Published in final edited form as:

Cell Tissue Res. 2012 January ; 347(1): 203–223. doi:10.1007/s00441-011-1241-3.

Transforming growth factor *beta* signaling in adult cardiovascular diseases and repair

Thomas Doetschman^{1,2}, Joey V. Barnett³, Raymond B. Runyan², Todd D. Camenisch^{4,5,6}, Ronald L. Heimark⁷, Henk L. Granzier⁸, Simon J. Conway⁹, and Mohamad Azhar^{1,2,*}

¹BIO5 Institute, University of Arizona, Tucson, Arizona 85724, USA

²Department of Cellular and Molecular Medicine, University of Arizona, Tucson, Arizona 85724, USA

³Department of Department of Pharmacology, Vanderbilt University Medical Center, Nashville, Tennessee 37232-6600, USA

⁴Department of Pharmacology and Toxicology, University of Arizona, Tucson, Arizona, 85724, USA

⁵Steele Children's Research Center, University of Arizona, Tucson, Arizona, 85724, USA

⁶Southwest Environmental Health Sciences Center, University of Arizona, Tucson, Arizona, 85724, USA

⁷Cancer Biology Graduate Interdisciplinary Program, and Department of Surgery, University of Arizona Health Sciences Center, 1501 North Campbell Avenue, Tucson, Arizona, 85724, USA

⁸Molecular Cardiovascular Research Program, Sarver Heart Center, Department of Physiology, University of Arizona, Tucson, Arizona, 85724, USA

⁹Program in Developmental Biology and Neonatal Medicine, Wells Center for Pediatric Research, Indiana University School of Medicine, Indianapolis, Indiana 46202

Abstract

The majority of children with congenital heart disease now live into adulthood due to the remarkable surgical and medical advances that have taken place over the past half century. Because of this, the adults now represent the largest age group with adult cardiovascular diseases. They include patients with heart diseases that were not detected or not treated during childhood, those whose defects were surgically corrected but now need revision due to maladaptive responses to the procedure, those with exercise problems, and those with age-related degenerative diseases. Because adult cardiovascular diseases in this population are relatively new, they are not well understood. It is therefore necessary to understand the molecular and physiological pathways involved if we are to improve treatments. Since there is a developmental basis to adult cardiovascular disease, transforming growth factor *beta* (TGF β) signaling pathways that are essential for proper cardiovascular development may also play critical roles in the homeostatic, repair and stress response processes involved in adult cardiovascular diseases. Consequently, we have chosen to summarize the current information on a subset of TGF β ligand and receptor genes and related effector genes that when dysregulated are known to lead to cardiovascular diseases and adult cardiovascular deficiencies and/or pathologies. A better understanding of the TGF β signaling network in cardiovascular disease and repair will impact genetic and physiologic investigations of cardiovascular diseases in elderly patients and lead to an improvement in clinical interventions.

*Correspondence to Mohamad Azhar, PhD, 1656 E Mabel St, PO Box 245217, Tucson, Arizona. Fax: 5206267600, Telephone: 5206261861, azharm@email.arizona.edu.

Keywords

TGFbeta; aging; disease; cardiovascular; heart

Introduction

Cardiovascular disease is extremely common in elderly patients and is their leading cause of morbidity and death, more so than (Jackson and Wenger 2011)cancer (Roger et al. 2011). Congenital heart disease represents the largest class of birth defects and affects about 0.8% of all babies born (Supino et al. 2006). As a result of improved medical and surgical management of congenital cardiovascular malformations, 90% of children born with congenital heart diseases now live into adulthood. Because of this, adult congenital cardiovascular disease now represents the largest population in the United States living with the consequences of the repaired congenital cardiovascular malformations (Khairy et al. 2008). The aging affects other organs and the higher susceptibility of the elderly patients to other diseases such as diabetes is other factors that can influence cardiovascular complications (Roger et al. 2011). Common long-term problems include rhythm problems, valve problems, heart failure and aortic aneurysm (Pearson et al. 2008;Markwald et al. 2010) (Hinton and Yutzey 2011;Force et al. 2010;Lipshultz et al. 2003). Once developed, however, these cardiovascular tissues must undergo homeostasis to maintain function throughout life, and to undergo repair and remodeling following stress and injury. It stands to reason that these homeostatic and repair functions would use many of the same mechanisms that were involved in the development and remodeling of those tissues during embryogenesis. Thus, many genes associated with transforming growth factor *beta* (TGF β) signaling that lead to congenital heart disease when disrupted (Arthur and Bamforth 2011;Derynck and Akhurst 2007;Dunker and Kriegelstein 2000) would therefore be ideal candidates for homeostatic, stress response and repair processes in the adult (Fig. 1).

Genetic mutations in TGF β pathway genes and TGF β signaling dysregulation have emerged as a major molecular pathway involved in adult cardiovascular diseases (Table 1) (Gordon and Blobe 2008;ten Dijke and Arthur 2007;Jain et al. 2009) A striking number of these cardiovascular defects are modeled in mice with mutations or dysregulation in TGF β signaling and effector genes (Arthur and Bamforth 2011). Unfortunately, the complexity of TGF β signaling often plays itself out in seemingly inconsistent fashion when one investigates its roles in human cardiovascular disease, in mouse models of cardiovascular disease, and in TGF β -based pre-clinical and clinical therapeutic outcomes. A critical barrier to progress in the field of adult cardiovascular diseases, in which TGF β and related pathways are potential therapeutic targets, is the poor understanding of the interrelated pathways by which three TGF β ligands can signal through multiple combinations of TGF β receptors, and then transduce signals through several SMAD-dependent and -independent pathways (Fig. 1) (Kang et al. 2009;Moustakas and Heldin 2009). In this review we will provide a summary of the potential involvement of TGF β signaling molecules in cardiovascular diseases and cardiovascular stress-related complications, and the prospect of the TGF β -based therapeutics in the field.

TGF β signaling and functional complexity

TGF β s 1, 2 & 3 control a plethora of cellular processes, including epithelial mesenchymal transformation, cell growth, apoptosis, differentiation, extracellular matrix (ECM) production and remodeling, all in a highly context-dependent manner (Sporn 2006;Barnard et al. 1990;Sporn and Roberts 1990;Massague et al. 1994;Mercado-Pimentel and Runyan 2007;Derynck and Akhurst 2007). TGF β 2 interacts with TGF β R3 to allow high affinity

binding to TGF β R2 and TGF β R1 receptor complex (Lopez-Casillas et al. 1993). Endoglin is an accessory protein that cannot bind ligand on its own but interacts with the signaling receptor complex of multiple members of the TGF β superfamily (Barbara et al. 1999). Since TGF β R2 can only bind TGF β 1 and TGF β 3, endoglin signals for these ligands when it is present in the TGF β receptor complex (Yamashita et al. 1994). This ligand-receptor binding results in phosphorylation and activation of TGF β R1, which leads to phosphorylation-dependent activation SMAD2/3 (Fig. 1). In association with SMAD4 the phosphorylated SMAD2/3 molecules migrate to the nucleus (Wu and Hill 2009). In conjunction with corepressors and coactivators the SMAD complex regulates TGF β target genes, including periostin (*Postn*), connective tissue growth factor (*Ctgf*) and platelet activator inhibitor-1 (*Pai1*) (Ross and Hill 2008; Snider et al. 2009; Moustakas and Heldin 2009). Recent findings have highlighted some cross-utilization of ligand and receptors between TGF β and BMP subfamilies. As with the TGF β s, ligands of the BMP subfamily also signal through a variety of Type I and Type II receptors from which transduction occurs via SMADs 1, 5 & 8 (Miyazono et al. 2010; Wang et al. 2011). TGF β 1 can also signal through ALK1 (a BMP Type I receptor) in endothelial cells (Lebrin et al. 2005), and ALK2 (a BMP Type I receptor) in both endothelial cells and fibroblasts (Daly et al. 2008), thereby bringing about cross-talk between TGF β and BMP signaling pathways. It has also been well documented that TGF β R3, a TGF β 2-binding receptor, can also bind to BMP2 and lead to epithelial-mesenchymal transformation during heart development (Townsend et al. 2011). The inhibitory SMAD6 predominantly inhibits BMP signaling, whereas SMAD7 inhibits both TGF β and BMP signaling (Goto et al. 2007; Tang et al. 2010). It is clear that growth, differentiation, morphogenetic and matrix modeling properties of TGF β , properties that have been associated with TGF β function for decades (reviewed (Roberts et al. 1990a; Roberts et al. 1990b; Barnard et al. 1990)), are critical for the proper development of the heart (reviewed (Azhar et al. 2003; Barnett and Desgrosellier 2003; Runyan et al. 2005; Camenisch et al. 2010; Arthur and Bamforth 2011)) and for cardiac function (reviewed (Azhar et al. 2003; Snider et al. 2009)). Given the non-overlapping (Sanford et al. 1997), multifunctional nature of TGF β function (dozens of phenotypes among the 3 TGF β ligand knockout mice, reviewed (Doetschman 1999)), as well as the multiple TGF β signaling pathways that have been identified (Moustakas and Heldin 2009), there are numerous potential mechanisms through which TGF β affects the complex differentiation and morphogenetic processes required to develop the heart's components.

Consistent with the theme that TGF β signaling pathways that are essential for proper cardiovascular development also play critical roles in the cardiovascular system during the aging process, we have chosen to focus on TGF β ligand and receptor genes and related effector genes that when dysregulated or mutated are known to lead to congenital heart disease and cardiovascular deficiencies and/or pathologies during aging. A schematic depicting the interaction of the TGF β -associated genes is presented in Fig. 1 and is discussed (see below).

Genetic variation and dysregulated expression of TGF β ligand genes in cardiovascular diseases

Genetic mutations affecting TGF β signaling has been reported in patients with cardiovascular diseases, including arterial aneurysm, valvular heart disease, and cardiomyopathy (Table 1). Elevated TGF β signaling (pSMAD2 as an indicator) was reported in cardiovascular patients of Marfan syndrome (MFS: aortic aneurysm and dissection, mitral valve prolapse), Ehlers-Danlos syndrome (EDS: mitral valve prolapse, aortic root dilation, aneurysm), Cutis Laxa (aortic aneurysm and stenosis), Arterial Tortuosity syndrome (ATS: aneurysm and aortic stenosis), Bicuspid Aortic Valve with aortic aneurysm, Loeys-Dietz syndrome (LDS: aortic aneurysm, congenital heart defects),

and aortic aneurysms and dissections with early-onset osteoarthritis (AOS: aneurysm/dissection, valvular heart disease, congenital heart disease, left ventricular hypertrophy, atrial fibrillation) (Jain et al. 2009; Matt et al. 2009; Loeys et al. 2005; Coucke et al. 2006; Loeys et al. 2006; Renard et al. 2010; van, I et al. 2011). Notably, what makes these TGF β -associated cardiovascular diseases of children, youth and adults more challenging is that they affect many cardiovascular tissues in the same patients (Eckman et al. 2009; Das et al. 2006; De Backer 2009a). There are also many non-syndromic cardiovascular disorders in which TGF β signaling is increased. TGF β 1 is elevated in calcified human aortic valves (Jian et al. 2003), and increased levels of TGF β 1 are found in humans with chronic rheumatic heart disease (Kim et al. 2008) and carcinoid syndrome (Jian et al. 2002). Both elevated levels of TGF β 1 and pSMAD2 are associated with non-syndromic ascending aortic aneurysm in humans (Gomez et al. 2009).

Patients of both idiopathic hypertrophic cardiomyopathy and dilated cardiomyopathy have increased levels of *TGFB1* in the myocardium (Li et al. 1997; Pauschinger et al. 1999). TGF β 1 is also increased in myocardial remodeling in patients with valvular aortic stenosis as evidenced by left ventricular transcriptional changes of genes linked to myocardial hypertrophy and fibrosis, and by heart morphology and function (Villar et al. 2009). Consistent with these observations, mice subjected to transverse aortic constriction have elevated TGF β 1 (Villar et al. 2009), and in TGF β 1-deficient mice we found that Angiotensin II (Ang II)-induced cardiac hypertrophy is attenuated (Schultz et al. 2002). TGF β 1 also plays an important role in attenuating hypertension in mice (Zacchigna et al. 2006). In addition, there are elevated levels of TGF β 1–3 in aortas of Marfan syndrome patients associated with altered hyaluronan synthesis, increased apoptosis, impaired progenitor cell recruitment, and abnormal directional migration, suggesting that defective tissue repair is likely to contribute to aortic disease in these patients (Nataatmadja et al. 2006). Consistently, TGF β 1 levels are found to be elevated in valvular and aortic tissues of *Fibrillin-1*^{+C1039G} mice, a Marfan syndrome mouse model (Ng et al. 2004; Habashi et al. 2006). Genetic polymorphism (Leu(10)→Pro (codon 10)) in the human *TGFB1* gene is associated with end-stage heart failure caused by dilated cardiomyopathy (Holweg et al. 2001). Renal dysfunction is a severe late complication of heart transplantation, and renal function decreases in most heart transplantation recipients with time. Progression of renal insufficiency worsens in patients with Leu at codon 10 polymorphism in *TGFB1* (Lacha et al. 2001). In mouse, *Tgfb1* gene expression is seen predominantly in endocardium in the heart and endothelium of the arterial walls during cardiovascular development and at neonatal stages (Fig. 2) (Molin et al. 2003; Akhurst et al. 1990). Gene targeted disruption of *Tgfb1* has revealed variable genetic background-dependent phenotypes in mice (Sanford et al. 2001). Depending on the mouse genetic background the loss of *Tgfb1* could lead to embryonic lethality caused by either preimplantation defects or defective vasculogenesis and hematopoiesis of the extraembryonic tissues such as yolk sac, and an autoimmune disease at weaning age (Kallapur et al. 1999; Shull et al. 1992; Kulkarni et al. 1995; Dickson et al. 1995). Several genetic modifier loci have been identified that influence *Tgfb1* knockout mouse phenotypes (Bonyadi et al. 1997). Because of these complex phenotypes the exact physiological role of TGF β 1 in cardiovascular development and function has not been elucidated.

Genetic variation in *TGFB2* has also been reported in association with cardiovascular complications in patients of Kawasaki disease (Shimizu et al. 2011) (Table 1). Kawasaki disease is an inflammatory disease in which death is often caused by coronary artery aneurysm, myocardial infarction, ischemic heart disease and sudden cardiac death. TGFB2 levels are elevated in the myocardial tissue of the patients of dilated cardiomyopathy (Pauschinger et al. 1999). In mouse, *Tgfb2* expression has been reported in myocardium surrounding the valve forming regions, valve mesenchyme, and arterial wall of the

pharyngeal arch artery system during cardiovascular development (Fig. 2) (Dickson et al. 1993; Azhar et al. 2003; Molin et al. 2003). Gene targeted disruption of *Tgfb2* in mice has resulted in valvuloseptal and myocardial defects, enlarged valves, and defects in aortic arch artery development and remodeling. (Sanford et al. 1997; Bartram et al. 2001; Azhar et al. 2009a; Azhar et al. 2011). Furthermore, *TGFB2* is elevated in diseased mitral valves and aortas of Marfan syndrome patients in which TGF β signaling is also increased (Ng et al. 2004; Nataatmadja et al. 2006; Jain et al. 2009). The expression of *Tgfb2* in adult mouse cardiovascular tissues has not been determined, and therefore it is difficult to predict its possible role in adult cardiovascular structure and function. There are also no reports on *Tgfb2* transgenic overexpression in mouse cardiovascular tissues. Overall, the role of TGF β 2 in the adult cardiovascular system and in cardiovascular diseases remains to be understood.

Significant expression of the *TGFB3* gene has been detected in human hearts (Rampazzo et al. 2003). Importantly, genetic mutations are found in *TGFB3* in patients of arrhythmogenic right ventricular cardiomyopathy/dysplasia (ARVC/D or ARVD1) (Rampazzo et al. 2003; Beffagna et al. 2005; McNally et al.). In addition, a genetic polymorphism in *TGFB3* is associated with the abnormal left ventricular structure-function in hypertensive patients (Hu et al. 2010). ARVD is considered a leading cause of unexpected sudden cardiac death, particularly in the young and in athletes. ARVD is a genetic, progressive heart condition in which the muscle of the right ventricle is replaced by fat and fibrosis which cause abnormal heart rhythms. ARVD primarily affects the right ventricle; with time, it may also involve the left ventricle. Familial ARVD disease is common, and it is transmitted as an autosomal dominant disorder. ARVD has been linked to several chromosomal loci, and therefore, a single gene change is usually not sufficient for the development of ARVD, and genetic interaction among various genes is needed for an individual to actually develop symptoms of ARVD (Tran-Fadulu et al. 2009; Uddin et al. 2010). Although it is thought that the causative factor in ARVD1 could be paradoxically increased TGF β signaling (Beffagna et al. 2005), it is currently unclear how mutations in *TGFB3* actually cause ARVD. The only clues about TGF β 3 function at an organismal level have come from mouse gene targeting studies. *Tgfb3*^{-/-} mice died at birth due to cleft palate (Proetzel et al. 1995; Kaartinen et al. 1995), and they have a subtle myocardial and aortic phenotype (Azhar et al. 2003). Although *Tgfb3* expression appears much later than *Tgfb2* during cardiovascular development in wild-type embryos, its patterns of expression are strikingly similar to that of *Tgfb2* (Fig. 2) (Molin et al. 2003). Also, *Tgfb3* expression increases as compared to *Tgfb2* as cardiovascular development proceeds (Vrljicak et al. 2010). This raises a possibility whether TGF β 2 plays a dominant role during early stages of cardiovascular development; whereas, TGF β 3 could represent the predominant TGF β isoform during later stages of cardiovascular remodeling, a suggestion consistent with the mutations in *TGFB3* in adult cardiac disease patients. Such speculation is also consistent with the observation that genetic deletion of *Tgfb3* in *Tgfb2*^{-/-} embryos worsens embryonic developmental phenotypes of *Tgfb2*^{-/-} embryos (Dunker and Kriegelstein 2002). Consequently, determination of *Tgfb3* expression in adult cardiovascular tissues and its exact physiological function in the adult cardiovascular system will lead to a better understanding of the pathogenesis of adult cardiovascular diseases. Overall, direct contribution of specific TGF β ligands or combinations of different TGF β ligands to the TGF β signaling *in vivo* in cardiovascular disease/disorders remains a complete mystery that needs to be resolved.

Connection between TGF β receptor mutations and cardiovascular diseases

Genetic mutations in *TGFBR2* and *TGFBR1* are found in patients of arterial aneurysms, including Loeys-Dietz syndrome and other Marfan syndrome-like disorders (Table 1) (Loeys et al. 2006; Mizuguchi et al. 2004). The *TGFBR2* mutation is also described in patients of non-syndromic or familial thoracic aortic aneurysms and dissections (Jain et al.

2009). Many patients of Kawasaki disease, which have cardiovascular complications, also have a mutated *TGFBR2* (Shimizu et al. 2011). A dominant-negative effect was demonstrated for most mutations associated with Marfan syndrome-related disorders. By contrast, the R460C mutation, which has been found in familial aneurysms but not in syndromic patients, showed a less-severe dominant-negative effect and retained residual SMAD phosphorylation and transcriptional activity (Horbelt et al. 2010). In cell culture experiment mutations in *TGFBR2* can inhibit SMAD2/3 activation, those same mutations have the potential to cause increased SMAD1/5 activation (Bharathy et al. 2008). Quite paradoxically, the cardiovascular tissues from Loeys-Dietz syndrome patients, which have loss-of-function *TGFBR2/1* mutations, show elevated pSMAD2 and TGF β target gene expression (Loeys et al. 2005; Loeys et al. 2006). Although *Tgfb2* and *Tgfb1* conditional deletion in mice has revealed important tissue-specific roles of these receptors in cardiovascular development, these studies have failed to generate any direct information about their function in adult cardiovascular tissues and in the pathogenesis of cardiovascular diseases (Jiao et al. 2006; Choudhary et al. 2009; Sridurongrit et al. 2008; Robson et al. 2010) (Langlois et al. 2010). Overall, the cell autonomous and non-autonomous aspects of the genetic mutations in *TGFB* receptor genes remain unclear. Adult mouse models mimicking these TGF β receptor mutations will be useful to establish a clear cause-and-effect relationship between the mutations and the adult cardiovascular complications seen in patients. It is also important to note that individuals with many of these *TGFB* receptors gene mutation-containing syndromes, in which there is failed matrix regulation possibly due to elevated and/or reduced TGF β signaling, share phenotypes similar to those seen in TGF β 2-deficient mice; e.g., craniofacial/skeletal, cleft palate, ocular defects and congenital heart disease (Sanford et al. 1997; Saika et al. 2001; Paradies et al. 1998; Crowe et al. 2000; Jain et al. 2009; Horbelt et al. 2010; Mizuguchi et al. 2004). Conditional deletion of the *Tgfb1* and/or *Tgfb2* in different cardiovascular cell types has also produced similar results (reviewed in (Arthur and Bamforth 2011)).

Genetic variants have been identified in *TGFBR3* and *endoglin* in familial intracranial aneurysm (Table 1) (Santiago-Sim et al. 2009). Due to *ENDOGLIN* mutations in HHT patients the role of endoglin in cardiovascular development and disease has caught significant attention (Goumans et al. 2008; Nomura-Kitabayashi et al. 2009; Mercado-Pimentel et al. 2007; Arthur et al. 2000), and therefore has not been reviewed here. Despite our ever increasing understanding of TGF β signaling, especially downstream of the canonical receptors TGF β R2 & TGF β R1, the diverse and often contradictory actions of TGF β remain unexplained. A critical component of TGF β signaling that is not well understood and understudied is the contribution of TGF β R3 (Lopez-Casillas et al. 1993). Although TGF β R3 augments the canonical TGF β signaling pathway, several lines of evidence establish a unique role for TGF β R3 in mediating the actions TGF β . First, TGF β R3 binds all 3 TGF β ligands in addition to BMP's (notably BMP-2) (Kirkbride et al. 2008) and inhibin (Lewis et al. 2000). All these ligands are members of the TGF β superfamily of growth factors. Second, TGF β R3 localizes endocardial to mesenchymal transformation to the AV cushion, and its inhibition prevents endocardial transformation and migration (Brown et al. 1999). This system provided the first report demonstrating that TGF β R3 was specifically required to mediate a biological event. Third, targeted deletion of *Tgfb2*, which requires TGF β R3 for high affinity binding, results in embryos with a unique and nonoverlapping cardiovascular phenotype compared to *Tgfb1* and *Tgfb3* knockout embryos (Azhar et al. 2003). Finally, deletion of *Tgfb3* results in embryos with defects distinct from those seen in *Tgfb1* and *Tgfb2* knockout embryos, including defects in the outflow tract, valves, coronary vessels and myocardium (Stenvers et al. 2003; Compton et al. 2007; Arthur and Bamforth 2011). Comparison of heart defects in *Tgfb3* and *Tgfb2* knockout embryos indicates common and unique roles for TGF β R3 and TGF β 2 in heart development (Azhar et al. 2003; Stenvers et al. 2003; Compton et al. 2007). A human GWAS identified a *TGFBR3*

single nucleotide polymorphism with significantly altered bone mineral density (Xiong et al. 2009). Recent findings have indicated that BMP2 can also bind and act as a ligand for TGF β R3 (Townsend et al. 2011). BMP2 has well documented role in bone biology and aortic valve calcification (Wang et al. 2011; Miyazono et al. 2010; Hakuno et al. 2009). Since the heart and vascular wall of aorta and bone share similar molecular pathways (Rajamannan 2009; Lincoln et al. 2006), this suggests a potential role for *TGFBR3* in calcific cardiovascular diseases, including aortic valve and vascular calcification. Overall, these observations suggest an important role for TGF β R3 in TGF β signaling. Understanding the biological roles of TGF β R3 is essential to further our understanding of TGF β signaling and the role of different TGF β ligands, particularly of TGF β 2-TGF β R2 ligand-receptor pair, in development and disease. Even in conditions such as Marfan syndrome and aortic aneurysm where recent insights into the role of TGF β have significantly impacted clinical care and outcomes, there is general recognition that our understanding is inadequate to fully explain and leverage therapeutic opportunities (reviewed in (Dietz 2010)). Delineating TGF β ligand(s) and receptor(s) interaction pathways involved in specific aspects of cardiovascular development and adult cardiovascular function should inform novel therapeutic approaches with higher specificity and more limited side effects.

TGF β effector molecules in cardiovascular diseases

Canonical TGF β signaling is mediated by combination of various SMADs, which are widely expressed in cardiovascular tissues (Luukko et al. 2001). Aneurysms-osteoarthritis syndrome is a newly described syndrome, which is caused by mutations in *SMAD3* (van, I et al. 2011). These patients have aneurysms, dissections and tortuosity throughout the arterial tree in association with mild craniofacial features and skeletal and cutaneous anomalies. In contrast with other aneurysm syndromes, most of these affected individuals present with early-onset osteoarthritis (van, I et al. 2011; Jain et al. 2009). These patients also have other (congenital) heart diseases, such as persistent ductus arteriosus, atrial septal defect, pulmonary valve stenosis and atrial fibrillation. Mitral valve abnormalities, ranging from mild valve prolapse to severe regurgitation requiring valve replacement, and idiopathic mild-to-moderate predominantly concentric left ventricular hypertrophy have also been reported in these patients. Intriguingly, as in Marfan-syndrome like disorders, heterozygous *SMAD3* mutations in humans are also associated with increased aortic expression of several key players in the TGF β pathway, including pSMAD3. In addition, genetic variation in *SMAD3* is also associated with Kawasaki disease susceptibility, coronary artery aneurysm formation, aortic root dilatation, and intravenous immunoglobulin treatment response in different cohorts (Table 1) (Shimizu et al. 2011). SMAD3 is also an important player in T-cell activation and cardiovascular remodeling, both of which are important features of Kawasaki disease (Roberts et al. 2006). A recent mouse genetic study has shown that SMAD3 plays a beneficial role in delimiting cardiac hypertrophic response and that SMAD3 has a detrimental effect on myocardial fibrosis (Divakaran et al. 2009). Importantly, a highly specific pharmacological agent, SIS3, exists which can effectively block SMAD3 signaling without affecting SMAD2 activation (Jinnin et al. 2006). SIS3 has been shown to block *in vivo* SMAD3 signaling in a mouse model (Li et al. 2010). Collectively, these findings endorse the SMAD3 as a critical effector of TGF β signaling and as a potential pharmacological target for the development of new treatments for adult cardiovascular disease.

The phosphorylated SMAD3 forms a complex with SMAD4, which enters the nucleus. This SMAD complex binds promoters and causes the transcription of target genes (Moustakas and Heldin 2009; Ross and Hill 2008). A recent study has reported that mutations in *SMAD4* are also involved in aortopathy and mitral valve dysfunction, in addition to Juvenile polyposis syndrome (Andrabi et al. 2011). *SMAD4* mutations are a major cause of Juvenile

polyposis syndrome (JPS), a syndrome characterized by the appearance of multiple polyps in the gastrointestinal tract. Individuals with this condition due to mutations in *SMAD4* are at greater risk to manifest signs of Hereditary Hemorrhagic Telangiectasia (HHT), a clinical condition which can also be caused by other mutated genes that modulate TGF β signaling (e.g., *ENG*, *ALK1*) (ten Dijke and Arthur 2007). Partial deletion of *Smad4* (haploinsufficiency) results in premature death caused by the worsening of aortic aneurysm and rupture in mouse of model of Marfan syndrome (Holm et al. 2011). In addition, *Smad4* plays important roles in valves and myocardium during cardiac development (Azhar et al. 2010; Nie et al. 2008; Moskowitz et al. 2011). Furthermore, conditional loss of *Smad4* in myocardium can cause spontaneous cardiac hypertrophy (Song et al. 2007). Collectively, SMAD4 serves as a nodal point where the majority of canonical TGF β signaling can converge and regulate cardiovascular development and adult tissue homeostasis.

Recent genetic studies have demonstrated that an X-linked myxomatous valvular dystrophy (XMVD) can be attributed to mutations in the Filamin-A (*FLNA*) gene (Table 1) (Kyndt et al. 2007). Genetic mutations in *FLNA* are also associated with a cardiological phenotype with aortic or mitral regurgitation, coarctation of the aorta or other left-sided malformations (de Wit et al. 2011). The cytoskeletal protein filamin A (FLNA) connects the actin filament network to membrane receptors and acts as a scaffold protein for signal transduction molecules, including SMADs (Sasaki et al. 2001). In FLNA-deficient cells TGF β does not induce SMAD2 phosphorylation and subsequent SMAD translocation to the nucleus does not occur. Reintroduction of FLNA to these mutant cells restores TGF β responsiveness (Sasaki et al. 2001). The FLNA is robustly expressed in non-myocyte cells throughout cardiac morphogenesis including epicardial and endocardial cells, and mesenchymal cells derived by epithelial mesenchymal transition (EMT) from these two epithelia, as well as mesenchyme of neural crest origin. In adult hearts, expression of FLNA is significantly decreased in the atrioventricular (AV) and outflow tract valve leaflets and their suspensory apparatus (Norris et al. 2010). Overall, genetic mutation analysis and gene expression and biochemical studies suggest that FLNA is a critical TGF β effector molecule involved in the pathogenesis of cardiovascular diseases of adults. Overall, FLNA and SMADs are important downstream effectors of TGF β ligand-receptor signaling involved cardiovascular development, homeostasis and disease pathogenesis.

Role of TGF β target genes in cardiovascular diseases

In this section we will summarize the published literature on RUNX2 and periostin as two potential TGF β target molecules that may play important roles in pathogenesis of adult cardiac disease (Fig. 1). These genes are chosen because they regulate ECM remodeling and organization and calcification, both of which are critical properties of adult cardiovascular homeostasis. Involvement of TGF β in valvular calcification was first reported in the 1990s (Watson et al. 1994a), but roles of the TGF β superfamily members still remain obscure (Xu et al. 2010). Valve calcification in the adult is substantial. Degenerative valve disease occurs in approximately 25% of individuals over 65, and while there is a substantial literature describing calcium, protein and lipid components of valve pathology, the etiology of the process is not well understood. Aortic valve stenosis is the most frequent complication for valve repair or replacement surgery, and is commonly associated with pathologic calcification. Degenerative disease incidence does increase with age, but studies suggest that valve disease is not inevitable and that an understanding could be translated into preventive measures (Soler-Soler and Galve 2000). Aortic valve calcification is the predominant pathology in valve transplant patients (Subramanian et al. 1984). Also, mitral annular calcification is one of the most common cardiac defects found upon autopsy, it is occasionally found in Marfan syndrome patients, and it is significantly associated with aortic stenosis, aortic and mitral valve regurgitation, left ventricular hypertrophy and functional

deficiency (Movahed et al. 2007;De Backer 2009b). Analysis of calcified aortic valve cusps found increased TGF β 1 and matrix metalloproteinases (MMPs), suggesting that both calcification and associated tissue remodeling are induced by TGF β and its downstream remodeling genes (Jian et al. 2003;Clark-Greuel et al. 2007). It has been demonstrated that genetic inactivation of *Tgfb2* leads to severe bone defects (Sanford et al. 1997), that TGF β 1 stimulates calcification in cells with osteoblastic potential (Watson et al. 1994b), that explanted calcified nodules from transplanted tissues increase calcification in response to TGF β 1 (Mohler, III et al. 1999), and that TGF β 1 induces calcification in porcine and ovine valve interstitial cells (Walker et al. 2004;Clark-Greuel et al. 2007). The mean concentration of TGF β 1 in patients with valvular heart disease is found to be significantly lower (18.67 μ g/l) than in the control group, suggesting that the lower plasma concentration of TGF β 1 in patients with valvular heart disease, and the lack of its regulatory effect may be involved in the increased inflammation and calcification seen in valvular heart disease. Although TGF β signaling is implicated in calcific and non-calcific (e.g., myxomatous degeneration and prolapse of mitral valve) valve diseases, the maturation of valve cells and the etiology of these pathologies, in relation to TGF β , are not understood. With the exception of a few recent studies on the role of TGF β in valve development (Azhar et al. 2011;Todorovic et al. 2011), there has not been a systematic examination of heart valve disease in mouse models related to TGF β pathway genes that would shed light on the etiology of valve disease.

Runx2 (*Cbfa1*) is a TGF β - and BMP-regulated gene, required for EMT, that also forms a transcription complex with SMAD proteins to regulate target gene expression (Franceschi et al. 2003). RUNX2 has been described as a “master regulator” of osteoblast differentiation and is required for chondrocyte hypertrophy (Stein et al. 2004;Napierala et al. 2008). *Runx2* is also named *Cbfa1*, *Aml3*, *Osf2* and *Pepb2a* (OMIM). In humans there are a variety of mutations in *RUNX2*, many of which affect the DNA binding domain or cause truncations of the C terminus and cause cleidocranial dysplasia (CCD) in heterozygous form (Otto et al. 2002). Truncations in the C terminus prevent transcriptional regulation of target genes as they prevent interaction with TGF β and BMP SMADs and block targeting to the nuclear matrix (Zhang et al. 2000). *Runx2* is expressed in up to nine splice forms, but few are understood. *Runx2(II)* includes exon 1 (activation domain 1) while *Runx2(I)* begins with exon 2 and activation domain 2 (Li and Xiao 2007). Alternative splicing appears to regulate the expression of a short nuclear localization sequence or a nuclear matrix/SMAD binding domain in either the I or II isoforms (Makita et al. 2008). *Runx2(II)* isoform is expressed in bone and cartilage. The significance of two isoforms was examined in bone and cartilage development and differences are observed in formation of different bones (Xiao et al. 2004), but the heart was not examined. It is clear that the RUNX2(I) isoform preferentially interacts with the co-regulator CBF β (Kundu et al. 2002) and alters its transcriptional activity. CBF β is necessary for breast cancer invasion (Mendoza-Villanueva et al. 2010). *Runx2* also induces expression of metalloproteinases in cancer cells that also play role in valvulogenesis (Pratap et al. 2005;Alexander et al. 1997).

A number of mouse mutations in *Runx2* have been examined but, except for one mutant, they have all been mutations of both *Runx2* isoforms and the analysis focused on the skeletal system as loss of *Runx2* expression results in neonatal lethality and failure of bone formation (Otto et al. 1997). One mutant has a deleted first exon that enables expression of only *Runx2(I)*, but analysis focused on a finding that commitment to the osteoblastic lineage was normal but osteoblastic differentiation was impaired, and only some bones were produced (Xiao et al. 2004). At the cell biological level, there has been investigation of RUNX2 role in cell proliferation (Pratap et al. 2003), and it is known that STAT1 can bind and sequester *Runx2* in the cytoplasm (Kim et al. 2003). It was seen that *Runx2(I)* is expressed in prostate, breast and melanoma cancers, but the focus on this expression has been related to the tendency of these cancers to metastasize to bone (Pratap et al. 2006). Although RUNX2

expression in tumor cells and regulation of genes such as *Mmp9* or *Col 1a1* are consistent with a role in EMT, that relationship has not been exploited in the developing heart.

Another transcription factor with a described role in EMT, TWIST1, is known to inhibit RUNX2 activity and is regulated by BMP and TGF β isoforms (Kronenberg 2004). Twist1, a class II basic-helix-loop-helix (bHLH) transcription factor, is expressed during early endocardial cushion development and is down-regulated later during valve remodeling. The reported expression of *Runx2* in the murine cardiac cushions warrants an investigation into a link between RUNX2 and TWIST1 in heart (Gitler et al. 2003). The requirements for Twist1-deficiency in the remodeling valves and the consequences of prolonged Twist1 activity were examined in mice. *Twist1*^{-/-} mice have defective EMT (Shelton and Yutzey 2008) and abnormal valve remodeling (Chakraborty et al. 2010a). Persistent Twist1 expression in the remodeling valves leads to increased valve cell proliferation, increased expression of *Tbx20*, and increased ECM gene expression, characteristic of early valve progenitors. Increased *Twist1* expression also leads to dysregulation of fibrillar collagen and periostin expression, as well as enlarged hypercellular valve leaflets prior to birth. In human diseased aortic valves, increased *Twist1* expression and cell proliferation are observed adjacent to nodules of calcification (Chakraborty et al. 2010b). Furthermore, *Runx2* is upregulated in the *ApoE* knockout mouse model of aortic valve disease (Aikawa et al. 2007), and also in human degenerative mitral valve and calcified aortic valve disease (Rajamannan et al. 2003; Caira et al. 2006). Thus, the presence of both *Runx2* and *Twist1* in calcified valves provides an avenue for TGF β -mediated calcific valve disease.

Periostin has emerged as another major TGF β -target gene involved in cardiac development and various adult cardiovascular pathologies (Fig. 1). Periostin is a secreted fasciclin-domain-containing protein, which is involved in both valve development and valvular heart disease, and cardiomyopathy (Snider et al. 2008; Litvin et al. 2006; Hamilton 2008; Teekakirikul et al. 2010). *In vitro* studies indicate that *Postn* can be induced by TGF β 1 (Horiuchi et al. 1999; Zhou et al. 2010). TGF β 2-deficiency results in reduced periostin in cardiac fibroblasts (Snider et al. 2009). Periostin gene expression is also induced in chick AV cushion cultures by TGF β 3-treatment (Norris et al. 2009). Periostin is specifically found in cardiac cushion mesenchyme of developing mouse embryos and valve interstitial cells in adult mice as well as adult myocardial tissue (Kruzynska-Frejtag et al. 2001; Snider et al. 2008; Conway and Molkentin 2008; Teekakirikul et al. 2010). Periostin deficiency in mice leads to valvular defects (Norris et al. 2008; Snider et al. 2008). Normal adult human valves have a well defined tissue organization of the ECM: elastin-enriched atrialis in mitral and tricuspid valves or ventricularis in semilunar valves, glycosaminoglycans proteoglycans-enriched spongiosa, and collagen-enriched fibrosa (Wirrig and Yutzey 2011). In valve disease this ECM organization is disrupted, which includes fragmented collagen and excess accumulation of proteoglycans (Lucas, Jr. and Edwards 1982; Tamura et al. 1995; Grande-Allen et al. 2003) (Hinton and Yutzey 2011). Interestingly, this well-structured ECM organization gets disrupted by a complete deficiency of periostin in adult mice (Snider et al. 2008). In addition to an important role in maintaining valve structure, periostin also suppresses the unwanted presence of various non-valvular cell-lineages (i.e., cardiomyocyte-, smooth muscle-, and cartilage-like cells) in valves (Norris et al. 2008; Snider et al. 2008).

Striking resemblance in the gene expression patterns of *periostin* and *Tgfb2* is found in the developing heart (Table 1) (Kruzynska-Frejtag et al. 2001; Snider et al. 2008; Molin et al. 2003; Azhar et al. 2003). Notably, deficiency of TGF β 2 or periostin results in defective mesenchymal differentiation during valve remodeling process (Azhar et al. 2011; Norris et al. 2008; Snider et al. 2008). Lack of periostin has been suggested to causes derepression of the osteogenic potential of mesenchymal cells, calcium deposition, and calcific aortic valve

disease in mice (Markwald et al. 2010). Consistently, reduced levels of periostin are correlated with valve disease in pediatric patients (Snider et al. 2008). A recent study has provided another *in vivo* correlation between TGF β and periostin in adult mouse model of hypertrophic cardiomyopathy (Teekakirikul et al. 2010). Both TGF β signaling and periostin levels are elevated in non-myocyte cells of this mouse model of hypertrophic growth. Surprisingly, administration of TGF β -neutralizing antibodies has attenuated cardiac fibrosis, reduced periostin expression, and diminished non-myocyte proliferation in this mouse model. Collectively, periostin may be an important mediator and target of TGF β signaling during heart development and in the pathogenesis of cardiovascular diseases. Consequently, determination of the genetic regulation of periostin by specific TGF β ligands *in vivo* during heart development and heart disease will lead to a better understanding of the pathogenesis of adult cardiovascular diseases and repair processes.

Integrated signaling network of TGF β , Ang II, and mitogen-activated protein kinase pathways in cardiovascular diseases

It has recently become evident that there is a interrelationship of TGF β , Ang II and mitogenactivated protein kinase (MAPK) pathways that plays a critical role in the pathogenesis of cardiovascular diseases and cardiovascular tissue repair (Fig. 1) (Lim and Zhu 2006; Rosenkranz 2004; Akat et al. 2009) (Lindsay and Dietz 2011). Ang II is the main active peptide of the renin-angiotensin system and acts through G-protein coupled receptors (Kumar et al. 2008). Two main Ang II receptor subtypes, AT1R and AT2R, have been identified through selective affinity for peptide and nonpeptide reagents. The type I receptor (1a) or (AT1a) knockout mice are hypotensive and appear to have a central nephrogenic mechanism contributing to the phenotype (Li et al. 2009). Two independent AT2R-deficient lines were created on different genetic backgrounds (reviewed in (Johren et al. 2004)). Both have increased basal blood pressure likely due to elevated AT1R expression in the absence of AT2R. Although both AT2R-deficient strains live into adulthood, they are susceptible to cardiac rupture after myocardial infarction. Many studies have indicated opposite function of AT1 and AT2 receptors in the cardiovascular system (Jones et al. 2004). AT2R-deficient mice have reduced cardiac fibrosis following pressure overload or Ang II-induced hypertension supporting a role for AT2R in cardiac remodeling. AT2R appears to function separately from AT1R which regulates adult cardiovascular function, fluid and electrolyte homeostasis. AT2R is upregulated in response to pathophysiological conditions including myocardial infarction suggesting that AT2R is involved in tissue remodeling, EMT, and fibrosis (Johren et al. 2004).

It has been well documented that both TGF β s and Ang II can induce MAPK signaling (Fig. 1) (Moustakas and Heldin 2009; Lee et al. 2007; Ulmasov et al. 2009; Rodriguez-Vita et al. 2005). The role of MAPKs in cardiovascular development and cardiovascular diseases has been extensively reviewed (Craig et al. 2008; Kehat and Molkentin 2010). Briefly, an upstream mitogen or growth factor signal initiates a module of three kinases: a MAPKKK that phosphorylates and activates a MAPKK (e.g., MEK) and finally activation of MAPK (e.g., ERK). MAPKs are critical effectors that regulate extracellular stimuli into cellular responses, such as differentiation, proliferation and apoptosis, all of which function during cardiovascular development, disease pathogenesis and tissue repair. There are 21 characterized MAP3Ks that activate known MAP2Ks, and they function in many aspects of cardiovascular biology. MAPKs function in sequential transduction of protein kinase cascades (Garrington and Johnson 1999). We have identified the MEKK3 and MEKK4 as important MAPKs which are involved in heart development (Stevens et al. 2008; Stevens et al. 2006). Similar to the Fibrillin-1 connection with TGF β activity, significant advances have been made in defining the integration of ECM, especially hyaluronan, with growth factor pathways for normal cardiac EMT (Camenisch et al. 2000; Camenisch et al. 2002a). TGF β 2

activity is essential in mouse cardiac EMT (Camenisch et al. 2002b; Azhar et al. 2009a). It is also reported that MEKK3 is sufficient for the production of cardiac mesenchyme (Stevens et al. 2008); (Stevens et al. 2006). MEKK3 induction of TGF β 2 signaling drives cushion EMT and that it is consistent with cardiovascular defects in mice lacking MEKK3 (Yang et al. 2000). Collectively, these findings link downstream signaling effectors to ECM production and TGF β pathways which both promote developmental EMT and valve maturation. Similarly, it is possible that MEKK3 along with ERKs, p38 and JNK1 are key nodal points in the TGF β signaling network that drives cardiac remodeling in adult disease. There also appears to be selective expression of AT2R over AT1R in the embryonic heart with AT2R being ten-times more highly expressed in the embryonic heart than in the adult heart (Burrell et al. 2001). Additional evidence of the anatomical existence of the Ang II receptors in the brain areas that are critical for cardiovascular and fluid regulatory functions *in utero* has also been found (Hu et al. 2004). This is consistent with Ang II functioning early in cardiovascular development. Consistent with this observation, ACE inhibitors (ACEi) cause septal defects, patent ductus arteriosus and pulmonary valve stenosis during the first trimester (Cooper et al. 2006). This is highly significant considering the recent reporting of a 5% incidence of congenital heart disease (Pierpont et al. 2007). Although the molecular mechanism is unknown, this suggests a previously unrecognized role for TGF β , Ang II and MAPK signaling in cardiac morphogenesis.

With regard to the involvement of TGF β , Ang II and MAPKs in cardiovascular pathologies, it has been shown that ERKs and SMAD2 are activated in a mouse model of Marfan syndrome and that blocking TGF β signaling inhibits both SMAD2 and ERK signaling (Holm et al. 2011). Inactivation of the AT2R gene results in increased aortic aneurysm in these mice (Habashi et al. 2011). Losartan (AT1R blocker) but not ACEi (blocks both AT1R & AT2R) abrogates this aneurysm progression in the combined mice. Both AT1R and ACEi attenuate canonical TGF β signaling in the aorta, but losartan, which targets AT1R and not AT2R, specifically blocks activation of ERKs. This suggests a detrimental role for AT1R and a protective role for AT2R in the pathogenesis of aortic disease. The role of non-canonical TGF β signaling has caught significant attention in adult cardiovascular pathology. Particularly, it has been well documented that TGF β ligands can induce signaling through non-canonical or SMAD-independent pathways, including the MAPK and JNK1 pathways (Lee et al. 2007; Yamashita et al. 2008; Moustakas and Heldin 2009). With regard to the interrelationship of TGF β , AngII and MAPK pathways, it is noteworthy that the Ang II signaling through AT1R has been shown to increase the expression of TGF β ligands, receptors and activators, SMAD and MAPK signaling (Fig. 1) (Habashi et al. 2006; Akishita et al. 1999). Importantly, Ang II can activate the intracellular SMAD pathway in vascular smooth muscle cells, in a TGF β -independent fashion, via the p38 MAPK pathway (Rodriguez-Vita et al. 2005). Ang II-infusion causes cardiac fibrosis and it also induces periostin expression in cardiac fibroblasts through a p38 MAPK-dependent pathway. Moreover, Ang II can induce TGF β 1 gene expression, SMAD activation, and periostin expression through the ERK1/2 pathway (Li et al. 2011). In another recent study it has been demonstrated that AT1R stimulation drives ERK1/2 activation; whereas, AT2R stimulation inhibits it *in vivo* in mouse aortic tissue. Furthermore, losartan attenuates ERK1/2 activation by blocking the AT1R cascade while simultaneously shunting signaling through the AT2R (Habashi et al. 2011). In summary, these findings are consistent with an integrated signaling network of TGF β , Ang II and MAPK regulatory pathways in cardiovascular homeostatic, repair and stress response processes, as well as in cardiac function.

TGF β -based therapies and its potential complications for cardiovascular repair

The hope for therapeutic targeting of TGF β is clear when one looks at the number of cardiovascular and related diseases in which it is implicated. The concern is that because of the complexity of TGF β signaling as indicated by unique and overlapping gene expression of TGF β ligands and its integration with numerous other signaling pathways, targeting one TGF β pathway could trigger severe unintended effects through other TGF β pathways (Fig. 3). With respect to therapeutic approaches, systemic delivery of pan-TGF β neutralizing antibodies (TGF β NAb), which non-specifically bind to all three TGF β ligands, prevents aortic aneurysm (Habashi et al. 2006), developmental emphysema (Neptune et al. 2003), skeletal myopathy (Cohn et al. 2007), and mitral valve prolapse (Ng et al. 2004) in the Marfan syndrome mouse model. These therapeutic effects were reproduced by losartan, which also non-specifically inhibits TGF β ligand expression, receptors, and activators such as thrombospondin and matrix metalloproteinases (Habashi et al. 2006). Similar studies have treated the hypertrophic cardiomyopathy in a cardiac hypertrophy mouse model by pan-TGF β antibodies and losartan. Both of these treatments were effective in preemptively blocking the cardiomyopathy in this model (Teekakirikul et al. 2010). Increased TGF β signaling is also associated with elastic fiber disruption and hypertension phenotypes in Emillin-1-deficient mice. Heterozygous deletion of *Tgfb1* has effectively rescued both elastic fiber defects and hypertension in these mice (Zacchigna et al. 2006). Since these studies suggest that reducing TGF β signaling is a possible effective therapy for cardiovascular diseases, a multicenter clinical trial is currently underway to treat aneurysmal patients with Marfan syndrome (Lacro et al. 2007; Brooke et al. 2008).

Many studies have raised concerns about therapies that non-specifically target the TGF β signaling as an attempt to treat cardiovascular diseases (Fig. 3). Unexpectedly, a partial genetic reduction of *Smad4* in the Marfan syndrome mouse model causes exacerbation of aortic aneurysm and dissection and premature death of the Marfan mice (Holm et al. 2011). Surprisingly, a dramatic rise in JNK1 levels, which are normal in aortic tissue of the Marfan mice, is associated with this premature death in the combined heterozygous *Smad4* Marfan mice. Interestingly, a JNK1 antagonist blocks aortic aneurysm in the Marfan mice that have reduced or fully retained *Smad4*, suggesting that the JNK1 pathway is a major player in the pathogenesis of aneurysm in Marfan mice. Since SMAD4 is co-SMAD molecule for both TGF β and BMP signaling, the direct role of TGF β signaling in the regulation of JNK1 signaling in the Marfan mouse model remains unclear. Similarly, strategies that would target SMAD4 may also be detrimental for overall health since conditional tissue-specific deletion of *Smad4* has revealed its important role in cardiac development and cardiac hypertrophy, and cancer (Azhar et al. 2010; Nie et al. 2008; Yang and Yang 2010; Wang et al. 2005) (Song et al. 2011; Kim et al. 2006). Collectively, the concerns about the undesirable outcome of non-specific targeting of TGF β pathway molecules may be valid, and a better knowledge of the function of TGF β ligands, receptors and effectors in the adult cardiovascular system is an essential prerequisite before embarking on any TGF β -based therapy to treat cardiovascular diseases.

Remarkably, TGF β NAb antibodies that neutralize TGF β 1/2/3 rescue the mitral valve (MV) prolapse, aortic aneurysm and hypertrophic cardiomyopathy in mouse models (Ng et al. 2004; Habashi et al. 2006; Teekakirikul et al. 2010). However, the same TGF β NAb antibody treatment induced lethal cardiovascular pathologies in mouse models of aneurysm and atherosclerosis (Wang et al. 2010; Mallat et al. 2001). Furthermore, reduced TGF β signaling is found in progressive valve disease in genetic mouse models, which should caution against any uncontrolled reduction in TGF β signaling as a therapeutic measure (Hinton et al. 2010; Snider et al. 2008). Numerous cardiovascular developmental malformations are

reported in mice with disrupted TGF β ligands signaling (Choudhary et al. 2009;Jiao et al. 2006;Wang et al. 2006) (Azhar et al. 2009a;Azhar et al. 2011;Azhar et al. 2010). Briefly, secondary effects on skeleton, bone repair, eyes, hearing and wound healing could occur from reducing TGF β 2 signaling (Sanford et al. 1997;Saika et al. 2001;Paradies et al. 1998;Crowe et al. 2000). TGF β 1 is a potent anti-inflammatory molecule in mice (Shull et al. 1992;Bommireddy and Doetschman 2007). It protects against Ang II-induced cardiac hypertrophy (Schultz et al. 2002); however, preemptive blocking of TGF β ligands in an Ang II-induced mouse model leads to premature death caused by accelerated aortic dissection and rupture (Wang et al. 2010). Gene targeted disruption of latent TGF β -binding proteins, which are critical for TGF β activation, also causes cardiovascular as well as other developmental abnormalities and cancer (Todorovic et al. 2011;Yoshinaga et al. 2008). A recent study has identified genetic mutations in *GATA4* that are associated with the AV septal defects in humans (Moskowitz et al. 2011). It turns out that these mutations in *GATA4* disable *GATA4* interaction with SMAD4. Endocardial *Smad4* conditional knockout embryos develop severe defects in AV cushion formation and septation, and heterozygous *Smad4* deletion causes AV septation defects in *GATA4*-deficient mice (Song et al. 2011;Moskowitz et al. 2011). Together, these studies highlight a beneficial role of TGF β and/or BMP signaling in cardiovascular development. In another study it has been found that transaortic constriction of *Smad3*^{-/-} mice causes cardiac hypertrophy (detrimental effect) and reduced fibrosis (beneficial effect) (Divakaran et al. 2009). Finally, increased TGF β signaling is found to be associated with the cardiovascular complications in the experimental mouse model of Kawasaki disease. Unexpectedly, non-specific blocking of TGF β by administration of TGF β NAb worsens the cardiovascular disease in these mice. This detrimental effect is attributed to a reduction in the proteolytic inhibitor and TGF β target molecule, plasminogen activator inhibitor-1 (PAI1), and an associated increase in matrix metalloproteinase activity (Alvira et al. 2011). It is noteworthy that many detrimental effects of TGF β -based therapy can be attributed, in major part, to the role of TGF β signaling in autoimmunity (Fig. 3) (Yoshimura et al. 2010;Shull et al. 1992;Azhar et al. 2009b;Anuurad et al. 2011;Alvira et al. 2011;King et al. 2009;Tieu et al. 2009;Daugherty et al. 2010;Wang et al. 2010;Mallat et al. 2001). Consequently, although targeting TGF β ligands and their signaling offers hope for treating valve, aortic and myocardial and inflammatory cardiovascular complications in both syndromic and non-syndromic patients, serious concerns remain whether a non-specific preemptive blocking of TGF β ligands aimed to treat a single cardiovascular manifestation could result in undesirable side-effects on other aspects of cardiovascular remodeling or physiology.

Perspectives

Current medical and surgical advances are likely to increase the number of elderly persons with cardiovascular disease in future. Clinical trials enrolling elderly patients are limited. Drug-based and/or less invasive strategies are better options to treat elderly patients. Numerous published studies indicate that TGF β and its associated signaling pathways are highly significant effectors of cardiovascular development, function and pathogenesis of cardiovascular diseases. Consequently, in adult cardiovascular tissues in which first-time repair or revision is required, the response of the tissue to surgical intervention is most likely going to involve TGF β signaling pathways. These pathways must be understood so that the long-term response to surgical repair can be predicted and so that TGF β -based therapies can be developed for more selective targets and with a better understanding of potential complications. Thus, it is important to determine the mechanisms by which TGF β and its effector genes develop functional cardiovascular structures during development, and to maintain those functions during aging, under normal, stress and repair conditions. Genetic manipulation of the TGF β pathway genes discussed above will reveal these mechanisms. In particular, the response of heterozygous genetic mouse models encoding mutated TGF β

pathway genes to the stresses of hypertension, exercise and age will be highly useful. Cardiovascular stress through transaortic constriction, exercise and aging and tissue transplantation through surgical advances such as artery by-pass or grafting in the context of these genetic manipulations will identify the stress and repair response mechanisms affected by these genes.

Serious concerns are emerging with respect to TGF β -based therapies for cardiovascular diseases. It is therefore imperative that we develop a better understanding of the complexities of TGF β signaling in the cardiovascular system during both embryonic development and aging so that highly specific, context-dependent, targeted therapies can provide the desired effects without complication. Finally, there is no solid evidence that establishes a direct cause-and-effect relationship between the TGF β pathway genes and the disease process during aging. Most studies highlighting the involvement of ECM disruption on increased TGF β signaling *in vivo* in disease pathogenesis are correlative. In fact, numerous studies based on direct mutation of TGF β pathway genes in mouse indicate that TGF β signaling is critical for both cardiovascular development and function. Thus, delineating TGF β signaling networks involved in specific aspects of cardiovascular development and cardiovascular structure and function during the aging process should inform novel therapeutic approaches with higher specificity and more limited side effects. For example, instead of targeting TGF β signaling in general for treatment of cardiovascular disease in patients, blocking ERKs and JNK1, which work well for the treatment of aortic aneurysm in Marfan mice, are other possible avenues that should be further explored in mouse models of other cardiovascular diseases. Finally, the projection that TGF β signaling inhibition is the holy grail for cardiovascular diseases seems premature and has been perhaps overstated. Overall, therapeutic approaches to non-specifically reduce TGF β signaling, applied in those with various mouse models, are unlikely to be beneficial and could be harmful in elderly patients. Because of the critical importance of TGF β signaling in cardiovascular development and disease, an improved understanding of TGF β pathway genes under whole animal settings (both conditional overexpression and deletion models) in longitudinal study, and the effects of TGF β -based therapeutics under those settings, will by necessity have a high impact on the positive and negative consequences of TGF β -based therapeutics.

Acknowledgments

This work was supported, in parts, by funds from the National Institutes of Health Grants - HL070174, HL92508, HL085708, HL077493, HL82851-03, and HL105280. Additional funding was provided by Arizona Biomedical Research Commission (ABRC #0901) and The Stephen Michael Schneider/The William J. "Billy" Gieszl Award.

List of abbreviations

MFS	Marfan syndrome
EDS	Ehlers-Danlos syndrome
ATS	Arterial Tortuosity syndrome
LDS	Loeys-Dietz syndrome
AOS	Aortic aneurysms and dissections with early-onset osteoarthritis
ARVC/D or ARVD1	Arrhythmogenic right ventricular cardiomyopathy/dysplasia
XMVD	X-linked myxomatous valvular dystrophy
JPS	Juvenile polyposis syndrome

HHT	Hereditary hemorrhagic telangiectasia
CCD	Cleidocranial dysplasia
TGFβNAb	Pan-TGF β neutralizing antibodies
EMT	Epithelial mesenchymal transition

References

- Aikawa E, Nahrendorf M, Sosnovik D, Lok VM, Jaffer FA, Aikawa M, Weissleder R. Multimodality molecular imaging identifies proteolytic and osteogenic activities in early aortic valve disease. *Circulation*. 2007; 115:377–386. [PubMed: 17224478]
- Akat K, Borggrete M, Kaden JJ. Aortic valve calcification: basic science to clinical practice. *Heart*. 2009; 95:616–623. [PubMed: 18632833]
- Akhurst RJ, Lehnert SA, Faissner A, Duffie E. TGF beta in murine morphogenetic processes: the early embryo and cardiogenesis. *Development*. 1990; 108:645–656. [PubMed: 1696875]
- Akishita M, Ito M, Lehtonen JY, Daviet L, Dzau VJ, Horiuchi M. Expression of the AT2 receptor developmentally programs extracellular signal-regulated kinase activity and influences fetal vascular growth. *J Clin Invest*. 1999; 103:63–71. [PubMed: 9884335]
- Alexander SM, Jackson KJ, Bushnell KM, McGuire PG. Spatial and temporal expression of the 72-kDa type IV collagenase (MMP-2) correlates with development and differentiation of valves in the embryonic avian heart. *Dev Dyn*. 1997; 209:261–268. [PubMed: 9215641]
- Alvira CM, Guignabert C, Kim YM, Chen C, Wang L, Duong TT, Yeung RS, Li DY, Rabinovitch M. Inhibition of transforming growth factor beta worsens elastin degradation in a murine model of Kawasaki disease. *Am J Pathol*. 2011; 178:1210–1220. [PubMed: 21356372]
- Andrabi S, Bekheirnia MR, Robbins-Furman P, Lewis RA, Prior TW, Potocki L. SMAD4 mutation segregating in a family with juvenile polyposis, aortopathy, and mitral valve dysfunction. *Am J Med Genet A*. 2011; 155A:1165–1169. [PubMed: 21465659]
- Anuurad E, Enkhmaa B, Gungor Z, Zhang W, Tracy RP, Pearson TA, Kim K, Berglund L. Age as a Modulator of Inflammatory Cardiovascular Risk Factors. *Arterioscler Thromb Vasc Biol*. 2011
- Arthur HM, Bamforth SD. TGFbeta signaling and congenital heart disease: Insights from mouse studies. *Birth Defects Res A Clin Mol Teratol*. 2011; 10
- Arthur HM, Ure J, Smith AJ, Renforth G, Wilson DI, Torsney E, Charlton R, Parums DV, Jowett T, Marchuk DA, Burn J, Diamond AG. Endoglin, an ancillary TGFbeta receptor, is required for extraembryonic angiogenesis and plays a key role in heart development. *Dev Biol*. 2000; 217:42–53. [PubMed: 10625534]
- Azhar M, Brown K, Gard C, Chen H, Rajan S, Elliott DA, Stevens MV, Camenisch TD, Conway SJ, Doetschman T. Transforming growth factor Beta2 is required for valve remodeling during heart development. *Dev Dyn*. 2011; 10
- Azhar M, Runyan RB, Gard C, Sanford LP, Miller ML, Andringa A, Pawlowski S, Rajan S, Doetschman T. Ligand-specific function of transforming growth factor beta in epithelial-mesenchymal transition in heart development. *Dev Dyn*. 2009a; 238:431–442. PMC2805850. [PubMed: 19161227]
- Azhar M, Schultz JE, Grupp I, Dorn GW, Meneton P, Molin DG, Gittenberger-de Groot AC, Doetschman T. Transforming growth factor beta in cardiovascular development and function. *Cytokine Growth Factor Rev*. 2003; 14:391–407. [PubMed: 12948523]
- Azhar M, Wang PY, Frugier T, Koishi K, Deng C, Noakes PG, McLennan IS. Myocardial deletion of Smad4 using a novel alpha skeletal muscle actin Cre recombinase transgenic mouse causes misalignment of the cardiac outflow tract. *Int J Biol Sci*. 2010; 6:546–555. [PubMed: 20877696]
- Azhar M, Yin M, Bommireddy R, Duffy JJ, Yang J, Pawlowski SA, Boivin GP, Engle SJ, Sanford LP, Grisham C, Singh RR, Babcock GF, Doetschman T. Generation of mice with a conditional allele for transforming growth factor beta 1 gene. *Genesis*. 2009b; 47:423–431. [PubMed: 19415629]

- Barbara NP, Wrana JL, Letarte M. Endoglin is an accessory protein that interacts with the signaling receptor complex of multiple members of the transforming growth factor-beta superfamily. *J Biol Chem*. 1999; 274:584–594. [PubMed: 9872992]
- Barnard JA, Lyons RM, Moses HL. The cell biology of transforming growth factor beta. *Biochim Biophys Acta*. 1990; 1032:79–87. [PubMed: 2194569]
- Barnett JV, Desgrosellier JS. Early events in valvulogenesis: a signaling perspective. *Birth Defects Res C Embryo Today*. 2003; 69:58–72. [PubMed: 12768658]
- Bartram U, Molin DG, Wisse LJ, Mohamad A, Sanford LP, Doetschman T, Speer CP, Poelmann RE, Gittenberger-de GA. Double-outlet right ventricle and overriding tricuspid valve reflect disturbances of looping, myocardialization, endocardial cushion differentiation, and apoptosis in *Tgfb2* knockout mice. *Circulation*. 2001; 103:2745–2752. [PubMed: 11390347]
- Beffagna G, Occhi G, Nava A, Vitiello L, Ditadi A, Basso C, Bauce B, Carraro G, Thiene G, Towbin JA, Danieli GA, Rampazzo A. Regulatory mutations in transforming growth factor-beta3 gene cause arrhythmogenic right ventricular cardiomyopathy type 1. *Cardiovasc Res*. 2005; 65:366–373. [PubMed: 15639475]
- Bharathy S, Xie W, Yingling JM, Reiss M. Cancer-associated transforming growth factor beta type II receptor gene mutant causes activation of bone morphogenic protein-Smads and invasive phenotype. *Cancer Res*. 2008; 68:1656–1666. [PubMed: 18339844]
- Bommireddy, R.; Doetschman, T. Transforming growth factor-beta: From its effect in T cell activation to a role in dominant tolerance. In: Graca, Luis, editor. *The Immune Synapse as a Novel Target for Therapy*. Basel: Birkhauser Verlag; 2007. p. 155-168.
- Bonyadi M, Rusholme SA, Cousins FM, Su HC, Biron CA, Farrall M, Akhurst RJ. Mapping of a major genetic modifier of embryonic lethality in TGF beta 1 knockout mice. *Nat Genet*. 1997; 15:207–211. [PubMed: 9020852]
- Brooke BS, Habashi JP, Judge DP, Patel N, Loeys B, Dietz HC III. Angiotensin II blockade and aortic-root dilation in Marfan's syndrome. *N Engl J Med*. 2008; 358:2787–2795. [PubMed: 18579813]
- Brown CB, Boyer AS, Runyan RB, Barnett JV. Requirement of type III TGF-beta receptor for endocardial cell transformation in the heart. *Science*. 1999; 283:2080–2082. [PubMed: 10092230]
- Burrell JH, Hegarty BD, McMullen JR, Lumbers ER. Effects of gestation on ovine fetal and maternal angiotensin receptor subtypes in the heart and major blood vessels. *Exp Physiol*. 2001; 86:71–82. [PubMed: 11429622]
- Caira FC, Stock SR, Gleason TG, McGee EC, Huang J, Bonow RO, Spelsberg TC, McCarthy PM, Rahimtoola SH, Rajamannan NM. Human degenerative valve disease is associated with up-regulation of low-density lipoprotein receptor-related protein 5 receptor-mediated bone formation. *J Am Coll Cardiol*. 2006; 47:1707–1712. [PubMed: 16631011]
- Camenisch TD, Molin DG, Person A, Runyan RB, Gittenberger-de Groot AC, McDonald JA, Klewer SE. Temporal and Distinct TGFbeta Ligand Requirements during Mouse and Avian Endocardial Cushion Morphogenesis. *Dev Biol*. 2002b; 248:170–181. [PubMed: 12142029]
- Camenisch, TD.; Runyan, RB.; Markwald, RR. Molecular regulation of cushion morphogenesis. In: Harvey, R.; Rosenthal, N., editors. *Heart Development and Regeneration*. Academic Press; 2010. p. 363-388.
- Camenisch TD, Schroeder JA, Bradley J, Klewer SE, McDonald JA. Heart-valve mesenchyme formation is dependent on hyaluronan-augmented activation of ErbB2–ErbB3 receptors. *Nat Med*. 2002a; 8:850–855. [PubMed: 12134143]
- Camenisch TD, Spicer AP, Brehm-Gibson T, Biesterfeldt J, Augustine ML, Calabro AJ, Kubalak S, Klewer SE, McDonald JA. Disruption of hyaluronan synthase-2 abrogates normal cardiac morphogenesis and hyaluronan-mediated transformation of epithelium to mesenchyme. *J Clin Invest*. 2000; 106:349–360. [PubMed: 10930438]
- Chakraborty S, Combs MD, Yutzey KE. Transcriptional regulation of heart valve progenitor cells. *Pediatr Cardiol*. 2010a; 31:414–421. [PubMed: 20039031]
- Chakraborty S, Wirrig EE, Hinton RB, Merrill WH, Spicer DB, Yutzey KE. Twist1 promotes heart valve cell proliferation and extracellular matrix gene expression during development in vivo and is expressed in human diseased aortic valves. *Dev Biol*. 2010b

- Choudhary B, Zhou J, Li P, Thomas S, Kaartinen V, Sucov HM. Absence of TGFbeta signaling in embryonic vascular smooth muscle leads to reduced lysyl oxidase expression, impaired elastogenesis, and aneurysm. *Genesis*. 2009; 47:115–121. [PubMed: 19165826]
- Clark-Greuel JN, Connolly JM, Sorichillo E, Narula NR, Rapoport HS, Mohler ER III, Gorman JH III, Gorman RC, Levy RJ. Transforming growth factor-beta1 mechanisms in aortic valve calcification: increased alkaline phosphatase and related events. *Ann Thorac Surg*. 2007; 83:946–953. [PubMed: 17307438]
- Cohn RD, van Erp C, Habashi JP, Soleimani AA, Klein EC, Lisi MT, Gamradt M, ap Rhys CM, Holm TM, Loeys BL, Ramirez F, Judge DP, Ward CW, Dietz HC. Angiotensin II type 1 receptor blockade attenuates TGF-beta-induced failure of muscle regeneration in multiple myopathic states. *Nat Med*. 2007; 13:204–210. [PubMed: 17237794]
- Compton LA, Potash DA, Brown CB, Barnett JV. Coronary vessel development is dependent on the type III transforming growth factor beta receptor. *Circ Res*. 2007; 101:784–791. [PubMed: 17704211]
- Conway SJ, Molkentin JD. Periostin as a heterofunctional regulator of cardiac development and disease. *Curr Genomics*. 2008; 9:548–555. [PubMed: 19516962]
- Cooper WO, Hernandez-Diaz S, Arbogast PG, Dudley JA, Dyer S, Gideon PS, Hall K, Ray WA. Major congenital malformations after first-trimester exposure to ACE inhibitors. *N Engl J Med*. 2006; 354:2443–2451. [PubMed: 16760444]
- Coucke PJ, Willaert A, Wessels MW, Callewaert B, Zoppi N, De Backer J, Fox JE, Mancini GM, Kambouris M, Gardella R, Facchetti F, Willems PJ, Forsyth R, Dietz HC, Barlati S, Colombi M, Loeys B, De Paepe A. Mutations in the facilitative glucose transporter GLUT10 alter angiogenesis and cause arterial tortuosity syndrome. *Nat Genet*. 2006; 38:452–457. [PubMed: 16550171]
- Craig EA, Stevens MV, Vaillancourt RR, Camenisch TD. MAP3Ks as central regulators of cell fate during development. *Dev Dyn*. 2008; 237:3102–3114. [PubMed: 18855897]
- Crowe MJ, Doetschman T, Greenhalgh DG. Delayed wound healing in immunodeficient TGF-beta1 knockout mice. *J Invest Dermatol*. 2000; 115:3–11. [PubMed: 10886500]
- Daly AC, Randall RA, Hill CS. Transforming growth factor beta-induced Smad1/5 phosphorylation in epithelial cells is mediated by novel receptor complexes and is essential for anchorage-independent growth. *Mol Cell Biol*. 2008; 28:6889–6902. [PubMed: 18794361]
- Das BB, Taylor AL, Yetman AT. Left ventricular diastolic dysfunction in children and young adults with Marfan syndrome. *Pediatr Cardiol*. 2006; 27:256–258. [PubMed: 16463128]
- Daugherty A, Rateri DL, Charo IF, Owens Iii AP, Howatt DA, Cassis LA. Angiotensin II infusion promotes ascending aortic aneurysms: attenuation by CCR2 deficiency in apoE $-/-$ mice. *Clin Sci (Lond)*. 2010
- De Backer J. Cardiovascular characteristics in Marfan syndrome and their relation to the genotype. *Verh K Acad Geneeskd Belg*. 2009b; 71:335–371. [PubMed: 20232788]
- De Backer J. The expanding cardiovascular phenotype of Marfan syndrome. *Eur J Echocardiogr*. 2009a; 10:213–215. [PubMed: 19106173]
- de Wit MC, de Coe IF, Lequin MH, Halley DJ, Roos-Hesselink JW, Mancini GM. Combined cardiological and neurological abnormalities due to filamin A gene mutation. *Clin Res Cardiol*. 2011; 100:45–50. [PubMed: 20730588]
- Derynck R, Akhurst RJ. Differentiation plasticity regulated by TGF-beta family proteins in development and disease. *Nat Cell Biol*. 2007; 9:1000–1004. [PubMed: 17762890]
- Dickson MC, Martin JS, Cousins FM, Kulkarni AB, Karlsson S, Akhurst RJ. Defective haematopoiesis and vasculogenesis in transforming growth factor-beta 1 knock out mice. *Development*. 1995; 121:1845–1854. [PubMed: 7600998]
- Dickson MC, Slager HG, Duffie E, Mummery CL, Akhurst RJ. RNA and protein localisations of TGF beta 2 in the early mouse embryo suggest an involvement in cardiac development. *Development*. 1993; 117:625–639. [PubMed: 7687212]
- Dietz HC. TGF-beta in the pathogenesis and prevention of disease: a matter of aneurysmic proportions. *J Clin Invest*. 2010; 120:403–407. [PubMed: 20101091]

- Divakaran V, Adroge J, Ishiyama M, Entman ML, Haudek S, Sivasubramanian N, Mann DL. Adaptive and maladaptive effects of SMAD3 signaling in the adult heart after hemodynamic pressure overloading. *Circ Heart Fail.* 2009; 2:633–642. [PubMed: 19919989]
- Doetschman T. Interpretation of phenotype in genetically engineered mice. *Lab Anim Sci.* 1999; 49:137–143. [PubMed: 10331542]
- Dunker N, Krieglstein K. Targeted mutations of transforming growth factor-beta genes reveal important roles in mouse development and adult homeostasis. *Eur J Biochem.* 2000; 267:6982–6988. [PubMed: 11106407]
- Dunker N, Krieglstein K. Tgfbeta2 $-/-$ Tgfbeta3 $-/-$ double knockout mice display severe midline fusion defects and early embryonic lethality. *Anat Embryol (Berl).* 2002; 206:73–83. [PubMed: 12478370]
- Eckman PM, Hsich E, Rodriguez ER, Gonzalez-Stawinski GV, Moran R, Taylor DO. Impaired systolic function in Loeys-Dietz syndrome: a novel cardiomyopathy? *Circ Heart Fail.* 2009; 2:707–708. [PubMed: 19919997]
- Force T, Bonow RO, Houser SR, Solaro RJ, Hershberger RE, Adhikari B, Anderson ME, Boineau R, Byrne BJ, Cappola TP, Kalluri R, LeWinter MM, Maron MS, Molkentin JD, Ommen SR, Regnier M, Tang WH, Tian R, Konstam MA, Maron BJ, Seidman CE. Research priorities in hypertrophic cardiomyopathy: report of a working group of the national heart, lung, and blood institute. *Circulation.* 2010; 122:1130–1133. [PubMed: 20837938]
- Franceschi RT, Xiao G, Jiang D, Gopalakrishnan R, Yang S, Reith E. Multiple signaling pathways converge on the Cbfa1/Runx2 transcription factor to regulate osteoblast differentiation. *Connect Tissue Res.* 2003; 44(Suppl 1):109–116. 109–116. [PubMed: 12952183]
- Garrington TP, Johnson GL. Organization and regulation of mitogen-activated protein kinase signaling pathways. *Curr Opin Cell Biol.* 1999; 11:211–218. [PubMed: 10209154]
- Gitler AD, Lu MM, Jiang YQ, Epstein JA, Gruber PJ. Molecular markers of cardiac endocardial cushion development. *Dev Dyn.* 2003; 228:643–650. [PubMed: 14648841]
- Gomez D, Al Haj ZA, Borges LF, Philippe M, Gutierrez PS, Jondeau G, Michel JB, Vranckx R. Syndromic and non-syndromic aneurysms of the human ascending aorta share activation of the Smad2 pathway. *J Pathol.* 2009; 218:131–142. [PubMed: 19224541]
- Gordon KJ, Blobel GC. Role of transforming growth factor-beta superfamily signaling pathways in human disease. *Biochim Biophys Acta.* 2008; 1782:197–228. [PubMed: 18313409]
- Goto K, Kamiya Y, Imamura T, Miyazono K, Miyazawa K. Selective inhibitory effects of Smad6 on bone morphogenetic protein type I receptors. *J Biol Chem.* 2007; 282:20603–20611. [PubMed: 17493940]
- Goumans MJ, Liu Z, ten Dijke P. TGF-beta signaling in vascular biology and dysfunction. *Cell Res.* 2008
- Grande-Allen KJ, Griffin BP, Ratliff NB, Cosgrove DM, Vesely I. Glycosaminoglycan profiles of myxomatous mitral leaflets and chordae parallel the severity of mechanical alterations. *J Am Coll Cardiol.* 2003; 42:271–277. [PubMed: 12875763]
- Habashi JP, Doyle JJ, Holm TM, Aziz H, Schoenhoff F, Bedja D, Chen Y, Modiri AN, Judge DP, Dietz HC. Angiotensin II type 2 receptor signaling attenuates aortic aneurysm in mice through ERK antagonism. *Science.* 2011; 332:361–365. [PubMed: 21493863]
- Habashi JP, Judge DP, Holm TM, Cohn RD, Loeys BL, Cooper TK, Myers L, Klein EC, Liu G, Calvi C, Podowski M, Neptune ER, Halushka MK, Bedja D, Gabrielson K, Rifkin DB, Carta L, Ramirez F, Huso DL, Dietz HC. Losartan, an AT1 antagonist, prevents aortic aneurysm in a mouse model of Marfan syndrome. *Science.* 2006; 312:117–121. [PubMed: 16601194]
- Hakuno D, Kimura N, Yoshioka M, Fukuda K. Molecular mechanisms underlying the onset of degenerative aortic valve disease. *J Mol Med.* 2009; 87:17–24. [PubMed: 18766323]
- Hamilton DW. Functional role of periostin in development and wound repair: implications for connective tissue disease. *J Cell Commun Signal.* 2008; 2:9–17. [PubMed: 18642132]
- Hinton RB, Adelman-Brown J, Witt S, Krishnamurthy VK, Osinska H, Sakthivel B, James JF, Li DY, Narmoneva DA, Mecham RP, Benson DW. Elastin haploinsufficiency results in progressive aortic valve malformation and latent valve disease in a mouse model. *Circ Res.* 2010; 107:549–557. [PubMed: 20576933]

- Hinton RB, Yutzey KE. Heart valve structure and function in development and disease. *Annu Rev Physiol.* 2011; 73:29–46. 29–46. [PubMed: 20809794]
- Holm TM, Habashi JP, Doyle JJ, Bedja D, Chen Y, van Erp C, Lindsay ME, Kim D, Schoenhoff F, Cohn RD, Loeys BL, Thomas CJ, Patnaik S, Marugan JJ, Judge DP, Dietz HC. Noncanonical TGFbeta signaling contributes to aortic aneurysm progression in Marfan syndrome mice. *Science.* 2011; 332:358–361. [PubMed: 21493862]
- Holweg CT, Baan CC, Niesters HG, Vantrimpont PJ, Mulder PG, Maat AP, Weimar W, Balk AH. TGF-beta1 gene polymorphisms in patients with end-stage heart failure. *J Heart Lung Transplant.* 2001; 20:979–984. [PubMed: 11557193]
- Horbelt D, Guo G, Robinson PN, Knaus P. Quantitative analysis of TGFBR2 mutations in Marfan-syndrome-related disorders suggests a correlation between phenotypic severity and Smad signaling activity. *J Cell Sci.* 2010; 123:4340–4350. [PubMed: 21098638]
- Horiuchi K, Amizuka N, Takeshita S, Takamatsu H, Katsuura M, Ozawa H, Toyama Y, Bonewald LF, Kudo A. Identification and characterization of a novel protein, periostin, with restricted expression to periosteum and periodontal ligament and increased expression by transforming growth factor beta. *J Bone Miner Res.* 1999; 14:1239–1249. [PubMed: 10404027]
- Hu BC, Li L, Sun RH, Gao PJ, Zhu DL, Wang JG, Chu SL. The association between transforming growth factor beta3 polymorphisms and left ventricular structure in hypertensive subjects. *Clin Chim Acta.* 2010; 411:558–562. [PubMed: 20083094]
- Hu F, Morrissey P, Yao J, Xu Z. Development of AT(1) and AT(2) receptors in the ovine fetal brain. *Brain Res Dev Brain Res %19.* 2004; 150:51–61.
- Jackson CF, Wenger NK. Cardiovascular Disease in the Elderly. *Rev Esp Cardiol.* 2011; 64:697–712. [PubMed: 21723657]
- Jain D, Dietz HC, Oswald GL, Maleszewski JJ, Halushka MK. Causes and histopathology of ascending aortic disease in children and young adults. *Cardiovasc Pathol.* 2009
- Jian B, Narula N, Li QY, Mohler ER III, Levy RJ. Progression of aortic valve stenosis: TGF-beta1 is present in calcified aortic valve cusps and promotes aortic valve interstitial cell calcification via apoptosis. *Ann Thorac Surg.* 2003; 75:457–465. [PubMed: 12607654]
- Jian B, Xu J, Connolly J, Savani RC, Narula N, Liang B, Levy RJ. Serotonin mechanisms in heart valve disease I: serotonin-induced up-regulation of transforming growth factor-beta1 via G-protein signal transduction in aortic valve interstitial cells. *Am J Pathol.* 2002; 161:2111–2121. [PubMed: 12466127]
- Jiao K, Langworthy M, Batts L, Brown CB, Moses HL, Baldwin HS. Tgfbeta signaling is required for atrioventricular cushion mesenchyme remodeling during in vivo cardiac development. *Development.* 2006; 133:4585–4593. [PubMed: 17050629]
- Jinnin M, Ihn H, Tamaki K. Characterization of SIS3, a novel specific inhibitor of Smad3, and its effect on transforming growth factor-beta1-induced extracellular matrix expression. *Mol Pharmacol.* 2006; 69:597–607. [PubMed: 16288083]
- Johren O, Dendorfer A, Dominiak P. Cardiovascular and renal function of angiotensin II type-2 receptors. *Cardiovasc Res.* 2004; 62:460–467. [PubMed: 15158138]
- Jones ES, Black MJ, Widdop RE. Angiotensin AT2 receptor contributes to cardiovascular remodelling of aged rats during chronic AT1 receptor blockade. *J Mol Cell Cardiol.* 2004; 37:1023–1030. [PubMed: 15522279]
- Kaartinen V, Voncken JW, Shuler C, Warburton D, Bu D, Heisterkamp N, Groffen J. Abnormal lung development and cleft palate in mice lacking TGF-beta 3 indicates defects of epithelial-mesenchymal interaction. *Nat Genet.* 1995; 11:415–421. [PubMed: 7493022]
- Kallapur S, Ormsby I, Doetschman T. Strain dependency of TGFbeta1 function during embryogenesis. *Mol Reprod Dev.* 1999; 52:341–349. [PubMed: 10092113]
- Kang JS, Liu C, Derynck R. New regulatory mechanisms of TGF-beta receptor function. *Trends Cell Biol.* 2009
- Kehat I, Molkenin JD. Extracellular signal-regulated kinase 1/2 (ERK1/2) signaling in cardiac hypertrophy. *Ann N Y Acad Sci.* 2010; 1188:96–102. 96–102. [PubMed: 20201891]

- Khairy P, Hosn JA, Broberg C, Cook S, Earing M, Gersony D, Kay J, Landzberg MJ, Nickolaus MJ, Opatowsky S, Valente AM, Warnes C, Webb G, Gurvitz MZ. Multicenter research in adult congenital heart disease. *Int J Cardiol.* 2008; 129:155–159. [PubMed: 18573551]
- Kim BG, Li C, Qiao W, Mamura M, Kaspercak B, Anver M, Wolfrum L, Hong S, Mushinski E, Potter M, Kim SJ, Fu XY, Deng C, Letterio JJ. Smad4 signalling in T cells is required for suppression of gastrointestinal cancer. *Nature.* 2006; 441:1015–1019. [PubMed: 16791201]
- Kim L, Kim dK, Yang WI, Shin DH, Jung IM, Park HK, Chang BC. Overexpression of transforming growth factor-beta 1 in the valvular fibrosis of chronic rheumatic heart disease. *J Korean Med Sci.* 2008; 23:41–48. [PubMed: 18303197]
- Kim S, Koga T, Isobe M, Kern BE, Yokochi T, Chin YE, Karsenty G, Taniguchi T, Takayanagi H. Stat1 functions as a cytoplasmic attenuator of Runx2 in the transcriptional program of osteoblast differentiation. *Genes Dev.* 2003; 17:1979–1991. [PubMed: 12923053]
- King VL, Lin AY, Kristo F, Anderson TJ, Ahluwalia N, Hardy GJ, Owens AP III, Howatt DA, Shen D, Tager AM, Luster AD, Daugherty A, Gerszten RE. Interferon-gamma and the interferon-inducible chemokine CXCL10 protect against aneurysm formation and rupture. *Circulation.* 2009; 119:426–435. [PubMed: 19139386]
- Kirkbride KC, Townsend TA, Bruinsma MW, Barnett JV, Blobe GC. Bone morphogenetic proteins signal through the transforming growth factor-beta type III receptor. *J Biol Chem.* 2008; 283:7628–7637. [PubMed: 18184661]
- Kronenberg HM. Twist genes regulate Runx2 and bone formation. *Dev Cell.* 2004; 6:317–318. [PubMed: 15030754]
- Kruzynska-Frejtak A, Machnicki M, Rogers R, Markwald RR, Conway SJ. Periostin (an osteoblast-specific factor) is expressed within the embryonic mouse heart during valve formation. *Mech Dev.* 2001; 103:183–188. [PubMed: 11335131]
- Kulkarni AB, Ward JM, Yaswen L, Mackall CL, Bauer SR, Huh CG, Gress RE, Karlsson S. Transforming growth factor-beta 1 null mice. An animal model for inflammatory disorders. *Am J Pathol.* 1995; 146:264–275. [PubMed: 7856732]
- Kumar R, Singh VP, Baker KM. The intracellular renin-angiotensin system: implications in cardiovascular remodeling. *Curr Opin Nephrol Hypertens.* 2008; 17:168–173. [PubMed: 18277150]
- Kundu M, Javed A, Jeon JP, Horner A, Shum L, Eckhaus M, Muenke M, Lian JB, Yang Y, Nuckolls GH, Stein GS, Liu PP. Cbfbeta interacts with Runx2 and has a critical role in bone development. *Nat Genet.* 2002; 32:639–644. [PubMed: 12434156]
- Kyndt F, Gueffet JP, Probst V, Jaafar P, Legendre A, Le Bouffant F, Toquet C, Roy E, McGregor L, Lynch SA, Newbury-Ecob R, Tran V, Young I, Trochu JN, Le Marec H, Schott JJ. Mutations in the gene encoding filamin A as a cause for familial cardiac valvular dystrophy. *Circulation.* 2007; 115:40–49. [PubMed: 17190868]
- Lacha J, Hubacek JA, Potmesil P, Viklicky O, Malek I, Vitko S. TGF-beta I gene polymorphism in heart transplant recipients--effect on renal function. *Ann Transplant.* 2001; 6:39–43. [PubMed: 11803605]
- Lacro RV, Dietz HC, Wruck LM, Bradley TJ, Colan SD, Devereux RB, Klein GL, Li JS, Minich LL, Paridon SM, Pearson GD, Printz BF, Pyeritz RE, Radojewski E, Roman MJ, Saul JP, Stylianou MP, Mahony L. Rationale and design of a randomized clinical trial of beta-blocker therapy (atenolol) versus angiotensin II receptor blocker therapy (losartan) in individuals with Marfan syndrome. *Am Heart J.* 2007; 154:624–631. [PubMed: 17892982]
- Langlois D, Hneino M, Bouazza L, Parlakian A, Sasaki T, Bricca G, Li JY. Conditional inactivation of TGF-beta type II receptor in smooth muscle cells and epicardium causes lethal aortic and cardiac defects. *Transgenic Res.* 2010; 19:1069–1082. [PubMed: 20213136]
- Lebrin F, Deckers M, Bertolino P, ten Dijke P. TGF-beta receptor function in the endothelium. *Cardiovasc Res.* 2005; 65:599–608. [PubMed: 15664386]
- Lee MK, Pardoux C, Hall MC, Lee PS, Warburton D, Qing J, Smith SM, Derynck R. TGF-beta activates Erk MAP kinase signalling through direct phosphorylation of ShcA. *EMBO J.* 2007; 26:3957–3967. [PubMed: 17673906]

- Lewis KA, Gray PC, Blount AL, MacConell LA, Wiater E, Bilezikjian LM, Vale W. Betaglycan binds inhibin and can mediate functional antagonism of activin signalling. *Nature*. 2000; 404:411–414. [PubMed: 10746731]
- Li J, Qu X, Yao J, Caruana G, Ricardo SD, Yamamoto Y, Yamamoto H, Bertram JF. Blockade of endothelial-mesenchymal transition by a Smad3 inhibitor delays the early development of streptozotocin-induced diabetic nephropathy. *Diabetes*. 2010; 59:2612–2624. [PubMed: 20682692]
- Li L, Fan D, Wang C, Wang JY, Cui XB, Wu D, Zhou Y, Wu LL. Angiotensin II increases periostin expression via Ras/p38 MAPK/CREB and ERK1/2/TGF- β 1 pathways in cardiac fibroblasts. *Cardiovasc Res*. 2011; 91:80–89. [PubMed: 21367774]
- Li RK, Li G, Mickle DA, Weisel RD, Merante F, Luss H, Rao V, Christakis GT, Williams WG. Overexpression of transforming growth factor- β 1 and insulin-like growth factor-I in patients with idiopathic hypertrophic cardiomyopathy. *Circulation*. 1997; 96:874–881. [PubMed: 9264495]
- Li XC, Shao Y, Zhuo JL. AT1a receptor knockout in mice impairs urine concentration by reducing basal vasopressin levels and its receptor signaling proteins in the inner medulla. *Kidney Int*. 2009; 76:169–177. [PubMed: 19387470]
- Li YL, Xiao ZS. Advances in Runx2 regulation and its isoforms. *Med Hypotheses*. 2007; 68:169–175. [PubMed: 16901655]
- Lim H, Zhu YZ. Role of transforming growth factor- β in the progression of heart failure. *Cell Mol Life Sci*. 2006; 63:2584–2596. [PubMed: 17013566]
- Lincoln J, Lange AW, Yutzey KE. Hearts and bones: shared regulatory mechanisms in heart valve, cartilage, tendon, and bone development. *Dev Biol*. 2006; 294:292–302. [PubMed: 16643886]
- Lindsay ME, Dietz HC. Lessons on the pathogenesis of aneurysm from heritable conditions. *Nature* %19. 2011; 473:308–316.
- Lipshultz SE, Sleeper LA, Towbin JA, Lowe AM, Orav EJ, Cox GF, Lurie PR, McCoy KL, McDonald MA, Messere JE, Colan SD. The incidence of pediatric cardiomyopathy in two regions of the United States. *N Engl J Med*. 2003; 348:1647–1655. [PubMed: 12711739]
- Litvin J, Blagg A, Mu A, Matiwala S, Montgomery M, Berretta R, Houser S, Margulies K. Periostin and periostin-like factor in the human heart: possible therapeutic targets. *Cardiovasc Pathol*. 2006; 15:24–32. [PubMed: 16414453]
- Loeys BL, Chen J, Neptune ER, Judge DP, Podowski M, Holm T, Meyers J, Leitch CC, Katsanis N, Sharifi N, Xu FL, Myers LA, Spevak PJ, Cameron DE, De Backer J, Hellemans J, Chen Y, Davis EC, Webb CL, Kress W, Coucke P, Rifkin DB, De Paepe AM, Dietz HC. A syndrome of altered cardiovascular, craniofacial, neurocognitive and skeletal development caused by mutations in TGFBR1 or TGFBR2. *Nat Genet*. 2005; 37:275–281. [PubMed: 15731757]
- Loeys BL, Schwarze U, Holm T, Callewaert BL, Thomas GH, Pannu H, De Backer JF, Oswald GL, Symoens S, Manouvrier S, Roberts AE, Faravelli F, Greco MA, Pyeritz RE, Milewicz DM, Coucke PJ, Cameron DE, Braverman AC, Byers PH, De Paepe AM, Dietz HC. Aneurysm syndromes caused by mutations in the TGF- β receptor. *N Engl J Med*. 2006; 355:788–798. [PubMed: 16928994]
- Lopez-Casillas F, Wrana JL, Massague J. Betaglycan presents ligand to the TGF β signaling receptor. *Cell*. 1993; 73:1435–1444. [PubMed: 8391934]
- Lucas RV Jr, Edwards JE. The floppy mitral valve. *Curr Probl Cardiol*. 1982; 7:1–48. [PubMed: 7116912]
- Luukko K, Ylikorkala A, Makela TP. Developmentally regulated expression of Smad3, Smad4, Smad6, and Smad7 involved in TGF- β signaling. *Mech Dev*. 2001; 101:209–212. [PubMed: 11231077]
- Makita N, Suzuki M, Asami S, Takahata R, Kohzaki D, Kobayashi S, Hakamazuka T, Hozumi N. Two of four alternatively spliced isoforms of RUNX2 control osteocalcin gene expression in human osteoblast cells. *Gene*. 2008; 413:8–17. [PubMed: 18321663]
- Mallat Z, Gojova A, Marchiol-Fournigault C, Esposito B, Kamate C, Merval R, Fradelizi D, Tedgui A. Inhibition of transforming growth factor- β signaling accelerates atherosclerosis and induces an unstable plaque phenotype in mice. *Circ Res*. 2001; 89:930–934. [PubMed: 11701621]

- Markwald RR, Norris RA, Moreno-Rodriguez R, Levine RA. Developmental basis of adult cardiovascular diseases: valvular heart diseases. *Ann N Y Acad Sci.* 2010; 1188:177–83. 177–183. [PubMed: 20201901]
- Massague J, Attisano L, Wrana JL. The TGF- β family and its composite receptors. *Trends in Cell Biology.* 1994; 4:172–178. [PubMed: 14731645]
- Matt P, Schoenhoff F, Habashi J, Holm T, van Erp C, Loch D, Carlson OD, Griswold BF, Fu Q, De Backer J, Loeys B, Huso DL, McDonnell NB, Van Eyk JE, Dietz HC. Circulating Transforming Growth Factor- β in Marfan Syndrome. *Circulation.* 2009
- McNally E, MacLeod H, Dellefave L. Arrhythmogenic Right Ventricular Dysplasia/Cardiomyopathy, Autosomal Dominant.
- Mendoza-Villanueva D, Deng W, Lopez-Camacho C, Shore P. The Runx transcriptional co-activator, CBF β , is essential for invasion of breast cancer cells. *Mol Cancer.* 2010; 9:171–171. [PubMed: 20591170]
- Mercado-Pimentel ME, Hubbard AD, Runyan RB. Endoglin and Alk5 regulate epithelial-mesenchymal transformation during cardiac valve formation. *Dev Biol.* 2007; 304:420–432. [PubMed: 17250821]
- Mercado-Pimentel ME, Runyan RB. Multiple transforming growth factor- β isoforms and receptors function during epithelial-mesenchymal cell transformation in the embryonic heart. *Cells Tissues Organs.* 2007; 185:146–156. [PubMed: 17587820]
- Miyazono K, Kamiya Y, Morikawa M. Bone morphogenetic protein receptors and signal transduction. *J Biochem.* 2010; 147:35–51. [PubMed: 19762341]
- Mizuguchi T, Collod-Beroud G, Akiyama T, Abifadel M, Harada N, Morisaki T, Allard D, Varret M, Claustres M, Morisaki H, Ihara M, Kinoshita A, Yoshiura K, Junien C, Kajii T, Jondeau G, Ohta T, Kishino T, Furukawa Y, Nakamura Y, Niikawa N, Boileau C, Matsumoto N. Heterozygous TGFBR2 mutations in Marfan syndrome. *Nat Genet.* 2004; 36:855–860. [PubMed: 15235604]
- Mohler ER III, Chawla MK, Chang AW, Vyavahare N, Levy RJ, Graham L, Gannon FH. Identification and characterization of calcifying valve cells from human and canine aortic valves. *J Heart Valve Dis.* 1999; 8:254–260. [PubMed: 10399657]
- Molin DG, Bartram U, Van der HK, Van Iperen L, Speer CP, Hierck BP, Poelmann RE, Gittenberger-de-Groot AC. Expression patterns of Tgfbeta1–3 associate with myocardialisation of the outflow tract and the development of the epicardium and the fibrous heart skeleton. *Dev Dyn.* 2003; 227:431–444. [PubMed: 12815630]
- Moskowitz IP, Wang J, Peterson MA, Pu WT, Mackinnon AC, Oxburgh L, Chu GC, Sarkar M, Berul C, Smoot L, Robertson EJ, Schwartz R, Seidman JG, Seidman CE. Transcription factor genes Smad4 and Gata4 cooperatively regulate cardiac valve development. [corrected]. *Proc Natl Acad Sci U S A.* 2011; 108:4006–4011. [PubMed: 21330551]
- Moustakas A, Heldin CH. The regulation of TGF β signal transduction. *Development.* 2009; 136:3699–3714. [PubMed: 19855013]
- Movahed MR, Saito Y, Ahmadi-Kashani M, Ebrahimi R. Mitral annulus calcification is associated with valvular and cardiac structural abnormalities. *Cardiovasc Ultrasound.* 2007; 5:14–14. [PubMed: 17359540]
- Napierala D, Sam K, Morello R, Zheng Q, Munivez E, Shivdasani RA, Lee B. Uncoupling of chondrocyte differentiation and perichondrial mineralization underlies the skeletal dysplasia in tricho-rhino-phalangeal syndrome. *Hum Mol Genet.* 2008; 17:2244–2254. [PubMed: 18424451]
- Nataatmadja M, West J, West M. Overexpression of transforming growth factor- β is associated with increased hyaluronan content and impairment of repair in Marfan syndrome aortic aneurysm. *Circulation.* 2006; 114:I371–I377. [PubMed: 16820603]
- Neptune ER, Frischmeyer PA, Arking DE, Myers L, Bunton TE, Gayraud B, Ramirez F, Sakai LY, Dietz HC. Dysregulation of TGF- β activation contributes to pathogenesis in Marfan syndrome. *Nat Genet.* 2003; 33:407–411. [PubMed: 12598898]
- Ng CM, Cheng A, Myers LA, Martinez-Murillo F, Jie C, Bedja D, Gabrielson KL, Hausladen JM, Mecham RP, Judge DP, Dietz HC. TGF- β -dependent pathogenesis of mitral valve prolapse in a mouse model of Marfan syndrome. *J Clin Invest.* 2004; 114:1586–1592. [PubMed: 15546004]

- Nie X, Deng CX, Wang Q, Jiao K. Disruption of Smad4 in neural crest cells leads to mid-gestation death with pharyngeal arch, craniofacial and cardiac defects. *Dev Biol.* 2008; 316:417–430. [PubMed: 18334251]
- Nomura-Kitabayashi A, Anderson GA, Sleep G, Mena J, Karabegovic A, Karamath S, Letarte M, Puri MC. Endoglin is dispensable for angiogenesis, but required for endocardial cushion formation in the midgestation mouse embryo. *Dev Biol.* 2009; 335:66–77. [PubMed: 19703439]
- Norris RA, Moreno-Rodriguez R, Wessels A, Merot J, Bruneval P, Chester AH, Yacoub MH, Hagege A, Slaugenhaupt SA, Aikawa E, Schott JJ, Lardeux A, Harris BS, Williams LK, Richards A, Levine RA, Markwald RR. Expression of the familial cardiac valvular dystrophy gene, filamin-A, during heart morphogenesis. *Dev Dyn.* 2010; 239:2118–2127. [PubMed: 20549728]
- Norris RA, Moreno-Rodriguez RA, Sugi Y, Hoffman S, Amos J, Hart MM, Potts JD, Goodwin RL, Markwald RR. Periostin regulates atrioventricular valve maturation. *Dev Biol.* 2008; 316:200–213. [PubMed: 18313657]
- Norris RA, Potts JD, Yost MJ, Junor L, Brooks T, Tan H, Hoffman S, Hart MM, Kern MJ, Damon B, Markwald RR, Goodwin RL. Periostin promotes a fibroblastic lineage pathway in atrioventricular valve progenitor cells. *Dev Dyn.* 2009; 238:1052–1063. [PubMed: 19334280]
- Otto F, Kanegane H, Mundlos S. Mutations in the RUNX2 gene in patients with cleidocranial dysplasia. *Hum Mutat.* 2002; 19:209–216. [PubMed: 11857736]
- Otto F, Thornell AP, Crompton T, Denzel A, Gilmour KC, Rosewell IR, Stamp GW, Beddington RS, Mundlos S, Olsen BR, Selby PB, Owen MJ. Cbfa1, a candidate gene for cleidocranial dysplasia syndrome, is essential for osteoblast differentiation and bone development. *Cell.* 1997; 89:765–771. [PubMed: 9182764]
- Paradies NE, Sanford LP, Doetschman T, Friedman RA. Developmental expression of the TGF betas in the mouse cochlea. *Mechs of Develop.* 1998; 79:165–168.
- Pauschinger M, Knopf D, Petschauer S, Doerner A, Poller W, Schwimmbeck PL, Kuhl U, Schultheiss HP. Dilated cardiomyopathy is associated with significant changes in collagen type I/III ratio. *Circulation.* 1999; 99:2750–2756. [PubMed: 10351968]
- Pearson GD, Devereux R, Loeys B, Maslen C, Milewicz D, Pyeritz R, Ramirez F, Rifkin D, Sakai L, Svensson L, Wessels A, Van Eyk J, Dietz HC. Report of the National Heart, Lung, and Blood Institute and National Marfan Foundation Working Group on research in Marfan syndrome and related disorders. *Circulation.* 2008; 118:785–791. [PubMed: 18695204]
- Pierpont ME, Basson CT, Benson DW Jr, Gelb BD, Giglia TM, Goldmuntz E, McGee G, Sable CA, Srivastava D, Webb CL. Genetic basis for congenital heart defects: current knowledge: a scientific statement from the American Heart Association Congenital Cardiac Defects Committee, Council on Cardiovascular Disease in the Young: endorsed by the American Academy of Pediatrics. *Circulation.* 2007; 115:3015–3038. [PubMed: 17519398]
- Pratap J, Galindo M, Zaidi SK, Vradii D, Bhat BM, Robinson JA, Choi JY, Komori T, Stein JL, Lian JB, Stein GS, van Wijnen AJ. Cell growth regulatory role of Runx2 during proliferative expansion of preosteoblasts. *Cancer Res.* 2003; 63:5357–5362. [PubMed: 14500368]
- Pratap J, Javed A, Languino LR, van Wijnen AJ, Stein JL, Stein GS, Lian JB. The Runx2 osteogenic transcription factor regulates matrix metalloproteinase 9 in bone metastatic cancer cells and controls cell invasion. *Mol Cell Biol.* 2005; 25:8581–8591. [PubMed: 16166639]
- Pratap J, Lian JB, Javed A, Barnes GL, van Wijnen AJ, Stein JL, Stein GS. Regulatory roles of Runx2 in metastatic tumor and cancer cell interactions with bone. *Cancer Metastasis Rev.* 2006; 25:589–600. [PubMed: 17165130]
- Proetzel G, Pawlowski SA, Wiles MV, Yin M, Boivin GP, Howles PN, Ding J, Ferguson MW, Doetschman T. Transforming growth factor-beta 3 is required for secondary palate fusion. *Nat Genet.* 1995; 11:409–414. [PubMed: 7493021]
- Rajamannan NM. Calcific aortic stenosis: lessons learned from experimental and clinical studies. *Arterioscler Thromb Vasc Biol.* 2009; 29:162–168. [PubMed: 19023094]
- Rajamannan NM, Subramaniam M, Rickard D, Stock SR, Donovan J, Springett M, Orszulak T, Fullerton DA, Tajik AJ, Bonow RO, Spelsberg T. Human aortic valve calcification is associated with an osteoblast phenotype. *Circulation.* 2003; 107:2181–2184. [PubMed: 12719282]

- Rampazzo A, Beffagna G, Nava A, Occhi G, Bause B, Noiato M, Basso C, Frigo G, Thiene G, Towbin J, Danieli GA. Arrhythmogenic right ventricular cardiomyopathy type 1 (ARVD1): confirmation of locus assignment and mutation screening of four candidate genes. *Eur J Hum Genet.* 2003; 11:69–76. [PubMed: 12529708]
- Renard M, Holm T, Veith R, Callewaert BL, Ades LC, Baspinar O, Pickart A, Dasouki M, Hoyer J, Rauch A, Trapane P, Earing MG, Coucke PJ, Sakai LY, Dietz HC, De Paepe AM, Loeys BL. Altered TGF β signaling and cardiovascular manifestations in patients with autosomal recessive cutis laxa type I caused by fibulin-4 deficiency. *Eur J Hum Genet.* 2010
- Roberts AB, Flanders KC, Heine UI, Jakowlew S, Kondaiah P, Kim SJ, Sporn MB. Transforming growth factor- β : multifunctional regulator of differentiation and development. *Philos Trans R Soc Lond B Biol Sci.* 1990a; 327:145–154. [PubMed: 1969655]
- Roberts AB, Heine UI, Flanders KC, Sporn MB. Transforming growth factor- β . Major role in regulation of extracellular matrix. *Ann N Y Acad Sci.* 1990b; 580:225–232. [PubMed: 2186691]
- Roberts AB, Tian F, Byfield SD, Stuelten C, Ooshima A, Saika S, Flanders KC. Smad3 is key to TGF β -mediated epithelial-to-mesenchymal transition, fibrosis, tumor suppression and metastasis. *Cytokine Growth Factor Rev.* 2006; 17:19–27. [PubMed: 16290023]
- Robson A, Allinson KR, Anderson RH, Henderson DJ, Arthur HM. The TGF β type II receptor plays a critical role in the endothelial cells during cardiac development. *Dev Dyn.* 2010; 239:2435–2442. [PubMed: 20652948]
- Rodriguez-Vita J, Sanchez-Lopez E, Esteban V, Ruperez M, Egido J, Ruiz-Ortega M. Angiotensin II activates the Smad pathway in vascular smooth muscle cells by a transforming growth factor- β -independent mechanism. *Circulation.* 2005; 111:2509–2517. [PubMed: 15883213]
- Roger VL, Go AS, Lloyd-Jones DM, Adams RJ, Berry JD, Brown TM, Carnethon MR, Dai S, De Simone G, Ford ES, Fox CS, Fullerton HJ, Gillespie C, Greenlund KJ, Hailpern SM, Heit JA, Ho PM, Howard VJ, Kissela BM, Kittner SJ, Lackland DT, Lichtman JH, Lisabeth LD, Makuc DM, Marcus GM, Marelli A, Matchar DB, McDermott MM, Meigs JB, Moy CS, Mozaffarian D, Mussolino ME, Nichol G, Paynter NP, Rosamond WD, Sorlie PD, Stafford RS, Turan TN, Turner MB, Wong ND, Wylie-Rosett J. Heart disease and stroke statistics--2011 update: a report from the American Heart Association. *Circulation.* 2011; 123:e18–e209. [PubMed: 21160056]
- Rosenkranz S. TGF- β 1 and angiotensin networking in cardiac remodeling. *Cardiovasc Res.* 2004; 63:423–432. [PubMed: 15276467]
- Ross S, Hill CS. How the Smads regulate transcription. *Int J Biochem Cell Biol.* 2008; 40:383–408. [PubMed: 18061509]
- Runyan, RB.; Heimark, RL.; Camenisch, TD.; Klewer, SE. Epithelial-mesenchymal transformation in the embryonic heart. In: Savagner, P., editor. *Rise and Fall of the Epithelium.* Kluwer/Plenum; 2005. p. 40-55. (<http://www.eurekah.com>)
- Saika S, Liu CY, Azhar M, Sanford LP, Doetschman T, Gendron RL, Kao CW, Kao WW. TGF β 2 in Corneal Morphogenesis during Mouse Embryonic Development. *Dev Biol.* 2001; 240:419–432. [PubMed: 11784073]
- Sanford LP, Kallapur S, Ormsby I, Doetschman T. Influence of genetic background on knockout mouse phenotypes. *Methods Mol Biol.* 2001; 158:217–225. [PubMed: 11236659]
- Sanford LP, Ormsby I, Gittenberger-de GA, Sariola H, Friedman R, Boivin GP, Cardell EL, Doetschman T. TGF β 2 knockout mice have multiple developmental defects that are non-overlapping with other TGF β knockout phenotypes. *Development.* 1997; 124:2659–2670. [PubMed: 9217007]
- Santiago-Sim T, Mathew-Joseph S, Pannu H, Milewicz DM, Seidman CE, Seidman JG, Kim DH. Sequencing of TGF- β pathway genes in familial cases of intracranial aneurysm. *Stroke.* 2009; 40:1604–1611. [PubMed: 19299629]
- Sasaki A, Masuda Y, Ohta Y, Ikeda K, Watanabe K. Filamin associates with Smads and regulates transforming growth factor- β signaling. *J Biol Chem.* 2001; 276:17871–17877. [PubMed: 11278410]

- Schultz JJ, Witt SA, Glascock BJ, Nieman ML, Reiser PJ, Nix SL, Kimball TR, Doetschman T. TGF-beta1 mediates the hypertrophic cardiomyocyte growth induced by angiotensin II. *J Clin Invest.* 2002; 109:787–796. [PubMed: 11901187]
- Shelton EL, Yutzey KE. Twist1 function in endocardial cushion cell proliferation, migration, and differentiation during heart valve development. *Dev Biol.* 2008; 317:282–295. [PubMed: 18353304]
- Shimizu C, Jain S, Davila S, Hibberd ML, Lin KO, Molkara D, Frazer JR, Sun S, Baker AL, Newburger JW, Rowley AH, Shulman ST, Davila S, Burgner D, Breunis WB, Kuijpers TW, Wright VJ, Levin M, Eleftherohorinou H, Coin L, Popper SJ, Relman DA, Fury W, Lin C, Mellis S, Tremoulet AH, Burns JC. Transforming Growth Factor- β Signaling Pathway in Patients With Kawasaki Disease. *Circ Cardiovasc Genet.* 2011; 4:16–25. [PubMed: 21127203]
- Shull MM, Ormsby I, Kier AB, Pawlowski S, Diebold RJ, Yin M, Allen R, Sidman C, Proetzel G, Calvin D, Doetschman T. Targeted disruption of the mouse transforming growth factor-beta 1 gene results in multifocal inflammatory disease. *Nature.* 1992; 359:693–699. [PubMed: 1436033]
- Snider P, Hinton RB, Moreno-Rodriguez RA, Wang J, Rogers R, Lindsley A, Li F, Ingram DA, Menick D, Field L, Firulli AB, Molkentin JD, Markwald R, Conway SJ. Periostin is required for maturation and extracellular matrix stabilization of noncardiomyocyte lineages of the heart. *Circ Res.* 2008; 102:752–760. [PubMed: 18296617]
- Snider P, Standley KN, Wang J, Azhar M, Doetschman T, Conway SJ. Origin of cardiac fibroblasts and the role of periostin. *Circ Res.* 2009; 105:934–947. PMC2786053. [PubMed: 19893021]
- Soler-Soler J, Galve E. Worldwide perspective of valve disease. *Heart.* 2000; 83:721–725. [PubMed: 10814642]
- Song L, Yan W, Chen X, Deng CX, Wang Q, Jiao K. Myocardial smad4 is essential for cardiogenesis in mouse embryos. *Circ Res.* 2007; 101:277–285. [PubMed: 17585069]
- Song L, Zhao M, Wu B, Zhou B, Wang Q, Jiao K. Cell autonomous requirement of endocardial Smad4 during atrioventricular cushion development in mouse embryos. *Dev Dyn.* 2011; 240:211–220. [PubMed: 21089072]
- Sporn MB. The early history of TGF-beta, and a brief glimpse of its future. *Cytokine Growth Factor Rev.* 2006; 17:3–7. [PubMed: 16290110]
- Sporn MB, Roberts AB. The transforming growth factor-betas: past, present, and future. [Review]. *Annals of the New York Academy of Sciences.* 1990; 593:1–6. [PubMed: 2197958]
- Sridurongrit S, Larsson J, Schwartz R, Ruiz-Lozano P, Kaartinen V. Signaling via the Tgf-beta type I receptor Alk5 in heart development. *Dev Biol.* 2008; 322:208–218. [PubMed: 18718461]
- Stein GS, Lian JB, van Wijnen AJ, Stein JL, Montecino M, Javed A, Zaidi SK, Young DW, Choi JY, Pockwinse SM. Runx2 control of organization, assembly and activity of the regulatory machinery for skeletal gene expression. *Oncogene.* 2004; 23:4315–4329. [PubMed: 15156188]
- Stenvers KL, Tursky ML, Harder KW, Kountouri N, Amatayakul-Chantler S, Grail D, Small C, Weinberg RA, Sizeland AM, Zhu HJ. Heart and liver defects and reduced transforming growth factor beta2 sensitivity in transforming growth factor beta type III receptor-deficient embryos. *Mol Cell Biol.* 2003; 23:4371–4385. [PubMed: 12773577]
- Stevens MV, Broka DM, Parker P, Rogowitz E, Vaillancourt RR, Camenisch TD. MEKK3 initiates transforming growth factor-beta 2-dependent epithelial-to-mesenchymal transition during endocardial cushion morphogenesis. *Circ Res.* 2008; 103:1430–1440. [PubMed: 19008476]
- Stevens MV, Parker P, Vaillancourt RR, Camenisch TD. MEKK4 regulates developmental EMT in the embryonic heart. *Dev Dyn.* 2006; 235:2761–2770. [PubMed: 16894626]
- Subramanian R, Olson LJ, Edwards WD. Surgical pathology of pure aortic stenosis: a study of 374 cases. *Mayo Clin Proc.* 1984; 59:683–690. [PubMed: 6482513]
- Supino PG, Borer JS, Preibisz J, Bornstein A. The epidemiology of valvular heart disease: a growing public health problem. *Heart Fail Clin.* 2006; 2:379–393. [PubMed: 17448426]
- Tamura K, Fukuda Y, Ishizaki M, Masuda Y, Yamanaka N, Ferrans VJ. Abnormalities in elastic fibers and other connective-tissue components of floppy mitral valve. *Am Heart J.* 1995; 129:1149–1158. [PubMed: 7754947]
- Tang S, Snider P, Firulli AB, Conway SJ. Trigenic neural crest-restricted Smad7 over-expression results in congenital craniofacial and cardiovascular defects. *Dev Biol.* 2010

- Teekakirikul P, Eminaga S, Toka O, Alcalai R, Wang L, Wakimoto H, Naylor M, Konno T, Gorham JM, Wolf CM, Kim JB, Schmitt JP, Molkentin JD, Norris RA, Tager AM, Hoffman SR, Markwald RR, Seidman CE, Seidman JG. Cardiac fibrosis in mice with hypertrophic cardiomyopathy is mediated by non-myocyte proliferation and requires Tgf-beta. *J Clin Invest.* 2010; 42028
- ten Dijke P, Arthur HM. Extracellular control of TGFbeta signalling in vascular development and disease. *Nat Rev Mol Cell Biol.* 2007
- Tieu BC, Lee C, Sun H, Lejeune W, Recinos A III, Ju X, Spratt H, Guo DC, Milewicz D, Tilton RG, Brasier AR. An adventitial IL-6/MCP1 amplification loop accelerates macrophage-mediated vascular inflammation leading to aortic dissection in mice. *J Clin Invest.* 2009; 119:3637–3651. [PubMed: 19920349]
- Todorovic V, Finnegan E, Freyer L, Zilberberg L, Ota M, Rifkin DB. Long form of latent TGF-beta binding protein 1 (Ltbp1L) regulates cardiac valve development. *Dev Dyn.* 2011; 240:176–187. [PubMed: 21181942]
- Townsend TA, Robinson JY, Deig CR, Hill CR, Misfeldt A, Blobe GC, Barnett JV. BMP-2 and TGFbeta2 Shared Pathways Regulate Endocardial Cell Transformation. *Cells Tissues Organs.* 2011
- Tran-Fadulu V, Pannu H, Kim DH, Vick GW III, Lonsford CM, Lafont AL, Boccalandro C, Smart S, Peterson KL, Hain JZ, Willing MC, Coselli JS, LeMaire SA, Ahn C, Byers PH, Milewicz DM. Analysis of multigenerational families with thoracic aortic aneurysms and dissections due to TGFBR1 oRTGFB2 mutations. *J Med Genet.* 2009; 46:607–613. [PubMed: 19542084]
- Uddin MJ, Crews BC, Blobaum AL, Kingsley PJ, Gorden DL, McIntyre JO, Matrisian LM, Subbaramaiah K, Dannenberg AJ, Piston DW, Marnett LJ. Selective visualization of cyclooxygenase-2 in inflammation and cancer by targeted fluorescent imaging agents. *Cancer Res.* 2010; 70:3618–3627. [PubMed: 20430759]
- Ulmasov B, Xu Z, Tetri LH, Inagami T, Neuschwander-Tetri BA. Protective role of angiotensin II type 2 receptor signaling in a mouse model of pancreatic fibrosis. *Am J Physiol Gastrointest Liver Physiol.* 2009; 296:G284–G294. [PubMed: 19033539]
- van dL I, Oldenburg RA, Pals G, Roos-Hesselink JW, de Graaf BM, Verhagen JM, Hoedemaekers YM, Willemsen R, Severijnen LA, Venselaar H, Vriend G, Pattynama PM, Collee M, Majoor-Krakauer D, Poldermans D, Frohn-Mulder IM, Micha D, Timmermans J, Hilhorst-Hofstee Y, Bierma-Zeinstra SM, Willems PJ, Kros JM, Oei EH, Oostra BA, Wessels MW, Bertoli-Avella AM. Mutations in SMAD3 cause a syndromic form of aortic aneurysms and dissections with early-onset osteoarthritis. *Nat Genet.* 2011; 43:121–126. [PubMed: 21217753]
- Villar AV, Cobo M, Llano M, Montalvo C, Gonzalez-Vilchez F, Martin-Duran R, Hurle MA, Nistal JF. Plasma levels of transforming growth factor-beta1 reflect left ventricular remodeling in aortic stenosis. *PLoS One.* 2009; 4:e8476. [PubMed: 20041033]
- Vrljick P, Chang AC, Morozova O, Wederell ED, Niessen K, Marra MA, Karsan A, Hoodless PA. Genomic analysis distinguishes phases of early development of the mouse atrioventricular canal. *Physiol Genomics.* 2010; 40:150–157. [PubMed: 19952280]
- Walker GA, Masters KS, Shah DN, Anseth KS, Leinwand LA. Valvular myofibroblast activation by transforming growth factor-beta: implications for pathological extracellular matrix remodeling in heart valve disease. *Circ Res.* 2004; 95:253–260. [PubMed: 15217906]
- Wang J, Greene SB, Martin JF. BMP signaling in congenital heart disease: New developments and future directions. *Birth Defects Res A Clin Mol Teratol.* 2011; 91:441–448. [PubMed: 21384533]
- Wang J, Nagy A, Larsson J, Dudas M, Sucov HM, Kaartinen V. Defective ALK5 signaling in the neural crest leads to increased postmigratory neural crest cell apoptosis and severe outflow tract defects. *BMC Dev Biol.* 2006; 6:51–51. [PubMed: 17078885]
- Wang J, Xu N, Feng X, Hou N, Zhang J, Cheng X, Chen Y, Zhang Y, Yang X. Targeted Disruption of Smad4 in Cardiomyocytes Results in Cardiac Hypertrophy and Heart Failure. *Circ Res.* 2005; 97:821–828. [PubMed: 16151019]
- Wang Y, Ait-Oufella H, Herbin O, Bonnin P, Ramkhalawon B, Taleb S, Huang J, Offenstadt G, Combadiere C, Renia L, Johnson JL, Tharaux PL, Tedgui A, Mallat Z. TGF-beta activity protects against inflammatory aortic aneurysm progression and complications in angiotensin II-infused mice. *J Clin Invest.* 2010; 120:422–432. [PubMed: 20101093]

- Watson KE, Bostrom K, Ravindranath R, Lam T, Norton B, Demer LL. TGF-beta 1 and 25-hydroxycholesterol stimulate osteoblast-like vascular cells to calcify. *J Clin Invest.* 1994b; 93:2106–2113. [PubMed: 8182141]
- Watson KE, Bostrom K, Ravindranath R, Lam T, Norton B, Demer LL. TGF-beta 1 and 25-hydroxycholesterol stimulate osteoblast-like vascular cells to calcify. *J Clin Invest.* 1994a; 93:2106–2113. [PubMed: 8182141]
- Wirrig EE, Yutzey KE. Transcriptional regulation of heart valve development and disease. *Cardiovasc Pathol.* 2011; 20:162–167. [PubMed: 20705485]
- Wu MY, Hill CS. Tgf-beta superfamily signaling in embryonic development and homeostasis. *Dev Cell.* 2009; 16:329–343. [PubMed: 19289080]
- Xiao ZS, Hjelmeland AB, Quarles LD. Selective deficiency of the "bone-related" Runx2-II unexpectedly preserves osteoblast-mediated skeletogenesis. *J Biol Chem.* 2004; 279:20307–20313. [PubMed: 15007057]
- Xiong DH, Liu XG, Guo YF, Tan LJ, Wang L, Sha BY, Tang ZH, Pan F, Yang TL, Chen XD, Lei SF, Yerges LM, Zhu XZ, Wheeler VW, Patrick AL, Bunker CH, Guo Y, Yan H, Pei YF, Zhang YP, Levy S, Papasian CJ, Xiao P, Lundberg YW, Recker RR, Liu YZ, Liu YJ, Zmuda JM, Deng HW. Genome-wide association and follow-up replication studies identified ADAMTS18 and TGFBR3 as bone mass candidate genes in different ethnic groups. *Am J Hum Genet.* 2009; 84:388–398. [PubMed: 19249006]
- Xu S, Liu AC, Gotlieb AI. Common pathogenic features of atherosclerosis and calcific aortic stenosis: role of transforming growth factor-beta. *Cardiovasc Pathol.* 2010; 19:236–247. [PubMed: 19942455]
- Yamashita H, Ichijo H, Grimsby S, Moren A, ten Dijke P, Miyazono K. Endoglin forms a heteromeric complex with the signaling receptors for transforming growth factor-beta. *J Biol Chem.* 1994; 269:1995–2001. [PubMed: 8294451]
- Yamashita M, Fatyol K, Jin C, Wang X, Liu Z, Zhang YE. TRAF6 mediates Smad-independent activation of JNK and p38 by TGF-beta. *Mol Cell.* 2008; 31:918–924. [PubMed: 18922473]
- Yang G, Yang X. Smad4-mediated TGF-beta signaling in tumorigenesis. *Int J Biol Sci.* 2010; 6:1–8. [PubMed: 20087440]
- Yang J, Boerm M, McCarty M, Bucana C, Fidler IJ, Zhuang Y, Su B. Mekk3 is essential for early embryonic cardiovascular development. *Nat Genet.* 2000; 24:309–313. [PubMed: 10700190]
- Yoshimura A, Wakabayashi Y, Mori T. Cellular and molecular basis for the regulation of inflammation by TGF-beta. *J Biochem.* 2010; 147:781–792. [PubMed: 20410014]
- Yoshinaga K, Obata H, Jurukovski V, Mazziere R, Chen Y, Zilberberg L, Huso D, Melamed J, Prijatelj P, Todorovic V, Dabovic B, Rifkin DB. Perturbation of transforming growth factor (TGF)-beta1 association with latent TGF-beta binding protein yields inflammation and tumors. *Proc Natl Acad Sci U S A.* 2008; 105:18758–18763. [PubMed: 19022904]
- Zacchigna L, Vecchione C, Notte A, Cordenonsi M, Dupont S, Maretto S, Cifelli G, Ferrari A, Maffei A, Fabbro C, Braghetta P, Marino G, Selvetella G, Aretini A, Colonnese C, Bettarini U, Russo G, Soligo S, Adorno M, Bonaldo P, Volpin D, Piccolo S, Lembo G, Bressan GM. Emilin1 links TGF-beta maturation to blood pressure homeostasis. *Cell.* 2006; 124:929–942. [PubMed: 16530041]
- Zhang YW, Yasui N, Ito K, Huang G, Fujii M, Hanai J, Nogami H, Ochi T, Miyazono K, Ito Y. A RUNX2/PEBP2alpha A/CBFA1 mutation displaying impaired transactivation and Smad interaction in cleidocranial dysplasia. *Proc Natl Acad Sci U S A.* 2000; 97:10549–10554. [PubMed: 10962029]
- Zhou HM, Wang J, Elliott C, Wen W, Hamilton DW, Conway SJ. Spatiotemporal expression of periostin during skin development and incisional wound healing: lessons for human fibrotic scar formation. *J Cell Commun Signal.* 2010; 4:99–107. [PubMed: 20531985]

Fig. 1. TGF β signaling pathway and its integration with other major pathways that is likely to be involved in the pathogenesis of cardiovascular diseases in elderly patients. The canonical TGF β pathway involves SMADs. The non-canonical TGF β pathways include several SMAD-independent signaling cascades such as calcium-calcineurin and MAPK pathways. The Ang II pathway can interact with TGF β signaling through both SMAD-dependent and SMAD-independent pathways (see text for details).

Cardiovascular tissue type	Embryonic development		Adult
	E9.5-E11.5	E12.5-E18.5	
Endocardium	+++ +	+++	+++ ND ND
Myocardium (valve-forming region)	+++	+	ND ND ND
Myocardium (atrial/ventricular)	+		ND ND ND
Cushion/valve mesenchyme	+ +++	+ ++ +++	+ ND ND
Epicardium	+++	+ + +++	ND ND ND
Walls of aorta	+++ +	+++ +++ +++	ND ND ND

Fig. 2.

Summary of gene expression patterns of the three TGF β ligand genes in cardiovascular tissues during embryonic development and adulthood. The data is collected from various *in situ* hybridization studies (see text for details). Blue, *Tgfb1*; Brown, *Tgfb2*; Yellow, *Tgfb3*. +, presence of gene expression; ++ higher intensity of gene expression; +++, highest level of gene expression; ND, not determined.

Fig. 3.

Integrated signaling of TGFβ, Ang II, and MAPK pathways in cardiovascular diseases of elderly patients. The proposed model summarizes the published information about the TGFβ-based therapies and their potential complications for cardiovascular disease-treatment and repair. Black color highlights genes that are found mutated in patients of cardiovascular diseases. Induction or inhibition arrows in solid line indicate the presence of sufficient experimental evidence to support their involvement in cardiovascular disease. The dotted line arrows indicate that there is no direct evidence as yet available and that these require serious investigation.

Table 1

Genetic mutation or variation associated with human cardiovascular diseases

Gene	Disease	Cardiovascular complications (Reference)
TGFB Ligands		
<i>TGFB2</i>	Kawasaki disease	Coronary artery aneurysm, ischemic heart disease, myocardial infarction, sudden cardiac death (Shimizu et al. 2011)
<i>TGFB3</i>	Arrhythmogenic right ventricular-cardiomyopathy/ dysplasia	Fibro-fatty replacement of myocardium, abnormal rhythms, RV and LV dysfunction, sudden cardiac death (Beffagna et al. 2005)
TGFB Receptors		
<i>TGFB1</i>	Loeys-dietz syndrome,	Aortic aneurysm, valvuloseptal defects, cardiac dysfunction, other congenital heart defects (Loeys et al. 2006)
	Kawasaki disease	See above
<i>TGFB2</i>	Loeys-dietz syndrome	See above
	Marfan syndrome-related disorders	Aortic aneurysm, mitral valve disease, cardiac dysfunction (Mizuguchi et al. 2004)
	Familial thoracic ascending aortic aneurysm and dissection	Ascending aortic aneurysm and aortic dissection or rupture (Jain et al. 2009)
<i>TGFB3</i>	Familial intracranial aneurysm	Cerebral artery aneurysm (Santiago-Sim et al. 2009)
<i>ENDGLIN</i>	Hereditary hemorrhagic telangiectasia	Vascular dysplasia leading to telangiectases and arteriovenous malformations, aortic aneurysm (ten Dijke and Arthur 2007)
TGFB effectors		
<i>FLNA</i>	X-linked cardiac valvular disease	Valvular hypertrophy (Kyndt et al. 2007)
<i>SMAD3</i>	Aortic aneurysm-osteoarthritis	Arterial aneurysm, congenital heart disease, valve and myocardial disease (van, I et al. 2011)
<i>SMAD4</i>	Juvenile polyposis syndrome with cardiovascular complication	Aortopathy, mitral valve disease (Andrabi et al. 2011)
	Hereditary hemorrhagic telangiectasia	See above