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Chemokinetics

Richard J. Miller and **Phuong B. Tran**
Dept of Molecular Pharmacology and Biological Chemistry Northwestern University Feinberg School of Medicine 303 East Chicago Avenue Chicago, Illinois 60611

Abstract

How do migrating neural progenitor cells and growing axons know where to go? In this issue of *Neuron*, two papers (Knaut et al. and Lieberam et al.) demonstrate that activation of Cxcr4 chemokine receptors by the chemokine SDF1/Cxcl12 can direct both of these tasks.

During the development of the nervous system, neural progenitor cells migrate from the germinal epithelia where they are born to their final destinations. Upon arrival they stop, extend axons, and become functionally integrated into neuronal circuits. Elucidating the molecules that regulate these different stages of development, as well as their mechanisms of action, is a key goal in understanding how the nervous system develops. Two fascinating and important papers in this issue of *Neuron*, both of which utilize the full palette of available genetic and anatomical techniques, add to the growing body of evidence suggesting that chemokines are of key importance in at least two of these processes—neural progenitor cell migration (Knaut et al., 2005) and axon guidance (Lieberam et al., 2005).

Chemokines (CHEMOtactic cytoKINES) are small, secreted proteins about 100 amino acids in length. They have been widely studied due to their important role in orchestrating leukocyte migration under normal conditions and during inflammatory responses (Tran and Miller, 2003). There are approximately 50 chemokines that are categorized into several subfamilies based on certain structural motifs. All of the known effects of chemokines are transduced through the activation of an extended family of G protein-coupled receptors. Although many different chemokines contribute to inflammatory responses, most of the excitement concerning their role in development surrounds the properties of SDF1 (also known as Cxcl12), the unique ligand for the chemokine receptor Cxcr4. This is not the first time that Cxcr4 receptors have taken center stage: they have also been shown to be the major receptors that mediate the infection of B-lymphocytes by HIV-1, and so they have been the subject of intense scrutiny in that context. It is clear that a rapid expansion of the chemokine family accompanied the evolution of a sophisticated immune system. However, phylogenetic analysis has shown that prior to that time the SDF1/Cxcr4 chemokine receptor system was still widely expressed, indicating that Cxcr4-mediated signaling presumably has functions beyond those in the immune system (Huising et al., 2003).

It now appears that chemokines and their receptors have numerous roles to play in the nervous system as well. In 1998, two groups reported that mice deficient in either Cxcr4 or SDF1 died in late gestation (Ma et al., 1998; Zou et al., 1998). Among several phenotypes noted in these mice, it was observed that the internal granule cell layer (IGL) of the cerebellum was malformed owing to an inappropriately early inward migration of granule neuron progenitors from the external granule cell layer (EGL), where they normally proliferate prior to migration. Subsequently, it has been shown that Cxcr4 null embryos also exhibit other phenotypes that reflect the abnormal migration of neuronal progenitors, including malformation of the dentate gyrus (Lu et al., 2002), lack of interneuron migration to the cortex (Stumm et al., 2003) and, most recently, malformation of the dorsal root ganglia (Belmadani et al., 2005).

This last observation dovetails very nicely with the paper published by Knaut et al. in this issue of *Neuron*, demonstrating how SDF1/Cxcr4 signaling guides the formation of the sensory trigeminal ganglia in zebrafish embryos. The authors observed that in wild-type embryos, the progenitors for trigeminal ganglion sensory neurons (TgSNs) were arranged as single cells or small clusters along the anterior-posterior axis lateral to the midbrain and the midbrain-hindbrain boundary. Using time-lapse imaging, as well as other techniques, they observed that the more anterior cells were highly motile, migrating toward the midbrain-hindbrain boundary and eventually coalescing with posteriorly placed cells to form the ganglion. Knaut et al. then asked: What was responsible for the guided movement of these cells? In answering this question, they demonstrated that all TgSNs expressed one of two Cxcr4 receptor types found in zebrafish (Cxcr4b), whereas SDF1 was expressed by cells positioned posteriorly to the final position of the coalesced ganglion and about 100 μM from the most anteriorly placed TgSNs. Manipulation of the expression patterns of Cxcr4 and SDF1 elegantly demonstrated the role of SDF1/Cxcr4 signaling in ganglion formation. In *odysseus* (*ody*) mutant embryos, in which Cxcr4b is inactivated, it was observed that TgSNs formed two miniganglia, instead of a single coalesced ganglion. The first of these miniganglia was found in the same position as the normal ganglion, but the second was situated ectopically at an anterior position and eventually disappeared. Similar patterns were observed when either Cxcr4 or SDF1 was inactivated in morpholino-injected wild-type embryos. On the other hand, forced expression of SDF1 in abnormal locations produced additional ectopic clusters of TgSNs. Overall, the data suggest a model in which the normal posterior source SDF1 plays two roles. First, it attracts the more anterior TgSNs that have to move posteriorly toward the coalescing ganglion in the face of an anterior bulk flow of cells in the embryo. Secondly, SDF1 maintains the position of posteriorly situated cells that are already in the correct position for ganglion formation.

Echoes of these two actions, involving the maintenance and attraction of cells, are found when considering the role of SDF1/Cxcr4 signaling in the development of the mouse brain (Tran and Miller, 2003). For example, in the case of cerebellar development, the source of SDF1 located in the overlying pia mater, ensures the maintenance of granule neuron progenitors in their proliferative niche, the EGL. When SDF1 expression ceases, the cells are free to migrate away from the EGL to their final destination, the IGL. In contrast, in development of the dentate gyrus, the source of SDF1 in the meningeal cells acts as a chemoattractant for granule cell progenitors migrating from the wall of the lateral ventricle to the rudimentary dentate gyrus. In this case, lack of SDF1 synthesis results in stalled migration of progenitors and lack of a properly formed dentate gyrus. Similarly, in the development of the mouse DRG, SDF1 acts as a chemoattractant for sensory neuron progenitors migrating from the neural tube (Belmadani et al., 2005). Interestingly, as in the case of the zebrafish, insufficient Cxcr4 signaling in the mouse results in malformed ganglia and the occurrence of ectopic clusters of sensory neurons. It is known that the zebrafish trigeminal sensory ganglion originally evolved from two distinct ganglia named the profundal and trigeminal sensory ganglia. A particularly interesting speculation offered by Knaut et al. (2005) is that it is possible that in the absence of Cxcr4 signaling the zebrafish trigeminal complex returns to this primitive state of organization.

The second paper in this issue of *Neuron* concerns the other identified effect of chemokine signaling during neural development—axon guidance. Several papers have demonstrated that SDF1/Cxcr4 signaling can regulate axon pathfinding in vitro. This effect manifests itself primarily as an ability to reduce the effects of axon repellants such as Slits and semaphorins. Indeed, Chalasani et al. (2003) previously demonstrated that Cxcr4 knockout mice appeared to have aberrantly projecting axons in the spinal cord. Now Lieberam et al. (2005) have used this principle to explain a mystery in developmental neurobiology. The mystery is this. Newly generated motor neurons send their growing axons from the neural epithelium into

the periphery along two distinct paths. In one of these paths, ventral motor neurons (vMNs), generated in the spinal cord and caudal hindbrain, send their axons through the ventral neural tube where they emerge at ventral exit points. Conversely, dorsal motor neurons (dMNs), generated in the hindbrain and rostral spinal cord, send their axons dorsally to emerge at dorsal exit points. Once in the periphery, vMNs take a path that avoids sensory ganglia. In contrast, the axons of dMNs follow a path that is close to incoming sensory fibers after which they traverse sensory ganglia in close proximity to their points of exit.

How are these two separate pathways specified? It was previously shown that in mice that lack the homeo-domain protein Pax6, vMNs behave like dMNs and their axons behave like those of dMNs. Presumably, Pax6 directs the expression of cell surface signaling molecules that help to direct axons to the ventral route. Lieberam et al. (2005) now show that Cxcr4 is the molecule that is responsible for this behavior. First, the authors noted that Cxcr4 was strongly, but transiently, expressed in the ventral neural tube by the pool of developing MNs at a time following their initial migration when they are just starting to extend axons. As motor neurons mature, the expression of Cxcr4 wanes. Thus, the receptor is expressed by only the most recently generated neurons. At the same time, it was observed that SDF1 was expressed by mesenchymal cells that line the ventral spinal cord. Further analysis matched Cxcr4 expression with different subclasses of motor neurons, identified through their selective expression of particular transcription factors, and revealed that Cxcr4 was expressed by vMNs, but not dMNs. The one exception, which proved to be very instructive, was the expression of Cxcr4 by developing trigeminal motor neurons, normally a dMN type. Lieberam et al. demonstrated that developing vMNs in culture transiently expressed Cxcr4 in their growth cones and that SDF1 expanded the size of the growth cone, thereby suggesting this as a site of Cxcr4 signaling in developing motor neurons. Analysis of the phenotypes of Cxcr4 and SDF1 knockout mice showed that, unlike DRG neurons or the sensory neurons studied by Knaut et al. (2005), the initial migration of motor neuron cell bodies seemed quite normal. However, these mice did exhibit a striking axonal guidance phenotype: vMNs had turned into dMNs! In Cxcr4-deficient mutants, the axons of many would-be vMNs now exhibited a dorso-lateral trajectory. At levels of the spinal cord where dMNs are also generated, aberrantly projecting vMNs exited the CNS along with dMNs. For example, in mutants some vMNs at a rostral cervical level of the spinal cord eventually innervated the acromiotrapezius muscle, which is usually innervated by motor neurons in the X1 cranial nerve. In addition, vMNs from embryos deficient in Cxcr4 signaling also showed dMN-like trajectories once they had emerged into the periphery. Normally, these axons follow a trajectory whereby they avoid sensory ganglia. Interestingly, this is also the case for trigeminal motor neurons, the only dMNs that express Cxcr4. Lack of Cxcr4 signaling caused many vMNs, and, significantly, also trigeminal motor neurons, to invade sensory ganglia. Thus, Cxcr4 signaling appears to control two distinct behaviors of motor neuron axonal pathfinding, whereby expression of Cxcr4 is responsible for both the initial axon trajectory and choice of exit point from the neural epithelium, as well as determining whether axons invade or avoid sensory ganglia.

Given the known effects of Cxcr4 signaling on axonal pathfinding, Lieberam et al. have come up with a very neat model to explain all of this. They point out that substances that repel motor neuron axons, such as semaphorins, are expressed by cells that flank the ventral neural tube. Normally, therefore, these substances will repel motor axons away from the floor plate. In other words, without other influences all motor neurons are dMN “wannabes” and would follow the dorsal trajectory. However, if axons also express Cxcr4, activation of this receptor will “turn down the volume” of repellant influences, allowing axons to follow a ventro-lateral course and exit the neural tube. This simple model beautifully explains the differences observed in the initial pathfinding of vMNs and dMNs. Perhaps similar factors

may also explain the choice to enter or avoid the sensory ganglia, clearly a question of interest.

Together, these two new papers convincingly illustrate the importance of Cxcr4 signaling in two phases of neural development. However, from the developmental point of view, this is probably only the tip of the iceberg. Patterns of Cxcr4/SDF expression flicker on and off all over the developing embryo, possibly acting as stop/go signals for the development of many parts of the nervous system and, indeed, of other tissues as well (McGrath et al., 1999; Stumm et al., 2003). It is also worth noting that in the adult brain, neurons and neural stem cells express numerous chemokine receptors in addition to Cxcr4, suggesting that they may be responsive to diverse chemokines whose synthesis is upregulated in the context of neuroinflammatory disease and brain repair (Tran et al., 2004). As a result, chemokines may be involved in regulating the migration of neural stem/progenitor cells during adult neurogenesis as well, thereby recapitulating events observed during embryogenesis. Furthermore, the expression of chemokine receptors by mature neurons in the adult nervous system may act as a conduit for decoding the effects of the diseased, inflamed brain on neuronal activity as well as on neuronal death and survival. Thus, chemokine signaling may be of great importance in the nervous system from its early development until its ultimate demise.

Selected Reading

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