
Retrovirus-mediated Cell Labeling

Chunmei Zhao

Laboratory of Genetics
Salk Institute for Biological Studies
La Jolla, California 92037

REPLICATION-INCOMPETENT RECOMBINANT RETROVIRUSES allow specific labeling of dividing cells and their progeny. Retrovirus-mediated cell labeling was first applied in the field of neurogenesis to understand the lineage relationship of different cell types and the migration pattern of clonally related cells. The combination of retrovirus vectors and live cell markers enables functional studies of newborn neurons in the adult mammalian brain. In addition, retrovirus vectors can be modified to manipulate the expression of a gene of interest, thus determining its role in the process of neurogenesis. This chapter summarizes our current understanding of neurogenesis based on studies using retrovirus-mediated cell labeling.

A BRIEF INTRODUCTION OF RETROVIRUSES

Retroviruses are (+)-stranded RNA viruses that are characterized by their ability to generate double-stranded DNA (dsDNA) from their RNA genome through reverse transcription. On entry into host cells through the specific interaction between viral surface glycoprotein and host-cell membrane receptor, the retroviral RNA genome is transcribed into a dsDNA in the cytoplasm of the host cell. The newly synthesized dsDNA enters the nucleus, where it integrates into the chromosomal DNA of the host cell and becomes a provirus. Although retroviruses package two copies of RNA genomes in their virions, it is believed that each virion makes a single provirus (Coffin et al. 1997).

Retroviruses have been classified in different ways. They are now divided into three subfamilies: orthoretrovirinae, spumaretrovirinae, and unclassified retroviridae (<http://www.ncbi.nlm.nih.gov/Taxonomy/Browser/>). There are six genera in the orthoretrovirinae subfamily, including alpharetrovirus, betaretrovirus, gammaretrovirus, deltaretrovirus, epsilonretrovirus, and lentivirus, of which the first five were previously known as oncoretroviruses. Oncoretroviruses, such as Moloney murine leukemia virus (Mo-MLV), enter the nucleus during the prophase-prometaphase transition at the onset of mitosis, when the nuclear envelope breaks down. Therefore, oncoretroviruses cannot transduce quiescent cells. In comparison, lentiviruses transduce both mitotic and quiescent cells (Coffin et al. 1997). Because of this fundamental difference in viral-genome transduction, recombinant vectors of oncoretroviruses and lentiviruses have different application potentials. Retroviral vectors in this chapter only concern those derived from oncoretroviruses.

RECOMBINANT RETROVIRUSES

Wild-type retroviral genomes mostly contain three genes encoding Gag, Pol, and Env—the structural, enzymatic, and envelope proteins, respectively. Most recombinant retroviruses are made replication incompetent through the separation of the viral genes *gag*, *pol*, and *env* from the recombinant viral genome. Therefore, the modified viruses cannot make new virus particles after they infect and integrate into a host cell. For the preparation of infectious virus particles, one could introduce the *gag*, *pol*, and *env* genes through separate plasmids or use a cell line that stably expresses these genes. Recombinant retroviruses can be pseudotyped with envelope proteins of other viruses. For example, VSV-G, the envelope glycoprotein of the rhabdovirus vesicular stomatitis virus, has been used for the preparation of recombinant Mo-MLV because VSV-G-pseudotyped virus has a broader host range and is more stable when concentrated through ultraspeed centrifugation (Burns et al. 1993).

Because the integration of oncoretroviruses is dependent on the M phase of the cell cycle, recombinant retroviruses that carry the coding sequences of histochemical markers (for example, the bacterial protein β -galactosidase [β -gal]) have been used to label dividing progenitors during neurogenesis. Because the marker gene is integrated into the host-cell genome, there is no concern of the signal being diluted out if the cell undergoes multiple rounds of cell division. The ubiquitous localization of the marker proteins, such as alkaline phosphatase (AP), β -gal, and

green fluorescent protein (GFP), allows the visualization of labeled cells. Furthermore, the expression of live fluorescent markers, such as GFP, enables the detection of labeled cells in live cultures or brain slices. Both the long terminal repeat (LTR) of the retrovirus and exogenous promoters have been used to drive the expression of these marker genes. Most of the retroviral vectors used in the field of neurogenesis are based on the backbone of Mo-MLV, with only a few exceptions (Table 1).

LINEAGE STUDIES USING RETROVIRUS-MEDIATED CELL LABELING

The uses of replication-incompetent recombinant retroviruses in studying neurogenesis were first reported in the late 1980s. The bacterial gene *lacZ* encoding β-gal was used to label dividing progenitors and their progeny to study the lineage relationships of different cell types during cortical neurogenesis and retinal development (Price 1987; Price et al. 1987; Turner and Cepko 1987; Gray et al. 1988; Luskin et al. 1988; Walsh and Cepko 1988). Labeled cells that were within a certain distance were considered as a clone derived from a single cell. This criterion has been challenged by other studies, which are discussed below. It was shown that different types of cells in the rodent retina may share the same progenitor and cells within a single clone have a radial arrangement (Turner and Cepko 1987). A similar phenomenon was observed in the chicken optic tectum (Gray et al. 1988). Studies on embryonic cortical neurogenesis in mice, on the other hand, confirmed previous views that neuronal and glial progenitors diverge quite early during development (Luskin et al. 1988). It has also been suggested that clonally related cells in the rodent embryonic cortex have a relative radial arrangement with certain degrees of spread (Price and Thurlow 1988; Walsh and Cepko 1988).

These early studies all used retroviruses at low multiplicity of infection (moi) so that clusters of labeled cells can be easily identified as clones. However, a problem associated with this assumption is that this analysis would exclude any cell that might have migrated away from a cluster. In fact, later studies did suggest that about 50% of the clonally related cells were quite dispersed in the rat cortex (Walsh and Cepko 1992). Here, they used a retrovirus library in which the modified viral genomes contain different DNA tags that can be distinguished by PCR. Cells that had the same DNA tag were frequently found in different areas of the cortex. This observation was confirmed later with a retrovirus library that expressed AP as a histochemical marker (Reid et al. 1995). There remains a possibility of two cells independently infected by two

Table 1. List of retroviral vectors discussed in this chapter

Name	Backbone	Replication competent?	Promoter	Additional features	References
BAG	Mo-MLV	no	LTR		Price et al. (1987); Turner and Cepko (1987); Price and Thurlow (1988); Walsh and Cepko (1988); Luskin (1993)
LZ1	Mo-MLV	no	SV40 early		Luskin et al. (1988)
Unnamed	RSV	no	LTR		Gray et al. (1988)
Unnamed	Mo-MLV	no	β-actin		Gray et al. (1988)
BAG library	Mo-MLV	no	LTR	genetic tags	Walsh and Cepko (1992); Reid et al. (1995)
CXL87	SNV	no	LTR	cytoplasmic	Kornack and Rakic (1995)
				LacZ	
LZ12	Mo-MLV	no	SV40 early	nuclear LacZ	Kornack and Rakic (1995)
RCAS	ALV	yes	LTR	control of viral entry ^a	Doetsch et al. (1999), Seri et al. (2001, 2004)
NIT-GFP	Mo-MLV	no	TRE	regulatable ^b	Noctor et al. (2001, 2002); van Praag et al. (2002); Espósito et al. (2005); Jakubs et al. (2006)

DAP	Mo-MLV	no	SV40 early		Petreanu and Alvarez-Buylla (2002)
LZRS-eGFP-GAP43	Mo-MLV	no	CA	membrane	Carleton et al. (2003)
gapEGFPm4	Mo-MLV	no	CA	GFP	Belluzzi et al. (2003)
CAG-GFP	Mo-MLV	no	CAG	WPRE	van Praag et al. (2005); Zhao et al. (2006); Laplagne et al. (2006)
Unnamed	Mo-MLV	no	EF1 α	shRNA	Ge et al. (2006)
CAG-GFP/Cre	Mo-MLV	no	CAG	WPRE	Tashiro et al. (2006)

(Mo-MLV) Moloney murine leukemia virus; (RSV) Rous sarcoma virus; (SNV) avian spleen necrosis virus; (ALV) avian leukosis virus; (LTR) long terminal repeat; (SV40) simian virus 40; (TRE) tetracycline (tet) response element; (CA) cytomegalovirus (CMV) enhancer and β -actin promoter; (CAG) CMV promoter, chicken β -actin promoter, and synthetic intron; (EF1 α) elongation factor 1 α ; (WPRE) woodchuck hepatitis virus posttranscriptional regulatory element; (shRNA) small hairpin RNA (also known as siRNA, small inhibitory RNA). The marker genes were not listed here because the same viral vector can be easily modified to express different genes. Only direct studies are cited here. The origin of these retroviral vectors can be found in the references cited in these studies.

^aThis virus was used in combination with a *trans*-genic mouse line GFAP-tva in which the receptor for ALV is expressed only in glial fibrillary acidic protein (GFAP) $^+$ cells.

^bIn this vector, the expression of GFP is initiated by binding of tet trans-activator (tTA) to the TRE, which can be inhibited by tet and tet derivatives such as doxycycline. The expression of tTA is driven by the LTR of Mo-MLV.

different virions with the same genetic tag, therefore creating a false dispersed clone. However, this is unlikely to account for the high incidence of widespread clones. These studies suggest that there are both radial and nonradial migration patterns for cortical neurogenesis. This view is in contrast to the “radial unit hypothesis” that considers radial arrangement as the dominant mode of cortical neuron migration (Rakic 1995). Lineage analysis of rhesus monkey cortical neurogenesis suggests that there might be two different modes of cell divisions, asymmetric and symmetric divisions, which gave rise to radial and horizontal arrangements of clonally related cells, respectively (Kornack and Rakic 1995). It has also been suggested that the tangential movement of dividing cortical progenitors within the proliferative zone might have caused some spread of clonally related cells (Fishell et al. 1993; Rakic 1995). The wide spread of clones in the neocortex could be explained by the fact that a subpopulation of the cortical interneurons reach the neocortex from the proliferative zone of the basal ganglia through tangential migration (Anderson et al. 1997). Furthermore, as we now know, new neurons generated in the postnatal subventricular zones (SVZs) migrate 5–8 mm tangentially before they integrate into the olfactory bulb (OB) (Doetsch and Alvarez-Buylla 1996).

A recent study by Noctor et al. (2001) showed that radial glia cells are responsible for the radial migration pattern of cortical neurogenesis. By using both conventional confocal microscopy and time-lapse imaging, Noctor et al. (2001, 2002) showed that radial glia cells gave rise to neurons that migrate along the long thin fiber of the parent radial glia and that the only mitotic cells labeled were of radial-glia morphology. This study, along with others, established that radial glia cells are neuronal progenitor cells during embryonic cortical neurogenesis. In addition, it has been suggested that after the completion of cortical neurogenesis and neuronal migration, radial glia cells transform into astrocytes (Voigt 1989; Rakic 2003). This is inconsistent with the notion that neuronal and glial progenitors diverge early during development. Indeed, some retrovirus-labeled clones contained both neurons and astrocytes (Walsh and Cepko 1992; Reid et al. 1995).

Retrovirus-mediated cell labeling has also been used in a lineage study of adult SVZ neurogenesis. It has been suggested that SVZ progenitors bear characteristics of astrocytes and express the glial fibrillary acidic protein (GFAP). To determine whether GFAP-expressing cells could give rise to neurons, Doetsch et al. (1999) used a transgenic mouse line that expresses the receptor for the avian leukosis virus (also a retrovirus) under the human GFAP promoter and injected the avian leukosis virus

containing the reporter gene alkaline phosphatase into the SVZ. Their results show that new neurons can be derived from a population of dividing cells in which the human GFAP promoter is active. A replication-competent virus was used in this study, which might have increased the efficiency of cell labeling (Doetsch et al. 1999). Because neuronal progenitors in the adult SVZ have been suggested to be relatively quiescent (Morshead et al. 1994; Doetsch et al. 1999), the likelihood of these cells being transduced by retrovirus is presumably quite low. A replication-competent retrovirus whose entry into host cells depends on a specific promoter allows the continuous labeling of a specific population of cells. The same virus was used to target progenitors in the subgranular zone (SGZ) of the adult hippocampus, and labeled granule neurons were found 4 weeks after viral injection, suggesting that the human GFAP promoter is also active in at least a subpopulation of progenitors in the adult hippocampus (Seri et al. 2001).

In summary, three different ways have been developed for lineage analysis in the nervous system using retrovirus-mediated cell labeling: *in vivo* clonal analysis, time-lapse imaging, and promoter-dependent lineage tracing. Clonal analysis can be used to determine the relative time window when the progenitors of different cell types in the nervous system diverge, and this technique can be applied at any time during development. However, this approach is flawed by the fact that retroviruses are often silenced in host cells, and we know little about the epigenetic regulations of retroviruses in neural progenitors. Time-lapse imaging of labeled progenitors in cultured brain slices gives a direct view of progenitor proliferation, differentiation, migration, and morphogenesis. Yet, brain slice cultures might not fully recapitulate the condition in the intact brain. Promoter-dependent lineage tracing reveals whether a certain cell type, defined by the specific promoter, can serve as the precursor of another cell type. It is critical to use a promoter of high specificity.

INTEGRATION OF RETROVIRUS-LABELED NEWBORN NEURONS

Because retrovirus-mediated cell labeling results in permanent labeling of dividing cells and their progeny, this technique is frequently used for studying the morphological and physiological development of newborn neurons during postnatal and adult neurogenesis (Fig. 1). The first evidence of functional integration of newborn neurons in an adult rodent brain came from a study in the adult hippocampus using the retroviral vector NIT-GFP, which expresses the neomycin phosphotransferase (neo) and the tetracycline (tet) *trans*-activator protein (tTA) through a

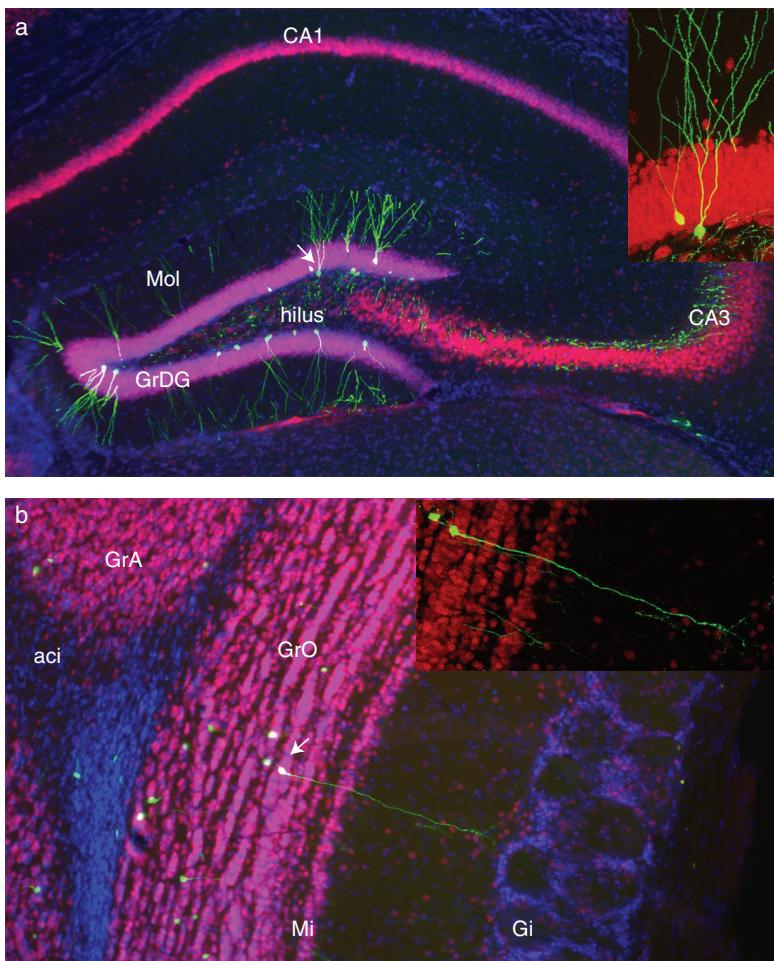


Figure 1. Retrovirus-mediated labeling of newborn neurons in the adult mouse brain. (a) Newborn neurons in adult hippocampus 56 days after viral injection. Note the growth of dendritic processes into the molecular layer and the projection of mossy fiber axons into field CA3 of hippocampus. (GrDG) Granule cell layer of the dentate gyrus (DG); (Mol) molecular layer of the DG. (b) Newborn granule neurons in adult olfactory bulb (OB) 15 days after viral injection. (aci) Anterior commissure, intrabulbar part; (Gl) glomerular layer of the OB; (GrA) granule cell layer of the accessory OB; (GrO) granule cell layer of the OB; (Mi) mitral cell layer of the OB. Insets are higher-magnification views of the cells indicated by arrows. (Blue) dapi; (green) GFP; (red) NeuN. Bars, 100 μ m.

bicistronic cassette neo-ires-tTA, and GFP through the binding of tTA to the tet responsive element (TRE) (van Praag et al. 2002). At 4 weeks post-injection, a subpopulation of GFP⁺ cells displayed morphological features typical of granule neurons, which can be evoked by perforant path stimulation. It was later shown that these newborn neurons follow a similar maturation process compared to granule neurons generated in the early postnatal hippocampus, being that they develop slow GABAergic responses, glutamatergic and fast GABAergic responses sequentially (Esposito et al. 2005).

Although the NIT-GFP vector provided invaluable advances in the field of neurogenesis (Noctor et al. 2001; van Praag et al. 2002; Esposito et al. 2005), the frequency of neuronal labeling by NIT-GFP is not consistent with that resulted from bromodeoxyuridine (BrdU) incorporation. For example, only 2.2% of GFP⁺ cells expressed the panneuronal marker NeuN at 4 weeks after viral injection into the adult hippocampus, whereas 50–80% of BrdU-labeled cells were found to be positive for NeuN (Kempermann et al. 1997a; van Praag et al. 1999, 2002).

To more efficiently mark newborn neurons, we and other investigators have tried to use other ubiquitous promoters to drive the expression of GFP using the same Mo-MLV retroviral backbone (Ge et al. 2006; Zhao et al. 2006). We found that the compound promoter CAG, which contains the chicken actin promoter, minimum cytomegalovirus (CMV) enhancer, and a large synthetic intron, allowed efficient labeling of newborn neurons (Zhao et al. 2006). For example, more than 400 newborn neurons can be labeled by a single injection of CAG-GFP (10^5 colony forming unit) into the SGZ of 7–10-week mice. Furthermore, about 72% of GFP⁺ cells are of neuronal phenotype, consistent with observations made with BrdU labeling. This vector allowed more systematic analyses of newborn neurons in the adult hippocampus. These neurons go through distinct stages of morphogenesis. They project mossy fiber axons into the distal area of CA3 within 16 days after birth, which is followed by a rapid growth of dendritic spines. Further structural modifications of newborn neurons can take place for several months and can be regulated by experience. In addition, newborn neurons in the adult appear to mature much slower compared to those in the early postnatal hippocampus (Zhao et al. 2006).

By labeling dentate granule neurons born in early embryonic/postnatal and adult brains with two different fluorescent proteins (GFP and red fluorescent protein [RFP]), Laplagne et al. (2006) were able to compare two distinct populations of neurons in the same mouse

brain. Extensive electrophysiological studies showed that mature granule neurons exhibited similar GABAergic and glutamatergic afferent connectivities, whether they were born in embryonic, early postnatal, or adult brains.

The development of newborn neurons in the SVZ has also been studied in depth through retrovirus-mediated cell labeling. Luskin (1993) used the BAG β -gal-at-gag virus and showed that newly generated neurons in the postnatal rat SVZ took a restricted migrating route (which is later called the rostral migratory stream [RMS]) reaching the OB, where they become periglomerular and granule neurons. By 14 days after virus infection at P0, labeled periglomerular neurons in the rat OB displayed electrophysiological properties similar to unlabeled cells (Belluzzi et al. 2003). Newborn neurons in the OB develop functional GABA receptors before glutamate receptors (Belluzzi et al. 2003; Carleton et al. 2003). Detailed morphological analyses in the adult brain showed that new neurons originating from SVZ go through distinct stages of migration and morphogenesis. These neurons develop dendritic spines between 13 and 15 days after viral injection. Interestingly, there is a sharp decrease of newborn OB neurons 15 days after [3 H]thymidine labeling. The survival of newborn granule neurons after the formation of dendritic spines appears to depend on the input from olfactory activity (Petreanu and Alvarez-Buylla 2002).

Retrovirus-mediated cell labeling has enabled the examination of morphological development and functional integration of newborn neurons *in vivo*. The electrophysiological properties of new neurons have been characterized extensively at the input level. No study has attempted to address the functional output from these cells.

RETROVIRUS-MEDIATED GENE MANIPULATIONS

Retrovirus-mediated gene transfer not only allows the visualization of dividing cells and their progeny through the expression of molecular markers, but it can also be modified to alter the expression of a gene of interest so that the function of the gene can be studied in the context of progenitor proliferation, differentiation, and neuronal maturation. For example, one could overexpress different forms of a protein of interest, including wild-type, constitutively active, and dominant-negative forms. One could also knock down a gene of interest. Two recent studies have used different ways to down-regulate a gene of interest and studied the activity-dependent modulation of newborn neurons.

In the first study, Ge et al. (2006) used retrovirus-mediated expression of a short hairpin RNA (shRNA) that was designed to target the Na^+/K^+ - 2Cl^- transporter NKCC1. Here, the vector expresses the shRNA under the human U6 promoter and GFP under the promoter of elongation factor 1 α . In mature granule neurons, the neurotransmitter GABA elicits a hyperpolarization response through the flow of the negatively charged chloride ion (Cl^-) into the cells. In contrast, in immature granule neurons, GABA leads to the depolarization of the cells because these cells have a higher $[\text{Cl}^-]$ compared to the extracellular environment. The expression of NKCC1 is believed to be responsible for the high $[\text{Cl}^-]$ within immature cells. By down-regulating NKCC1 through shRNA, Ge et al. (2006) showed that newborn neurons with less NKCC1 indeed have lower $[\text{Cl}^-]$ compared to control cells and that these neurons hyperpolarized in response to tonic GABA activation. Interestingly, the targeted neurons do not mature normally, which is reflected in dramatic defects in both dendritic development and glutamatergic responses (Ge et al. 2006). These results suggest that the early GABAergic depolarization response has an important role in the development of newborn neurons born in the adult hippocampus.

In the second study, Tashiro et al. (2006) used a mouse line in which the DNA fragment encoding the transmembrane domain and the carboxy-terminal region of the *N*-methyl-D-aspartate (NMDA) receptor NR1 sub-unit is flanked by two *loxP* sites. A retrovirus expressing GFP/Cre fusion protein was delivered into the SGZ of adult hippocampus and induced the excision of the floxed *NR1* fragment only in neuronal progenitors (Tashiro et al. 2006). By coinjection with the CAG-RFP virus, Tashiro et al. (2006) showed that deletion of NR1 in immature neurons increased cell death during the third week after virus injection, suggesting that functional NMDA receptor is important for the survival of newborn neurons. Furthermore, the survival rate of NR1 knockout neurons increased when an NMDA receptor antagonist, 3-(2-carboxypiperazin-4-yl) propyl-1-phosphonic acid (CPP), was administered to reduce the activity of wild-type cells, indicating a competitive mechanism of activity-dependent survival of new neurons. The timing of NMDA receptor-dependent survival coincides with dendritic spine growth and the onset of functional glutamatergic responses (Esposito et al. 2005; Ge et al. 2006; Tashiro et al. 2006; Zhao et al. 2006).

Both strategies allowed cell-autonomous analysis of individual neurons because only small populations of cells are altered and their surrounding environment is intact. These methods will no doubt be valuable for future mechanistic studies.

STUDYING NEUROGENESIS IN DISEASE MODELS

It is well known that the proliferation of neuronal progenitors and the survival of new neurons can be regulated by physiological and pathological conditions (Kempermann et al. 1997b; Gould et al. 1999; van Praag et al. 1999; Abrous et al. 2005; Ming and Song 2005). However, it is less clear how the integration of new neurons is affected by these conditions and whether the alteration of newborn neuron integration is involved in these processes.

Hippocampal neurogenesis decreases with aging, and this can be rescued to a certain extent by voluntary exercise. A pilot study showed that new dentate granule neurons in running old mice display morphological characteristics similar to those in young adult mice, suggesting that newborn neurons can also stably integrate in old hippocampus (van Praag et al. 2005).

Epilepsy is a pathological condition that has recently been associated with a dramatic increase in progenitor proliferation in the hippocampus (Parent et al. 1997; Jessberger and Kempermann 2003). Morphological abnormalities of seizure-induced newborn neurons have been reported, such as the growth of basal dendrites and ectopic migration of granule neurons (Parent et al. 1997; Shapiro and Ribak 2006). To study the function of seizure-induced neurogenesis, Jakubs et al. (2006) labeled new cells in the hippocampus with the retroviral vector NIT-GFP in a rat model of status epilepticus (SE). They showed that 4–6 weeks after viral labeling, seizure-induced new neurons displayed reduced excitatory input and increased inhibitory input compared to neurons generated in running rats (Jakubs et al. 2006). It remains to be determined whether this represents a mechanism of self-repair through the decreased excitability of newborn neurons; it is not known whether new neurons in epileptic brain also have lower excitability than those in a sedentary rat brain.

Using the retrovirus vector CAG-GFP, we examined the integration of newborn neurons in kainic acid (KA)-induced epilepsy in rats. A subpopulation of newborn neurons displayed growth of basal dendrites, consistent with earlier findings using immature neuronal markers. Furthermore, new neurons with aberrant dendrites seem to be stably integrated in the epileptic brain, as a similar percentage of labeled neurons were found to bear basal dendrites even 3 months after viral infection (Jessberger et al. 2007). Moreover, seizure activity appears to have differential effects on mature neurons, as we found that neurons born before seizure never developed basal dendrites. In contrast, we observed mossy fiber sprouting only in neurons at least 4 weeks old at the time of KA administration (Jessberger et al. 2007). This is consistent

with the finding that mossy fiber sprouting was observed in GFP[−] cells in POMC-GFP transgenic mice, in which GFP is expressed in immature neurons in the adult SGZ (Overstreet-Wadiche et al. 2006).

LENTIVIRUS-MEDIATED CELL LABELING

Because the integration of retrovirus depends on the mitotic activity of host cells, retrovirus-mediated cell labeling is biased toward cells with an active cell cycle, for example, the transit-amplifying cells in the SVZ. This is supported by the observation that very few cells were detected in the RMS 15 days after viral injection (Petreanu and Alvarez-Buylla 2002). In contrast to recombinant retroviruses, the newly transcribed DNA from a lentivirus could enter the host-cell nucleus at any time during the cell cycle. Therefore, lentiviral vectors can be used to target relatively quiescent cells. Using lentivirus-mediated expression of GFP in SVZ cells, Consiglio et al. (2004) showed that GFP-labeled cells could be found in the RMS even 6 months after viral injection, confirming that newborn olfactory neurons originate from the SVZ. However, this vector cannot distinguish neuronal progenitors from other cell types in the SVZ, because a ubiquitous promoter was used and all cells at the injection site expressed the GFP marker. Therefore, this approach cannot be adapted for studying hippocampal neurogenesis, because newborn neurons do not migrate far away from the site of proliferation. Instead, specific promoters of putative stem cell markers have been used to confer specificity to lentiviral vectors (H. Suh et al., unpubl.).

DISADVANTAGES OF RETROVIRUS-MEDIATED CELL LABELING

Retrovirus-mediated cell labeling in the adult rodent brain requires stereotaxic surgery to deliver the virus to the area of interest. The surgery itself can induce local inflammatory reactions. It has been reported that the combination of retrovirus and microglia proliferation could stimulate the fusion of labeled microglia with existing postmitotic neurons in the postnatal neocortex (Ackman et al. 2006). Therefore, one should take caution interpreting results obtained from retroviral labeling. Fortunately, it is relatively easy to determine whether newborn neurons identified through retroviral labeling are indeed derived from dividing progenitors in the adult brain. Because neurons go through extensive morphogenesis before maturation, a time-course study of retrovirus-labeled neurons provided unambiguous evidence that retrovirus-labeled cells were in fact derived

from progenitor cells (Petreanu and Alvarez-Buylla 2002; Carleton et al. 2003; Esposito et al. 2005; Ge et al. 2006; Zhao et al. 2006).

Retroviruses are often silenced in host cells such as embryonic stem cells, embryonic carcinoma cells, and hematopoietic stem cells. This is complicated by the fact that two daughter cells from the same parent cell could undergo differential silencing and that expressing cells could also be silenced during differentiation (Ellis 2005). It has not been carefully investigated whether retroviral vectors are also silenced in neuronal stem cells or during their differentiation, but the low level of neuronal labeling of the NIT-GFP vector does suggest that the expression of retroviral vectors is not homogeneous in all cell types. Therefore, retrovirus and lentivirus vectors at the current stage are not optimal for lineage analysis in the nervous system, as one cannot be certain whether all progenies of a neuronal stem cell can be traced.

SUMMARY

Retrovirus-mediated gene expression can specifically label dividing cells and their progeny, making it an ideal tool for studying neurogenesis, especially in the adult brain, in which only a small population of cells are mitotic. However, one should bear in mind that we know little about epigenetic and promoter regulations of retroviruses in adult neuronal progenitors, especially when retroviral vectors are used in lineage and fate-mapping studies. Nevertheless, marker gene expression can be maintained in neurons, which allowed long-term systematic studies of newborn neuron integration. Furthermore, with the technical advancement in molecular biology and imaging, it is hoped that retrovirus-mediated cell labeling will help us understand the molecular mechanisms which regulate the self-renewal and differentiation of adult neuronal progenitors, as well as the maturation and integration of newborn neurons.

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