Published in final edited form as:

FEBS Lett. 2010 May 3; 584(9): 1694–1699. doi:10.1016/j.febslet.2009.08.026.

Membrane Glycolipids in Stem Cells

Robert K. Yu*, Yusuke Suzuki, and Makoto Yanagisawa

Institute of Molecular Medicine and Genetics and Institute of Neuroscience, Medical College of Georgia, Augusta, Georgia, 30912, USA

Abstract

Stem cells, such as embryonic stem cells, hematopoietic stem cells, neural stem cells, mesenchymal stem cells, and very small embryonic-like stem cells, are undifferentiated cells that are endowed with a high potential for proliferation and the capacity for self-renewal with retention of pluri/multipotency to differentiate into their progenies. Recently, studies regarding the biological functions of glycolipids and cell surface microdomains (caveolae, lipid rafts, or glycolipid-enriched microdomains) in stem cells are emerging. In this review, we introduce the expression patterns of glycolipids and the functional roles of cell surface microdomains in stem cells.

1. Introduction

Glycolipids are lipid molecules linked with one or more carbohydrate units. Based on their core lipid moiety, glycolipids are classified into glycosphingolipids (containing ceramide) and glyceroglycolipids (containing glycerol). Glycosphingolipids (GSLs) containing sialic acid residue(s) are referred to as gangliosides. Glycolipids are found in virtually all vertebrate cells and body fluids and are localized primarily, but not exclusively, on the plasma membrane. One of notable characteristics of glycolipids is the structural diversity; so far, 172 neutral GSLs, 24 sulfated GSLs, and 188 gangliosides with variations in the carbohydrate chain have been reported in vertebrate tissues and organs [1]. Their complexity may increase manifold when variations in their lipophilic components are taken into consideration. The expression patterns of glycolipids are known to change drastically during development or cellular differentiation. Therefore, glycolipids have been frequently used as important developmental marker molecules.

Glycolipids have also been suggested to play important biological functions. Mouse embryos deficient in glucosylceramide synthase, which catalyzes the initial step of GSL biosynthesis, are able to differentiate into endoderm, mesoderm, and ectoderm but are unable to form more differentiated tissues and die during midgastrulation by apoptosis in the

*Correspondence to: Robert K. Yu, Ph.D., Med.Sc.D., Institute of Molecular Medicine and Genetics and Institute of Neuroscience, Medical College of Georgia, 1120 15th Street, Augusta, GA 30912, USA; Tel, +1-706-721-0699; Fax, +1-706-721-8727; ryu@mcg.edu.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

ectoderm [2]. Although neural cell-specific disruption of glucosylceramide synthase does not impair late embryonic development, all glucosylceramide synthase-deficient mice die within 3 weeks after birth by dysfunction of cerebellum and peripheral nerves, associated with structural defects. This strongly indicates that GSLs are required for brain maturation after birth [3]. Mice deficient in ganglioside synthases are basically viable but exhibit multiple defects such as a lethal sound-induced seizure in GD3 synthase-/GM2 synthase-double knockout mice and a hearing loss in GM3 synthase-knockout mice [4, 5] (for further details and discussions of the mice deficient in ganglioside synthases, see [6]). Also in humans, a nonsense mutation of GM3 synthase causes autosomal recessive infantile-onset symptomatic epilepsy syndrome [7]. These studies revealed that mutation of GSL-synthesis enzymes leading to absence of specific GSL structures and accumulation of certain precursor GSLs. The consequences are manifested with developmental abnormalities, leading to certain clinical consequences and death. They further reinforce the concept that plasma membrane glycolipids are crucial in developmental events by playing important biological functions, such as modulation of cell signaling and cell-cell interaction. On the plasma membrane, glycolipids are believed to cluster with other membrane lipids, such as cholesterol and sphingomyelin, to form specialized microdomains termed caveolae, lipid rafts, or glycolipid-enriched microdomains [8–11]. In these microdomains, signaling and cell-adhesion molecules are localized, implicating that these domains may form platforms for signal transduction and cell adhesion.

Although they are still few, studies regarding the biological functions of glycolipids and cell surface microdomains in stem cells are emerging. A stem cell is defined as an undifferentiated cell endowed with a high potential for proliferation and the capacity for self-renewal with retention of pluripotency or multipotency to differentiate into their progenies. Stem cells represent cellular reservoirs for formation of tissues and organs during development and for replacement of cells lost during normal cellular turnover in adulthood. Stem cells are roughly classified into two types: embryonic stem cells and somatic stem cells. An embryonic stem cell is derived from epiblasts in the inner cell mass of blastocysts and has a pluripotency to generate all cells in three germ layers, endoderm, mesoderm and ectoderm. Somatic stem cells are multipotent cells which can restrictively differentiate into a related group of cells. As the representative somatic stem cells, hematopoietic stem cells, neural stem cells and mesenchymal stem cells are well known. In addition to these stem cells, induced pluripotent stem cells (iPS cells), pluripotent stem cells artificially generated from mouse somatic cells such as fibroblasts by introducing Oct3/4, Sox2, c-Myc, and Klf4 have recently been established [12]. These stem cells have attracted a great interest lately, owing to their potential in unlocking specific cellular events leading to differentiation, proliferation, and fate determination. Furthermore, it has been hypothesized that there is a sub-population of particular cancer cells having stem cell-like characteristics such as self-renewal and differentiation capacity and continually sustaining tumorigenesis in primary tumors. These stem cell-like cancer cells are referred to as cancer stem cells. Recently, cancer stem cells have been isolated from tumors. Cancer stem cells, namely tumor-initiating cells or tumor-propagating cells, can be important targets for cancer treatment.

In the stem cells, it has been expected that glycolipids and cell surface microdomains play important roles as markers and functional molecules. In this review, we will introduce the

expression patterns of glycolipids and the functional roles of the cell surface microdomains in stem cells. The structures of the glycolipids and the glycolipid expressions in stem cells referred to in this paper are presented in Table 1 and 2.

2. Glycolipids expressed in stem cells

2.1 Glycolipids expressed in embryonic stem cells

In undifferentiated E14 mouse embryonic stem cells, it has been biochemically elucidated that a-series gangliosides, GM3, GM1 and GD1a, are expressed [13]. On the other hand, it has also been reported that GM1 is exclusively expressed in J1 mouse embryonic stem cells [14]. During differentiation, the concentration of gangliosides is increased and the expression pattern of gangliosides becomes more complex, with GD3 predominating initially and a- and b-series emerging at the expense of GD3 [13]. One of the unusual differentiation-related gangliosides in E14 ES cells is GalNAc-GD1a, which was identified by fast-atom bombardment mass spectrometry [13]. With respect to neutral GSLs expressed in undifferentiated mouse embryonic stem cells, lactosylceramide, ceramide trihexoside, globoside and forssman antigen have been identified [13]. In H7 human embryonic stem cells, a flow cytometric study revealed that globo-series GSLs, stage specific embryonic antigen-3 (SSEA-3) and SSEA-4, are also expressed [15]. These antigens are down-regulated following induction of differentiation with concomitant increase in expression of the b-series gangliosides, GD3, 9-O-acetyl-GD3 and GD2, and c-series gangliosides.

SSEA-1 is a trisaccharide antigen recognized by a monoclonal antibody against embryonal carcinoma cells [16]. It is well recognized that mouse embryonic stem cells express the SSEA-1 antigen [17, 18]. It has also been reported that iPS cells are positive for SSEA-1 [12]. The SSEA-1 carbohydrate epitope is carried by glycoproteins, proteoglycans and glycolipids [19]. However, in the teratocarcinoma cell lines, glycoproteins rather than glycolipids are the major carrier molecules of SSEA-1 [20]. Interestingly, SSEA-1 is not expressed in human embryonic stem cells; SSEA-3 and SSEA-4 are expressed in these cells [21]. In contrast, SSEA-3 and SSEA-4 are not expressed in mouse embryonic stem cells.

Glycolipids expressed in embryonal carcinoma cells, stem cells in teratocarcinoma, have also been reported. In mouse P19 embryonal carcinoma cells, trace amounts of GM3 and GD3 are expressed [22, 23]. However, after neuronal differentiation induced by retinoic acid, the total ganglioside amount was elevated, and expression of GM2, GM1, GD1a, GD1b, GT1b and GQ1b was upregulated in these cells. In 2102Ep human teratocarcinoma cells, globo-series GSLs such as globotriaosylceramide, globotetraosylceramide (globoside), globopentaosylceramide (SSEA-3), fucosyl-globopentaosylceramide (SSEA-3) and sialyl-globopentaosylceramide (SSEA-3 and SSEA-4) have been characterized [24]. In addition, fucosyl-globoside and sialyl-globoside have been found in HT-E (833K) human teratocarcinoma cells [25]. It is interesting to note that globo-series GSLs are related to cellular differentiation [26].

2.2 Glycolipids expressed in neural stem cells

Neural stem cells are multipotent cells generating brain-forming cells such as neurons and glial cells; the latter include astrocytes and oligodendrocytes [27]. Neural stem cells appear

in the neuroepithelium during development and are located in the subventricular zone of the lateral ventricles and the subgranular layer of the dentate gyrus in the hippocampus in adulthood [28, 29]. There are studies reporting the expression of glycolipids in these cells [27].

In mouse and human neural stem cells, the expression of a b-series ganglioside, GD2, was detected by flow cytometric analysis [30]. By a biochemical study of the mouse neuroepithelial cells that are known to be rich in neural stem cells, it was determined that another b-series ganglioside, GD3, is the predominant gangliosides [31]. In addition, GQ1b, GT1b and Chol-1 α gangliosides (GT1 α and GQ1 α) are also expressed in the neuroepithelial cells in much lesser amount [31–33]. Interestingly, a-series gangliosides such as GM1 and GD1a are not detectable in the neuroepithelial cells, but are up-regulated after neuronal differentiation [32]. In mouse and human neural stem cells, a fucosylated carbohydrate antigen, SSEA-1 is also expressed [30, 34]. It has been found that the SSEA-1 antigen in neuroepithelial cells is carried not only by glycoproteins, but also by glycolipids [33].

2.3 Glycolipids expressed in cancer stem cells

Recently, glycolipids characterizing cancer stem cells in brain tumors have been reported. In human glioma such as glioblastoma, astrocytoma, oligodendroglioma and ependymoma, the cancer stem cells are positive for A2B5 antigens, c-series gangliosides such as GT3, GT2, GQ1c and GP1c [35, 36]. It has been confirmed by xenograft experiments that A2B5⁺ cells isolated from human glioblastoma by fluorescence-activated cell sorting (FACS) or magnetic cell sorting can form tumors in brains of nude rats or mice. These studies clearly indicate the usefulness of c-series gangliosides as marker molecules to identify brain cancer stem cells. In addition to c-series gangliosides, SSEA-1 has also been found in cancer stem cells. Tumors from Patched mutant mice, a mouse model of medulloblastoma, has been reported to highly express SSEA-1 [37, 38]. SSEA-1⁺ cells collected from the tumors by FACS gave rise to tumors in the brains of all to be positive for SSEA-1. Also, in human glioblastoma multiforme cells, SSEA-1-positive populations have been reported to be the cancer stem cells [39]. In this study, it was found that SSEA-1⁺ cells sorted from human glioblastoma multiforme by magnetic cell sorting and injected into the brains of NOD-SCID mice generated tumors more efficiently than SSEA-1[−] cells. These findings suggest that SSEA-1 is a general marker antigen of cancer stem cells in brain tumors. The carrier molecules of SSEA-1 in these tumor stem cells have not yet been characterized, but there might be a possibility that they are glycolipids.

2.4 Glycolipids expressed in hematopoietic stem cells

Hematopoietic stem cells are multipotent cells which can give rise to cells forming blood and immune systems, such as myeloids (erythrocytes, basophils, eosinophils, neutrophils, monocytes, macrophages, megakaryocytes, and platelets) and lymphoids (T-cells, B-cells, and natural killer cells). Hematopoietic stem cells originally appear in the aorta-gonad-mesonephros in embryos and then are located in the bone marrow in adults.

So far there has not been biochemical analysis of glycolipids expressed in hematopoietic stem cells, probably because they are in very low abundance in bone marrow (mouse bone marrow hematopoietic stem cells account for 0.004% of bone marrow mononuclear cells) [40]. However, in rat lymphocyte progenitor cells prepared from bone marrow by Ficoll-Paque density centrifugation, the expression of gangliosides has been quantified by biochemical analysis [41]. The contents of each ganglioside fraction are as follows: monosialogangliosides, 22.8 ± 1.8 %; disialogangliosides-1, 17.7 ± 1.6 %; disialogangliosides-2, 15.8 ± 1.3 %; trisialogangliosides, 19.3 ± 2.0 %; and tetrasialogangliosides, 24.4 ± 1.9 %. The molecular species of these gangliosides has not yet been identified. Furthermore, it has been found that cholera toxin B subunit, a molecular probe recognizing GM1, are reactive with mouse hematopoietic stem cells [42–44], which suggests the presence of GM1 in these cells. However, it is known that the cholera toxin B-subunit is reactive with not only GM1, but also to a much lesser extent with other glycolipids, such as GD1a, asialo-GM1, fucosyl-GM1, GM2, GD1b, GT1b, and GM3. The possibility that hematopoietic stem cells express these gangliosides cannot be ruled out. To identify *bona fide* gangliosides expressed in hematopoietic stem cells, systematic biochemical studies are required.

2.5 Glycolipids expressed in mesenchymal stem cells

Mesenchymal stem cells are multipotent stromal cells which can differentiate into cells of the mesodermal lineage such as myocytes, osteocytes, adipocytes and chondrocytes. These cells can be isolated from all mesenchymal tissues, especially bone marrow. It has been reported that the SSEA-4 carbohydrate epitope is expressed in adult mouse and human mesenchymal stem cells and is useful for isolating these cells from bone marrow [45]. It was found in this study that SSEA-4⁺ cells isolated from mouse and human bone marrow cells by FACS have a high proliferative ability and a multipotency to differentiate into adipocytes and osteoblasts. Furthermore, an immunocytochemical and flow cytometric analysis revealed that GD2 is specifically expressed in human mesenchymal stem cells isolated from bone marrow and adipose tissue, but not in unfractionated bone marrow cells, CD45⁺ leukocytes, CD34⁺ hematopoietic progenitor cells, CD33⁺ myeloid cells, CD3⁺ T-lymphocytes, and CD19⁺ B-lymphocytes [46]. It has been confirmed that GD2⁺ cells isolated from bone marrow using immunomagnetic beads have the characteristics of mesenchymal stem cells such as the morphology, plastic adherence ability, and multipotency to differentiate into osteoblasts, adipocytes, and chondroblasts. In addition, mesenchymal stem cells isolated from human umbilical cord blood have been reported to be positive for GD2 [47]. These findings clearly indicate the expression of GD2 in human mesenchymal stem cells and its usefulness as a marker molecule. However, it has also been reported that GM1 is expressed in human mesenchymal stem cells prepared from periodontal bone marrow [14]. Recently, it also has been reported that GM3, GM2, and GD1a are expressed in human dental pulp-derived stem cells which share molecular and cellular characteristics with bone marrow-derived mesenchymal stem cells; during neuronal differentiation, GD3 is up-regulated [48]. It still remains to be undetermined whether the predominant ganglioside expressed in human mesenchymal stem cells is GD2 or other gangliosides.

2.6 Glycolipids expressed in very small embryonic-like stem cells

Very small embryonic-like stem cells (VSELs) are adult versatile cells, which were originally found in mouse bone marrow and human umbilical cord blood [49–51]. VSELs express embryonic stem cell markers such as Oct3/4 and Nanog and have pluripotency to differentiate into cells of all three germ layers (i.e., cardiomyocytes, neurons, astrocytes, oligodendrocytes, and pancreatic cells) in vitro like embryonic stem cells [49]. The most interesting characteristic of these cells is the size; the diameter of the cell body is just 2–4 μm in mice and 3–5 μm in human. VSELs were identified not only in bone marrow, but also in organs such as brains, kidneys, pancreas and skeletal muscles in mice [51].

VSELs are found in a population of CXCR4⁺ Sca-1⁺ lineage marker[−] CD45[−] cells in adult mouse bone marrow, and a population of CXCR4⁺ CD133⁺ CD34⁺ lineage marker[−] CD45[−] cells in human umbilical cord blood [49, 50]. In addition to these markers, SSEA-1 and SSEA-4 are expressed in mouse and human VSELs, respectively. It is interesting that the expression patterns of these antigens are similar to those in mouse and human embryonic stem cells. It is not yet clear whether the carrier molecule of SSEA-1 in the mouse VSELs is a glycolipid, but the molecular characteristics are expected to be similar with those in mouse embryonic stem cells. The immunoreactivity of SSEA-1 in mouse VSELs after permeabilization with Triton X-100 which can easily wash out glycolipid antigens on the cell surface [49, 50] suggests that the SSEA-1 carrier molecules in these cells are mainly glycoproteins.

3. Cell surface microdomains in stem cells

3.1 Cell surface microdomains in hematopoietic stem cells

Long-term hematopoietic stem cells in the bone marrow niche are in a noncycling state. In this hibernation or dormancy state of hematopoietic stem cells, cell surface microdomains play an important role [42]. In CD34[−] c-Kit⁺ Sca-1⁺ lineage marker[−] hematopoietic stem cells freshly isolated from the bone marrow niche, cell surface microdomains positive for GM1 are diffusely distributed [44]. Stimulation by cytokines, stem cell factor and thrombopoietin, induces clustering of cell surface microdomains and re-entry into the cell cycle, probably via segregation and concentration of the activated cytokine receptors. The factor from the niche which inhibits cell surface microdomain clustering and induces the hibernation of hematopoietic stem cells has been identified as transforming growth factor- β [52]. These studies clearly indicate the importance of cell surface microdomain as platforms to integrate the signals for the cells to maintain in a hibernation state.

Rac GTPases are known to be essential for filamentous-actin assembly and chemotaxis of hematopoietic stem/progenitor cells. It has been reported that disruption of cell surface microdomains with methyl- β -cyclodextrin suppresses the translocation of Rac1 to cell surface microdomains and the assembly of filamentous-actin in c-Kit⁺ hematopoietic progenitor cells derived from mouse low-density bone marrow [43]. This study suggests that cell surface microdomains are also involved in chemotaxis of hematopoietic stem/progenitor cells.

It has been suggested that the number of stem cells in physiological condition is regulated by a balance between symmetric and asymmetric cell division. Cell surface microdomains have been implicated also in polarization of human CD34⁺ hematopoietic stem/progenitor cells [53].

3.2 Cell surface microdomains in neural stem cells

In mouse neuroepithelial cells, cell surface microdomains have been reported to be involved in signal transduction and cell adhesion [31]. In these cells, disruption of cell surface microdomains with methyl- β -cyclodextrin suppressed basic fibroblast growth factor-induced activation of the Ras-mitogen-activated protein kinase pathway that is essential for cellular proliferation. In addition, disruption of cell surface microdomains inhibited the cell adhesion via β 1 integrin which is localized in these microdomains. It has also been suggested that cell surface microdomains in mouse neurospheres act as integrators of the signal crosstalk by integrin, growth factors, and Notch, which are important for the cell fate regulation [54].

In mid-embryonic to early-postnatal stages of developing mouse cortex, astroglial lineage cell surface microdomains are characterized by enrichment of phosphatidylglucoside (PtdGlc), a phosphoglycerolipid containing glucose linked to phosphatidic acid [55, 56]. It has been recently suggested that the PtdGlc-rich cell surface microdomains are involved in astrocytogenesis during development [57]. Treatment of mouse neural stem cells with anti-PtdGlc antibody causes recruitment of epidermal growth factor receptor into cell surface microdomain compartments with subsequent activation of the downstream signaling pathway. Therefore, PtdGlc-rich cell surface microdomains are physiologically coupled and regulate the activation of epidermal growth factor receptor on neural stem cell during development.

4. Closing remark

In recent years, major progresses have been made in stem cell biology, including generation of iPS cells from somatic cells. Although many unresolved issues remain, such as low success rate and safety concerns, application of cell-based transplantation using stem cells is a very attractive and promising strategy for regenerative and restorative medicine. The identification of stem cell marker, especially those located on the plasma membrane, is becoming increasingly important for the isolation and classification of specific populations of stem cells. As we described above, specific glycolipids detected on each stem cell type are used for this purpose. Additionally, understanding the functional role of glycolipids during cellular differentiation and proliferation remains to be an important area of research. Such information will undoubtedly stimulate the progresses in developing stem cell-based therapeutic strategies for a variety of degenerative diseases or tissue damage.

Acknowledgments

This work was supported by USPHS grants NS11853, NS26994, AG027199 and a grant from the Childrens' Medical Research Foundation, Chicago, IL to RKY.

References

1. Yu, RK.; Yanagisawa, M.; Ariga, T. Glycosphingolipid Structures. In: Kamerling, JP., editor. Comprehensive Glycoscience. Elsevier; Oxford, UK: 2007. p. 73-122.
2. Yamashita T, Wada R, Sasaki T, Deng C, Bierfreund U, Sandhoff K, Proia RL. A vital role for glycosphingolipid synthesis during development and differentiation. *Proc Natl Acad Sci U S A*. 1999; 96:9142–9147. [PubMed: 10430909]
3. Jennemann R, et al. Cell-specific deletion of glucosylceramide synthase in brain leads to severe neural defects after birth. *Proc Natl Acad Sci U S A*. 2005; 102:12459–12464. [PubMed: 16109770]
4. Kawai H, et al. Mice expressing only monosialoganglioside GM3 exhibit lethal audiogenic seizures. *J Biol Chem*. 2001; 276:6885–6888. [PubMed: 11133999]
5. Yoshikawa M, et al. Mice lacking ganglioside GM3 synthase exhibit complete hearing loss due to selective degeneration of the organ of Corti. *Proc Natl Acad Sci U S A*. 2009; 106:9483–9488. [PubMed: 19470479]
6. Allende ML, Proia RL. Lubricating cell signaling pathways with gangliosides. *Curr Opin Struct Biol*. 2002; 12:587–592. [PubMed: 12464309]
7. Simpson MA, et al. Infantile-onset symptomatic epilepsy syndrome caused by a homozygous loss-of-function mutation of GM3 synthase. *Nat Genet*. 2004; 36:1225–1229. [PubMed: 15502825]
8. Anderson RG. The caveolae membrane system. *Annu Rev Biochem*. 1998; 67:199–225. [PubMed: 9759488]
9. Simons K, Toomre D. Lipid rafts and signal transduction. *Nat Rev Mol Cell Biol*. 2000; 1:31–39. [PubMed: 11413487]
10. Hakomori S, Handa K, Iwabuchi K, Yamamura S, Prinetti A. New insights in glycosphingolipid function: “glycosignaling domain,” a cell surface assembly of glycosphingolipids with signal transducer molecules, involved in cell adhesion coupled with signaling. *Glycobiology*. 1998; 8:xi–xix. [PubMed: 9840984]
11. Sonnino S, Mauri L, Chigorno V, Prinetti A. Gangliosides as components of lipid membrane domains. *Glycobiology*. 2007; 17:1R–13R.
12. Takahashi K, Yamanaka S. Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. *Cell*. 2006; 126:663–676. [PubMed: 16904174]
13. Kimber SJ, Brown DG, Pahlsson P, Nilsson B. Carbohydrate antigen expression in murine embryonic stem cells and embryos. II Sialylated antigens and glycolipid analysis. *Histochem J*. 1993; 25:628–641. [PubMed: 7693623]
14. Kwak DH, et al. Dynamic changes of gangliosides expression during the differentiation of embryonic and mesenchymal stem cells into neural cells. *Exp Mol Med*. 2006; 38:668–676. [PubMed: 17202843]
15. Draper JS, Pigott C, Thomson JA, Andrews PW. Surface antigens of human embryonic stem cells: changes upon differentiation in culture. *J Anat*. 2002; 200:249–258. [PubMed: 12033729]
16. Solter D, Knowles BB. Monoclonal antibody defining a stage-specific mouse embryonic antigen (SSEA-1). *Proc Natl Acad Sci U S A*. 1978; 75:5565–5569. [PubMed: 281705]
17. Brown DG, Warren VN, Pahlsson P, Kimber SJ. Carbohydrate antigen expression in murine embryonic stem cells and embryos. I Lacto and neo-lacto determinants. *Histochem J*. 1993; 25:452–463. [PubMed: 7689550]
18. Muramatsu T, Muramatsu H. Carbohydrate antigens expressed on stem cells and early embryonic cells. *Glycoconj J*. 2004; 21:41–45. [PubMed: 15467397]
19. Eggens I, Fenderson B, Toyokuni T, Dean B, Stroud M, Hakomori S. Specific interaction between Lex and Lex determinants. A possible basis for cell recognition in preimplantation embryos and in embryonal carcinoma cells. *J Biol Chem*. 1989; 264:9476–9484. [PubMed: 2470757]
20. Childs RA, Pennington J, Uemura K, Scudder P, Goodfellow PN, Evans MJ, Feizi T. High-molecular-weight glycoproteins are the major carriers of the carbohydrate differentiation antigens I, i and SSEA-1 of mouse teratocarcinoma cells. *Biochem J*. 1983; 215:491–503. [PubMed: 6197967]

21. Thomson JA, Itskovitz-Eldor J, Shapiro SS, Waknitz MA, Swiergiel JJ, Marshall VS, Jones JM. Embryonic stem cell lines derived from human blastocysts. *Science*. 1998; 282:1145–1147. [PubMed: 9804556]
22. Osanai T, Watanabe Y, Sanai Y. Glycolipid sialyltransferases are enhanced during neural differentiation of mouse embryonic carcinoma cells, P19. *Biochem Biophys Res Commun*. 1997; 241:327–333. [PubMed: 9425271]
23. Liour SS, Kapitonov D, Yu RK. Expression of gangliosides in neuronal development of P19 embryonal carcinoma stem cells. *J Neurosci Res*. 2000; 62:363–373. [PubMed: 11054805]
24. Kannagi R, Cochran NA, Ishigami F, Hakomori S, Andrews PW, Knowles BB, Solter D. Stage-specific embryonic antigens (SSEA-3 and -4) are epitopes of a unique globo-series ganglioside isolated from human teratocarcinoma cells. *EMBO J*. 1983; 2:2355–2361. [PubMed: 6141938]
25. Schwarting GA, Carroll PG, DeWolf WC. Fucosyl-globoside and sialosyl-globoside are new glycolipids isolated from human teratocarcinoma cells. *Biochem Biophys Res Commun*. 1983; 112:935–940. [PubMed: 6847688]
26. Ariga T, Yu RK. The role of globo-series glycolipids in neuronal cell differentiation--a review. *Neurochem Res*. 1998; 23:291–303. [PubMed: 9482241]
27. Yanagisawa M, Yu RK. The expression and functions of glycoconjugates in neural stem cells. *Glycobiology*. 2007; 17:57R–74R.
28. Doetsch F, Caille I, Lim DA, Garcia-Verdugo JM, Alvarez-Buylla A. Subventricular zone astrocytes are neural stem cells in the adult mammalian brain. *Cell*. 1999; 97:703–716. [PubMed: 10380923]
29. Seri B, Garcia-Verdugo JM, McEwen BS, Alvarez-Buylla A. Astrocytes give rise to new neurons in the adult mammalian hippocampus. *J Neurosci*. 2001; 21:7153–7160. [PubMed: 11549726]
30. Klassen H, Schwartz MR, Bailey AH, Young MJ. Surface markers expressed by multipotent human and mouse neural progenitor cells include tetraspanins and non-protein epitopes. *Neurosci Lett*. 2001; 312:180–182. [PubMed: 11602340]
31. Yanagisawa M, Nakamura K, Taga T. Roles of lipid rafts in integrin-dependent adhesion and gp130 signalling pathway in mouse embryonic neural precursor cells. *Genes Cells*. 2004; 9:801–809. [PubMed: 15330857]
32. Ngamukote S, Yanagisawa M, Ariga T, Ando S, Yu RK. Developmental changes of glycosphingolipids and expression of glycogenes in mouse brains. *J Neurochem*. 2007; 103:2327–2341. [PubMed: 17883393]
33. Yanagisawa M, Taga T, Nakamura K, Ariga T, Yu RK. Characterization of glycoconjugate antigens in mouse embryonic neural precursor cells. *J Neurochem*. 2005; 95:1311–1320. [PubMed: 16219035]
34. Capela A, Temple S. LeX/ssea-1 is expressed by adult mouse CNS stem cells, identifying them as nonependymal. *Neuron*. 2002; 35:865–875. [PubMed: 12372282]
35. Ogden AT, et al. Identification of A2B5+CD133- tumor-initiating cells in adult human gliomas. *Neurosurgery*. 2008; 62:505–514. discussion 514–505. [PubMed: 18382330]
36. Tchoghadjian A, Baeza N, Colin C, Cayre M, Metellus P, Beclin C, Ouafik L, Figarella-Branger D. A2B5 Cells from Human Glioblastoma have Cancer Stem Cell Properties. *Brain Pathol*. 2009
37. Read TA, Fogarty MP, Markant SL, McLendon RE, Wei Z, Ellison DW, Febbo PG, Wechsler-Reya RJ. Identification of CD15 as a marker for tumor-propagating cells in a mouse model of medulloblastoma. *Cancer Cell*. 2009; 15:135–147. [PubMed: 19185848]
38. Ward RJ, et al. Multipotent CD15+ cancer stem cells in patched-1-deficient mouse medulloblastoma. *Cancer Res*. 2009; 69:4682–4690. [PubMed: 19487286]
39. Son MJ, Woolard K, Nam DH, Lee J, Fine HA. SSEA-1 is an enrichment marker for tumor-initiating cells in human glioblastoma. *Cell Stem Cell*. 2009; 4:440–452. [PubMed: 19427293]
40. Osawa M, Hanada K, Hamada H, Nakauchi H. Long-term lymphohematopoietic reconstitution by a single CD34-low/negative hematopoietic stem cell. *Science*. 1996; 273:242–245. [PubMed: 8662508]
41. Zakaryan A, Karageuzyan K, Hovsepyan L, Karabashyan L, Zakaryan G. Quantitative analysis of phospholipids and gangliosides in bone marrow progenitors of lymphocytes, thymocytes and

- mature lymphocytes in tumor-bearing animals. *Int Immunol.* 2001; 13:1141–1145. [PubMed: 11526094]
42. Yamazaki S, Iwama A, Morita Y, Eto K, Ema H, Nakauchi H. Cytokine signaling, lipid raft clustering, and HSC hibernation. *Ann N Y Acad Sci.* 2007; 1106:54–63. [PubMed: 17442772]
 43. Chae HD, Lee KE, Williams DA, Gu Y. Cross-talk between RhoH and Rac1 in regulation of actin cytoskeleton and chemotaxis of hematopoietic progenitor cells. *Blood.* 2008; 111:2597–2605. [PubMed: 18089848]
 44. Yamazaki S, Iwama A, Takayanagi S, Morita Y, Eto K, Ema H, Nakauchi H. Cytokine signals modulated via lipid rafts mimic niche signals and induce hibernation in hematopoietic stem cells. *EMBO J.* 2006; 25:3515–3523. [PubMed: 16858398]
 45. Gang EJ, Bosnakovski D, Figueiredo CA, Visser JW, Perlingeiro RC. SSEA-4 identifies mesenchymal stem cells from bone marrow. *Blood.* 2007; 109:1743–1751. [PubMed: 17062733]
 46. Martinez C, Hofmann TJ, Marino R, Dominici M, Horwitz EM. Human bone marrow mesenchymal stromal cells express the neural ganglioside GD2: a novel surface marker for the identification of MSCs. *Blood.* 2007; 109:4245–4248. [PubMed: 17264296]
 47. Xu J, et al. Neural ganglioside GD2 identifies a subpopulation of mesenchymal stem cells in umbilical cord. *Cell Physiol Biochem.* 2009; 23:415–424. [PubMed: 19471109]
 48. Ryu JS, Ko K, Lee JW, Park SB, Byun SJ, Jeong EJ, Ko K, Choo YK. Gangliosides are involved in neural differentiation of human dental pulp-derived stem cells. *Biochem Biophys Res Commun.* 2009
 49. Kucia M, Zuba-Surma E, Wysoczynski M, Dobrowolska H, Reca R, Ratajczak J, Ratajczak MZ. Physiological and pathological consequences of identification of very small embryonic like (VSEL) stem cells in adult bone marrow. *J Physiol Pharmacol.* 2006; 57(Suppl 5):5–18.
 50. Kucia M, et al. Morphological and molecular characterization of novel population of CXCR4+ SSEA-4+ Oct-4+ very small embryonic-like cells purified from human cord blood: preliminary report. *Leukemia.* 2007; 21:297–303. [PubMed: 17136117]
 51. Zuba-Surma EK, Wu W, Ratajczak J, Kucia M, Ratajczak MZ. Very small embryonic-like stem cells in adult tissues-potential implications for aging. *Mech Ageing Dev.* 2009; 130:58–66. [PubMed: 18377952]
 52. Yamazaki S, Iwama A, Takayanagi S, Eto K, Ema H, Nakauchi H. TGF-beta as a candidate bone marrow niche signal to induce hematopoietic stem cell hibernation. *Blood.* 2009; 113:1250–1256. [PubMed: 18945958]
 53. Giebel B, et al. Segregation of lipid raft markers including CD133 in polarized human hematopoietic stem and progenitor cells. *Blood.* 2004; 104:2332–2338. [PubMed: 15231568]
 54. Campos LS, Decker L, Taylor V, Skarnes W. Notch, epidermal growth factor receptor, and beta1-integrin pathways are coordinated in neural stem cells. *J Biol Chem.* 2006; 281:5300–5309. [PubMed: 16332675]
 55. Nagatsuka Y, Kasama T, Ohashi Y, Uzawa J, Ono Y, Shimizu K, Hirabayashi Y. A new phosphoglycerolipid, 'phosphatidylglucose', found in human cord red cells by multi-reactive monoclonal anti-i cold agglutinin, mAb GL-1/GL-2. *FEBS Lett.* 2001; 497:141–147. [PubMed: 11377429]
 56. Nagatsuka Y, et al. Phosphatidylglucoside exists as a single molecular species with saturated fatty acyl chains in developing astroglial membranes. *Biochemistry (Mosc).* 2006; 45:8742–8750.
 57. Kinoshita MO, et al. Lipid rafts enriched in phosphatidylglucoside direct astroglial differentiation by regulating tyrosine kinase activity of epidermal growth factor receptors. *Biochem J.* 2009; 419:565–575. [PubMed: 19170657]
 58. Svennerholm L. Chromatographic Separation of Human Brain Gangliosides. *J Neurochem.* 1963; 10:613–623. [PubMed: 14066623]

Table 1

Structures and nomenclature of glycosphingolipids referred to in this paper. The nomenclature for gangliosides is based on that of Svennerholm [58].

Abbreviation	Series	Structure
Asialo-GM1	asialo-series	Gal β 1,3GalNAc β 1,4Gal β 1,4Glc β 1,1Cer
Fucosyl-GM1		Fuca1,2Gal β 1,3GalNAc β 1,4(Neu5Ac α 2,3)Gal β 1,4Glc β 1,1Cer
GM3	a-series	Neu5Ac α 2,3Gal β 1,4Glc β 1,1Cer
GM2	a-series	GalNAc β 1,4(Neu5Ac α 2,3)Gal β 1,4Glc β 1,1Cer
GM1	a-series	Gal β 1,3GalNAc β 1,4(Neu5Ac α 2,3)Gal β 1,4Glc β 1,1Cer
GD1a	a-series	Neu5Ac α 2,3Gal β 1,3GalNAc β 1,4(Neu5Ac α 2,3)Gal β 1,4Glc β 1,1Cer
GalNAc-GD1a	a-series	GalNAc β 1,4(Neu5Ac α 2,3)Gal β 1,3GalNAc β 1,4(Neu5Ac α 2,3)Gal β 1,4Glc β 1,1Cer
GT1a	a-series	Neu5Ac α 2,8Neu5Ac α 2,3Gal β 1,3GalNAc β 1,4(Neu5Ac α 2,3)Gal β 1,4Glc β 1,1Cer
GT1a α	a-series	Neu5Ac α 2,3Gal β 1,3(Neu5Ac α 2,6)GalNAc β 1,4(Neu5Ac α 2,3)Gal β 1,4Glc β 1,1Cer
GD3	b-series	Neu5Ac α 2,8Neu5Ac α 2,3Gal β 1,4Glc β 1,1Cer
9-O-acetyl-GD3	b-series	Neu5,9Ac α 2,8Neu5Ac α 2,3Gal β 1,4Glc β 1,1Cer
GD2	b-series	GalNAc β 1,4(Neu5Ac α 2,8Neu5Ac α 2,3)Gal β 1,4Glc β 1,1Cer
GD1b	b-series	Gal β 1,3GalNAc β 1,4(Neu5Ac α 2,8Neu5Ac α 2,3)Gal β 1,4Glc β 1,1Cer
GT1b	b-series	Neu5Ac α 2,3Gal β 1,3GalNAc β 1,4(Neu5Ac α 2,8Neu5Ac α 2,3)Gal β 1,4Glc β 1,1Cer
GQ1b	b-series	Neu5Ac α 2,8Neu5Ac α 2,3Gal β 1,3GalNAc β 1,4(Neu5Ac α 2,8Neu5Ac α 2,3)Gal β 1,4Glc β 1,1Cer
GQ1b α	b-series	Neu5Ac α 2,3Gal β 1,3(Neu5Ac α 2,6)GalNAc β 1,4(Neu5Ac α 2,8Neu5Ac α 2,3)Gal β 1,4Glc β 1,1Cer
GT3	c-series	Neu5Ac α 2,8Neu5Ac α 2,8Neu5Ac α 2,3Gal β 1,4Glc β 1,1Cer
GT2	c-series	GalNAc β 1,4(Neu5Ac α 2,8Neu5Ac α 2,8Neu5Ac α 2,3)Gal β 1,4Glc β 1,1Cer
GT1c	c-series	Gal β 1,3GalNAc β 1,4(Neu5Ac α 2,8Neu5Ac α 2,8Neu5Ac α 2,3)Gal β 1,4Glc β 1,1Cer
GQ1c	c-series	Neu5Ac α 2,3Gal β 1,3GalNAc β 1,4(Neu5Ac α 2,8Neu5Ac α 2,8Neu5Ac α 2,3)Gal β 1,4Glc β 1,1Cer
GP1c	c-series	Neu5Ac α 2,8Neu5Ac α 2,3Gal β 1,3GalNAc β 1,4(Neu5Ac α 2,8Neu5Ac α 2,8Neu5Ac α 2,3)Gal β 1,4Glc β 1,1Cer
SSEA-1		Gal β 1,4(Fuca1,3)GlcNAc β 1,3Gal β 1,4Glc β 1,1Cer
SSEA-3		Gal β 1,3GalNAc β 1,3Gal α 1,4Gal β 1,4Glc β 1,1Cer
SSEA-4		Neu5Ac α 2,3Gal β 1,3GalNAc β 1,3Gal α 1,4Gal β 1,4Glc β 1,1Cer

Table 2

Glycolipids expressed in stem cells

Stem cells	Species	Glycolipid	Reference
Embryonic stem cell	Mouse	GM3	[13]
		GM1	[13, 14]
		GD1a	[13]
		Lactosylceramide	[13]
		Ceramide trihexoside	[13]
		Globoside	[13]
		Forssman antigen	[13]
		SSEA-1	[17, 18]
		SSEA-3	[15, 21]
		SSEA-4	[15, 21]
Induced pluripotent stem cell	Mouse	SSEA-1 (glycoprotein/proteoglycan?)	[12]
Neural stem cell	Mouse, human	GD2	[30]
	Mouse	GD3	[31]
		GQ1b	[31]
		GT1b	[31]
		Chol-1 α (GT1a α and GQ1b α)	[32]
	Mouse	SSEA-1 (glycoprotein/proteoglycan?)	[30, 34]
	Human	SSEA-1 (glycoprotein/proteoglycan?)	[30]
	Human	A2B5 antigens (c-series gangliosides)	[35, 36]
	Mouse	SSEA-1 (glycoprotein/proteoglycan?)	[37, 38]
	Human	SSEA-1 (glycoprotein/proteoglycan?)	[39]
Hematopoietic stem cell	Mouse	GM1?	[42–44]
Mesenchymal stem cell	Human	GD2	[46]
		GM1	[14]
		GM3	[48]
		GM2	[48]
		GD1a	[48]
	Mouse, human	SSEA-4	[45]
	Mouse	SSEA-1 (glycoprotein/proteoglycan?)	[49]
	Human	SSEA-4	[50]