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Epigenetic regulation of ganglioside expression in neural stem cells and neuronal cells

Yutaka Itokazu[1,2], **Yi-Tzang Tsai**[1,2], and **Robert K. Yu**[1,2]

[1]Department of Neuroscience and Regenerative Medicine, Medical College of Georgia, Augusta University, Augusta, GA 30912, USA

[2]Charlie Norwood VA Medical Center, Augusta, GA 30904, USA

Abstract

The structural diversity and localization of cell surface glycosphingolipids (GSLs), including gangliosides, in glycolipid-enriched microdomains (GEMs, also known as lipid rafts) render them ideally suited to play important roles in mediating intercellular recognition, interactions, adhesion, receptor function, and signaling. Gangliosides, sialic acid-containing GSLs, are most abundant in the nerve tissues. The quantity and expression pattern of gangliosides in brain change drastically throughout development and these changes are mainly regulated through stage-specific expression of glycosyltransferase genes. We previously demonstrated for the first time that efficient histone acetylation of the glycosyltransferase genes in mouse brain contributes to the developmental alteration of ganglioside expression. We further demonstrated that acetylation of histones H3 and H4 on the *N*-acetylgalactosaminyltransferase I (*GalNAcT*, GA2/GM2/GD2/GT2-synthase; *B4galnt1*) gene promoter resulted in recruitment of trans-activation factors. In addition, we showed that epigenetic activation of the *GalNAcT* gene was detected and accompanied by an apparent induction of neuronal differentiation of neural stem cells (NSCs) responding to an exogenous supplement of ganglioside GM1. Most recently, we found that nuclear GM1 binds with acetylated histones on the promoters of the *GalNAcT* as well as on the *NeuroD1* genes in differentiated neurons. Here, we will introduce epigenetic regulation of ganglioside synthase genes in neural development and neuronal differentiation of NSCs.

Keywords

Brain development; DNA methylation; Epigenetics; Glycosyltransferase; Ganglioside; Histone acetylation; Neural stem cell; Neuronal progenitor cell; Neuronal differentiation

Compliance with ethical standards

Conflicts of interest

The authors declare that they have no conflicts of interest.

Ethical approval

This article does not contain any studies with human participants performed by any of the authors.

Introduction

Gangliosides, sialic acid–containing glycosphingolipids (GSLs), are composed of a ceramide moiety linked to an oligosaccharide chain (Fig. 1). Gangliosides are expressed primarily, but not exclusively, on the outer leaflet of the plasma membrane of cells in all vertebrates. They are particularly abundant in nervous tissues. 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 [1]. In later developmental stages, more complex gangliosides prevail, particularly GM1, GD1a, GD1b, and GT1b, which account for more than 80 % of the total gangliosides. Thus, the expression of neural gangliosides changes dramatically during cellular differentiation and brain development, indicating that ganglioside expression and neurodevelopmental events are closely correlated. This unique expression pattern of specific gangliosides can be used for specific cell lineage markers and may reflect the functional roles they play in a specific developmental stage. Abundant evidence supports the notion that GSLs, including ganglio-sides, serve regulatory roles in cellular events, including proliferation and neural differentiation, as exemplified by neuritogenesis, axonogenesis, and synaptogenesis [2, 3]

The brain tissue is composed of a variety of neural cells, including neurons and glia; those cells are generated from progenitor cells that are recognized as neural stem cells (NSCs). NSCs are undifferentiated neural cells characterized by their high proliferative potential and the capacity for self-renewal with retention of multipotency to differentiate into neurons and glia. Specific gangliosides are considered to have critical roles in the regulation of NSC cell-fate determination (Fig. 2). We have shown that GD3 is the predominant ganglioside in NSCs [4]. The interaction of GD3 with epidermal growth factor receptor (EGFR) plays a crucial role in maintaining the self-renewal capacity of NSCs by directing the EGFR through the recycling pathway rather than through the degradative pathway after endocytosis [5]. Although Sialyltransferase II (*ST-II*, GD3-synthase; *St8sia1*) knockout (KO) mice and *N*-acetylgalactosaminyltransferase I (*GalNAcT*, GA2/GM2/GD2/GT2-synthase; *B4galnt1*) KO mice do not have explicit phenotypes in early development [6], it has been revealed that GD3 is required for long-term maintenance of NSCs populations in postnatal mouse brain [5, 7]. Deficiency in GD3 leads to developmental and behavior deficits, such as depression [7]. GalNAcT-KO mice exhibit impaired movement and have virtually all the neuropathological symptoms of Parkinson disease [8]. For specialization of neuronal cells, GM1 potentiates nerve growth factor and regulates neurite outgrowth to contribute to neuronal differentiation. Intriguingly, our recent studies revealed that epigenetic activation of the *GalNAcT* gene, but not the *ST-II* gene, occurred and was accompanied by an apparent induction of neuronal differentiation in NSCs responding to an exogenous supplement of ganglioside GM1 [9]. Supplemental GM1 enhanced GalNAcT expression of mRNA, while exogenous GD3 had no significant effect in this expression. The binding of acetylated histone H4 (AcH4) on the *GalNAcT* promoter region was elevated by GM1 addition, but neither by GD3 nor GD1b supplementations. Unlike GD3 and GD1b, GM1 has epigenetic effect for neuronal differentiation. This report apparently showed that the epigenetic regulation in neuronal cells by GM1 did not influence on the genome at random, but at specifically controlled pinpoint loci [9].

Epigenetic regulation of gene expression involving DNA methylation, histone modifications, chromatin remodeling, and non-coding RNAs, is an important mechanism governing stage-specific gene expression in developing mammalian brains as well as other tissues [10, 11]. The most common post-translational histone modification is acetylation, which occurs by addition of acetyl groups to the lysine residues of the amino-terminal tails of core histones. Acetylation of his-tone H3 or H4 can alter the interaction between histones and DNA to allow for relaxation of chromatin. Thus, changes in histone acetylation status are often associated with transcriptional activation and repression [12, 13]. In NSCs, induction of histone acetylation promotes neuronal differentiation and inhibits glial differentiation through up-regulation of neurogenic transcription factors [14]. Histone acetylation is controlled by histone acetyl transferases (HATs) and histone deacetylases (HDACs) [15]. Intriguingly, recent studies have suggested that glycans can contribute to epigenetic gene regulation as modulators. For example, a Bcarbohydrate-like^ molecule, inositol tetraphosphate (IP4; inositol 1, 3, 4, 5– tetrakisphosphate) acts as a stimulator to the activity of class I HDACs (HDAC1/2/3) [16–18]. Additionally, OGlcNAcylation has been reported to regulate localization, activity, and stability of many transcription factors, as well as their interactions with other proteins and DNA [19, 20]. As such, glycosyl modification, such as *O*-GlcNAcylation, is now considered as an important modulatory mechanism to epigenetic control of gene expression [21].

We have previously reported that efficient histone acetylation of glycosyltransferase (GT) genes in mouse brain contributes to the developmental alteration of ganglioside expression [22]. Further, we have demonstrated that acetylation of histones H3 and H4 on the *GalNAcT* gene promoter leads to recruitment of trans-activation factors SP1 and AP-2 [9]. More recently, we found that nuclear GM1 binds with acetylated histones on the promoters of the *GalNAcT* as well as on the neurogenic transcription factor, *NeuroD1* gene, in differentiated neurons [23]. Here, we will further elaborate the importance of epigenetic regulation of ganglioside synthase genes in neural development and neuronal differentiation of NSCs.

Histone acetylation and gene activation of the 5′ region of *GalNAcT* and *ST-II* genes during brain development

The quantity and expression pattern of gangliosides in brain change drastically throughout development and are mainly regulated through stage-specific expression of GT genes [1, 24, 25]. A diagram of the biosynthetic pathways of gangliosides is shown in Fig. 1. Of particular importance is the regulation of GTs located at the branching points that govern pathway shifts for ganglioside biosynthesis. For example, regulation of the two key GTs at the branching point leading to GD3 and GM2 synthesis constitutes one of the critical steps for the expression of GD3 and *N*-acetylgalactosaminyl-containing “brain-type” gangliosides in the a- and b- series. Our analyses of the whole brain have shown that the *GalNAcT* gene encounters drastic activation since the late embryonic stage of mice, and its expression level is further elevated from birth through adulthood; transcription of the sialyltransferase II (*ST-II*, GD3-synthase; *St8sia1*) gene is also increased during brain development, albeit less pronounced in comparison [22, 25]. We have demonstrated that acetylated histone H3 (AcH3) of the chromatin in the 5′ region of the mouse *GalNAcT* and *ST-II* genes is highly

correlated to the developmental changes of their mRNA levels in the mouse brain. In that study, the 5′ DNA regions of the *GalNAcT* and *ST-II* loci appeared to be unmethylated in either the embryonic or postnatal mouse brain. These observations suggest that transcriptional activation of *GalNAcT* and *ST-II* genes in developing mouse brains is not regulated by DNA methylation, at least in the 5′ DNA regions [22]. Subsequently, Taniguchi's group suggested that for the brain-specific synthesis of branched *O*-mannose glycan that was catalyzed by N-acetyl-glucosaminyltransferase-IX (GnT-IX), the mRNA expression of GnT-IX could be triggered by the neurogenic transcription factor NeuroD1 as well as an insulator protein CTCF as a consequence of active histone marks deposited around GnT-IX gene's transcription start site [26, 27].

Histone acetylation recruits transcription factors onto the promoters of glycosyltransferase genes in NSCs

We have further elucidated the differential regulatory mechanisms underlying epigenetic activation for the *GalNAcT* and *ST-II* genes during neuronal differentiation in a NSC culture system. This study has showed that during neuronal differentiation of NSCs, not only AcH3, but also AcH4, exhibited a pattern of increment similar to that of enhanced mRNA expression of GalNAcT and ST-II. It has been reported that GT promoters are often controlled by transcription factors Sp1 and AP-2 [28–30]. The promoter activity of the human *GalNAcT* gene is known to be activated by Sp1 and AP-2, and that for the *ST-II* gene by Sp1 by way of promoter association [30]. A putative binding site for the transcription factor AP-2 was found within the 5′ untranslated region (5′ UTR) of the *GalNAcT* gene and in the first exon of the ST-II gene, while a binding site for the transcription factor Sp1 was present only in the 5′ UTR of the *ST-II* gene [31]. When the cellular HDAC activity was globally inhibited by valproic acid (VPA), more GalNAcT or ST-II mRNA was detected, which could be triggered due to a loading boost of the transcription factors AP-2 and Sp1 on the promoter region [9]. Individually knocking down HDAC1 and HDAC2 gene expression increased the levels of AcH3 and AcH4. However, *GalNAcT* and *ST-II* genes did not show any corresponding change in the histone acetylation status, promoter binding abilities for the transcription factors, or mRNA expression level. Intriguingly, when both HDAC1 and HDAC2 were knocked down, the expression of GalNAcT mRNA was upregulated and AP-2- or Sp1-loading was significantly increased, reflecting the elevated level of histone acetylation on the *GalNAcT* gene. This was not the case for the *ST-II* gene, however, which remained unchanged with respect to the levels of mRNA, the binding of transcription factors, or the AcH status. Our results indicated that transcription of *GalNAcT* and *ST-II* could be regulated by different HDAC isoforms, since double-knockdown of HDAC1 and HDAC2 led to *GalNAcT* gene *trans*-activation, but not *ST-II*.

Nuclear GM1 at the nuclear periphery is associated with acetylated histones in neurons

Glycolipid-enriched microdomains (GEMs) or lipid rafts on the cell plasma membrane surface are increasingly recognized as an important site for signaling transduction. Nuclear lipid domains on the nuclear envelope have also recently been suggested to play a similar

role [32]. Both GM1 and GD1a have been detected in the inner and outer nuclear membranes [33]. The nuclear distribution of gangliosides in the developing brain reflects their composition in the total brain [2, 34], i.e., GD3 is abundant in the nuclear membranes of the embryonic brain and adult brain type gangliosides (GM1, GD1a, GD1b, GT1b) become more abundant in the nuclei of postnatal brain cells [34]. With regard to gangliosides associated with chromatins, GD3 is reported to interact with histone H1 in the nucleus [35]. It has also been shown that nuclear sphingolipids participate in epigenetic regulation of gene expression by controlling histone acetylation [36]. For these reasons, it is reasonable to assume that membrane lipids, including GSLs, may contribute, in a stage- and cell-specific manner, to modulate gene expression as happening on the nuclear membrane. The nuclear envelope, including the nuclear lamina and nuclear pore complexes, is a key structure to maintain chromatin architecture and cell-specific gene expression [37]. We have confirmed that GM1 is indeed present and colocalized with lamin B1 and nucleoporin at the nuclear periphery of neuronal cells derived from NSCs (Fig. 3a). Our co-immunoprecipitation experiments have clearly revealed that nuclear GM1 genuinely interacts with AcH3 and AcH4, which are both active epigenetic marks, but not with H3K27me3, an inactive epigenetic mark. This result strongly suggests that GM1-engaged chromatins are transcriptionally active. We have further investigated the interaction between GM1 and putative genetic loci, including the *GalNAcT* and *ST-II* genes. ChIP assay was performed using anti-GM1 antibody, and revealed that GM1 was increased at the promoter region of the *GalNAcT* gene in neurons (Fig. 3b). Such a tendency did not appear for the *ST-II* gene loci. Interestingly, after neuronal differentiation, more GM1 was accumulated on the promoter of the neurogenic transcription factor *NeuroD1* gene, which commits the transition of NSCs to neuronal progenitor cells (NPCs). These results are consistent with the notion that GM1 is involved in the chromatin complex that promotes neuronal differentiation.

GM1 promotes neuronal differentiation with enhanced histone acetylation

It is known that GM1 enhances neurite outgrowth, and up-regulation of GM1 in the nuclear membrane accompanies the process of neurite outgrowth [38, 39]. Ledeen *et al.* have proposed that the elevated level of GM1 has a modulatory effect on Ca^{2+} homeostasis in the nucleus, which is mediated by a tight association of GM1 with the Na^{+}/Ca^{2+} exchanger [8, 40]. The supplement of GM1 in our NSC culture led to neuronal differentiation as well. A notable observation from our own study was that GM1-induced NSCs produced more *GalNAcT* transcripts as well as a higher level of acetylated histones on the gene promoter where more transcription factors are recruited. On the other hand, the *ST-II* gene did not show any significant change. Given that the global acetylation state of histones was not changed, the GM1-triggered epigenetic activation appears to be specific for certain targeted genes, including *GalNAcT*, in this enhanced neurogenic process. This result might represent a potential mechanism accounting for the correlations between the ganglioside pattern shift and epigenetic modifications of ganglioside synthase expression during neuronal differentiation and neural development. In this regard, GM1 might play a crucial role in modulating the “pathway switch” in ganglioside expression in the developing brain. We propose that GM1 may generate a positive feedback loop (Fig. 2) for NSCs to enhance neuronal differentiation and thereby to produce more GM1 and other “brain-type”

gangliosides, such as GD1a, GD1b and GT1b by increasing the *GalNAcT* message level. Since the content of GM1 in the nuclear membrane is increased during neuronal differentiation [39], it is possible that the nuclear GM1-lipid domains may serve as a docking site at the nuclear periphery specifically for active chromatins. Our working model on the role of nuclear gangliosides, which are to epigenetically modulate the gene expression in neuronal cells is shown schematically in Fig. 4. In the nuclear envelope, GM1 and GD1a are present [8, 41] and sialidase activity has also been identified [42, 43]. Further, it has been reported that neuraminidases (sialidases) Neu3 and Neu1 are present in the inner and outer nuclear membranes, respectively [33]. Since these neuraminidases can convert GD1a to GM1, nuclear GD1a may serve as a precursor of GM1 on site. Alternatively, GM1 is found to be associated with lamin B1, nucleoporin, and chromosomes during a breakdown of the nuclear envelope at mitosis of NSCs. Thus, GM1 may hitchhike into the reforming nucleus with nuclear envelope vesicles or with chromosomes as a passenger. In either case, GM1 can gain access to the chromosomes for modulating transcriptional activity of neurogenic genes, such as *GalNAcT* and *NeuroD1*.

Conclusion and future studies

Although recent evidence of GSLs, including gangliosides, has shed light on their roles in modulating signaling pathways during cellular differentiation and reprograming, mice deficient in some of these molecules show only subtle phenotypic abnormalities compared with the wild-type animals in early development. Clearly, the biological function of one glycoconjugate can be substituted by another, albeit with less efficiency. However, the aberrant ganglioside expression becomes progressively more serious in adult stage and pathogenic conditions. The “biological redundancy” can be considered for more important functions of these molecules. Epigenetic regulation is an important regulatory mechanism for controlling the expression pattern of gangliosides during cellular differentiation and brain development. Most interestingly, our data suggest that nuclear gangliosides themselves can modulate epigenetic gene expression, presumably by a feed-back mechanism. It would be interesting if different gangliosides can modulate a certain gene expression during different stages of development. Ganglioside expression profiles and glycogene expression patterns are associated not only with CNS development but also with pathogenic mechanisms of neurodegenerative diseases in CNS, such as Alzheimer’s disease [44–46], Parkinson disease [47], Huntington’s disease [48], amyotrophic lateral sclerosis [49, 50], and multiple sclerosis [51, 52]. In addition, the aberrant and elevated expression of gangliosides has been also observed in many types of cancer cells, thereby promoting tumor survival. Epigenetic regulations for the ganglioside expression, and *vice versa*, should provide clues underlying the pathogenic mechanisms, which are useful in developing novel strategies for disease treatment and tissue damage repair. Futures studies will prove fruitful in this regard.

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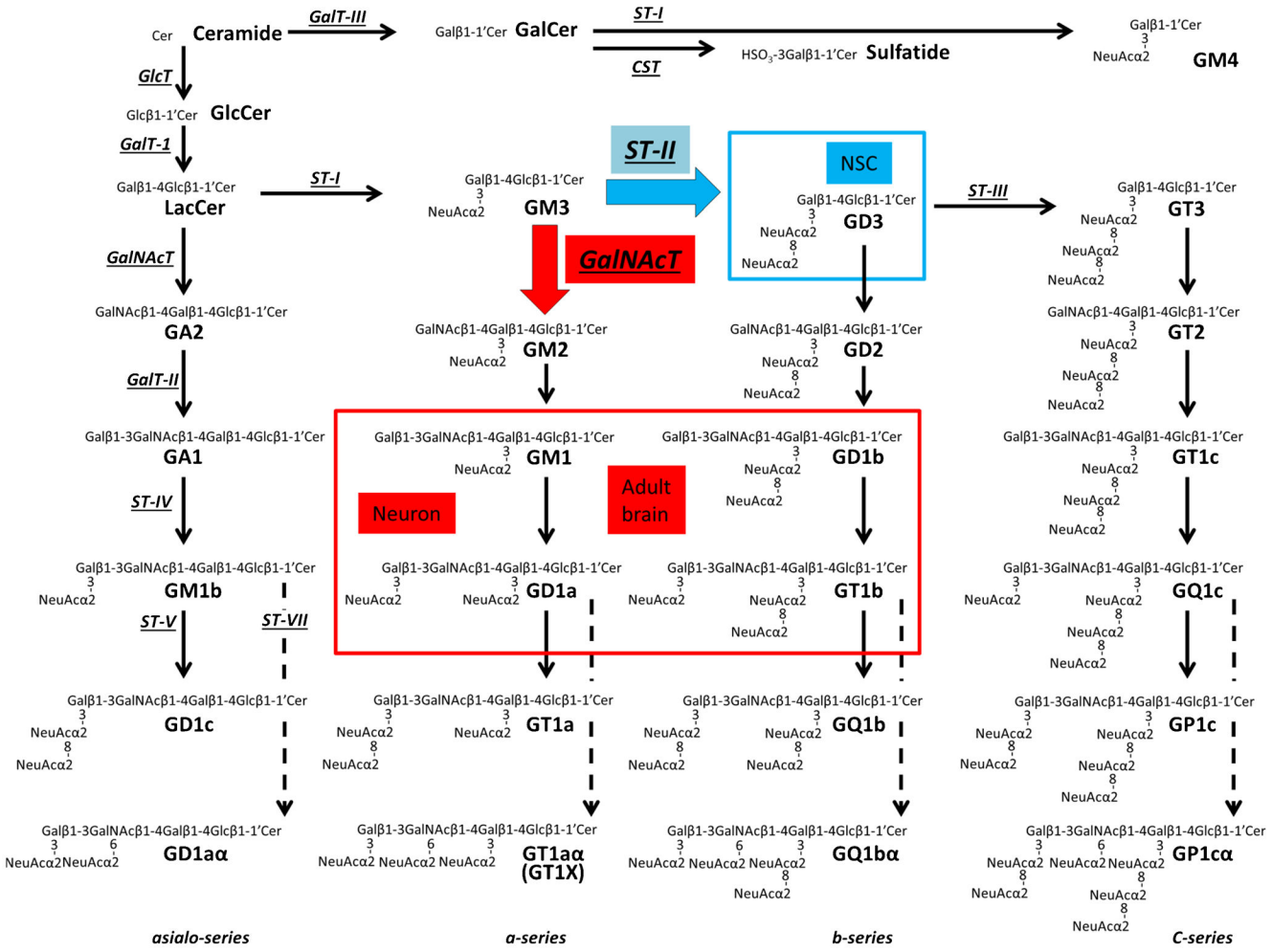

Fig. 1.

Structures and biosynthetic pathways of glycosphingolipids (GSLs). Cer, ceramide; CST, cerebroside sulfotransferase (*Gal3st1*, sulfatide synthase); GalNAc-T, *N*-acetylgalactosaminyltransferase I (*B4galnt1*, GA2/GM2/GD2/GT2 - synthase); G a l T- 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 (*St 3 gal5*, GM3-synthase); ST-II, sialyltransferase II (*St8Sia1*, GD3-synthase); ST-III, sialyltransferase III (*St8Sia3*, GT3-synthase); ST-IV, sialyltransferase IV (*St 3 gal2*, 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

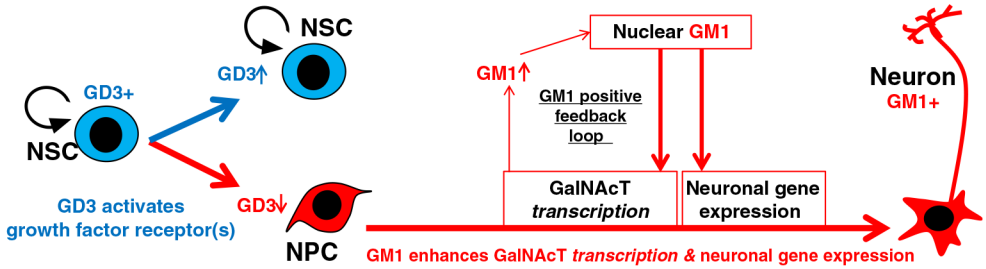

Fig. 2.

Specific gangliosides modulate NSC function and neuronal differentiation. Our published data suggest that GD3 maintains NSC's characteristics and GM1 epigenetically promotes neuronal differentiation of NSCs. ST-II (GD3S) and GalNAcT (GM2/GD2S) are located at a key branching point of a- and b-series ganglioside biosynthetic pathways (Fig. 1). GD3 modulates NSC proliferation by interacting with growth factor receptors and regulating growth factor-induced signaling. The synthesis of GD3 is switched into the synthesis of complex gangliosides (GM1, GD1a, GD1b, and GT1b), resulting in terminal differentiation and loss of the "stemness" of NSCs. During neuronal differentiation, the expression of complex a-series gangliosides, especially GM1, is augmented by a GM1-modulated epigenetic gene regulation mechanism of GalNAcT. Nuclear GM1 modulates transcriptional activity of neuronal genes, such as Neuro D1. NSC, neural stem cell; NPC, neuronal progenitor cell

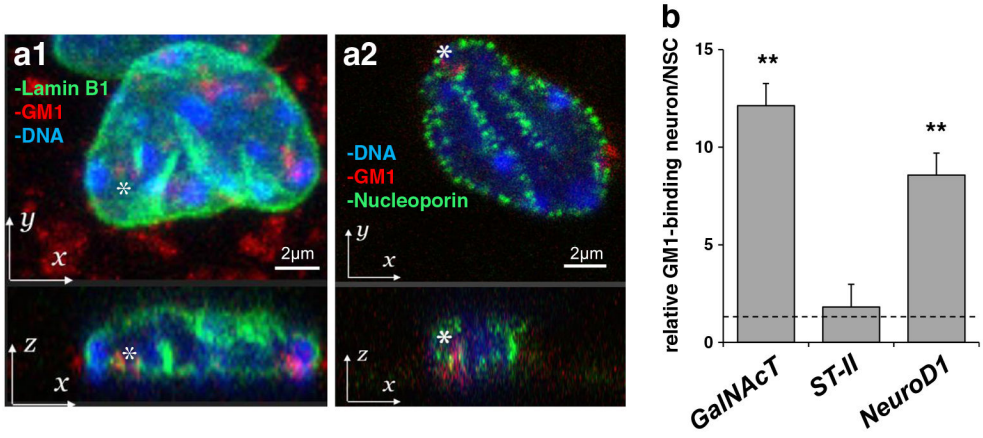

Fig. 3.

Nuclear GM1 in at the nuclear periphery and is accumulated in the activated *GalNAcT* and *NeuroD1* genes in neurons. (**a**) Localization of GM1 at the nuclear periphery in neurons. Differentiated neurons were fixed, stained with fluorescent cholera toxin B subunit (CtxB, red fluorescent) to determine the localization of GM1. Cells were co-stained with lamin B1 (green fluorescence in a1) or nucleoporin (green fluorescence in a2). Nuclear DNA was counterstained with Hoechst 33,258. Z-projection: bottom panel is x-z plane. (**b**) GM1 is accumulated in the activated GalNAcT and NeuroD1 genes. The nuclear fraction of differentiated neurons with prior formaldehyde cross-linking was used for anti-GM1 immunoprecipitation. The amount of specific DNA fragments co-precipitated with GM1 was analyzed by quantitative real-time PCR. The data indicate the relative GM1 binding ability in neurons. The value of NSC samples is defined as 1.0 and represented with a dashed line. Each bar represents mean ± SD of 3 to 4 independent experiments ($n = 3–4$). ** ($p < 0.01$) indicates the level of significance in two-tailed *t*-tests of differences between differentiated neurons versus NSCs

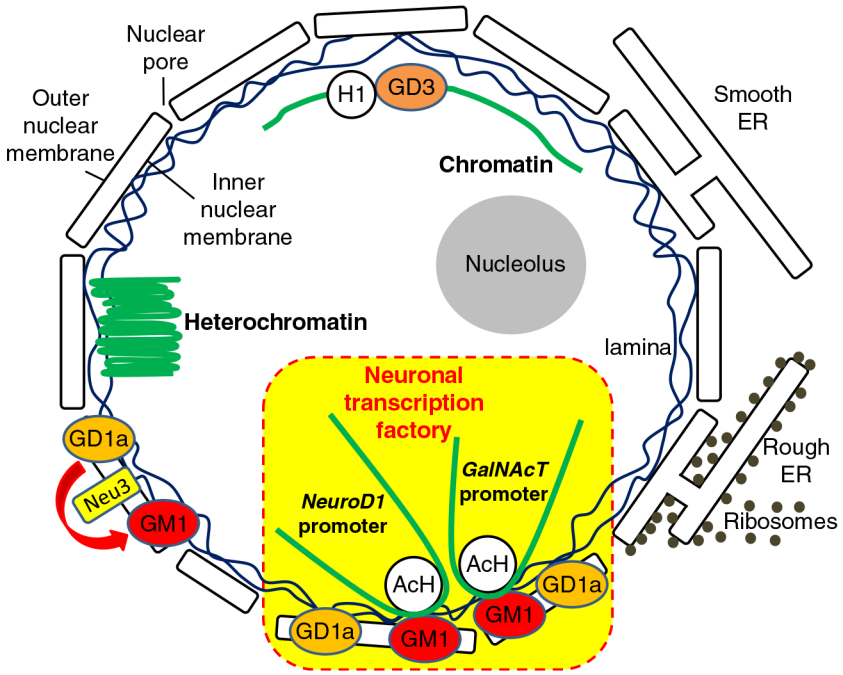

Fig. 4.

A model depicting the regulation of glycosyltransferase *GalNAcT* gene by gangliosides for neuronal differentiation. Nuclear GM1 interacts with acetylated histones (AcH) which are active epigenetic marks. GM1 binds with the *GalNAcT* and the *NeuroD1*genes, and GM1 enhances histone acetylation on the promoters of the *GalNAcT* as well as on the NeuroD1 genes in differentiated neurons. These associations of GM1 and these genes were found in differentiated neurons, but not in undifferentiated NSCs. The interaction of GM1 and the *GalNAcT* gene promoter occurs in a differentiation stage-specific manner. At a later differentiation stage, the nuclear GM1-lipid domains may serve as a docking site at the nuclear periphery for specific active chromatins for neuronal differentiation