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Reprogramming of neonatal SVZ progenitors by Islet-1 and Neurogenin-2

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ABSTRACT

The subventricular zone (SVZ) lining the lateral walls of the lateral ventricles is one of the major neurogenic areas in the postnatal brain. Precursor cells in the SVZ migrate via the rostral migratory stream to the olfactory bulb where they differentiate into neurons. Cell replacement strategies utilizing the recruitment of these endogenous progenitors and their progeny to different areas of the brain hold great promise for the future, but much research is needed in order to understand the sequence of molecular signals necessary to induce proliferation, migration and site-specific differentiation of these cells.

In this study we show that the SVZ cells can be redirected from their normal migration route and directed towards other brain regions when they are infected with retroviruses encoding the developmentally important transcription factors Islet-1 and Neurogenin-2. After co-transduction with these transcription factors, transduced cells could be detected in several areas of the brain. When located in the striatum, the reprogrammed cells displayed neuroblast-like morphology. Once removed from the striatal parenchyma and allowed to further differentiation *in vitro* they developed into β-III-tubulin positive neurons.

Introduction

Neurogenesis occurs during the embryonic period and is completed around birth in most brain regions. Some neurogenesis, however, persist in the postnatal and adult brain, but it is restricted to two regions: the subventricular zone of the lateral ventricles (SVZ) and the hippocampus (Alvarez-Buylla and Lim, 2004). The progenitor cells within these regions generate migrating neuroblasts. Neuroblasts originating from the SVZ migrate over long distance; via the rostral migratory stream (RMS) to olfactory bulb (OB), where they differentiate into interneurons (Lois and Alvarez-Buylla, 1994). This migration pattern can be visualized by retroviral labeling of dividing cells within the SVZ (Rogelius et al., 2005; Yamada et al., 2004). Under normal conditions the neuroblasts migrate specifically to the OB and no significant migration to other areas such as cortex, striatum, or external capsule (CE) occurs. However, neurogenesis and migration from the postnatal and adult SVZ can be influenced under experimental conditions by changes in the brain environment such as by brain lesions (Arvidsson et al., 2002; Parent et al., 2002; Yamashita et al., 2006), addition of trophic factors (Benraiss et al., 2001; Chmielnicki et al., 2004; Pencea et al., 2001), or expression of transcription factors important during embryogenesis (Hack et al., 2005; Rogelius et al., 2006). Taken together, these studies illustrate the plasticity and capacity of the SVZ cells to be recruited to alternative fates which make cell replacement therapies utilizing these endogenous progenitors a possible alternative to transplantation of *in vitro* expanded neural stem cells in the future.

Our group has previously studied what effect ectopic expression of the LIM homeodomain transcription factor Islet-1 (Isl1) in the SVZ cells has on their migration and final differentiation (Rogelius et al., 2006). In the ventral forebrain, Isl1 expression is restricted to the embryonic period where it is expressed in the SVZ from embryonic day 12 to birth in cells that differentiate into striatal projection neurons (Olsson et al., 1995; Toresson et al., 2000; Wang and Liu, 2001). Viral-driven ectopic expression of Isl1 in the neonatal SVZ cells resulted in a new migration pattern as the Isl1 expressing cells, in addition to the OB, also migrated specifically to the striatum. The newly generated cells in the striatum however, did not differentiate into striatal neurons, instead they adopted a Ng2 positive glial phenotype.

In the present study we hypothesized that the proneural basic helix-loop-helix transcription factor Neurogenin-2 (Ngn2), which during the embryonic development contribute to neurogenesis, may be sufficient to direct the progeny from ectopic Isl1 expressing SVZ progenitors in the striatum and further instruct them to adopt a neuronal fate. Ngn2 is an activator of neuronal gene expression (Morrison, 2001), participates in neuronal specification (Fode et al., 2000), and is known to promote neuronal differentiation of embryonic and adult neural stem cells in culture (Berninger et al., 2007; Falk et al., 2002; Jensen and Parmar, 2006). To test our hypothesis we injected two retroviruses into the

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Table 1
Presence of transduced cells in the olfactory bulb

	Score
Rv.GFP, *2w*	++
Rv.ISL, *2w*	++
Rv.Ngn2, *2w*	–
Rv.Isl/Rv.Ngn2, *2w*	+

– = 0 cells, + = 1–50 cells, ++ = 51–100 cellsand +++ = more than 100 cells.

lateral ventricle of neonatal rats, one encoding Isl1and the other Ngn2, thus infecting the proliferating cells of the SVZ with both Isl1 and Ngn2 at the same time.

The results presented in this study show that ectopic expression of Ngn2 in combination with Isl1 in the proliferating SVZ cells results in the appearance of cell populations primarily in the striatum, CE, and septum in addition to the OB. When the newly formed cells in the striatum were isolated and further differentiated *in vitro* they displayed neuronal morphology and expressed the neuronal marker β-III-tubulin. Thus, our results suggest that Ngn2 prevents the cells from adopting a glial fate and further that Ngn2 and Isl1 act synergistically and their combined expression affects which migratory cues and differentiation signals that the cells responds to.

Results

Retroviral injections to the lateral ventricles of neonatal rats target the proliferating cells of the SVZ (Rogelius et al., 2005). In this study we injected four combinations of retrovirus; the control virus containing the marker gene GFP only (Rv.GFP), the Rv.Ngn2 virus which contain the coding sequence for Ngn2 and IRES-GFP (Falk et al., 2002), the Rv.Isl1 virus (Rogelius et al., 2006) which also contain IRES-GFP and finally co-injections of Rv.Ngn2 and Rv.Isl1 (Rv.Ngn2/Rv.Isl1).

Ectopic expression of Ngn2 in combination with Isl1 results in recruitment of cells to the striatum, external capsule, and septum

The normal fate of the neuroblasts in the SVZ is to migrate to the OB and form interneurons (Lois and Alvarez-Buylla, 1994; Luskin, 1993). As we have previously reported (Rogelius et al., 2006), and also shown in this study, this same migration and differentiation pattern were seen in the control (Rv.GFP) animals 2 weeks after injection. Also in agreement with previous observations, the animals in the control group did not have transduced GFP positive cells in the striatum, CE, or septum (not shown). In the co-injected group that received both Rv. Ngn2 and Rv.Isl1 (Rv.Ngn2/Rv.Isl1) the animals had the normal migration of cells in the RMS to the OB, as detected by their expression of GFP. However, the number of cells in the OB was reduced compared to control-injected animals (Table 1). In addition to this, and in contrasts to the control animals, an extensive amount of GFP-expressing cells could be detected in both the striatum and in the CE 2 weeks after transduction in the co-injected group. Small populations of GFP positive cells could also be detected in the septum, contralateral striatum and contralateral CE 6 weeks after injection (Table 2). Within the striatum, the newly formed cells derived from the co-transduced SVZ were evenly distributed in all regions of the striatum, whereas in the CE the cells were clustered in tight association with each other (Fig. 1A). 6 weeks after injection, the cells in the striatum had a tendency to be concentrated within the striatal fiber tracts. In agreement with this, co-staining for GFP and the neuronal marker MAP2 show that the majority of the GFP-expressing cells reside in the low-MAP2 expressing fiber bundles (Figs. 2A and 4A).

We also studied the distribution of cells in animals that received injections with only Rv.Ngn2 or only Rv.Isl1. When Ngn2 is expressed alone, GFP-expressing cells were mainly found in the striatum close to the SVZ and in the CE (Table 1) but not in the OB. In the Rv.Isl1 injected group the migration of cells to the OB was undisturbed (Table 1). In addition, a large population of SVZ-derived cells had migrated to the striatum. In some animals (3/12) a few cells could also be detected in CE (Table 2). The distribution of cells in the striatum was markedly different, though, between animals injected with Isl1 only and Isl1/Ngn2 in combination. In the co-injected group the cells were found evenly distributed in all areas of the striatum, whereas the cells in the Isl1-only group were primarily located in the ventrolateral striatum (Figs. 1A and B).

Taken together, ectopic expression of Isl1 and Ngn2 affects the migration of the cells, and expression of both Ngn2 and Isl1 in combination contributes to recruitment of cells from the SVZ mainly to the to the striatum, CE and septum.

Cells recruited to striatum after co-expression of Isl1 and Ngn2 have a neuroblast-like morphology

In the control-injected group transduced cells in the RMS and OB were indistinguishable from non-transduced cells based on morphology, localization and immunostainings for DCX and Er81. In the RMS the cells had elongated cell bodies and a leading process where as the cells in the OB displayed differentiated neuronal morphologies (not shown and Rogelius et al., 2006). In the co-injected group, the transduced cells in the RMS and OB had the same morphology as the cells in the control (Fig. 2C). Interestingly, the transduced cells recruited to the striatum and CE also had an elongated cell body and a long process, thus resembling a migrating neuroblast (Figs. 2A and B). In the Rv.Ngn2 group, the striatal and CE cell populations had the same neuroblast-like morphology (Figs. 2D and E), as the cells that received injections of both Rv.Ngn2 and Rv.Isl1. In the RV.Isl1 only group, however, the striatal cells did not have a neuroblast-like morphology but had a typical glial morphology (Fig. 2F) and expressed Ng2 as previously shown (Rogelius et al., 2006). No such cells with this glia morphology and Ng2 expression could be detected in any of the co-injected animals.

In this study, GFP is the marker gene for the transduced cells in both the Rv.Ngn2 and Rv.Isl1 vectors. Thus we cannot easily determine if a cell is in fact expressing from both viruses or just from one in the co-injected group. However, after immunohistochemistry analysis followed by confocal analysis of transduced cells in striatum, CE and RMS in the Rv.Ngn2/Rv.Isl1 group, the Isl1 protein was co-detected with GFP in virtually all cells indicating that the great majority of GFP-expressing cells have received Rv.Isl1 viruses (Fig. 3). Further, if the transduced cells in the Rv.Ngn2/Rv.Isl1 group only had received the Rv.Isl1 vector and not the Rv.Ngn2 one would expect the presence of some GFP positive, Ng2 expressing glia-like cells in the striatum as this is the only cell type present in the animals that received only Isl1 injections. As this cell phenotype was never detected in the co-transduced group, we can therefore conclude that a vast majority of the GFP-expressing cells in the Rv.Ngn2/Rv.Isl1 injected group have received both viruses.

Table 2
Relative distribution of cells in different brain regions after genetic manipulation of SVZ cells

	Striatum	CE	cl CE	cl striatum	Septum	Average total number of cells per animal
Rv.ISL, *2w*	96±5%	4±3%	0	0	0	873
Rv.Ngn2, *2w*	73±30%	27±26%	0	0	≤1%	317
Rv.Isl/Rv.Ngn2, *2w*	87±5%	13±5%	0	0	≤1%	1005
Rv.ISL, *6w*	100±0%	0	0	0	0	1334
Rv.Ngn2, *6w*	na	na	na	na	na	na
Rv.Isl/Rv.Ngn2, *6w*	46±14%	34±22%	3±2%	11±12%	7±7%	1073

CE = Capsula Externa, cl = side contralateral to injection, na = not analyzed.