

Chemokine signaling in embryonic cell migration: a fisheye view

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Chemokines and their receptors were discovered about twenty years ago as mediators of leukocyte traffic. Over the past decade, functional studies of these molecules have revealed their importance for cell migration processes during embryogenesis, which, in addition to providing mechanistic insights into embryonic development, could complement information about chemokine function in the immune system. Here, we review the roles of the chemokine stromal cell-derived factor 1 (SDF-1/CXCL12) and its receptor CXCR4 during zebrafish and mouse embryonic development, and discuss their function in regulating the interactions of cells with their extracellular environment, in directing their migration, and in maintaining their location.

Introduction

Chemokines (chemotactic cytokines) are a group of vertebrate-specific small (8-14 kDa) proteins that, depending on the presence and the position of conserved cysteine residues, are categorized into four subgroups (C, CC, CXC and CX3C). The chemokines (of which there are at least 46 in humans) interact with a smaller number of G-protein-coupled seven-transmembrane receptors (of which there are at least 18 in humans) (DeVries et al., 2006; Zlotnik et al., 2006). These chemokine receptors are classified based upon the type of chemokines they bind to; their name includes the letter 'R' and a number that signifies the chronological order in which they were identified. The best-characterized role of chemokines is in the control of cell trafficking and activation as part of the immune response (Luster, 1998), where they direct the movement of responsive cells towards higher concentrations of their ligand in the environment (a process termed chemotaxis). This function is shared by practically all members of the chemokine superfamily.

Chemokines can be classified into two groups: inflammatory chemokines, which recruit leukocytes to inflamed tissues; and homeostatic chemokines, which are constitutively produced and control homeostatic leukocyte traffic, secondary lymphoid organ structure and processes not related to the immune system. Chemokines and their receptors have attracted special attention in recent years as they are involved in a range of clinical disorders, such as Human immunodeficiency virus (HIV) infection, autoimmune conditions, inflammatory diseases and cancer, making them attractive potential targets for drug development (Viola and Luster, 2008; Zlotnik, 2008).

Whereas the primary role of chemokines is in the immune system, the homeostatic chemokine SDF-1/CXCL12 and its receptors CXCR4 and CXCR7 have been found to play crucial

roles in a wide range of developmental processes. The first evidence that chemokines have a function beyond the control of leukocyte trafficking was obtained from the analysis of mice in which *Sdf-1/Cxcl12* or its receptor *Cxcr4* were knocked out, resulting in disturbed vascular development, hematopoiesis and cardiogenesis (Nagasawa et al., 1996; Tachibana et al., 1998; Zou et al., 1998). These findings, and subsequent *in vivo* and *in vitro* studies in mice, fish and chick, have expanded our knowledge of the biological significance of chemokines, and have demonstrated their roles in muscle patterning, heart development, melanophore patterning, blood vessel formation, neuronal patterning and neurotransmission, cell migration in embryogenesis, and the homing of hematopoietic cells during ontogeny (Chong et al., 2007; Horuk et al., 1997; Knaut et al., 2005; Nagasawa et al., 1996; Sparmann and Bar-Sagi, 2004; Svetic et al., 2007; Vasyutina et al., 2005; Ara et al., 2003b; David et al., 2002; Doitsidou et al., 2002; Herpin et al., 2008; Hesselgesser et al., 1998; Knaut et al., 2003; Limatola et al., 2000; Nair and Schilling, 2008; Sasado et al., 2008; Stebler et al., 2004). In this review, we discuss several examples of the roles that SDF-1/CXCL12 and its receptors play in early development. As results concerning the role of chemokines in mouse embryonic development have been recently reviewed elsewhere (Cardona et al., 2008; Li and Ransohoff, 2008; Tiveron and Cremer, 2008), we discuss them only briefly here and focus instead on recent studies of chemokine signaling in zebrafish embryogenesis.

The optical clarity and extra-uterine development, combined with its genetic tractability, make the zebrafish an excellent model for studying chemokine-dependent cellular and developmental processes at an unprecedented resolution. As we review here, these characteristics have enabled studies of the function of CXCL12 proteins (as a result of gene duplication, zebrafish possess two CXCL12-encoding genes, *cxcl12a* and *cxcl12b*) and their receptors (CXCR4a, CXCR4b and CXCR7b in zebrafish) in controlling the migration of cells during gastrulation, primordial germ cell (PGC) migration, and the migration of cell clusters during the development of the zebrafish lateral line organ.

Controlling cell interactions with the extracellular environment

A central theme in inflammation and immunity is leukocyte extravasation, the movement of leukocytes out of the circulatory system during normal immune surveillance or in the course of responses to local tissue damage or infection. The recruitment of leukocytes to specific sites in the body involves the function of adhesion molecules (such as selectins) and their ligands, as well as that of chemokines and their receptors (for reviews, see Alon and Dustin, 2007; Ley et al., 2007). The signaling cascade that leads to leukocyte recruitment is initiated by selectin-mediated adhesive interactions of the leukocytes with endothelial cells at regions where extravasation should occur. These interactions tether the circulating

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cells to the blood vessel wall, facilitating the firmer attachment of the leukocytes to these sites. The increased cell-extracellular matrix (ECM) adhesion in these locations is mediated by integrins, ECM receptors that are activated by chemokine signaling.

A role for chemokine signaling that is conceptually similar to the one it plays in integrin activation in circulating leukocytes was revealed by studies of germ-layer morphogenesis during zebrafish gastrulation (Mizoguchi et al., 2008; Nair and Schilling, 2008), in which the roles of the chemokine receptor CXCR4a and the ligand CXCL12b were investigated (Fig. 1).

The position of mesodermal and endodermal cells in the embryo is determined by the coordinated movement of these germ layers during gastrulation. The coordination between endoderm and mesoderm migration thus controls the proper location and morphology of the tissues and organs that develop from these germ layers. During gastrulation, *cxcl12b* is expressed in mesodermal cells, whereas *cxcr4a* is expressed in the endodermal cell layer. By analyzing zebrafish embryos deficient for the function of this chemokine and chemokine receptor, Nair and Schilling discovered that severe defects in endodermal organ development occur in their absence, including pancreas, liver and intestine duplications (Nair and Schilling, 2008). In their investigation of the underlying basis of these defects, the authors observed that the function of CXCR4a and CXCL12b is required to coordinate the movement of endodermal cells with that of the mesodermal germ layer (Fig. 1A). Specifically, the lack of CXCL12b-CXCR4a signaling resulted in

the ‘untethering’ of the endodermal layer such that it was displaced towards the animal pole of the embryo relative to the mesodermal germ layer (Fig. 1B,C).

Nair and Schilling went on to show that the ‘tethering’ of endodermal cells requires CXCR4a-dependent integrin $\beta 1$ function. The suggested course of events is thus that maternally and mesodermally provided CXCL12b activates the CXCR4a receptor, which in turn enhances integrin-dependent endodermal cell adhesion to the extracellular matrix. In this instance, the precise mechanism by which the activation of the chemokine receptor leads to an increase in integrin $\beta 1$ function is not known, but could involve transcriptional activation of this gene (Nair and Schilling, 2008). It would be interesting to determine whether the activation of integrin function also occurs at the protein level, in which case endodermal cell migration would constitute an accessible model for studying certain events in leukocyte extravasation.

Directing cell migration

The importance of chemokines for *in vivo* chemotaxis was first shown in the context of the immune system, in relation to the accumulation of lymphocytes at sites of immune and inflammatory reactions (reviewed by Baggiolini, 1998). The first chemokines to which lymphocytes were reported to respond with chemotactic activity were RANTES (CCL5), MIP-1 α (CCL3) and MIP-1 β (CCL4) (Baggiolini et al., 1994). A subsequent wave of studies established that chemokines play a role in numerous cell trafficking events in the immune system (reviewed by Sallusto and Baggiolini, 2008).

Consistent with the *in vitro* and *in vivo* activity of chemokines within the immune system, chemokines were found to guide cell migration *in vivo* during development and disease. For example, the distinctive metastatic pattern of breast cancer has been suggested to involve the attraction of CXCR4-expressing tumor cells to organs in which CXCL12 is found, such as the lung, liver and bone marrow (Muller et al., 2001). During normal brain development in mouse, the migration of cortical interneurons is controlled by CXCL12 (Stumm et al., 2003; Tiveron et al., 2006). In this setting, the interneurons follow stereotypic migration routes in the cortex, along which *Cxcl12* RNA is expressed, suggesting that this chemokine prefigures the route of cell migration. Similarly, CXCL12 was shown to act as a chemoattractant for cortical neurons (Stumm et al., 2003). The proper migration of these cells *in vivo* depends on CXCR4 function, as revealed by the analysis of mice that lack the normal expression of either *Cxcr4* or *Cxcl12* RNA (Stumm et al., 2003; Tiveron et al., 2006).

One of the best-characterized examples of chemokine-guided single-cell migration in development is that of PGCs in fish, mouse and chick (Ara et al., 2003a; Doitsidou et al., 2002; Herpin et al., 2008; Knaut et al., 2003; Molyneaux et al., 2003; Stebler et al., 2004). In zebrafish, the process by which CXCR4b-expressing germ cells (Doitsidou et al., 2002; Knaut et al., 2003) arrive at their target – the position where the gonad develops – is regulated by the ligand-receptor pair CXCL12a-CXCR4b (Doitsidou et al., 2002), a combination distinct from the CXCL12b-CXCR4a pair that is required for the process of endoderm tethering mentioned above. CXCL12a is expressed in tissues that serve as the PGC migration targets, and both CXCL12a and its receptor CXCR4b were shown to be essential for the normal migration of these cells (Fig. 2A-C) (Doitsidou et al., 2002; Knaut et al., 2003). Specifically, in embryos that lack the activity of either CXCL12a or CXCR4b, the PGCs failed to migrate directionally towards their targets (Fig. 2B,C). Consistently, cells engineered to express CXCL12a readily attract

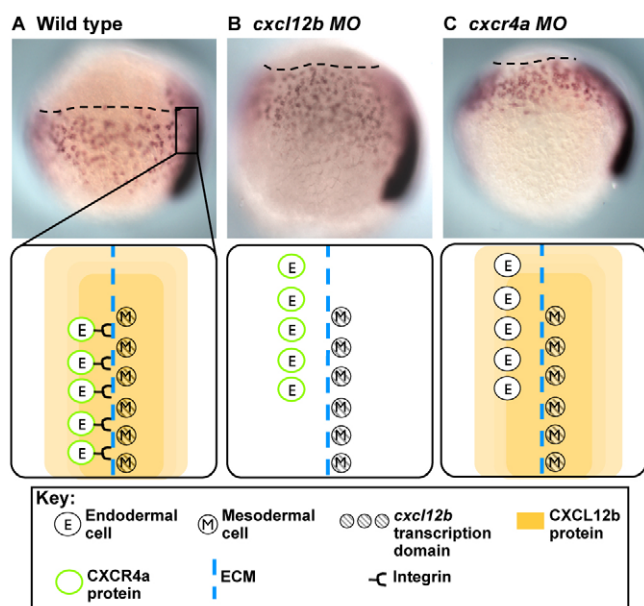


Fig. 1. Chemokines in zebrafish gastrulation. (A-C, top) Zebrafish embryos at 8 hours post-fertilisation (hpf), anterior to the top, labeled for expression of *foxa2*. Dotted lines represent the endodermal cell front. (Bottom) Schematic cross-section representing the mesoderm-endoderm interactions during gastrulation. In wild-type zebrafish embryos (A), chemokine signaling coordinates the migration of the endoderm with that of the mesoderm during gastrulation. The endodermal cell front of gastrulating zebrafish embryos is abnormally displaced in embryos in which CXCL12b (B) or CXCR4a (C) is knocked down, and integrin-based tethering between the endodermal and mesodermal germ layers, which depends on attachment to the extracellular matrix (ECM), is reduced. [Top panels adapted, with permission, from Nair and Schilling (Nair and Schilling, 2008)].

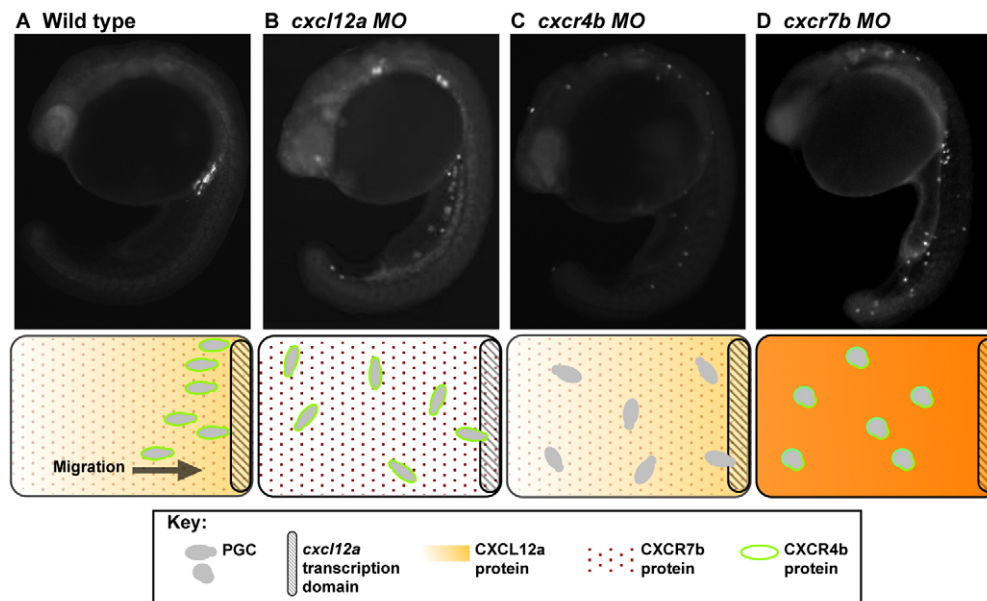


Fig. 2. Chemokines in individual cell migration. (A-D, top) Zebrafish embryos at 20-23 hpf, anterior to the top, with primordial germ cells (PGCs) expressing green fluorescent protein (GFP). (Bottom) Schematic representation of PGC target-tissue interactions during PGC migration. (A) In the wild-type, the directional migration of individual PGCs is controlled by chemokines. (B) In the absence of CXCL12a, no chemokine gradient is formed, and the PGCs fail to migrate towards their target. (C) Similarly, in the absence of the corresponding CXCR4b receptor, the cells fail to respond to the chemotactic gradient formed by CXCL12a. (D) Lower levels of CXCR7b, which usually sequesters CXCL12a, result in abnormally high levels of CXCL12a and in defects in PGC polarization and directional migration. [Top panel in B adapted, with permission, from Doitsidou et al. (Doitsidou et al., 2002)].

CXCR4b-expressing PGCs, supporting the notion that CXCL12a constitutes the actual guidance cue for these cells (Blaser et al., 2005). Interestingly, the CXCL12-CXCR4 axis has been found to play a role in controlling PGC migration in other organisms as well. A correlation between the expression pattern of CXCL12 and the migration path of PGCs has also been demonstrated in mouse, chick and medaka (Herpin et al., 2008; Molyneaux et al., 2003; Stebler et al., 2004). Furthermore, CXCL12 is capable of influencing the migration path of PGCs (in the cases of mouse and chick), and reducing the function of the receptor or its ligand results in abnormal cell migration (in the cases of mouse and medaka).

This conceptually simple scheme, according to which controlling the expression pattern of CXCL12a is sufficient to account for the migration path of zebrafish PGCs en route to their target, had to be revised following the identification of CXCR7, a second receptor for this chemokine, in a number of species (Balabanian et al., 2005; Burns et al., 2006; Thelen and Thelen, 2008). Surprisingly, whereas knocking down CXCR7b in zebrafish embryos resulted in a strong PGC migration phenotype (Fig. 2D), *cxcr7b* RNA was not visibly expressed in the PGCs, unlike *cxcr4b* RNA, nor was it required for the mobilization of calcium, a general second messenger in chemokine receptor signaling (Boldajipour et al., 2008). These observations are consistent with the features attributed to decoy receptors – receptors that merely bind their ligand without inducing downstream signaling (Mantovani et al., 2006). Indeed, CXCR7b was shown to be dynamically expressed in various somatic tissues and to function as a sink for CXCL12a. This conclusion was based on the observation that CXCR7b-expressing cells exhibit strong CXCL12a internalization activity and on the fact that PGCs show reduced active migration and polarization in a CXCR7b-depleted environment (Boldajipour et al., 2008). This cellular response is in agreement with the idea that, in the absence of CXCR7b, CXCL12a

levels are significantly increased, thus interfering with the formation of an informative chemotactic gradient (Fig. 2D). The finding that *Cxcl12a* mRNA levels are controlled by microRNAs (Giraldez et al., 2006) suggests that the restriction of CXCL12a production by controlling RNA stability and translation could represent yet another mechanism for regulating the shape of the CXCL12a gradient.

The CXCL12a-CXCR4b ligand-receptor pair has been shown to coordinate not only single cell migration, but also that of cells moving as a cluster. Collective cell migration, defined as the migration of cells that maintain cell-cell interactions during their movement, is a common theme in morphogenesis and disease (Friedl et al., 2004; Friedl and Wolf, 2008; Lecaudey and Gilmour, 2006). The invasion of solid tumors, such as human fibrosarcoma, into their surrounding tissue also involves the formation of multicellular strands. The migration of such strands is pioneered by cells (cancer cells or activated fibroblasts) at the front of the strand that are followed by tumor cells that maintain cell-cell contact (Friedl et al., 2004; Friedl and Wolf, 2008; Gaggioli et al., 2007; Wolf et al., 2007). Examples of collective cell migration in normal development include branching morphogenesis for duct formation (e.g. in *Drosophila* tracheal development), border cell migration (during *Drosophila* oogenesis), and morphogenetic movements during early embryonic development, such as convergence-extension movements in vertebrate gastrulation (Affolter and Caussinus, 2008; Montell, 2006; Rohde and Heisenberg, 2007; Rorth, 2007; Solnica-Krezel, 2005).

In the zebrafish, the collective migration of a group of cells called the posterior lateral line primordium (PLL) has been shown to require CXCL12a-CXCR4b signaling (David et al., 2002; Ghysen and Dambly-Chaudiere, 2007; Haas and Gilmour, 2006) (Fig. 3A-C). The PLL is a cohesive mass of approximately 100 cells that migrates from the anterior to the posterior of the embryo along a path

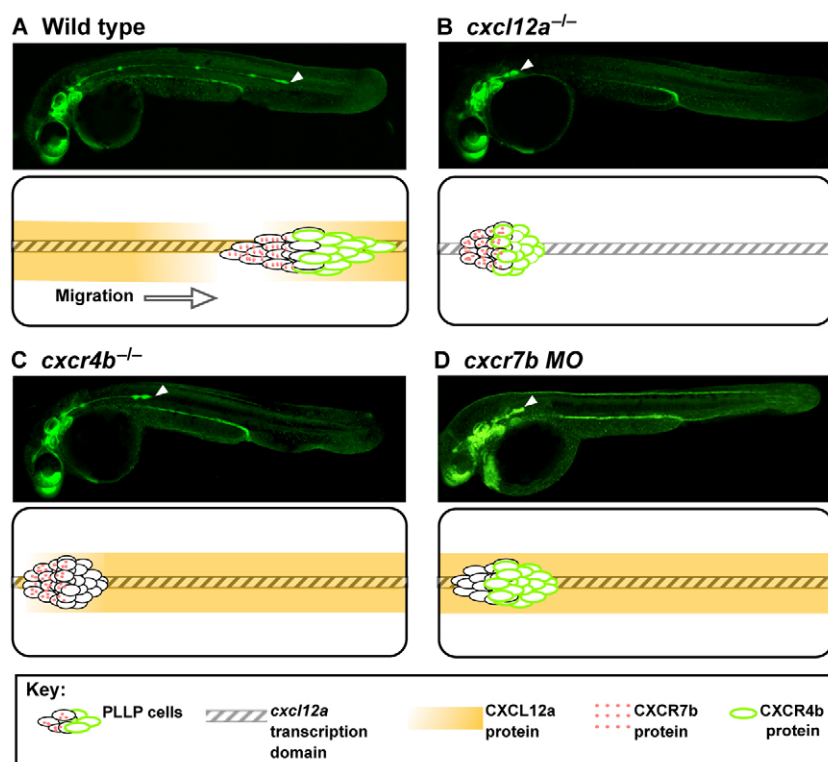


Fig. 3. Chemokines in collective cell migration in the zebrafish lateral line primordium. (A–D, top) *Cldnb::lynGFP* transgenic zebrafish embryos at 42 hpf, anterior to the left, with the posterior lateral line primordium (PLL) labeled with GFP (arrowheads). (Bottom) Schematic representation of chemokine signaling in the collective migration of the PLL. (A) In the wild-type, cells at the back of the PLL (left) express CXCR7b (red dots), which sequesters CXCL12a (dark yellow), thereby generating a chemokine gradient over the migrating cluster. (B) In *Cxcl12a*^{-/-} embryos, the PLL cluster fails to polarize and does not move. (C) Similarly, in the PLL clusters of *Cxcr4b*^{-/-} embryos, the CXCL12a gradient is not interpreted by cells at the front of the cluster, and the PLL does not migrate. (D) PLL clusters in which CXCR7b is knocked down fail to generate the CXCL12a gradient and therefore do not migrate. [Fluorescence images adapted, with permission, from Valentin et al. (Valentin et al., 2007)].

defined by the expression of the chemokine CXCL12a (Ghysen and Dambly-Chaudiere, 2007). As was clearly demonstrated by knockdown experiments and mutant analysis, CXCR4b function is required for the movement of the primordium along the path defined by CXCL12a (David et al., 2002; Haas and Gilmour, 2006). However, this description of the system does not provide a mechanistic explanation for the observed directionality of PLLP migration through the embryo. Specifically, the transcription of *cxcl12a* is not polarized along the migration axis and thus cannot provide the required positional information pattern by itself (David et al., 2002). The migration of the primordium can, however, be guided experimentally by an ectopic CXCL12a source, or by CXCL12a expressed in other tissues of the embryo (Haas and Gilmour, 2006; Li et al., 2004), suggesting that directional migration does involve CXCR4 signaling. Very intriguing is the observation that the PLLP can, under certain experimental conditions, migrate in the opposite direction (from the posterior to the anterior) along the CXCL12a path, hence suggesting that polar migration is directed by the organization of the primordium itself, rather than by the polarized production of chemokines in the environment (Haas and Gilmour, 2006).

This intrinsic polarity of the PLLP in the context of migration is potentially explained by recent reports that demonstrate that the CXCL12a receptors CXCR4b and CXCR7b are asymmetrically expressed in the PLLP. In these studies, *cxcr4b* expression is detected at the leading part of the migrating PLLP, whereas expression of *cxcr7b* is confined to the trailing part of the cell cluster (Dambly-Chaudiere et al., 2007; Valentin et al., 2007). This expression pattern appears to be dictated by Wnt/ β -catenin and FGF signaling (Aman and Piotrowski, 2008), and might involve antagonistic interactions between the two receptors (Dambly-Chaudiere et al., 2007) that together result in the observed polarity in the expression of the two molecules. The functional significance of these observations lies in the suggestion that, as a non-signaling

receptor, CXCR7b expression at the back of the cell cluster is responsible for sequestering CXCL12a, thereby generating an effective gradient of the chemokine across the PLLP (Dambly-Chaudiere et al., 2007; Ghysen and Dambly-Chaudiere, 2007) (Fig. 3D). This hypothesis, which is in line with the suggested role for CXCR7b in controlling PGC migration as discussed above (Boldajipour et al., 2008), can account for the behavior of the cluster in wild-type, as well as in manipulated or mutated, zebrafish embryos. It would be interesting to determine whether in other examples of collective migration a similar strategy is in place to generate an intrinsic chemotactic gradient in an otherwise uniform attractant field.

In contrast to the examples mentioned above, the precise role of CXCR7 in additional CXCR7-dependent processes, such as cardiac development, vasculogenesis and angiogenesis in mouse or PGC and PLLP migration in medaka (Miao et al., 2007; Sasado et al., 2008; Sierro et al., 2007), is not known. It would be interesting to determine whether CXCR7 acts as a decoy receptor that controls the distribution of its ligands in these cases as well.

Keeping cells in place

An interesting variation on the theme of chemokine function in directing cell movement occurs when a chemokine gradient appears to instruct cells to maintain their position. In contrast to their role in coordinating the migration speed of one tissue relative to another population of moving cells (e.g. during zebrafish gastrulation, see Fig. 1), chemokines can be engaged in anchoring cells at a certain stationary position within a developing tissue. This specific function of chemokines was revealed in an analysis of the multiple roles that CXCR4 and CXCL12 play in the development of various mouse brain structures (Cardona et al., 2008; Li and Ransohoff, 2008; Tiveron and Cremer, 2008). The first migration process in the brain shown to depend on CXCL12–CXCR4 signaling was the migration of small neurons called granule cells in the mouse cerebellum (Fig.

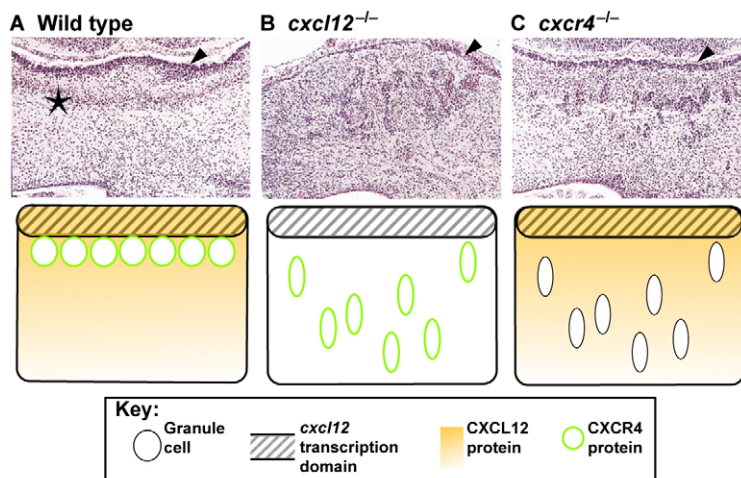


Fig. 4. Chemokines in cell positioning in the mouse cerebellum. (A-C, top) Coronal sections of mouse embryonic day 18.5 cerebellar tissue stained with Hematoxylin and Eosin. Arrowheads indicate the external granule layer, asterisk indicates the Purkinje cell layer. (Bottom) Schematic representation of chemokine function in granule cell precursor localization in the developing mouse cerebellum. (A) In the wild-type, granule precursor cells are initially maintained at the external granule layer (upper domain of the box) of the cerebellum. (B,C) In *Cxcl12*^{-/-} or *Cxcr4*^{-/-} mice, granule precursor cells migrate prematurely into deeper layers of the cerebellum (lower regions in the boxes). [Top panels adapted, with permission, from Ma et al. (Ma et al., 1998)].

4A-C) (Ma et al., 1998; Zou et al., 1998). Interestingly, in the cerebellum of *Cxcl12*- or *Cxcr4*-deficient mice (Fig. 4B,C), cells from the external granular layer (EGL, an external cell layer of the cerebellum) migrate prematurely into more internal layers of the cerebellum. The analysis of the CXCL12 and CXCR4 expression patterns, and of the effect of CXCL12 on granule cells, has uncovered the basis of this phenotype (Reiss et al., 2002; Zhu et al., 2002). These studies have shown that CXCL12 serves as an attractant for neuronal cells at the EGL. CXCL12 is provided to the granule cells by meningeal cells that are located at external parts of the cerebellum, thereby anchoring granule cells close to the brain surface. Intriguingly, in the later (postnatal) stages of mouse cerebellar development, the migration of granule cells away from the CXCL12-expressing peripheral layer correlates with the loss of CXCR4 expression from the surface of the migrating cells. An analogous role for CXCL12-CXCR4 signaling in anchoring cells to a specific location in the brain has been demonstrated for other cell types. For example, the Cajal-Retzius cells of the marginal zone in the developing brain require CXCL12 signaling to prevent them from being displaced into deeper cortical layers (Paredes et al., 2006; Tiveron and Cremer, 2008).

Another example of a possible role for CXCR4 in maintaining migratory cells at a specific location presents itself in the colonization of the gonad by PGCs. In this case, CXCL12 is expressed at high levels at the final destination of the PGCs in the developing gonads of mouse, chick and zebrafish (Ara et al., 2003a; Doitsidou et al., 2002; Knaut et al., 2003; Molyneaux et al., 2003; Stebler et al., 2004). These findings are consistent with the idea that the CXCR4-expressing PGCs are retained at their location at the target site through the influence of CXCL12. To confirm this possibility would require observing the response of PGCs to the loss of the chemokine receptor or ligand function after PGCs arrive at their target site, which remains a technical challenge. Nevertheless, it is worth noting that high-resolution analysis of cell behavior at the site of the gonad supports this proposition. Specifically, the examination of zebrafish PGCs when clustered at their target site, where peak levels of CXCL12 are expressed, has revealed that the cells extend small protrusions in all directions and remain in this location (Reichman-Fried et al., 2004). Indeed, the cessation of PGC migration upon their arrival at the target site can be experimentally recapitulated by generating CXCL12-rich ectopic domains within the zebrafish embryo, which retain the PGCs that reach these domains (Reichman-Fried et al., 2004).

The involvement of chemokines in controlling the positioning of cells at a specific location emerges as a common theme in both normal and disease contexts. For example, human and murine hematopoietic stem cells (HSCs) are maintained in the bone marrow through CXCL12-CXCR4 interactions (Petit et al., 2002). The reduction of CXCL12 levels in the bone marrow of mice or humans by granulocyte colony-stimulating factor (G-CSF) treatment, coupled with an increase in CXCR4 expression and maintenance of CXCL12 levels in the blood, results in HSCs becoming mobilized and entering the blood stream. The role of the CXCL12-CXCR4 signaling pathway in regulating cell compartmentalization has been further demonstrated by knocking out CXCR4 function selectively in mouse B cell precursors (Nie et al., 2004). *Cxcr4*-deficient B cell precursors were shown to migrate out of their normal niches in the bone marrow prematurely. Similar results were obtained when studying certain pathological conditions. For example, the bias of certain malignancies to metastasize to specific distant organs suggests that, in addition to the directed migration of tumor cells (as discussed in the previous section), the microenvironment within the target organ might assist the establishment of metastases at these locations (Ben-Baruch, 2008). Interestingly, various parameters important for the formation of tumors at metastatic sites were shown to depend on inflammatory and homeostatic chemokines and their receptors (Ben-Baruch, 2006; Ben-Baruch, 2008; O'Hayre et al., 2008). For example, the homing of metastatic cells, the infiltration of tumor-associated macrophages that can support the tumor by providing growth factors and inhibiting the anti-tumor immune response, the regulation of angiogenesis at the tumor milieu, tumor cell proliferation and tumor cell malignancy are all positively regulated by chemokines.

Conclusions

Chemokines appear to function in two main modes: they can either provide positional information in the form of a gradient or act as a switch that is responsible for changing certain cellular properties. Chemokine gradients can be experimentally generated in vitro and in vivo, and they effectively direct the migration of cells that express the proper receptors (e.g. Blaser et al., 2005; Bleul et al., 1996; Doitsidou et al., 2002; Knaut et al., 2003). Such gradients are postulated to form and function in the intact developing organism; for example, during the migration of cells towards their targets, and in the retention of cells at one location after their arrival at their migration target. In generating such positional information, the transcription pattern of the RNA that encodes the chemokine

normally plays a crucial role in shaping the gradient, with the interesting possibility of gradient formation at the posttranslational level through the action of CXCR7 within the zebrafish PLLP (Ghysen and Dambly-Chaudiere, 2007). The intracellular signaling pathways that are responsible for the directional migration of cells towards domains of high chemokine expression, or for maintaining cell location in such developmental contexts, are poorly understood. PGC migration appears to involve the polarization of intracellular calcium and the contraction of myosin, which drive a specific type of cellular protrusions that are powered by the flow of cytoplasm in the direction of migration (termed blebs) (Blaser et al., 2006). Chemokine-directed migration in the development of other morphologically different cell types could, however, involve other biochemical pathways. In these cases, one should examine the possible involvement of signaling cascades that have previously been shown to control the migration of HSCs in mammals in response to chemokine signaling (reviewed by Thelen and Stein, 2008). Specifically, molecules that control F-actin formation at the leading edge, such as PI3K γ , DOCK2 and RAC, and molecules involved in the remodeling of the actin cytoskeleton, such as ARP2/3, formins, WASP and N-WASP, are among the effectors that might play a role in cell polarization and migration in the instances discussed above.

Another effect of chemokines on cell migration involves their control of integrin-mediated adhesion (Ley et al., 2007; Luo et al., 2007). This effect is considered to be mediated by the rapid modulation of the affinity of integrin receptors for their substrates through a conformational change that requires the RAP1, talin and kindlin proteins (Thelen and Stein, 2008), as well as by alterations in the amount or distribution of integrin receptors on the cell surface (Carman and Springer, 2003). The control of integrin-mediated cell adhesion by chemokines does not depend on graded chemokine distribution, but leads to an increased interaction or tethering of the responding cells with their environment. Similarly, CXCL12b, the signal that controls the tethering of zebrafish endodermal cells to the mesodermal cell layer, is initially provided in the form of uniformly distributed RNA (Nair and Schilling, 2008) and could thus function in a gradient-independent manner. Whereas chemokines can induce extremely rapid (subsecond) changes in integrin activity in the context of leukocyte adhesion to ensure cell arrest under flow conditions at sites where the cells transmigrate into the vascular system, developmental pathways might not require such fast reaction times. Consistently, the CXCL12b-CXCR4a-mediated control of zebrafish endodermal cell adhesion is associated with changes in integrin transcription levels, which represents a slow response to chemokine signaling.

Over the past decade, and particularly during the last few years, we have witnessed an impressive increase in the number of migration processes in development and disease that have been shown to be controlled by chemokines. Interestingly, whereas the cell types and the tissue contexts differ, the roles that chemokines play in the immune system and in development are remarkably similar on a conceptual level. Therefore, attempts to find answers to open questions that concern the mechanisms of chemokine gradient formation, the intracellular events that translate the chemokine gradient into directed migration and the processes by which chemokines control integrin-mediated cell adhesion will benefit from combining the insights gained in these two disciplines.

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