Chapter 9
Glycolipid and Glycoprotein Expression During Neural Development

Robert K. Yu and Yutaka Itokazu

Abstract In mammals, the central and peripheral nervous systems are developmentally derived from cells in the neural plate. Specific ectodermal cells in this area form the neural tube and neural crest during the early developmental stage. The neural tube is the origin of the central nervous system which consists of both the brain and spinal cord, whereas neural crest cells are precursors of the peripheral nervous system. During neural tube formation and neural crest development, carbohydrate-rich molecules, including glycolipids, glycoproteins, and proteoglycans, are expressed primarily on the outer surface of cell plasma membranes. The structural diversity of their carbohydrate moieties coupled with their expression at different stages of development makes these molecules excellent biomarkers for various cell types. In addition, these molecules play crucial functional roles in cell proliferation, differentiation, interaction, migration, and signal transduction. In this chapter, we discuss the expression profiles and potential functional roles of glycoconjugates during neural development.

Keywords Neural stem cell • Neural development • Neurogenesis • Gliogenesis • Glycolipid • Carbohydrate • Glycoconjugate • Glycosphingolipid • Ganglioside • Glycoprotein • Proteoglycan

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Abbreviations

BLBP	Brain lipid-binding protein
BMB	Bone morphogenetic protein
CD	Cluster of differentiation
Cer	Ceramide
CNS	Central nervous system
CNTF	Ciliary neurotrophic factor
CS	Chondroitin sulfate
CSPG	Chondroitin sulfate proteoglycan
CST	Cerebroside sulfotransferase
Dll1	Delta-like1
EGF	Epidermal growth factor
FABP7	Fatty acid-binding protein 7
FGF	Fibroblast growth factor
Fuc	Fucose
FUT	Fucosyltransferase
GAG	Glycosaminoglycan
GalCer	Galactosylceramide
GalNAcT	*N*-acetylgalactosaminyltransferase
GalT	Galactosyltransferase
GFAP	Glial fibrillary acidic protein
GlcAT-P	UDP-glucuronyltransferase-P
GlcCer	Glucosylceramide
GlcT	Glucosyltransferase
GRP	Glial-restricted precursor
GSL	Glycosphingolipid
HA	Hyaluronic acid
HNK-1	Human natural killer-1 antigen
HS	Heparin sulfate
HSPG	Heparin sulfate proteoglycan
IL-6	Interleukin 6
INP	Intermediate neuronal progenitor cell
IPC	Intermediate progenitor cell
JAK-STAT	Janus kinase (JAK)-signal transducer and activator of transcription 3 (STAT3)
LacCer	Lactosylceramide
Lfng	Lunatic fringe
MA	Cerebral mantle
mAb	Monoclonal antibody
MAPK	Mitogen-activated protein kinase
MZ	Marginal zone
NEC	Neuroepithelial cell
NG-2	Nerve/glial antigen 2

NRP	Neuronal restricted progenitor
NSC	Neural stem cell
OPC	Oligodendrocyte precursor cell
PDGF	Platelet-derived growth factor
PG	Proteoglycan
PHA-E4	*Phaseolus vulgaris* erythroagglutinating lectin
PNA	Peanut agglutinin
PNS	Peripheral nervous system
PSA-NCAM	Polysialic acid-neural cell adhesion molecule
PST	ST8SiaIV
RGC	Radial glial cell
SGZ	Subgranular zone
SSEA	Stage-specific embryonic antigen
ST	Sialyltransferase
STX	ST8SiaII
SVZ	Subventricular zone
VZ	Ventricular zone

9.1 Introduction

During neural development, dramatic and consistent changes in the composition of glycoconjugates, including glycolipids, glycoproteins, and proteoglycans (PGs), occur (Ngamukote et al. 2007; Yanagisawa and Yu 2007; Yu et al. 1988). It is known that changes in the expression of glycolipids, including gangliosides, in the nervous system correlate with neurodevelopmental events (Yu et al. 2009). For example, in fertilized eggs, the globo-series of glycolipids are robustly expressed. As cell division proceeds, the lacto-series glycosphingolipids (GSLs) are expressed, followed by the ganglio-series GSLs in the developing brain. The lipid portion of GSLs, including gangliosides, is the ceramide, which is synthesized in the endoplasmic reticulum (ER) from a sphingosine base and a fatty acid residue. Ceramide is transferred to the Golgi apparatus where it is modified by the sequential addition of carbohydrate moieties (Fig. 9.1) (Yu et al. 2012). Each step is catalyzed by a unique, specifically controlled glycosyltransferase. In early embryonic rodent brains, the pattern of ganglioside expression is characterized by the expression of a large amount of simple gangliosides, such as GM3 and GD3. In the later developmental stages, more complex gangliosides prevail, particularly GM1, GD1a, GD1b, and GT1b (Fig. 9.2). Correlations between ganglioside expression in the nervous system and neurodevelopmental events are summarized schematically in Fig. 9.3. This unique expression pattern suggests that the presence of specific gangliosides may reflect the functional roles they play at specific developmental stages. Abundant evidence supports the notion that GSLs, including gangliosides, serve regulatory roles in cellular events, including proliferation and neural differentiation, as exemplified by neuritogenesis, axonogenesis, and synaptogenesis (Bieberich et al. 2001;

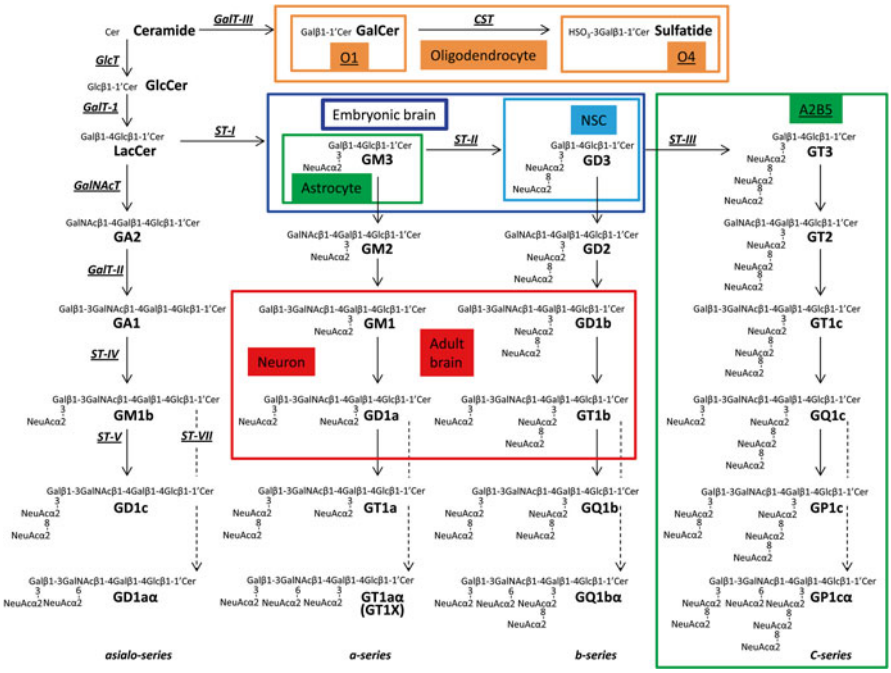

Fig. 9.1 Structures and biosynthetic pathways of glycosphingolipids (GSLs). The nomenclature for gangliosides and their components are based on that of Svennerholm (1963) and the IUPAC–IUBMB Joint Commission on Biochemical Nomenclature (1977). *Cer* ceramide, *CST* cerebroside sulfotransferase (*Gal3st1*, sulfatide synthase), *GalNAc-T N*-acetylgalactosaminyltransferase I (*B4galnt1*, GA2/GM2/GD2/GT2 synthase), *GalT-I* galactosyltransferase I (*B4galt6*, lactosylceramide synthase), *GalT-II* galactosyltransferase II (*B3galt4*, GA1/GM1/GD1b/GT1c synthase), *GalT-III* galactosyltransferase III (*Ugt8a*, galactosylceramide synthase), *GlcT* glucosyltransferase (Ugcg, glucosylceramide synthase), *ST-I* sialyltransferase I (*St3gal5*, GM3 synthase), *ST-II* sialyltransferase II (*St8Sia1*, GD3 synthase), *ST-III* sialyltransferase III (*St8Sia3*, GT3 synthase), *ST-IV* sialyltransferase IV (*St3gal2*, GM1b/GD1a/GT1b/GQ1c synthase), *ST-V* sialyltransferase V (*St8sia5*, GD1c/GT1a/GQ1b/GP1c synthase), *ST-VII* sialyltransferase VII (*St6galnac6*, GD1aα/GT1aα/GQ1bα/GP1cα-synthase). Official symbols of genes are represented in *italics* in this figure legend. GM3 and GD3 are abundant in embryonic brain (*blue*) and NSCs express GD3 (*light blue*). c-series gangliosides are A2B5 antigens (*green*) and astrocytes express GM3 (*green*). GM1, GD1a, GD1b, and GT1b are the most abundant ganglioside species in adult mammalian brain (*red*). Oligodendrocyte markers O1 and O4 are GalCer and sulfatide, respectively (*orange*)

Fang et al. 2000; Ngamukote et al. 2007; Wu et al. 1998, 2001; Yu et al. 2004, 2009). In recent years, with the advent of contemporary molecular genetics and biology, several lines of genetically modified mice have been established in which the expression of gangliosides and other GSLs has been altered or depleted, and this has greatly facilitated the unraveling of their biological functions. For example, GM2/GD2 synthase (GalNAcT) is one of the key enzymes needed for synthesis of the major "brain-type" gangliosides, including GM1, GD1a, GD1b, and GT1b. Mice lacking this enzyme do not express GalNAc-containing gangliosides. As a result

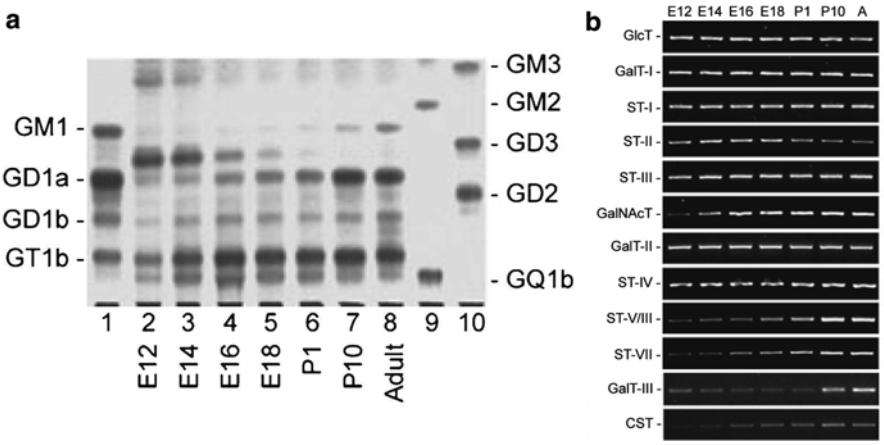

Fig. 9.2 Ganglioside and glycosyltransferase expression in the developing mouse brain. (**a**) Ganglioside expression patterns analyzed by thin-layer chromatography. Expression in mouse brain shift, with age, from simple gangliosides such as GM3 and GD3 to complex gangliosides such as GM1 and GD1a. (**b**) Glycosyltransferases expressed in developing mouse brains analyzed by RT-PCR. During early development, the message levels of GalNAcT (GA2/GM2/GD2/GT2 synthase) and ST-II (GD3 synthase) are developmentally regulated. "A" indicates adult mouse brain (reproduced from Ngamukote et al. 2007)

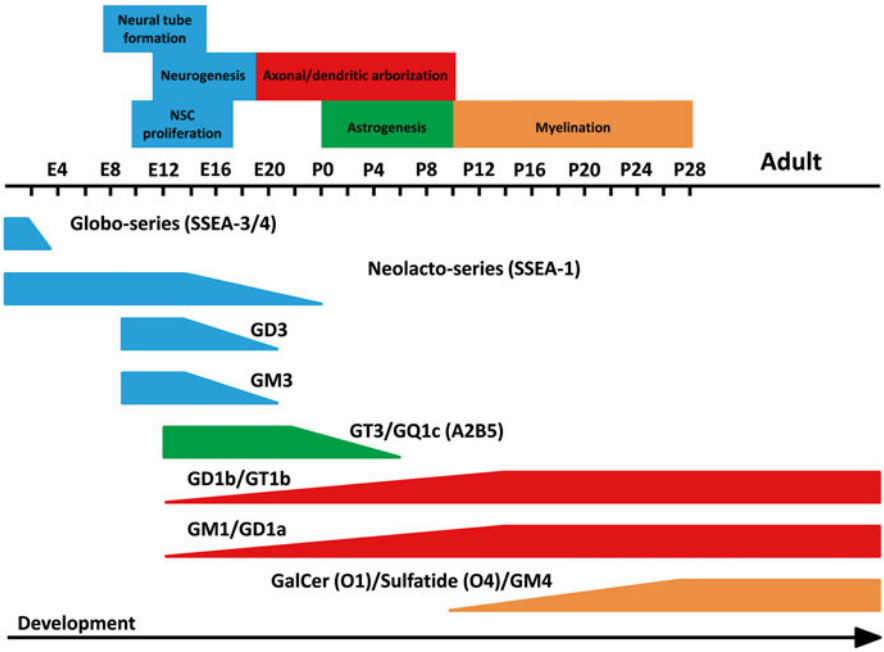

Fig. 9.3 Neurodevelopmental events and concurrent changes in GSL expression. "E" denotes embryonic day and "P" postnatal day

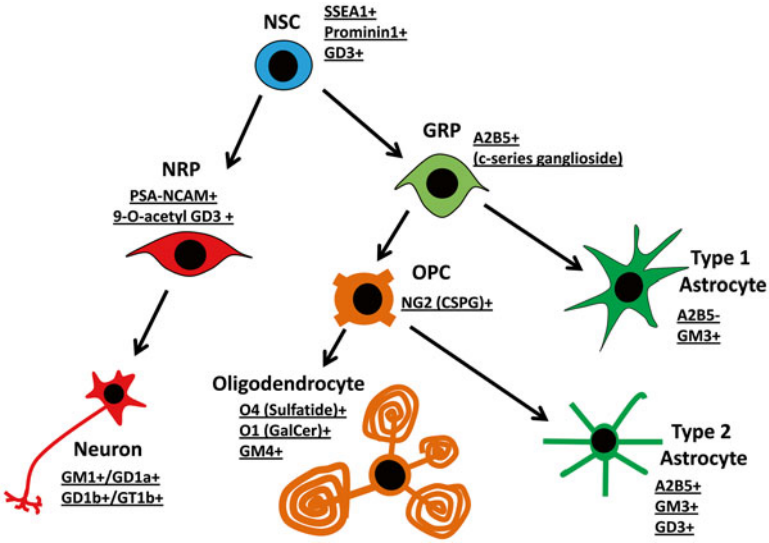

Fig. 9.4 A model for neural cell lineages derived from mouse neural stem cells (NSCs). The known glycoconjugate markers are *underlined. NSC* neural stem cell, *NRP* neuronal restricted precursor, *GRP* glial restricted precursor, *OPC* oligodendrocyte precursor cell

they are developmentally abnormal and appear to have neurological problems such as axonal degeneration; sensory, motor, and behavioral deficits; and other neurological dysfunctions (Furukawa et al. 2008; Sheikh et al. 1999; Sugiura et al. 2005; Susuki et al. 2007; Takamiya et al. 1996; Wu et al. 2011). During brain development, gangliosides are assumed to modulate ceramide (Cer)-induced apoptosis and to maintain cellular survival and differentiation (Bieberich et al. 2001). GM3 synthase (sialyltransferase I, ST-I) is a critical enzyme for the synthesis of all complex gangliosides. Mutation of GM3 synthase is associated with human autosomal recessive infantile-onset symptomatic epilepsy syndrome (Simpson et al. 2004). This study clearly demonstrated that deletion of complex gangliosides can be associated with human diseases. A lack of b- and c-series gangliosides results in clear and subtle developmental and behavioral deficits with mice lacking these gangliosides exhibiting sudden death from audiogenic seizures (Kawai et al. 2001). Both GalNAcT- and ST-I-deficient mice, which lack all gangliosides, die soon after weaning at 3 weeks of age (Yamashita et al. 1999). Combined these observations clearly indicate that gangliosides have important biological functions in the developing nervous system.

In addition to glycolipids, proteoglycans and glycoproteins are also known to modulate cellular proliferation and differentiation by participating in signal transduction in response to external stimuli and in mediating cell–cell interactions and adhesion. In this chapter, we will introduce these glycoconjugates expressed during neural development (Fig. 9.4).

9.2 Glycobiology During Early Embryogenesis

After fertilization, the fertilized egg undergoes cleavage to 2-, 4-, and 8-cell stages. From the 8-cell to 32-cell stage, the spherical cells undergo changes in morphology to a cubic shape. The cells bind tightly to each other forming compact spheres, and this stage is called the compaction stage (Purves and Lichtman 1985). At this stage, cell surface glycoconjugate markers, composed of fucose, *N*-acetyllactosamine, stage-specific embryonic antigen-1 (SSEA-1), and others, start to emerge (Fig. 9.5). SSEA-1 is also known as Lewis X antigen, belonging to cluster of differentiation (CD) 15. Strictly, "SSEA-1" is not equal to "Lewis X." Lewis X structure is defined by a minimal Lewis X motif consisting of the structural element Galβ1-4(Fucα1-3) GlcNAcβ-. The structure of SSEA-1 is shown in Fig. 9.5. However, in this chapter we will describe both as SSEA-1 because SSEA-1 and Lewis X have not been clearly distinguished in the literature. Since Lewis haptens have been reported to inhibit the cell compaction process in mouse embryos (Fenderson et al. 1984; Solter and Knowles 1978), it is believed that SSEA-1 may play an important role in early embryogenesis. Other stage-specific antigens, such as SSEA-3 and SSEA-4, are

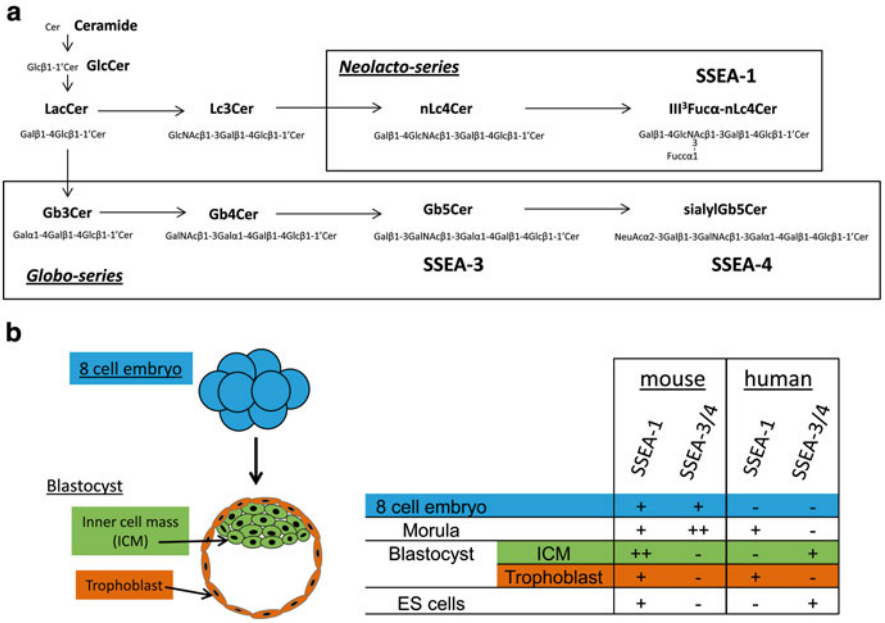

Fig. 9.5 Expression of SSEAs and their biosynthetic pathways in early embryogenesis. (**a**) Structures and synthetic pathway of globo- and neolacto-series glycosphingolipids (GSLs). The abbreviations for GSLs follow the nomenclature systems of IUPAC–IUBMB Joint Commission on Biochemical Nomenclature (1977) and Svennerholm (1963). SSEA-1 is carried not only by neolacto-series GSLs but also by proteoglycans, glycoproteins, and lacto-, ganglio-, and globo-series GSLs. (**b**) A summary of the expression patterns of SSEAs in mouse early embryogenesis and embryonic stem (ES) cells

also expressed at the early stages of mouse embryogenesis. The expression of SSEA-3 usually peaks at the 4- to 8-cell stages, whereas SSEA-4 peaks at the morula and early blastocyst stages with some overlap with that of SSEA-3 (Fenderson et al. 1990). The expression patterns of these stage-specific antigens are different in human and mouse (Fig. 9.5). Thus, SSEA-1 has been utilized as a specific marker of mouse embryonic stem (ES) cells. SSEA-1 is not expressed in human ES cells. Instead, human ES cells express SSEA-3, SSEA-4, and keratan sulfate antigens (TRA)-1-60, TRA-181, GCTM2, and GCT343 (Adewumi et al. 2007; Muramatsu and Muramatsu 2004).

Analysis of mice deficient in SSEA-1 [fucosyltransferase 9 (FUT9)-deficient mice] revealed increased anxiety-like behavior, but no distinguishable morphological phenotypes in brain development (Kudo et al. 1998, 2007). While mice deficient in SSEA-3 and SSEA-4 expression (α1,4galactosyltransferase-deficient mice) were resistant to Shiga-like toxins, they showed no apparent abnormality in development (Okuda et al. 2006). These studies suggest that the functions of SSEAs may be compensated for by other carbohydrate molecules or are not essential for neural development.

9.3 Neural Tube Formation

Both the central nervous system (CNS) and peripheral nervous system (PNS) originate from ectodermal cells in the neural plate (Purves and Lichtman 1985). Cells at the neural plate undergo a series of divisions and morphological changes, and they form a neural groove that has neural folds on either side (Fig. 9.6). Cells in the neural folds constitute the precursors of neural crest cells. In mice, these neural folds approach each other in the median plane, become fused at embryonic day (E) 8.5, and eventually form the neural tube. All the cells for the CNS emanate from the neural tube. On the other hand, the PNS originates from the neural crest cells. With respect to neural tube formation, glycosaminoglycans (GAGs) and high molecular weight unbranched polysaccharides made up of repeating disaccharide subunits of an amino sugar and a uronic acid play an important role in its genesis. In addition, nonsulfated GAGs, hyaluronans, or hyaluronic acid (HA)-containing glycoconjugates support structural and tensile strengths during neural tube folding and closure (Morris-Wiman and Brinkley 1990a, b). Enzymatic degradation of the HA matrix in the neural plate with exogenous hyaluronidase leads to incomplete closure of the neural tube in chick embryos (Schoenwolf and Fisher 1983). Other GAGs are also important for early embryogenesis. For example, mice deficient in heparin sulfate (HS) die by E8.5, indicating that HS also has an important role(s) in early embryogenesis. In addition, mice deficient in glucuronyltransferase I (GlcAT-1), an enzyme required for the synthesis of the linkage tetrasaccharide for both HS and chondroitin sulfate (CS), fail to express either, and these knockout mice die before the 8-cell stage as the result of cytokinesis failure (Izumikawa et al. 2010). To identify the contributor of this lethality, specific glycanase treatments were performed. Treatment

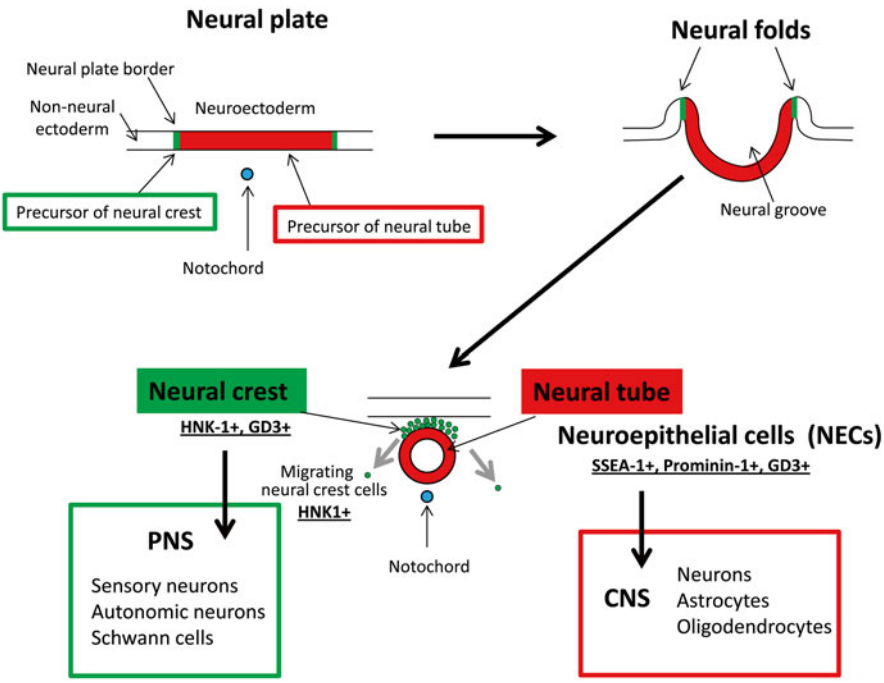

Fig. 9.6 Neural tube and neural crest formation. Cells in the neural plate undergo a series of divisions and form a neural groove that has neural folds on both sides. Cells in the neural fold are the future neural crest cells. The neural folds then approach each other in the median plane, become fused, and eventually form the neural tube. All the cells for the CNS are derived from neuroepithelial cells (NECs) of the neural tube. The PNS originates from neural crest cells. The precursors of neural crest cells reside in the dorsal neural tube, and these cells undergo epithelial to mesenchymal transition (EMT) and delaminate from the neural tube as neural crest cells. The known carbohydrate markers are *underlined*

of 2-cell embryos with chondroitinase ABC which degrades CS had marked effects on cell division were observed. At the same time, many heparitinase, which specifically degrade HS, treated embryos normally developed to blastocysts. Thus, CS is indispensable for embryonic cell division. These examples underscore the importance of glycoconjugates in embryonic cell division.

9.4 Neuroepithelial Cells and Radial Glial Cells

9.4.1 Neural Stem Cells in Development

Neuroepithelial cells (NECs) proliferate by repeating symmetric cell division at the wall of the neural tube, and as NECs accumulate the wall gradually becomes thicker. At first, an NEC elongates its fibers and becomes a radial glial cell (RGC) whose

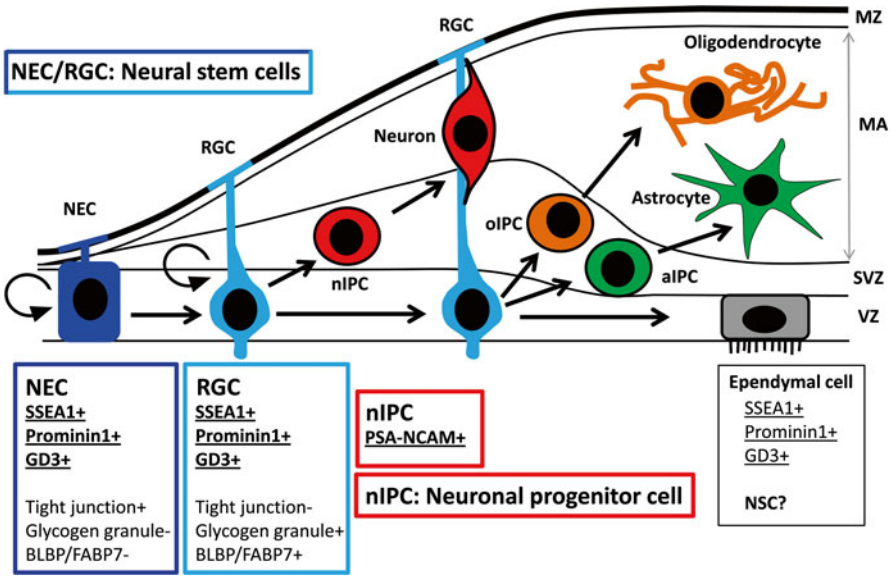

Fig. 9.7 Neuroepithelial cells (NECs) proliferate at the wall of the neural tube, and NECs elongate their fibers and become radial glial cells (RGCs). An RGC generates an RGC and an intermediate progenitor cell (IPC). *nIPC* neuronal intermediate progenitor (also called basal progenitor), *oIPC* oligodendrocyte IPC (also called oligodendrocyte precursor cell, OPC), *aIPC* astrocyte IPC, *MZ* marginal zone, *MA* mantle, *SVZ* subventricular zone, *VZ* ventricular zone. The known carbohydrate markers are *underlined*. Whether aIPCs are involved in this pattern is not well known

cell body lines the ventricular zone (VZ) and the apical surface meets the ventricles with the radial fibers reaching the pial surface (Fig. 9.7). Previously, RGCs were thought of as specialized glial cells whose function was to guide neuronal migration (Alvarez-Buylla et al. 2001; Fishell and Kriegstein 2003, 2005; Fujita 2003; Gotz and Huttner 2005; Miller and Gauthier 2007; Shimojo et al. 2011). Recently, RGCs were recognized as the precursors of neurons and glia. By asymmetric cell division, an RGC generates another RGC and an intermediate progenitor cell (IPC) or immature neuron (Malatesta et al. 2000; Miyata et al. 2001; Noctor et al. 2001). IPCs stay in the subventricular zone (SVZ) to proliferate and give rise to more neurons. Immature neurons migrate along with radial fibers into the cortical plate and then become mature neurons. At first, RGCs give rise to inner layer neurons and later to outer layer neurons. RGCs also give rise to oligodendrocytes and ependymal cells and can eventually differentiate into astrocytes. Both NECs and RGCs are considered NSCs (Franco and Muller 2013; Shimojo et al. 2011). The NSC niche is a specialized microenvironment that maintains stem cells in a multipotent and undifferentiated state. The NSC niche hosts a variety of stem/progenitor cells, such as NECs, RGCs and IPCs. Altogether, these versatile progenitors cooperate for neurogenesis and gliogenesis in the developing CNS (Fig. 9.7). In the following sections, some of the key glycoconjugate biomarkers are described.

Notch

Notch receptors are transmembrane proteins whose signaling has been shown to regulate a wide range of developmental processes (Hori et al. 2013; Koch et al. 2013). Notch signaling plays essential roles in neurogenesis, including inhibition of neurogenesis and oligodendrocyte differentiation, maintenance of the RGC pool, and promotion of astrocyte differentiation (de la Pompa et al. 1997; Gaiano and Fishell 2002). Notch signaling is activated by interaction with ligand molecules, such as *Delta-like1* (*Dll1*, *Delta* in *Drosophila*) or *Jagged* 1 (*Serrate* in *Drosophila)*. Neuronal IPCs (nIPCs) or intermediate neuronal progenitor cells (INPCs) are known to be a source of Dll1 to activate Notch signaling in RGCs (Mizutani et al. 2007). nIPCs/INPCs provide intrinsic neuronal differentiation information to new neurons by themselves and by extrinsic inhibitory signals to maintain the stemness of RGCs. *Fringe* is a major regulator of Notch signaling, serving as a promoter of Delta–Notch signaling and as an inhibitor of *Serrate*–Notch signaling in *Drosophila wing* (Hou et al. 2012; Panin et al. 1997). In mammals, there are three *Fringe* genes (*Lfng, Mfng, Rfng*) expressed in different populations of cells in the developing cortex. *Lunatic fringe* (*Lfng*) is expressed in immature cells, presumed to be NECs and RGCs, in the VZ (Ishii et al. 2000; Kato et al. 2010). It is known that *O*-glycosylation of the Notch extracellular domain is essential for Notch activity by affecting protein folding, ligand interaction, and endocytosis of the Notch receptor (Okajima et al. 2008). The Notch receptor contains epidermal growth factor (EGF)-like repeats, which have *O*-fucose glycan modifications on the serine or threonine residues (Haines and Irvine 2003). These *O*-fucose glycans modulate protein–protein interactions and their resultant functional roles in regulating Notch signaling (Haines and Irvine 2003; Luther and Haltiwanger 2009; Stanley and Okajima 2010). The synthesis of the *O*-Fuc glycan is initiated by *O*-fucosyltransferase (OFUT) catalyzing the O-linked fucosylation of serine or threonine residues. Knockdown of *Drosophila* OFUT1 by RNA interference (RNAi) leads to defects in Notch signaling, indicating the importance of *O*-Fuc or the *O*-Fuc glycan in this process (Okajima and Irvine 2002). In cell culture, RNAi of OFUT1 inhibits both Delta–Notch and Serrate–Notch binding, whereas OFUT1 overexpression increases *Serrate*–Notch binding but inhibits Delta–Notch binding (Okajima et al. 2003). Deletion of OFUT1 in *Drosophila* prompts a severe Notch-like phenotype, exemplified by an overabundance of neurons due to failure of Notch-dependent lateral inhibition (Sasamura et al. 2003). Elimination of OFUT1 in mice causes the embryos to die in midgestation with defects in neurogenesis, somitogenesis, vasculogenesis, and cardiogenesis. The knockout mice present similar phenotypes as other mutants of Notch signaling molecules (Shi and Stanley 2003), suggesting that *O*-Fuc modification is conserved in various animal species. Interestingly, in addition to its role in glycosylation, OFUT 1 has been reported to have a distinct function as a molecular chaperone of Notch molecules (Okajima et al. 2005). *O*-Fuc residues are further modified by a series of glycosyltransferases, including β1-3*N*-acetylglucosaminyltransferase, β1-4galactosyltransferase, and α2-3sialyltransferase. *O*-Fuc glycan (SAα2-3Galβ1-4GlcNAcβ1-3Fuc-Ser/Thr) is synthesized by sequential addition of sugar residues,

depending on the activities of these enzymes (Moloney et al. 2000). Intriguingly, *Fringe* protein, a promoter of *Delta* and an inhibitor of *Serrate*, has *N*-acetylglucosaminyl (GlcNAc) transferase activity and is required for modulation of Notch signaling (Bruckner et al. 2000; Moloney et al. 2000). Because the elongated *O*-Fuc glycans by *Fringe* leads to a higher affinity for Notch to *Delta* than to *Serrate*, the promoter activities of *Fringe* for *Delta* and inhibitor activities for *Serrate* are presumed to be modulated by the elongated O-Fuc glycans on Notch (Okajima et al. 2003). Recently it was reported that *Lfng*, which is distinctly expressed in the VZ, enhances the self-renewal of NSCs in the developing mouse brain (Kato et al. 2010). *Lfng* was also reported to be associated with neurogenesis in the chick spinal cord (Skaggs et al. 2011). These studies clearly indicate the importance of carbohydrate chains in the regulation of stem cell self-renewal and differentiation via Notch signaling.

9.4.2 Neuroepithelial Cells, Radial Glial Cells, and Intermediate Progenitor Cells

NECs

In the brain, neurons and glia originate from NSCs derived from the neuroectoderm. These cells have many epithelial cell characteristics and are known as NECs. Around E8, NECs undergo rapid proliferation by symmetric division to expand the progenitor pools (Smart 1973). From E9 to E10, the anterior portion of the neural tube, which later becomes the telencephalon, closes to form the lateral ventricle. Proliferative NECs are layered at the lateral ventricles as a pseudostratified neuroepithelium with epithelial apicobasal polarity. Tight junctions and adherent junctions are present at the most lateral end of the lateral plasma membrane. At the pial surface, NSCs make contact with the basal lamina (Aaku-Saraste et al. 1996; Gruss-Porta et al. 2001; Lui et al. 2011; Smart 1973).

RGCs

NECs begin to transform into RGCs at E9.5. NECs lose some of their epithelial properties in favor of certain glial characteristics, but retain contacts with the ventricular and pial surfaces that give them their radial morphology. NEC-to-RGC transition is characterized by the loss of tight junctions, acquisition of glycogen storage granules, and the expression of astroglial genes, such as brain lipid-binding protein (BLBP) or fatty acid-binding protein 7 (FABP7), astrocyte-specific glutamate transporter (GLAST), and tenascin-C. RGCs still retain many NEC characteristics, such as adherent junctions, apical surface at ventricles, basal lamina contact, and expression of nestin, an NSC selective marker. During this period of development, the two cell types, NECs and RGCs, coexist. Although it was believed that

mitotic cells in the VZ were the progenitors that generate neurons, astrocytes, and oligodendrocytes, more recent investigations have provided evidence that RGCs are the progenitors of most neurons, astrocytes, and oligodendrocytes in the CNS. The primary role of NECs is to expand the progenitor pool before transitioning to RGCs (Aaku-Saraste et al. 1996; Bruckner and Biesold 1981; Franco and Muller 2013; Hartfuss et al. 2001).

IPCs

Before their transformation to RGCs, only a small population of postmitotic neurons are generated directly from NECs. An RGC tends to divide asymmetrically and generates a RGC and a non-RGC daughter cell (Noctor et al. 2002). Only about 10 % of asymmetrically dividing RGCs are directly transformed into neurons (Attardo et al. 2008). Most RGCs divide into RGCs and IPCs. Unlike NEC and RGC, an IPC can undergo symmetric terminal division into two neurons. To generate more IPCs, certain IPCs can also undergo a limited number of additional symmetric divisions to paired IPCs (Noctor et al. 2004). The majority of RGCs can produce only neuronal or glial precursor cells (Malatesta et al. 2003). Occasionally, but rarely, RGCs host multipotent progenitor cells that generate both neurons and glia. The glial-specific progenitors typically generate either astrocytes or oligodendrocytes, but not both in vivo (McCarthy et al. 2001).

SSEA-1

SSEA-1 is expressed on NECs at early stages of development and the expression remains by E19 in the VZ and SVZ, where the NSC populations reside (Capela and Temple 2006; Hennen et al. 2011; Mai et al. 1998). This suggests a functional role for SSEA-1 in sustaining stem and progenitor cell growth. SSEA-1 can bind and regulate fibroblast growth factor 2 (FGF-2), which is known as a mitogen that maintains the stemness of NSCs (Dvorak et al. 1998; Jirmanova et al. 1999). In addition, the SSEA-1 epitope is also associated with chondroitin sulfate proteoglycan (CSPG) (Kabos et al. 2004), β1 integrin, glycolipids (Yanagisawa et al. 2005), lysosome-associated membrane protein 1 (LAMP-1) (Yagi et al. 2010a), extracellular matrix protein tenascin-C (Hanjan et al. 1982), phosphacan (Hanjan et al. 1982; Tole et al. 1995), and Wnt-1 (Capela and Temple 2006). Strong SSEA-1 expression can be observed during embryonic development on NSCs in neurogenic regions, such as the hippocampal primordium and the embryonic cerebral cortex; its expression remains clearly visible until E19 (Hennen et al. 2011; Mai et al. 1998). SSEA-1+ cells typically have bipolar morphology, radial orientation, and glial processes, and they resemble a subtype of RGCs (E12-E14) (Mai et al. 1998; Mo et al. 2007). In vitro experiments revealed that blockage of SSEA-1 by anti-SSEA-1 antibody inhibits cell migration from neurospheres, but does not affect cellular proliferation (von Holst et al. 2006; Yanagisawa et al. 2005). Recently, knockdown of FUT9

(a key enzyme for the biosynthesis of SSEA-1) in mouse NSCs was shown to downregulate Musashi-1 expression and NSC proliferation (Yagi et al. 2012). Musashi-1 plays a crucial role in maintaining the undifferentiated state of NSCs via activation of the Notch signaling pathway (Imai et al. 2001; Okano et al. 2005). SSEA-1 may regulate proliferation of NSCs via modulation of the expression of Musashi-1 (Yagi et al. 2012).

Prominin-1

Prominin-1, also known as CD133 or AC133 (the human homologue), is a pentaspan membrane glycoprotein originally identified as an antigen expressed on the apical surface of mouse NECs at E8.5 (Marzesco et al. 2005; Shmelkov et al. 2005; Weigmann et al. 1997). Prominin-1 is specifically associated with plasma membrane protrusions that have a microvilli-like structure on the apical surface of NECs (Weigmann et al. 1997). During development at E10.5–12.5, the apical plasma membrane protrusions containing prominin-1 are released into the lumen of the neural tube as a novel class of extracellular membrane particles (Marzesco et al. 2005). After E12.5, the release of prominin-1-containing extracellular particles is decreased (Marzesco et al. 2005; Yanagisawa et al. 2004a). At the same time, NEC proliferation decreases and NECs transit into RGCs. Prominin interacts with cholesterol and gangliosides in the plasma membrane to modulate the membrane microdomains (lipid rafts) at the membrane protrusions (Huttner and Zimmerberg 2001; Janich and Corbeil 2007; Roper et al. 2000). Analysis of mice deficient in prominin-1 revealed progressive degeneration of mature photoreceptors with complete loss of vision, but no other obvious abnormalities in brain development (Zacchigna et al. 2009). In prominin-1-deficient mice, upregulation of prominin-2, which is structurally related to prominin-1, was detected, and it seems that prominin-2 compensates for the loss of prominin-1.

Gangliosides

Expression of GD3 ganglioside (CD60a) in neural tubes early in development was detected using the GD3-specific monoclonal antibody (MAb) R24 (Rosner et al. 1992). Upon closer examination it was found to be expressed in NECs in neural tubes, in RGCs in the VZ of embryos, and in the SVZ of postnatal and adult rodents (Bannerman et al. 1996; Cammer and Zhang 1996a, b; Goldman et al. 1984; Nakatani et al. 2010). $GD3^+$ cells are also co-localized with SSEA-1 in the SVZ of mouse brains (Nakatani et al. 2010). In mouse neurosphere cultures, GD3 is the predominant ganglioside species (Nakatani et al. 2010; Yanagisawa et al. 2004b), accounting for more than 80 % of the total gangliosides. For this reason, it has been proposed that it can serve as a biomarker for mouse NSCs (Nakatani et al. 2010).

Heparin Sulfate Proteoglycans and Chondroitin Sulfate Proteoglycans

Proteoglycans, the major components of extracellular matrices (ECM), are a class of glycosylated proteins possessing covalently linked GAGs, sulfated carbohydrate chains made of repeating disaccharides. Proteoglycans are categorized into a number of subclasses, based on the components of disaccharides. For example, proteoglycans containing heparan sulfate GAGs are classified as heparin sulfate proteoglycans (HSPGs), whereas proteoglycans containing chondroitin sulfate GAGs are classified as CSPGs. Both HSPGs and CSPGs are known to be expressed in NSCs. (See Chap. 5 for more details about HSPGs and CSPGs.)

9.5 Neurogenesis

Neurons and astrocytes are generated in the CNS by a defined temporal sequence. At early developmental stages, a preplate consisting of the earliest-born neurons and possibly other cell types are formed between the VZ and meninges at the brain surface. The VZ is a densely packed cell layer formed by morphologically homogeneous RGCs, and the SVZ is a second proliferative layer. Newly generated neurons migrate radially out of the proliferative zones and form a new laminar structure. This preplate is subsequently split into the marginal zone and subplate by waves of migrating neurons. The neurons in the lower layers VI and V are born first, followed by those in layers IV, III, and II in the cortex. During development, the VZ becomes smaller, and after neurogenesis is completed, the VZ is replaced by an ependymal cell layer. Postnatally, most of the SVZ disappears except along the lateral wall of the lateral ventricles, where it is considered an NSC niche in the adult state (Franco and Muller 2013; Pinto and Gotz 2007; Qian et al. 2000).

9.5.1 Polysialic Acid–Neural Cell Adhesion Molecule

The polysialic acid (PSA) carbohydrate structure (Finne et al. 1983), carried exclusively by the neural cell adhesion molecule (NCAM), is expressed in neuronal precursor cells (nIPCs, INPs). PSA is a linear homopolymer containing up to 200 α2–8-linked sialic acid residues (SAα2-8SAα2-). Polysialyltransferases, ST8SiaII (also known as STX) and ST8SiaIV (also known as PST), are the responsible enzymes catalyzing the synthesis of PSA (Angata and Fukuda 2003; Kleene and Schachner 2004; Rutishauser and Landmesser 1996). PSA has interesting properties, including its highly negative charges, a high level of hydration, and an excellent ability to bind cations. Its remarkable structure enables PSA-NCAM to regulate myelination, axon guidance, synapse formation, and

functional plasticity of the nervous system (Angata and Fukuda 2003; Aubert et al. 1995; Charles et al. 2000; Kleene and Schachner 2004; Seki and Rutishauser 1998). PSA-NCAM is prominently expressed during neural development; enzymatic deletion of PSA represses cell migration and induces premature neuronal differentiation as seen in the sprouting of axons, outgrowth of dendrites and axons, and dendritic branching (Durbec et al. 2001; Petridis et al. 2004; Yamamoto et al. 2000). Polysialyltransferase-deficient mice show developmental and behavioral defects, such as reduction of long-term potentiation and long-term depression, misguidance of mossy fibers, and ectopic synapse formation in the hippocampus (Angata et al. 2004; Eckhardt et al. 2000). In mouse NSC overexpressing PSA, cell migration is enhanced and oligodendrocyte genesis is suppressed (Franceschini et al. 2004). Thus, it is considered that the chemical structure of PSA-NCAM may modify cell fate.

9.5.2 9-O-Acetyl GD3

Ganglioside 9-O-acetyl GD3 (CD60b) was detected in neuroblasts during neural development using the JONES antibody (Blum and Barnstable 1987; Mendez-Otero et al. 1988). 9-O-acetyl GD3 is expressed in the SVZ and along the rostral migration stream (RMS) in both embryonic and adult brains (Mendez-Otero and Cavalcante 1996). Most of migrating neuroblasts expressing 9-O-acetyl GD3 also express PSA-NCAM (Miyakoshi et al. 2012). A more recent study casts some doubt on the importance of 9-O-acetyl GD3 in these studies. GD3 synthase knockout mice, in which GD3 and its downstream products, including 9-O-acetyl GD3, are missing, appear "grossly" normal in development (Yang et al. 2007). This raises the intriguing question whether the 9-O-acetyl sialic acid residue is conjugated with a protein and it functions in a similar manner as 9-O-acetyl GD3.

9.5.3 Gangliosides

During neuronal differentiation, the concentration of GD3, which is the predominant ganglioside in NSCs, is rapidly decreased. Concomitantly, the levels of GM1, GD1a, GD1b, and GQ1b continuously increase in young animals, reaching a plateau during adulthood (Hirschberg et al. 1996; Nakatani et al. 2010; Ngamukote et al. 2007). This pattern change follows closely with the upregulation of *N*-acetylgalactosaminyltransferase (GalNAcT) expression (Ngamukote et al. 2007). The dramatic changes in the expression profile of gangliosides during neuronal cell differentiation clearly reflect the biological needs at the particular stages during brain development (Fig. 9.3).

9.6 Gliogenesis

9.6.1 Oligodendrogenesis

Oligodendrocytes, the chief myelin-forming cells in the CNS, are derived from RGCs. The myelin structures provide efficient axon insulation and facilitate conduction of nerve impulses. At E12.5, the earliest oligodendrocyte progenitor cells (OPCs) are located in the developing cerebral cortex. The number of OPCs in the cortex increases between E16 and birth. However, most of the early generated oligodendrocytes disappear after birth. This suggests that most of the oligodendrocytes present in the adult cortex are generated at a later stage (Kriegstein and Alvarez-Buylla 2009; Rowitch and Kriegstein 2010). Many glial cell biomarkers are glycoconjugates and are described below.

A2B5

The first ganglioside antigen expressed in cells of glial lineage is the A2B5 antigen. A2B5 is a monoclonal antibody originally developed by Eisenbarth et al. using embryonic retina cells as the immunogen (Eisenbarth et al. 1979). The antigens recognized by the A2B5 monoclonal antibody have been established as the c-series gangliosides, including GQ1c, GT1c, and GT3 (Kasai and Yu 1983; Saito et al. 2001). These c-series gangliosides are abundant in fish brains and in mammalian embryonic, but not adult brains (Ando and Yu 1979; Freischutz et al. 1994, 1995; Rosner et al. 1988; Yu and Ando 1980). During development, the expression of c-series gangliosides is diminished in favor of the a- and b-series gangliosides, and the rate-limiting enzyme appears to be ST-III (Freischutz et al. 1994). Glial-restricted precursors (GRPs) have been recognized by the expression of A2B5 (Rao and Mayer-Proschel 1997). It is uncertain, however, whether GRPs exist in vivo.

NG2

Nerve/glial antigen 2 (NG2)/CSPG4 is one of the important CSPGs and was originally identified in rat (Stallcup 1981). The mouse homologue is also known as AG2. NG2 is a CSPG highly expressed in embryonic and adult brains (Jones et al. 2002). NG2+ cells are considered to be committed OPCs in developing brain. O-2A progenitor cells, glial precursor cells capable of differentiating into oligodendrocytes and Type 2 astrocytes, are positive for NG2 (Levine and Stallcup 1987; Raff et al. 1983b). O-2A progenitors exist in the ventricular germinal zones of the embryonic CNS and proliferate, migrate, and disseminate throughout the developing CNS (Richardson et al. 2011). Although the number of O-2A progenitors is decreased after birth, they are still found albeit in smaller numbers in the adult nervous system. O-2A progenitors are uniformly distributed throughout the CNS and are associated

with axons where they generate myelinating oligodendrocytes (Dawson et al. 2003; Ffrench-Constant and Raff 1986a, b). Since O-2A cells exclusively generate oligodendrocytes during normal development, the term O-2A has been replaced as oligodendrocyte precursor (OLP) or OPCs. It has been reported that $NG2^+$ cells in postnatal mouse brain exhibit characteristics of NSCs, such as multipotency to differentiate into oligodendrocytes and astrocytes as well as neurons; this claim, however, has not been confirmed. More recently, Cre-lox fate mapping experiments revealed that embryonic $NG2^+$ cells generate mainly oligodendrocytes and some astrocytes, but not neurons, in the ventral zone (Zhu et al. 2011). None of the cells express either astrocyte or oligodendrocyte lineage markers, suggesting that at least two distinct types (either oligodendrocyte precursors or astrocyte precursors) of $NG2^+$ cells exist in the embryonic CNS. On the other hand, postnatal $NG2^+$ cells generate only oligodendrocytes in vivo (Zhu et al. 2011).

With respect to its functional roles, NG2 has been shown to have a high affinity for FGF-2 and platelet-derived growth factor-AA (PDGF-AA); both are important mitogens for OPCs (Goretzki et al. 1999). The high affinity between NG2 and growth factors is similar to that of HSPGs, which possess a strong affinity for FGF2. NG2 is required for the responsiveness of PDGFα-receptor to PDGF-induced cell proliferation or migration. Interestingly, NG2 knockout mice do not exhibit an obvious mutant phenotype during CNS development (Grako et al. 1999; Thallmair et al. 2006). However, the observation that mice deficient in the Olig2 basic helix–loop–helix (bHLH) transcription factor exhibit severe defects in $NG2^+$ cells in the developing CNS (Ligon et al. 2006) indicates that development of $NG2^+$ cells requires Olig transcription factors, especially Olig2.

O4 and O1

As oligodendrocyte development proceeds, unique GSLs appear on the oligodendrocyte plasma membrane and myelin. These GSLs include the O4 (sulfatide; HSO_3-3Galβ1-1′Cer) and O1 antigens (galactosylceramide; GalCer; Galβ1-1′Cer), which also have been used as specific markers to define immature and mature oligodendrocytes, respectively (Zhang 2001). The O1 and O4 antigens play important roles as modulators of oligodendrocyte development and function as well as major components of the myelin sheath to facilitate nerve conduction. (Please see Chap. 12.) A series of studies have clearly shown that knockout mice deficient in GalCer synthase or sulfatide synthase present severe neurological deficits, such as tremor, progressive ataxia, and reduction of nerve conduction velocity (Bosio et al. 1996; Coetzee et al. 1996; Honke et al. 2002). In these knockout mice, morphologically normal-appearing compact myelin is preserved, but paranodal loops are absent from the axon, and paranodal junctions are abnormal (Honke et al. 2002). The number of oligodendrocytes is increased in sulfatide knockout mice, indicating that the O4 antigen, sulfatide, is a critical molecule for the negative regulation of terminal differentiation of oligodendrocytes (Hirahara et al. 2004). GalCer expression

factor-1, a rat homologue of hepatocyte growth factor-regulated tyrosine kinase substrate, has been cloned as an inducer of O1 antigen expression (Ogura et al. 1998). Overexpression of this molecule causes suppression of cell proliferation, causing dramatic change in morphology to become fibroblast-like in appearance (Ogura and Tai 2002). Although GalCer expression factor-1 may regulate the expression of O1 and O4 antigens during glial development, the function of GalCer expression factor-1 in NSC and glial precursor cells remains to be investigated.

9.6.2 Astrogliogenesis

The cell bodies of RGCs remain in the VZ throughout the period of neurogenesis and neuronal migration. At the end of cortical development, most RGCs lose their ventricular attachment and migrate toward the cortical plate by a process of somal translocation. Most RGCs transform into astrocytes. Some astrocytes may divide locally before terminal differentiation as a population of astrocyte IPCs is present in embryonic and postnatal stages (Hajos et al. 1981; Ichikawa et al. 1983). On the day of birth [postnatal day (P) 0], most astrocyte precursors are found in the inner half of the cortical width. On P4, the majority of astrocyte precursors are distributed in the outer half of the cortical width. The pattern of gliogenesis in the early postnatal rat thus shows an inside-out tendency, in analogy to neurogenesis (Kriegstein and Alvarez-Buylla 2009; Rowitch and Kriegstein 2010).

gp130

The cell surface glycoprotein gp130, also known as CD130, is a receptor component and signal transducer of interleukin (IL)-6 (Taga et al. 1989). This molecule mediates signaling activated by all of the eight members of the IL-6 family of cytokines: IL-6, IL-11, leukemia inhibitory factor (LIF), ciliary neurotrophic factor (CNTF), oncostatin M, cardiotrophin-1, cardiotrophin-like cytokines/novel neurotrophin-1/B-cell stimulating factor 3, and neuropoietin. The signaling pathways that are activated by the IL-6 family of cytokines via gp130 include the following: the Janus kinase (JAK)-signal transducer and activator of transcription 3 (STAT3) pathway, the Ras–MAPK pathway, and the phosphatidylinositol 3 kinase–Akt pathway (Fukuda and Taga 2005). The IL-6 family of cytokines induces astrocyte differentiation of NSCs via activation of gp130 and the JAK–STAT pathway (Bonni et al. 1997). Thus, gp130 is involved primarily in the induction of astrocytic differentiation. Cardiotrophin-1 is proposed to be a bona fide inducer of astrocytic differentiation via the gp130 pathway in the developing brain (Barondes et al. 1994). Deletion of gp130 results in reduction of the number of astrocytes in the developing mouse brain (Nakashima et al. 1999a). Astrocytic differentiation, however, is not regulated only by the IL-6 family of cytokines, gp130, and downstream JAK–STAT

pathway signaling molecules. For instance, positive and negative cross talk between gp130 signaling and that of bone morphogenetic proteins (BMP) (Nakashima et al. 1999b), or Notch-hairy-enhancer of split (HES) signaling (Kamakura et al. 2004), as well as with neurogenin-2 (a bHLH transcription factor) (Sun et al. 2001), has been identified. Also, the epigenetic status of astrocytic genes in NSCs is critical for astrocyte differentiation via the gp130 pathway (Takizawa et al. 2001). In addition, gp130 is involved in maintenance of the proliferation of NSCs. CNTF maintains embryonic and adult NSCs in an undifferentiated state by blocking differentiation via gp130 signaling in cultured NSCs (Shimazaki et al. 2001). Conversely, CNTF lacking a secretory signal sequence is localized in the cytosol. Therefore, CNTF is not considered a secreted cytokine during brain development. Another member of the IL-6 family of cytokines, neuropoietins, was postulated to share the biological functions of CNTF (Derouet et al. 2004). Thus, NSC proliferation may be maintained by more than one IL-6 cytokine. Gp130 signaling has also been reported to support NSC survival via activation of the phosphatidylinositol 3 kinase–Akt pathway (Chang et al. 2004). Recently, unglycosylated gp130 present on the outer surface of the plasma membrane was found to be unable to form a heterodimer with the LIF receptor resulting in failure for signaling, because the unglycosylated gp130 could not be phosphorylated in response to LIF stimulation (Yanagisawa and Yu 2009). The above examples clearly show the N-glycans of gp130 are crucial for its activation, but not its cellular localization.

PtdGlc

A phosphoglycerolipid, phosphatidylglucoside (PtdGlc), is expressed in astrocytes and radial glia in rat embryonic brain (Nagatsuka et al. 2001). PtdGlc is localized in the lipid rafts, which are thought to operate as sorting platforms that bring together molecules for efficient cross talk that controls cellular signaling cascades, including those regulating cell proliferation and differentiation (Nagatsuka et al. 2003). A PtdGlc monoclonal antibody DIM21 has been developed that labels RGCs at E12.5-E14.5, astrocytes in late embryo to early postnatal stages, and RGC-like cells in the adult SVZ (Kinoshita et al. 2009a, b; Nagatsuka et al. 2006). In an in vitro study, the association of EGF receptors and PtdGlc-enriched lipid rafts was confirmed in NSCs, and PtdGlc-enriched lipid rafts were found to control NSC to astrocyte differentiation through EGF signaling (Kinoshita et al. 2009a). (See also Chap. 4.)

Gangliosides

GM3 and GD3 represent about 70 % and 10–20 %, respectively, of the total gangliosides in astrocytes bulk-isolated from neonatal rat brains (Asou et al. 1989; Murakami et al. 1999; Sbaschnig-Agler et al. 1988). Both Type 1 and Type 2 astrocytes express GM3. On the other hand, a recent study indicated that GD3 is

expressed only in Type 2, but not in Type 1 astrocytes (Murakami et al. 1999). Type 2 astrocytes are known to express c-series gangliosides, which are recognized by mAb A2B5. The expression of GD3 and c-series gangliosides ($A2B5^+$) in Type 2 astrocytes indicates that these cells might have more similar properties as progenitor or immature cells than Type 1 astrocytes. mAb A2B5 has been recognized to identify GRPs (Rao and Mayer-Proschel 1997). On the other hand, Type 1 astrocytes are $GFAP^+/A2B5^-$, whereas Type 2 astrocytes express both GFAP and A2B5 (Raff et al. 1983a). For other cells of glial lineage, OPCs have been identified by $NG2^+$/PDGFR-α^+ (Goretzki et al. 1999). In addition, immature oligodendrocytes express NG2 and can be identified by the phenotypic marker O4 (sulfatide), whereas mature oligodendrocytes express the O1 antigen (GalCer) (Fig. 9.4).

9.7 Adult NSCs and Niche

Although neurogenesis is mostly complete at the time of the development in most mammals, it continues to occur at a much slower pace and in a limited manner throughout the entire adult life. In the adult brain of mammals, neurogenesis persists primarily in two germinal zones, the SVZ of the lateral ventricles (Doetsch et al. 1997, 1999) and the subgranular zone (SGZ) in the dentate gyrus of the hippocampus (Seri et al. 2001; Suhonen et al. 1996).

9.7.1 SVZ

In the adult SVZ, four distinct cell types are present (Fig. 9.8). Type B cells are RGC-like cells and have been considered as NSCs. Type B NSCs are slow dividing (duration of cell cycle >15 days) and express GFAP. Type C cells are transient amplifying cells that are rapidly proliferating (duration of cell cycle about 13 h) and express the transcription factor Mash1. Type A cells are neuronal precursors that have already committed to differentiate into neurons, and these cells express PSA-NCAM on the cell surface (duration of cell cycle about 13 h) (Morshead 2004). Ependymal cells are lined on the wall of the ventricle and have multi-motile cilia, which are important for controlling the flow of cerebrospinal fluid (CSF). Multipotency of the ependymal cells has been reported (Johansson et al. 1999), although this is not settled (Chiasson et al. 1999; Doetsch et al. 1999; Laywell et al. 2000). Recently, ependymal cells were shown to be the most quiescent type of NSCs whose cell cycle is strictly regulated and reinitiated under specific circumstances. In certain restricted situations, a subpopulation of ependymal cells may develop into neurons, and these cells are considered as NSCs (Carlen et al. 2009; Coskun et al. 2008).

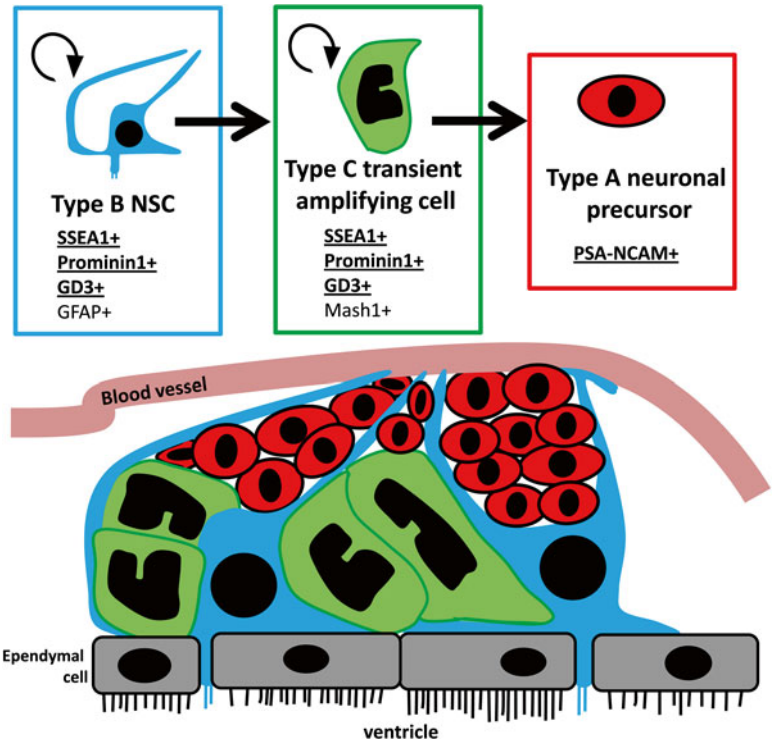

Fig. 9.8 Neural stem cell niche at the subventricular zone (SVZ) on the surface of the lateral ventricle in the adult mouse brain. Type B cells are radial glial cell (RGC)-like cells and have been considered as neural stem cells (NSCs). Type C cells are transient amplifying cells that are rapidly proliferating. Type A cells are neuronal precursors that have already committed to becoming neurons expressing PSA-NCAM on the cell surface. The known carbohydrate markers are *underlined*

9.7.2 SGZ

Five types of cells have been described in the SGZ (Filippov et al. 2003). Type 1 cells are considered quiescent neural progenitors that are RGC-like cells and largely equivalent to Type B NSCs in the SVZ. Type 2 cells express nestin, and this cell type has been classified into two cell populations: Type 2a cells are amplifying neural progenitors that are similar to Type C transient amplifying cells in the SVZ; Type 2b and 3 cells are neuroblasts that express PSA-NCAM (Encinas et al. 2006; Steiner et al. 2006). The other type of cells is mature granule neurons. Recently, it has been reported that Mash1^{+} cells do not amplify and are therefore not Type 2a amplifying neural progenitors that can directly differentiate into early neuroblasts without mitosis (Lugert et al. 2012).

9.7.3 Glycoconjugates in Adult NSCs

Ganglioside GD3, SSEA-1, and prominin-1 are expressed in Type B NSCs in the SVZ and in Type 1 quiescent neural progenitors in the SGZ of the adult brain (Beckervordersandforth et al. 2010; Cammer and Zhang 1996a; Capela and Temple 2002; Nakatani et al. 2010; Walker et al. 2013). The intensity of prominin-1 expression in the SGZ is heterogeneous. Cells that do not express prominin-1 are not NSCs, but cells with intermediary or low levels of prominin-1 expression possess NSC properties. Analysis of cells deficient in prominin-1 indicates that there is no difference in the number of astrocytes, oligodendrocytes, neural precursor cells, or adult-born early postmitotic neurons, nor is there any difference in the ability for neurosphere formation.

9.7.4 Lectins

Lectins are carbohydrate-binding proteins that do not act enzymatically on their corresponding ligands. They are found in all kinds of organisms, including plants, microorganisms, animals, and humans. Each lectin specifically recognizes a monosaccharide or oligosaccharide structure and binds to glycoconjugates present in insoluble or soluble form (Sharon 2008; Sharon and Lis 1972). As neural and glial cells express various glycoconjugates, specific lectins can be used effectively for histochemical identification or sorting of specific cell types from heterogeneous NSC populations (Yanagisawa and Yu 2007). For example, the low expression of peanut agglutinin (PNA) ligand and the heat stable antigen (HSA, CD24a) in adult NSCs permit them to be effectively separated by negative selection (Rietze et al. 2001). PNA binds to the Galβ1-3GalNAc structure that is part of the ganglio-series ganglioside structure. For this reason, PNA is useful in recognizing those gangliosides. Another lectin, *Phaseolus vulgaris* erythroagglutinating lectin (PHA-E4), which binds to biantennary complex type N-glycans, has been used to isolate embryonic and adult NSCs by positive selection (Hamanoue et al. 2009), while *Ricinus communis* agglutinin (RCA), which binds to Galβ1-4GlcNAc-, has been used to detect Type A neuronal precursors (Kitada et al. 2011). Other lectins, such as *Agaricus bisporus* agglutinin (ABA) that shows specificity for Galβ1-3GalNAcα1, and PHA-E4 and wheat germ agglutinin (WGA) that show specificity for GlcNAc1-4GlcNAc recognize Type B NSCs and Type C transient amplifying cells in the SVZ as well as Type 1 quiescent neural progenitors and Type 2a amplifying neural progenitors in the SGZ (Kitada et al. 2011). These lectins are useful for the identification and purification of specific populations of NSCs.

9.8 Neural Crest Cells

The precursors of neural crest cells reside in the dorsal neural tube, and these cells undergo epithelial to mesenchymal transition (EMT) and detach from the neural tube and migrate during development to diverse locations (Fig. 9.6) (Anderson 1997; Sauka-Spengler and Bronner-Fraser 2008). Neural crest cells contain a population of neural crest stem cells (Bronner-Fraser and Fraser 1988; Morrison et al. 1999; Stemple and Anderson 1992). Neural crest stem cells are capable of self-renewal and have the multipotency to differentiate into Schwann cells upon induction with glial growth factor (Shah et al. 1994), autonomic neurons by induction with BMP (Shah et al. 1996), and smooth muscles by induction with transforming growth factor-β (Shah et al. 1996).

9.8.1 HNK-1

The human natural killer-1 (HNK-1) antigen (CD57) is a carbohydrate antigen whose structure has been established as HSO3-3GlcAβ1-3Galβ1-4GlcNAc- (Ariga et al. 1987; Chou et al. 1986). HNK-1 is distributed on the surface of neural crest cells and is required for their proper migration during development in avian, rat, and human (Bronner-Fraser 1987; Holley and Yu 1987; Nagase et al. 2003; Tucker et al. 1988). However, mouse neural crest cells are negative for HNK-1 expression by immunohistochemistry in fixed cryo-sections (Tucker et al. 1988). In a careful study using synthetic model compounds, the minimal carbohydrate unit for the HNK-1 epitope was shown to reside in the terminal disaccharide structure HSO3-3GlcAβ1- (Tokuda et al. 1998). A commonly used HNK-1 immunoreagent is an IgM monoclonal antibody, e.g., mouse mAb Leu 7, whose large molecular size (about 970 kDa) could sterically hinder its ability to cross-react with epitopes in fixed whole-mount tissues (Abo and Balch 1981). It is well known that fixed cells and living cells have far different staining patterns in studies using immunohistochemistry and flow cytometry. Loss of antigenicity with fixation could be caused by the conditions of immunohistochemical detection. Neural crest stem cells are isolated not only from the neural fold and neural tube but also from fetal peripheral nerve (Morrison et al. 1999) and fetal and postnatal gut (Bixby et al. 2002). Most recently Walters and colleagues found murine living neural crest stem cells do express HNK-1 (Walters et al. 2010). Less than half of murine HNK-1$^+$ cells express SRY (sex determining region Y)-box 10 (Sox10), known to be expressed in neural crest stem cells. Thus, the expression of HNK-1 alone is not sufficient to isolate a population of pure neural crest stem cells. The HNK-1 epitope is associated with a number of cell adhesion molecules (Jungalwala 1994; Kruse et al. 1984). Of particular interest is the fact that carrier molecules of the HNK-1 epitope can be a glycoprotein or a glycolipid. Among the glycoprotein antigens are L1, P0, MAG, and NCAM (Kruse et al. 1984),

while the glycolipid antigens include just the two sulfated glucuronosyl glycolipids (SGGLs), sulfated glucuronosyl paragloboside (SGPG), and sulfated glucuronosyl lactosaminyl paragloboside (SGLPG) (Ariga et al. 1987; Chou et al. 1986). Interestingly, certain proteoglycans, e.g., CSPGs (Domowicz et al. 1995; Margolis et al. 1987; Pettway et al. 1996), cross-react with the HNK-1 antibody. Because of its wide distribution on various glycoconjugates, the HNK-1 epitope is expected to have important roles in neural development. So far, studies of brains from mice deficient in glucuronyltransferase P (GlcAT-P) or HNK-1 sulfotransferase, the two key enzymes of HNK-1 antigen synthesis, have not revealed any overt defect in brain development (Yamamoto et al. 2002). However, adult mice deficient in GlcAT-P or HNK-1 sulfotransferase exhibit reduced long-term potentiation and defective spatial memory formation, suggesting a functional role of the HNK-1 antigen in synaptic plasticity of the hippocampus, but not in brain development. Recently, HNK-1 expression in mouse embryonic NPCs was confirmed, and the HNK-1 epitope was present almost exclusively on tenascin-C (Yagi et al. 2010b; Yanagisawa et al. 2005).

9.8.2 PSA-NCAM

As a marker of neuronal precursor cells, PSA-NCAM is expressed not only in the CNS but also in the PNS. Sensory and autonomic neurons of rodents express PSA-NCAM (Boisseau et al. 1991; Stemple and Anderson 1992). Also PSA-NCAM expression is seen in the development of the enteric nervous system. PSA-NCAM$^+$ precursor cells from vagal, sacral, and rostral truncal regions of the neural crest migrate to the gut, stop at appropriate locations, proliferate and differentiate into the many different phenotypes of enteric neurons, form two ganglionated plexuses, and establish correct interconnections (Epstein et al. 1991; Heuckeroth et al. 1998; Le Douarin and Teillet 1973). The expression of high PSA-NCAM is restricted to early neuronal lineage cells derived from neural crest cells (Boisseau et al. 1991). In the rat gut, polysialyltransferases PST and STX are highly expressed at E14 to E18 and then downregulated postnatally (Faure et al. 2007). Approximately 30 % of neuron-committed cells in the myenteric layer express PSA-NCAM at E12. The number of PSA-NCAM$^+$ cells in the mesenteric plexus increases to 50 % at E14 and E16 and 80 % at E18 to E20 and then declines gradually during postnatal life. About 50 % of the cells committed to neuronal differentiation in the submucosal layer are PSA-NCAM$^+$ at E18 to E20. At P14 to P24, less than 10 % of the cells express PSA-NCAM in the submucosal plexus. In the development of the enteric nervous system, BMP enhances migration, neurite fasciculation, and clustering of neuronal cells via promotion of polysialylation of NCAM in the enteric nervous system formed from neural crest cells (Faure et al. 2007; Fu et al. 2006).

9.8.3 *Other Glycoconjugates*

Other glycoconjugate markers reported to be present in neural crest stem cells and neural crest-derived cells include GD3 in mouse neural crest cells (Stainier et al. 1991); SSEA-1 in cells committed to differentiating into sensory neurons in quail (Sieber-Blum 1989); B30 gangliosides, which are unidentified gangliosides recognized by the B30 antibody (one migrates between GM2 and GM1 and the other migrates between GD3 and GD1a on thin-layer chromatography) in mouse sensory neurons (Stainier et al. 1991); and O4 antigen (sulfatide) in Schwann cells and their precursors (Dong et al. 1999; Stemple and Anderson 1992).

9.9 Future Studies

Since their discovery, progress in the biology of NSCs has been made owing to their importance in the development of the nervous system. NSCs are characterized by their capacity for self-renewal and their ability to differentiate into neurons and glia (multipotency). Remarkably, they can be isolated not only from embryonic brains (Stemple and Anderson 1992) but from adult CNS tissue as well (Reynolds and Weiss 1992). NSCs cultured using the floating culture method with EGF and FGF-2 in a defined serum-free medium form neurospheres, which consist not only of NSCs, but of rather heterogeneous undifferentiated cell populations. A more homogenous population of cells can be prepared using monolayer, serum-free culture. NSCs from neurospheres or monolayer cultures can be induced to differentiate into multiple neural lineages upon growth factor withdrawal (Reynolds and Weiss 1992). The availability of relatively pure NSCs in culture has greatly enhanced current knowledge about the molecular mechanisms underlying cell fate determination and ultimately brain development. Moreover, cell reprogramming studies have indicated that lineage-restricted neuronal and glial precursors can display acquired properties that are not evident in vivo (Gabay et al. 2003; Kondo and Raff 2000; Palmer et al. 1999). For example, treatment of cells with fetal serum or BMPs can reprogram NG2 positive cells to generate NSCs containing reprogrammed multipotential stem cells that can differentiate into neurons, astrocytes, and oligodendrocytes (Kondo and Raff 2000). During reprogramming from OPCs to NSCs, chromatin remodeling and histone modifications occurred (Kondo and Raff 2004). The ability to manipulate NSC cell fate determination in vitro has greatly facilitated understanding of the properties and regulatory mechanisms of NSCs in the developing nervous system and adult brain that would have been difficult to decipher in vivo.

Glycoconjugates, including glycolipids and glycoproteins, are predominantly expressed on the cell surface. Because of their structural diversity, they have been used effectively as cell surface biomarkers for identification and isolation of specific cell types. During neural development and neuronal/glial cell differentiation, these glycoconjugates frequently undergo dramatic qualitative and quantitative changes

that correlate with cellular changes. There is an urgent need to answer the question of whether these changes are merely consequences of differentiation needed to satisfy biological needs, such as cell–cell recognition, migration, and adhesion. More importantly, recent evidence has shed light on their roles in modulating signaling pathways during cellular differentiation and reprogramming. For example, we found that cell surface SSEA-1 modulates NSC proliferation mediated by the Notch signaling pathway and migration (Yagi et al. 2012; Yanagisawa et al. 2005). In addition, it was shown that GM3 can modulate EGF receptor function by inhibiting its tyrosine kinase activity. Most recently, we showed that GD3 associated with EGF receptor to modulate NSC proliferation (unpublished data). Additionally, GSLs have been shown to play an important role in the epithelial mesenchymal transition (EMT); changes in cell surface glycolipid expression by inhibition of glucosylceramide synthesis convert epithelial cells to a fibroblastic morphology (Guan et al. 2009). Conversely, overexpression of prominin in fibroblasts induces an epithelial cell-like phenotype with an abundance of microvilli-like protrusions (mesenchymal epithelial-like cell transition; MET) (Singh et al. 2013). These studies indicate that cell surface glycoconjugates may control cell fate in order to effect transdifferentiation of one cell type into another. Clearly this represents a fruitful area of future research.

Another critical area for future investigation is the basis of induced pluripotent stem cell (iPSC) generation. Cell surface glycoconjugates again occupy an important area for study. For example, in human fibroblasts, less than 1 % of the cells express SSEA-3 and SSEA-3^+ dermal fibroblast-enhanced iPSC generation, while no iPSCs could be generated from the SSEA-3^- fraction (Reijo Pera et al. 2009; Wakao et al. 2011). SSEA-3^+ fibroblast and bone marrow stromal cells host a multipotent stem cell population that can generate the three germ layers without Yamanaka factors, such as Oct3/4, Sox2, c-Myc, and Klf4 (Kuroda et al. 2010). It clearly shows that SSEA-3 plays a crucial role during reprogramming of fibroblasts to stem cells in maintaining stemness. Although SSEA-1, SSEA-3, SSEA-4, GD3, and prominin-1 are all expressed in stem cells, mice deficient in one of these molecules show only subtle phenotypic abnormalities compared with the wild-type animals. Clearly, the biological function of one glycoconjugate can be substituted by another, albeit with less efficiency. The "biological redundancy" phenomenon governing cellular events needs to be further defined. Future studies in this regard should contribute greatly to regenerative and reparative biology.

Conflict of Interest The authors declare no conflicts of interest.

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