The "Cardiac Neural Crest" Concept Revisited

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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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T. Nakanishi et al. (eds.), *Etiology and Morphogenesis of Congenital Heart Disease*,
DOI 10.1007/978-4-431-54628-3_30

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.

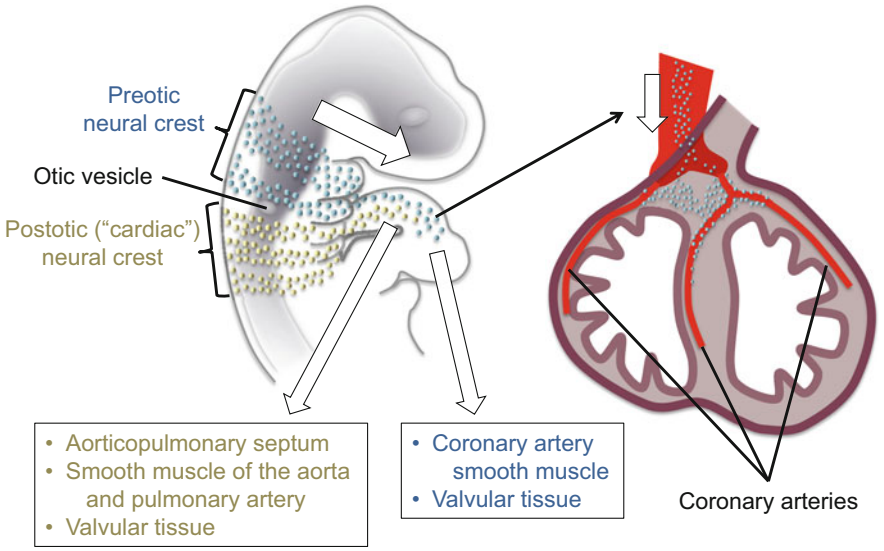

Fig. 30.1 Different contributions of preotic and postotic NCCs to craniofacial and cardiovascular development. Preotic NCCs migrate into the first and second pharyngeal arches to differentiate into the bone, cartilage, teeth, and connective tissue, a part of which further migrates into the heart to differentiate 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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