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Abstract

Chemokines regulate proliferation and migration of various types of normal stem and progenitor cells, including precursor cells of neuroectodermal origin. Based on this it is conceivable that the established role of chemokines in cancer cell proliferation and organ specific-metastasis might also be associated with stem cell-like cells present in the tumor. Such cancer stem cells (CSCs) represent a small subpopulation of tumor cells that are thought to initiate and sustain tumor formation. More recently, characteristics of stem cells have also been observed in metastatic cancer cells, and it has been suggested that CSCs might play a crucial role in the metastatic process as such. Intriguingly, first evidence has been provided that the metastatic spread of specific CSCs is driven by chemokine signaling. Thus it is possible that chemokine-mediated CSC regulation might be a general feature of metastasis formation.

Keywords: Neuroectoderm, CNS, neural crest, EMT, chemokines, cancer stem cells, metastasis.

Abbreviations: NC: neural crest; CSCs: cancer stem cells; EMT: epithelial-mesenchymal transition; CNS: central nervous system.
Introduction

A wealth of data suggests that chemokine signaling can contribute to tumorigenesis and metastases [1]. In particular, a strong correlation between chemokine receptor expression and organ-specific metastasis formation has been described for many different types of solid cancers [2-10]. It is assumed that chemokine receptor-expressing cancer cells home to organs that express high levels of the appropriate ligand. Thereby, the chemokine axis involved appears to be specific both for the cancer cell type and the target organ. For instance, in breast cancer, expression of both CXCR4 and CCR7 predicts lung and lymph node metastases [11]. In melanoma, however, CXCR4 is highly associated with pulmonary [12,13] and liver [14] metastasis formation, whereas CCR7 and CXCR3 were associated with lymph node metastases [12,15-18]. In addition, CCR10 is predominantly expressed by keratinocytes and associated with melanoma-derived skin metastasis [12], while CCR9 has been linked to small intestinal melanoma metastases [19,20]. In neuroblastoma, CXCR4 expression is strongly correlated with bone and bone marrow metastases [21,22]. Studies of Zhang and colleagues recently demonstrated an influence of tissue stromal cells on CXCR4 expression levels in neuroblastoma cells [22]. Moreover, neuroblastoma cells showed CXCR4 up-regulation when cultivated with liver and bone marrow preconditioned media suggesting that the specific microenvironment plays a pivotal role to promote metastases outgrowth [22].

Recently, however, it has been shown that CXCR4 has no significant influence on the number or pattern of neuroblastoma metastasis. Rather, a strong growth-promoting effect was observed, potentially representing the main role of CXCR4 in neuroblastoma progression [23]. Moreover, intracranial glioblastoma and medulloblastoma xenografts treated with a CXCR4 antagonist (AMD3100) showed reduced cell growth and increased tumor cell apoptosis [24]. Thus, although chemokine signaling in cancer has predominantly been associated with organ-specific metastasis, other cellular processes also appear to be controlled by these molecules. Likely, the mode of chemokine action depends on the cancer cell type affected, as cell intrinsic properties determine the cells response to a specific chemokine ligand/receptor pair. Similarly, during development, cell fates are regulated by the combinatorial activities of intrinsic cues and extracellular factors [25]. Therefore, given the parallels between tumor formation and embryonic development and in view
of the recent discovery of tumor-initiating cells sharing certain properties of normal stem cells [26], it might be valuable to consider chemokine function in cancer with the eyes of a developmental biologist. In this review, we will first give a brief overview on neuroectodermal development and discuss how chemokines affect precursor cells originating from the neuroectoderm and other tissues. We will then extend this discussion to possible associations between chemokine signaling and metastasizing cancer stem cells.

**Precursor cells in neuroectodermal development**

Development of the central nervous system (CNS) begins as an epithelial sheet called the neuroectoderm that is composed of primary neuroepithelial precursor cells displaying stem cell properties. During neurulation, the edges of this sheet fold together to form the neural tube, with the fluid-filled center later becoming the ventricular system and spinal canal. Neuroepithelial cells divide symmetrically to increase the pool of stem cells. Later they give rise to so-called radial glial cells that divide either symmetrically or asymmetrically, generating a stem cell that remains in the ventricular zone and a daughter cell that radially migrates outward [27]. In the adult mammalian brain, neural stem cells give rise to specialized cell types through generation of more committed transiently amplifying intermediate progenitors [28,29]. Neural cells with stem cell properties have been identified in four distinct anatomical areas of the postnatal mammalian brain: the subventricular zone below the lateral wall of the lateral ventricles [28], the subgranular zone in the dentate gyrus of the hippocampus [30], and more recently also in the subcallosal zone between the hippocampus and the corpus callosum [31] and in the cerebellum at the boundary between internal granular layer and white matter [32].

A second neuroectodermal cell population, called the neural crest (NC), arises during development from the dorsal neural tube at the time of neural tube closure [33]. This highly migratory and transient population of cells gives rise to specialized cells throughout the body, including Schwann cells, neuronal cells of the peripheral nervous system (enteric, parasympathetic, sympathoadrenal, and sensory neurons), melanocytes in the skin, endocrine cells, and cells forming connective tissue of the face and neck (Fig. 1A) [33]. Initially, NC cells are a part of the neuroectodermal
epithelium, and thus maintain an epithelial character. Subsequently, NC cells undergo an epithelial-mesenchymal transition (EMT) [34], delaminate from the epithelium, and extensively migrate throughout the embryo before differentiation. EMT is a key biological process occurring during embryonic development, tissue remodeling, and wound repair, allowing epithelial cells to escape from the rigid structural constraints provided by the tissue architecture and to adopt a phenotype more permissive for cell migration [34]. Essentially, during this event, epithelial cells actively downregulate cell-cell adhesion, lose their polarity, and acquire a mesenchymal phenotype with reduced intercellular interactions and increased migratory capacity. EMT of NC cells represents a paradigmatic example during embryogenesis. During EMT, NC cells lose N-cadherin-mediated cell-cell adhesion while becoming excluded from the neural epithelium [35]. When NC cells are released from the neural epithelium, they concomitantly upregulate genes required for a mesenchymal phenotype and migratory ability. These include transcription factors such as Slug or Snail, AP-2, Foxd3, PAX3, Twist, Sox9, and others [36]. Furthermore, NC cell induction and delamination are promoted by specific signaling pathways, including BMP4 and Wnt signal activation [37-39].

Immediately after delamination, NC cells start to migrate either ventrally close to the neural tube or dorso-laterally in proximity to the somatic ectoderm [40,41]. At least a subpopulation of migratory NC cells display stem cell properties, being multipotent and able to self-renew [42]. By largely unknown mechanisms, which may depend on their progenitor state, NC cells stop migrating and subsequently differentiate into specialized cell types. However, there is multiple evidence that multipotent cells with stem cell features (so-called NC-derived stem cells, NCSCs) persist in NC target structures, including in the adult organism. Notably, cells very similar to embryonic NCSCs in terms of marker expression and differentiation program have been isolated from the adult heart [43], gut [44], bone marrow [45], and skin [46-48]. The presence of postnatal stem cells derived from neuroectodermal structures raises the question of whether such cells might contribute to the formation of neuroectodermal tumors.

Chemokines regulating neural precursor cell migration and proliferation
The fate of CNS and NC stem cells is largely dependent on a finely tuned, stage- and area-specific interplay between the extracellular environment and intracellular regulatory cues. Various growth factors including sonic hedgehog (Shh), Wnt, and transforming growth factor (TGF)β are known to control the balance between stem cell proliferation and differentiation both in the CNS and during NC development [49-51]. In addition, factors often act in combination to elicit specific cellular responses not achieved by the individual factors alone. For instance, Wnt together with BMP support maintenance and expansion of dorsal CNS stem cells and early migratory NCSCs, while the individual factors alone promote cell cycle exit and differentiation [52,53].

With respect to chemokines, comprehensive studies of neural stem cell-specific functions are scarce. However, several reports point to a role of chemokine signaling at early cellular stages, indicating an involvement in precursor cell proliferation and migration. Examination of embryonic neural progenitor cells in culture has confirmed that these cells express CXCR4 and that its unique ligand CXCL12 can act as a chemoattractant [54-57]. Deletion of the genes for the CXCR4 chemokine receptor or for CXCL12 is lethal at a relatively late stage of embryogenesis [58,59]. CXCR4 or CXCL12-deficient embryos exhibit a large number of phenotypes, including defects in the cardiovascular, gastrointestinal, and nervous systems, in addition to the immune system [58-61]. In the nervous system, the development of the cerebellum, hippocampal dentate gyrus, cortex, and dorsal root ganglia (DRG) are all affected [59,62-65] and deficits in the migration and proliferation of oligodendrocyte progenitors have also been observed [66,67]. The expression patterns of CXCL12 and CXCR4 during embryogenesis [68,69] indicates that all of these developmental phenotypes could possibly be explained on the basis of deficits in CXCL12-mediated attraction of embryonic neural precursor cells. This is best illustrated by the cerebellar phenotype of CXCL12 and CXCR4 knockout mice [58,59]. During development of the cerebellum, cerebellar granule cell progenitors are restricted to the external granule cell layer, where they proliferate. Once the pool of progenitors has expanded, post-mitotic cells migrate to the internal granule cell layer, the normal location for mature granule cells. In CXCR4 and CXCL12-deficient mice granule cell progenitors proceeded with their migration at an improperly early time, causing the progenitors to locate ectopically within the Purkinje cell layer. In this system, CXCL12 signal
maintains progenitors within the external granule cell layer [70,71]. Upon inactivation of CXCR4 the progenitors escape the confines of the external granule cell layer, leading to their inappropriately early migration.

Similarly, in the hippocampal dentate gyrus, deletion of CXCR4 results in ectopically placed granule cells along the normal way of granule cell progenitor migration [62,63]. Granule cell progenitors move from the wall of the lateral ventricle immediately after birth driven by meningeal cells secreting CXCL12. Abolishing CXCR4 expression on the granule cell progenitors, therefore, delays migration, explaining the observed phenotype. Other neuronal phenotypes apparent in CXCR4 knockout mice include defects in the placement of a specific subpopulation of forebrain neurons, Cajal–Retzius cells, and cortical GABAergic interneurons [64,72-75]. In all of these situations, the progenitors for these neurons employ CXCL12-mediated chemoattraction to accomplish their final positioning, and lack of CXCR4 signaling results, therefore, in interrupted progenitor migration.

During NC cell development, the chemokine CCL5 (RANTES) has been described to drive migration of primary neurons in DRGs [76]. Similarly, CXCR4 expressed on migratory cells regulates migration of NC-derived sensory neuronal progenitor cells into DRGs (Fig. 1B) [65]. CXCR4 or CXCL12 are also involved in cardiac development. During development of the heart, a subpopulation of cardiac NC cells migrate to colonize the outflow tract endocardial cushions prior to septation, the process through which a single outflow vessel, the truncus arteriosus, develops into the ascending aorta and the pulmonary trunk [77]. However, in both CXCR4 and CXCL12-deficient mice the region of the ventricular septum is abnormal [59,78]. Since CXCL12 is expressed in the developing heart tissue [68] and CXCR4 is expressed in migrating cells of the NC [65], it is conceivable that disrupted ‘homing’ of mutant cells to their target organ brings about this phenotype (Fig. 1B), consistent with the defects observed in CXCR4-mutant DRG. Likewise, mutation of CXCL12, a chemoattractant for NC-derived melanophores in zebrafish, results in ectopic melanophore migration and disturbed pigment patterning in this system (Fig. 1B) [79].

Apart from migration, chemokine signaling can also influence the number of neural precursor cells. For instance, CXCL12 is a mitogenic cue for postnatal mouse neural progenitor cells [80] and various human neural progenitor cell lines [81]. However, in
human hippocampal neural precursors, CXCL12 has also been reported to promote survival and maintenance in a quiescent state, rather than inducing cell cycle progression [54]. The use of cell lines from different stages or brain areas might possibly explain the discrepancy between these studies, which emphasizes again that one and the same chemokine can elicit different effects in distinct cell types of neuroectodermal origin.

The role of chemokines in regulating precursor cell development is not exclusive to the nervous system. Functional CXCR4 is found on the surface of several tissue committed stem/progenitor cells, such as haematopoietic stem cells [82], primordial germ cells [83], skeletal muscle satellite progenitor cells [84], liver progenitor cells [85], and retinal pigment epithelium progenitors [86]. Functional CXCR4 is also expressed on murine embryonic stem cells. In mice deficient for CXCL12 or CXCR4, primitive hematopoietic stem cells fail to localize to the bone marrow, the site of hematopoiesis at postnatal stages [58,59]. A further interesting phenotype identified in CXCL12/CXCR4 knockout mice is a deficiency in blood vessel development, as initially observed in the gastrointestinal system [60]. Consistent with these observations, CXCR4 is expressed by hemangioblasts [87] and endothelial cells derived from embryonic stem cells in culture [88]. In the latter situation the endothelial cells expressed CXCR4 and migrated towards a CXCL12 gradient. Therefore, there is strong indication that CXCR4 signaling in endothelial progenitors has a substantial impact on vascularization in the embryo. Finally, analysis of primordial germ cell migration in zebrafish, in which genes for CXCL12 or CXCR4 have been deleted, shows aberrant colonization of the gonads by these cells [89]. The combined data suggest that chemokine signaling might play a general role during tissue regeneration and repair, e.g. by chemoattraction of CXCR4-positive stem or progenitor cells to a specific tissue.

**Cancer stem cells in neuroectodermal tumors**

The fundamental functions of chemokines in spread and proliferation of normal stem and progenitors cells raises the hypothesis that chemokines might elicit similar effects in specific cancer cell subpopulations, the so-called cancer stem cells (CSCs). Growing evidence over the past few years has revealed that distinct populations of
cancer cells with features of stem cells play a crucial role in the development and progression of tumors [90,91]. As currently defined, a CSC represents a cell within a tumor that is able to self-renew in vitro and in vivo, is exclusively tumorigenic even at low cell numbers, and is capable of reproducing the cellular heterogeneity found in the parental tumor [90]. Therefore, it should be achievable to identify the cells responsible for de novo tumor formation based on their distinct phenotypic and functional characteristics [92]. Indeed, tumor-initiating CSCs with specific properties and marker expression have been identified in several malignancies including blood, skin, breast, ovarian, prostate cancers, and neuroectodermal tumors [93-105].

As their name implies, neuroectodermal tumors arise from adult tissue originating from the embryonic neuroectoderm. As derivatives of CNS cells, neuroectodermal tumors include medulloblastoma, oligodendroglioma, oligodendrocytoma, and astrocytoma. Another class of neuroectodermal tumors have a NC cell origin and comprise, among others, neurofibroma, schwannoma, neuroblastoma, malignant nerve sheath tumor, and melanoma [106,107]. Clinically, the classification of neurological tumors is based on the predominant cell type(s), which is generally determined by morphological and immunohistochemical criteria (Fig. 2). Although not all neuroectodermal neoplasms have yet been shown to contain CSCs, the existence of brain tumor stem cells has, for instance, been described for both adult and pediatric gliomas and for malignant medulloblastomas [108-110]. Interestingly, CSCs from these brain tumors express many genes characteristic of CNS stem cells, including Sox2, musashi-1, bmi-1, and CD133 (Prominin-1) [109-111].

An involvement of CSCs in tumor initiation and progression, chemoresistance and therapeutic failure has also been reported for human melanoma, the most fatal skin cancer [112-117]. Similar to normal tissues, melanomas consist of phenotypically heterogeneous cell populations in accordance with the idea that these tumors arise from multipotent cells [112,113,118-120]. Indeed, various cell culture and in vivo transplantation experiments indicated the existence melanoma cell subpopulations with stem cell-like differentiation capacity and increased tumorigenic potential [112,116,120]. Thus, malignant melanoma stem cells may contribute to natural progression and therapeutic failure in this disease.

Despite the growing evidence for CSCs underlying neuroectodermal tumor formation, their cellular origin remains unclear. One possibility is that they arise by
transformation of neural stem cells. Concurring with this hypothesis, normal CNS stem cells can be found in the adult brain, and NCSCs associated with the melanocytic lineage are present in the skin, the site of melanoma formation [29,48]. Nonetheless, CSCs could also arise by de-differentiation upon transformation of more mature cells. For instance, normal pigmented melanocytes can give rise to less differentiated cells, although these have a somewhat more limited potential than NCSCs [121]. Recently, neurofibroma—a tumor arising around peripheral nerves—has been suggested to develop from differentiated adult cells rather than stem cells [122,123]. Neurofibroma is caused by a defective version of the protein neurofibromin (NF1) that normally acts as a brake preventing cells in the nervous system from overproliferation [106]. Neurofibromas are composed of a mixture of cells, consistent with an involvement of a multipotent cell in tumor formation. However, NF1-mutant NCSCs differentiate into the expected cell types and are unable to initiate tumor formation upon transplantation into adult nerves [122]. Moreover, inactivation of the NF1 gene induced a marked increase in proliferation of non-myelinating Schwann cells rather than of undifferentiated stem cells [123]. Still, these data do not exclude that other tumors, as for instance melanoma, might originate from transformation of normal stem cells, as it is well conceivable that different tumor types with different aggressiveness might arise from distinct cell types.

Chemokines in cancer stem cells and metastasis

CSCs may not only be associated with tumor initiation and growth but also with metastasis formation: if CSCs represent the only cell population with tumor-initiating potential, one would hypothesize that solely CSCs are capable of generating tumor metastases [124]. It has even been proposed that cancer cells adopt stem cell features only upon undergoing EMT. Indeed, induction of EMT in immortalized human mammary epithelial cells resulted in the expression of stem-cell markers, the gain of mesenchymal behavior, and phenotypes associated with CSCs [125]. These findings illustrated a direct link between EMT and gain of properties characteristic for migratory stem cells (Fig. 3). It remains to be shown, however, whether this is a general phenomenon of metastasis formation. For instance, in tumors derived from NC structures, CSCs in primary tumors might share properties with normal NCSCs that are already endowed with an intrinsic capacity to migrate. This hypothesis could
offer an explanation for why neuroectodermal tumors of NC cell origin, such as melanoma, are highly metastatic and aggressive.

As mentioned above, EMT is characterized by the expression of various factors responsible for mediating this process at the molecular level. It is interesting but not surprising that many of these molecules have also been associated with tumor progression [126]. Little is known regarding the type of EMT induced by chemokine system(s) despite abundant evidence of the diverse malignant behaviors of such systems in cancer cells. In a model of EMT in colon carcinoma an upregulation of CXCL8 and its cognate chemokine receptor CXCR1 has been observed. In this study, both CXCL8 and CXCR1 were involved in the chemokinetic and chemotactic migration of colon carcinoma cells as assessed by antibody inhibition, establishing that the regulated expression of a specific chemokine and its receptor are linked to EMT [127]. Similarly, Onoue and colleagues demonstrated that CXCL12/CXCR4 signaling induces EMT in oral squamous cell carcinoma (SCC) [128]. In addition, it has been suggested that EMT induced by the CXCL12/CXCR4 system might also be involved in lymph node metastasis of oral SCC [4].

From a functional perspective, these studies highlight that two chemokine/chemokine-receptor systems (CXCL8/CXCR1 and CXCL12/CXCR4) are associated with EMT and linked with a more invasive tumor cell phenotype. Given the likely involvement of CSCs in metastasis and the roles of chemokines both in metastasis and in normal precursor cell migration and proliferation it follows that chemokines could specifically drive metastasis of CSCs. Not until very recently, though, experimental evidence for this has been provided [96]. Human pancreatic cancer tissue contains CSCs expressing CD133 that are tumorigenic and highly resistant to standard chemotherapy. Remarkably, in the invasive front of pancreatic tumors, a distinct subpopulation of CD133+/CXCR4+ CSCs was identified that appears to determine the metastatic phenotype of the individual tumor. Depletion of the CSC pool for this migrating CSC subpopulation effectively abrogated the metastatic capacity of pancreatic cancer cells without affecting their tumorigenic potential [96]. In this study, Hermann and colleagues for the first time demonstrated that not only CSCs are underlying metastasis formation, but that expression of a chemokine receptor defines a metastatic subpopulation within the CSC pool. Moreover, at least in breast cancer, CXCR4 expression appears to correlate with the CSC content, and thus the
aggressiveness, of cancer cell lines. For example, relative to non-metastatic MCF-7 breast cancer cells, highly metastatic MDA-MB-231 cells have a larger proportion of CSCs and express higher levels of CXCR4 [129]. All together, these results suggest that CSCs may indeed be involved in metastasis formation and, furthermore, be partially controlled by chemokine signaling (Fig. 4). These preliminary data together with the reported role of various chemokines in metastasis strongly support the idea that migrating CSCs could play a key function in chemokine-mediated metastasis of cancer. Interestingly, AMD3100, a specific pharmacological inhibitor of the CXCR4 receptor, strongly inhibits metastatic activity of unselected murine pancreatic cancer cells [130] as well as purified CSCs [96], and the fact that AMD3100 is already evaluated in ongoing clinical trials make it a candidate for further experimental studies on CSC-mediated metastasis [131,132].

**Conclusion**

In recent years, the field of cancer research has gained many novel insights into the cellular and molecular processes underlying tumor growth, dissemination of tumor cells, and metastasis. Tumor-initiating cancer cells with stem cell properties have been identified in a wide range of cancers including several neuroectodermal tumors, and there is good evidence that CSCs might be implicated in the generation of metastases. This model would also explain the low efficiency of the metastatic process, since only a small fraction of cells would be responsible for initiating and sustaining a new metastatic lesion. However, there remain at least as many questions to be addressed as have been resolved to date. In pancreatic cancer—the only tumor for which a direct association between CSCs, chemokine signaling, and metastasis has been provided so far—the CSC in question was reported to express the stem cell marker CD133 [96]. However, in colon cancer, cells negative for CD133 recently turned out to have a metastatic potential comparable to the one of CD133-positive cells, and metastatic CD133-negative cells form even more aggressive tumors [133]. In melanoma, several markers have been associated with stem cells [112,116,117,120], but a strict correlation between marker expression, self-renewal, and high tumorigenicity remains to be established [105]. Likewise, the exact identity and biological features of metastatic cells from melanoma or other neuroectodermal tumors are unknown. Such knowledge will be necessary, though, to be able to
understand the mode of action of factors influencing cancer cell metastasis, because these cues act for sure in cell type and context-specific manners. To this end we propose to thoroughly consult developmental biology and to elucidate the nature of normal stem cells, and the mechanisms controlling their proliferation and migration, as a basis for the study of tumor biology. Indeed, metastatic cells appear to share many similarities with normal stem cells, including the use of chemokine signaling for migration and proliferation. It would thus stand to reason that chemokine signaling plays a pivotal role in CSC-driven metastasis. Efforts should be undertaken to address this hypothesis, because if confirmed, chemokines and their receptors would represent a powerful target for eradicating metastasis formation.
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Figure Legends

Fig. 1: **Neural crest migration and chemokines.** A, Neural crest cells arise from the dorsal part of the neural tube. Subsequently, they migrate through the tissue of the developing embryo and differentiate into a wide array of cell types, including neurons and glia of the peripheral nervous system, cartilage and bone of the face, connective tissue, and melanocytes. B, Chemokine signaling regulates proper migration of neural crest cells that give rise to sensory neurons of the dorsal root ganglia (DRG) and smooth muscle cells in the outflow tract of the heart in mammalian. Melanophores in zebrafish also rely on chemokine signaling for correct migration.

Fig. 2: **Developmental scheme of neuroectodermal cells and the classification of neuroectoderm-derived tumors.** A, Multipotent neural stem cells in the ventricular/subventricular zones of the embryonic neural tube give rise to three main cell types in the mature CNS: neurons, oligodendrocytes, and astrocytes. The classification of neurological tumors is based on their predominant cell type(s). Astrocytoma is composed primarily of astrocytes, oligodendroglioma is composed primarily of oligodendrocytes, and oligoastrocytoma contains both astrocytic and oligodendrogial components. B, Neural crest cells are neural tube-derived multipotent stem cells, which give rise to various cell types. Neuroblastoma mainly occurs in children and originates from the sympathoadrenal lineage. Neurofibroma is a heterogeneous tumor that contains Schwann cells, fibroblasts, perineural cells, and neuronal cells. Schwannoma is a more homogeneous tumor that is composed of Schwann cells. Note that the arrows indicate a lineage relationship and do not imply that tumor cells arise from differentiated cells. In fact, tumors could originate from transformation of stem and/or progenitor cells.

Fig. 3: **Cancer stem cells in metastasis formation.** A, Progressive epigenetic and genetic alterations drive the transformation of normal human cells into malignant cancer stem cells (CSC, red), which generate locally a tumor. Additional changes lead to a local dissemination of CSCs, possibly via an epithelial-mesenchymal transition (EMT). The migrating cells intravasate into lymph or blood vessels. The cells are passively transported to distant organs, where single cells can extravasate and form a new tumor via a mesenchymal-epithelial transition (MET). B, An alternative model suggests that CSCs are generated in primary tumor from cancer cells during EMT.
Fig. 4: **Potential implication of cancer stem cells in metastasis.** The CSC model assumes that intra-tumor heterogeneity is mainly caused by cell differentiation and that only CSCs (red), but not differentiated cells, emigrate and form metastases. In the CSC model, metastatic cancer cells undergo differentiation programs that closely resemble those observed in the corresponding primary tissue. As in normal stem cells, chemokine signaling may drive migration, and thus metastasis, of CSCs.
**Fig. 1**

A  
Neural crest cells  
PNS glia  
PNS neurons  
Endocrine and paraendocrine cells  
Neural tube  
Melanocytes  
Smooth muscle  
Connective tissue  
Cartilage and Bone  

B  
Neural crest cells  
CXCR4  
CXCL12  
CCL5  
DRG neurons  
Neural tube  
CXCL12  
Melanophores  
CXCR4  
Smooth muscle cells in heart outflow tract
Fig. 2

![Diagram showing cell lineages and tumors]

A

- Neural stem cells
- Oligodendrocytes → Oligodendroglioma
- Astrocytes
- Neurons → Medulloblastoma

B

- Neural crest cells
- Melanocytes → Melanoma
- PNS glia → Schwannoma
- Other cells (fibroblasts, perineural cells) → Neurofibroma
- PNS neurons → Neuroblastoma
Fig. 4

![Diagram showing the relationship between Cancer stem cell, Chemokines, Liver, and Metastasis. The diagram indicates a differentiation process and the possible involvement of Chemokines in the metastasis process.]