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Developmental basis of adult cardiovascular diseases:

Valvular heart diseases

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Abstract

In this chapter, we review the working hypothesis that the roots of adult valvular heart disease (VHD) lie in embryonic development. Valvulogenesis is a complex process in which growth factors signal the process of endocardium-to-mesenchyme transformation (EMT) resulting in formation of preavalvular “cushions.” The post-EMT processes, whereby cushions are morphogenetically remodeled into valve leaflets, are less well understood, but they require periostin. Mice with targeted deletion of periostin develop degenerative changes similar to human forms of VHD. Mitral valves are also abnormally elongated in hypertrophic cardiomyopathy (HCM), which plays an important role in clinical disease expression. However, the mechanism for this is unclear, but correlates with enhanced expression of periostin in a specific population of ventricular cells derived from the embryonic proepicardial organ, which accumulate at sites where valvular endocardial EMT is reactivated. Collectively, these findings suggest that developmental mechanisms underlie adult valve responses to genetic mutations in degenerative VHD and HCM.

Keywords

valvulogenesis; differentiation; mitral valves; periostin; cardiomyopathy; EMT; stem cells

Introduction

Adult valve heart disease (VHD) is a common medical condition that increases with age and, despite improvements in treatment options, continues to convey elevated mortality and morbidity. In view of the growing aging population, VHD is an increasing public health concern. New therapeutic strategies are needed to stimulate endogenous repair pathways, including formation of new progenitor cells. To devise such strategies will require understanding the morphogenetic processes driving embryonic valve formation and how their disruption might result in age-related progressive degenerative diseases or maladaptive responses of valve leaflets to myocardial injury or hypertrophy.

The morphogenetic events in vertebrate valvulogenesis are summarized in Figure 1. A key first step is endocardial-to-mesenchyme transformation (EMT), which is restricted to the junctional regions of the heart, where the future inlet and outlet valves will be formed.

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Junctional myocardium secretes growth factors of the TGF supergene family (TGF β 1–3 and BMP2 and 4), which induces the endocardial endothelium to downregulate cell–cell adhesion molecules and upregulate motility genes that cause the intervening extracellular matrix to become mesenchymal. Proliferation of the mesenchyme produces “buds” or expansions into the lumen of the heart called endocardial cushions, which constitute the primordia for both septa and valves.¹ A hallmark of endocardial cells that have been induced to transform is their initial co-expression of endothelial and mesenchymal markers.² Once seeded into the extracellular matrix (ECM), post-EMT cushion cells downregulate their endothelial markers, sustain their mesenchymal markers, and proliferate. However, they do not immediately express lineage-specific markers. What regulates post-EMT differentiation and remodeling of the cushions into mature valve leaflets and cusps having organized lamellae of fibrous connective tissue is a current topic of investigation. Basing our work on microarray analyses and *in situ* mRNA and protein expression results, we have identified periostin as a candidate gene for regulating post-EMT cushion remodeling into mature leaflets and cusps. In the case of the atrioventricular (AV) mitral and tricuspid valves, the periostin gene is associated with the regulatory genetic network that directs formation of the tendinous cords connecting the valve leaflets to the papillary muscles.^{3,4} To determine the *in vivo* role of periostin in valvulogenesis, periostin gene knockout studies were performed.^{4,5} Full-length sense and antisense viral vectors to overexpress or inhibit periostin secretion were also studied in isolated and cultured prevalvular cushion mesenchymal cells.

Valve phenotype of periostin-null mice

Two groups have generated periostin-null mice and analyzed them for developmental cardiac defects.^{6,7} Most null mice were viable, although about 20% died either before or after birth, depending on the mouse line. Loss of periostin gene function did not affect EMT, but altered post-EMT morphogenesis. Both inlet and outlet cushions were affected. At 3 months, valve leaflets were hypertrophied and abnormally shortened compared with the attenuated and sculpted leaflets of their wild-type littermates. The tendinous cords of the AV valves were either truncated or absent. Histologically, null AV leaflets lacked an organized fibrous matrix; this correlated with null cells being either undifferentiated or expressing chondrogenic or cardiomyocyte markers.^{4,7} Introducing a cre lineage marker driving GFP in periostin-null mice confirmed that differentiation of cushion cells was misdirected into a cardiomyocyte lineage permanently in the absence of periostin.⁷ Conversely, for the outlet semilunar valves, based on differential selective subtraction hybridization studies, loss of periostin function suppressed notch 1 signaling, which correlates with the expression of preosteoblastic markers and the early onset of calcification in adult heart valves (unpublished observations). These findings suggest the hypothesis that periostin normally promotes differentiation of prevalvular mesenchyme into collagen-producing fibroblastic cells termed “valve interstitial cells,” whose enhanced secretion and compaction of collagen promotes sculpting and attenuation of the cushions into cusps and mature leaflets.^{4,7,8} Another interpretation of the data is that periostin inhibits differentiation of cushion cells into non-fibroblastic lineages (e.g., cardiomyocyte, osteoblastic, chondrogenic). This “periostin hypothesis” as based on *in vivo* studies is summarized in Figure 2.

In vitro assays to test the periostin hypothesis

To test the hypothesis that periostin promotes differentiation of prevalvular mesenchyme into a fibroblastic lineage and/or inhibits differentiation into other mesodermal lineages (Fig. 2), we transfected isolated chick or mouse wild-type embryonic cushion cells with viral vectors either to promote or to inhibit periostin expression. Inhibiting periostin expression in chick prevalvular mesenchyme maintained in hanging drop cultures significantly increased the number of cells that expressed cardiomyocyte markers compared with controls.⁹ Almost

80% of the cells infected with the inhibitory vector expressed a muscle phenotype, whereas those transfected with the sense vector (overexpressors) were fibroblastic and increased expression of collagen.⁹ We also supplemented null mouse cushion cells with purified periostin protein to attempt a “rescue” experiment: null AV cushion cells not receiving the purified protein expressed myocardial markers and greatly reduced levels of fibroblast markers; whereas periostin null cultures receiving the purified protein reversed these results, with myosin markers being downregulated and fibroblastic markers upregulated.⁴ These data are consistent with recent studies by Niu and colleagues, which indicate that genetically inhibiting a transcription factor (e.g., serum response factor) that promotes expression of contractile proteins in cardiogenic mesoderm in E8.5 mouse embryos removed a “brake” on periostin expression and promoted differentiation into fibroblastic/osteoblastic lineages.¹⁰ These data suggest that periostin functions as a “nodal” switch whose expression in mesodermal or mesenchymal cells promotes their differentiation into a fibroblastic lineage, whereas blocking its expression results in aberrant differentiation into cardiac muscle or other non-fibroblastic phenotypes.

What is periostin?

Periostin is a multifunctional protein that has clearly emerged as a major candidate for regulating valve differentiation and maturation. Maturation refers to the compaction and alignment of the extracellular matrix, especially collagen, which correlates with sculpting and attenuation of primitive preavalvular cushions into mature cusps and leaflets, as shown in Figure 1. Periostin expression in the neonatal heart and re-expression in adult hearts correlates with pathophysiologic remodeling, which is often manifested in part by increased collagen production (fibrosis). As such, periostin is frequently envisioned as an adhesive, profibrogenic protein.¹¹ The ability to affect differentiation, migration, matrix production, and maturation is consistent with periostin's belonging to a growing list of matricellular proteins (e.g., sparc, thrombospondin, osteopontin, fibulin, and tenascin) and connective tissue growth factors like CCN1 and CCN2. Like all matricellular proteins, periostin can bind to structural ECM proteins like collagen or to cell surface integrin receptors. Binding to integrins (e.g., α V, β 3, β 1) initiates intracellular signaling through phosphorylation of Akt1, PI3 kinase, Rho kinase, and focal adhesion kinases. Activation of these signal mediators, and other potential intracellular transduction pathways modifies cell behaviors related to migration, differentiation, and survival or apoptosis, but not proliferation. It is important to note that these intracellular signals (especially Rho) are essential for promoting migration and cell-shape changes, but have also been shown to: (1) block muscle differentiation and (2) mediate cardiac fibrosis in an ischemic/reperfusion cardiomyopathy model by regulating fibroblast precursor cell differentiation.¹² In the context of the periostin-null mouse, decreased expression or phosphorylation of Rho and/or FAK could be anticipated to decrease cell migration and formation of filopodia, as well as disrupt normal processes of differentiation. In addition, valvular precursor cells contribute to the reorganization of ECM required for sculpting the cushions into fibrous cusps and leaflets. Uncontrolled valve interstitial cell migration, proliferation, differentiation, and remodeling could result in a random distribution of ECM components and therefore impair leaflet attenuation and shape that ultimately dictate function. Thus, the leaflet must remodel itself during embryonic and fetal life to acquire the proper geometry. This might occur through the potential of valve precursor cells to compact and align collagen fibers; it has recently been shown that cushion cells increase collagen compaction through a periostin-dependent, integrin-based signaling mechanism.^{7,13}

As the valve fibroblasts compact and align the collagen fibers to achieve a mature fibrous leaflet, cross-linking of the collagen fibrils is an additional important event ensuring proper biomechanical stability of the valve structure. In periostin-null mice, connective tissues in

skin, tendon, myocardial wall, and heart valves have significantly altered biomechanical and biophysical properties. These changes can largely be attributed to decreased collagen cross-linking and/or reduced numbers of collagen-secreting fibroblasts caused by altered differentiation. As a result, the connective tissues of the periostin-null mice are more compliant (less stiff) than in wild-type mice and more prone to rupture.⁵

Relationship of periostin to human adult VHD

The loss of periostin expression in mice results in a phenotype similar to the degenerative changes often seen in prolapsed human mitral valves or bicuspid aortic leaflets.^{4,7} Interestingly, in one patient subpopulation, premature onset of calcifying aortic valve disease was linked to a mutation in the notch 1 gene,¹⁴ which we have recently examined as a potential downstream target of periostin signaling (unpublished observations). However, the human periostin gene, located on chromosome 13, has not yet itself been directly linked in human population studies to mitral valve prolapse, but may be implicated as a downstream effector, as discussed below.

One lesson learned from the Marfan syndrome is that dysregulation of an important growth factor like TGF β can cause mitral valve degeneration and prolapse even though the growth factor/cytokine gene itself is normal. In the case of Marfan syndrome, the mutation is in an extracellular protein, fibrillin-1, that normally binds the latent precursor form of TGF β .¹⁵ Mutations in fibrillin-1 lead to excess TGF β signaling, which over time contributes to increased production of metalloproteinases and degenerative changes characteristically seen in the mitral valves of patients with the disorder.^{16,17} An innovative therapy for Marfan syndrome is directed at neutralizing TGF β activity by the angiotensin II antagonist, losartan. Because periostin is a downstream target of TGF β , it is possible that dysregulation of TGF β signaling might modify periostin expression and function even though the periostin gene is normal. Please note that the mouse fibrillin-1 knockout model closely phenocopies human Marfan syndrome. This adds further support to the basic premise that adult valve diseases can result from mutations in proteins normally secreted in embryonic life, but act over a prolonged time, so that it might take decades for the effect of the mutation to become manifested.

Hypertrophic cardiomyopathy (HCM)

Valve diseases are not always linked to mutations in genes expressed in developing valve progenitor cells. Elongated and displaced mitral valves are also seen in hypertrophic human and mouse hearts with mutations in sarcomeric proteins expressed by the cardiomyocytes; they are also found in patients with HCM, in whom they contribute to left ventricular outflow tract obstruction and clinical symptoms through systolic anterior motion of the elongated valves that contact the hypertrophied upper interventricular septum (IVS). How a mutation in cardiomyocytes can lead to pathologic changes in the growth and structure of mitral valves is unclear. Again, developmental biology may provide one possible explanation for how valves can become pathologically elongated in HCM. In this case, the root of the paradox may lie with the abnormal differentiation of a subset of extracardiac mesenchymal cells that are derived from coelomic mesothelium and called epicardial-derived cells (EPDCs).^{18–20} EPDCs migrate from the surface of the heart into the ventricular and atrial walls and normally differentiate into fibroblasts. Based on cre-lineage markers, EPDCs that migrate into the muscular IVS and around the mitral–aortic continuity region, uniquely differentiate into cardiomyocytes, not fibroblasts.^{21,22} One explanation for why a mutation in sarcomeric proteins can affect valve development is that during development, EPDCs—rather than becoming hypertrophic like other cardiomyocytes—differentiate or revert into fibroblastic-like cells. If this is true, one would expect increased levels of

periostin production in hypertrophic hearts since the hallmark of fibroblastic differentiation is expression of periostin. Consistent with this hypothesis, markedly elevated levels of periostin are indeed expressed in HCM mice.^{23,24} Recently, it has been suggested that periostin can be part of a nodal molecular switch that determines whether a mesodermal progenitor cell becomes a cardiomyocyte or fibroblast. Specifically, results published from the Schwarz laboratory have demonstrated that blocking expression of the serum response factor (SRF) transcription factor, which is required for initiating contractile protein synthesis, causes upregulation of periostin expression, with diminished differentiation of cardiac mesoderm into myocytes.¹⁰ Consistent with this hypothesis, we have preliminary evidence that the pattern of periostin expression in HCM mice exactly correlates with the hypothesis that EPDC-derived cardiomyocytes become fibroblasts when a gene encoding a contractile protein is mutated. On the basis of the periostin hypothesis (Fig. 2), one effect of elevated levels of periostin in close proximity to the mitral valve could be to increase production of collagen, as in normal valvulogenesis. Collagen synthesis and periostin expression in the AV heart valve will then be increased in HCM mice. As in normal valvulogenesis, the increased expression of periostin in and around valve interstitial cells could reasonably be expected to result in elongation of the leaflets, constituting one explanation for the changes seen in the mitral valves of patients with HCM.

Another explanation is that new valve tissue may be added to existing valves through reawakening of the embryonic process of endothelial–mesenchymal transformation or EMT (Fig. 2). However, unlike the normal EMT process, the transformed cells may neither sustain the expression of the alpha smooth muscle actin mesenchyme marker nor express periostin. Therefore, although new cells will be added to the valve complex, those cells might not behave as normal cells, potentially resulting in altered cell cycling, differentiation, or migration. The potential of adult mitral valve endocardium to rekindle its potential for EMT after TGF β stimulation has in fact been recently shown both *in vitro* and *in vivo*.^{25–29} These findings as a whole suggest that adult heart valves can adapt to changes in their environment through either abnormal development (as with EPDCs in mice with sarcomeric protein mutations) or reactivation of embryonic valvulogenic processes (e.g., EMT).

Summary

Valvulogenesis involves a series of cellular transformations under the influence of a family of molecular signals generated by the valve itself as well as by the surrounding myocardium. That some of these same signals can affect adult valves is indicated by reactivation of EMT in adult valve endocardial lining cells during (1) degenerative (myxomatous) diseases, (2) in the presence of sarcomeric protein mutations in HCM, and (3) when the valve leaflets are stretched using a surgical model of mitral valve regurgitation due to leaflet stretch and tethering.

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Figure 1.

Schematic representation of valvular morphogenesis. AV and outflow tract (semilunar) valvulogenesis commences with an EMT event, yielding cushion mesenchymal cell (blue), followed by a remodeling process whereby mesenchyme differentiates into collagen-secreting (black lines) interstitial valve fibroblasts (yellow). This remodeling event ultimately leads to leaflet compaction, attenuation, and formation of fibrous continuities that are indicative of the mature valve tissue.

Figure 2.

The periostin hypothesis: Periostin expression is regulated in part by TGF β signals and secreted by cushion mesenchymal cells (*middle cell*). This secretion promotes a mesenchymal to fibroblast differentiation (*left cell*) and is important for promoting collagen fibrillogenesis and the material properties of forming valves. In its absence, aberrant differentiation of alternative cell types (e.g., myocytes) are observed (*right cell*). Thus, periostin promotes differentiation of preavalvular mesenchyme into a fibroblastic lineage and/or inhibits differentiation into other mesodermal lineages.