The Endocardium as a Master Regulator of Ventricular Trabeculation

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Xianghu Qu and H. Scott Baldwin

Abstract

Ventricular chamber morphogenesis, characterized by trabeculae formation, is crucial for cardiac function and embryonic viability and depends on cellular interactions between the endocardium and myocardium. The *ang1-Tie2* pathway is required for normal heart chamber development, but its molecular effectors are not well defined. Here we show that loss of *Tie2* in endocardial cells (EC) results in mid-gestation lethality due to heart defects including hyperplastic myocardium trabeculation (fewer but thicker trabeculae), thinner compact myocardium and simplified endocardial network. The thicker trabeculae phenotype results from enhanced proliferation of trabecular cardiomyocytes. Meanwhile, endocardial loss of *Tie2* reduces proliferation and production of ECs and impairs endocardial sprouting, which provides necessary support for trabecular assembly and extension, thus resulting in a dramatically simplified trabecular meshwork. These findings reveal two seemingly apposing functions of *Tie2* in myocardium trabeculation: ensuring normal trabeculation by supporting EC proliferation and sprouting while preventing hypertrabeculation by inhibiting trabecular cardiomyocyte proliferation.

X. Qu (✉)

Department of Pediatrics (Cardiology), Vanderbilt University Medical Center,
Nashville, TN, USA

e-mail: Xianghu.qu@vumc.org

H. S. Baldwin

Department of Pediatrics (Cardiology), Vanderbilt University Medical Center,
Nashville, TN, USA

Department of Cell and Development Biology, Vanderbilt University Medical Center,
Nashville, TN, USA

e-mail: scott.baldwin@vumc.org

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52.1 Introduction

Congenital heart diseases are some of the most common human birth defects. In many congenital heart diseases, genetic defects lead to impaired embryonic heart development or growth. A key development process in cardiac ontogeny is ventricular chamber formation. One of the first signs of chamber development is the formation of trabeculae [1]. Lack of trabeculation frequently causes embryonic lethality in mice and excess trabeculation causes cardiomyopathy and heart failure in humans [2]. A number of signaling molecules from myocardium, endocardium and cardiac extracellular matrix (ECM) have been implicated in trabeculation and an intimate communication between endocardium and myocardium promotes cardiomyocyte (CM) proliferation and differentiation leading to trabeculae formation [2]. The Notch signaling pathway has been the most intensely investigated in this process with Bmp10, Nrg1 and EphrinB2 identified as downstream targets of Notch signaling during trabeculation [3]. However, the molecular and cellular mechanisms controlling trabeculation are still poorly understood.

The Tie (tyrosine kinase with immunoglobulin-like and EGF homology) receptors, Tie1 and Tek/Tie2, are type 1 transmembrane protein receptor tyrosine kinases (RTKs) [4]. Along with the vascular endothelial growth factor (VEGF) receptors, these are the only known endothelial cell (EC)-specific RTKs. Although deficiency in Tie1 results in cardiovascular demise in utero [5–7], Tie1 is dispensable for cardiac trabeculation. Mutations in Tie2 have been described to cause ventricular septal defects and cutaneomucosal venous malformations in humans [8, 9]. Previous reports have suggested a role for Tie2 signaling in trabeculation because Tie2-null embryos have no trabeculae [5, 10] and deficiency of *Ang1*; the primary agonist for Tie2 results in a marked reduction in cardiac trabeculation [11, 12]. However, the molecular basis for these phenotypes has remained elusive. In addition, *Tie2*-deficient mouse embryos with abnormal trabeculation also had lethal vascular defects raising the question of whether the cardiac defects observed were primary or secondary to more global vascular compromise. Here, we show that loss of endocardial Tie2 results in embryonic heart failure that is characterized by impaired endocardial growth and hyperplastic trabeculation.

52.2 Mouse Models for Endocardial Specific Gene Deletion

In order to allow temporal and tissue-specific gene inactivation, we have developed a conditional allele of the mouse *Tie2* gene by introducing Cre recombinase recognition sites (*loxP*) into the *Tie2* locus. This strategy involved introducing a *loxP* site

Fig. 52.1 *Nfatc1^{Cre}* mediates *loxP* excision exclusively in the endocardium. X-gal staining of *R26^{slz};Nfatc1^{Cre}* heart sections at E12.5 shows that *Nfatc1^{Cre}*-mediated recombination is restricted to the embryonic atrial and ventricular endocardium but not in the endothelial cells (ECs) of the peripheral vasculature. RA right atrium, RV right ventricle, LA left atrium, LV left ventricle, IVS, interventricular septum

within the first intron, and another *loxP* site just upstream of the minimal promoter region. To specifically determine the role of *Tie2* in cardiac trabeculation, we utilized this floxed *Tie2* allele and an endocardial specific Cre transgenic line previously generated in our lab (*Nfatc1^{Cre}*) [13]. We first analyzed Cre activity by X-gal staining of *R26^{slz};Nfatc1^{Cre}* heart sections showing that Cre expression was restricted to the endocardium from E9.0 but not in the ECs of the peripheral vasculature (Fig. 52.1).

52.3 Tie2 Is Essential for Ventricular Chamber Development

Mice with a floxed *Tie2* allele were crossed with the *Nfatc1^{Cre}* line. We refer to *Nfatc1^{Cre/+};Tie2^{fl/fl}* mice as *Tie2* conditional knockout (*Tie2-cko*). Similar to the global deletion of *Tie2*, no endocardial *Tie2-cko* animals survived to birth. *Tie2-cko* embryos were grossly indistinguishable from their littermates until E12.5 at which point most began to show dorsal edema that increased until demise at E13.0–E13.5. Histological analysis of their hearts confirmed abnormal trabeculation, thin compact myocardium and ventricular septal defects compared with littermate controls (*Tie2^{+fl/fl}* and *Tie2^{fl/fl}*). Dual-fluorescence staining of heart sections with antibodies for troponin T (myocardial marker) and endomucin (which identifies ECs) (Fig. 52.2) revealed fewer but thicker trabeculae in *Tie2-cko* hearts. In addition, the endomucin-positive endocardial network and sproutings were abnormal.

To determine if the thicker trabeculae in *Tie2-cko* hearts were due to enhanced proliferation rate in myocardium, we assessed MCs proliferation rate by BrdU pulse labeling. There is no significant difference in the percentage of BrdU-positive cardiomyocytes in the compact zone between the control and *Tie2-cko* hearts at E11.5 and E12.5. However, in the trabecular zone the percentage of BrdU-positive MCs in

Fig. 52.2 Endocardial specific deletion of *Tie2* results in abnormal trabeculation. (a, b) Gross images of control (a) and *Tie2-cko* (b) embryos at E12.5 showing pericardial effusions (arrow) and edema (arrowhead) in the mutant embryos. (c, d) Control and *Tie2-cko* ventricular sections at E12.5 were stained with troponin T (myocardial marker, red) and endomucin (endocardial marker, green) antibodies. Compared to the control, the mutant heart had fewer but thicker trabeculae (arrows), thinner compact myocardium and simplified endocardial network

the *Tie2-cko* was significantly higher than that in the control, 36.6% vs. 24.6% at E11.5 and 32.4 vs. 11.8% at E12.5. Co-immunostaining for endomucin and Erg (EC-specific ETS transcription factor) revealed that the reduced endocardial sproutings in *Tie2-cko* mice was accompanied by a decreased number of ECs; the total endocardial EC number was determined by counting cells positive for Erg (Fig. 52.3). BrdU analysis also revealed that EC proliferation in the *Tie2-cko* endocardium was reduced by nearly 50% compared with control mice at E10.5 and E11.5. Thus, endocardial attenuation of *Tie2* leads to impaired endocardial growth and hyperplastic trabeculation.

In summary, we propose a model of trabeculation regulation by Endocardial *Tie2* (Fig. 52.4). In wild-type mice, at E9.0, the first stage or initiation of trabeculation is characterized by endocardial sprout formation and touchdown coincident with

Fig. 52.3 Endocardial attenuation of Tie2 results in decreased EC number and simplified endocardial network. Dual immunostaining of control and *Tie2-cko* heart sections (left ventricle) for Erg (red) and endomucin (green) at E11.5 reveals a significant decrease in Erg-positive endocardial cell number in the mutant ventricles (**b**) compared to control ventricles (**a**) following endocardial loss of Tie2. DAPI, blue

Fig. 52.4 Proposed model of trabeculation regulation by endocardial Tie2. In wild-type mice, myocardial trabeculation develops as a three-step model: stage 1 at E9.0, initiation of trabeculation, stage 2 at E9.25, trabecular assembly and stage 3 at E9.5, trabecular extension. In *Tie2-cko* mice, only limited endocardial sproutings reached the outer layer myocardium. Although the myocardial wall in mutant ventricles was able to thicken to a multicellular layer and form sheet-like protrusions (myocardial lamina), assembly and extension of trabeculae were impaired, thus leading to thicker but reduced trabeculae. *CJ* cardiac jelly, *CM* cardiomyocytes, *End*, endocardium

myocardial delamination. Next endocardial sproutings progress laterally beneath the myocardial lamina resulting in isolated clusters of myocardial cells within a “bubble” of cardiac ECM, covered by endocardium. By E9.25 the endocardial sprouting-enveloped myocardial lamina assemble to isolated short trabecular clusters (stage 2: assembly), which is followed by trabecular extension (stage 3) at E9.5, leading to finger-like long trabecular structures. In *Tie2-cko* mice, only limited

endocardial sproutings reached the outer myocardial layer. Although the myocardial wall in mutant ventricles was able to thicken and form multicellular sheet-like protrusions (myocardial lamina), assembly and extension of trabeculae were impaired, thus leading to thicker but reduced trabeculae.

52.4 Future Directions and Clinical Implications

Despite the absolute requirement for functional Tie2 signaling in normal cardiovascular development as well as its essential role in the progression of adult heart and vascular diseases, there is a paucity of information delineating the mechanism(s) of Tie2 activation or the subsequent signaling events that modulate heart ontogeny. This work reveals a critical role for the endocardium in the orchestration of ventricular trabeculation which is dependent on endocardial RTK Tie2 signaling. Tie2 not only plays a primary autocrine function in support of EC proliferation and angiogenic sprouting and touchdown but also is instrumental in the generation of paracrine signaling that results in inhibition of trabecular CM proliferation that prevents hypertrabeculation. Further investigation on this project will focus on delineating downstream targets of Tie2 activation in both endocardium (cell autonomous effect) and myocardium (paracrine effect) during ventricular trabeculation. These new insights will be important in further defining mechanisms of disease pathology related to ventricular chamber morphogenesis and function and will ultimately be critical in elucidating the etiology of congenital heart diseases.

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