Cardiac Neural Crest Cells

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Cardiac neural crest cells (NCCs), a specified subpopulation of the neural crest (NC), are vital for normal cardiovascular development, as they significantly contribute to the pharyngeal arch arteries, the developing cardiac outflow tract (OFT), cardiac valves, and interventricular septum. Various signaling pathways and factors are shown to orchestrate the proper migration, compaction, and differentiation of cardiac NCCs during cardiovascular development. Any loss or dysregulation of various signaling components in cardiac NCCs can lead to abnormal cardiovascular development during embryogenesis, resulting in abnormalities categorized as congenital heart defects (CHDs).

Keywords: neural crest cells (NCCs) ; cardiac neural crest ; heart development ; outflow tract (OFT) ; congenital heart defects (CHDs)

1. Introduction

Neural crest cells (NCCs) are a multipotent, and highly migratory, transient vertebrate cell population originating in the dorsal region of the neural tube. During embryogenesis, NCCs, upon neural plate folding, arise from either side of the neural plate at a region called the neural plate border, situated between the neuroectoderm and non-neuroectoderm [1][2]. During and after the neural plate closes, NCCs undergo epithelial-to-mesenchymal transition (EMT) in which they obtain their migratory potential and disperse from the neural tube, relocating to specific locations throughout the embryo, to differentiate into a wide variety of cell types, such as osteoblasts and smooth muscle cells [2][3][4]. Although NCCs arise sequentially during embryo development, they are specified into four main subpopulations based on their anteroposterior axis position, differential abilities, and corresponding terminal locations [5]: cranial neural crest (NC), contributing to the majority of bone and cartilage formation of the head [6]; vagal NC, aiding in the formation of the thymus, lung, enteric nervous system and cardiovascular system [7]; trunk NC, contributing to the peripheral nervous and endocrine systems [8]; sacral NC, aiding in the development of neurons and glia of the enteric nervous system [9]. However, studies on these subpopulations have indicated that the vagal NC consists of a smaller specified group of cells deemed the cardiac NC, known to significantly contribute to cardiovascular development, along with aiding in the development of the thymus, thyroid glands, and cardiac ganglia [7][10][11][12][13].

NC ablation studies have demonstrated the importance of NCCs for proper heart formation. Deficiencies in these cells result in a variety of cardiac malformations during embryonic development categorized as congenital heart defects (CHDs) [14]. CHDs are the most common birth defect and the leading cause of birth defect-related deaths [15][16][17], with a variety of phenotypes ranging from mild forms, accompanied by minimal cardiac complications, to severe and life-threatening forms, resulting in extreme cardiac impediments and death. The most common defects, seen in approximately 30% of human cases, are atrial and ventricular septal defects (ASD/VSD), caused by a hole along the interatrial septum or interventricular septum, respectively [18]. Other common CHDs include malformations of the cardiac outflow tract (OFT), which normally gives rise to the proper vessel development and septation of the semilunar valves (leading to the aorta and pulmonary valves). OFT defects result in common CHD phenotypes such as transposition of the great arteries (TGA), aortic or pulmonary artery stenosis, and patent ductus arteriosus (PDA), as well as rarer CHD phenotypes, such as persistent truncus arteriosus (PTA), characterized by the absence of aortopulmonary septation, overriding aorta, in which the aorta is centralized over the VSD and open to both ventricles, and double outlet right ventricle (DORV), where the aorta is connected to the right, instead of left, ventricle [19][20][21]. Multiple congenital human syndromes, such as CHARGE, Treacher Collins, and DiGeorge, are associated with a variety of CHD phenotypes. Although substantial progress has been made in uncovering the origin of congenital cardiac diseases, details regarding how NCCs contribute to heart development, and how NC deficiencies cause CHDs, is still under investigation.

2. Neural Crest Associated Cardiac Congenital Abnormalities in Humans

Cardiac NC deficiency phenotypes are consistent with the CHDs present in a variety of human diseases. In recent years, the prevalence of CHDs has increased due to improved pediatric screening and increased survival [18]. However, further investigation is needed to determine the pathogenic and/or novel genetic deficiencies contributing to these cardiac defects, to improve diagnostic screening and treatment. Although many CHDs are considered nonsyndromic, multiple genetic syndromes, including DiGeorge and CHARGE, involve NC deficiencies with known associated cardiac phenotypes. Not only do NC deficiencies produce heart defects, they are also known to contribute to various craniofacial defects common among human cardio-craniofacial syndromes, such as the most common multiple anomaly syndromes DiGeorge, Noonan, and Velo-cardio-facial [22][23]. However, more studies are needed to determine how cardiac NC contributions are similar and/or different between multiple syndromes, and what NC regulatory pathways and factors are altered in such diseases.

2.1. DiGeorge Syndrome

DiGeorge syndrome, also known as 22q11.2 deletion, has a well-defined phenotype consisting of characteristic facial features, immunodeficiencies, CHDs, hypocalcemia, and developmental delays. Commonly associated CHDs include interrupted aortic arch, VSD, TOF, and PTA [24][25][26]. Tbx1, a region of Df1, the first targeted region homologous to human 22q11, is expressed in the pharyngeal region and is necessary for OFT and aortic arch development [27][28]. Although Tbx1 has shown not to be expressed by NCCs [27][29], the loss of *Tbx1* has shown to be associated with VSD and tetralogy of fallot (TOF) in patients [30]. Calmont and colleagues found that in mice, Tbx1 drives downstream expression of genes such as Gbx2, to regulate cardiac NCC migration to the pharyngeal arches by a non-cell-autonomous effect, and that the combinational disruption of *Tbx1* and *Gbx2* results in abnormal pharyngeal arch development, along with aortic arch interruption (**Figure 1**), suggestively due to cardiac NC migration deficiencies [31]. However, further studies are needed to determine the specifics regarding the onset and longevity of such NC migration deficiencies, along with determining whether other various CHD phenotypes arise. It has been shown that a loss of *Tbx1* in mice, negatively impacts the development of the second heart field (SHF), partly due to a lack of cell proliferation, which can produce OFT hypoplasia and vascular trunk mislocalization [32][33]. SHF progenitors are a multipotent cardiac progenitor population important for heart tube looping, along with myocardium, smooth muscle, and endothelial cell formation. Given that SHF progenitors and cardiac NCCs closely interact and make major contributions to the development of the OFT, it is of great interest to determine the impact of such *Tbx1* depletions in the SHF on cardiac NCCs during heart development.

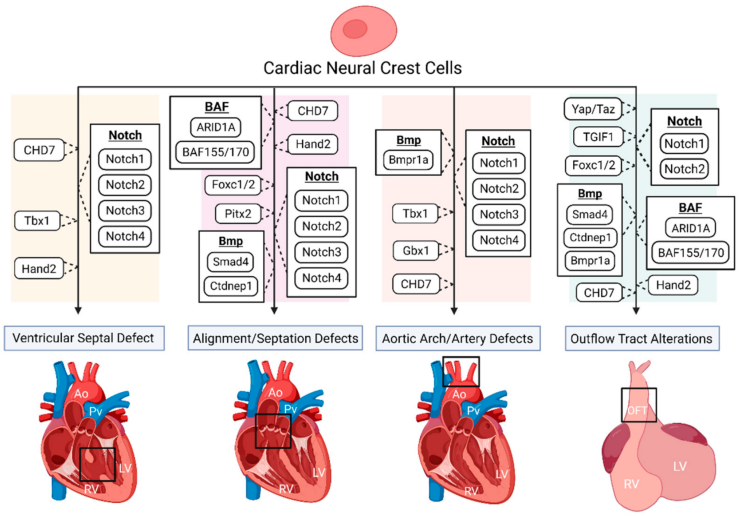

Figure 1. The disruption of various genes and signaling pathways, indicated to be important for proper cardiac neural crest (NC) contribution, results in numerous congenital heart defect (CHD) phenotypes.

2.2. CHARGE Syndrome

CHARGE syndrome affects multiple organ systems and is an acronym for coloboma, heart defects, atresia choanae, growth and mental retardation, genital abnormalities, and ear abnormalities [34]. The most common CHD associated with CHARGE is TOF, detected in approximately 33% of human cases, followed by VSD and aortic arch abnormalities, suggesting that NC development is possibly affected during embryogenesis [35]. A study by Bajpai and colleagues showed that CHD7, the only mutated gene known to cause CHARGE syndrome, is essential for NC migration and the promotion of key transcriptional regulatory genes such as Sox9, Twist1, and Slug, both in vivo (Xenopus) and in vitro (human embryonic stem cells), while a deficiency of *CHD7* caused cardiac OFT defects in Xenopus embryos, resulting in vascular septation defects such as PTA (**Figure 1**) [36]. A recent study by Yan and colleagues found that the deletion of *CHD7* in NCCs of mice, by the use of the Wnt1-Cre2 neural crest-specific driver, not only results in severe conotruncal defects

(VSD and DORV), interrupted aortic arch, and perinatal lethality, but inhibits the OFT invasion of cardiac NCCs (**Figure 1**) [37]. The authors suggest that such cardiac defects are due to the establishment that CHD7 directly regulates key NC regulatory genes such as Foxc2 and Hand2, through ATP-dependent and -independent functions, clarifying the molecular etiology of CHD7-related cardiac defects [37].

2.3. Treacher Collins Syndrome

Cardiac malformations can occur in patients with Treacher Collins syndrome. Treacher Collins is most commonly caused by a mutation within the TCOF1 gene. TCOF1 is involved in mRNA formation in NCCs during embryogenesis, largely associated with NC depletions in pharyngeal arches 1 and 2 [38]. Treacher Collins is normally associated with craniofacial abnormalities, but the depletion of NCCs has also been indicated in producing human CHD phenotypes such as VSD, ASD, and PDA [39]. Studies have found that haploinsufficiency of *TCOF1* in mice results in a reduced number of migrating NCCs, leading to severe craniofacial defects [38]. More recently, Sanchez and colleagues found that the knockdown of one of the only genes known to be involved in Treacher Collins, *POLR1B* (RNA polymerase1 subunit B), results in notable cardiac edema, reduced NC migration, and embryonic death in zebrafish [40]. Serrano and colleagues, using human pluripotent stem cell (HPSC)-derived NCCs, found that disruption of *TCOF1*, by siRNA, confirmed previous conclusions that NC migration is impaired, but also found that NC proliferation was reduced [41]. Although many in vivo TCOF1 studies focus on cranial NC contributions, their findings, along with those from in vitro experiments, indicate a potential role of TCOF1 in cardiac NC function and cardiac formation, which requires further investigation.

3. Conclusions and Future Perspective

NCCs are known for their multipotent and migratory potentials, contributing to various cell types for organ and tissue development. It is well-known that the cardiac NC is highly regulated by a variety of pathways, and that insufficient NC contribution to the heart results in CHDs involving the OFT, great vessels, and cardiac septa.

Although progress has been made in understanding the regulatory networks of NC-derived cardiovascular formation, NCC developmental processes are exceedingly complex. Further investigation is needed to determine the impact that known contributors have at different developmental stages, regarding NC specification, migration, and differentiation, and how genetic deficiencies of these pathways lead to CHDs.

Recent studies showed that the dysregulation of various gene regulatory networks negatively impacts proper NCC function and is associated with human diseases such as CHARGE (*CHD7*), Treacher Collins (*TCOF1*, *POLR1B*), and DiGeorge (*Tbx1*) [31][35][38][40]. Although there are common cardiac congenital defects seen between these syndromes, mainly VSD and OFT malformations, each syndrome is the result of different regulatory deficiencies associated with cardiac NCCs. These findings indicate the critical role of NCCs in proper cardiac formation, while also highlighting the significance of studying NCC deficiencies in human diseases.

To enhance current therapies for patients with heart defects, various studies have begun investigating the potential role of NCCs for cardiac regeneration. Cardiac regeneration is currently at the forefront of cell-renewal research, with the effort to repair irreversibly damaged heart tissue. Cardiac NCCs are known to contribute to the cardiomyocytes of the trabecular myocardium in zebrafish, chicks, and mice [42][43][44]. Tang and colleagues have found a novel contribution of cardiac NCCs for zebrafish heart regeneration, by the reactivation of genes such as *sox10* and *tfap2a* after the surgical removal of a portion of the ventricle [42]. This novel regeneration capability of zebrafish cardiac NCCs poses the possibility for regeneration capability in birds and mammals; however, further examination is needed to determine whether this capability is indeed found in various species, along with the determination of the mechanisms regulating such renewal capabilities.

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