The Role of Cell Autonomous Signaling by BMP in Endocardial Cushion Cells in AV Valvuloseptal Morphogenesis

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Atrioventricular (AV) canal • Endocardial cushion • BMP-2 • BMP receptors • Valvuloseptal morphogenesis

Distal outgrowth and fusion of the mesenchymalized AV endocardial cushions are essential morphogenetic events in AV valvuloseptal morphogenesis. BMP-2 myocardial conditional knockout (cKO) mice die by embryonic day (ED) 10.5 [1] at the initial stage for the formation of endocardial cushions, hampering investigation of the role of BMP-2 in AV valvuloseptal morphogenesis at the later stages. In our previous study, we localized BMP-2 and type I BMP receptors, *BMPR1A* and *Alk2*, in AV endocardial cushions [2, 3]. Based on their expression patterns, we hypothesize that autocrine signaling by BMP-2 within mesenchymalized AV cushions plays a critical role during AV valvuloseptal morphogenesis. To test this hypothesis, we employed recently generated endocardial/endocardial cushion-specific cre-driver line *Nfact1*^{Cre}. Unlike a previously generated *Nfatc1*^{enCre} line whose cre-mediated recombination is restricted to AV

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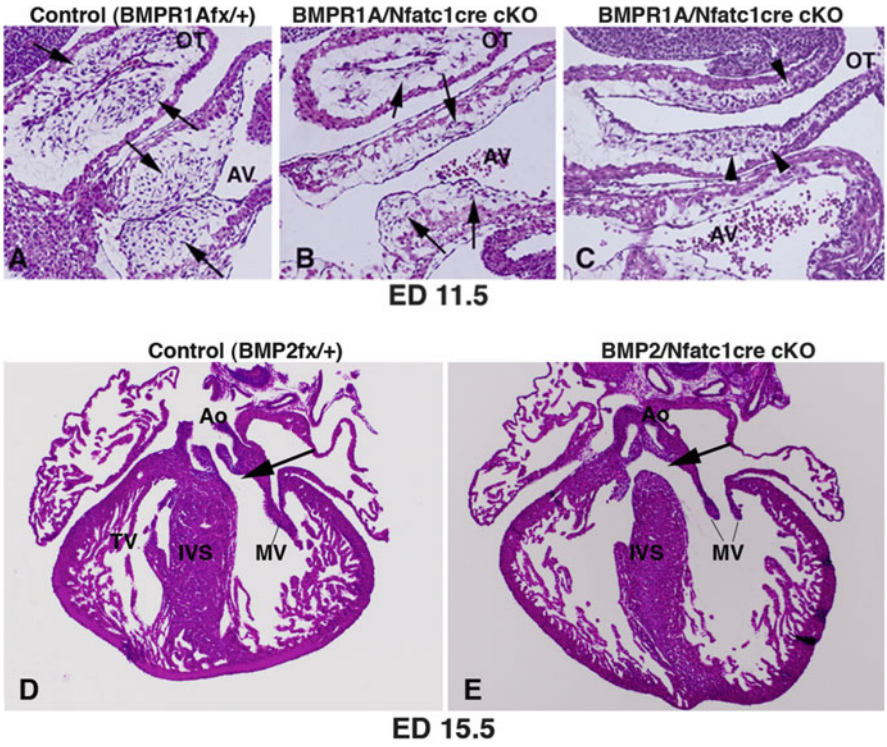

Fig. 22.1 (**a**–**c**) *BMPR1A cKO*^{Endo} mouse embryos exhibit failure of cellularization in AV cushions. *Arrows* indicate abundant mesenchymal cells in AV and outflow tract (OT) cushions in a control heart (**A**). *Arrows* show a few mesenchymal cells in AV and OT in a mutant heart (**B**). *Arrowheads* indicate abundant cells at the distal part of the mutant OT, which appear to be neural crest-derived and not reduced by *BMPR1A* endocardial/cushion-specific inactivation (**C**). (**d**, **e**) Endocardial/cushion-specific deletion of BMP-2 results in perimembranous septal defects (*arrow* in **E**), whereas control mouse heart shows well-formed ventricular septum (*arrow* in **D**). *Ao* aorta, *IVS* interventricular septum, *TV* tricuspid valves, *MV* mitral valves

and OT endocardium, this *Nfatc1*^{Cre} line confers cre-mediated recombination within the endocardial cells as well as their mesenchymal progeny. Using the *Nfactc1*^{Cre} driver line, we disrupted BMPR1A (Alk3) and BMP-2 specifically from AV endocardium and endocardial cushions. *BMPR1A* endocardial cushion cKO (*cKO*^{Endo}) mouse embryos died by ED 12.5 and exhibited failure of cellularization of AV cushions (Fig. 22.1a–c) and disruption of extracellular matrix (ECM) protein deposition in the cushion mesenchyme. On the other hand, AV cushion formation occurred in the *BMP-2 cKO*^{Endo} mice that survived beyond the AV cushion formation stage because BMP-2 expression remained intact in the AV myocardium during AV cushion formation. *BMP-2 cKO*^{Endo} mice exhibited perimembranous ventricular septal defects (VSDs) (Fig. 22.1d, e), defective deposition of ECMs in the membranous septum, and AV mitral valve dysplasia, suggesting the cell autonomous requirement of BMP-2 in AV endocardial cushions.

BMP-2 cKO^{Endo} did not exhibit muscular VSDs. These data strongly support our hypothesis that cell autonomous signaling by BMP-2 in the endocardial lineage plays a critical role in mesenchymalized AV cushions during AV valvuloseptal morphogenesis.

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