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Developmental Biology 294 (2006) 292–302
DEVELOPMENTAL BIOLOGY
www.elsevier.com/locate/ydbio

Review

Hearts and bones: Shared regulatory mechanisms in heart valve, cartilage, tendon, and bone development☆

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Received for publication 16 February 2006; revised 6 March 2006; accepted 19 March 2006
Available online 27 April 2006

Abstract

The mature heart valves are dynamic structures composed of highly organized cell lineages and extracellular matrices. The discrete architecture of connective tissue within valve leaflets and supporting structures allows the valve to withstand life-long functional demands and changes in hemodynamic forces and load. The dysregulation of ECM organization is a common feature of heart valve disease and can often be linked to genetic defects in matrix protein structure or developmental regulation. Recent studies have identified specific regulatory pathways that are active in the developing valve structures and also control cartilage, tendon, and bone development. This review will focus on the regulatory hierarchies that control normal and abnormal heart valve development in parallel with other connective tissue cell types.

Keywords: Heart; Valve; Extracellular matrix; Disease; Cartilage; Tendon; Bone

Introduction

Developmental defects of heart valves represent 20–30% of all cardiovascular malformations, and heart valve disease is a leading cause of morbidity and mortality in adults (Hoffman and Kaplan, 2002; Supino et al., 2004). The etiology of heart valve disease is relatively undefined, but there is recent evidence to suggest that valve disease discovered later in life may be related to developmental defects in valvulogenesis (Cripe et al., 2004; Garg et al., 2005). During embryonic development, the heart valves arise from endocardial cushions that elongate and remodel into the valve leaflets and their supporting apparatus (Armstrong and Bischoff, 2004; Schroeder et al., 2003). Recent work has contributed to a basic understanding of the cellular and molecular regulation of endocardial cushion formation, but less is known about how the highly organized extracellular matrix (ECM) in the mature valves develops (Armstrong and Bischoff, 2004; Person et al., 2005; Schroeder et al., 2003).

There is increasing evidence that valve leaflets and supporting structures are composed of diversified ECM compartments with characteristics of different types of connective tissues (Rabkin-Aikawa et al., 2005). Recent work has demonstrated that regulatory hierarchies active in developing cartilage, tendon, and bone also are important in heart valve maturation and ECM remodeling (Garg et al., 2005; Lange and Yutzey, 2006; Lincoln et al., 2006). In diseased heart valves, there is a distinct loss of ECM organization associated with changes in mechanical properties that ultimately leads to dysfunction (Rabkin et al., 2001; Schoen, 2005). However, the molecular events that result in aberrant ECM expression and distribution characteristic of valve disease are largely unknown. In many tissues, including heart muscle and bone, the regulatory pathways required for normal development also contribute to pathogenesis later in life (Chien and Karsenty, 2005). The structural properties and regulatory hierarchies common to the heart valves and other connective tissues will be the focus of this review in the context of normal development and disease mechanisms.

☆ Grant Sponsors: P50-HL074728 SCCOR in Pediatric Heart Development and Disease (KEY), AHA Postdoctoral Fellowship Award 0425366B (JL), AHA Predoctoral Award 0415234B (AWL).
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doi:10.1016/j.ydbio.2006.03.027

The heart valves are a complex connective tissue

In a given individual, the heart beats approximately 100,000 times a day, and the heart valves must coordinately open and close with each beat to achieve unidirectional blood flow. Normally, the semilunar (SL) valves of the outflow tract (OFT) and mitral and tricuspid valves of the atrioventricular (AV) canal function without obstruction or regurgitation during the cardiac cycle to direct blood flow through the cardiac chambers and great vessels. Normal SL valves are composed of three cusps, whereas the AV valves consist of two or three leaflets supported by chordae tendineae that insert into the ventricular papillary muscles. Although the gross anatomy of the SL and AV valves is distinct, the mechanical requirements for elasticity, compressibility, stiffness and strength, as well as durability throughout the lifespan of an individual, are similar for both sets of valves (Rabkin-Aikawa et al., 2005; Schoen, 2005; Vesely, 2005). This is achieved in large part from highly organized and compartmentalized ECM composition of the valves and their supporting structures.

Electron microscopy and immunohistochemical studies of human valve tissue identified three distinct layers of ECM within the valve leaflets: the fibrosa, spongiosa and atrialis (AV)/ventricularis (SL) (Fig. 1) (Garcia-Martinez et al., 1991; Gross and Kugel, 1931; Rabkin-Aikawa et al., 2005). The orientation of these matrix layers is the same relative to blood flow for the SL and AV valves, with the fibrosa layer situated away from blood flow, the atrialis or ventricularis adjacent to blood flow and the spongiosa layer in between. Each of the matrix layers provides specific mechanical properties to the valve leaflet due to the composition and organization of ECM proteins present in each compartment. The fibrosa layer provides strength, whereas the atrialis/ventricularis layers are more elastic. The spongiosa layer in between allows movement between the surface cell layers and acts as a shock absorber during valve closure. Together, these matrix layers and their associated interstitial cells provide the cellular and molecular components for development and maintenance of normal valve structure and function throughout life.

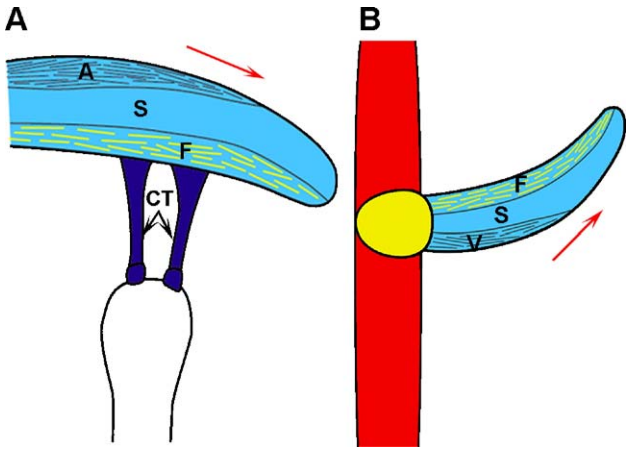

Fig. 1. Schematic representation of extracellular matrix compartmentalization of the mature atrioventricular (AV) and semilunar (SL) heart valves. ECM of AV (A) and SL (B) valves is organized in stratified layers relative to blood flow (red arrows). The atrialis (A) in the AV valve or the ventricularis (V) in the SL is composed of elastin fibers (gray lines). Proteoglycans (blue) are predominant in the spongiosa (S) layer, while collagen fibers arranged in parallel networks (yellow) are present in the fibrosa (F) layer. The supporting chordae tendineae (CT) (black arrows) of the AV valve are composed of collagen and elastic fibers. In panel B, muscle is indicated in red and collagen-rich fibrous annulus is yellow.

The connective tissue matrix within the fibrosa layer is predominantly composed of parallel, dense collagen fibers that confer stiffness and strength to the valve leaflets (Icardo and Colvee, 1995; Rabkin-Aikawa et al., 2005). During development, collagen types I, II, III, V, VI and XI are expressed in the immature remodeling valves, although extensive collagen fiber networks are not yet apparent (Klewer et al., 1998; Lincoln et al., 2004; Lincoln and Hinton, unpublished). During postnatal development in mice, the type I collagen fiber network becomes more extensive and is predominant in the fibrosa layer of the valves (Hinton, unpublished). The structural assembly of these fibers provides resilience and rigidity to the valve leaflet in order to close the valve orifices effectively without prolapse into the adjacent chamber (Gross and Kugel, 1931; Rabkin-Aikawa et al., 2005). The structural and mechanical properties provided by specific collagen fibers are also apparent in other connective tissue systems. Fiber alignment of collagen types I, III and V is apparent in remodeling tendons and bones (Birk, 1997; Zhang et al., 2005). In tendon, the assembly of collagen matrix is required for flexibility with varying degrees of stiffness, while in the bone, type I collagen provides a substratum for mineralization (Silver et al., 2002). In addition to collagen fibers, the fibrosa layer also expresses tenascin, a glycoprotein used as a ‘classic’ marker of tendon development (Hurle et al., 1989; Kardon, 1998; Lincoln et al., 2004, 2006). Thus, the fibrosa matrix layer of the heart valves shares cellular and mechanical properties with tendon and bone ECM that confer both stiffness and strength.

The spongiosa matrix layer of the mature valve is rich in proteoglycans, with a lower abundance of collagens than the fibrosa layer, thereby providing a more compressible matrix and allowing for changes in leaflet shape during the cardiac cycle (Rabkin-Aikawa et al., 2005). In the mouse and chicken, there are several proteoglycans and collagens expressed in the spongiosa layer during remodeling, including aggrecan, versican, perlecan, cartilage-link protein, and type II and XI collagen, but there are differences in expression patterns between species (Costell et al., 2002; Henderson and Cobb, 1998; Hurle et al., 1994; Lincoln et al., 2004, 2006; Lincoln, unpublished; Mjaatvedt et al., 1998). Although not exclusive to the heart valves, these proteoglycans and collagens are characteristic of cartilage and provide properties to resist compression and maintain structural integrity (Poole et al., 2001). Therefore, shared molecular and structural properties of cell matrix of the spongiosa layer and cartilage cell types are reflected in the protein composition of these tissues.

The atrialis/ventricularis matrix layer is predominantly composed of aligned fibers of elastin interspersed with short collagen fibers in a radial arrangement (Schoen, 1997; Scott and Vesely, 1995). This type of elastic cell matrix also is characteristic of vascular smooth muscle and could be related to

directional blood flow across this layer of the valves, although this has not yet been demonstrated (Laurent et al., 2005). The elasticity of the ECM on the surface of the valves is important for the leaflet's ability to stretch and retract during the cardiac cycle (Rabkin-Aikawa et al., 2005; Vesely, 1998). Elastic fibers are also present in the chordae tendineae that support the AV valve leaflets through insertion into the papillary muscles of the ventricles (Liao and Vesely, 2004). Tissue flexibility and extensibility provided by elastic fibers are apparent in several tissue types including arterial walls, skin, tendons, and ligaments, as well in the valves and their supporting structures (Kielty et al., 2002).

Heart valve disease and connective tissue disorders

Histological changes in valve ultrastructure and histopathologic alterations in ECM organization are characteristic of many forms of valve disease and dysfunction (Table 1) (Akhtar et al., 1999; Nasuti et al., 2004; Ng et al., 2004; Rabkin et al., 2001; Tamura et al., 1995; Walker et al., 2004). Valve dysfunction can be characterized as stenosis, defined as failure of the valve leaflet to fully open often due to mechanical stiffness, or as insufficiency, caused by inefficient closing of the valve associated with myxomatous or thickened valve tissue (Rabkin-Aikawa et al., 2005). In the United States, 95,000 diseased valves required replacement in 2003, and valve calcification was frequently observed (Thom et al., 2006). These disease lesions occur with the deposition of mineralized calcium leading to progressive valve stiffening, incomplete valve opening, and subsequent valve stenosis (Mohler et al., 2001; Rajamannan et al., 2003). Congenital heart valve disease often includes histological changes in proteoglycan, collagen, and elastic fibers resulting in myxomatous or "floppy" valves, associated with mitral valve prolapse (MVP) (Ng et al., 2004; Rabkin et al., 2001). These thickened valves loose their rigidity and resilience due to loss of ECM stratification and are the most common cause of valve insufficiency (Akhtar et al., 1999; Nasuti et al., 2004; Tamura et al., 1995). Disorganization of ECM layers also occurs with aortic valve stenosis resulting from bicuspid aortic valve (BAV), which is the most common congenital valve anomaly and is also associated with valve calcification later in life (Cripe et al., 2004; Garg et al., 2005; Hinton and Benson, unpublished). This finding suggests that congenital and adult valve disease may actually be a continuum of dysfunction resulting from developmental anomalies that lead to progressively abnormal distribution and organization of ECM proteins over time.

In the human population, heart valve dysfunction is a frequent manifestation of connective tissue disorders that affect cartilage, tendon, bone, and skin (Table 1). Marfan syndrome, caused by mutations in *Fibrillin-1*, is characterized by skeletal malformations and cardiovascular anomalies, including BAV and heart valve insufficiency (Dietz et al., 2005; Lee et al., 1991; Weyman and Scherrer-Crosbie, 2004). Ehlers–Danlos syndrome associated with mutations in several collagen or tenascin genes, and Stickler syndrome, caused by mutations in type II or XI collagen, both include premature connective tissue fragility in skin and joints (Burch et al., 1997; Liberfarb and Goldblatt, 1986; Schalkwijk et al., 2001). Heart valve dysfunction, including MVP, also has been reported in these patient populations (Liberfarb and Goldblatt, 1986). Histological analyses of these affected valves have not been reported, but it is predicted that mutations in ECM protein genes would affect the organization of stratified valve layers resulting in valve insufficiency. Nonsyndromic mutations in several ECM protein genes also have been reported for a variety of limb and skin malformations (Table 1). Since the ECM and cell lineages that make up these affected tissues are similar to those in developing heart valves, these individuals also may provide insights into previously unappreciated forms of valve disease. In addition, a more complete understanding of the developmental regulation of these specific connective tissue cell types will likely be informative of valve disease mechanisms.

Valvulogenesis

The process by which valve precursors remodel into mature valves is an active area of recent research using animal model systems. In chicken embryos, ECM stratification within the valve leaflets is well defined in the AV and SL valves (Lincoln et al., 2004, 2006; Hinton, unpublished). Therefore, avian embryos provide an excellent system for examination of regulatory hierarchies and remodeling events in isolated precursor populations under controlled experimental conditions (Lincoln et al., 2006). In the mouse, the valve leaflets are comparatively thinner than in the chick and ECM stratification is less well-defined (Hinton, unpublished). Nevertheless, transgenic and gene targeting approaches in mice have advanced our understanding of cellular and molecular mechanisms required for valve remodeling and maturation in vivo (Armstrong and Bischoff, 2004; Person et al., 2005; Yutzey et al., 2005). Additional genes required for early stages of valvulogenesis have been identified in zebrafish, however, the basic ECM organization in the mature fish valve has yet to be determined (Beis et al., 2005; Walsh and Stainier, 2001). Collectively, these systems have been used to define regulatory processes that control the initial formation and maturation of the heart valves during development.

Valvulogenesis begins with the formation of endocardial cushions in the AV canal and OFT during looping stages of the heart tube that correspond to Hamburger–Hamilton stage 14 in chicken embryos and E9.5 in mice (Person et al., 2005). Cushion formation is initiated with expansion of the cardiac jelly and epithelial–mesenchymal transformation (EMT) of endocardial cells (Eisenberg and Markwald, 1995, 1977; Person et al., 2005). The resulting endocardial cushions contain an undifferentiated mesenchymal cell population of valve progenitor cells surrounded by ECM composed predominantly of the proteoglycan glycosaminoglycan hyaluronan (HA) (Lincoln et al., 2004; Schroeder et al., 2003). The molecular and cellular regulation of EMT has been extensively studied and thoroughly reviewed in recent publications (Armstrong and Bischoff, 2004; Person et al., 2005; Schroeder et al., 2003). The regulatory events that occur after cushion formation to produce highly

organized valve structures are less well understood but are an increasingly active area of research.

Heart valve differentiation is characterized by the expression of specific signaling molecules, transcription factors, ECM proteins, and associated regulatory enzymes in the developing leaflets and supporting structures. Recent work has demonstrated that the precursors for these diversified structures are present in a common population of progenitor cells within the endocardial cushions (de Lange et al., 2004; Lincoln et al., 2004, 2006). AV valve cell lineage diversification begins when the cushions fuse to separate the right and left channels of the AV canal and form the valve primordia (Lincoln et al., 2006). Following fusion, the valve primordia begin to express transcription factors and structural proteins associated with differentiation of the valve leaflets and supporting structures (Hurle et al., 1994; Lincoln et al., 2004, 2006; Montero et al., 2002). Strikingly, many of these regulatory genes and structural proteins also are expressed in developing cartilage, tendon, and bone cell lineages (Brent and Tabin, 2002; Karsenty and Wagner, 2002).

Heart valve differentiation also is associated with a decrease in precursor cell proliferation in the valve primordia. The mesenchymal cells of the endocardial cushions exhibit a uniformly high proliferation index (de Lange et al., 2004; Lincoln et al., 2004). After fusion, differential rates of proliferation are observed in the valve primordia with the highest levels present at the distal tips, consistent with a distal outgrowth mechanism for valve elongation. Signaling through the Ras-MAPK pathway is a critical component of valve progenitor cell cycle regulation as indicated by the presence of activated dpERK throughout the endocardial cushion mesenchyme and localized at the distal tips of the more mature valve primordia (Gitler et al., 2003b; Liberatore and Yutzey, 2004; Yutzey et al., 2005). Likewise, aberrant function of the SHP-2 phosphatase modulates Ras activation through ERK1/2 and alters valve progenitor proliferation in genetically modified mice or cultured chicken embryo valve progenitors (Araki et al., 2004; Krenz et al., 2005). Mutations in the human gene encoding SHP-2, *PTPN11*, have been implicated in congenital heart defects, including valve malformations, associated with Noonan syndrome (Tartaglia et al., 2001). Remodeling of the valve primordia is accompanied by dramatic reduction in proliferation, and mutant mice with increased or decreased cellularity of the endocardial cushions exhibit premature lethality (Armstrong and Bischoff, 2004; Lincoln et al., 2004; Yutzey et al., 2005). Therefore, cell cycle regulation is crucial for establishing a pool of progenitor cells in the endocardial cushions that then withdraws from the cell cycle and differentiates to form the diversified structures of the valve leaflets and supporting apparatus.

Heart valve remodeling includes both the deposition and proteolysis of ECM proteins and results in a stereotypic stratified ECM that is the structural basis for valve function throughout life (Rabkin-Aikawa et al., 2005; Schoen, 2005). Valve maturation is characterized by increasing complexity of ECM protein expression in different compartments of the valves that develop into leaflet fibrosa, spongiosa, and atrialis/ventricularis layers and supporting structures. Mutagenesis studies in mice have demonstrated the requirements for a variety of ECM proteins in heart valve formation and function (Table 1). Loss of the proteoglycans perlecan or versican results in midgestational lethality from endocardial cushion maturation defects (Costell et al., 2002; Mjaatvedt et al., 1998). Additional mutations in collagen or fibrillin genes are less severe and lead to valve malformations detected postnatally (Ng et al., 2004; Lincoln, unpublished). Matrix remodeling enzymes, such as matrix metalloproteinases (MMPs), and their inhibitors, tissue inhibitors of metalloproteinases (TIMPS), also are expressed in the remodeling valves (Barth et al., 2005; Dreger et al., 2002; Rabkin et al., 2001). The functions of MMPs and TIMPs in the developing valves have not been fully characterized, but increased expression of several of these proteins is observed with ECM disorganization associated with human myxomatous valve disease (Fondard et al., 2005; Rabkin et al., 2001; Soini et al., 2001). The balance of ECM protein expression and remodeling underlies heart valve structure and function during development and likely is important for valve homeostasis throughout life. However, the regulatory hierarchies that control the development of valve cell lineages with characteristics of distinct connective tissues are just beginning to be uncovered. Much more extensive information is available regarding the development of related connective tissue cell types in other organ systems, and there is increasing evidence for conserved regulatory pathways in the heart valves with those of developing cartilage, tendon, and bone (Fig. 2).

Remodeling heart valves share regulatory pathways with developing cartilage, tendon, and bone

Recent studies have demonstrated that regulatory interactions between specific signaling molecules, transcription factors, and structural genes or remodeling enzymes occur in related connective tissue cell types with diverse developmental origins. The relationships between these regulatory hierarchies are complex and involve multiple feedback mechanisms, but defined pathways involved in specific aspects of valve formation are beginning to emerge. Three of these regulatory pathways are reviewed below.

BMP2–Sox9–aggrecan

In the developing limb buds, the transcription factor Sox9 is required for differentiation of chondrogenic cell lineages, and mice with conditional inactivation of Sox9 display an array of skeletal malformations (Akiyama et al., 2002; Bi et al., 1999; Chimal-Monroy et al., 2003; Kist et al., 2002). In the heart, Sox9 has been implicated in endocardial cushion formation, and predominant expression in the remodeling valve leaflets is suggestive of a role in later stages of valve remodeling (Akiyama et al., 2004; Lincoln et al., 2004, 2006; Lincoln, unpublished; Montero et al., 2002). Sox9 target genes in cartilage cell lineages encode type II and XI collagen, aggrecan and cartilage-link protein, and several of these ECM proteins also are expressed in the mature valves (Bell et al., 1997; Bridgewater et al., 1998; Chimal-Monroy et al., 2003; Kou and

Table 1
Extracellular matrix proteins and molecular regulatory pathways involved in connective tissue and heart valve development and disease

	Role in connective tissue development	Role in cardiac development	Associated human disease
A. Extracellular matrix proteins			
Elastin	Expressed in periphery of tendons and fibrils anchoring tendons to skeletal elements (Ros et al., 1995)	Required for arterial development; expressed atrialis/ventricularis of mature AV and SL valves (Li et al., 1998; Rabkin et al., 2001; Hinton, unpublished)	Williams syndrome supravalvular aortic stenosis (Ewart et al., 1993a,b; Morris et al., 1993); cutis laxa (Zhang et al., 1999)
Fibrillin-1	Connective tissue microfibril; key component of elastic fibers (Kielty et al., 1998)	Marfan syndrome models display mitral valve prolapse (Brown et al., 1975; Glesby and Pyeritz, 1989; Ng et al., 2004)	Marfan syndrome (Dietz et al., 2005; Lee et al., 1991); Weill–Marchesani syndrome (Faivre et al., 2003); MASS syndrome (Glesby and Pyeritz, 1989)
Perlecan	60% mutant mice die at birth with skeletal and chondrodysplasia (Arikawa-Hirasawa et al., 1999)	Expressed in AV and outflow tract (OFT) endocardial cushions; mutant mice have OFT anomalies (Costell et al., 2002; Handler et al., 1997)	Schwartz–Jampel syndrome (Nicole et al., 2000)
Tenascin	Expressed in tendon progenitor and mature tendon cells (Chiquet and Fambrough, 1984)	Expressed in supporting apparatus of AV and SL valve structures (Lincoln et al., 2004, 2006)	Ehlers–Danlos syndrome type 3 (Burch et al., 1997)
Type I collagen	Mutant mice die at E12.5 with vascular defects (Lohler et al., 1984)	Expressed in AV and OFT endocardial cushions and mature valves (Lincoln et al., 2004)	Ehlers–Danlos syndrome classic type; osteogenesis imperfecta (Nuytinck et al., 2000; Sykes et al., 1985)
Type II collagen	Required for chondrogenesis (Vandenberg et al., 1991)	Expressed in developing chick and mouse heart valves (Lincoln et al., 2004; Montero et al., 2002)	Spondyloepiphyseal dysplasia; achondrodysplasia; Stickler syndrome; osteogenesis imperfecta; osteoarthritis (Ahmad et al., 1991; Lee et al., 1989; Snead et al., 1994; Tsipouras et al., 1983; Vissing et al., 1989)
Type III collagen	Required for type I collagen fibrillogenesis (Liu et al., 1997)	Mutant mice die in adulthood due to blood vessel rupture; expressed in fibrosa layer of mature AV and SL valves (Hinton, unpublished) (Liu et al., 1997)	Ehlers–Danlos syndrome Type III, IV (Superti-Furga et al., 1988)
Type XI collagen	Required for formation of cartilage collagen fibrils (Li et al., 1995)	Expressed in remodeling valve structures; mitral valve prolapse in Stickler syndrome (Ahmad et al., 2003; Liberfarb and Goldblatt, 1986) (Lincoln et al., unpublished) (Yoshioka et al., 1995)	Stickler syndrome Marshall syndrome (Griffith et al., 1998; Snead et al., 1996)
Versican	Required for precartilaginous aggregation and cartilage differentiation (Williams et al., 2005)	Mutant mice die at E10.5 with hypoplastic endocardial cushions and right cardiac chamber malformations (Mjaatvedt et al., 1998)	
B. Regulatory pathways in connective tissue development			
BMP	Promotes chondrogenesis; regulates sox9 expression (Yoon and Lyons, 2004)	Required for EMT; regulates sox9 and aggrecan expression (Lincoln et al., 2006; Ma et al., 2005; Sugi et al., 2004)	
Sox9	Required for cartilage formation through BMP2 signaling (Bi et al., 1999; Yoon and Lyons, 2004)	Required for EMT; regulated by BMP2 in chick EC cells (Akiyama et al., 2004; Lincoln et al., 2006)	Campomelic dysplasia (Bi et al., 2001; Foster et al., 1994)
Aggrecan	Mutant mice die at birth with skeletal malformations (Watanabe et al., 1997)	Expressed in chicken valve leaflet (Lincoln et al., 2006)	Spondyloepiphyseal dysplasia (Gleghorn et al., 2005)
Cartilage-link protein	Mutant mice die at birth with cartilage defects (Watanabe and Yamada, 1999)	Expressed in AV and OFT endocardial cushions and mature valves (Lincoln, unpublished)	
FGF4	Regulates tendon formation in somitic compartment (Brent and Tabin, 2004)	Involved in EC proliferation and expressed in EC and AV myocardium (Sugi et al., 2003)	
Scleraxis	Expressed in tendon progenitors; regulates tenascin (Edom-Vovard et al., 2002)	Expressed in supporting apparatus of AV and OFT valve (Lincoln et al., 2004, 2006)	
RANKL	Required for osteoclast development (Mizuno et al., 1998)	Expressed in endocardial cells of remodeling valves (Lange and Yutzey, 2006)	Osteopetrosis (Kong et al., 1999)

Table 1 (*continued*)

	Role in connective tissue development	Role in cardiac development	Associated human disease
B. Regulatory pathways in connective tissue development			
NFATc1	Required for osteoclast differentiation (Hirotani et al., 2004)	Required for heart valve formation (de la Pompa et al., 1998; Ranger et al., 1998)	

(continued on next page)

Ikegawa, 2004; Lefebvre et al., 1997; Liu et al., 2000; Ng et al., 1997; Yoshioka et al., 1995). The direct regulation of these genes by Sox9 in the valve primordia has not yet been demonstrated, but the expression of these matrix proteins in regions where Sox9 is present is consistent with a role for Sox9 in the development of proteoglycan-rich compartments of the developing valves.

Upstream regulators of *sox9* gene expression were first identified in limb bud chondrogenic precursors, and the main inducing factor of *sox9* expression in these cells is BMP2 (Chimal-Monroy et al., 2003). BMP2 is also expressed in the AV myocardium adjacent to the developing valves that express *sox9* and has been implicated in cushion formation as well as later events in valve remodeling (Delot, 2003; Lincoln et al., 2006; Ma et al., 2005). In the limb cartilage, one of the best-characterized downstream target genes of Sox9 is the chondroitin sulfate proteoglycan *aggrecan* (Chimal-Monroy et al., 2003). Strikingly, BMP2 induction of *sox9* and *aggrecan* expression also occurs in chick valve precursor cells treated in culture (Lincoln et al., 2006). In avian embryos, *aggrecan*-expressing valve cells with characteristics of cartilage cell types occupy the spongiosa layer of the remodeled valve, consistent with the compressible property of the proteoglycan matrix (Lincoln et al., 2006). The observation that BMP2 activates *sox9* and *aggrecan* in both cartilage and valve precursor cells represents surprising cellular and molecular similarities between the two systems.

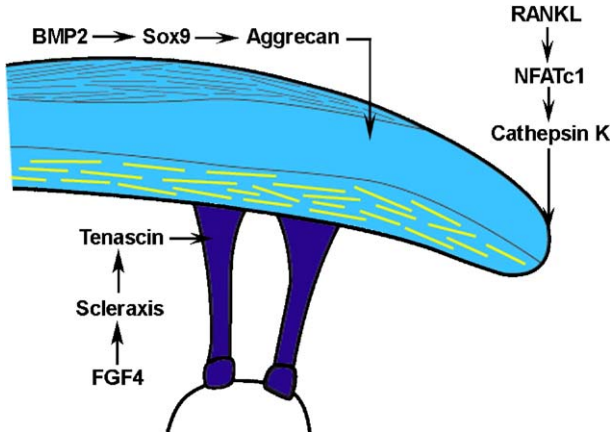

Fig. 2. Regulatory pathways required for chondrogenesis, tendon formation, and osteoclast differentiation are active in remodeling AV heart valves. Simplified regulatory hierarchies involved in aspects of heart valve compartmentalization and remodeling are represented. BMP signaling, Sox9, and *aggrecan* are present in developing valve leaflets and cartilage cell lineages. Tenascin and *scleraxis* expression is affected by FGF signaling in valve supporting structures and tendon precursors. RANKL expression, NFATc1 activation, and cathepsin K are apparent in endothelial cells of at the distal tips of the remodeling valves and also in differentiating osteoclasts involved in bone matrix remodeling.

FGF–scleraxis–tenascin

In the developing tendons, FGF4 activates the MAPK (ERK1/2) signaling cascade to promote expression of the bHLH transcription factor *scleraxis* (Edom-Vovard et al., 2002; Smith et al., 2005). Scleraxis subsequently induces expression of *tenascin*, a glycoprotein prominent in tendon cell types (Edom-Vovard et al., 2002; Hurle et al., 1989; Kardon, 1998; Ros et al., 1995; Schweitzer et al., 2001). All of the components of this regulatory pathway are present in remodeling valves (Karabagli et al., 2002; Liberatore and Yutzey, 2004; Sugi et al., 2003). In particular, *scleraxis* and tenascin are distinctly expressed in the supporting structures of the valve leaflet and chordae tendineae (Lincoln et al., 2004, 2006). Mice null for *scleraxis* die from mesoderm defects during gastrulation; therefore, further targeted genetic studies are necessary to determine a requirement for scleraxis during cell lineage differentiation of valve precursor cells (Brown et al., 1999). However, insights have been gained from in vitro studies in which FGF4, through ERK1/2 signaling, is able to induce expression of *scleraxis* and tenascin in valve progenitor cells from chicken embryos (Lincoln et al., 2006). This conservation in the regulation of *scleraxis* and *tenascin* expression represents further shared regulatory pathways in heart valve precursors and other connective tissue cell types, including tendon.

In the limb buds, diversification of cartilage and tendon cell lineages from a common precursor population is regulated by antagonism between BMP and FGF signaling pathways (Brent and Tabin, 2002; Tickle, 2002). In these cells, BMP2 not only promotes chondrogenesis, but also inhibits tendon development, while FGF4 promotes tendon differentiation at the expense of chondrogenesis (Edom-Vovard and Duprez, 2004; Edom-Vovard et al., 2002). Likewise, inhibitors of BMP signaling promote tendon differentiation, and FGF inhibition increases chondrogenesis. A similar antagonistic relationship between these signaling pathways in the differentiation of cell lineages characterized by *aggrecan* or tenascin expression also was observed in heart valve progenitor cells (Lincoln et al., 2006). In addition, the BMP inhibitor Noggin represses *sox9* and *aggrecan* while inducing expression of *scleraxis* and tenascin in both systems (Capdevila and Johnson, 1998; Lincoln et al., 2006; Schweitzer et al., 2001). The antagonism between these BMP and FGF pathways extends to the level of the signaling intermediates, Smad1/5 and ERK1/2, since corresponding changes in phosphorylation of these proteins were observed with the different signaling conditions in valve

progenitor cells (Lincoln et al., 2006). Together, these studies have defined an antagonistic relationship between BMP and FGF signaling pathways that controls cartilage versus tendon cell determination in limb bud progenitors and also functions in diversification of heart valve precursor cells.

RANKL–NFATc1–cathepsin K

Expression of the transcription factor NFATc1 is required for heart valve remodeling in mice; however, the initial reports did not provide information regarding the molecular mechanism by which this occurs (de la Pompa et al., 1998; Ranger et al., 1998). NFATc1 also is required for osteoclast lineage development, and it has been useful to apply knowledge of NFAT function obtained in this cell type to the analysis of heart valve regulatory mechanisms (Boyle et al., 2003; Lange and Yutzey, 2006). Osteoclasts are specialized cells derived from the macrophage lineage that resorb and remodel the bone matrix during development and homeostasis. In osteoclasts, receptor activator of NFκB ligand (RANKL), a member of the TNF ligand family, signals through its receptor RANK to promote differentiation of bone-resorbing osteoclasts (Boyle et al., 2003). NFATc1 is a necessary transcriptional effector of RANKL signaling in osteoclasts through direct regulation of enzymes involved in bone matrix degradation and remodeling, such as *tartrate-resistant acid phosphatase* (*TRAP*/*Acp5*) and the cysteine protease *cathepsin K* (*Ctsk*) (Ikeda et al., 2004; Matsumoto et al., 2004; Takayanagi et al., 2002). Thus, in osteoclasts, NFATc1 is responsible for the transcriptional activation of specific extracellular matrix degrading enzymes, and the heart phenotype in *nfatc1*$^{-/-}$ mouse embryos is consistent with a similar regulatory function in valve remodeling.

During cardiac development, *RANKL*, its receptor *RANK*, and the downstream matrix protease *Ctsk* are co-expressed with NFATc1 in the endocardium of the remodeling heart valves (Lange and Yutzey, 2006). This pathway appears to be active during valve development since RANKL treatment of cultured hearts stimulates cardiac expression of *NFATc1* and *Ctsk*, and *Ctsk* expression is lost in remodeling valves of *nfatc1*$^{-/-}$ embryos (Lange and Yutzey, 2006). Together, these findings provide initial evidence for RANKL-mediated NFATc1 function in the regulation of genes directly involved in heart valve matrix remodeling. While RANKL signaling and Ctsk are known to be essential for osteoclast development, abnormalities in heart valve formation have not been reported with mutation of these genes in mice or humans (Gelb et al., 1996; Gowen et al., 1999; Li et al., 2000). Therefore, additional regulatory signals may contribute to NFATc1 activation and ECM remodeling during valve leaflet formation, or there may be valve defects associated with these gene mutations that have not been fully appreciated. Further studies are necessary to determine the exact role of RANKL signaling and Ctsk in heart valve remodeling as well as to identify additional pathways that affect NFATc1 activation. Of interest, the balance of RANKL and its receptor antagonist osteoprotegerin (OPG) controls the extent of bone mineralization and contributes to vascular calcification (Bucay et al., 1998; Theoleyre et al., 2004). Therefore, signaling through the RANK receptor and control of NFATc1 activation may have important regulatory roles, not only in heart valve development, but also in adult valve homeostasis and disease mechanisms associated with aging.

Conclusions and perspectives

Recent research has revealed striking similarities in the cellular and molecular mechanisms that control heart valve cell differentiation with those of developing cartilage, tendon, and bone. It is likely that these findings do not represent the full regulatory conservation of these tissue types, and that additional pathways originally characterized in developing cartilage, tendon, and bone will be found to be important for heart valve lineage differentiation and remodeling. For example, there is initial evidence that Notch and canonical Wnt signaling pathways are active during valve remodeling, but the specific cells and processes affected by these pathways are not well-characterized (Garg et al., 2005; Gitler et al., 2003a). Insights into the functions of these pathways in the valves could be provided by extensive mechanistic studies of cartilage, tendon, and bone development, already in the literature. The conservation in genetic mechanisms between these systems has certainly advanced our understanding of heart valve remodeling thus far and will likely provide exciting avenues of research for the future.

There is an increasing understanding of the specific connective tissue cell types that form the diverse structures of the heart valves and emerging evidence for dysregulation of these cell types associated with valve disease. Transcriptional regulators, such as Sox9, scleraxis, and NFATc1, are expressed during valve lineage differentiation and patterning, but further studies are necessary to determine the precise functions of these genes during valvulogenesis in vivo. It is tempting to speculate that these critical regulators of valve development also have a role in aberrant ECM expression and organization associated with valve disease, but induction of these factors during valve pathogenesis has not yet been demonstrated. In addition, appropriate viable animal models for molecular and cellular studies of myxomatous or calcified valves are not widely available. Human genetic studies are beginning to provide evidence that developmental pathways important in valvulogenesis also contribute to adult valve disease (Cripe et al., 2004; Garg et al., 2005). However, more research is necessary to determine if valve disorders characterized by abnormal or mineralized ECM include reactivation of developmental gene programs or if these molecular mechanisms can be exploited for new clinical therapies.

Acknowledgments

We thank D. Woodrow Benson and Robert Hinton for critical reading of the manuscript and communication of data prior to publication. Figures were generated with the help of Andreas Lange. Research support was provided by AHA Ohio Valley Affiliate post-doctoral (#0425366B to JL) and pre-doctoral

(#0415234B to AWL) fellowships and NIH/NHLBI SCCOR in Pediatric Heart Development and Disease (P50 HL074728).

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