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Preview

BMPing up endocardial angiogenesis to generate coronary vessels

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Understanding how coronary vessels develop is important for designing better strategies to repair ischemic hearts. In this issue of *Developmental Cell*, D'Amato et al. report that BMP2 and CXCL12/CXCR4 act sequentially on endocardial cells to drive coronary angiogenesis and artery morphogenesis.

Coronary arteries are responsible for supplying blood to the heart tissue; their occlusion causes tissue ischemia, and this may result in myocardial infarction and heart failure. Therapies aiming to promote coronary angiogenesis or artery formation have been sought after as a possible treatment for ischemic disease, but to date, these attempts have not been successful.[1] A better understanding of the processes underlying the development and response to injury of cardiac vessels has the potential of yielding new, promising strategies for treating ischemic heart disease.

Endothelial cells (ECs) which form the coronary vessels (CVs) can arise from two distinct sources during development: ECs from the sinus venosus (SV) and from the endocardium. The first studies using lineage tracing and clonal analyses revealed a contribution from the endocardium to the capillaries and arteries of the myocardium, particularly in and near the septum.[2] Subsequent studies using temporally controlled lineage labeling showed that the embryonic endocardium contributes mostly to septal CV ECs and only minimally to CV ECs of ventricular free walls, and these studies proposed a second wave of differentiation and CV development from the endocardium shortly after birth.[2,3] Another more recent study challenged this view and proposed that endocardial-derived angiogenesis and contribution to CV ECs occurs prior to E16.5 and not postnatally.[4] However, important questions still remained unanswered: what are the early temporal dynamics of the endocardial to CV EC differentiation, and what are the molecular drivers of this process?

In a recent study published in this issue of *Developmental Cell*,[5] D'Amato et al. elegantly tackled these questions by combining the Bmx-CreERT2 and Rosa26$^{LSL\text{-}tdTomato}$ alleles with distinct floxed alleles and scRNAseq to label, fate map, and profile wild-type and mutant cells derived from endocardial (Bmx^{+}) cells.[5] They found that, at E11.5, the first endocardial-derived CV ECs arose and localized exclusively in the septum, whereas at E12.5 and E13.5, they were observed in the ventral myocardium, representing 80% of CV ECs in this area. Endocardial cells showed very little contribution to CV ECs when labeled after E11.5, and this is evidence that endocardial cells differentiate into CV ECs mainly during early stages of development. The authors also labeled endocardial cells at E17.5 and found little contribution to inner myocardial wall CV ECs (2.5%), a finding that suggests the absence of a major second wave of differentiation.

Given this data, D'Amato et al. proposed that endocardial-derived septal CV ECs differentiate and form between E8.5 and 10.5, and that these are the precursors of ventral CV ECs. After E11.5, there is minimal endocardial-to-CV fate conversion. These data are in contrast with a previous model that indicated that a second wave of CV ECs derives from postnatal endocardial *in situ* differentiation,[3] but they are consistent with a more recent report that demonstrated that this cell fate transition occurs mostly prior to E16.5[4].

To characterize the molecular mechanisms responsible for this process, D'Amato et al. first evaluated whether chemokine signaling mediated by the ligand-receptor pair CXCL12-CXCR4 was responsible for the migration of CV ECs from the septum to the ventral myocardium. This hypothesis was supported by the expression of the gene *Cxcl12* in the myocardium and *Cxcr4* in the septal CV ECs and by previous evidence of this signaling stimulating CV EC migration.[6,7] Interestingly, endocardial-derived ECs with deletion of *Cxcr4* still migrated and populated the ventral side of the heart, but subsequent coronary artery development was severely stunted. The use of a new Cxcr4 signaling reporter mouse generated by the authors confirmed that the pathway is activated in 90% of differentiated arteries ECs and only in 10% of CV ECs. scRNAseq data analysis allowed the authors to find that *Cxcr4* deletion does not affect the differentiation of endocardial-derived capillaries to pre-artery ECs, but rather their final migration and assembly to form coronary arteries.

In light of these results, the authors sought alternative pathways involved in the differentiation of endocardial cells to CV ECs and their sprouting. Through scRNAseq data analysis, they identified a population of transitory endocardial-derived CV ECs that are present in early but not later development stages and that express high levels of *Bmp2*. Expression of its receptor subunit *Bmpr1a* was found specifically in endocardial cells. Based on these results, D'Amato et al. hypothesized that the BMP2 ligand expressed by this cluster of newly formed CV ECs could signal to the BMPR1A receptor expressed by the adjacent endocardium and promote endocardial-to-CV ECs differentiation and sprouting. BMP signaling was previously involved in endothelial-to-mesenchymal transition and downregulation of endocardial genes.[8]

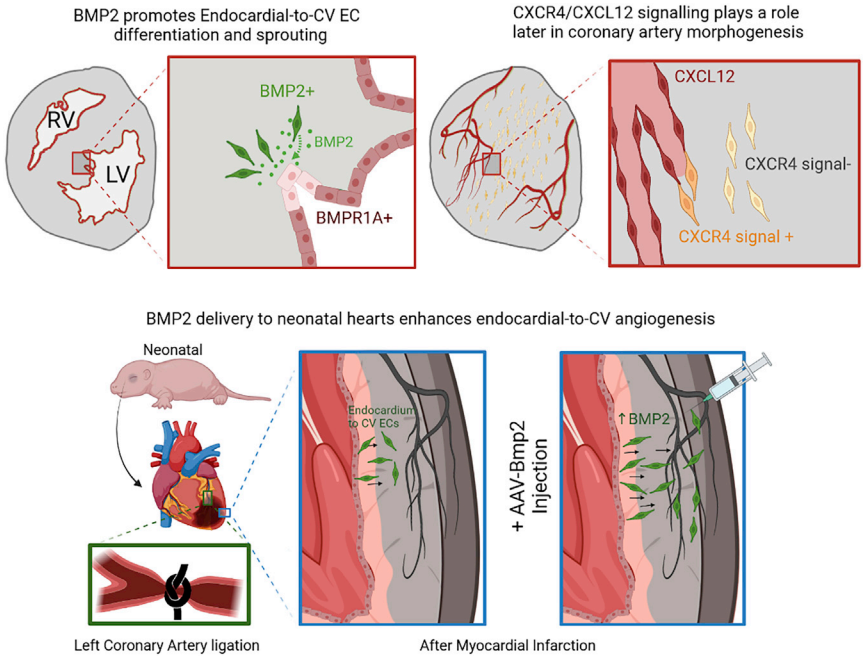

Figure 1. New proposed mechanisms for coronary artery development and regeneration

Upper panels: Proposed model for how BMP2-BMPR1A and CXCR4-CXCL2 signaling sequentially regulate coronary artery development from endocardial cells. BMP2 secreted from new endocardial-derived sprouts stimulates BMPR1A receptor signaling on the endocardium to promote endocardial-to-endothelial differentiation and sprouting. The CXCR4-CXCL12 signaling axis only plays a role later on, during the final steps of artery morphogenesis and maturation.

Lower panels: BMP2 delivery to infarcted mouse neonatal hearts promotes endocardial sprouting angiogenesis and heart regeneration.

To test this model, D'Amato et al. performed a combination of *in vitro* coronary sprouting assays together with *in vivo* studies where E9.5 embryos were treated with BMP receptor antagonist LDN-193189. Their results indicated that BMP2 signaling alone does not induce CV EC outgrowth, but it does greatly enhance VEGFA-stimulated endocardial sprouting and angiogenesis. Finally, the authors tested the potential role of BMP2 in promoting endocardial to CV vessel formation in ischemic hearts. They administered adeno-associated viral vectors (AAVs) expressing *Bmp2* into neonatal hearts subjected to myocardial infarction (MI). This promoted endocardial angiogenesis after MI. Overall, D'Amato et al. propose a model where BMP2 secreted from CV ECs of newly formed sprouts further stimulates BMPR1A receptor signaling and this is able to promote endocardial differentiation and sprouting when VEGFA is also present, such as in early embryogenesis or after MI (Figure 1).

Endocardial angiogenesis has previously been shown to occur also in injured adult hearts overexpressing Vegfb in cardiomyocytes[9] or Vegfr2 in endocardial cells.[10] Therefore, to build on the findings from this work, future studies should seek to characterize the impact and safety of BMP2 or BMP2+VEGFA delivery to adult mice infarcted hearts. This can result in a significant pro-regenerative response and provide a new therapeutic strategy for cardiac neovascularization after coronary occlusion.

DECLARATION OF INTERESTS

The authors declare no competing interests.

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