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Molecular Interaction between Projection Neuron Precursors and Invading Interneurons via Stromal-Derived Factor 1 (CXCL12)/CXCR4 Signaling in the Cortical Subventricular Zone/Intermediate Zone

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Most cortical interneurons are generated in the subpallial ganglionic eminences and migrate tangentially to their final destinations in the neocortex. Within the cortex, interneurons follow mainly stereotype routes in the subventricular zone/intermediate zone (SVZ/IZ) and in the marginal zone. It has been suggested that interactions between invading interneurons and locally generated projection neurons are implicated in the temporal and spatial regulation of the invasion process. However, so far experimental evidence for such interactions is lacking.

We show here that the chemokine stromal-derived factor 1 (SDF-1; CXCL12) is expressed in the main invasion route for cortical interneurons in the SVZ/IZ. Most SDF-1-positive cells are proliferating and express the homeodomain transcription factors Cux1 and Cux2. Using MASH-1 mutant mice in concert with the interneuron marker DLX, we exclude that interneurons themselves produce the chemokine in an autocrine manner. We conclude that the SDF-1-expressing cell population represents the precursors of projection neurons during their transition and amplification in the SVZ/IZ. Using mice lacking the SDF-1 receptor CXCR4 or Pax6, we demonstrate that SDF-1 expression in the cortical SVZ/IZ is essential for recognition of this pathway by interneurons. These results represent the first evidence for a molecular interaction between precursors of projection neurons and invading interneurons during corticogenesis.

Key words: neocortex; migration; tangential; Cux1; Cux2; pallium; subpallium

Introduction

Whereas glutamatergic projection neurons of the rodent neocortex are generated locally in the dorsal forebrain (or pallium), GABAergic interneurons are generated in the ganglionic eminences (GEs) of the ventral forebrain (or subpallium) and invade the cortex via tangential long-distance migration, thereby using well defined migration routes in the subventricular zone/intermediate zone (SVZ/IZ) and the marginal zone (MZ) (for review, see Marin and Rubenstein, 2003). During their journey, they encounter radially migrating projection neurons, and there is evidence that the birthdates of principal neurons and interneurons are partially correlated within a particular cortical layer (for review, see Metin et al., 2006). Thus, the process of interneuron invasion is spatially and temporally regulated, suggesting interactions between both cell populations.

Several factors have been identified as potential regulators of cortical interneuron migration. These include semaphorins, Neuregulin-1, and glial cell line-derived neurotrophic factor (for review, see Metin et al., 2006). Furthermore, it has been reported that stromal-derived factor 1 (SDF-1) is a chemoattractant for cortical interneurons that is expressed not in the cortex itself but exclusively by meningeal cells (Stumm et al., 2003). However, several groups have reported SDF-1-expressing cells in the deeper aspect of the embryonic neocortex (Tham et al., 2001; Tissir et al., 2004), and this expression was associated with invading prospective interneurons (Daniel et al., 2005). In this work, we identify the deep expression domain of SDF-1 in the developing cortex as the SVZ/IZ and investigate how this major pathway for interneurons is defined.
SDF-1 transcripts in the SVZ/IZ colocalize widely with Cux1 and Cux2, transcription factors that identify upper-layer cortical neurons and their precursors (Nieto et al., 2004; Zimmer et al., 2004; for review, see Rash and Grove, 2006), and exclude that SDF-1 expression is an autocrine function of interneurons via the use of specific markers and Mash-1 mutant mice. Furthermore, we show through the analysis of CXCR4 and Pax6 mutant mice that SDF-1 is necessary for guidance of interneurons in the SVZ/IZ pathway.

These results provide the first demonstration of a molecular interaction between cortical projection neurons and interneurons before these reach their final destinations in the cortical layers.

Materials and Methods

Animals. All animals were treated according to protocols approved by the French Ethical Committee. CD1 mice (Ifa-Credo, Lyon, France) were used for expression pattern analyses. CXCR4 (Zou et al., 1998), Mash1 (Guillen et al., 1993), and Pax6

Expression of SDF-1 and Cux2 in the developing forebrain. ISH for SDF-1 (a, c, e, g) and Cux2 (b, d, f, h) on frontal serial forebrain vibratome sections at E14.5 (a, b) and cryostat sections at E14.5 (c, d), E15.5 (e, f), and E18.5 (g, h). a, c, e, g at E13.5 and E14.5. SDF-1 expression is confined to the meninges and a cell population deep within the pallium. b, d, f, h Cux2 labels the SVZ/IZ, interneurons in the MZ, and the first cohort of upper-layer neurons. Cux2 staining extends into the ventral forebrain. The deep SDF-1 expression domain (a, c) is mainly overlapping with the Cux2 domain, demarcating the SVZ/IZ (b, d, e, g). At E14.5 (e) and E18.5 (g), SDF-1 expression is strong in the meninges but is confined to the deep aspect in the SVZ/IZ (arrowhead), f, h. At the same time, Cux2 expression is found in upper-layer neurons of the developing CP. Furthermore, cells in the SVZ/IZ and radially oriented cells traversing the already formed deep layers express the transcription factor. Scale bars: a, b, e–h, 200 μm; c, d, 100 μm. Me, Meninges; AA, amygdala anlage; MGE, medial GE.

Projection neuron precursors express SDF-1

We aimed at identifying the SDF-1-expressing cell populations in the SVZ/IZ. Recent studies have shown that precursors of CP

neurons undergo divisions in the SVZ/IZ (Haubensak et al., 2004; Miyata et al., 2004; Noctor et al., 2004; Wu et al., 2005). The transcription factors Cux2 and Cux1 are expressed in upper-layer neurons as well as their proliferating precursors in the SVZ/IZ (Nieto et al., 2004; Zimmer et al., 2004; Rash and Grove, 2006). Therefore, we asked whether the transcription factors were localized with SDF-1 at the cellular level.

In the absence of functional antibodies against Cux2 or SDF-1, we used FISH (Fig. 2a,b), allowing the detection of both transcripts on the same section. Low-magnification imaging verified the general overlap of Cux2 and SDF-1 expression at E13.5 mainly in the deep aspect of the SVZ/IZ, whereas more superficial areas were positive for Cux2 only. High-magnification confocal images showed the punctiform appearance of the respective fluorescent labels as separate signals within individual cells, because of the local precipitation of fluorescent substrate (Fig. 2b). Such high-magnification images in concert with phase-contrast imaging and DAPI nuclear staining allowed the identification of individual cells and their content of Cux2- and SDF-1-specific precipitates. Quantitative analyses revealed that 52.2 ± 3.9% of all cells in the SVZ/IZ expressed both SDF-1 and Cux2. A total of 39.0 ± 8.9% of the cells expressed only Cux2. These were mainly, but not exclusively, localized in the superficial aspect of the SVZ/IZ. Finally, 8.8 ± 4.9% expressed SDF-1 only (Fig. 2c).

In the next step, we combined immunohistochemistry for Cux1 with ISH for SDF-1. At E15.5, Cux1 was strongly expressed in the developing upper layers of the CP and more weakly in the SVZ/IZ as well as in the VZ (Fig. 2d), whereas it was confined to individual radially oriented cells in the deep layers (Nieto et al., 2004). SDF-1 colocalized with Cux1 in the SVZ/IZ but was absent from the strongly Cux1-positive cells in the upper cortical layers (Fig. 2d,e). Examination of 410 individual SDF-1-positive cells in the SVZ/IZ revealed that 94.9% coexpressed Cux1.

Thus, SDF-1 in the SVZ/IZ was mainly, but not exclusively, present in cells that coexpressed the transcription factors Cux2 and Cux1, indicating that precursors of principal neurons are responsible for expression of the chemokine. Because such precursors have been shown to divide in the SVZ/IZ, we combined ISH for SDF-1 with immunolabeling for the cell-cycle marker PCNA (Fig. 2f) (Takahashi and Caviness, 1993). Analyses of 906 cells in the SVZ/IZ revealed that 68.9% of the SDF-1-positive cells in the SVZ/IZ were proliferating, in agreement with a projection neuron precursor identity of the SDF-1-expressing cell population.

Cux2 is expressed by precursors of principal neurons in the SVZ/IZ as well as by interneurons invading from the GE (Zimmer et al., 2004). Therefore, we asked whether SDF-1 expression is also a property of interneurons during their migration in the SVZ/IZ. We combined SDF-1 ISH with immunolabeling using a pan-DLX antibody. The complete absence of double-positive cells (10 sections from two embryos analyzed) (Fig. 2g) indicated SDF-1 expression in cells other than interneurons. We used a genetic model to further exclude a possible implication of interneurons in SDF-1 expression. Mash1 knock-out mice show a severe reduction in neuronal progenitors in the ventral telencephalon (Casarosa et al., 1999), leading to a loss of cortical interneurons, as identified by Dlx5 expression, in the MZ as well as in the SVZ/IZ (Fig. 2i,k). However, SDF-1 expression was not apparently reduced in the SVZ/IZ of E13.5 Mash1 mutants (Fig. 2h,i).

Altogether, the findings that the majority of SDF-1 cells in the SVZ/IZ (1) expressed the transcription factors Cux1 and Cux2,


The observation that in the specific absence of Lhx6, identifying them as GE-derived interneurons in the SVZ/IZ (Fig. 4).

Double FISH analyses demonstrated that the chemokine receptor in the MZ and in the SVZ/IZ (Fig. 4) in Lhx6-positive interneurons disappear from the SVZ/IZ (d), whereas larger amounts of these cells appear in the MZ pathway. Scale bar, 100 μm. Me, Meninges.

(2) were proliferating, and (3) were non-interneurons identified them as precursors of cortical projection neurons.

**SDF-1/CXCR4 signaling is essential for correct use of the SVZ/IZ pathway**

In the next step, we analyzed the functional implication of SDF-1 expression within the SVZ/IZ in interneuron migration. Pax6 mutant mice show a variety of forebrain defects including alterations in patterning, migration, and differentiation (Manuel and Price, 2005). We analyzed SDF-1 expression and interneuron migration in Pax6 mutants. For identification, we used ISH for Lhx6, a marker for pallial interneurons (Lavdas et al., 1999). ISH on brain sections of E13.5 Pax6−/− embryos showed a total absence of SDF-1 transcripts from the SVZ/IZ (Fig. 3a,b), whereas expression in the meninges was normal. In parallel, Lhx6-expressing interneurons were absent from the SVZ/IZ in Pax6 mutants but overrepresented in the MZ pathway (Fig. 3c,d). The observation that in the specific absence of SDF-1 from the SVZ/IZ more Lhx6-expressing cells were found in the MZ suggests that cells that migrate normally in the SVZ/IZ join now the MZ route. This indicates a functional implication of SDF-1 in the routing of cortical interneurons in the SVZ/IZ.

We investigated this role of SDF-1 more directly. The SDF-1 receptor CXCR4 has been functionally implicated in neuronal routing of cortical interneurons in the SVZ/IZ (Price, 2005). We analyzed the functional implication of SDF-1/CXCR4 signaling in the pallium of CXCR4 mutant mice. In the wild-type (WT) E14.5 cortex, the majority of invading interneurons expressing Lhx6 in the WT cortex (e) are concentrated in the SVZ/IZ and the MZ. At E18.5, interneurons are relatively evenly distributed over the entire CP in the wild type (e), while in the mutants (f) they accumulate in intermediate positions. Quantitative analysis showed that in CXCR4 mutants (f), both SVZ/IZ and MZ contain significantly less interneurons, whereas intermediate routes are preferentially used (*p < 0.05; significant differences between bins in wild type and mutant). Scale bars: a, c–f, 100 μm; b, 10 μm. UL, Upper-layer neurons; DL, deep-layer neurons.

We investigated CXCR4-deficient mice to gain direct insight into the functional implication of SDF-1/CXCR4 signaling in the pallium. In the wild-type (WT) E14.5 cortex, the majority of interneurons were confined to the SVZ/IZ pathway, whereas a smaller population was localized to the MZ (Fig. 4c). In CXCR4 mutant mice, this pattern was strikingly altered. Here, the invading interneurons did not show a clear preference for the MZ or SVZ/IZ routes but migrated in a more dispersed manner and in intermediate regions of the pallium (Fig. 4d). Quantitative analysis of the distribution of Lhx6-expressing neurons in the pallium of WT and CXCR4 mutant E14.5 forebrain. At E14.5, invading interneurons expressing Lhx6 in the WT cortex (e) are concentrated in the SVZ/IZ and the MZ. At E18.5, interneurons are relatively evenly distributed over the entire CP in the wild type (e), while in the mutants (f) they accumulate in intermediate positions. Quantitative analysis showed that in CXCR4 mutants (f), both SVZ/IZ and MZ contain significantly less interneurons, whereas intermediate routes are preferentially used (*p < 0.05; significant differences between bins in wild type and mutant). Scale bars: a, c–f, 100 μm; b, 10 μm. UL, Upper-layer neurons; DL, deep-layer neurons.

**Figure 3.** SDF-1 expression in the SVZ/IZ is necessary for correct interneuron migration. a–d, ISH for SDF-1 (a, b) and for the interneuron marker Lhx6 (c, d) in the wild type (a, c) and in the Pax6 mutant (b, d) at E14.5. In Pax6 mutants, SDF1 mRNA expression is absent in the SVZ/IZ but remains unchanged in the meninges. At the same time, Lhx6-positive interneurons disappear from the SVZ/IZ (d), whereas larger amounts of these cells appear in the MZ pathway. Scale bar, 100 μm. Me, Meninges.

**Figure 4.** Altered interneuron migration in the pallium of CXCR4 mutant mice. a, ISH for CXCR4 at E15.5. CXCR4 is strongly expressed in cells in the MZ and in the SVZ/IZ. b, Double FISH for Lhx6 (red) and CXCR4 (green) in the SVZ/IZ. Lhx6-positive interneurons coexpress CXCR4 (arrows). c–f, ISH for Lhx6 in WT (c, e) and CXCR4 mutant (d, f) mice at E14.5 (c, d) and E18.5 (e, f). g, Quantitative analysis of the distribution of Lhx6-expressing neurons in the pallium of WT and CXCR4 mutant E14.5 forebrain. At E14.5, invading interneurons expressing Lhx6 in the WT cortex (e) are concentrated in the SVZ/IZ and the MZ. At E18.5, interneurons are relatively evenly distributed over the entire CP in the wild type (e), while in the mutants (f) they accumulate in intermediate positions. Quantitative analysis showed that in CXCR4 mutants (f), both SVZ/IZ and MZ contain significantly less interneurons, whereas intermediate routes are preferentially used (*p < 0.05; significant differences between bins in wild type and mutant). Scale bars: a, c–f, 100 μm; b, 10 μm. UL, Upper-layer neurons; DL, deep-layer neurons.
number of cells per section was not significantly different between control and mutant animals (wild type, 114.8 ± 21.8; mutant, 85.9 ± 18.7).

CXCR4-deficient mice die shortly after birth. Therefore, we investigated the positioning of interneurons in the absence of the SDF-1 receptor at the latest possible time point, E18.5. At this age, Lhx6-expressed cells in wild types were still concentrated in the MZ, whereas few cells were visible in the SVZ/IZ (Fig. 4e). Furthermore, individual Lhx6-positive cells were distributed over the entire CP. In CXCR4 mutants, the cortex was reduced in size (Fig. 4f) but showed a generally normal organization (data not shown). Here, neither the MZ nor the SVZ/IZ showed a particular accumulation of interneurons, but Lhx6-expressing cells were found predominantly in an intermediate position within the CP (Fig. 4f).

In conjunction with our observations in Pax6 mutants, these results demonstrate that SDF-1/CXCR4 signaling is functionally implicated in defining the SVZ/IZ and the MZ as immigration routes for interneurons in the pallium.

Discussion
We show here that the chemokine SDF-1 is transiently expressed by the precursors of projection neurons in the SVZ/IZ of the neocortex. This expression is essential for the correct routing of interneurons that invade the pallium from the underlying sub-pallium via this major pathway.

SDF-1/CXCR4 signaling in interneuron migration
During corticogenesis, SDF-1 has been shown to be specifically expressed in the meninges above the MZ as well as in a deep domain (Tham et al., 2001; Tissir et al., 2004; Daniel et al., 2005). Stumm et al. (2003) examined the effects of SDF-1 or CXCR4 knock-out on interneuron migration at a late stage of embryonic development (E18.5) and attributed the observed misrouting of CXCR4- or Reelin-expressing interneurons exclusively to the lack of chemoattraction by meningeal SDF-1. We identify here the deep expression domain of SDF-1 as the deep aspect of the SVZ/IZ, which represents the major entry route for interneurons in the pallium at this stage. Furthermore, we show that misrouting in CXCR4 knock-out or Pax6-deficient mice is detectable already at early stages of corticogenesis (at least by E14.5).

Expression of SDF-1 by projection neuron precursors in the SVZ/IZ
SDF-1 expression above the superficial pathway has been unambiguously attributed to meningeal cells (Tham et al., 2001), whereas its expression in the SVZ/IZ has so far not been positively attributed to a particular cell type. We show here that SDF-1 transcripts are localized in a deep domain within the SVZ/IZ. However, the SVZ/IZ is a highly heterogeneous region containing different cell populations from various origins and destined for different locations and differentiation pathways.

We considered different cellular sources for the expression of the chemokine. SDF-1 could be expressed by migratory interneurons themselves, thereby supporting their own migration into the cortex. An autocrine loop implicating SDF-1 and CXCR4 leading to actin reorganization and the promotion of cell migration has fostered the idea that one population might compete with others for a particular cell type. We show here that SDF-1 expression above the superficial pathway has been unambiguously attributed to meningeal cells (Tham et al., 2001), whereas its expression in the SVZ/IZ has so far not been positively attributed to a particular cell type. We show here that SDF-1 transcripts are localized in a deep domain within the SVZ/IZ. However, the SVZ/IZ is a highly heterogeneous region containing different cell populations from various origins and destined for different locations and differentiation pathways.

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In contrast, a considerable body of evidence allowed us to conclude that SDF-1 is expressed by the precursors of a subpopulation of cortical projection neurons during their amplification in the SVZ/IZ. First, SDF-1-expressing cells in this compartment are proliferative. Over the past years, it became clear that the SVZ/IZ is a major neurogenic compartment in which intermediate neuronal progenitors divide and supply neurons for the CP (Haubensak et al., 2004; Miyata et al., 2004; Noctor et al., 2004; Wu et al., 2005). Therefore, our finding that SDF-1-positive cells in the SVZ/IZ express the cell-cycle marker PCNA indicated an expression of the chemokine in principal neuron precursors.

Additional evidence for this scenario is provided by comparing SDF-1 expression to that of Cux1 and Cux2. Cux1 has been shown to be expressed in the VZ, in the SVZ/IZ, and very strongly in the upper layers of the CP (Nieto et al., 2004). Our analyses show colocalization of SDF-1 and nuclear Cux1 protein exclusively in the SVZ/IZ. In contrast, Cux2 expression is absent from the VZ but, like Cux1, labels upper-layer progenitors in the SVZ/IZ and mature neurons of layers II–IV in the CP (Nieto et al., 2004; Zimmer et al., 2004). We found colocalization of SDF-1 and Cux2 in cells located in the SVZ/IZ.

Control of interneuron migration by proliferating precursors of principal neurons: a new element for understanding corticogenesis
The fact that, during their migration, interneurons in the cortex are in close contact with other neopallial cells, some of which are also migrating, has fostered the idea that one population might influence the behavior of the other. However, no precise evidence for a molecular cross talk between these populations has yet been presented. In the present study, we show that prospective principal neurons in the SVZ/IZ express SDF-1 at the right time and the right place to act on tangentially migrating prospective interneurons, which express the SDF-1 receptor CXCR4. We furthermore demonstrate that the laminar distribution of Lhx6-positive interneurons invading from the GE is radically altered when SDF-1 expression in the SVZ/IZ is lacking, which is the case in Pax6 knock-out mice. Altogether, our data represent the first evidence that precursors of the two principal cell populations in the neocortex, glutamatergic projection neurons and GABAergic interneurons, are molecularly linked during their amplification/migration phase.

References


