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Review Article

The response to valve injury. A paradigm to understand the pathogenesis of heart valve disease

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Abstract

Human heart valve diseases have become an important topic in cardiovascular pathology and medicine. These diseases have different etiologies and manifestations. However, the most common ones including calcific aortic stenosis have histopathological features that are best characterized as a "response to tissue injury" similar to ones seen in numerous tissues and organs. The valve interstitial cell is the prevalent cell type in the valve and is likely the master cell which ultimately regulates cell and molecular repair processes within the valve that involve autocrine and paracrine processes as well as interactions with the matrix components of the valve. This presentation explores the concept of "response to tissue injury" in understanding the pathogenesis of calcific aortic stenosis. © 2011 Published by Elsevier Inc.

Keywords: Valve interstitial cells; Heart valve disease; Valve repair; Alpha-smooth muscle actin; TGF-b; FGF-2; Adherens junctions

1. Human heart valve disease

Heart valve disease has several different etiologies (Table 1) and manifests as several different clinical conditions including calcific aortic stenosis (CAS), bicuspid aortic valve stenosis, acute and chronic rheumatic valve disease, valve prolapse (e.g., mitral valve prolapse), and annular calcification (Fig. 1) [5,6]. Although each clinical condition has distinct gross and microscopic features, there are several histopathological changes that are common to most heart valve diseases (Table 2). Characteristic features include a change in the number of valve interstitial cells (VICs), accumulation of myxomatous and/or dense connective tissue matrix, inflammation with or without immune activity, neovascularization, necrosis, calcification (Fig. 2), and lipid deposition. These tissue changes are due to cellular processes that, taken together, suggest that a "response to tissue injury" is occurring in the valve and may be regulated by VICs, the most prevalent cells in the valve (Fig. 3). This is supported by the observation that VICs become activated in response to injury. In diseased valves they differentiate into myofibroblast type cells, expressing the marker alpha-smooth muscle actin (a-SMA) (Fig. 4) [7]. These activated VICs likely play a key role in the pathogenesis of human clinical disease when the "response to tissue injury" becomes excessive and leads to disruption of the leaflets/cusps with excessive remodeling, scarring, and calcification through unknown mechanisms. Thus the tissue and cell processes activated in both the pathobiology of valve disease and the biology of engineered replacement valves are best studied and understood as a response to tissue injury [8]. Within this paradigm, the VIC appears to be the master cell, which regulates both the maintenance of the normal valve structure/function and the response to injury.

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Table 1
Etiology of human heart valve disease

Congenital
Bicuspid aortic stenosis
Genetic
Noonan syndrome [1]
Notch I mutation [2]
Osteogenesis imperfecta [3]
Williams–Beuren syndrome [4]
Others
Infectious
Infective endocarditis
Inflammatory/immune
Rheumatic valve disease, acute/chronic
Rheumatoid arthritis
Systemic lupus erythematosus
Ankylosing spondylitis
Scleroderma (progressive systemic sclerosis)
Polyarteritis nodosa
Drugs
Fen-phen
Other
Carcinoid heart valve disease
Calcification
Calcific aortic valve disease
Mitral valve annular calcification

We will focus on CAS since it is the most common heart valve condition in adults in Western society [9]. It is a condition with high morbidity and mortality that is very costly to the health care system [10-12]. It is a chronic disease that is slowly progressive and has precursor lesions that may remain asymptomatic for some time [13]. During the initial stages of the disease, aortic valve sclerosis occurs which results in cusp thickening without creating obstruction to the left ventricular outflow [13]. This gradually progresses to CAS, obstructing flow [13]. While 20–30% of individuals over the age of 65 and 48% of individuals over 85 are affected by sclerosis, only 2% of individuals over 65 and 4% of individuals over 85 end up with CAS [9,11,14]. Risk factors for CAS include hypertension, elevated low-density lipoprotein, male gender, smoking, and diabetes mellitus [9], which are similar to those of atherosclerosis [15]. Statins, well-known therapeutic agents that target atherosclerosis, are being tested in prospective clinical trials on CAS [16-19].

Despite research efforts focused on understanding the pathogenesis of CAS, the knowledge gained has not been sufficient to better prevent and treat the disease. The mainstay of treatment for CAS is still surgical valve replacement, which is costly and carries a certain amount of risk. Catheter-based aortic valve implantation is a relatively new technique which is gaining acceptance in patients unsuitable for conventional open-heart surgery.

CAS is no longer considered to occur due to passive degeneration secondary to aging. Research has shown that the pathogenesis involves active cell and tissue processes that occur in a tissue response to injury including inflammation, neovascularization, oxidative stress, activation of the renin–angiotensin system, matrix remodeling, and calcification and osteogenesis [7,13,20-23]. These processes develop as the valve cusps thicken due to fibrosis. Irregular calcified nodules develop initially at sites exposed to high mechanical force in the fibrosa layer of the valve.

The valve is normally avascular, thought to be due, at least in part, to the presence of chondromodulin-I, an antiangiogenic factor found to be abundantly expressed in normal valves but markedly down-regulated in diseased valves [24].

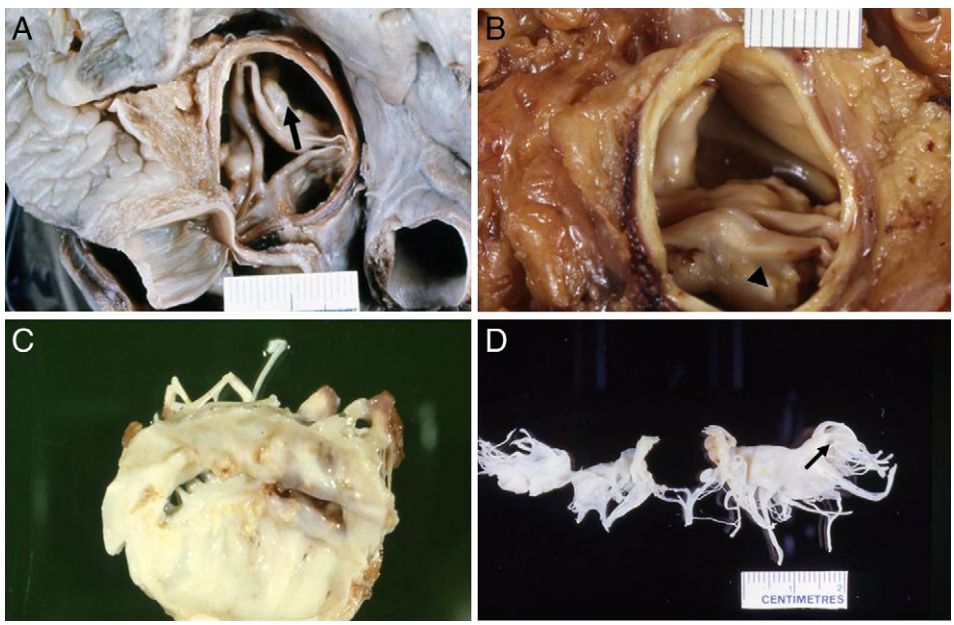

Fig. 1. Gross morphology of (A) CAS (arrow, calcified nodules). (B) Calcified bicuspid aortic valve (arrowhead, calcified nodules). (C) Chronic rheumatic mitral valve with prominent fibrosis distorting valve architecture and narrowing valve orifice. (D) Mitral valve prolapse with thinning of leaflets (arrow) and chordae tendineae (arrowhead).

Table 2
Histopathology of diseased native valves

VIC Accumulation
Matrix accumulation (myxomatous and/or fibrosis) and degradation
Inflammation (±immune activity)
Neovascularization
Calcification
Bone/cartilage (osteogenesis/chondrogenesis)
Necrosis
Lipid deposition

Its absence or down-regulation results in enhanced expression of angiogenic factors such as vascular endothelial growth factor (VEGF) as well as matrix metalloproteinase (MMP)-1, MMP-2, and MMP-13, promoting angiogenesis [24,25]. In advanced lesions, histopathology of the valves shows numerous new blood vessels associated with the expression of angiogenic factors such as VEGF and endothelial nitric oxide synthase [25,26]. The origin of the cells to form these new vessels is poorly understood. It is hypothesized that some of the endothelial cells that form these neovessels may originate from several sources including surface valve endothelial cells (VECs), circulating endothelial progenitor cells, and/or resident VICs [25,27]. In early lesions, new-forming vascular sprouts are present associated with VICs expressing a-SMA, VEGF receptor-2, and Tie-2. This suggests the presence of a transitional phenotype from mesenchymal- to endothelial-type cells, forming new capillaries with interendothelial junctions and partial basement membrane-like structures [27].

2. Valve interstitial cells

The connective tissue of human heart valves serves to maintain proper structure in a hemodynamic flow [28,29] and a heterogeneous population of constituent cellular elements regulates complex functions to give flexibility and durability to the leaflets [30]. The four types of cells found in valves are surface VECs, VICs, and towards the base of the valve, cardiac muscle cells and smooth muscle cells (SMCs) [31,32]. A confluent monolayer of VECs lines the surface of the valve. Underneath the endocardium are VICs embedded in matrix which they secrete and which form the three histologically distinct layers of the valve—the fibrosa, the spongiosa, and the ventricularis [32]. The fibrosa is rich in collagen; the spongiosa contains mainly glycosaminoglycans (GAGs) and proteoglycans; and the ventricularis is composed of elastin, collagen, and GAGs. These provide the valve with a zone of stiffness, a zone which is compressible, and a zone which is elastic in nature, respectively [30]. It is assumed that physical forces at the valve, which have not been well characterized, play an important role in organizing the trilaminar structure of the adult valve [33,34]. Cyclic flexure and bending occur during the constant opening and closing of the cusps. Compressive strains and tension (stretch) are exerted on the closed leaflet due to a pressure gradient across the valve. Under such constant physical forces, the structural integrity of the valve is maintained by VICs.

As well, VICs play a pivotal role in valve function, both under physiological and pathological conditions [7]. Based on their cellular/molecular functions and residing environment, we proposed that VICs show at least five distinct phenotypes: embryonic progenitor endothelial/mesenchymal cells, progenitor VICs, osteoblastic VICs, quiescent VICs (qVICs), and activated VICs (aVICs), all of which are critical regulators of valve pathobiology [7]. Of particular interests are qVICs that maintain normal structure and function in healthy adult valves as well as aVICs which regulate numerous processes in diseased or developing fetal valves [35]. Since elevated chemokines and growth factors are found in disease conditions, we proposed that activation of VICs is largely due to the stimulation by these factors released from activated VECs, macrophages, and dendritic activated T lymphocytes upon injury or hemodynamic/mechanical stress [36]. Activated VICs are characterized by increased extracellular matrix (ECM) secretion, proliferation, migration, and cytokine production, all of which are key aspects of wound healing [7].

The field of valve research has now transformed from the days of static histopathology studies which provided the research questions that are now being examined by dynamic mechanistic cellular and molecular biology investigations [37]. New insights into valve biology and pathogenesis of

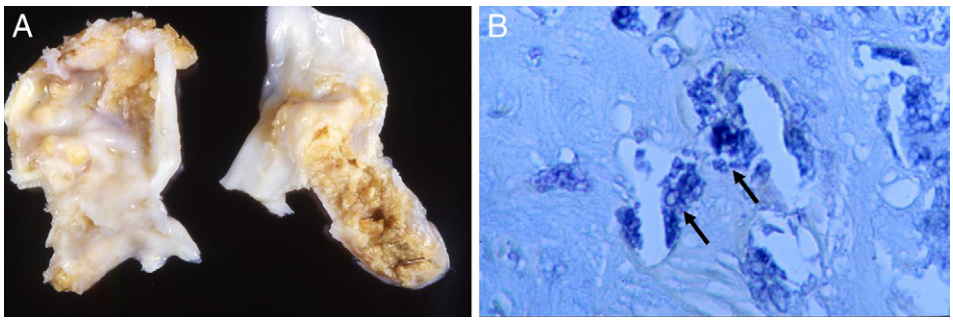

Fig. 2. Aortic valve calcification. (A) Gross morphology of two surgically removed calcified aortic valve cusps showing cusp distortion due to extensive nodular calcification and fibrosis. (B) Photomicrograph of valve calcification (arrow).

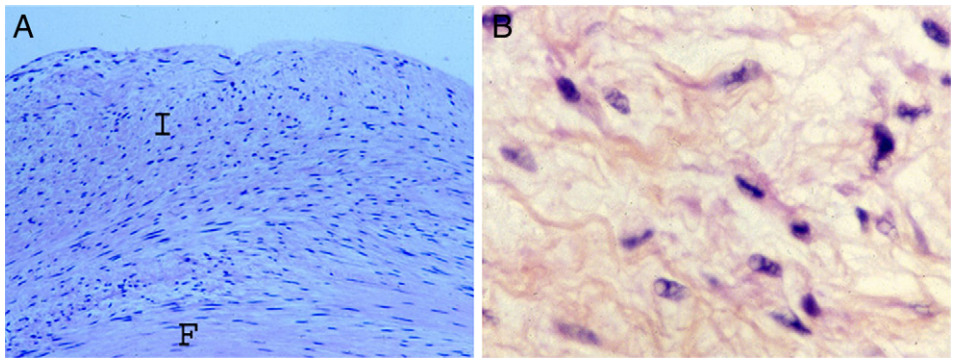

Fig. 3. Photomicrographs of (A) a thickened porcine mitral valve showing the abundance of VICs in an injured valve. Note: Fibrosa layer of valve with new VICs toward the surface (I) and VICs within the original fibrosa area (F). (B) Single VICs in the matrix of the leaflet (hematoxylin and eosin stain).

valve disease would not have been possible without the success in harvesting and culturing VICs [28,38] and the availability of in vitro experimental models [8]. We are one of the first to characterize VIC cultures. Briefly, porcine hearts were obtained from a local abattoir and the distal one-third of mitral valve was used for cell culture. After the complete removal of VECs, the valve tissue was cut, plated, and incubated in standard media with fetal bovine serum at 37°C in a humidified atmosphere of 95% air and 5% CO_2. By 3 weeks, cells growing out of the explants were detached and subcultured [38]. Others have acquired VICs released from porcine aortic valves using collagenase digestion [28]. Regardless of the methods of obtaining the cells, similar set of characteristics was observed. Like fibroblasts, VICs in vitro have long cytoplasmic extensions, lack a basal lamina [31], and secrete components of ECM such as fibronectin, chondroitin sulfate, and prolyl-4-hydroxylase [28]. Moreover, VICs display serum-dependent growth similar to fibroblasts [28,38]. Like myofibroblasts, VICs show prominent expression of a-SMA and myosin heavy chain [8], and contract upon stimulation by epinephrine or angiotensin II [31]. In confluent monolayers, the "hill and valley" configurations reminiscent of SMC cultures are not observed [38,39]. Single VICs in culture are activated and show basal levels of a-SMA expression. These single cells show different cell morphologies which are associated with differences in single cell motility and adhesion. Tailed and spindle-shaped VICs are characterized by high motility and less focal adhesion, whereas round and rhomboid VICs are less motile and more adhesive [37]. These features are likely important in the repair process.

3. Heart valve wound repair

Mesenchymal wound healing is considered to occur in several stages beginning with injury/inflammation followed by fibroblast differentiation, migration, proliferation, neovascularization, fibrosis, and tissue remodeling. Inflammation is initially acute consisting of an influx of neutrophils, and within a few days, an extensive macrophage infiltrate appears. Fibroblasts are the main cell type present at the site of a wound and are important in multiple aspects of wound healing. Migration is an essential process in wound repair as mesenchymal cells migrate into the wound from surrounding tissue and become activated to transform into myofibroblasts. In numerous migrating cell types, signal transduction pathways involving small GTPase Rho, Rac, and CDC44 are

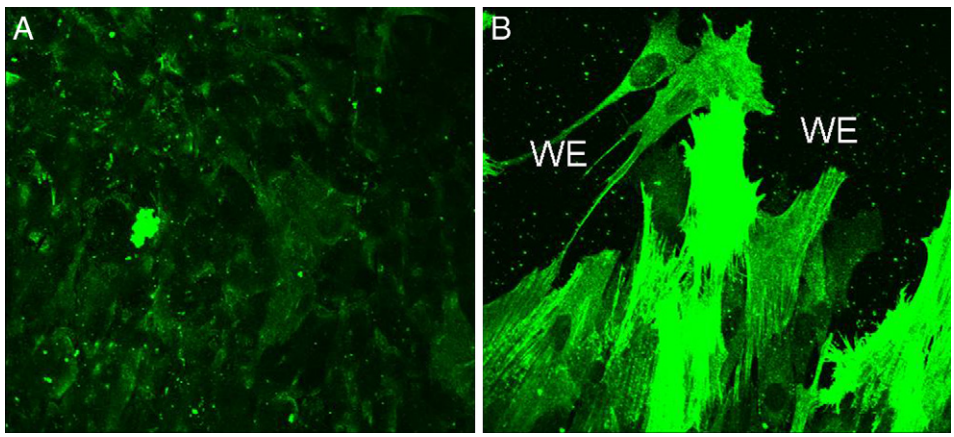

Fig. 4. A 1-mm linear wound is made in a confluent VIC monolayer. The cultures are fixed and stained for a-SMA 24 h post-wounding. Photomicrographs depict the area in the monolayer (A) well away from the wound edge, which is not activated, and (B) along the wound edge (WE), where VICs are activated.

activated that regulate the distribution and activity of the cytoskeleton [40,41] and its associated proteins.

VICs are required in many aspects of wound healing including migration, proliferation, apoptosis, and ECM remodeling. The "response to tissue injury" theory is now one of the favorite paradigms to study valve pathology [8,42]. In brief, it is now postulated that injury to the endocardium initiates a cascade of events [43] that elicit a response in the underlying VICs and via the cellular processes described above promote accumulation of cell and matrix material, inflammation, and calcification [8]. One of the events that occur immediately after injury is the release of various growth factors and cytokines that initiate proliferation and myofibroblast transformation. Such factors include fibroblast growth factor (FGF) and transforming growth factor-beta (TGF-b) [43].

Endogenous FGFs are known to regulate wound repair [43]. More specifically, they enhance proliferation of various cell types at the wound site including fibroblasts and keratinocytes, regulate cell migration and differentiation, and stimulate angiogenesis [43]. Mouse studies have shown that reduced FGF-2 expression/responsiveness is correlated with reduced angiogenic response, decreased collagen deposition, and overall impaired wound healing [43,44]. In experimental wound models, FGF-2 has also been reported to promote VIC wound repair [28].

TGF-b is a cytokine with diverse regulatory activities of numerous cell processes, especially inflammation, proliferation, and wound healing. The effects of TGF-b are cell and context dependent, and thus TGF-b exhibits marked diversity in its transcriptional response. Diseased valves exhibit increased levels of TGF-b. TGF-b [7,45-53] has effects on VIC differentiation [46,53] by increasing the expression of a-SMA, smooth muscle myosin, and calponin [54]. Upon binding to its receptor I/II complex, TGF-b elicits a signaling pathway that involves the phosphorylation of Smad2/3 complex, its association with Smad4, and subsequent translocation to the nucleus to mediate transcription. Diverse cellular responses are regulated by the TGF-b pathway, including proliferation, migration, cytokine secretion, ECM synthesis and degradation, and myofibroblast differentiation [55].

Currently, there are many wound models present. In addition to the organ culture model of valve injury [56], we and others also use a well-characterized in vitro mechanical wound model where a spatula tip is dragged across a confluent monolayer to create a 1-mm linear wound [8]. Using this model, we studied wound repair in association with FGF-2 and TGF-b1. In these studies the cells secreted their own matrix. These cells were not plated on specific matrixes nor was the thickness or the stiffness of the matrix studied. FGF-2 and its receptor FGFR1 were found up-regulated at the wound edge during early repair, and the single cells migrating into the wound showed prominent FGF-2 and FGFR1. Moreover, FGF-2 concentration in the conditioned media of wounded culture increased by sixfold compared to nonwounded cultures. When neutralizing antibody was added to the culture, wound closure was significantly delayed [45]. In the study involving TGF-b1, we found that the expression of this molecule was significantly up-regulated 24 h after wounding, accompanied by increased VIC activation, proliferation, apoptosis, and enhanced wound closure. When exogenous TGF-b1 was added to the culture, wound closure was further facilitated. In contrast, the opposite was observed when neutralizing antibody to TGF-b1 was added [46]. Walker et al. [57] reported that TGF-b1 increased VIC contractility and force transmission to the ECM. Collectively, these data suggest that growth factors and cytokines regulate VIC wound repair (Fig. 5).

4. Cell–cell contacts in VICs

Activation of VICs is not well understood and remains one of the important areas of heart valve disease to investigate. Our current studies suggest that it would be

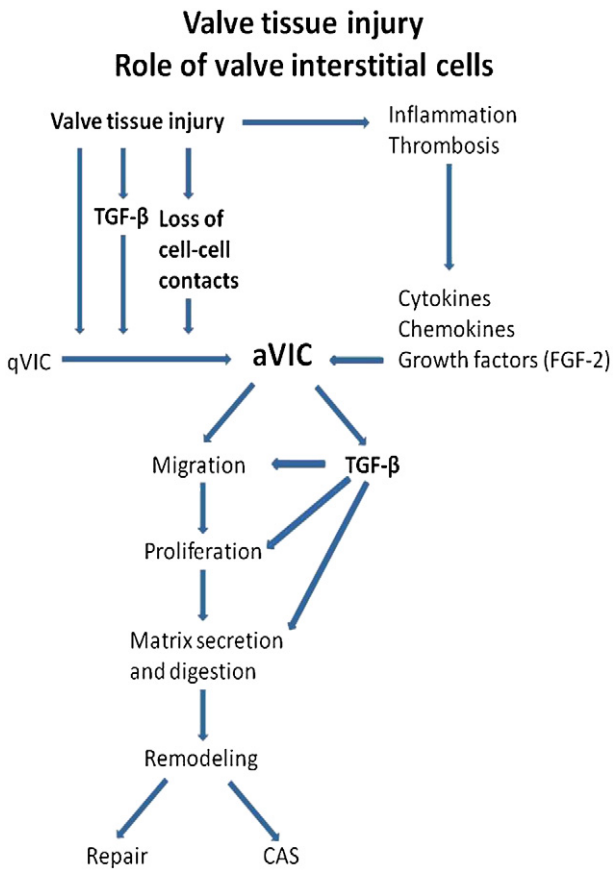

Fig. 5. A concept describing the role of TGF-b and loss of cell–cell contact in the activation of VIC in the "response to valve injury" paradigm. Valve tissue injury, resulting in increased TGF-b, loss of cell–cell contacts, and inflammation, leads to the activation of VICs that can further increase TGF-b and mediate wound repair. Eventually, the response to valve injury can lead to successful repair or CAS. At present, what determines whether repair or disease occurs is not understood.

useful to investigate the role of cell–cell adhesion as a regulator of VIC activation. We have shown that adhesion junctions are present between VICs in vivo in the normal valve. VICs are activated and express a-SMA at the wound edge in response to a mechanical injury made in an early-passage confluent monolayer culture where cell–cell adhesion is disrupted [46]. We also reported that early-passage single VICs cultured at low density are activated. There are no cell–cell contacts between these cells [37]. Taken together, our published data suggest that absence of cell–cell junctions may either signal or create a condition in which VICs become activated and express a-SMA and other activation genes, while formation of cell–cell contacts in monolayer culture may regulate the phenotypic switch of VICs from the activated to the quiescent phenotype. A similar paradigm is reported in a model of epithelial–mesenchymal transformation in which contact disassembly of renal tubular epithelial cells results in transformation of these epithelial cells to a-SMA-expressing myofibroblasts [58].

Cell–cell adhesion junctions are present in vivo and in vitro in VICs [38]. However, there appear to be more junctions in vitro in confluent monolayers than there are in the normal in vivo valve [59]. They provide focal contact between adjacent cells through homophilic links and include several proteins including cadherins which are transmembrane glycoproteins that promote calcium-dependent homophilic cell–cell adhesion, and associated a- and b-catenin. The E-cadherins in epithelial cells and the N-cadherins in mesenchymal cells bind to each other across the cell membrane [56]. Each cadherin molecule binds to catenins which bind to actin microfilaments [60]. b-Catenin and N-cadherin are shown to be present at the adherens junctions of VICs [59]. However, besides being a component of adherens junctions, b-catenin is also a well-known mediator of the Wnt signaling pathway [61-63]. Normally, the level of free cytoplasmic b-catenin is kept low by the axin complex, which includes the scaffolding protein axin, the tumor suppressor adenomatous polyposis coli gene product (APC), casein kinase 1 (CK1), and glycogen synthase kinase 3 (GSK3). CK1 and GSK3 sequentially phosphorylate the amino terminal region of b-catenin, resulting in b-catenin recognition by b-Trcp (beta-transducin repeat-containing protein), an E3 ubiquitin ligase subunit, leading to subsequent b-catenin ubiquitination and proteasomal degradation [61-63]. However, in situations where free cytoplasmic b-catenin is stabilized, such as upon activation of the Wnt pathway, b-catenin can travel to the nucleus to act as a transcriptional co-activator [61-63]. The main partner for b-catenin in gene regulation is the T-cell factor/lymphoid enhancer factor family of proteins [61-63]. They are high-mobility group DNA-binding factors which cause significant DNA bending that may alter local chromatin structure and thus affect gene transcription [61-63]. Beta-catenin is known to regulate processes such as cell fate determination and proliferation [61-63]. The Wnt/b-catenin pathway has been implicated in the development of many tissues and organs, including heart valves where it was shown to regulate proliferation and endothelial–mesenchymal transition [64-66]. Dysregulation of this pathway has been implicated in many diseases, the most well known being colorectal cancer, which results from constitutively activated b-catenin signaling, due to APC deficiency or b-catenin mutations that prevent its degradation, leading to excessive stem cell renewal or proliferation that predisposes cells to tumorigenesis [63]. In the lung and kidney, Wnt/b-catenin signaling has been implicated in fibrosis [67,68]. In heart valves, increased Wnt pathway gene expression has been associated with CAS, more specifically LRP5 expression and stabilized b-catenin [22,69]. However, research on b-catenin and its role in cell–cell adhesion and transcription in VICs has been very limited and requires further investigation.

This article offers the concept that the core processes that result in valve disease are part of the universal tissue repair processes present in human tissues and organs. A paper by Wirrig and Yutzey [70] in the previously mentioned symposium offers an interesting theory that aortic valve stenosis may be due to the reactivation of developmental gene programs that in the adult lead to acute valve calcification. These two concepts are not mutually exclusive and do overlap.

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