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Review Article

Roles of TGFβ and BMP during valvulo–septal endocardial cushion formation

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Abstract The primordia of valves and the atrioventricular septum arise from endocardial cushion tissue that is formed in the outflow tract (OFT) and in the atrioventricular (AV) regions during cardiogenesis. Abnormal development of the endocardial cushion results in various congenital heart diseases. Endocardial epithelial–mesenchymal transformation (EMT) is a critical process in cushion tissue formation and is regulated by many factors, such as growth factors, intercellular signaling molecules, transcription factors, and extracellular matrices. A signal that is produced by the myocardium of the AV and OFT regions and transferred to the adjacent endocardium across the extracellular matrix mediates EMT. Studies in vitro and genetic analyses have shown that transforming growth factor β and bone morphogenetic protein play central roles in the regulation of EMT during cushion tissue formation.

Keywords Bone morphogenetic protein · Cushion tissue · Epithelial–mesenchymal transformation · Heart development · Growth factors · Transforming growth factor β

Abbreviations

AV	Atrioventricular canal
BMP	Bone morphogenetic protein
MCM	Myocardial conditioned medium
ODN	Oligodeoxynucleotide
OFT	Outflow tract
TGFβ	Transforming growth factor β

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Endocardial cushion tissue formation during heart development

The prospective heart region in vertebrates is formed bilaterally in the anterior lateral portion of the mesoderm at the tri-laminar germinal disc stage. As the embryo folds ventrally, the right and left splanchnic mesoderm containing future heart cells fuses in the ventral midline and forms a primitive heart tube. Progenitors of the endocardial cells arise from the splanchnic mesoderm during this process. The primitive heart tube consists of myocardium (outer layer) and endocardium (inner layer) that are separated by an expanded extracellular matrix, namely cardiac jelly. As development proceeds, the heart tube bends towards the right, and cardiac segments begin to appear in an anterior–posterior sequence: truncus arteriosus and conus cordis (outflow tract; OFT), bulbus cordis (presumptive right ventricle), primitive ventricle (left ventricle), atrioventricular (AV) canal, primitive atrium, and sinus venosus (De La Cruz et al. 1989). Endocardial cushion tissue is formed in the OFT and AV regions of the embryo by endocardial epithelial–mesenchymal transformation (EMT) of the endocardium to mesenchyme (Markwald et al. 1975, 1977) (Fig. 1). In addition to the endocardial-derived mesenchyme, mesenchymal cells from the cardiac neural crest migrate into the OFT region via the pharyngeal arches and contribute to the formation of the septum of the OFT region (Waldo et al. 1998). The cushion tissue of the OFT region subsequently gives rise to the aorticopulmonary septum and semilunar valves and that of the AV region generates AV valves and AV septum. As development proceeds, the

aorticopulmonary, outflow, interventricular, AV, and primary atrial septa align and fuse, resulting in the formation of a four-chambered heart. Consequently, thus, abnormal development of endocardial cushion tissues causes various heart malformations, such as transposition of the great arteries and AV septum defect (Sakabe et al. 2005).

The myocardium of the OFT and AV regions is considered to be important in the formation of endocardial cushion tissues. Bernanke and Markwald (1982) developed an in vitro model of cushion tissue using a three-dimensional collagen gel culture system (Fig. 2) in which the EMT occurs similarly to that observed in vivo, thus allowing the mechanism regulating this process to be analyzed. The results from other studies using this system suggest that only AV or OFT endothelial cells are able to transform and invade the gel lattice when cultured with associated myocardium—but not with ventricular myocardium (Krug et al. 1987; Mjaatvedt et al. 1987). Therefore, region-specific myocardial signals are important in the regulation of EMT during cardiogenesis. This review focuses on signaling regulated by transforming growth factor β (TGFβ) and bone morphogenetic protein (BMP) and reviews the mechanisms regulating valvo-septal endocardial EMT.

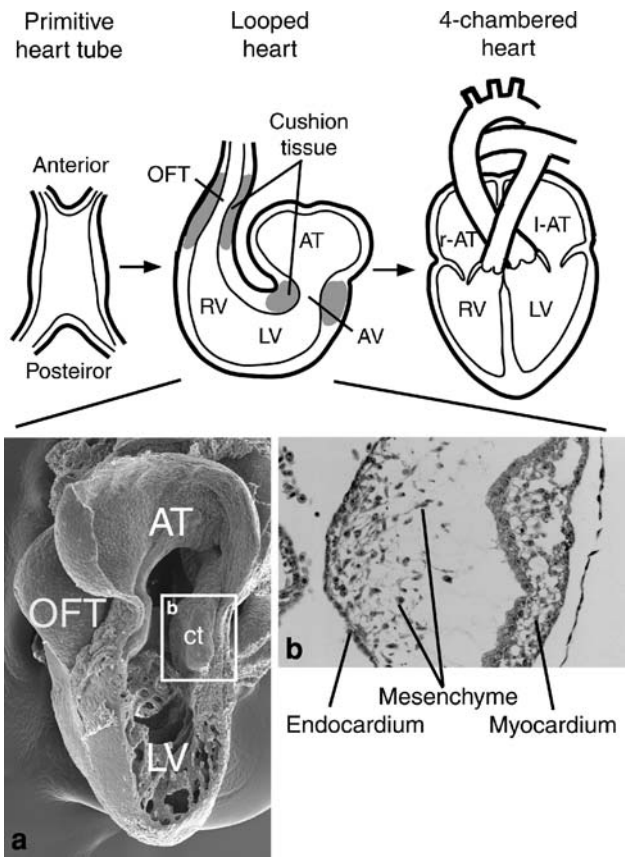

Fig. 1 Endocardial cushion tissue development. The primitive heart tube is formed through the fusion of the right and left precardiac mesoderm migrating into the ventral midline. As such, it generates the right-side bend (d-loop), and presumptive heart segments are established segmentally: outflow tract (*OFT* or conus cordis), right ventricle (*RV* or bulbus cordis), left ventricle (*LV* or primitive ventricle), atrioventricular canal (*AV*), and atrium and sinus venosus (*AT*). As development proceeds, valvoseptal endocardial cushion tissue is developed in the OFT and AV regions, and cardiac neural crest simultaneously migrates into the OFT region and contributes to OFT septation. Trabeculation is generated in the primitive ventricle, and right and left ventricular cavities are expanded progressively, resulting in the formation of the muscular septum between the RV and LV. OFT septa, including the aorticopulmonary and AV septa and the base of the primary atrial septum, align and fuse, resulting in a complete four-chambered heart. **a** Sagittal section of 3-day chick embryonic heart. Endocardial cushion tissue (*ct*) is being formed. **b** Frontal section of *boxed area* in **a**. Many mesenchymal cells are formed by endocardial transformation in cardiac jelly between endocardium and myocardium

Role of TGFβs during cushion tissue formation

The TGFβ superfamily comprises several subgroups, one of which is a class of secreted dimeric proteins. Over 30 members of the TGFβ superfamily have been identified from invertebrates or vertebrates, and they can be structurally categorized into various subgroups, such as activins/inhibins, nodals, BMPs, growth and differentiation factors (GDFs), and Müllerian inhibiting substance (MIS) (Miyazawa et al. 2002). Members of the TGFβ superfamily regulate numerous cellular functions, including growth, adhesion, migration, differentiation, and apoptosis. TGFβ1, 2, and 3 have been identified in mammals and birds, whereas TGFβ5 is found only in amphibians (Kingsley 1994). Several descriptions of TGFβ expression during embryogenesis suggest that this family plays important roles in the regulation of cell growth, differentiation, and cell–cell interaction during development (Heine et al. 1987; Lehnert and Akhurst 1988; Pelton et al. 1989, 1990, 1991; Akhurst et al. 1990; Flanders et al. 1991; Dickson et al. 1993; Millan et al. 1991; Roelen et al. 1994). During mouse heart development, TGFβ1 is initially expressed in the endocardium and then regionalizes to the endothelial cells overlying the cardiac cushion tissue (Lehnert and Akhurst 1988; Akhurst et al. 1990; Camenisch et al. 2002a, b; Molin et al. 2003). The endocardium and myocardium of the OFT and AV regions express TGFβ2 during cushion tissue formation (Millan et al. 1991; Dickson et al. 1993; Camenisch et al. 2002a, b; Molin et al. 2003), but TGFβ3 is expressed in the endocardium and mesenchymal cells after the onset of EMT (Millan et al. 1993; Pelton et al. 1990; Camenisch et al. 2002a, b; Molin et al. 2003). During chick endocardial cushion formation, both TGFβ2 and TGFβ3 are expressed in the endocardium, mesenchyme, and myocardium during cushion tissue formation (Boyer et al.

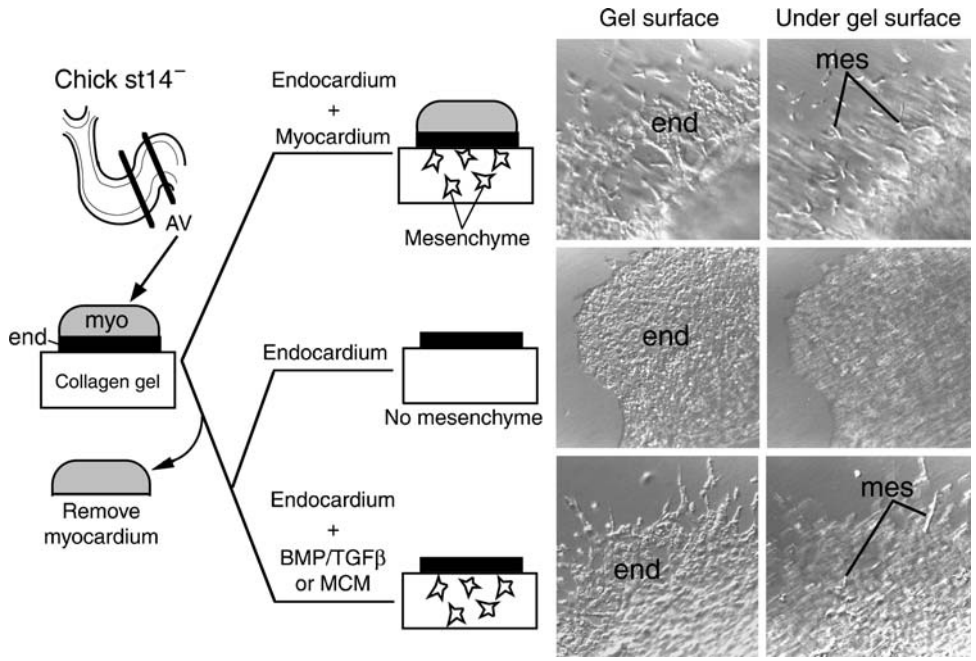

Fig. 2 Three-dimensional collagen gel bioassay. Explants of cardiac tissues collected from atrioventricular (*AV*) canal or outflow tract (*OFT*) regions of chick stage 14⁻ (or ED 9–9.5 mouse) hearts were placed on the surface of collagen gels for 24–48 h. Explants project many mesenchymal cells (*mes*) into the collagen gel lattice. The removal of explanted myocardium (*myo*) from the gel does not change the endothelial monolayer (*end*) phenotype to mesenchyme. Endothelial monolayers cultured with transforming promoters, such as embryonic myocardial conditioned medium (*MCM*), transforming growth factor β (*TGFβ*), and bone morphogenetic protein (*BMP*), develop mesenchymal cells in the collagen gel lattice

1999a, b; Nakajima et al. 1999; Yamagishi et al. 1999a, b). Thereafter, the expression profiles of TGFβs differ between mice and chicks, indicating that different TGFβs are required during murine and avian cushion tissue formation (Camenisch et al. 2002a, b).

The results of assays in vitro using a three-dimensional collagen gel culture system indicate that TGFβ plays an important role in EMT of the endocardium during cushion tissue formation (Potts and Runyan 1989; Potts et al. 1991; Nakajima et al. 1994; 1997b, 1998; Brown et al. 1996, 1999; Boyer et al. 1999a) (Fig. 2). Potts et al. (1991) demonstrated that the antisense oligodeoxynucleotide (ODN) to TGFβ3 can inhibit the EMT in AV endocardium co-cultured with associated myocardium. Nakajima et al. (1994) demonstrated that the expression of TGFβ3 in AV endocardium is induced by myocardially derived signals other than TGFβ3 and that TGFβ3 functions in an autocrine manner to induce phenotypic changes in endothelial cells.

The activation of the AV endocardium to form mesenchyme is a morphologically multistep process comprising cellular hypertrophy, cell–cell separation, lateral cell mobility, polarization of the Golgi apparatus, formation of migratory appendages, and invasion of the cardiac jelly (Markwald et al. 1975). The direct addition of TGFβ3 to pre-activated AV endothelial cells in a collagen culture system causes initial phenotypic changes in EMT, such as the loss of cell–cell contact, cellular hypertrophy, and migration to the gel surface, and, at this time, also induces the expression of α-smooth muscle actin (SMA), which is essential for migratory appendage formation in the activated endothelial cells (Nakajima et al. 1997a, 1999). However, TGFβ3 itself does not stimulate the invasion of endothelial cells into the gel lattice. Thus, based on the finding that TGFβ2 is expressed in the cushion tissue, TGFβ2 and TGFβ3 together would seem to synergistically act to induce EMT. However, the biological effect of TGFβ2 alone and in combination with TGFβ3 on pre-activated AV endocardium is similar to that of TGFβ3 (Nakajima et al. 1998).

Although in vitro experiments using endocardial cushion models support the notion that TGFβ is essential for epithelial–mesenchymal transformation during endocardial cushion tissue formation, neither TGFβ1- nor TGFβ3-null mice have any apparent cardiac malformation (Shull et al. 1992; Kulkarni et al. 1993, 1995; Proetzel et al. 1995). In the absence of maternal TGFβ1, cardiac abnormalities develop in TGFβ1-null mice, including poorly formed ventricular lumina and disorganized ventricular muscle and valves (Letterio et al. 1994). The development of the OFT

region is abnormal in TGFβ2-null mice (Sanford et al. 1997; Bartram et al. 2001). These results suggest that TGFβ isoforms are functionally redundant in the regulation of EMT and that a specific TGFβ isoform may act in a spatiotemporal-specific manner during cardiogenesis.

The secretion and activation of TGFβs is regulated by association with latent TGFβ binding proteins (LTBPs; LTBP1, LTBP 3, and LTBP 4) that belong to the LTBP/fibrillin family of extracellular matrix proteins (Koli et al. 2001). During mouse endocardial cushion formation, LTBP1 is expressed in transforming endothelial/mesenchymal and migrating mesenchymal cells and colocalizes with the mature region of TGFβ1 (Nakajima et al. 1997b). Congenital heart defects consisting of abnormal septation of the cardiac OFT develop in LTBP1-null mice (Todorovic et al. 2007). Therefore, it can be concluded that extracellular regulation of TGFβ activity is also essential for EMT during endocardial cushion tissue formation.

Role of BMPs during cushion tissue formation

Bone morphogenetic proteins are members of the TGFβ superfamily, and over 20 related proteins have been identified (Ducy and Karsenty 2000; Miyazono et al. 2005). Recent studies have demonstrated that BMPs are essential for embryogenesis and organogenesis. During murine cardiogenesis, BMP2 and BMP4 are expressed in the myocardium of the OFT and AV regions (Lyons et al. 1990; Jones et al. 1991), and BMP6 (Vgr-1) transcripts are localized in the myocardium of the OFT region and endothelial/mesenchymal cells of the AV region (Jones et al. 1991; Kim et al. 2001). BMP7 is expressed in the OFT and AV myocardium but not in the endocardium (Lyons et al. 1995; Dudley and Robertson 1997; Solloway and Robertson 1999). During chick heart development, BMP2 and BMP5 are expressed in the OFT and AV myocardium, and BMP7 is found throughout the myocardium (Wall and Hogan 1995; Yamagishi et al. 1999a, 2001; Yamada et al. 1999; Somi et al. 2004).

The expression profiles of BMP2 and BMP4 suggest that BMPs can be included in the group of myocardially derived signals that are required to regulate EMT. To understand the role of BMP, we isolated BMP2, BMP5, BMP6, BMP7, dorsalin-1, and GDF6/7 from the chick embryonic heart. Anti-BMP properties, such as an antisense ODN, dominant negative BMP type I receptor and noggin, have been found to inhibit EMT in AV explant cultures in vitro (Yamagishi et al. 1999a; Okagawa et al. 2007). To understand the biological effects of BMP2 on the AV endocardium, pre-activated AV endothelial monolayers were cultured with recombinant BMP2 protein. By itself, BMP2 does not induce phenotypic changes associated with EMT in the pre-activated AV endocardium; however, BMP2 does enhance TGFβ-induced initial phenotypic changes in EMT (Yamagishi et al. 1999a; Nakajima et al. 2000) (Fig. 2). Therefore, BMP appears to be one of the myocardially derived inductive molecules that may regulate EMT. Recent studies have revealed that the Rho–ROCK pathway plays a critical role in mesenchymal cell invasion/migration during EMT (Zhao and Rivkees 2004; Sakabe et al. 2006) and that TGFβ3, but not BMP2, can induce ROCK1 in transforming cells (Sakabe et al. 2008). These findings suggest that an additional myocardially derived signal is required to complete EMT. However, BMP2 could substitute for myocardium to induce EMT, induce TGFβ2 expression, and initiate EMT in cultured mouse AV endothelial monolayers (Sugi et al. 2004). The regulation of EMT is probably species-specific and thus different between mice and chicks.

Embryos deficient in BMP2 or BMP4 show embryonic lethality before cushion tissue formation (Winnier et al. 1995; Zhang and Bradley 1996). Therefore, the conditional knockout mouse generated using the Cre–loxP system has recently been used in genetic approaches to BMPs and their receptors. Cardiac-specific BMP2 deletion mice (Nkx2.5–Cre) have less cardiac jelly and insufficient AV cushion formation, but a normal OFT (Ma et al. 2005; Rivera-Feliciano and Tabin 2006). Less BMP2 is expressed in the OFT than in the AV region, thus other BMPs, such as BMP4 and BMP7, may be involved in the formation of OFT cushion tissue. Cardiac-specific BMP4 deletion mice (Nkx2.5–Cre) show abnormal morphogenesis of the pharyngeal arch arteries and defective OFT septation, suggesting that BMP4 functions in the local proliferation and migration of cardiac neural crest cells (Liu et al. 2004). Studies of mice with a myocardially specific BMP4 deletion (TnT–Cre) have shown that while BMP4 is dispensable for the initiation of cushion formation, it is specifically required for proper AV septation after cushion formation (Jiao et al. 2003). Although the hearts of BMP5-, BMP6- or BMP7-null mutant embryos develop normally, overall cell density and trabeculation are reduced, and the endocardial cushions are missing due to delayed heart growth and differentiation in the BMP5/7 double mutant (Solloway and Robertson 1999), and the formation of OFT septation is delayed, and valve and chamber morphogenesis is defective in the BMP6/7 double mutant embryo (Kim et al. 2001). Heterodimers composed of the DPP subfamily (BMP2, BMP4) and 60A (BMP5, BMP6, BMP7, BMP8 and PC-8), such as BMP2/7 and BMP4/7, exhibit a significantly higher level of biological activity than an equivalent amount of either of the relevant homodimers (Hazama et al. 1995; Suzuki et al. 1997a, b; Nishimatsu and Thomsen 1998). Consequently, a heterodimer of a

different BMP subfamily might be required to control EMT during cardiogenesis in vivo.

Signaling of TGFβ and BMP during cushion tissue formation

Members of the TGFβ superfamily bind to two distinct type II and type I serine/threonine kinase receptors, both of which are required for signal transduction (Schmierer and Hill 2007). TGFβ binds to TGFBR2 (TGFβRII: type II receptor) in combination with TGFBR1 (ALK5: type I receptor) or ACVRL1 (ALK1: type I receptor). TGFBR2 is expressed in the endocardium and myocardium during chick or mouse embryonic heart development (Brown et al. 1996; Mariano et al. 1998; Jiao et al. 2006). Low levels of TGFBR1 are expressed throughout the chick and mouse embryo, including the endocardium (Mariano et al. 1998; Desgrosellier et al. 2005), and ACVRL1 is expressed in the mouse embryonic endocardium (Roelen et al. 1997).

Embryos deficient in TGFBR2, TGFBR1, and ACVRL1 develop embryonic lethality (Oshima et al. 1996; Oh et al. 2000; Larsson et al. 2001), whereas the myocardial-specific deletion of TGFBR2 (TnT–Cre) results in a low incidence of cardiac defects (Jiao et al. 2006). Myocardial TGFβ signaling may not be essential for normal heart development. Endocardial-specific deletion of the TGFBR2 (Tie2–Cre) results in normal AV cushion development (Jiao et al. 2006). However, assays using the embryonic heart of this mutant mouse in vitro show that the endocardium does not cause EMT activation and transformation. In collagen culture assays using the chick embryonic heart, the addition of anti-TGFBR2 antibody blocks endocardial cell activation and subsequent migration during EMT (Brown et al. 1996). These results indicate that complementary mechanisms compensate for the loss of TGFBR2 in live embryos in support of EMT. A recent study using the AV culture system in vitro discovered that TGFBR1 mediates endothelial cell proliferation and activation according to developmental stage (Mercado-Pimentel et al. 2007). Additionally, mice with a neural crest-specific TGFBR2 deletion (Wnt1–Cre) or a TGFBR1 deletion (Wnt1–Cre) develop OFT defects (Choudhary et al. 2006; Wang et al. 2006). Reception of the TGFβ signal by the neural crest might be essential for cardiogenesis. Cushion tissue is not formed in ACVRL1-null mice (Sorensen et al. 2003). Complexes of type I and II receptors interact with co-receptors on the cell surface, such as the TGFβ type III receptor (betaglican) or endoglin. The TGFβ type III receptor is localized in the endocardium and mesenchyme of the AV cushion during chick heart development, and anti-TGFβ III antibody inhibits mesenchyme formation and migration in AV explants (Brown et al. 1999). Endoglin is expressed in the endocardium of chick and mouse embryonic heart, and the inhibition of its expression results in the perturbation of EMT in vitro: endoglin-null mice display embryonic lethal and cardiac malformation, including cushion tissue defects (Bourdeau et al. 1999; Arthur et al. 2000; Mercado-Pimentel et al. 2007).

The target genes of TGFβ during cushion tissue formation remain uncertain. Romano and Runyan (1999, 2000) showed that one target of TGFβ2 signaling is snail2 (slug) during chick endocardial cushion formation. Snail2, a zinc finger transcription factor of the Snail superfamily, is thought to be involved in epithelial–mesenchymal transitions (Barrallo-Gimeno and Nieto 2005). During mouse endocardial cushion tissue formation, Snail1, which is expressed in AV endothelial and mesenchymal cells, suppresses the expression of VE-cadherin, suggesting that TGFβs initiate EMT via snail gene induction (Timmerman et al. 2004).

ACVR1 (ALK2), BMPR1A (ALK3), and BMPR1B (ALK6) are type I receptors that transduce BMP signals and locate in the endocardial and mesenchymal cells during endocardial cushion tissue formation (Gu et al. 1999; Wang et al. 2005). The BMPR1A gene is ubiquitously expressed in the heart, whereas BMPR1B is not found in the developing heart (Dewulf et al. 1995). During chick cushion tissue formation, ACVR1, BMPR1A, and BMPR1B are expressed in the endocardium of the developing heart (Desgrosellier et al. 2005; Okagawa et al. 2007).

Mouse embryos deficient in ACVR1, BMPR1A, and BMPR2 (BMPRII: type II receptor) die before cardiac development (Mishina et al. 1995, 1999; Gu et al. 1999; Beppu et al. 2000). Endothelial-specific deletion of the ACVR1 (Tie2–Cre) causes defects in the AV septa and valves because of failure of the EMT (Wang et al. 2005). This mouse also expresses lower levels of msx1 and snail1 as well as reduced phosphorylation of BMP and TGFβ Smads. Studies of AV explant cultures of the chick embryonic heart in vitro have shown that anti-ACVR1 antibody inhibits EMT and that mis-expression of the constitutive active ACVR1 in non-transforming ventricular endocardial cells induces EMT (Lai et al. 2000; Desgrosellier et al. 2005). An ACVR1 deletion in the neural crest (Wnt1–Cre) results in cardiovascular defects, including persistent truncus arteriosus and abnormal maturation of the aortic arch, as well as the inability to express msx1 (Kaartinen et al. 2004). A deletion of BMPR1A only in the myocardium (αMHC–Cre) results in heart defects involving the interventricular septum, trabeculae, and AV cushion and decreased TGFβ2 expression specifically in the myocardium adjacent to the AV canal but not in the OFT (Gaussin et al. 2002). A deletion of BMPR1A in endothelial cells (Tie1–Cre) results in severely impaired EMT in the AV canal region (Song et al. 2007), whereas

that in the neural crest (Wnt1–Cre) causes a shortened cardiac OFT with defective septation, and embryos die in mid-gestation with reduced proliferation of the ventricular myocardium (Stottmann et al. 2004). Knockout mice expressing BMPR2 altered to reduce signaling capacity have defective septation of the conotruncus, whereas the atrioventricular valves are apparently unaffected (Délot et al. 2003).

Smad proteins are major intracellular mediators of signaling activated by TGFβ superfamily ligands. The Smad1, Smad2, Smad3, Smad5, and Smad8 proteins (receptor-regulated Smad: R-Smad) are phosphorylated and activated by type I receptors; they are also associated with the common partner, Smad4, to trigger transcriptional responses. Inhibitory Smad6 and Smad7 (inhibitory Smad: I-Smad) are associated with type I receptors and prevent R-smad activation (Miyazawa et al. 2002). The Smad6 mutant mouse develops multiple cardiovascular abnormalities, such as hyperplasia of the cardiac valves and OFT septation defects, indicating that Smad6 is functionally important in the regulation of endocardial cushion formation (Galvin et al. 2000). Smad6 expression is induced by the BMP signal via ACVR1 and preferentially inhibits BMP signaling (Yamada et al. 1999; Desgrosellier et al. 2005). Therefore, Smad6 appears to act as a negative regulator of EMT during endocardial cushion tissue formation.

Little is understood about the target genes of BMP signals during cushion tissue formation. The homeodomain transcription factors, msx1 and msx2, are BMP target genes (Chen et al. 1996; Watanabe and Le Douarin 1996; Barlow and Francis-west 1997; Suzuki et al. 1997b; Bei and Maas 1998), and msx1 is expressed in endocardial and mesenchymal cells during EMT (Chan-Thomas et al. 1993; Yamagishi et al. 2005). Mice deficient in BMP2 (Nkx2.5–Cre) or ACVR1 (Tie2–Cre; Wnt1–Cre) express lower levels of msx1 (Kaartinen et al. 2004; Ma et al. 2005; Wang et al. 2005), suggesting that BMP regulates msx1 via ACVR1. Preactivated AV endothelial cells cultured with BMP express msx1, but to a lesser extent than that induced by associated myocardium (Yamagishi et al. 2005), suggesting that in addition to BMP, other factors are required for msx1 expression. Although antisense ODN to chick Msx1 inhibited the invasion of mesenchymal cells in culture (Yamagishi et al. 2005), mouse embryos with a deletion of msx1 develop a normal heart (Satokata and Maas 1994). Double msx1/2 mutant mice have defective OFT development (Ishii et al. 2005; Chen et al. 2007). Thus, signal(s) via msx1/2 may regulate not only endocardial EMT, but also survival and expansion during cardiogenesis. To date, signal regulation of endocardial EMT is largely unknown. Further studies are required to fully elucidate the mechanisms regulating endocardial EMT.

Other signaling pathways regulating cushion tissue formation

The results from genetic analyses using gain- or loss-of-function have revealed that there are several signaling pathways regulating endocardial EMT. Vascular endothelial growth factors (VEGFs) regulate the blood vessel formation of angiogenesis as well as vasculogenesis during development (Ferrara et al. 2003). During mouse cushion tissue formation, VEGF-A mRNA is expressed in the myocardium and endocardium (Dor et al. 2001). Overexpression of VEGF-A in mice induces malformation of heart development caused by abnormal cushion tissue formation (Miquerol et al. 2000; Dor et al. 2001). Expression of myocardial VEGF-A mRNA is repressed by NFATs (nuclear factor of activated T cells), and this event is essential for the endocardium to transform into mesenchyme (Chang et al. 2004). The epidermal growth factor (EGF)–ErbB signaling network contributes to cushion tissue development (Iwamoto and Mekada 2006): null mice of HB-EGF or EGFR display hyperplasia of mesenchyme in cushion tissue (Chen et al. 2000; Iwamoto et al. 2003). In addition, the deletion of ErbB3, neuregulin-1, or has-2 (hyaluronan synthase-2) results in hypoplasia of mesenchyme in cushion tissue (Meyer and Birchmeier 1995; Erickson et al. 1997; Camenisch et al. 2000, 2002a, b). The Notch signaling pathway controls cell fate and has been demonstrated to be essential for cardiovascular development, including cushion tissue formation (High and Epstein 2008; Niessen and Karsan 2008). Null mice of Notch1, Rbpj, or Hey1/L (Notch target genes) fail to form cushion tissue (Timmerman et al. 2004; Fischer et al. 2007). Wnt/β–catenin signaling is known to control many developmental processes, but its roles in cushion tissue formation are still obscure. Wnt9a regulates mesenchymal cell proliferation during avian cushion tissue development, and the endothelial deficiency of β-catenin conditional knockout mouse leads to a lack of heart cushion (Liebner et al. 2004; Person et al. 2005). During zebrafish heart development, overexpression of Apc (adenomatous polyposis coli) and Dickkopf 1 (Wnt antagonist) blocks cushion tissue formation (Hurlstone et al. 2003). Neurofibromatosis type 1 (NF1), which encodes neurofibromin and is the responsible gene for the human genetic disorder neurofibromatosis can downregulate ras-activity. Therefore, the NF1 that is expressed in the endocardial cushions controls ras activity, and mice null-mutant for *Nf1* show an overabundance of cushion tissue in the OFT and AV regions (Lakkis and Epstein 1998). In addition to the above-mentioned signaling pathways, several transcription factors, cell adhesion molecules, and proteases have been reported to play a role in the regulation of endocardial EMT during cushion tissue formation.

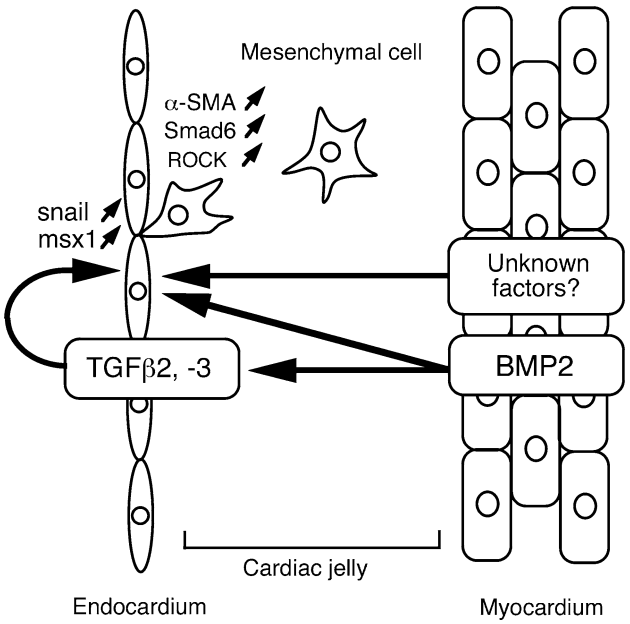

Fig. 3 Model of endocardial–mesenchymal transformation (EMT) in atrioventricular canal. Unknown factors and bone morphogenetic protein (*BMP*) secreted from the myocardium act on the endocardium across the cardiac jelly and synergistically induce EMT with endocardial transforming signals, such as transforming growth factor β (*TGFβ*). The resulting induced transcription factors (such as snail and msx1) in the endocardium translate the mRNA of mesenchymal-specific genes, such as α-smooth muscle actin (*α-SMA*), ROCK, and Smad6

Conclusion

Figure 3 shows a schematic model of EMT during cushion tissue formation. Both TGFβ and BMP play critical roles in the initial phenotypic change in the endocardium in EMT. Despite considerable insight, many questions must be answered to clarify the molecular mechanisms of cushion tissue formation. A few reports have addressed the target genes that are induced by BMP or TGFβ. Numerous factors, such as VEGF, NFATc, ErbB, Notch, β-catenin, and NF1, regulate endocardial EMT (Armstrong and Bischoff 2004). However, the signaling network among these factors during cushion tissue formation remains unknown. Further dissection of the mechanisms regulating valvo–septal cushion formation may provide valuable data towards furthering our understanding of the etiology of congenital heart defects as well as contributing to the development of therapeutic strategies aimed at combating heart diseases.

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