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Bmp Signaling in Congenital Heart Disease: New Developments and Future Directions

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Abstract

Congenital heart malformations are the most common of all congenital human birth anomalies. During the past decade, research with zebrafish, chick and mouse models have elucidated many fundamental genetic pathways that govern early cardiac patterning and differentiation. This review highlights the roles of the Bmp signaling pathway in cardiogenesis and how defective Bmp signals can disrupt the intricate steps of cardiac formation and cause congenital heart defects.

Keywords

cardiac development; Bmp signaling; congenital heart disease

Overview of Bmp signaling

The Bone morphogenetic proteins (Bmp) are members of the evolutionarily conserved transforming growth factor-β (Tgf-β) superfamily that signals via a heterodimeric complex composed of type I and type II receptors. Bmps were originally discovered by their ability to induce the formation of bone and cartilage, but additional studies have implicated their important roles in multiple aspects of embryogenesis (Hogan, 1996a; Hogan, 1996b). In the canonical Bmp signaling pathway, Bmp ligands bind to the type II receptor, such as Bmpr2, and then activate a type I receptor, such as Bmpr1a, to phosphorylate the Bmp receptor regulated Smads (R-Smad) signal transducers, Smad1, Smad5 or Smad8 (Derynck and Zhang, 2003; Shi and Massague, 2003). Following release from the receptor complex, phosphorylated R-Smad associates with the common Smad4 to form a trimeric complex composed of two R-Smads and Smad4, which can then induce transcription of downstream genes (Fig.1). In addition, Bmp signaling is also mediated via non-Smad signaling pathways such as a MAPK-signaling pathway (Aubin et al., 2004; Nohe et al., 2004; Xu et al., 2008).

Recent findings further indicate that Bmp signals could regulate gene expression through microRNA (miRNA)-mediated pathways (Davis et al., 2008). In some contexts, Bmp signaling regulates miRNA expression via the canonical effector pathway (Li et al., 2008). In addition, Smad1/5 forms a protein-protein interaction with the Drosha complex to regulate processing of the primary (pri-) miRNA to the precursor (pre-) miRNA (Fig.1). This interaction between Smad and Drosha complex is both Smad4 independent and C-terminal phosphorylation independent (Davis et al., 2008). Furthermore, very recent work has identified an RNA Smad-binding element (R-SBE) within the stem sequence of a subset of pri-miRNAs which binds to the MH1 domain of Smad1/5 and is necessary and sufficient

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for Drosha cleavage (Davis et al., 2010). Together these new findings indicate that Smad1/5 is a multifunctional molecule that in addition to transcriptional regulation also functions as part of an enzymatic complex to promote miRNA processing.

Overview of Bmp ligand and receptor germ line loss of function studies in mice

Mice with functional disruption (knockout) of *Bmp2* and *4* are nonviable. *Bmp2* knockout mice die at 7.5~10.5 days post coitum (dpc) and have defects in amnion/chorion and cardiac development (Zhang and Bradley, 1996). *Bmp4* knockout mice die at 6.5~9.5dpc and show defective mesodermal differentiation (Winnier et al., 1995). Both *Bmp5* and *Bmp6* knockout mice are viable and fertile with no gross cardiac abnormalities (Kingsley et al., 1992; Solloway et al., 1998). *Bmp7* deficient mice die shortly after birth and display severe defects of kidney and eye development, as well as minor skeletal defects (Dudley et al., 1995; Luo et al., 1995). Since *Bmp5*, *Bmp6* and *Bmp7* are in the same Bmp family subgroup and individual knockout mice failed to uncover a role in cardiogenesis, functional redundancy may underlie the observed phenotypes of individual knockouts. Indeed, the double knockout mice have more severe phenotypes than the individual knockouts. *Bmp5* and *Bmp7* double knockout mice are embryonic lethal at 10.5dpc with defective cardiac cushion formation, and show other defects within co-expressing tissues (Solloway and Robertson, 1999). *Bmp5* and *Bmp6* double mutant animals have slightly exacerbated sternal defects compared to the *Bmp6* knockout mice (Solloway et al., 1998). *Bmp6* and *Bmp7* double knockout mice die at 10.5 ~15.5dpc due to cardiac defects (Kim et al., 2001).

Bmpr1a knockout mice die at 9.5dpc due to a lack of mesoderm formation and morphological defects are first detected at 7.5dpc, which suggests the critical role of *Bmpr1a* in mesoderm induction during gastrulation (Mishina et al., 1995). *Bmpr1b* is not expressed during early cardiac development and knockout mice are viable but exhibit defects in the appendicular skeleton (Yi et al., 2000). *Bmpr2* is required for mouse gastrulation and its knockout is early embryonic lethal (Beppu et al., 2000).

Germ line loss of function studies have also uncovered roles for *Smad1* and *Smad5* in embryogenesis. *Smad1* knockout mice die at 10.5dpc due to failure to form umbilical-placenta connections (Tremblay et al., 2001). *Smad5* knockout mice die at 10.5~11.5dpc due to defects in angiogenesis and cardiac looping that are secondary to aberrant left-right asymmetry signaling (Chang et al., 2000; Yang et al., 1999). *Smad8* knockout mice are viable and fertile, but have defective pulmonary vascular remodeling (Huang et al., 2009). A nonsense mutation of *Smad8* resulting in a truncated protein in human patients is associated with pulmonary arterial hypertension (Shintani et al., 2009). *Smad4* knockout mice die before 7.5dpc and have growth retardation, failure of gastrulation and abnormal visceral endoderm development (Sirard et al., 1998).

Bmp signaling function in cardiogenic mesoderm specification

During gastrulation, heart precursor cells are generated within bilateral fields in the anterior lateral plate mesoderm (Fig.2) and the cardiogenic regions receive inductive cues that promote cardiac differentiation. Insight into the function of Bmp signaling in specification of cardiogenic mesoderm was initially found through studies of *Dpp*, which is a Drosophila orthologue of vertebrate *Bmp2*. *Dpp* is required for heart-inducing activity during gastrulation and mesoderm migration. *Dpp* deficient embryos failed to form progenitor cells of the dorsal vessel, the Drosophila cardiac organ, while mutant embryos with ectopic *Dpp* expression resulted in ectopic formation of dorsal vessel cells (Frasch, 1995; Yin and Frasch, 1998).

Data from zebrafish, chick and mouse models also provided solid evidence that Bmp signals have essential roles in cardiac induction. *Bmp2* is a cardiac specification factor that elicits ectopic expression of the early cardiac markers *Nkx2.5* and *Gata4* when it is ectopically expressed (Andree et al., 1998; Brand, 2003; Jamali et al., 2001; Liberatore et al., 2002; Lien et al., 2002; Reiter et al., 2001; Schlange et al., 2000; Schultheiss et al., 1997; van Wijk et al., 2007). *Nkx2.5* has an evolutionarily conserved Smad binding site in its enhancer and maintenance of *Nkx2.5* expression requires regulation by Bmp signaling in Xenopus, chick, and mouse embryonic hearts (Andree et al., 1998; Jamali et al., 2001; Liberatore et al., 2002; Lien et al., 2002; Reiter et al., 2001; Schlange et al., 2000; Schultheiss et al., 1997; van Wijk et al., 2007). Analysis of zebrafish mutant for the type I serine/threonine kinase receptor *Alk8* (Lost-a-fin), also known as *Acvr1*, which is required for Bmp 2, 4 and 7 signaling, indicated that Bmp activity is required for cardiac progenitor specification (Marques and Yelon, 2009). Moreover, data from studies using Bmp inhibitors including Noggin, truncated versions of Bmp receptors, and inhibitory Smad6 further confirmed the heart-inducing activity of Bmp signaling (Schlange et al., 2000; Schultheiss et al., 1997; Shi et al., 2000).

Bmp signaling function in second heart field (SHF)

Two major cardiac progenitor pools, the primary/first heart field (PHF/FHF) and the anterior/second heart field (AHF/SHF), contribute to the heart and give rise to cardiomyocyte, smooth muscle and endothelial cells, the major cardiac lineages (Fig.2) (for detailed review see (Abu-Issa and Kirby, 2008; Buckingham et al., 2005; Dyer and Kirby, 2009)). The FHF mainly contributes to atrial and left ventricular (LV) myocardium. The SHF, located dorsomedial to cardiac crescent at 7.5dpc, starts extending anterior and dorsal at 8.0-8.5dpc to add to form the outflow tract (OFT) and right ventricular (RV) myocardium and endocardium. The SHF also contributes to the inflow tract and smooth muscle walls of the intra-pericardial aortic and pulmonary trunks (Fig.2) (Abu-Issa and Kirby, 2008; Buckingham et al., 2005; Cai et al., 2003; Meilhac et al., 2004a; Meilhac et al., 2004b; Waldo et al., 2005; Zaffran et al., 2004). The FHF and SHF express both distinct and overlapping molecular markers: the *Hand1* and *Tbx5* transcriptional regulators mark FHF while *Fgf10* and the Lim-homeobox gene *Isl1* mark SHF, and the *Nkx2.5* homeobox gene is expressed in both FHF and SHF (Bruneau et al., 2001; Cai et al., 2003; Kelly et al., 2001; Schwartz and Olson, 1999; Srivastava and Olson, 1997; Takeuchi et al., 2003; Yuan and Schoenwolf, 2000).

Bmp signaling plays a critical role in SHF specification, regulation of proliferation, and induction of myocardial differentiation. *Nkx2.5* null embryos have up-regulated Bmp2 expression and activity that is associated with expanded SHF specification (Prall et al., 2007). *Nkx2.5* mutants also had drastically reduced cardiac progenitor proliferation that could be rescued by *Smad1* loss of function indicating Bmp signaling limits progenitor cell growth (Prall et al., 2007). After progenitor cell specification and expansion, Bmp signaling is still required for SHF differentiation. Based on data from chick embryos, Bmp2 induced SHF explants to differentiate into myocardium (Waldo et al., 2001). Very recent work using mouse embryos indicated that Bmp signaling regulates the *miR-17-92* cluster as a mechanism to promote myocardial differentiation (Wang et al., 2010). The oncogenic miRNA *miR-17-92* cluster, also known as *oncomir-1*, has essential functions not only in tumor formation but also in normal embryonic development, and has specifically been shown to be required for lung, heart and immune system development (Hayashita et al., 2005; He et al., 2005; Lu et al., 2007; Ventura et al., 2008; Xiao et al., 2008). The *miRNA 17-92* cluster encodes six miRNAs (*miR-17*, *miR-18a*, *miR-19a*, *miR-20a*, *miR-19b-1*, and *miR-92a-1*) that are processed from a common primary transcript, Bmp signaling directly regulates transcription of the *miRNA-17-92* cluster via Smad binding sites in the 5′ flanking

region of *miRNA-17-92*. In normal embryos, the *Bmp-miRNA-17-92* regulatory pathway down-regulates cardiac progenitor genes such as *Isl1* and *Tbx1* to enhance myocardial differentiation, while in *Bmp* mutant embryos, cardiac progenitor genes failed to be down-regulated and myocardial differentiation was defective (Wang et al., 2010).

Bmp signaling events during chamber and cushion tissue morphogenesis

Cardiac looping brings the future chamber forming areas into an alignment with the atrioventricular canal separating the atrial and ventricular regions. As cardiac remodeling occurs chamber septation is completed by 12.5dpc, forming the distinct left and right ventricles as well as the left and right atria. Chamber formation involves septation of the atrial and ventricular chambers and septation of the primary heart tube into left and right components. The myocardially-derived septums of the inter-atrial and inter-ventricular regions fuse with the endocardial cushion-derived atrioventricular septum, resulting in complete separation of the chambers (for detailed review see (Harvey, 2002)).

Clinically, heterozygous deletion of *BMP2* within 20p12.3, has been found to predispose human patients to Wolff-Parkinson-White syndrome (WPW), a pre-excitation syndrome that is often asymptomatic, but in some presents as tachycardia as a result of abnormal connection between the atria and ventricles (Lalani et al., 2009). While germline deletion of *Bmp2* in mouse models leads to embryonic lethality, some mutants fail to form a heart, while some formed hearts in the exocoelomic cavity (Zhang and Bradley, 1996). Conditional deletion of *Bmp4* in cardiomyocyte in mice results in ventricular septal defects (VSD), atrioventricular canal defect (AVCD) and double outlet right ventricle (DORV) (Jiao et al., 2003). *Bmp2* and *Bmp4* compound heterozygous mice embryos also have VSD (Goldman et al., 2009; Uchimura et al., 2009). Additionally, *Bmp6* and *Bmp7* deficient mice display defective chamber septation and hypoplastic ventricles with reduced trabeculations (Kim et al., 2001). Likewise, compound *Bmp5* and *Bmp7* mutant mice have defective chamber septation with myocardial and pericardial abnormalities (Solloway and Robertson, 1999). Interestingly, as chamber formation begins around 9.0dpc, *Bmp10* expression can first be detected in the ventricular myocardium, when its expression is restricted to the trabeculated part of the common ventricular chamber and the bulbus cordis of the developing heart, and after 12.5dpc can be detected in the atrial wall (Neuhaus et al., 1999). Mice deficient for this novel member of the Tgf-β family die around 9.0dpc due to incomplete ventricular development including profound hypoplastic ventricular walls and absence of ventricular trabeculae, and abnormal cushion proliferation in both the OFT and atrioventricular canal (AVC) (Chen et al., 2004). Endocardial Notch1 expression has been shown to be required for myocardial Bmp10 expression during ventricular chamber formation (Grego-Bessa et al., 2007).

Moreover, analysis of Bmp receptors further determined that Bmp signaling has essential roles in development of chamber myocardium. Data from zebrafish mutant for *Alk8* indicated that Bmp activity is required for chamber formation, regulation of overall heart size, and atrial and ventricular fate (Marques and Yelon, 2009). Further, conditional deletion of *Bmpr2* in the developing heart in mice results in cardiac defects including DORV, VSD, and AV cushion defects (Beppu et al., 2009).

By using *Smad4* conditional loss of function alleles in conjunction with several different *Cre* lines, Smad4 deletion in cardiomyocytes resulted in mice with severe cardiac defects including ventricular myocardium defects and VSD (Azhar et al., 2010; Qi et al., 2007; Song et al., 2007; Wang et al., 2005). Despite variations in the timing and efficiency of the myocardial specific *Smad4* inactivation, these mouse models mutant for the common Smad4 further demonstrated the essential roles of Bmp signaling in myocardium.

During myocardial patterning, Bmp signaling is required for precise regulation of many myocardial target genes. Myocardial patterning within the AVC is tightly regulated by Bmp signaling in the AV myocardium where Bmp2 induces *Tbx2* transcription, which has precise expression patterns in chamber formation (Ma et al., 2005). *Tbx2* induction is required to inhibit chamber-specific genes such as *Natriuretic precursor peptide type A (Nppa)*, *Connexin (Cx) 40*, *Cx43*, and *Chisel* in AVC myocardium, suggesting a role for *Bmp2* in myocardial differentiation (Christoffels et al., 2004). Bmp2 directly regulates *Tbx2* through canonical transcriptional pathway (Shirai et al., 2009). In addition, an inhibitory protein-protein interaction between Smad1 and Tbx20, a Tbx2 repressor, in AVC myocardium, is permissive for *Tbx2* transcription in AVC myocardium (Singh et al., 2009). Interestingly, there is further evidence in Xenopus that Bmp signaling also regulates *Tbx20* transcription via a canonical Smad1/4 pathway suggesting a complex regulatory network involving Bmp-regulated *Tbx* expression in AVC myocardium (Mandel et al., 2010).

Defects in cardiac cushion derived structures, including cardiac valves and associated structures, are the most common subtype of cardiovascular malformations and account for 25% to 30% of defects (Loffredo, 2000). The mouse heart tube at 8.5dpc is composed of an outer myocardial layer and an inner monolayer of specialized endothelium, the endocardium. The two layers are separated by a thick extracellular matrix named cardiac jelly, which is secreted by myocardial cells. At about 9.5dpc, a subset of endocardial cells in the atrioventricular (AV) canal and OFT regions undergo epithelial-mesenchymal transition (EMT) in response to regionalized myocardial signals. These cells delaminate from endocardium and invade the cardiac jelly to form the endocardial cushions, which will contribute to cardiac valve development and heart chamber formation. EMT, essential in numerous developmental processes, can be induced by a number of signal pathways such as Tgf-β and Notch, and transcription effectors such as Snail, Twist, and FoxC2 (Mani et al., 2007; Perez-Pomares and Munoz-Chapuli, 2002; Thiery and Sleeman, 2006; Timmerman et al., 2004; Yang et al., 2004) .

Data from mouse embryos showed that *Bmp2* is important for enhancing cardiac jelly formation, endocardial EMT, and patterning the AV myocardium (Ma et al., 2005; Rivera-Feliciano and Tabin, 2006; Sugi et al., 2004). *Bmp2* is required for myocardial expression of *Has2*, which is a crucial component of the cardiac jelly matrix and required for endocardial EMT (Camenisch et al., 2000; Ma et al., 2005). In addition, in the endocardium, Bmp2 signals via Bmpr1a to promote expression of *Twist1*, which was a reported inducer of EMT (Ma et al., 2005; Yang et al., 2004). Data from chick models showed that Bmp2 protein is expressed in the endocardial cushions of the OFT and AV, as well as adjacent myocardial layers (Keyes et al., 2003). Other data indicate that *Bmp2* is important for the formation of endocardial cushion tissue and acts synergistically with Tgf-β3 to regulate EMT (Boyer et al., 1999; Nakajima et al., 2000; Yamagishi et al., 1999).

Bmp4 has also been implicated in AV cushion morphogenesis. *Bmp2* and *Bmp4*, encoding highly related peptides with 92% identity in the C-terminal mature ligand domain, have overlapping functions in heart development (Goldman et al., 2009; Uchimura et al., 2009). Inactivation of *Bmp4* in the heart using a *cardiac troponin T (cTnT) Cre* transgene, a cardiomyocyte-specific *Cre*, and a hypomorphic *Bmp4* conditional allele (Kulessa and Hogan, 2002), indicated that *Bmp4* regulates proliferation of atrioventricular cushion mesenchyme (Jiao et al., 2003). In *Bmp6* and *Bmp7* deficient mice, formation of the OFT endocardial cushions was markedly delayed, providing further evidence for the central role of Bmps in cushion development (Kim et al., 2001).

Bmp receptors are expressed in both OFT and AV cushions of the chick embryo (Keyes et al., 2003). Conditional deletion of the *Alk3/ Bmpr1a* in AV myocardium resulted in

abnormal cushion formation, which indicated a feedback loop with Tgf-β2 in cushion development (Gaussin et al., 2002). Endocardial deletion of *Bmpr1a* resulted in failed cushion EMT, which indicated a direct requirement for Bmp signaling in cushion forming endocardium (Ma et al., 2005). Moreover, studies with inhibitors of Bmp signaling further demonstrated the essential roles of Bmp signaling in cushion morphogenesis. Targeted mutation of *Smad6* resulted in hyperplasia of the cardiac valves and OFT septation defects, which suggests the role of Bmp signaling in promoting endocardial cushion transformation (Galvin et al., 2000). Mouse explant experiments indicated that when Noggin was added to AV endothelial cells co-cultured with associated myocardium, it blocked endothelial cells undergoing EMT (Sugi et al., 2004).

Bmp2, which is critical for the induction of cushion EMT, also has roles during later stages in cushion and valve morphogenesis. *Bmp2* has persistent expression in cushion mesenchyme at 13.5-16dpc during valve remodeling and in the valve tissue of adult mice (Sugi et al., 2004). Very recent studies with mouse models suggest the interaction between Notch1 in the endocardium and myocardial Bmp2 coordinately regulates endocardial EMT. In this model Bmp2 promotes endocardial cell invasiveness within the valve forming region between the chambers (Luna-Zurita et al., 2010). Data from mouse model also indicated that an Fgf- Bmp signaling axis is required for regulation of OFT valve remodeling, mainly through promoting the differentiation of cranial neural crest cells in OFT cushion (Zhang et al., 2010).

Bmp signaling in the outflow tract: the second heart field (SHF) and cranial neural crest (CNC)

Unseptated initially, the OFT divides into the pulmonary trunk (PT) and aorta, which is critical for separation of postnatal pulmonary and systemic circulation. Malformations in human OFT development include double outlet right ventricle (DORV) where both of the great arteries completely or partially connect to the right ventricle and transposition of the great arteries (TGA), which results in reversed ventriculoarterial connections. Other malformations in OFT development including persistent truncus arteriosus (PTA) where truncus arteriosus never properly divides into the pulmonary artery and aorta, patent ductus arteriosus (PDA) wherein a neonate's ductus arteriosus fails to close after birth. Congenital OFT malformations are found in 4 per 10,000 live births and are commonly lethal (Webb, 2003), therefore insight into the genetic pathways regulating OFT development is critical for developmental biology and clinical medicine.

The OFT myocardium receives a cellular input from SHF (Fig.2) and defective development of the SHF may cause malformations in OFT development including DORV, TGA, PTA or overriding aorta (the aorta straddles the interventricular septum instead of positioned over the left ventricle). *Bmp4* is expressed in SHF and within the OFT myocardium itself, suggesting a role in OFT morphogenesis (Abdelwahid et al., 2001). In mouse conditional deletion studies, inactivation of *Bmp4* in SHF, OFT endocardium, pharyngeal endoderm, and OFT myocardium using *Nkx2.5 Cre* resulted in defects in OFT septation with aorto-pulmonary (AP) window and also defects in vascular smooth muscle recruitment to forming vessels (Liu et al., 2004). It has also been reported that feedback repression of Bmp2/Smad1 signaling by Nkx2.5 regulates OFT morphogenesis (Prall et al., 2007). The *Nkx2.5* mutants have dramatically narrowed and shortened OFT, while specific inactivation of *Smad1* in anterior mesoderm using *Mesp1 Cre*, results in increased OFT length. Importantly, *Smad1* deletion in *Nkx2.5* mutants partially rescued the OFT anomalies in *Nkx2.5* mutants, potentially because the suppression of BMP2/Smad1 signaling by Nkx2-5 is permissive for SHF proliferation that drives OFT morphogenesis.

In addition, the developing OFT receives a cellular contribution from the cardiac neural crest (CNC) (Fig.2). The CNC is a migratory cell population that originates from the dorsal neural tube in the hindbrain region at the level of rhombomeres 6, 7, and 8. The CNC precursors migrate to the caudal 3rd, 4th and 6th pharyngeal arches and a subpopulation of these neural crest–derived cells in these pharyngeal arches continue to migrate into the outflow tract of the developing heart and contribute extensively to cardiac OFT cushion formation (Kirby et al., 1983; Kirby and Waldo, 1995). The CNC-derived aorticopulmonary septum is required for septation of the single outflow vessel into two trunks while CNC cells in the OFT cushions and truncal valves appear to make little contribution to the resulting structures postnatally (Webb et al., 2003). Ablation of neural crest in chick embryos resulted in failure of cardiac cushion formation and OFT defects including PTA and anomalies of aortic arches (Kirby et al., 1983; Kirby and Waldo, 1995). In mouse embryos, lineage tracing experiments have validated the notion that the CNC contributes to OFT cushions and the aorto-pulmonary septum (Jiang et al., 2000). Important roles of Bmp proteins, particularly Bmp2 and Bmp4, in induction, maintenance, migration and differentiation of neural crest cells have been indicated in different model organisms (Aybar and Mayor, 2002; Christiansen et al., 2000; Knecht and Bronner-Fraser, 2002).

Analysis of Bmp receptors provides further insight into functions of Bmp signaling in CNC cells during OFT morphogenesis. Conditional inactivation of *Bmpr1* in cardiac neural crest, using the *Wnt1Cre* transgenic driver to direct *Cre* activity, led to severe OFT defects such as shortened OFT and PTA (Kaartinen et al., 2004; Stottmann et al., 2004). Additionally, analysis of a hypomorphic allele of the ubiquitously expressed *Bmpr2*, containing a partial ectodomain deletion, revealed defective proximal OFT septation in mutant mouse embryos (Delot et al., 2003). The hypomorphic mutants undergo normal gastrulation but die at midgestation with major OFT phenotype PTA. Moreover, specific inactivation of common *Smad4* in CNC resulted in increased cell death and reduced contribution of CNC cells to the developing OFT (Jia et al., 2007; Nie et al., 2008). *Smad4* mutants had decreased expression of genes that are critical for neural crest cells development, such as *Msx1* and *Msx2*, and displayed severe OFT defects including defective OFT elongation and positioning, cushion hypoplasia and PTA. Lastly, over-expression of *Noggin* in chick embryos resulted in OFT abnormalities such as PTA and DORV. Data from these Bmp inhibitor studies suggested that Bmp signaling is required for both migration of CNC cells into the developing OFT and proliferation of OFT mesenchyme (Allen et al., 2001)

Bmp signaling related to epicardium development and differentiation

The proepicardium (PE) is a transient extracardiac mesothelial rudiment located at the cardiac venous pole and emigrates onto the looping heart tube to form the epicardium, the epithelial outer lining of the heart (Fig.2). The PE derived from the mesodermal lining of the prospective pericardial cavity, expresses specific marker genes *Tbx18*, *Wt1*, and *Cfc*. PE contributes multiple cell types to the heart, giving rise to cardiac fibroblasts and coronary smooth muscle cells, also perhaps contributing to the cardiac myocyte lineage (Cai et al., 2008; Christoffels et al., 2009; Ishii et al., 2010; Ishii et al., 2009; Kruithof et al., 2006; Mikawa and Fischman, 1992; Mikawa and Gourdie, 1996; Perez-Pomares et al., 2002; van Wijk et al., 2009; Winter and Gittenberger-de Groot, 2007; Zhou et al., 2008).

Although the signals that control the epicardium morphogenetic events are still largely unknown, the essential roles of Bmp signaling during different events of epicardium morphogenesis have been indicated in different animal model studies. In the chick, a distinct level of Bmp signaling is required for PE identity and expression of PE-specific marker genes (Schlueter et al., 2006). PE and the inflow myocardium separate from the same precursor under the regulation of proper crosstalk between Bmp and Fgf signals, but a

misbalance between them could cause a developmental arrest of the epicardium and enhancement of myocardium formation (van Wijk et al., 2009). Data from zebrafish also indicate that Bmp signaling in conjunction with Tbx5 is essential for PE specification (Liu and Stainier, 2010). In chick, myocardium-derived Bmp signals promote the PE protrusion toward and the attachment to the looping heart tube, and ectopic expression of Bmp signals resulted in ectopic attachment of PE (Ishii et al., 2010). In contrast, expression of Bmp antagonist, Noggin, resulted in diminished PE attachment to the heart tube (Ishii et al., 2010).

V. Concluding remarks

Although much has been learned Bmp signaling in cardiac development there are many areas for fruitful investigation. The role of Bmp signaling in miRNA processing is an important area for future studies. Further insight into what Bmp-mediated cardiac functions are due to defective miRNA activity is needed. Currently, it is unclear how generally important this mechanism will be in the heart. It will also be important to determine how many miRNAs are regulated by the canonical Bmp pathway and to integrate this information into a larger model for heart development.

Recent experiments have uncovered the importance of feedback loops in cardiac development (Prall et al., 2007). This mechanism is undoubtedly of broad importance in many different aspects of cardiac development and will need to be intensively investigated in order to solidify our understanding of heart development. Lastly, the integration of Bmp signaling and other important signaling pathways, such as Wnt and Notch signaling, will need to be understood with much more clarity.

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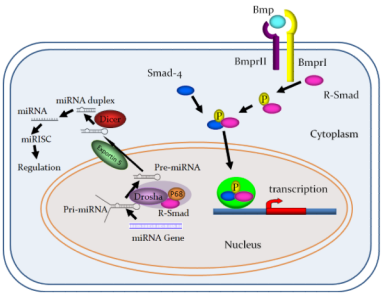

Figure 1. Summary of *Bmp*-signaling pathway

In the canonical pathway, Bmp ligands bind to type II receptors then activate type I receptors. Activated type I receptors phosphorylate R-Smads (Smad 1,5,8) and following release from the type I receptors, phosphorylated R-Smads associate with the common Smad4 to form complex, which can then initiate transcription of target genes. Recent findings indicate that Bmp signals regulate gene expression through pathways mediated by microRNAs. Bmp signaling can regulate miRNA expression via the canonical pathway, and in addition, Smad1/5 can bind to a sequence specific site in the primary (pri)-miRNA to recruit the Drosha complex and promote the processing of the pri-miRNA to the precursor (pre)-miRNA (Davis et al., 2008; Davis et al., 2010; Li et al., 2010).

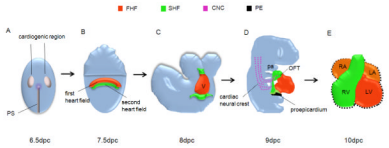

Figure 2. Summary of cardiac development

(A) Cardiac progenitor stage. About 6.5dpc, myocardial progenitor cells, which originate in the primitive streak (PS), start to migrate to the anterior bilateral fields of the embryo. (B) Cardiac crescent stage. About 7.5dpc, myocardial progenitor cells form the cardiac crescent and the first heart field (FHF) starts to give rise to differentiated myocardial cells. The second heart field (SHF) is located medially to the cardiac crescent at this stage. Shown in red: FHF location and contribution; Shown in green: SHF location and contribution (the same color coding as here for the lineage contributions to the heart at later stages in C to E). (C) Linear heart tube stage. About 8dpc, the cardiac crescent fuses at the midline of the embryo and forms a linear cardiac tube. SHF cells start extending anterior and dorsal at 8-8.5dpc to add to the heart tube. (D) Heart looping stage. The linear heart tube subsequently undergoes looping at about 8.5dpc. About 9dpc, the cardiac neural crest (CNC) cells (shown in purple) start to migrate into the outflow tract (OFT) and contribute to the developing heart tube. Proepicardium (PE) (shown in dark) located at the cardiac venous pole also start to emigrate onto the looping heart tube to form the epicardium. (E) Chamber formation stage. About 10dpc, the heart starts to form well-defined four chambers and chamber septation is completed by 12.5dpc (Buckingham et al., 2005).

pa, pharyngeal arch; LA, left atrium; LV, left ventricle; OFT, outflow tract; PS, primitive streak; RA, right atrium; RV, right ventricle.