Part VI

Vascular Development and Diseases

Perspective

H. Scott Baldwin

While the critical pathways that are important for normal cardiac development have focused extensively on transcriptional regulation of myocyte differentiation, critical mediators of vascular development have received much less attention. One reason for this has been the inability in the past to manipulate gene expression in a temporal and tissue-specific manner. There is no doubt that both normal vascular and normal myocardial development are essential for early embryonic survival and the two are inextricably linked; normal vascular development requires normal flow, and maturation of the myocardium requires simultaneous maturation and remodeling of the extracardiac vasculature. Ubiquitous or global gene deletions, resulting in both cardiac and extracardiac mutations, have resulted in numerous "chicken and egg" quandaries: Did the heart fail because of a primary defect in heart development, or were the defects merely secondary to upstream perturbations in extracardiac vascular defects? In this section, investigators used tissue-specific mutagenesis strategies as well as a focus on cell membrane and extracellular matrix regulation to begin to elucidate important aspects of extracardiac vascular development that are particularly relevant to human disease. Sakebe et al. generated an endothelial-specific deletion of Hrt2/Hey2, repressors of Notch signaling, to demonstrate that both Hrt1 and Hrt2 are essential for vascular development independent of their role in myocardial development. Furthermore, they suggest that the

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endothelial or vascular processes mediated by these factors, rather than the defects in myocardial development, might be the primary mechanism for embryonic demise. Exploring the role of calcium signaling in extraembryonic vascular development, Uchida and colleagues were able to document dramatic defects in placentation as early as E9.5 in the mouse as a result of combinatorial deletion of the inositol IP3 receptors. This work clearly establishes a role for calcium handling in cardiovascular viability. Changing the focus to later stages of vascular development, Dr. Imanaka-Yoshida provides a detailed description of the role of the extracellular matrix protein, tenascin-C, in smooth muscle cell recruitment of both the descending aorta and coronary arteries and provides in vitro evidence that tenascin-C promotes SMC precursor expansion and differentiation by augmenting PDG-BB/PDGFR-β signaling. Finally, Yoshikane et al. show the potential importance of delineating the role of tenascin-C in normal and abnormal coronary artery remodeling as they discuss a model of the most common acute systemic vasculitis in children, Kawasaki disease. By studying the inflammatory and abnormal vascular remodeling induced by *Candida albicans*, they demonstrate accentuation of tenascin-C expression associated with aneurysm formation. Furthermore, they document that inhibition of JNK signaling attenuated aneurysm formation potentially providing a mechanistic link between JNK signaling and tenascin-C signaling that could provide a therapeutic target for treatment of Kawasaki disease. In summary, the investigations presented in this section provide an overview of exciting work that expands the focus of cardiovascular development and disease beyond myocyte transcriptional regulation and provides new insights into extracardiac vascular development and remodeling while emphasizing the importance that the extracellular matrix is ontogeny of cardiovascular disease.

Extracellular Matrix Remodeling in Vascular Development and Disease

29

Kyoko Imanaka-Yoshida

Abstract

Blood vessels constantly subjected to mechanical stress have well-developed elastic fiber-rich frameworks, which contribute to the elasticity and distensibility of the vascular wall. Destruction of the fibrous structure due to genetic predisposition as well as acquired disorders such as Kawasaki disease often induces irreversible dilation of blood vessels, e.g., aneurysm formation. In addition to their structural role, extracellular matrix molecules also provide important biological signaling, which influences various cellular functions. Among them, increased attention has been focused on matricellular proteins, a group of non-structural extracellular matrix (ECM) proteins highly upregulated in active tissue remodeling, serving as biological mediators by interacting directly with cells or regulating the activities of growth factors, cytokines, proteases, and other ECM molecules. Tenascin-C (TNC) is a typical matricellular protein expressed during embryonic development and tissue repair/regeneration in a spatiotemporally restricted manner. Various growth factors, pro-inflammatory cytokines, and mechanical stress upregulate its expression. TNC controls cell adhesion, migration, differentiation, and synthesis of ECM molecules. Our recent results suggest that TNC may not only play a significant role in the recruitment of smooth muscle/mural cells during vascular development, but also regulate the inflammatory response during pathological remodeling. TNC may be a key molecule during vascular development, adaptation, and pathological tissue remodeling.

Keywords

Tenascin • Extracellular matrix • Coronary artery • Aorta

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29.1 Introduction

Tissue, including the cardiovascular system, is composed of diverse cells and the extracellular matrix (ECM) synthesized by those cells. Several ECM molecules form a fibrous framework and provide structural support for the tissue. Blood vessels constantly subjected to mechanical stress have a well-developed fibrous framework, which contributes to the elasticity and distensibility of the vascular wall in concert with vascular smooth muscle cells. Highly ordered structures consisting of cells and fibrous elements are formed during development and are remodeled during tissue repair/regeneration after injury. In addition to their physical role, several ECM molecules provide important biological signaling, which influences various cellular functions in physiological and pathological tissue remodeling. In particular, ECM, termed matricellular protein, has attracted increasing attention as a biological mediator. Tenascin-C (TNC) is a prototype matricellular protein expressed during embryonic development and tissue repair after injury. This chapter will focus on the role of TNC in vascular development, especially coronary arteries and the aorta.

29.2 Extracellular Matrix in Vascular Wall

Blood vessels have abundant fibrous matrix tissue: well-developed elastic fibers in the medial layer and rich collagen fibers in adventitia. It is known that several gene mutations related to these fibrous components cause vascular fragility, eventually leading to aneurysm formation or dissection. For example, the collagen gene and fibrillin-1 gene, which is important for microfibril formation, have been identified as the genes responsible for Ehlers-Danlos syndrome (reviewed in [1]) and Marfan's syndrome [2], respectively. In addition to genetic predisposition, inflammation of blood vessels in acquired disease may induce fragmentation and destruction of normal elastic fibers in the vascular wall and causes irreversible dilation of blood vessels. For example, coronary aneurysm formation is sometimes seen in patients with Kawasaki vasculitis, one of the most common acquired heart diseases in children. Evidently, the structural support by fibrous ECM is essential to maintain the proper morphology and function of blood vessels.

Besides these fibrous elements, unique ECM molecules, matricellular protein [3], have attracted considerable attention. The matricellular proteins have common unique properties: (1) do not contribute directly to structures such as fibrils or basement membranes; (2) high levels of expression during embryonic development and in response to injury; and (3) binding to many cell surface receptors, components of ECM, growth factors, cytokines, and proteases [4]. This is a growing family originally including SPARC, tenascin, and thrombospondin [3].

29.3 Tenascin-C in Vascular System

Tenascins are a family of four multimeric extracellular matrix glycoproteins: tenascin-C, X, R, and W [5]. The first member, tenascin-C (TNC), is a typical matricellular protein. It is a huge molecule of about 220–400 kDa as an intact monomer and is assembled with a hexamer. The molecule consists of an N-terminal assembly domain, followed by EGF-like repeats, constant and alternatively spliced fibronectin type III repeats, and a C-terminal fibrinogen-like globular domain. Several receptors including integrins, EGFR, annexin II, syndecan-4, and toll-like receptor 4 (TLR-4) bind to the respective domains of TNC and transmit multiple signals (see [6]). Numerous studies have shown that TNC can control the balance of cell adhesion and de-adhesion, cell motility, proliferation, differentiation, and survival (reviewed in [5–7]). Recently, the role of TNC in the modulation of inflammation is highlighted [8].

Tenascin-C is found in many developing organs, including the cardiovascular system, but is often restricted transiently to specific sites, for example, near migrating cells and at sites of epithelial–mesenchymal/mesenchymal–epithelial transition. In normal adults, tenascin-C expression is sparsely detected; however, marked expression is seen in injury, regeneration, and cancer at sites where the tissue structure is being dynamically remodeled. Various factors, including growth factors and pro-inflammatory cytokines, can activate TNC expression (reviewed in [9]). It is particularly of interest that mechanical stress is an important inducer of TNC. Moreover, it is also noteworthy that TNC itself is an elastic molecule and may contribute to tissue elasticity [10].

As well as in other tissue, the expression of TNC in the normal vascular wall is low and upregulated in pathological conditions. The major source is medial smooth muscle cells [11]. However, TNC in the vascular system appears more complex in contrast to the heart [7]. For example, constitutive expression of TNC is observed in the medial layer of the abdominal aorta of normal adult mice but not in the thoracic aorta [12].

29.3.1 Development of Aorta and Tenascin-C

The origin of vascular smooth muscle cells (VSMC) of the aorta is heterogeneous [13]. The second heart field gives rise to VSMC of the root of the aorta. The cardiac neural crest contributes ascending and arch portions of the aorta. The origin of VSMC of the descending aorta is more complex. Primitive VSMC of the thoracic aorta originate from the lateral plate mesoderm and are replaced by cells derived from the paraxial mesoderm (somites). Moreover, individual somites build up restricted spatial domains of the "segmental" aortic wall. However, no evident segmental expression pattern of TNC is observed during development of the aorta. In E12–13 mouse embryos, very weak expression of TNC is observed in the ascending aorta and pulmonary truncus. Whereas elastic fibers in the medial layer of the aorta become mature around E12–13, the expression of TNC is upregulated after ED14–15 (Fig. 29.1) when the systemic circulatory system is established. This upregulated expression of TNC may reflect the increased hemodynamic stress on the aortic wall.

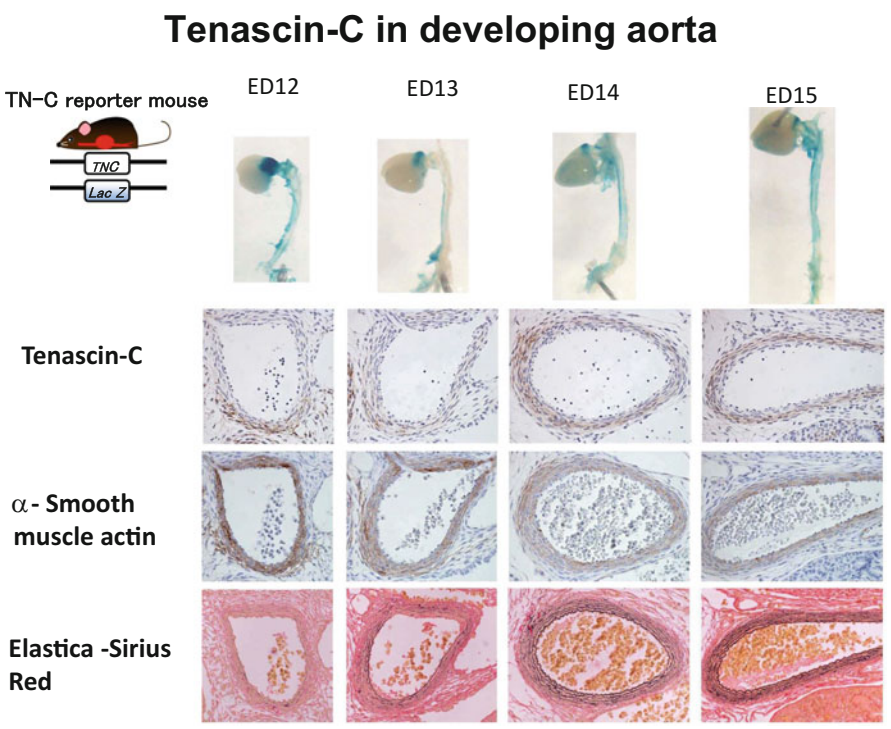

Fig. 29.1 Expression pattern of TNC during development of the aorta. Whole mount lacZ staining and histological sections of descending portion of the thoracic aorta of TNC-reporter mouse embryos at ED12–15. The sections were immunostained with anti-TNC or anti-α-smooth muscle actin or stained with elastica sirius red

29.3.2 Development of Coronary Artery and Tenascin-C

Coronary vessels are formed with the cells originating mostly from extracardiac tissue known as the proepicardial organ (PE) (see [14] for review). During coronary development, strong expression of TNC is observed, closely associated with thickening of the medial layer when the primitive coronary vasculature connects with the aortic sinuses [15], suggesting a significant role of TNC in maturation of the wall of coronary arteries. Indeed, TNC accelerates the differentiation of mesenchymal cells of PE to smooth muscle cells in culture [15]. Maturation of the vascular wall is regulated by various signaling pathways. In particular, the PDGF-BB/PDGFR-β signaling loop is known to be a key regulator of smooth muscle cell recruitment. In vitro, TNC amplifies crosstalk signaling between integrin αvβ3 and PDGF receptor (PDGFR) -β in smooth muscle cells, followed by enhancing cell proliferation and migration [16]. TNC may promote smooth muscle precursor expansion and differentiation in maturation of the vascular wall by enhancing PDGF-BB/PDGFR-β signaling (Fig. 29.2).

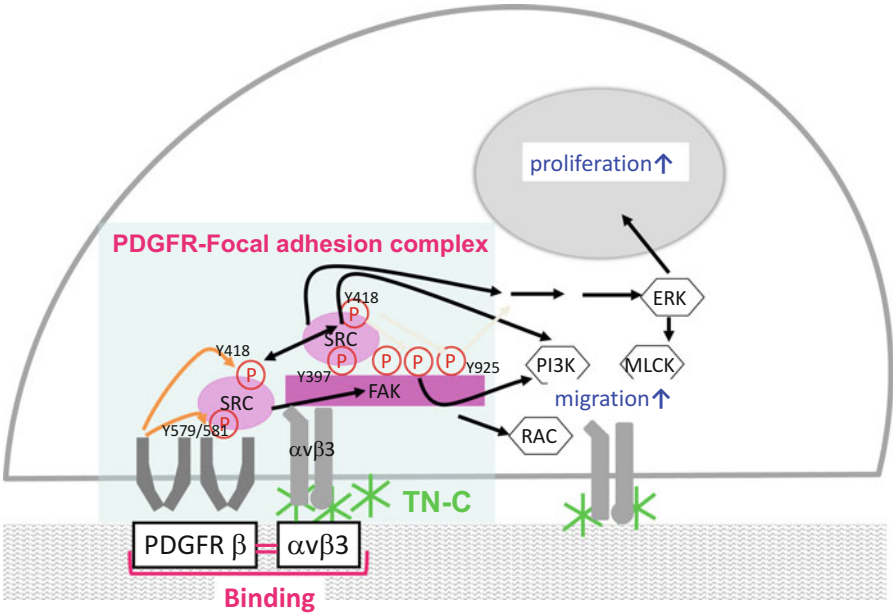

Fig. 29.2 Crosstalk signaling between TNC/integrin αvβ3 and PDGF-BB/PDGFR-β in vascular smooth muscle cells (Adapted from Ref. [7])

29.4 Future Direction and Clinical Implications

The characteristic spatiotemporally restricted expression of TNC has suggested its significant role during embryonic development. Several in vitro functional assays support this possibility. Although the grossly normal phenotype of knockout mice suggests the importance of redundancy and compensatory mechanisms during embryonic development, it is not straightforward to understand its molecular function. Meanwhile, TNC expression is linked to a range of vascular diseases, such as aortic aneurysm, acute aortic dissection, and Kawasaki disease (reviewed in [11, 17], also see Yoshikane et al. in this proceeding). Increasing numbers of studies have reported that TNC is highly upregulated, associated with inflammation and destruction of the vascular wall, suggesting that TNC may be a diagnostic biomarker. Furthermore, we have succeeded in endovascular treatment of a rat aneurysm model with a TNC-coated coil [18]. Although TNC could contribute to both favorable and undesirable effects during pathological processes in a context-dependent manner, it could be a potential therapeutic target for vascular disease.

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The "Cardiac Neural Crest" Concept Revisited

30

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Abstract

Neural crest cells (NCCs) are a unique stem cell population, which originate from the border between the neural plate and surface ectoderm and migrate throughout the body to give rise to multiple cell lineages during vertebrate embryonic development. The NCCs that contribute to heart development, referred to as the cardiac NCCs, have been assigned to the neural crest at the level of the postotic hindbrain. Recently, we found that the NCCs from the preotic region migrate into the heart and partially differentiate into coronary artery smooth muscle cells. This finding indicates that the origin of the cardiac

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NCCs appears more widely extended to the anterior direction than Kirby et al. first designated.

Keywords
Neural crest • Preotic • Postotic • Coronary artery • Endothelin

30.1 Introduction

The neural crest (NC) was first identified by Wilhelm His as "Zwischenstrang," the intermediate cord, in 1868 [1], the year of Meiji Ishin, the westernizing revolution of Japan. It is located at the border between the developing neural plate and surface ectoderm and serves as a source of migratory cells spreading throughout the body. The NC research was greatly accelerated by the establishment of quail-chick chimera technique accomplished by Nicole Le Douarin [2]. This technique enabled tracing the origin and fate of the NC during embryonic development and revealed that NC cells (NCCs) differentiate into a wide variety of cell types including neurons, glia, pigment cells, and craniofacial bones and cartilages in different developmental contexts [2]. Thus, NCCs are nowadays regarded as a multipotent stem cell population with unique differentiation capacities.

30.2 Cardiac Neural Crest Arising from the Postotic Region

Since Margaret Kirby discovered that NCCs at the level of occipital somites 1–3 migrate to the region of the aorticopulmonary septum [3], the concept "cardiac neural crest" has prevailed to cover NCCs contributing to the formation of the heart and great vessels. NCCs arising from the postotic hindbrain posterior to the mid-otic vesicle, corresponding to rhombomeres (r) 6–8, migrate into the third, fourth, and sixth pharyngeal arches and contribute to the formation of the tunica media of pharyngeal arch artery-derived great vessels, the aorticopulmonary septum, and the outflow tract endocardial cushion as well as some noncardiac organs such as the thymus, parathyroid glands, and thyroid glands [4]. Ablation of the cardiac NC in chick embryos results in aortic arch anomalies and persistent truncus arteriosus [3, 5, 6]. In addition to direct contribution to the cardiovascular structure, cardiac NCCs affect the migration and alignment of myogenic precursors from the second heart field migrating into the outflow region.

Chromosome 22q11.2 deletion syndrome, formerly known as DiGeorge syndrome, velocardiofacial (Shprintzen) syndrome, and conotruncal anomaly face (Takao) syndrome, is a disease complex characterized by craniofacial, thymic, and parathyroid anomalies and cardiac manifestations including tetralogy of Fallot, persistent truncus arteriosus, and aortic arch anomalies [7]. This syndrome was formerly recognized as an NC disorder because of its resemblance to the avian phenotype of NC ablation. However, identification and analysis of the responsible

genes in the 22q11.2 locus such as *TBX1* and *CRKL* and related factors have revealed that the pathogenesis is far more complex, involving interaction among NCCs, second heart field, endoderm, and other cell components.

30.3 Endothelin Signal and Neural Crest Development

Endothelin (Edn)-1 (Edn1), originally identified as a potent vasoconstrictor peptide, is a key regulator of craniofacial and cardiovascular development, acting on NCCs expressing Edn receptor type A (Ednra), a G protein-coupled receptor [8–10]. Inactivation of Edn1-Ednra signaling causes homeotic-like transformation of the lower jaw into an upper jaw structure and cardiovascular anomalies similar to chromosome 22q11.2 deletion syndrome. The craniofacial and cardiovascular anomalies are attributed to the disordered development of cranial (preotic) and cardiac (postotic) NCCs, respectively. In craniofacial development, the Edn1-Ednra signaling activates $G\alpha_q$-/$G\alpha_{11}$-dependent pathway, resulting in the induction of *Dlx5*/*Dlx6*, homeobox genes critical to ventral (mandibular) identity of the pharyngeal arches [10–12]. In cardiovascular development, the *Edn1*-/*Ednra*-null phenotype of aortic arch anomalies is independent of *Dlx5*/*Dlx6* [13], indicating that the Edn1-Ednra signaling pathway appears differently involved in craniofacial and cardiac development.

30.4 Preotic Neural Crest Contributing to Heart Development

Recently, we identified an additional cardiac phenotype of *Edn1*-/*Ednra*-null mice in the coronary artery [14]. The mutant mice exhibit marked dilatation of the septal branch and abnormalities of orifice and proximal branch formation. Labeling of NCCs using *Wnt1-Cre;Rosa26R* reporter mice revealed that NCCs contribute to coronary artery smooth muscle cells in the proximal region and septal branch, and NCC-derived smooth muscle cells are hardly detected in the smooth muscle layer in *Edn1*-/*Ednra*-null embryos. Correspondingly, NCC-specific knockout of $G\alpha_{12}$/$G\alpha_{13}$ rather than $G\alpha_q$/$G\alpha_{11}$ results in similar dilatation of the coronary artery septal branch [15], indicating that Edn1/Ednra signaling is necessary for NCC recruitment to coronary artery formation via $G\alpha_{12}$/$G\alpha_{13}$ and downstream Rho signaling.

Here, we faced to a conundrum where the NCCs came from. It had long been controversial whether and how NCCs contribute to coronary artery formation. Although NC-derived cell clusters are formed in association with the proximal portion of coronary arteries, quail-chick chimera experiments have shown that the cardiac NCCs do not differentiate into coronary smooth muscle cells [16, 17]. In contrast, *Wnt1-Cre* mice have indicated the possible direct involvement of NCCs as the source of coronary artery smooth muscle cells [18]. The apparent discrepancy was sometimes ascribed to differences in species, but no definite explanation had been given for it.

This controversy was settled by quail-chick chimera experiments, in which different regions of the chick neural folds were homotopically replaced by quail tissues. When the cardiac (postotic) NC at the level of r6-r8 (posterior to the mid-otic vesicle) was replaced, no contribution of quail NCCs to the wall of coronary arteries was observed. In contrast, replacement of the NC by exchanging the midbrain and preotic hindbrain (r1-r5) neural folds anterior to the otic vesicle resulted in a significant number of quail NCCs distributing into the heart and differentiating into coronary artery smooth muscle cells. The intracardiac migration of preotic NCCs and their contribution to the coronary artery smooth muscle layer were also confirmed by experiments using *R4-Cre;Z/AP* reporter mice, in which r4-derived preotic NCCs were specifically and permanently labeled. Furthermore, ablation of the preotic NC in chick embryos caused abnormalities in coronary septal branch and orifice formation, reminiscent of the *Edn1-/Ednra*-null phenotype.

Are preotic and postotic NCCs spatially segregated within the heart region to play distinct roles? Double labeling with different dyes of premigratory NCCs at the levels of r3/4 (preotic) and somites 1/2 (postotic) in chick embryos revealed sequential migration of NCCs from preotic to postotic neural folds. Consequently, preotic and postotic NCCs distribute differently within the heart and great vessel-forming regions after migration, with anteroposterior order of NCCs corresponding to their proximodistal location within the heart. Preceding preotic NCCs are likely to differentiate into coronary artery smooth muscle cells, whereas subsequent postotic NCCs predominantly form the aorticopulmonary septum and the smooth muscle layer of the aorta and pulmonary artery (Fig. 30.1). In addition, both NCC populations differently distribute within semilunar valves, suggesting their distinct roles in valve formation (Fig. 30.1).

30.5 Future Direction and Clinical Implications

Identification of preotic NCCs as an origin of cardiac cellular components may provide a novel insight into cell lineage-based understanding of cardiac development, anatomy, and (patho-)physiology. The spatiotemporal pattern of preotic NCC migration and distribution suggests close interaction with second heart field-derived mesodermal cells. In coronary artery formation, interactions between preotic NCCs and other precursor cells from different origins such as the proepicardium and endocardium seem to be an important issue to be addressed. Considering endothelial and endocardial cells are major source of Edn1, the Edn signaling may play a role in these interactions.

From a clinical viewpoint, it is intriguing to pursue the relationship between the NC origin and susceptibility to atherosclerosis and calcification of the proximal coronary arteries. Preotic NCCs retain multipotent capacities including osteogenic and chondrogenic differentiation, leading us to speculate a possibility that these capacities may be related to the pathogenesis and progression of coronary artery diseases. Characterization of preotic NC-derived smooth muscle cells and other derivatives may open perspectives toward novel therapeutic strategies.

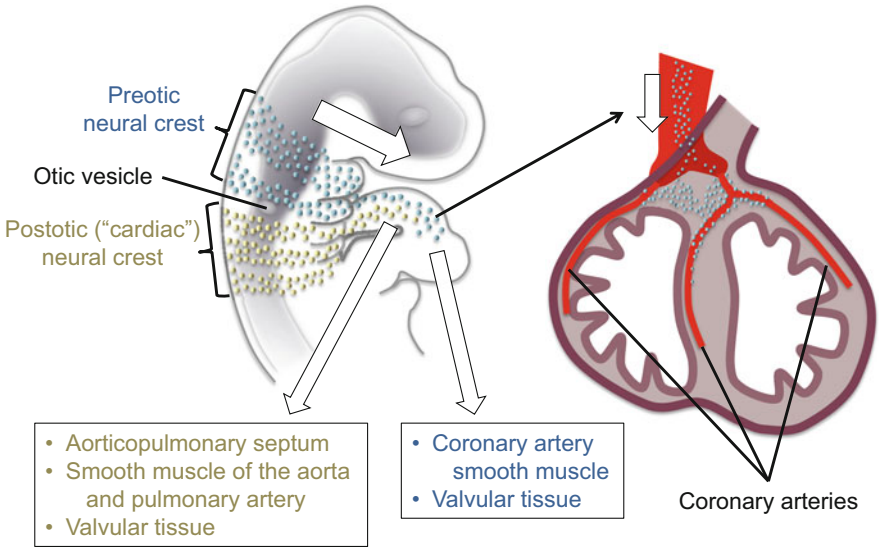

Fig. 30.1 Different contributions of preotic and postotic NCCs to craniofacial and cardiovascular development. Preotic NCCs migrate into the first and second pharyngeal arches to differentiate into the bone, cartilage, teeth, and connective tissue, a part of which further migrates into the heart to differentiate into the coronary artery smooth muscle and valvular tissues. Postotic NCCs follow preotic NCCs in migration and form the aorticopulmonary septum and the smooth muscle layer of the aorta and pulmonary artery with some contribution to the semilunar valves

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Roles of Endothelial Hrt Genes for Vascular Development

31

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Keywords
Notch signaling • Vascular development • Endothelial cells

Various cellular signaling pathways play essential roles in regulating embryonic vascular development. Among them, Notch signaling is implicated in arterial endothelium differentiation and vascular morphogenesis. Mice that lack Notch receptors or other signaling components die in utero due to severe vascular abnormalities. We previously identified the Hairy-related transcription (Hrt) factor family, also called Hey, Hesr, CHF, Herp, and Gridlock, as downstream mediators of Notch signaling in the developing vasculature [1]. The Hrt family proteins, Hrt1/Hey1, Hrt2/Hey2, and Hrt3/HeyL, mainly act as transcriptional repressors, by binding to consensus DNA elements or by associating with other DNA-binding transcription factors. The mice deficient for *Hrt2* showed perinatal lethality due to ventricular septal defects and mitral valve insufficiency, and cardiomyocyte-specific deletion of *Hrt2* caused abnormal expression of atrial-specific genes in the ventricle and cardiac dysfunction in adulthood [2].

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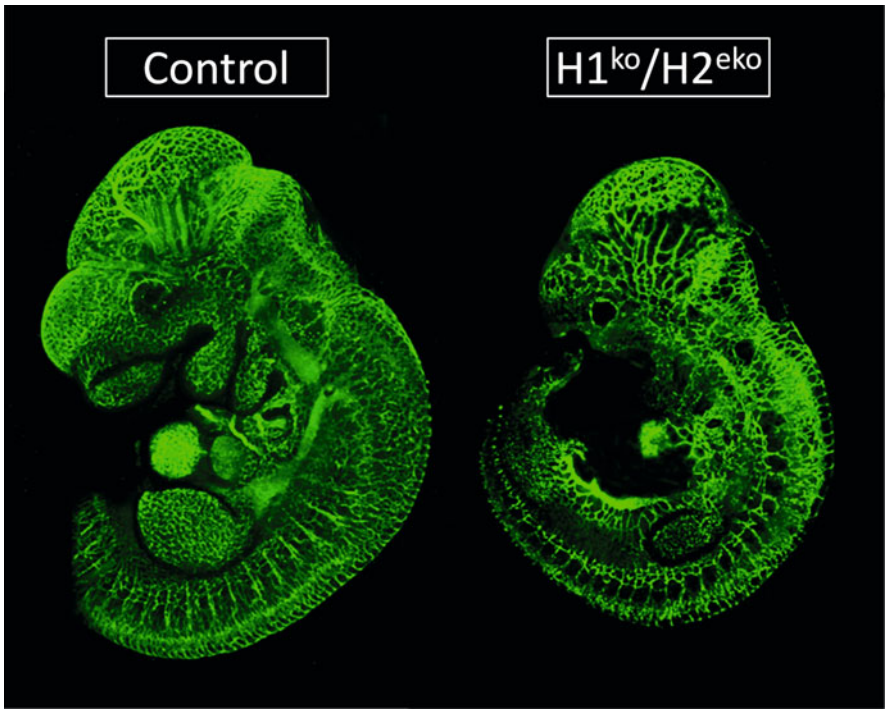

Fig. 31.1 The mice in which *Hrt2* was deleted specifically in endothelial cells with the global *Hrt1* null background ($H1^{ko}/H2^{eko}$) show embryonic lethality with severe defects of vascular morphogenesis. Whole mount PECAM1 immunostaining demonstrated impairment of vascular network formation in $H1^{ko}/H2^{eko}$ embryos

It was also reported that combined loss of *Hrt1* and *Hrt2* resulted in early embryonic lethality due to vascular demise similar to that observed in Notch signal-deficient embryos. While *Hrt1* and *Hrt2* are expressed in endothelial cells as well as smooth muscle cells of embryonic vasculature, it remained unclear which vascular cell type requires *Hrt1*/*Hrt2* functions. In the present study, we generated the mice with endothelial-cell-specific deletion of *Hrt2* combined with global *Hrt1* null mutation and analyzed their vascular phenotypes during embryonic development. The loss of endothelial *Hrt1*/*Hrt2* caused early vascular abnormalities virtually identical to those observed in the global *Hrt1*/*Hrt2* knockout mouse embryos (Fig. 31.1), suggesting that *Hrt* functions in endothelial cells are indispensable for normal vascular development.

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Inositol Trisphosphate Receptors in the Vascular Development

32

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Keywords
Intracellular calcium • Vascular development • Angiogenesis • Placenta

The placental circulation is crucial for the development of mammalian embryos [1]. The labyrinth layer in the placenta is created by extensive villous branching of the trophoblast and vascularization arising from the embryonic mesoderm. In the labyrinth, materials are exchanged between the maternal and embryonic circulation. Recently, we have found that inositol 1,4,5-trisphosphate (IP_3) receptors (IP_3Rs) may be required for the placental vascularization.

IP_3Rs are intracellular Ca^{2+} release channels that have three subtypes in mammals (IP_3R1, IP_3R2 and IP_3R3) [2]. We previously showed that IP_3R1 and IP_3R2 played an essential role in heart development from the analysis of mouse embryo double knockout for IP_3R1 and IP_3R2 [3]. A previous report on the

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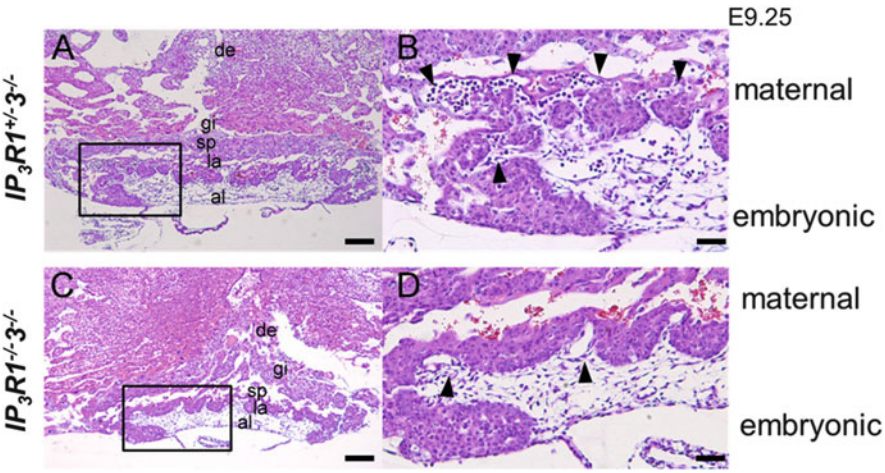

Fig. 32.1 Cross sections of E9.25 placentas from the $IP_3R1^{+/-}3^{-/-}$ (**a** and **b**) and $IP_3R1^{-/-}3^{-/-}$ (**c** and **d**) mice. (**b**) and (**d**) show higher-power fields of the *rectangular* areas of the labyrinth in (**a**) and (**c**), respectively. Embryonic vessels (*arrowheads*) fail to elongate to the maternal sinuses in the placenta of $IP_3R1^{-/-}3^{-/-}$ compared to that of $IP_3R1^{+/-}3^{-/-}$ (wild type). *al* allantois, *de* decidua, *gi* trophoblast giant cells, *la* labyrinth layer, *sp* spongiotrophoblast layer. Scale bars, 0.5 mm in (**a**) and (**c**) and 0.2 mm in (**b**) and (**d**)

requirement for phospholipase (PLC) δ1 and δ3 [4] that produce IP_3 for placentation led us to investigate the placental defects by deletion of any subtypes of IP_3Rs. Our preliminary result revealed that embryonic vasculature in the labyrinth was impaired in the placenta double knockout for IP_3R1 and IP_3R3 at E9.25 (Fig. 32.1). The detailed phenotype and the underlying mechanism how the intracellular Ca^{2+} signaling via IP_3Rs may be implicated in the development of extraembryonic vasculature are under investigation.

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Tissue Remodeling in Vascular Wall in Kawasaki Disease-Related Vasculitis Model Mice

33

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Keywords
Kawasaki disease • Tenascin-C • c-Jun N-terminal kinase • Aneurysm • Remodeling

Kawasaki disease is the most common acute systemic vasculitis of unknown etiology in children [1] and can cause inflammation of the coronary arteries leading to aneurysms. Tenascin-C, an extracellular matrix protein, and c-Jun N-terminal kinase (JNK), an intracellular signaling protein, are known to be associated with inflammation and tissue remodeling [2, 3]. The purpose of this study was to demonstrate tenascin-C and JNK might be involved in tissue remodeling in a *Candida albicans*-induced murine model of aneurysm.

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1. More than 80 % of the mice showed the macroscopic features of aneurysms in the aorta and/or iliac and coronary arteries.
2. Marked inflammatory cell infiltration was observed in vascular wall and perivascular connective tissue, accompanied by fragmentation of elastic fibers.
3. Expression of tenascin-C was highly observed in vascular wall, accompanied by active degradation of elastic fibers.
4. Pharmacologic inhibition of JNK attenuated the aneurysm formation in the mice model.

In conclusion, these findings suggest that both tenascin-C and JNK are involved in abnormal tissue remodeling and inflammation in the *Candida albicans*-induced Kawasaki disease murine model of aneurysm and that JNK inhibition may represent a novel therapeutic target for preventing a Kawasaki disease-related aneurysm.

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