Commentary & View

Multiple signaling interactions coordinate collective cell migration of the posterior lateral line primordium

Andy Aman and Tatjana Piotrowski*

Neurobiology and Anatomy Department; University of Utah; Salt Lake City, UT USA

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Collective migration of adherent cohorts of cells is a common and crucial phenomenon during embryonic development and adult tissue homeostasis. The zebrafish posterior lateral line primordium has emerged as a powerful in vivo model to study collective migration due to its relative simplicity and accessibility. While it has become clear that chemokine signaling is the primary guidance system responsible for directing the primordium along its migratory path it is not clear what mechanisms downstream of chemokine signaling coordinate migration of individual cells within the primordium. In this review, we summarize the cell signaling interactions that underlie collective migration of the primordium and discuss proposed mechanisms for the function of chemokine signaling in this tissue.

Cell migration is crucial for the embryonic development and homeostatic maintenance of multicellular animals. Although in many cases cells migrate as individuals, migration of adherent cellular clusters, sheets and chains is a fundamental morphogenetic process used to generate three-dimensional forms in the developing embryo. Collective cell migration underlies many important developmental events including gastrulation, the fusion of the two primary heart fields in vertebrate development, during blood vessel formation, wound healing, Drosophila tracheal development and border cell migration during oogenesis.1-7 Understanding collective migration also has clinical importance, as tumor invasion and metastasis often involves collective migration of cancer cells.7,8

The zebrafish posterior lateral line (pLL) has emerged as a powerful model for elucidating molecular genetic mechanisms that regulate collective cell migration in vivo. The pLL is a sensory system found in fish and amphibians comprised of mechanosensory organs called neuromasts that contain sensory hair cells very similar to the hair cells that enable hearing in terrestrial vertebrates.9 The neuromasts of the pLL are deposited along a stereotyped pathway by the migrating posterior lateral line placode (primordium), a cohesive cluster of over 100 cells that originates posterior to the ear and migrates along the embryonic trunk. During migration cells in the trailing two thirds of the primordium organize into two or three garlic bulb-shaped rosettes. These rosettes are periodically deposited from the trailing edge of the primordium and subsequently mature to form mechanosensory neuromasts. Several excellent reviews describe the embryology of the lateral line in detail.10-12

During the past seven years our knowledge about the regulation of pLL migration has increased significantly. The first clues into the molecular regulation of this process came in 2002 when David et al. discovered that the migrating primordium expresses the chemokine receptor cxcr4b and that the ligand cxcl12a (sdf1a) is expressed along the presumptive path of migration.13 Knocking down either gene caused a strong loss of migration. Intriguingly, it was shown that cxcr4b is most strongly expressed in the leading edge of the migrating primordium and is downregulated in cells about to be deposited from the trailing edge. Elegant gain of function studies showed that this signaling pathway is indeed the primary guidance system, as the primordium migrates toward an ectopic source of chemokine ligand.14 In a different study it was demonstrated that in the absence of chemokine signaling, cells in the primordium are still quite motile, but lose their coordination and their directional collective migration.15 Subsequently, two groups independently discovered the presence of a second Cxcl12a-binding chemokine receptor Cxcr7b expressed in trailing cells of the primordium. Importantly, polarized expression of both cxcr4b and cxcr7b is crucial for normal migration (Fig. 1A).16,17 Despite rapid progress into understanding the regulation of collective migration of the primordium, key questions remain about how chemokine signaling directs collective migration of this tissue. Here we summarize proposed mechanisms of chemokine receptor mediated collective migration of the primordium and suggest approaches to resolve some lingering questions regarding the underlying molecular mechanisms. For a recent comprehensive review of cell signaling interactions in the primordium see reference. 18.

*Correspondence to: Tatjana Piotrowski; Neurobiology and Anatomy Department; The Brain Institute; University of Utah; Piotrowski Lab; 417 Wintrobe, 20 N. 1900 E; Salt Lake City, UT 84132 USA; Tel.: 801.581.4233; Email: piotrowski@neuro.utah.edu

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With regard to Cxcr4b function, informative mosaic analyses have demonstrated that Cxcr4b is only required in a few tip cells, even though ccr4b is expressed rather broadly in the primordium (dark blue cells in Fig. 1B). Small clones of wildtype cells in otherwise ccr4b deficient primordia completely rescued primordium migration. This approach also revealed that all cells in these mosaic primordia, including cells that presumably lack chemokine signaling, extend lamellipodia and actively migrate. These results lead to the proposal of a mechanism wherein only a few cells at the leading edge respond to Cxcl12a stimulation causing the propagation of another signal or signals that polarize more trailing cells in the primordium (Fig. 1B; green arrows). This second, intra-primordium signal could involve the propagation of a chemical signal similar to the production of chemotactic cAMP in the leading edge cells in Dictyostelium slugs. Alternatively, migration of ccr4b expressing leading edge cells might stimulate the propagation of a mechanotactic signal similar to that observed in in vitro wound healing assays where tension on cells behind the leading edge is thought to stimulate ERK1/2 type MAP kinase signaling in more trailing cells. At leading edge is thought to stimulate ERK1/2 type MAP kinase signaling in more trailing cells. At this point it is unresolved if leading cells migrate posteriorly because they respond to a Cxcl12a gradient, are intrinsically polarized or are repelled by adjacent cells, as is the case of migrating neural crest cells.

Cxcl12a belongs to a family of proteins known as chemokines, named for the fact that they are chemotactic cytokines. In most in vitro and in vivo contexts these molecules guide the chemotaxis of cells up a gradient of ligand. It has been proposed that the primordium does not require a Cxcl12a gradient for directional migration but that asymmetric expression of the chemokine receptors Cxcr4b and Cxcr7b in the primordium direct migration along a homogenous stripe of Cxcl12a. While there is no obvious gradient of cxcl12a mRNA this finding does not preclude the possibility of an instructional gradient of Cxcl12a protein, especially given that chemokines have been shown to be post-transcriptionally regulated in other contexts.

The most recent experiment designed to evaluate the necessity of a chemokine gradient in primordium migration involved the fused somites (fss) mutant. fss homozygote embryos possess a truncated cxcl12a stripe that does not reach the tail tip. In these mutants, the primordium stalls upon reaching the end of the cxcl12a stripe. In the majority of cases mutant primordia migrate ventrally towards the cxcl12a expressing pronephros. However some primordia make ‘U-turns,’ double back on themselves dorsally, and migrate toward the head. These results demonstrate intrinsic polarity of the primordium but they do not rule out the presence of an instructive Cxcl12a gradient in wildtype embryos. As the mutant primordia reach the end of the cxcl12a stripe, it is conceivable that Cxcl12a protein continues to be produced by cells trailing of the primordium. This could lead to a reversal of the gradient with higher levels towards the head. Resolution of this issue will require assaying the concentrations of Cxcl12a protein along the anterior-posterior axis of fss-/- mutant embryos.

Regardless of whether an instructional gradient of Cxcl12a protein exists along the midline of the trunk, it has become abundantly clear that intrinsic polarity of chemokine receptors within the primordium is critical for coordinated collective migration. A compelling model explaining the necessity of chemokine receptor asymmetry was originally proposed by Dambly-Chaudiere and coworkers. In this model Cxcr7b in the trailing zone of the primordium does not signal but rather acts to sequester Cxcl12a protein, thereby establishing or amplifying a gradient of Cxcl12a protein across the tissue (Fig. 1B). Elegant work on the role of Cxcr4b and Cxcr7b during zebrafish germ cell migration has since demonstrated that Cxcr7b can act as an Cxcl12a sink that binds and internalizes Cxcl12a protein generating protein gradients in extracellular space. It is not clear whether Cxcr7b might also signal in response to Cxcl12a binding. CXCL12-CXCR7 interaction stimulates the AKT signaling pathway in prostate cancer cells, suggesting that CXCR7 can function as a signaling receptor in certain contexts.

Recent work from our laboratory has elucidated the complex cell signaling network underlying primordium polarity including the asymmetric expression of cxcr7b in the migrating primor-
dium.\textsuperscript{28} The network is based on feedback interactions between the Wnt/β-catenin and Fgf pathways that restrict activation of these two signaling pathways to opposite poles of the primordium. Wnt/β-catenin signaling is activated only in the first several rows of leader cells where it induces the expression of secreted Fgf3 and Fgf10 ligands. Simultaneously, Wnt/β-catenin signaling upregulates the membrane tethered Fgf signaling inhibitor dkk1, which restricts Wnt/β-catenin pathway activation to cells occupying the leading portion of the primordium (Fig. 1C).\textsuperscript{28} By manipulating Wnt/β-catenin signaling using both gain and loss of function strategies and assaying chemokine receptor expression we discovered that Wnt/β-catenin activation represses cxcr7b expression in leading cells. Importantly, ectopic activation of Wnt/β-catenin signaling in the trailing portion of the primordium abolishes expression of cxcr7b in these cells. As a result the primordium stalls similar to what is observed in cxcr7b-depleted embryos.\textsuperscript{16,17,28}

These studies also revealed that Wnt/β-catenin signaling not only regulates chemokine receptor expression but simultaneously influences morphogenesis of the lateral line. Neurogenesis/rosette formation depends on the Fgf-dependent expression of proneural genes and cell shape changes that drive rosette formation.\textsuperscript{28,30,31} Wnt/β-catenin activation restricts Fgf-dependent neurogenesis to the trailing portion of the primordium and keeps the leading portion unpatterned. Based on the analysis of Fgf depleted primordia which simultaneously lose rosettes and stop migrating it was postulated that rosette formation is indispensable for migration.\textsuperscript{30,31} However, our analysis of embryos in which Wnt/β-catenin and Fgf signaling was inhibited revealed that primordia migrate normally in the absence of rosettes and that stalling in Fgf depleted primordia is due to ectopic Wnt/β-catenin signaling and the resulting loss of cxcr7b expression.

This Wnt/β-catenin-Fgf feedback mechanism maintains the stable asymmetric expression of chemokine receptors as the primordium migrates and deposits rosette clusters from the trailing edge.\textsuperscript{28} Thus, interactions between the Wnt/β-catenin and Fgf pathways provide an elegant mechanism to couple forward migration with the periodic generation of sensory organs. A more complete understanding of collective migration of the primordium will require a mechanistic understanding of chemokine signaling in the developing lateral line.

It is clearly important to resolve whether an instructional gradient of Cxcl12a protein exists along the horizontal myoseptum and how Cxcl12a protein distribution is affected by the passage of the primordium. We speculate that leading cells perceive a shallow Cxcl12a gradient that is amplified within the primordium by the intrinsic polarity of chemokine receptor expression. The isolation of a specific Cxcl12a antibody would allow the visualization of Cxcl12a protein distribution around wildtype primordia and primordia that have lost chemokine receptor function. This approach would also aid in determining the role of Cxcr7b in shaping an extracellular Cxcl12a protein gradient around the primordium.

It is also significant to establish the molecular functions of cxcr7b in the primordium. It is possible that, in addition to acting as a Cxcl12a sink, signaling via cxcr7b may also actively facilitate rosette deposition. This hypothesis is supported by the observation that cxcr7b is specifically expressed in cells fated to be deposited and that Cxcl12-CXCR7 binding facilitates cell adhesion and survival in vitro.\textsuperscript{16,17,29,32} Evaluating the role of Cxcr7b as a Cxcl12a sink can be accomplished using approaches similar to those employed for the analysis of germ cell migration in.\textsuperscript{24} Such experiments include for example following the intracellular fate of tagged Cxcl12a and Cxcr7b protein. If Cxcr7b acts as a Cxcl12a sink, these two proteins should co-localize in intracellular vesicles and these vesicles should co-localize with lysosome markers. Evaluating a possible signaling role for Cxcr7b in the primordium will be more challenging, as little is known about how signal transduction downstream of Cxcl12a-Cxcr7b binding occurs. Structure/function approaches aimed at identifying domains in the Cxcr7b protein necessary for receptor internalization and/or signaling will be necessary to evaluate the role of Cxcr7b-dependent signaling in the primordium.

Resolution of these issues promises to yield a wealth of information on how collective cell migration is achieved in vivo that will deepen our understanding of cellular mechanisms underlying morphogenesis and possibly also the spread of epithelial cancers.

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