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A Chemotactic Model of Trunk Neural Crest Cell Migration

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Short title: Trunk neural crest cell chemotaxis

Keywords: Wilhelm His, neural crest, migration, trunk, chemotaxis, mathematical model

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9 July, 2018

ABSTRACT

Trunk neural crest cells follow a common ventral migratory pathway but are distributed into two distinct locations to form discrete sympathetic and dorsal root ganglia along the vertebrate axis. Although fluorescent cell labeling and time-lapse studies have recorded complex trunk neural crest cell migratory behaviors, the signals that underlie this dynamic patterning remain unclear. The absence of molecular information has led to a number of mechanistic hypotheses for trunk neural crest cell migration. Here, we review recent data in support of three distinct mechanisms of trunk neural crest cell migration and develop and simulate a computational model based on chemotactic signaling. We show that by integrating the timing and spatial location of multiple chemotactic signals, trunk neural crest cells may be accurately positioned into two distinct targets that correspond to the sympathetic and dorsal root ganglia. In doing so, we honor the contributions of Wilhelm His to his identification of the neural crest and extend the observations of His and others to better understand a complex question in neural crest cell biology.

INTRODUCTION

Cells migrate long distances in the growing embryo and may be guided chemotactically by chemical gradients. However, models that propose a single chemotactic cue are problematic since diffusible signals are range-limited. This has suggested alternative scenarios including the hypothesis that cells readout several guidance cues that are coordinated across distinct microenvironments through which cells travel. The timing and location of multiple signals could in principle act to direct cellular traffic along a common migratory pathway but position cells into distinct peripheral locations. Unfortunately, the knowledge of where in the microenvironment to search for guidance signals and how they are coordinated in space and time to produce a complex pattern of cell distribution in several distinct embryonic cell migration phenomena has proved challenging. This is primarily due to the lack of an in vivo model and the marriage of experiment with computational modeling that could rapidly test potential mechanistic hypotheses. Knowledge of how multiple signals are coordinated to direct cells over long distances would have implications to our better understanding of embryonic germ cell and neural crest cell migration, cancer cell invasion, wound healing, and the immune response. Thus, there is a tremendous need to combine experiment and theory within an in vivo model of cell migration to study how cells interpret and respond to multiple guidance signals to reach precise targets.

Trunk neural crest cell migration is an excellent model system to study these questions since there is a subpopulation of trunk neural crest cells that travel along a common migratory pathway but distribute into two distinct locations (Figure 1). The initial neural

crest cells that exit the trunk dorsal neural tube follow a medioventral pathway between the neural tube and compact somite, as discovered from fluorescent dye labeling and static imaging in mouse (Serbedzija et al., 1992), Xenopus (Collazo et al., 1993), chick (Serbedzija et al., 1989) and zebrafish (Raible et al., 1992). The lead neural crest cells of the migratory stream reach a region near the dorsal aorta and coalesce to form the initial primary sympathetic ganglia of the peripheral nervous system. Time-lapse imaging in chick sagittal slice explants have revealed that neural crest cells that continue to exit the dorsal neural tube and follow leaders either contribute to the sympathetic ganglia or stop at a dorsal location to form the dorsal root ganglia of the sensory nervous system (Kasemeier-Kulesa et al., 2005). Together, these neural crest cell marking experiments and cell behavior analyses reveal that sympathetic and sensory nervous system assembly arise from a common neural crest cell subpopulation that is directed to two distinct locations within the embryo.

There is increasing evidence that guidance signals, such as vascular endothelial growth factor (VEGF), fibroblast growth factor (FGF), and chemokines including CXCL12 within the embryonic neural crest microenvironment direct neural crest cell behaviors throughout the head and trunk (reviewed in Thevenaeu and Mayor, 2012; Kulesa and Gammill, 2010; Kulesa and McLennan, 2015; Vega-Lopez et al., 2017). In the chick, chemokine signaling has been implicated in directing trunk neural crest cells to the ventral region near the dorsal aorta (Kasemeier-Kulesa et al., 2010; Saito et al., 2012); cells are then sculpted into primary sympathetic ganglia by other molecular signals (Kasemeier-Kulesa et al., 2006). Initial emerging trunk express the chemokine receptor

CXCR4 after exiting the dorsal neural tube (Kasemeier-Kulesa et al., 2010; Saito et al., 2012). The CXCL12 ligand for CXCR4 identified in situ hybridization is expressed by cells adjacent to the dorsal aorta at HH14 in chick (Kasemeier-Kulesa et al., 2010) and is initiated by BMP signals in the chick dorsal aorta (Saito et al., 2012). When ectopic sources of CXCL12 protein soaked beads are placed either lateral to the chick dorsal aorta (Kasemeier-Kulesa et al., 2010) or near the surface ectoderm (Saito et al., 2012), CXCR4-expressing neural crest cells are lured to incorrect locations. Blocking of CXCR4 by morpholino or shRNA in premigratory chick trunk neural crest cells leads to significantly fewer cells that reach the dorsal aorta and instead populate the dorsal root ganglia (Kasemeier-Kulesa et al., 2010).

Although these results clearly implicate the CXCR4/CXCL12 signaling axis to position the initial trunk neural crest cells to a ventral location in the embryo, they also raise two important questions. First, how do trunk neural crest cells that exit the dorsal neural tube readout a diffusible signal that is generated by cells in a ventral location several hundred microns away (Figure 1A)? In the chick, neural crest cells travel 350um in 24hrs to reach the primary sympathetic ganglia target during HH14-16 (Kasemeier-Kulesa et al., 2010). Ephrin-B ligands expressed along the dorsolateral pathway are thought to inhibit initial emerging trunk neural crest cells from entering and instead force cells to move onto but do not direct cells along the medio-ventral migratory pathway (Santiago and Erickson, 2002). In the absence of another directional signal, it is tempting to speculate that the mRNA expression of CXCL12 directly translates into secreted protein that is readout by initial emerging trunk neural crest cells. However,

CXCL12 mRNA expression is restricted to the ventral region in chick during HH14-16 and is found in the chick dorsal dermamyotome (Saito et al., 2012) but at a much later stage HH20 after neural crest cells have already formed the primary sympathetic ganglia. Further, it is not accurate to assume that mRNA expression directly translates into secreted protein amounts and no evidence that CXCL12 is diffused in a dorsal direction from the dorsal aorta.

Second, what signals direct some trunk neural crest cells to reverse direction, move towards and stop to form the dorsal root ganglia (Figure 1A)? Interestingly, later emerging trunk neural crest cells that either reach the sympathetic ganglia or contribute to the dorsal root ganglia do not express the CXCR4 receptor for CXCL12 (Kasemeier-Kulesa et al., 2010). Thus, although a common subpopulation of trunk neural crest cells migrate to form the dorsal root and sympathetic ganglia, there are gene expression and cell behavioral differences that appear to be driven by dynamic microenvironmental signals.

Agent-based computational models have emerged as a powerful approach to rapidly test mechanistic hypotheses based on a limited set of empirical data. We previously constructed a 2D agent-based model of chick cranial neural crest cell migration (McLennan, Dyson, et al., 2012) that incorporated domain growth based on empirical measurements and evidence of vascular endothelial growth factor (VEGF) as a cranial neural crest cell chemoattractant (McLennan et al., 2010). By simulating the model with the observed entry rate of cells into the 2D domain and the hypothesis that all cells

consume and move in response to VEGF, the model predicted that many follower cells remain near the entrance to the migratory domain since the level of VEGF signal is depleted by cell consumption and tissue growth (McLennan, Dyson, et al., 2012). When we revised the model to include lead cells that readout VEGF and transfer information to follower cells, we could better recapitulate the normal cranial neural crest cell migratory pattern (McLennan, Dyson, et al., 2012). Genomic profiling of migrating chick cranial neural crest cells showed distinct molecular signatures between leaders and followers, supporting the model prediction of distinct functional roles (McLennan et al., 2012; Morrison et al., 2017). Thus, a 2D computational agent-based model approach was able to test and verify a mechanistic hypothesis that led to new unique insights of cranial neural crest cell migration.

In this paper, we focus on trunk neural crest cell migration. We briefly review exciting recent discoveries within the context of the rich history of trunk neural crest cell migration. We present a hypothetical model for the distribution of a common subpopulation of trunk neural crest cells into two distinct target locations. We develop and simulate an agent-based computational model to test this hypothesis related to the interplay of chemokine signals that direct the neural crest cellular traffic.

RESULTS

How do trunk neural crest cells that exit the dorsal neural tube readout a diffusible signal that is generated in a ventral location?

There are at least three hypothetical scenarios that address the questions posed above (Figure 1B-D). First, repulsive signals from the roof plate or dorsal neural tube may act to repel and/or polarize neural crest cells towards the medioventral direction (Figure 1B). Second, tracking of endothelial cells through their intersomitic journey has revealed the exciting possibility that trunk neural crest cells use endothelial cells as a scaffold and are perhaps directed by endothelial cell signaling to move along the medioventral pathway (Figure 1C). Third, a local secreted or membrane bound factor within the tissue near the dorsal neural tube or somitic mesoderm may attract neural crest cells to move along a medioventral pathway (Figure 1D). Here, we discuss data that support and identify limitations of these three scenarios and develop and simulate a computational model based on a chemotactic model of trunk neural crest cell migration.

Do signals from the dorsal neural tube midline repel and polarize neural crest cells to move around the sides of the neural tube?

Whether trunk neural crest cells are repelled by signals at the dorsal midline that promote cells to move around the side of the neural tube and within range of a ventral guidance signal(s), such as CXCL12 in chick, is unclear (Figure 1B). When negative chemotaxis was examined in vitro using isolated quail neural crest cells, there was no evidence to support this mechanism (Erickson and Olivier, 1983). Slit/Robo signals that have been shown to restrict axons from midline crossings and are obvious candidates

to consider do not seem to fit this scenario. Slits1,2 are expressed in the chick trunk dorsal neural tube and in the notochord (Slit1) and floorplate (Slit2) at HH12-13 (Giovannone et al., 2012), just prior to the initial exit of trunk neural crest cells at the level of the forelimb. Robo receptors are expressed by premigratory neural crest cells at HH10-11 (Robo1) and in later migrating neural crest cells at HH16 (Robo2) that have reached the sympathetic ganglia (Giovannone et al., 2012). However, there is no evidence that migrating neural crest cells express either Robo1,2 receptors while traveling along the medio-ventral pathway from the dorsal neural tube to the dorsal aorta. When Slit signaling in chick was silenced by morpholino at HH13-14, there was an unexpected enhanced (rather than reduced) number of HNK1-positive neural crest cells along the medioventral pathway (Giovannone et al., 2012).

Another candidate repulsive signal and secreted protein is Draxin. Draxin is expressed by cells in the mouse roofplate, dorsal spinal cord, and dorsal root ganglia a day after (E10.5) the initial trunk neural crest cells exit the dorsal neural tube at E9.5 (Zhang et al., 2017). In chick, Tanaka and colleagues had previously observed a similar Draxin expression pattern in the trunk (Su et al., 2009). Together, this led to the speculative hypothesis that emerging neural crest cells are repelled by a Draxin midline signal.

Draxin protein in in vitro stripe assays repels mouse neural crest cells exiting from trunk neural tube explants and reduces cell polarity in vitro (Zhang et al., 2017); a similar behavior observed in chick neural tube explants cultured in the presence of Draxin protein (Su et al., 2009). Draxin expression in the roofplate, lateral neural tube, and

dorsal lip of the dermamyotome in chick suggests neural crest cells move through a medioventral corridor to avoid Draxin inhibition, being repulsed initially from the dorsal midline (Su et al., 2009; Zhang et al., 2017). Chick trunk neural crest cells are slower and less directed in culture with Draxin cultured media (Su et al., 2009). However, over-expression of Draxin in the chick neural tube and migrating neural crest cells at HH12-13 shows a relatively normal migration pattern (Zhang et al., 2017). Further, Draxin-negative neural crest cells follow the dorsolateral migratory pathway at HH18 similar to the normal migratory pattern of the later emerging melanocyte precursors (Zhang et al., 2017). Comparison of migrating mouse neural crest cell positions by anti-p75 staining (to mark early neurogenesis by neural crest cells) at E10.5 in wildtype versus Draxin knockout mice did not show significant changes in the cell migration pattern (Zhang et al., 2017). Thus, these results reduce support for Draxin as a strong neural crest cell repulsive signal to direct trunk neural crest cells to migrate along a medioventral pathway.

Neural crest/endothelial cell interactions may drive directed neural crest cell migration to ventral targets

Previous studies of the embryonic neural crest microenvironment have shown that the basal lamina and migratory pathways in the head and trunk are rich in fibronectin and laminin and conducive to cell migration (reviewed in Thiery et al., 1986; Perris and Perissinotto, 2000). However, there is no evidence that the pattern of fibronectin or laminin provide direction information to migrating neural crest cells. That is, for example little evidence that either of these molecules are expressed in a higher concentration in

the ventral portion of the trunk medio-ventral neural crest cell migratory pathways to suggest cells take advantage of a long-range adhesive gradient or display haptotaxis to reach the dorsal aorta. Further, a uni-directional adhesive gradient would not explain the reverse in direction of trunk neural crest cells to form the dorsal root ganglia.

Development of a transgenic quail model Tg(tie1:H2B-EYFP) that includes the fluorescent labeling of endothelial cells coupled with time-lapse imaging has enabled embryologists with a unique perspective into endothelial cell movements and blood vessel formation (Sato et al., 2010). When premigratory neural crest cells are fluorescently labeled within this transgenic quail model, the complex ballet between trunk (George et al., 2016) and cranial (McKinney et al., 2016) neural crest cells and endothelial cells has been visualized for the first time. In the trunk, visualization of cell behaviors in whole quail embryo explants has keenly observed that trunk neural crest cells migrate in the ventral direction along and in contact with endothelial cell streams that travel in the opposite dorsal direction within the intersomitic furrow (George et al., 2016). Static 3D imaging revealed that quail neural crest cells are preferentially juxtaposed to the posterior sides of the endothelial cell streams, sandwiching neural crest cells between the endothelial cell streams and rostral somite halves (George et al., 2016). These data raise the exciting possibility that trunk neural crest and endothelial cells may use each other as a migratory scaffold (Figure 1C).

Do chemokine factors near the dorsal neural tube and somitic mesoderm attract the initial emerging neural crest cells along a medioventral migratory pathway?

Trunk neural crest cells that initially exit the dorsal neural tube display a spatially-ordered migration within discrete streams, as observed in time-lapse imaging in chick (Kasemeier-Kulesa et al., 2005) and zebrafish (Boer et al., 2015; Richardson et al., 2016). Despite migration in discrete streams, trunk neural crest cells may move between streams enroute to ventral locations (Kasemeier-Kulesa et al., 2005) and change direction in response to ectopic chemoattractant sources (Kasemeier-Kulesa et al., 2010; Saito et al., 2012). This suggests that trunk neural crest cells may be responsive to changes in local microenvironmental signals. This is further supported by evidence showing that later emerging chick trunk neural crest cells that follow lead cells and populate the perimeter of each primary sympathetic ganglia may reverse direction and move towards the presumptive dorsal root ganglia (Kasemeier-Kulesa et al., 2005) or stop at the presumptive dorsal root ganglia. Thus, both lead and follower trunk neural crest cells appear capable of responding to dynamic changes in local directional signals.

These data raise the possibility that lead trunk neural crest cells sense directional signals and transfer information to the follower cells, as proposed in the model for cranial neural crest cell migration (McLennan et al., 2012, 2015; Morrison et al., 2017). In support of this, Raible and colleagues (1992) observed that the first emerging trunk neural crest cells in zebrafish have long filopodial processes. This was later confirmed by time-lapse imaging in the living zebrafish embryo (Jesuthasan, 1996). Experiments

that disrupt fascin1, an actin-bundling protein essential for filopodia formation, reveal that severe loss of filopodia in zebrafish trunk neural crest cells leads to significantly reduced numbers of sympathetic neurons (Boer et al., 2015). The dorsal root ganglia in fascin1 zebrafish mutants were normal (Boer et al., 2015). Together, this supports the hypothesis that initial trunk neural crest cells that move over long distances towards the dorsal aorta readout guidance signals through long filopodia. Later emerging trunk neural crest cells that travel shorter distances, such as from the dorsal neural tube to the dorsal root ganglia may simply remain motile by using short-range lamellipodia. Although the data offer a plausible possibility for initial trunk neural crest cells to inform followers where to go, there is still no evidence of a specific guidance factor(s) present in the paraxial mesoderm near the dorsal neural tube and within the somite that would attract cells to a subregion within range of a ventral chemoattractive signal (Figure 1D).

An agent-based model to test the hypothesis that multiple chemokine signals direct trunk neural crest cell migration

To begin to mechanistically explore the hypothesis that multiple chemokine signals direct trunk neural crest cell migration, we developed a mathematical agent-based model that exhibits the observed biological behavior of trunk neural crest cell migration (as proposed in Figure 1D). The migratory domain is the region that includes the pathway in the embryo along which the trunk neural crest cells migrate. We model the 2D domain as a rectangle that corresponds to the length and width of the typical chick trunk neural crest cell migratory pathway and allows for the distribution of cells into simulated sympathetic and dorsal root ganglia positions. We use a reaction-diffusion

partial differential equation (PDE) with no-flux boundary conditions to model production and diffusion of the chemoattractants (Figure 1D). Cells chemotax up gradients of a chemoattractant in the following way. At each timestep a cell samples the local chemoattractant level and the level in a randomly chosen direction. If the levels away from the current position are favorable then the cell moves, and otherwise remains stationary.

Model simulation results show that with only one chemoattractant, the cells fail to reach the distal end of the migratory domain (Model result 1)

The simplest initial assumption for the mechanism of migration posits a single chemoattractant that is produced at the end of the migratory pathway. This corresponds to the CXCL12/CXCR4 signaling axis discussed earlier to position trunk neural crest cells to form the primary sympathetic ganglia. Our model simulations demonstrate that the ligand does not diffuse quickly enough to enable the migratory cells to reach the end of the migratory domain (corresponding to the sympathetic ganglia) after 24 hours of migration (Figure 2, Movie 1).

Model simulation results show that a second chemoattractant produced partway along the migratory pathway enables more cells to reach ventral destinations (Model result 2)

A single chemoattractant does not enable the cells to reach the sympathetic ganglia by the end of migration, since it takes some time for the chemoattractant signal to diffuse to the entrance of the migratory route. We therefore hypothesize the existence of a second

chemoattractant signal that is produced partway along the route (Figure 1D). This hypothesis is supported by the observation discussed earlier that later-emerging chick trunk neural crest cells do not express the CXCR4 receptor, yet populate the perimeter of the sympathetic ganglia (Kasemeier-Kulesa et al., 2010). In this second model, we assume that the early emerging cells (colored blue) chemotax towards both chemoattractants (blue and green), with a preference for the blue chemoattractant (Figure 3A, Movie 2). Thus, early in migration these cells obtain directional guidance from the green chemoattractant that is produced closer to the dorsal neural tube, and this signal is later overridden as cells move close enough to sense the blue chemoattractant (Figure 3B, Movie 2). Simulations of this model demonstrate that more cells reach the distal destinations by 24 hours, with 15% of blue cells reaching the blue source and 40% of green cells reaching the green source (Figure 3B, Movie 2).

Model simulations demonstrate that three chemoattractants are sufficient to partition the neural crest cell migratory stream into two distinct locations, corresponding to the sympathetic ganglia and the dorsal root ganglia (Model result 3)

Model 2 allowed many of the cells to reach the end of the migratory pathway after 24 hours of migration (Figure 3B). However, the full migratory population is not seen experimentally to reach the sympathetic ganglia. Instead the population is split into two subpopulations, with early-emerging cells forming the core and perimeter of the sympathetic ganglia near the distal end of the migratory pathway, and later-emerging cells that either migrate partway along the route before reversing direction to form the

dorsal root ganglia midway along the migratory pathway or exit the dorsal neural tube and stop at the dorsal root ganglia (Figure 1A). We therefore extend our model to include a third hypothesized chemoattractant, which is colored red and is produced 12 hours into migration (Figure 4A). The first two subpopulations of cells (blue and green) behave as described in Model 2. The latest cells to emerge are colored red and respond to the red and green chemoattractants, with a preference for the red chemoattractant (Figure 4A). Simulations of this model show that this mechanism successfully splits the population into cells (green and blue) that reach the sympathetic ganglia and those (red cells) that change direction partway through migration and reach the dorsal root ganglia (Figure 4B, Movie 3). After 24 hours nearly all cells have reached their intended destinations (Figure 4B; 100% of red and green cells and 95% of blue cells).

DISCUSSION

We discussed two major questions underlying the migration pattern of trunk neural crest cells to deploy into the embryonic microenvironment and form the peripheral nervous system. We first asked how trunk neural crest cells are initially directed away from the dorsal neural tube and along a medioventral migratory pathway to within range of known ventral guidance signals (Figure 1A). Second, we asked how signals direct some ventral migrating trunk neural crest cells to reverse direction or later exit the dorsal neural tube, move towards and stop to form the dorsal root ganglia (Figure 1A)? We reviewed recent data to discuss the validity of three hypothetical scenarios as to how trunk neural crest cells that migrate along a common pathway are distributed into these two discrete locations. We described our development of an agent-based model from

empirical data in the quail and chick embryo research organisms and presented simulations that test the hypothesis that multiple, spatially-distinct chemotactic sources are coordinated to distribute trunk neural crest cells into two discrete locations.

One of the goals of our study was to review the current evidence and propose that computational modelling may be a way forward to start a discussion of trunk neural crest cell migration to pattern the peripheral nervous system. Previous reviews have covered hypothetical mechanisms to direct trunk neural crest cells along a medio-ventral migratory pathway, including a gradient of extracellular matrix rigidity and differential adhesion properties. However, both of these mechanisms are not time-varying and so would not reproduce the observed migration of cells backwards along the migratory pathway or stopping to form the dorsal root ganglia. Thus, although it is likely that there are more mechanisms at play in this system we focused on hypothetical scenarios that included recent experimental data and introduced a modeling framework to incorporate emerging quantitative information.

When we considered the possibility that repulsive signals such as Draxin or Slits direct trunk neural crest cells away from the dorsal neural tube midline (Figure 1B), we found inconclusive evidence to support this scenario. In the absence of Robo receptor expression on migrating trunk neural crest cells, protein expression analysis of Slit1,2 may help to assess where these secreted molecules are present in the trunk neural crest microenvironment. Further, in vivo bead experiments that place ectopic sources of Slit proteins adjacent to or ahead of the invasive leaders of trunk neural crest cell

migratory streams may help to assess their in vivo repulsive roles. In the case of Draxin, loss of function experiments and clever placement of ectopic Draxin-soaked beads to challenge trunk neural crest cell behaviors in vivo will help us better understand its role as a candidate trunk neural crest cell inhibitor.

The exciting visual observations in transgenic quail embryos that revealed the dynamic interplay between the trunk neural crest and endothelial cells (George et al., 2016) suggest rationale for further experiments to examine underlying cellular and molecular signals that guide cells in opposite directions. These data add to the close relationship already observed between trunk neural crest cells, the basal lamina surrounding the medio-ventral migratory corridor, and fibronectin/laminin molecules within the pathway (reviewed in Thiery et al., 1986; Perris and Perissinotto et al., 2000). Whether trunk neural crest cells readout signals from the endothelial cells that influence cell movements in the medioventral direction to within range of known ventral-located chemotactic factors is still unclear. The present data in quail cannot rule out that trunk neural crest cells travel along a medioventral migratory pathway 'prior' to the presence of endothelial cells in the intersomitic furrow since current time-lapse imaging data begins at HH16 (George et al., 2016), after the initial quail neural crest cells have reached the dorsal aorta. Further, in the absence of endothelial cell ablation, it is unknown whether trunk neural crest cells travel through the intersomitic furrow in the absence of endothelial cell streams. This scenario is also complicated by data in the zebrafish, where the medioventral migration of trunk neural crest cells is through the middle portion of the somite rather than through the rostral somite halve and thus not

juxtaposed to endothelial cells in the intersomitic furrow (Raible et al., 1992). Thus, follow up experiments that step back the timing of observations to begin as quail trunk neural crest cells exit the dorsal midline, together with tissue ablation experiments that inhibit endothelial cell sprouting from the dorsal aorta will help us better understand the dynamic interplay and reliance of each cell subpopulation on one another for guidance.

Chemokine signals that are coordinated in space and time as we presented in our computational model present a plausible hypothesis for further experimental testing. We are not arguing that this is the only possible mechanism, simply that three chemoattractants are sufficient to reproduce the sorting of the cells and fewer than three chemoattractants are not sufficient without other mechanisms. We have previously proposed a computational model of cranial neural crest cell migration based on a cell-induced gradient of VEGF (McLennan et al., 2015). However, this model is inadequate to describe the trunk neural crest cell migration pattern that segregates a common cell population into two distinct locations to form the sympathetic and dorsal root ganglia. Thus, our simplistic model of trunk neural crest cell migration presented here that is based on multiple chemotactic signals is distinct from our previous models.

In the chick, there is strong evidence to support the CXCR4/CXCL12 signaling axis to position initial lead trunk neural crest cells in a ventral location to form the primary sympathetic ganglia (Kasemeier-Kulesa et al., 2010; Saito et al., 2012). High resolution imaging data in chick and zebrafish support a role for filopodia on initial emerging trunk neural crest cells to readout out long-range signals (Raible et al., 1992; Jesuthasan,

1996; Kasemeier-Kulesa et al., 2005; Boer et al., 2015; Richardson et al., 2016). Genomic profiling of the chick trunk paraxial mesoderm and chemokine expression analyses may help shed light on the presence of local chemoattractive signals that direct newly exiting cells to within range of CXCL12.

Comparative analysis of embryo model systems may help to determine the role of CXCR4/CXCL12 and other chemokines in trunk neural crest cell migration. In the mouse, trunk neural crest cells express CXCR4 as cells exit the dorsal neural tube (Belmandani et al., 2005). CXCL12 is chemoattractive to migrating trunk neural crest cells from neural tube explants and is expressed in tissue along the trunk neural crest medioventral migratory pathway (beginning approximately at the mid-level of the dorsoventral length of the spinal cord) and expanding in the ventral direction to surrounding the neural tube and throughout the perinotochordal region (Belmandani et al., 2005). Although disruption of CXCR4 receptors in CXCR4 null mouse mutants showed smaller and malformed dorsal root ganglia, there was no mention of analysis of the sympathetic ganglia (Belmandani et al., 2005). Further, it cannot be ruled out that CXCL12 is expressed by either migrating mouse trunk neural cells or mesodermal cells within the presumptive dorsal root ganglia microenvironment to maintain cohesion of CXCR4-exressing neural crest cells; disruption of the CXCR4 receptor then leads to reduced cohesion and less compact dorsal root ganglia. In the zebrafish, CXCR4/CXCL12 is not present in the trunk (Olesnicky-Killian et al., 2009), so further analysis of both the dorsal and ventral tissues may help to determine whether there is a correlative chemokine signal(s). Thus, although other potential chemokine signals have

yet to be fully discovered, especially in the zebrafish trunk, the presence of chemokines in the chick (Sato et al., 2011; Martinez-Morales et al., 2011) and mouse trunk and observed long filopodial extensions on the initial exiting chick and zebrafish trunk neural crest cells support the hypothesis that multiple chemical signaling sources direct cellular traffic to form the peripheral nervous system.

The model hypothesis that multiple chemotactic signals direct trunk neural crest cellular traffic allows for all cells to make direction decisions, rather than just lead cells. Laser ablation of individual lead trunk neural crest cells in the zebrafish has shown that follower cells remain motile but do not establish directed migration to the ventral target site (Linker et al., 2016), suggesting only lead cells possess direction information that is either hardwired at the dorsal neural tube or acquired shortly after neural tube exit. However, this hypothesis is speculative for two reasons. If follower neural crest cells are unable to respond to directional cues, how do some of these cells stop at the dorsal root ganglia? Second, what is the fate of the neural crest cells that migrate to a subregion between the sympathetic and dorsal root ganglia since the migratory stream is continuous (Raible et al., 1992; Jesuthasan, 1996)? If only a single lead trunk neural crest cell provides direction information, it is conceivable that laser ablation of this cell (Linker et al., 2016) inadvertently corrupts the local microenvironment so as to not allow follower cells to readout guidance information and re-establish directed migration.

Given the developmental plasticity of the neural crest, it seems unlikely that the peripheral nervous system would rely so heavily on a single cell for proper navigation of

the migratory stream without adaptation of follower cells to respond to minor insults. Plasticity in phenotype and genotype has been observed in chick cranial neural crest cells after tissue transplantation of followers into the lead position that reveals follower cells express a genotype similar to leaders and are able to reach the branchial arches (McLennan et al., 2012). In addition, follower cranial neural crest cells can overcome physical barriers that impede leaders, and reach the branchial arches (Kulesa et al., 2005). Thus, these types of experiments performed in the zebrafish may offer clearer insights into the role and plasticity of lead trunk neural crest cells to reach the presumptive sympathetic ganglia region.

In summary, we have raised two important questions regarding trunk neural crest cell migration and the formation of the peripheral nervous system. By converging on a working mechanistic hypothesis for the distribution of a common pool of trunk neural crest cells into two distinct locations via the dynamic spatio-temporal expression of chemokine signaling, we are stimulated to identify these signals using newly developed technologies in in vivo imaging, genomics, and multiplexed mRNA expression detection (Morrison et al., 2017). We propose that computational modelling allows us to integrate this information into a quantitative framework and rapidly test mechanistic scenarios. As more chemotactic signals are identified, future experiments and computational model simulations will allow us to refine our mechanism to better explain trunk neural crest cell migration decisions. This will in turn have a broader impact on tissue patterning mechanisms that rely on the precise migration of cell populations in the presence of multiple directional signals.

METHODS

Agent-based model of trunk neural crest cell migration. The mathematical model is detailed here. The migratory domain is taken to be a non-growing 350um by 100um rectangle since there are no current empirical measurements of tissue growth and the three-dimensional depth of the migratory streams is small. Each simulation contains up to three chemoattractants, each of which is modelled using the following set of equations, which include production and diffusion terms:

$$\frac{\partial u}{\partial t} - D\Delta u = \chi\left(1 - \frac{u}{K}\right)$$

$$\nabla u = 0 \text{ on the boundaries}$$

$$u(x, 0) = 0$$

where, u is the concentration of the chemoattractant, $D = 1.2*10^2$μm/h is the diffusivity of the chemoattractant, χ = 0.1/h (within the source region for each chemoattractant, and 0 outside) is the rate of production of the chemoattractant, and K=1 is the carrying capacity. We take the diffusivity of the chemoattractants to be $D = 10^4 um^2/h$, based on the published values of the diffusivity of CXCL12 of 2.7 x 105um2/h and of CCL21 of 2.3 x 105um2/h in collagen (Wang and Irvine, 2011), reducing by an order of magnitude to account for the convoluted extracellular environment along the migratory route. Sensitivity analysis on the value χ (from 0.01 to 0.25) reveals that our results are robust to changes in this parameter. Changing D also does not affect our results for $D>10^3 um^2/h$. Taking D<103um2/h does significantly reduce the number of cells successfully migrating within 24 hours, however this is more than two orders of magnitude lower than the diffusivities of CXCL12 and CCL21 in collagen (Wang and

Irvine, 2011). Since each chemoattractant is independent of the other chemoattractants and the cell positions, we solve each equation numerically using the solvepde function in MATLAB before simulating the cell movements (discussed below).

Each cell is included as an individual at a given location with a single "filopodium". Rather than including multiple filopodia and enabling them to retract and extend in a continuous way to sample the environment we have simplified this to use a single sample in a random direction at each time point. Cell chemotaxis is implemented in the following way. At each timepoint each cell compares the chemoattractant concentration at its location to the concentration at the end of its filopodium (of length 5um) in a randomly chosen direction. If the detected concentration is greater or equal (to within a threshold sensitivity of 10^{-5}) than in the current location, then the cell will move in that direction with velocity v = 50um/h. If the detected concentration is lower then, 1% of the time, the cell will still move, to simulate the intrinsic motility of the neural crest in the absence of directional signals. Our results are insensitive to the magnitude of this intrinsic movement. Note that this is merely one way of modelling cell chemotaxis, and our results are insensitive to the exact specifications of the chemotactic model. Sensitivity analysis on the threshold sensitivity (from 10^{-8} up to 10^{-3}) demonstrates that the results are insensitive to changes in this parameter.

For simulations with more than one type of chemoattractant we include as many subpopulations of cells as there are chemoattractants. Thus with two chemoattractants (Model result 2), we include a "green" subpopulation that only consider the

concentration of the “green” chemoattractant, while the “blue” subpopulation considers the weighted sum: 0.99 blue + 0.01 green, so that the cells respond primarily to the “blue” chemoattractant, but can also follow gradients in the “green” chemoattractant when there is no other signal. With three chemoattractants (Model result 3), we also include a “red” chemoattractant, that begins being produced 12 hours into migration, and a “red” subpopulation that initially follows the “green” chemoattractant, and later follows the “red” chemoattractant after it is present.

Cells enter the domain at a rate of 20 per hour at the neural tube end, beginning with 10 blue cells initially. They are initiated at a random time through their cell cycle and thereafter divide once every 10 hours into two cells of the same subpopulation. The cells enter in the following order: blue cells in the first 1.76 hours; green cells entering between 1.76 and 2.39 hours; and red cells entering between 2.39 hours and 18 hours. No cells enter in the last 6 hours (but divisions still occur). The entry times were determined by setting the final proportion of the various subpopulations to match experimental observations.

Model pseudocode. For each timepoint, solve the chemoattractant equations to find the chemoattractant concentrations. Insert cells at the neural tube end of the domain at a rate of 20 per hour. Every cell that is at the division stage of their cell cycle divides into two cells of the same subpopulation. For every blue cell: pick a random direction and calculate 0.99 * blue chemoattractant + 0.01*green chemoattractant at 5μm away in that direction and at the cell body. If it is better or equal (up to the sensing threshold) 5μm in

that direction than at the cell body move in that direction at a speed of 50μm/h. With a probability of 0.01, move anyway, otherwise stay put. For every green cell: pick a random direction. If the green chemoattractant is better or equal (up to the sensing threshold) 5μm in that direction than at the cell body move in that direction at a speed of 50μm/h. With a probability of 0.01, move anyway, otherwise stay put. For every red cell pick a random direction xIf t < 12 hours and the green chemoattractant is better than or equal (up to the sensing threshold) to the concentration 5μm in that direction than at the cell body move in that direction at a speed of 50μm/h. With a probability of 0.01, move anyway, otherwise stay put. If t > 12 hours and the red chemoattractant is better or equal (up to the sensing threshold) 5um in that direction than at the cell body move in that direction at a speed of 50μm/h. With a probability of 0.1, move anyway, otherwise stay put.

ACKNOWLEDGEMENTS

PMK would like to thank the kind generosity of the Stowers Institute.

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FIGURE LEGENDS

Figure 1: Trunk neural crest cell migratory pathways and three distinct mechanistic hypotheses for patterning the peripheral nervous system. (A) Trunk neural crest cell migratory pathways to form the peripheral nervous system (Sympathetic and Dorsal Root Ganglia). (B) Scenario 1. Repulsive signals. (C) Scenario 2. Neural crest/endothelial cell interactions. (D) Scenario 3. Multiple chemoattractants.

Figure 2: Single Chemoattractant Model Simulations (Model 1 result). A simulation of our computational model showing the migratory domain, a single chemoattractant (in blue), where the darker color corresponds to higher chemoattractant levels. All cells in the simulation perform chemotaxis up gradients in the blue chemoattractant. Only 0.07% of cells reach the source of the blue chemoattractant after 24hrs of migration. Length and width of the migratory domain corresponds to distance in microns.

Figure 3: Two Chemoattractant Model Simulations (Model 2 result). A simulation of our computational model showing the migratory domain, two chemoattractants (in blue and green), where the darker color corresponds to higher chemoattractant levels. Green cells chemotax up gradients in the green chemoattractant. Blue cells chemotax up gradients in the weighted sum: 0.99 blue + 0.01 green. Blue (green) cells chemotax up gradients in the blue (green) chemoattractant. After 24hrs of migration 15% of blue cells reach the blue source and 40% of green cells reach the green source. Length and width of the migratory domain corresponds to distance in microns.

Figure 4: Three Chemoattractant Model Simulation (Model 3 result). A simulation of our computational model showing the migratory domain, three chemoattractants (in blue, green and red), where the darker color corresponds to higher chemoattractant levels. Green cells chemotax up gradients in the green chemoattractant. Blue cells chemotax up gradients in the weighted sum: 0.99 blue + 0.01 green. Prior to 12 hours of simulation, red cells also chemotax up gradients in the green chemoattracant. After 12 hours of simulation, red cells chemotax up gradients in the red chemoattractant. After 24hrs of migration 100% of red and green cells reach the red and green sources (respectively) and 99% of blue cells reach the blue source. Length and width of the migratory domain corresponds to distance in microns.

MOVIE LEGENDS

Movie 1: Simulations of the Single Chemoattractant Source. Model simulation considering only a single subpopulation of cells (blue). Only a small minority migrate to their desired location within 24 hours.

Movie 2: Simulations of the Two Chemoattractant Sources. Model simulation considering two distinct subpopulations of cells (blue and green). Only a minority of cells from each subpopulation migrate to their desired location within 24 hours.

Movie 3: Simulations of the Three Chemoatttractant Sources. Model simulation considering three distinct subpopulations of cells (bue, green and red). The majority of cells from each subpopulation migrate to their desired location within 24 hours.