arterial calcification score in type 2 diabetic patients. Cardiovasc Diabetol 16(1):24

Masjedi S, Amarnath A, Baily KM, Ferdous Z (2016) Comparison of calcification potential of valvular interstitial cells isolated from individual aortic valve cusps. Cardiovasc Pathol 25(3):185–194

Masoumi N, Johnson KL, Howell MC, Engelmayr GC (2013) Valvular interstitial cell seeded poly (glycerol sebacate) scaffolds: toward a biomimetic in vitro model for heart valve tissue engineering. Acta Biomater 9(4):5974–5988

Mathieu P, Voisine P, Pepin A, Shetty R, Savard N, Dagenais F (2005) Calcification of human valve interstitial cells is dependent on alkaline phosphatase activity. J Heart Valve Dis 14(3):353–357

Mathieu P, Bouchareb R, Boulanger MC (2015) Innate and adaptive immunity in calcific aortic valve disease. J Immunol Res 3:2015

Meng X, Ao L, Song Y, Babu A, Yang X, Wang M, Weyant MJ, Dinarello CA, Cleveland JC, Fullerton DA (2008) Expression of functional Toll-like receptors 2 and 4 in human aortic valve interstitial cells: potential roles in aortic valve inflammation and stenosis. Am J Physiol Cell Physiol 294(1):C29–C35

Meredith GT, Ramponi F, Scurry JP, Singh T (2013) Fatty infiltration of an aortic valve. Ann Thorac Surg 96(2):697–699

Miller JD, Weiss RM, Heistad DD (2011) Calcific aortic valve stenosis: methods, models, and mechanisms. Circ Res 108(11):1392–1412

Mohler ER, Gannon F, Reynolds C, Zimmerman R, Keane MG, Kaplan FS (2001) Bone formation and inflammation in cardiac valves. Circulation 103(11):1522–1528

Monzack EL, Masters KS (2011) Can valvular interstitial cells become true osteoblasts? A side-by-side comparison. J Heart Valve Dis 20(4):449

Mozaffarian D, Benjamin EJ, Go AS, Arnett DK, Blaha MJ, Cushman M, Das SR, de Ferranti S, Després JP, Fullerton HJ, Howard VJ (2016) Heart disease and stroke statistics—2016 update. Circulation 133(4):e38–e60

Nadlonek NA, Weyant MJ, Jessica AY, Cleveland JC, Reece TB, Meng X, Fullerton DA (2012) Radiation induces osteogenesis in human aortic valve interstitial cells. J Thorac Cardiovasc Surg 144(6):1466–1470

Nadlonek NA, Lee JH, Weyant MJ, Meng X, Fullerton DA (2013a) ox-LDL induces PiT-1 expression in human aortic valve interstitial cells. J Surg Res 184(1):6–9

Nadlonek N, Lee JH, Reece TB, Weyant MJ, Cleveland JC, Meng X, Fullerton DA (2013b) Interleukin-1 Beta induces an inflammatory phenotype in human aortic valve interstitial cells through nuclear factor kappa Beta. Ann Thorac Surg 96(1):155–162

Nagy E, Bäck M (2012) Epigenetic regulation of 5-lipoxygenase in the phenotypic plasticity of valvular interstitial cells associated with aortic valve stenosis. FEBS Lett 586(9):1325–1329

Ng LJ, Wheatley S, Muscat GE, Conway-Campbell J, Bowles J, Wright E, Bell DM, Tam PP, Cheah KS, Koopman P (1997) SOX9 binds DNA, activates transcription, and coexpresses with type II collagen during chondrogenesis in the mouse. Dev Biol 183(1):108–121

Nomura A, Seya K, Yu Z, Daitoku K, Motomura S, Murakami M, Fukuda I, Furukawa KI (2013) CD34-negative mesenchymal stem-like cells may act as the cellular origin of human aortic valve calcification. Biochem Biophys Res Commun 440(4):780–785

Okada K, Marubayashi S, Fukuma K, Yamada K, Dohi K (2000) Effect of the 21-aminosteroid on nuclear factor-κB activation of Kupffer cells in endotoxin shock. Surgery 127(1):79–86

Osako MK, Nakagami H, Shimamura M, Koriyama H, Nakagami F, Shimizu H, Miyake T, Yoshizumi M, Rakugi H, Morishita R (2013) Cross-talk of receptor activator of nuclear factor-κB ligand signaling with renin-angiotensin system in vascular calcification significance. Arterioscler Thromb Vasc Biol 33(6):1287–1296

Osman L, Yacoub MH, Latif N, Amrani M, Chester AH (2006) Role of human valve interstitial cells in valve calcification and their response to atorvastatin. Circulation 114(suppl 1):I-547

Osman N, Grande-Allen KJ, Ballinger ML, Getachew R, Marasco S, O'Brien KD, Little PJ (2013) Smad2-dependent glycosaminoglycan elongation in aortic valve interstitial cells enhances binding of LDL to proteoglycans. Cardiovasc Pathol 22(2):146–155

Passmore M, Nataatmadja M, Fung YL, Pearse B, Gabriel S, Tesar P, Fraser JF (2015) Osteopontin alters endothelial and valvular interstitial cell behaviour in calcific aortic valve stenosis through HMGB1 regulation. Eur J Cardiothorac Surg 48(3):e20–e29

Peacock JD, Levay AK, Gillaspie DB, Tao G, Lincoln J (2010) Reduced sox9 function promotes heart valve calcification phenotypes in vivo. Circ Res 106(4):712–719

Peltonen T, Ohukainen P, Ruskoaho H, Rysä J (2017) Targeting vasoactive peptides for managing calcific aortic valve disease. Ann Med 49(1):63–74

Persy V, D'Haese P (2009) Vascular calcification and bone disease: the calcification paradox. Trends Mol Med 15(9):405–416

Poggio P, Sainger R, Branchetti E, Grau JB, Lai EK, Gorman RC, Sacks MS, Parolari A, Bavaria JE, Ferrari G (2013) Noggin attenuates the osteogenic activation of human valve interstitial cells in aortic valve sclerosis. Cardiovasc Res 98(3):402–410

Poggio P, Branchetti E, Grau JB, Lai EK, Gorman RC, Gorman JH, Sacks MS, Bavaria JE, Ferrari G (2014) Osteopontin–CD44v6 interaction mediates calcium deposition via phospho-Akt in valve interstitial cells from patients with noncalcified aortic valve sclerosis significance. Arterioscler Thromb Vasc Biol 34(9):2086–2094

Qian S, Regan JN, Shelton MT, Hoggatt A, Mohammad KS, Herring PB, Seye CI (2017) The P2Y 2 nucleotide receptor is an inhibitor of vascular calcification. Atherosclerosis 257: 38–36

Quinlan AM, Billiar KL (2012) Investigating the role of substrate stiffness in the persistence of valvular interstitial cell activation. J Biomed Mater Res Part A 100(9):2474–2482

Rajamannan NM (2011) The role of Lrp5/6 in cardiac valve disease: experimental hypercholesterolemia in the ApoE$^{-/-}$/Lrp5$^{-/-}$ mice. J Cell Biochem 112(10):2987–2991

Rajamannan NM, Subramaniam M, Rickard D, Stock SR, Donovan J, Springett M, Orszulak T, Fullerton DA, Tajik AJ, Bonow RO, Spelsberg T (2003) Human aortic valve calcification is associated with an osteoblast phenotype. Circulation 107(17):2181–2184

Rajamannan NM, Bonow RO, Rahimtoola SH (2007) Calcific aortic stenosis: an update. Nat Clin Pract Cardiovasc Med 4(5):254–262

Reeves EK, Hoffman EP, Nagaraju K, Damsker JM, McCall JM (2013) VBP15: preclinical characterization of a novel anti-inflammatory delta 9, 11 steroid. Bioorg Med Chem 21(8):2241–2249

Rickelt S, Rizzo S, Doerflinger Y, Zentgraf H, Basso C, Gerosa G, Thiene G, Moll R, Franke WW (2010) A novel kind of tumor type-characteristic junction: plakophilin-2 as a major protein of adherens junctions in cardiac myxomata. Mod Pathol 23(11):1429–1437

Roy A, Brand NJ, Yacoub MH (2000) Molecular characterization of interstitial cells isolated from human heart valves. J Heart Valve Dis 9(3):459–464

Rush MN, Coombs KE, Hedberg-Dirk EL (2015) Surface chemistry regulates valvular interstitial cell differentiation in vitro. Acta Biomater 28:76–85

Sakamoto Y, Buchanan RM, Sacks MS (2016) On intrinsic stress fiber contractile forces in semilunar heart valve interstitial cells using a continuum mixture model. J Mech Behav Biomed Mater 54:244–258

Sakamoto Y, Buchanan RM, Sanchez-Adams J, Guilak F, Sacks MS (2017) On the functional role of valve interstitial cell stress fibers: A continuum modeling approach. J Biomech Eng 139(2). doi:10.1115/1.4035557

Sant S, Iyer D, Gaharwar AK, Patel A, Khademhosseini A (2013) Effect of biodegradation and de novo matrix synthesis on the mechanical properties of valvular interstitial cell-seeded polyglycerol sebacate–polycaprolactone scaffolds. Acta Biomater 9(4):5963–5973

Saremi A, Bahn G, Reaven PD (2012) VADT Investigators. Progression of vascular calcification is increased with statin use in the Veterans Affairs Diabetes Trial (VADT). Diabetes Care 35(11):2390–2392

Schoen FJ, Gotlieb AI (2016) Heart valve health, disease, replacement, and repair: a 25-year cardiovascular pathology perspective. Cardiovasc Pathol 25(4):341–352

Shimizu T, Tanaka T, Iso T, Matsui H, Ooyama Y, Kawai-Kowase K, Arai M, Kurabayashi M (2011) Notch signaling pathway enhances bone morphogenetic protein 2 (BMP2) responsiveness of Msx2 gene to induce osteogenic differentiation and mineralization of vascular smooth muscle cells. J Biol Chem 286(21):19138–19148

Simmons CA, Grant GR, Manduchi E, Davies PF (2005) Spatial heterogeneity of endothelial phenotypes correlates with side-specific vulnerability to calcification in normal porcine aortic valves. Circ Res 96(7):792–799

Song Y, Fullerton DA, Mauchley D, Su X, Ao L, Yang X, Cleveland JC, Meng X (2011) Microfilaments facilitate TLR4-mediated ICAM-1 expression in human aortic valve interstitial cells. J Surg Res 166(1):52–58

Song R, Fullerton DA, Ao L, Zheng D, Zhao KS, Meng X (2015) BMP-2 and TGF-β1 mediate biglycan-induced pro-osteogenic reprogramming in aortic valve interstitial cells. J Mol Med 93(4):403–412

Sun W, Zhao R, Yang Y, Wang H, Shao Y, Kong X (2013) Comparative study of human aortic and mitral valve interstitial cell gene expression and cellular function. Genomics 101(6):326–335

Sun F, Shi J, Chen S, Deng C, Hu X, Li H, Li G, Liu Y, Dong N (2015) Lazaroid U-74389G inhibits the osteoblastic differentiation of IL-1β-induced aortic valve interstitial cells through glucocorticoid receptor and inhibition of NF-κB pathway. J Ster Biochem Mol Biol 152:114–123

Sung DC, Bowen CJ, Vaidya KA, Zhou J, Chapurin N, Recknagel A, Zhou B, Chen J, Kotlikoff M, Butcher JT (2016) Cadherin-11 overexpression induces extracellular matrix remodeling and calcification in mature aortic valves. Arteriosclerosis, thrombosis, and vascular biology. ATVBAHA-116

Suzuki K, Takahashi S, Watanabe K, Fujioka D, Nakamura T, Obata JE, Kawabata KI, Katoh R, Matsumoto M, Kugiyama K (2014) The expression of groups IIE and V phospholipase A2 is associated with an increased expression of osteogenic molecules in human calcified aortic valves. J Atheroscler Thromb 21(12):1308–1325

Swindle MM, Makin A, Herron AJ, Clubb FJ, Frazier KS (2012) Swine as models in biomedical research and toxicology testing. Vet Pathol Online 49(2):344–356

Tandon I, Razavi A, Ravishankar P, Walker A, Sturdivant NM, Lam NT, Wolchok JC, Balachandran K (2016) Valve interstitial cell shape modulates cell contractility independent of cell phenotype. J Biomech 49(14):3289–3297

Taylor PM, Batten P, Brand NJ, Thomas PS, Yacoub MH (2003) The cardiac valve interstitial cell. Int J Biochem Cell Biol 35(2):113–118

Thayer P, Balachandran K, Rathan S, Yap CH, Arjunon S, Jo H, Yoganathan AP (2011) The effects of combined cyclic stretch and pressure on the aortic valve interstitial cell phenotype. Ann Biomed Eng 39(6):1654–1667

Treuting PM, Dintzis SM (2011) (eds) Comparative anatomy and histology: a mouse and human atlas (expert consult), 1st edn. Academic Press

Wahlin B, Meedt T, Jonsson F, Henein MY, Wållberg-Jonsson S (2016) Coronary artery calcification is related to inflammation in rheumatoid arthritis: a long-term follow-up study. BioMed Res Int. doi:10.1155/2016/1261582

Wang H, Tibbitt MW, Langer SJ, Leinwand LA, Anseth KS (2013) Hydrogels preserve native phenotypes of valvular fibroblasts through an elasticity-regulated PI3K/AKT pathway. Proc Natl Acad Sci 110(48):19336–19341

Wang B, Li F, Zhang C, Wei G, Liao P, Dong N (2016) High-mobility group box-1 protein induces osteogenic phenotype changes in aortic valve interstitial cells. J Thorac Cardiovasc Surg 151(1):255–262

Weiss RM, Lund DD, Chu Y, Brooks RM, Zimmerman KA, El Accaoui R, Davis MK, Hajj GP, Zimmerman MB, Heistad DD (2013) Osteoprotegerin inhibits aortic valve calcification and preserves valve function in hypercholesterolemic mice. PLoS One 8(6):e65201

White MP, Theodoris CV, Liu L, Collins WJ, Blue KW, Lee JH, Meng X, Robbins RC, Ivey KN, Srivastava D (2015) NOTCH1 regulates matrix gla protein and calcification gene networks in human valve endothelium. J Mol Cell Cardiol 84:13–23

Wirrig EE, Hinton RB, Yutzey KE (2011) Differential expression of cartilage and bone-related proteins in pediatric and adult diseased aortic valves. J Mol Cell Cardiol 50(3):561–569

Wirrig EE, Gomez MV, Hinton RB, Yutzey KE (2015) COX2 inhibition reduces aortic valve calcification in vivo. Arteriosclerosis, thrombosis, and vascular biology. ATVBAHA-114

Witt W, Jannasch A, Burkhard D, Christ T, Ravens U, Brunssen C, Leuner A, Morawietz H, Matschke K, Waldow T (2012) Sphingosine-1-phosphate induces contraction of valvular interstitial cells from porcine aortic valves. Cardiovasc Res 93(3):490–497

Witt W, Büttner P, Jannasch A, Matschke K, Waldow T (2014) Reversal of myofibroblastic activation by polyunsaturated fatty acids in valvular interstitial cells from aortic valves. Role of RhoA/G-actin/MRTF signalling. J Mol Cell Cardiol 74:127–138

Wu B, Wang Y, Xiao F, Butcher JT, Yutzey KE, Zhou B (2016) Developmental mechanisms of aortic valve malformation and disease. Annu Rev Physiol

Xie C, Shen Y, Hu W, Chen Z, Li Y (2016) Angiotensin II promotes an osteoblast-like phenotype in porcine aortic valve myofibroblasts. Aging Clin Exp Res 28(2):181–187

Xu S, Gotlieb AI (2013) Wnt3a/β-catenin increases proliferation in heart valve interstitial cells. Cardiovasc Pathol 22(2):156–166

Xu S, Liu AC, Kim H, Gotlieb AI (2012) Cell density regulates in vitro activation of heart valve interstitial cells. Cardiovasc Pathol 21(2):65–73

Yang X, Meng X, Su X, Mauchley DC, Ao L, Cleveland JC, Fullerton DA (2009a) Bone morphogenic protein 2 induces Runx2 and osteopontin expression in human aortic valve interstitial cells: role of Smad1 and extracellular signal-regulated kinase 1/2. J Thorac Cardiovasc Surg 138(4):1008–1015

Yang X, Fullerton DA, Su X, Ao L, Cleveland JC, Meng X (2009b) Pro-osteogenic phenotype of human aortic valve interstitial cells is associated with higher levels of Toll-like receptors 2 and 4 and enhanced expression of bone morphogenetic protein 2. J Am Coll Cardiol 53(6):491–500

Yu Z, Seya K, Daitoku K, Motomura S, Fukuda I, Furukawa KI (2011) Tumor necrosis factor-α accelerates the calcification of human aortic valve interstitial cells obtained from patients with calcific aortic valve stenosis via the BMP2-Dlx5 pathway. J Pharmacol Exp Ther 337(1):16–23

Zeng Q, Song R, Ao L, Weyant MJ, Lee J, Xu D, Fullerton DA, Meng X (2013) Notch1 promotes the pro-osteogenic response of human aortic valve interstitial cells via modulation of ERK1/2 and nuclear factor-κB activation significance. Arterioscler Thromb Vasc Biol 33(7):1580–1590

Zhan JK, Tan P, Wang YJ, Wang Y, He JY, Tang ZY, Huang W, Liu YS (2014) Exenatide can inhibit calcification of human VSMCs through the NF-kappaB/RANKL signaling pathway. Cardiovasc Diabetol 13(1):153

Zhang XW, Zhang BY, Wang SW, Gong DJ, Han L, Xu ZY, Liu XH (2014) Twist-related protein 1 negatively regulated osteoblastic transdifferentiation of human aortic valve interstitial cells by directly inhibiting runt-related transcription factor 2. J Thorac Cardiovasc Surg 148(4):1700–1708

Zhao Z, Rivkees SA (2000) Programmed cell death in the developing heart: regulation by BMP4 and FGF2. Dev Dyn 217(4):388–400

Zhou J, Zhu J, Jiang L, Zhang B, Zhu D, Wu Y (2016) Interleukin 18 promotes myofibroblast activation of valvular interstitial cells. Int J Cardiol 221:998–1003