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Invited Review

TGFβ and BMP signaling in cardiac cushion formation: Lessons from mice and chicken

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ABSTRACT

Cardiac cushion formation is crucial for both valvular and septal development. Disruption in this process can lead to valvular and septal malformations, which constitute the largest part of congenital heart defects. One of the signaling pathways that is important for cushion formation is the TGFβ superfamily. The involvement of TGFβ and BMP signaling pathways in cardiac cushion formation has been intensively studied using chicken in vitro explant assays and in genetically modified mice. In this review, we will summarize and discuss the role of TGFβ and BMP signaling components in cardiac cushion formation.

Contents

1. Introduction

Congenital heart disease occurs in approximately 5% of all life births and thereby are the most common birth defects (Pierpont et al., 2007). Valvular and septal malformations constitute the largest part of life births (Hoffman and Kaplan, 2002). A key process in both valvular and septal development is the proper formation of the cardiac cushions (Briggs et al., 2012; DeVlaming et al., 2012; Van den Akker et al., 2012). The regulation of the formation of the cardiac cushions consists of a complex interplay between molecular and mechanical factors. The Transforming Growth Factor β (TGFβ) and Bone Morphogenetic Protein (BMP) signaling pathways are known to be major players during development and disease and are required for several aspects of heart development, including myocardial differentiation, and the development of the atrioventricular conduction system. TGFβ and BMP belong to the TGFβ superfamily that consists of more than 30

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members. The involvement of the TGFβ and BMP signaling pathways in cardiac cushion formation has been shown by using chicken in vitro explant assays and in genetically modified mice. The initial germline deletion of members of the TGFβ and BMP signaling pathway often caused early lethality. To bypass this early lethality conditional gene-targeting have been employed, which revealed the spatiotemporal requirement of many TGFβ and BMP components in cardiac cushion formation (Table 1). In this review, we will summarize and discuss the role of TGFβ and BMP signaling components in cardiac cushion formation. Elucidation of the role of the TGFβ and BMP in cushion and subsequently in septum and valve formation gives a better understanding of the etiology of congenital septum and valve defects.

2. TGFβ/BMP signaling

On the molecular level, the TGFβ/BMP signaling pathway functions through activation and nuclear localization of the small mothers against decapentaplegic (Smad) proteins which results in the cell type-specific activation of target genes (Fig. 1). Starting upstream in the TGFβ/BMP pathway a TGFβ, activin or BMP ligand binds to a heterodimer of type I and type II serine-threonine kinase receptors. For subsequent downstream activation, the type II receptor transphosphorylates the type I receptor at the SGSGSG sequence or so called GS (glycine/serine) domain (Kang et al., 2009). To date, there are seven type I receptors (the activin receptor-like kinases 1 through 7; ALK1-7) and five type II receptors known in mammals (Heldin et al., 1997; Schmierer and Hill, 2007; Shi and Massague, 2003) which in different combinations can bind different ligands.

TGFβ is synthesized in a biological inactive form and is secreted as a multiproteincomplex, which includes the latent TGFβ binding protein (LTBP). In the extracellular matrix (ECM) the TGFβ complex binds to fibrillin to regulate the temporal and spatial control of TGFβ signaling. TGFβ is activated by proteolytic enzymes, however, this process is incompletely understood (Kaartinen and Warburton, 2003). On most cells, TGFβ (TGFβ1, β2 and β3 in mammals) binds with high affinity to the TGFβRII leading to activation of the ALK5 (TGFβRI) receptor and downstream signaling. In a similar fashion, the activins and Nodal signal through the activin receptor type IIA and IIB (ActRIIA/IIB) and the type I receptors ALK4 and ALK7, respectively. In contrast to TGFβ and activin, some BMP ligands have a high affinity for a particular type I receptor (Sebald et al., 2004). For activation of the BMP pathway, the type I receptor ALK1, ALK2 (ActR-IA, ACVRI), ALK3 (BMPR-IA, BRK-1) or ALK6 (BMPR-IB, BRK-2) forms a complex with the type II receptors BMPRII, ActRIIA or ActRIIB.

An active complex of two serine—threonine kinase receptors at the membrane leads to phosphorylation and activation of the Smad proteins. Originally, these proteins can be divided into the TGFβ/activin (Smad2 and Smad3) and BMP (Smad1,5,8) mediated Smads. However, it has become clear that Smad molecules do not intrinsically belong to the TGFβ or BMP pathway. Activation of one of both signaling pathways largely depends on the receptors available on the cell surface. Furthermore, a particular cellular response is dependent on the concentration of ligands present in the extracellular space.

An either activated Smad2/3 or Smad1/5 complex recruits the common Smad4 protein. This complex of Smad proteins transduces the signal to the nucleus by interacting with a variety of transcription factors (Ross and Hill, 2008).

To control the cellular response, the activation of the TGFβ/BMP signaling pathway can be modulated on several levels. In the extracellular space antagonists, like Noggin and Chordin, interfere with BMP signaling by capturing the ligand away from the receptor complex (Krause et al., 2011; Zakin and De Robertis, 2010). The different concentrations of both ligand and antagonist form a bioactive gradient, which is important for pattern formation during early development (Miyazono, 2000). Another level of regulation is provided by the receptors betaglycan (TGFβRIII) and endoglin (CD105) which by themselves do not have signaling activity. Betaglycan regulates the cellular response to the TGFβ isoforms and has been shown to make the cells more sensitive to TGFβ2 signaling (Sankar et al., 1995). Endoglin, on the other hand, facilitates the binding of TGFβ1/3 and BMP9 to the ALK1 receptor.

In the context of angiogenesis, in endothelial cells, TGFβ binds to a receptor complex consisting of ALK1 and ALK5 in which ALK1 counteracts ALK5 mediated signaling (Goumans et al., 2003). On these cells, endoglin is needed for TGFβ/ALK1 signaling leading to pSmad1/5 activation (Lebrin et al., 2004). This promotes the migration and proliferation of endothelial cells, while signaling by TGFβ/ALK5/Smad2/3 inhibits these processes (Goumans et al., 2002). Furthermore, BMP9 binds with high affinity to ALK1 and endoglin leading to inhibition of endothelial cell proliferation in vitro (Scharpfenecker et al., 2007). Thereby, endoglin seems to play an important role in fine-tuning the state of endothelial cells (Goumans et al., 2009).

Downstream of the receptors, the inhibitory Smads provide a negative-feedback loop mechanism. Smad6 inhibits a BMP response by forming an inactive Smad1–Smad6 complex which prevents Smad1 from binding to Smad4 (Hata et al., 1998). Smad7 is a more general antagonist and is able to interfere with both BMP and TGFβ/activin signaling. Smad7 interacts with the activated type I receptor. Thereby it prevents the activation of Smad proteins and either the Smad2/3 or Smad1/5/8 signaling route to occur (Hayashi et al., 1997; Nakao et al., 1997; Yan and Chen, 2011). In summary, these modulation mechanisms, together with interference of other signaling pathways, fine-tune a cell-specific response. As a consequence, one signaling pathway has often different outcomes depending on the state and place of the cell during development as well as in adult tissues.

3. Overview cushion development

The cardiac cushions are the primordia for the valves and septa in the developing heart. They form by a process called endocardial-to-mesenchymal transformation (EMT; see also review by De Vlaming et al., 2012). This occurs in the atrioventricular canal (AVC), which separates the atria and ventricles, and the outflow tract (OFT), which connects the ventricles with the aortic sac. Initially, the early heart tube consists of an outer myocardial layer and inner endocardial layer separated by ECM, called the cardiac jelly (Fig. 2a). The endocardial cells in the AVC and OFT will become hypertrophic, lose their apical–basal polarity and cell–cell contact and migrate as mesenchymal cells into the cardiac jelly (Fig. 2B; Eisenberg and Markwald, 1995). This process has been intensively studied in several mouse models (Table 1) and by using an in vitro explant assay (Fig. 3), initially for chicken and later also for mouse explants (Lencinas et al., 2011). In this assay, AVC explants are cut open and put on a collagen gel with the endocardial face down. The endocardial cells will grow out of the explant and form a monolayer (Bernanke and Markwald, 1979; Runyan and Markwald, 1983). In this in vitro assay, three phases can be distinguished in the process of EMT (Barnett and Desgrosellier, 2003). In the first phase, termed activation, the endocardial cells become activated displaying separation from neighboring endocardial cells and elongation. In the second phase, termed invasion, the mesenchymal cells invade the matrix and in the third phase, termed migration, the mesenchymal cells migrate through the matrix. The ability to undergo EMT is unique