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Seminars in Cell and Developmental Biology Model Systems for the Study of Heart Development and Disease Cardiac Neural Crest and Conotruncal Malformations

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Abstract

Neural crest cells are multipotential cells that delaminate from the dorsal neural tube and migrate widely throughout the body. A subregion of the cranial neural crest originating between the otocyst and somite 3 has been called “cardiac neural crest” because of the importance of these cells in heart development. Much of what we know about the contribution and function of the cardiac neural crest in cardiovascular development has been learned in the chick embryo using quail-chick chimeras to study neural crest migration and derivatives and using ablation of premigratory neural crest cells to study their function. These studies show that cardiac crest cells are absolutely required to form the aorticopulmonary septum dividing the cardiac arterial pole into systemic and pulmonary circulations. They support the normal development and patterning of derivatives of the caudal pharyngeal arches and pouches, including the great arteries and the thymus, thyroid and parathyroids. Recently, cardiac neural crest cells have been shown to modulate signaling in the pharynx during the lengthening of the outflow tract by the secondary heart field. Most of the genes associated with cardiac neural crest function have been identified using mouse models. These studies show that the neural crest cells may not be the direct cause of abnormal cardiovascular development but they are a major component in the complex tissue interactions in the caudal pharynx and outflow tract. Since cardiac neural crest cells span from the caudal pharynx into the outflow tract, they are especially susceptible to any perturbation in or by other cells in these regions. Thus, understanding congenital cardiac outflow malformations in human sequences of malformations as represented by the DiGeorge syndrome will necessarily require understanding development of the cardiac neural crest.

Keywords

heart development; cardiac neural crest; conotruncal malformations; secondary heart field

Introduction

Neural crest cells originate from the region of the neuroepithelium that borders the surface ectoderm [1,2]. The neuroepithelium gives rise to a neural tube that develops as the central nervous system. Neural crest cells originate along the neural tube from the mid-diencephalon to the most caudal extremity of the embryo. These crest cells are divided broadly into cranial and trunk based on their ability to give rise to ectomesenchyme: only cranial neural crest has

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this capacity [1]. Cranial neural crest cells originate from the mid-diencephalon to somite 5, and trunk crest originate from somite 5 to the caudal tip of the neural tube [2]. Cardiac crest is a subdivision of the cranial crest. It originates from the middle of the otic placode to the caudal border of somite 3, corresponding to rhombomeres 6, 7, and 8 [3]. The cardiac crest seems to represent a transitional region between the cranial and trunk crest because it shares some properties common to both regions. It generates ectomesenchyme like the more cranial crest, and it lacks the ability for regeneration like trunk crest [4].

Ectomesenchymal cells from the cardiac crest form the smooth muscle tunics of the great arteries and the connective tissue of glands in the neck, i.e. thymus, thyroid, and parathyroids [5,6]. In addition, ectomesenchyme from the cardiac crest forms the aorticopulmonary septum that divides the cardiac arterial pole into systemic and pulmonary outlets [7]. Cardiac neural crest-derived cells contribute to the semilunar and atrioventricular valves but the function of the crest cells in the valves is unknown [8,9]. Cardiac neural crest cells also provide all of the parasympathetic innervation to the heart [10] and they have been associated with maturation of the cardiac conduction system [9,11]. Trunk neural crest provides sympathetic innervation but does not contribute to the structural development of the heart [7].

Cardiac neural crest cells require a wide variety of environmental signals in order to be specified in the neural tube and then to migrate, proliferate, differentiate and survive. Wnts, fibroblast growth factors (FGFs), bone morphogenetic proteins (BMPs) and retinoic acid (RA), are required for neural crest induction (for review see [12]). Delamination from the neural tube is regulated via BMP-dependent Wnt1 activity [13], with Wnt1 expression turning off soon after the cells leave the neural tube. Many transcription factors and signaling molecules have been implicated in the later steps of migration, proliferation, survival and differentiation of the cardiac neural crest, some of which are discussed below.

Cardiac Neural Crest Ablation Phenotype

Much of what we now know about the role of cardiac neural crest cells in heart development and function has been learned from studying chick embryos after ablation of the premigratory cardiac neural crest [14]. This neural crest ablation model was the first reliable model of congenital heart defects in an experimental animal and has served as the “gold” standard for defining the pathogenesis of heart defects in other experimental models and in transgenic mice. Cardiac neural crest ablation leads to a number of cardiovascular and non-cardiovascular defects [15]. Non-cardiovascular phenotypes include hypoplasia or aplasia of the thymus, parathyroids and occasionally the thyroid gland [5]. The cardiovascular phenotypes include three distinct components: 1) defective development of the cardiac outflow tract including persistent truncus arteriosus and outflow malalignment, 2) abnormal patterning of the great arteries, and 3) abnormal myocardial function.

Cardiac Neural Crest and Outflow Septation

While several cardiac dysmorphologies have been reported after cardiac neural crest ablation, only those involving outflow or conotruncal defects are seen routinely, and these are the cardiac defects that have been investigated most thoroughly. The morphological defects include complete absence of outflow septation (persistent truncus arteriosus, common trunk) and overriding aorta [15]. Overriding aorta is an alignment defect rather than a problem of septation and is linked with abnormal looping during earlier heart development [16]. Overriding aorta is always accompanied by a ventricular septal defect and thus the aorta receives blood from both the right and left ventricles. Interestingly, alignment defects are also observed in embryos with persistent truncus arteriosus in that the common trunk is situated predominantly over the right ventricle.

Most of our knowledge of the distribution of cardiac neural crest cells in the heart comes from using quail-chick chimeras. These studies show that after the cardiac neural crest cells migrate into pharyngeal arches 3, 4 and 6, a subset of the cells continues migrating into the cardiac outflow cushions (Figure 1)[17]. The cushions themselves are bulges of cardiac jelly populated by a mixture of mesenchymal cells that migrate into the outflow tract from the pharynx and cells generated by epithelial-mesenchymal transformation of endocardial cells [18]. The neural crest cells follow these preformed cushions and collect as two prongs of condensed cells in the distal or truncal outflow cushions. The condensed cells do not extend into the conus although clusters of neural crest cells populate the conal cushions [19]. The prongs of condensed neural crest-derived mesenchyme are connected dorsal to the aortic sac between the origins of the 4th (systemic) and 6th (pulmonary) arch arteries (Figure 2). Together, the horseshoe-shaped prongs and shelf of tissue in the aortic sac are called the aortopulmonary septation complex. The complex initiates division between the systemic and pulmonary blood streams by the shelf protruding into the dorsal wall of the aortic sac. The shelf elongates into the distal outflow tract at the expense of the prongs and because the prongs themselves spiral, the elongating shelf spirals. The spiraling of the cushions is important for the correct positioning of the aorta and pulmonary trunk with respect to the ventricles. The closure of the proximal or conal cushions occurs in a zipperlike fashion from distal to proximal toward the ventricles. The most proximal part of the cushions closes concurrently with invasion of the cushions by myocardium. The invading myocardial cells cause the cushions to bulge and meet in the lumen. When they touch, the endocardium covering the cushions breaks down, allowing mixing of the underlying mesenchyme and myocardium and this brings about the fusion of the opposing cushions to form a septum. Because the neural crest cells were subendocardial prior to the fusion, they now appear as a seam where the two cushions fused (Figure 2)[19].

In mammals, the pattern of cardiac neural crest migration and role in outflow septation appear to be very similar to the chick. Marking neural cardiac neural crest cells in cultured rat embryos showed that neural crest cells did indeed migrate to the heart [20]. However, a major advance for our understanding of mammalian cardiac neural crest came with transgenic methods that allowed the neural crest cells to be marked genetically. Because neural crest cells express the gap junction protein Cx43, Lo and colleagues were able to use a portion of the Cx43 promoter driving lacZ to create a transgenic mouse with LacZ marked neural crest cells [21]. The pattern of neural crest migration and participation in cardiovascular development reflected quail-chick chimeras remarkably well [8]. Use of cre recombinase (cre-lox) technology further refined the ability to track neural crest cells by cell lineage tracing in mouse embryos. The most commonly used promoters to drive cre recombinase today are the Wnt1cre, Pax3cre, P0cre, and PlexinA2cre [22–24]. While there are some differences in the patterns of labeled neural crest cells in these models (they have never been carefully compared), it is difficult to understand the precise differences and what they might mean. However, all of the models confirm the major patterns of migration and contributions of cardiac neural crest to cardiovascular development that were described in quail-chick chimeras.

Studies of cardiac neural crest in zebrafish embryos have shown that neural crest cells migrating into the heart have the additional potential of adopting a myocardial phenotype [25,26]. Unlike chick and mouse the cardiac neural crest in zebrafish originate from a slightly different axial level, however, the migration and final destination appear very similar to that in mouse and chick. The fact that cardiac neural crest exists in the zebrafish is remarkable because the zebrafish cardiac output is directed to the gills. This makes the septation of the outflow tract into pulmonary and systemic circulations unnecessary.

Role of Cardiac Neural Crest in Secondary Heart Field Development

One of the earliest defects observed after neural crest ablation is abnormal cardiac looping because the outflow limb of the cardiac loop is shorter and straighter than normal [16]. During looping myocardium is added to the lengthening and looping heart tube from the splanchnic mesoderm located caudal to the attachment of the outflow tract to the ventral pharynx (Figure 3). This region has been called the “secondary” heart field although it is most likely part of the cardiogenic field that is added after the heart tube has already formed. The abnormal looping observed after neural crest ablation is due to a failure of addition of secondary heart field-derived myocardium to the outflow tract. In neural crest-ablated embryos, the cells that normally migrate from the secondary heart field proliferate rather than migrate and differentiate into myocardium [27,28].

During early heart looping the secondary heart field provides myocardium to the outflow portion of the heart tube and later the smooth muscle, i.e. the elements of the arterial pole where the myocardium of the ventricles and smooth muscle of the arterial trunks meet. Thus, the secondary heart field cells produce the myocardial-to-smooth muscle junction at the level of the pulmonary and aortic valves (Figure 3). In the cardiac neural crest-ablated embryo, abnormal heart looping occurs prior to the arrival of the neural crest cells into the outflow tract. This suggests that there is a factor negatively influencing normal heart development at time critical for normal addition of myocardium from the secondary heart field. Recently, FGF8 signaling has been shown to be elevated in the pharynx of neural crest-ablated embryos during the time when the myocardium is being added from the secondary heart field [29]. If the excess FGF8 in the pharynx is neutralized in neural crest-ablated embryos, normal development of the secondary heart field is restored leading to normal looping and normal outflow alignment. In addition, the myocardial calcium transient is also restored after reducing FGF8 signaling. By contrast, treatment of sham-operated embryos with reagents that inhibit FGF8 signaling also results in failure of myocardial development from the secondary heart field suggesting that these cells are extremely sensitive to FGF8 signaling levels [29].

The secondary heart field also generates smooth muscle that forms the tunica media of the proximal great arterial trunks as they exit the heart [27]. This smooth muscle appears not to be sensitive to neural crest ablation although the vessel walls are thickened in embryos that are treated with FGF8 antibody [29]. Neural crest cells form the smooth muscle tunics of the aortic arch artery derivatives and the distal continuations of the great arteries.

Cardiac Neural Crest and Pharyngeal Patterning

The arch artery patterning defects observed after cardiac neural crest ablation include hypoplasia or stenosis of variable combinations of the right and left brachiocephalic arteries, the aortic arch, and the bilateral ductus arteriosus (the chick has bilateral ductus during embryonic and fetal development)[30,31].

Cardiac neural crest cells are not required for the initial formation of the endothelial channels that form the arch arteries, but are required for the final patterning. In the absence of the cardiac neural crest, the vessels persist or regress in an unpredictable manner [32,31].

Cardiac crest cells first migrate into the circumpharyngeal ridge where they pause while pharyngeal arches 3, 4 and 6 are formed [33]. The crest cells migrate into each arch as it forms where they surround the endothelial cells that form the nascent aortic arch arteries [34]. Arches 1 and 2 are populated by non-cardiac, cranial neural crest cells which mostly develop into skeletal elements. Arches 3, 4 and 6 are populated by cardiac neural crest cells [18]. These more caudal arches are largely devoted to glandular and vascular development.

Endothelin signaling is thought to be involved in aortic arch artery patterning because mutations in the endothelin-1 (ET1) signaling pathway are associated with abnormal patterning of the great arteries [35]. Endothelin A (ETA), the receptor for ET1, is expressed by neural crest cells in the pharyngeal arches. ETA-null ES cells are excluded from the walls of the aortic arch arteries suggesting that neural crest cells require ETA to interact with the endothelium of the aortic arch arteries as they are forming [36,37]. In chick, ETA specific antagonists cause aortic arch mispatterning and outflow defects [38]. If premigratory neural crest cells are infected with a virus expressing the precursor protein for ET1 called preproET1, the neural crest cells undergo selective expansion of the adventitial cells but not the smooth muscle cells of the great arteries [39].

Little is known about the downstream effectors of endothelin signaling; however, the basic helix-loop-helix transcription factor Hand2 is one downstream effector of endothelin signaling in the pharyngeal arches [40]. The Hand2 gene has an evolutionarily conserved enhancer with four homeodomain binding sites that are required for endothelin-activated activity in the pharyngeal arches [41]. Dlx6, a member of the Distal-less family of homeodomain proteins, acts as an intermediary between endothelin signaling and Hand2 transcription. However, downstream effectors of Hand2 that mediate neural crest-endothelial interactions for normal patterning are not known.

Haploinsufficiency for the transcriptional regulator Tbx1 by contrast, affects the initial growth of arch artery 4 [42]. While there is no detectable defect in neural crest cells, the smooth muscle tunic of the 4th aortic arch artery is reduced, suggesting that proliferation of the crest cells might be suboptimal. Tbx1 is not expressed by the neural crest cells in the pharyngeal arches but rather by the pharyngeal endoderm, where it is an upstream regulator of Fgf8 expression [43]. Ectodermally expressed Fgf8 is necessary for initial development of the 4th aortic arch artery because deletion of Fgf8 expression specifically from the pharyngeal ectoderm prevents development of the 4th arch arteries, leading to interrupted arch [44].

Cardiac Neural Crest and Myocardial Function

Myocardial function defects comprise the third component of the cardiac neural crest ablation phenotype. Like the looping defect, myocardial dysfunction is first observed in neural crest-ablated embryos about the time neural crest cells should migrate into the caudal pharyngeal arches, several days prior the normal arrival of the neural crest cells into the outflow tract [45]. These defects include, depressed ejection fraction with decreased, calcium transient, L-type calcium current, excitation-contraction coupling and calcium sensitivity of the contractile apparatus. The embryos that survive do so by ventricular dilation to maintain cardiac output in the normal range [46,47]. Interestingly, levels of FGF signaling appear to also influence cardiac function. The myocardial calcium transient can be normalized when FGF signaling is reduced in hearts from neural crest-ablated embryos [48,29].

Thus cardiac neural crest cells influence heart development directly and indirectly. They are essential in building the outflow septum and arch artery patterning, while their presence in the pharynx is required for normal looping and lengthening of the heart tube and early myocardial function.

Mouse Models of Dysfunctional Cardiac Neural Crest

While chick is the developmental model of choice for observing tissue interactions, most of the genes associated with cardiac neural crest function have been identified using mouse models. Many genes have been associated with a neural crest-ablation phenotype in transgenic and mutant mice [49–51]. Many of these genes expressed by the neural crest or adjacent tissues are important in the migration, proliferation, differentiation and patterning of the cardiac neural

crest at all stages of their developmental program from the neural tube to their final destination in the pharynx and the outflow tract. Two naturally occurring mutants, Patch and Splotch, show cardiac phenotypes similar to the neural crest ablation phenotypes although the cardiac defects are part of a constellation of more severe non-cardiac abnormalities [52–58]. The Splotch mutation is in the Pax3 gene, a transcription factor. Homozygous Splotch embryos die by 14.5 dpc because of myocardial dysfunction similar to that seen after neural crest ablation [52]. They also have persistent truncus arteriosus, outflow malalignment, abnormally patterned aortic arch arteries, absent thymus, thyroid, and parathyroids, spina bifida, and abnormal pigmentation. Pax3 is highly expressed in the dorsal neural tube and migrating cardiac neural crest cells. Expression diminishes as the cells populate arches 3, 4, and 6, and the cardiac outflow tract. Cre-lox technology has been used to fate map the Pax-3-expressing cells in normal and Splotch mice [59,60]. While the neural crest cells migrate to the arches and outflow tract, there are fewer cells than normal. This suggests that Pax3 is not necessary for migration of the cardiac neural crest but may play a role in expansion of the neural crest cell population. Therefore, as in the chick ablation model, a critical number of cardiac neural crest cells must reach the pharynx for control of pharyngeal signaling and then the outflow tract for proper septation. The paucity of neural crest cells in arches 3, 4 and 6 may phenocopy the failure of addition of the myocardium from the secondary heart field, as seen in the chick ablation model, resulting in the malalignment defects observed in the Splotch mouse. Msx2, a homeobox gene regulating BMP signaling, has been shown to be a downstream effector of Pax3. Msx2 is upregulated in the Splotch mutant, and a loss of function Msx2 mutation rescues the cardiac defects of the Splotch mutant embryos, as well as defects in the dorsal root ganglia, thymus and thyroid [60]. The α subunit of the platelet-derived growth factor (PDGF) receptor is deleted in Patch mouse mutants. PDGF is important in regulating deposition and turnover of the extracellular matrix which in turn is required for cell proliferation, survival, morphology, and migration. Homozygous PATCH embryos have multiple defects including cleft face, and PTA both of which could be due to abnormal neural crest migration, proliferation and/or survival [61]. PDGF appears to regulate matrix metalloprotease (MMP)-2, an enzyme involved in extracellular matrix remodeling. MMP-2 is down regulated in neural crest cells in the PATCH mutant mice [62].

Targeted and/or conditional gene knockouts in mouse have provided many models that recapitulate all or portions of, the neural crest ablation phenotype in chick. The earliest knockout was of Hoxa3 [63]. The cardiac neural crest phenotype of this mouse is partial in that the glands derived from the third pharyngeal pouch are absent, and the third aortic arch artery is patterned abnormally [64,65]. However, the Hoxa3 mutant mouse does not have any cardiac defects. It was later confirmed in chick that disrupted hox expression is associated with abnormal patterning of the great arteries but not with outflow defects [66].

As mentioned previously the Wnt signaling pathway is involved in early specification of the neural crest. Many molecules in the Wnt signaling pathway are also implicated in the proliferation, migration and targeting of the cardiac neural crest cells. Because Wnt1 is expressed in early migrating neural crest cells and is turned off as the cells migrate away from the neural tube, Wnt1-cre mice have been widely used to specifically knock out genes expressed in the neural crest. Frizzled 2, a receptor in the Wnt signal transduction pathway, is expressed in the cardiac neural crest-populated regions of the outflow tract [67]. Mutations in the murine gene disheveled 2 (Dvl2), another member of the Wnt signaling pathway, results in double outlet right ventricle, transposition of the great arteries or persistent truncus arteriosus [68]. In these mutants, few neural crest cells are detected in the outflow and the pharyngeal arches, suggesting a lack of cardiac neural crest cells also causes abnormal development of the secondary heart field in mice resulting in outflow alignment defects.

BMP2 and 4, have been implicated as regulators of neural crest cell induction, maintenance, migration, differentiation and survival. One of the BMP receptors, *Bmpr1a* is expressed in the neural tube sufficiently early to be involved in neural crest specification and/or migration. Mice in which the *Bmpr1a* receptor has been ablated from the neural crest prior to their migration display a shortened cardiac outflow tract and defective septation. These mice also display reduced myocardial proliferation and die at midgestation due acute heart failure. Surprisingly, the mice do not have defects in the induction, delamination or initial migration of the neural crest [69]. Deletion of *Alk4*, another BMP receptor, in the cardiac neural crest also results in artery patterning defect and PTA [70]. In these mice not enough neural crest reach the outflow to effect normal septation.

The homeobox containing transcription factor, *Pitx2* is expressed in the cardiac neural crest and *Pitx2*-expressing cells exhibit defective proliferation and outflow defects in both *Wnt-1-cre/catenin-flox* and *Pitx2*^{-/-} mice [71]. *Pitx2* is directly induced by the *Wnt*-*Dsh*-catenin pathway suggesting that in both the *Pitx2* and *Dsh2* mutants there is a deficiency of cardiac neural crest cells arriving in the outflow tract [72]. *Pitx2* is also important for right-left patterning. Mice with an isoform-specific deletion of *Pitx2c* have defects in asymmetric remodeling of the aortic arch vessels [73].

Semaphorin 3C is a member of a family of secreted ligands used in axon guidance. It is also important in the migration and targeting of cardiac neural crest cells to the outflow tract. *Sema3C*-null mice have interrupted aortic arch and persistent truncus arteriosus [51]. Because other neural crest derivatives are normal in *Sema3C*-null embryos *Sema3C* signaling appears to be particularly important for cardiac neural crest. However, *Sema3C* is not expressed by cardiac crest cells but is expressed in outflow myocardium. The neural crest do express the receptors for semaphorin ligands which are multimeric complexes of Plexins and neuropilin (Np)1 and/or Np2 [74]. A complex of Np1 and PlexinA2 may be the functional receptor that signals the cardiac neural crest, as either PlexinA2 or Np1-null mice have PTA and interrupted aortic arch [22,74]. This suggests that cardiac neural crest cells use guidance cues to be targeted to the cardiac outflow tract. Interestingly, *GATA6*, a transcription factor expressed by vascular smooth muscle has been shown to regulate *Sema3C* expression in the outflow tract and vascular smooth muscle. Mice with targeted deletion of *GATA6* in the vascular smooth muscle have interrupted aortic arch and PTA [75].

Cx43, a gap junction protein, is another gene needed for normal neural crest cell migration [76,77]. Gap junctions are intracellular channels that allow the passage of small signaling molecules, low molecular weight metabolites and ions between cells. *Cx43*-null mice die soon after birth because of pouches in the outflow of the right ventricle that obstruct blood leaving the heart. This is a defect that is not seen in the neural crest-ablation phenotype. Cardiac neural crest cell migration in *Cx43* knockout mice show reduced directionality and speed, while cardiac neural crest cells over-expressing *Cx43* in a transgenic mice show increased directionality and speed [78]. Thus, a reduced number of neural crest cells arrive in the heart in *Cx43* knockout mice and an increased number of neural crest cells are found in the outflow tract of mice that overexpress *Cx43*. Both situations are associated with pouches in the right ventricular outflow tract [79]. Although these mice have outflow tract defects, these experiments show that not all neural crest migration defects result in outflow tract septation defects. Interestingly, a recent study by Gutstein and colleagues [80] found that conditional knockout of *Cx43* in the dorsal neural tube and the neural crest cells mediated by *Wnt1-Cre* failed to recapitulate the *Cx43*-null outflow obstruction phenotype. However, a broader conditional knockout mediated by a *Pax3-Cre* knocked out *Cx43* expression in a larger dorsoventral extent of the neural tube and neural crest. This resulted in outflow obstruction defects similar to those seen in *Cx43*-null hearts. The *Pax3-cre* mediated knockout resulted in

an increased number of neuroepithelial cells leaving the dorsal and lateral neural tube and an excess of cells migrating to the outflow tract.

Not all genes important for outflow septation are expressed by the cardiac neural crest cells. The transcriptional regulator, Sox4 is expressed by the non-neural crest-derived mesenchymal cells that populate the cushions of the outflow tract and atrioventricular canal [81]. Sox4-null mice suffer from lack of fusion of the outflow cushions, causing persistent truncus arteriosus in some embryos and a large infundibular septal defect in others. The transcriptional repressor in the forkhead family, Foxp1 is expressed in the myocardium and endocardium. Foxp1 mutant embryos have a very similar phenotype to the Sox4 mutant mice and Sox4 expression is reduced in the outflow cushions of the embryos [82].

While Foxp1 is important in outflow septation, other members of the forkhead family of transcription factors, Foxc1 and Foxc2, are required for arch artery patterning, outflow septation and secondary heart field development. These genes are coexpressed in the secondary heart field, the cardiac neural crest cells, the endocardium, and proepicardium. Embryos lacking either Foxc1 and Foxc2, as well as compound heterozygotes, have coarctation or interrupted aortic arch [83–86]. The cardiac neural crest cells in Foxc1 and Foxc2 compound heterozygous mice undergo abnormal apoptosis leading to outflow septation defects.

As discussed earlier, neural crest-ablated embryos have FGF8 over signaling in the pharynx which is detrimental to normal secondary heart field development. Interestingly, mice haploinsufficient for FGF8, display abnormal apoptosis of cardiac neural crest cells, and the typical neural crest ablation cardiovascular phenotype including PTA and outflow malalignment [87,88]. Outflow alignment defects, but not PTA, are observed in chick embryos when FGF8 levels are reduced to below normal levels during secondary heart field migration. These studies suggest that the secondary heart field is particularly sensitive to FGF8 signaling levels. It should be noted that FGF8 is produced by the pharyngeal ectoderm and the endoderm and not by the neural crest cells. In addition to levels of FGF8, the source of the FGF8 signal may also be important. Tissue specific ablation of Fgf8 in the pharyngeal arch ectoderm results in arch artery patterning defects but no arterial pole defects [44]. This suggests that the FGF8 produced by the pharyngeal endoderm may be more important for normal outflow development.

Tbx1 is a member of the T-box family of transcription factors that lies within the deleted region of chromosome 22q11, which is most commonly deleted in patients with the DiGeorge phenotype (see below [89]). Tbx1 has been shown to be haploinsufficient in several patients with conotruncal defects [90]. In mouse, tbx1 homozygous mutation recapitulates the cardiovascular and glandular defects common in DiGeorge syndrome, but mice haploinsufficient for Tbx1 have abnormal aortic arch patterning with normal outflow septation. One of the ways tbx1 appears to affect conotruncal development is by supporting proliferation of cells in the secondary heart field [91].

DiGeorge Syndrome and Neural Crest

The DiGeorge syndrome consists of a PTA, type B interrupted aortic arch, absent or hypoplastic thymus, craniofacial dysmorphology and cognitive or behavioral disorders [92,93]. It can also include absent or hypoplastic parathyroid and thyroid glands. A variant of the DiGeorge phenotype, called Sprintzen or Velocardiofacial syndrome, also includes cleft palate [94]. The DiGeorge syndrome was characterized originally as defective development of structures derived from the third and fourth pharyngeal pouches. After the discovery that neural crest provides the mesenchyme of the 3rd, 4th, and 6th arches, as well as the outflow septum, the DiGeorge Syndrome was recognized as resulting from defective development of the neural crest and while neural crest is most likely involved in development of the syndromic features,

it is not the causative agent. More recently, the phenotype has been linked to a microdeletion in chromosome 22q11 leading to assignment of yet another name to the phenotype: CATCH22 (Cardiac defects, Abnormal facies, Thymic hypoplasia, Cleft palate, and Hypocalcemia). Identification of the chromosome 22 microdeletion subsequently led to the recognition of Tbx1 as the gene that is most likely to underlie the phenotype, although it is generally recognized that the multiple forms of the phenotype are likely to involve other genetic loci and modifier genes (see below). Tbx1 is not expressed by cardiac neural crest cells while they are migrating or when they populate the pharyngeal arches, but instead it is expressed in the pharyngeal ectoderm, endoderm and in the secondary heart field mesenchyme [95]. Tbx1 expression in the endoderm may control Fgf8 expression, which affects some aspect of neural crest development, perhaps proliferation [96]. At all developmental times and in most tissues tested there is a crucial role for Tbx1. One of the more remarkable properties of Tbx1 is that different structures have different sensitivity to Tbx1 dose. Recently, Baldini and colleagues showed mesoderm-specific deletion of Tbx1 causes severe pharyngeal patterning and cardiovascular defects [97]. When mesoderm-specific Tbx1 expression was restored in a mutant background the outflow morphology was rescued but not the arch artery patterning.

While the chromosome 22 microdeletion is detected in many patients with diagnosed DiGeorge syndrome not all patients with the DiGeorge phenotype have the microdeletion and/or a mutation in Tbx1. Further, not all DiGeorge patients with the deletion have the same severity of malformations suggesting that there are other genetic or epigenetic modifiers. Many studies have revealed interactions between Tbx1 and FGF [98,99,91], Pitx2 [100] hedgehog [43], retinoic acid [101,102] and vascular endothelial growth factor [103] signaling. Crkl, an adaptor protein important for intercellular signaling, is also located within the 22 microdeleted region and homozygous mice null for Crkl have some but not all of the DiGeorge features [104]. However, Crkl and FGF8 compound heterozygous mice have increased incidence and severity of cardiovascular defects [105].

In conclusion, all of the tissues in the pharynx and outflow tract appear to perform highly specialized and coordinated functions in normal development of the outflow tract and great arteries. We have presented studies that highlight the intricacy of signaling and tissue interactions in the pharynx and outflow tract required for normal cardiovascular development. The cardiac neural crest represents a major population that spans both regions. Disrupted gene expression or behavior of any of these tissues impacts all the others. Since the cardiac neural crest cells span both regions, they are particularly central to any type of disrupted signaling or interactions. Thus the cardiac neural crest ablation phenotype is a key to understanding the embryogenesis of these defects even though it may not be the causative agent.

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Figure 1. Migration and distribution of cardiac neural crest cells from their origin to the caudal pharynx and from there into the outflow tract.

Figure 2. Schematic representation of cardiac neural crest cells during outflow septation. A) Horseshoe-shaped aorticopulmonary septation complex composed of condensed mesenchyme of cardiac neural crest derived cells (blue). B) Conus after septal closure showing a seam of neural crest cells remaining (stippled gray).

Figure 3.

Formation of the arterial pole from secondary heart field. A) Origin of the secondary heart field cells (yellow) in the ventral pharynx during cardiac looping stages. B) Myocardium (green) and smooth muscle (red) derived from the secondary heart field that form the arterial pole. Neural crest derived smooth muscle (tan) continues the tunica media distal to smooth muscle derived from secondary heart field.