



Signaling domains of cancer-associated glycolipids

Koichi Furukawa^{1,3} · Yuhsuke Ohmi² · Kazunori Hamamura⁴ · Yuji Kondo³ · Yuki Ohkawa⁵ · Kei Kaneko¹ · Noboru Hashimoto⁶ · Farhana Yesmin^{1,3} · Robiul H. Bhuiyan¹ · Orié Tajima¹ · Keiko Furukawa¹

Received: 18 December 2021 / Revised: 6 February 2022 / Accepted: 15 February 2022 / Published online: 22 March 2022
© The Author(s), under exclusive licence to Springer Science+Business Media, LLC, part of Springer Nature 2022

Abstract

Immunotherapy of malignant cancers is now becoming one of representative approaches to overcome cancers. To construct strategies for immunotherapy, presence of tumor-specific antigens should be a major promise. A number of cancer specific- or cancer-associated antigens have been reported based on various experimental sets and various animal systems. The most reasonable strategy to define tumor-specific antigens might be “autologous typing” performed by Old’s group, proposing three classes of tumor-antigens recognized by host immune systems of cancer patients. Namely, class 1, individual antigens that is present only in the patient’s sample analyzed; class 2, shared antigens that can be found only in some group of cancers in some patients, but not in normal cells and tissues; class 3, universal antigens that are present in some cancers but also in normal cells and tissues with different densities. Sen Hakomori reported there were novel carbohydrates in cancers that could not be detected in normal cells mainly by biochemical approaches. Consequently, many of class 2 cancer-specific antigens have been revealed to be carbohydrate antigens, and been used for cancer diagnosis and treatment. Not only as cancer markers, but roles of those cancer-associated carbohydrates have also been recognized as functional molecules in cancer cells. In particular, roles of complex carbohydrates in the regulation of cell signaling on the cell surface microdomains, glycolipid-enriched microdomain (GEM)/rafts have been reported by Hakomori and many other researchers including us. The processes and present status of these studies on cancer-associated glycolipids were summarized.

Keywords Cancer. Tumor antigen. Carbohydrate. Glycolipid. Antibody. Raft

Introduction

Despite a number of efforts to demonstrate tumor-specific antigens using murine and human tumor systems, no clear tumor-specific antigens had been reported for a long time. There were many studies on tumor antigens using murine leukemias and sarcomas [1–6], since genetic back grounds of experimental mice were complete and easy to be used for immunological reaction to tumor cells [7]. During these studies, many differentiation antigens and allogeneic antigens on immune cells were defined [7]. Then, various studies to reveal tumor-specific antigens in human cancers were performed. The most reasonable strategy to define tumor-specific antigens might be “autologous typing” performed by Old’s group with serological approach. They proposed three classes of tumor-antigens recognized by host immune systems of cancer patients (Table 1). Namely, class 1, individual antigens that are present only in the patient’s sample analyzed; class 2 shared antigens that can be found only in

✉ Koichi Furukawa
koichi@isc.chubu.ac.jp

¹ Department of Biomedical Sciences, Chubu University
College of Life and Health Sciences, Kasugai, Japan

² Department of Clinical Engineering, Chubu University
College of Life and Health Sciences, Kasugai, Japan

³ Department of Molecular and Cellular Biochemistry, Nagoya
University Graduate School of Medicine, Nagoya, Japan

⁴ Department of Pharmacology, Aichi Gakuin University
School of Dentistry, Nisshin, Japan

⁵ Department of Glyco-Oncology and Medical Biochemistry,
Osaka International Institute, Osaka, Japan

⁶ Department of Tissue Regeneration, Tokushima University
Graduate School Institute of Biomedical Sciences,
Tokushima, Japan

some group of cancers in some patients' samples, but not in normal cells and tissues; class 3, universal antigens that are present in some cancers but also in normal cells and tissues with different densities. Then, many of class 2 antigens have been elucidated to be carbohydrate epitopes. A representative example might be ganglioside GD2 detected by the serum of patient "AH" [8]. Here, GD2 was shown to be expressed in melanoma and glioma cell lines, but not in normal cells except fetal tissues. Combined with monoclonal antibody technique of mouse [9, 10] and human [11, 12], various cancer-associated glycosphingolipids were defined so far. Efforts to find and apply monoclonal antibodies reactive with gangliosides to clinical use were made by many researchers [13]. Some of them showed excellent achievements [14, 15].

Sen Hakomori reported aberrant glycosylation in transformed cells that were not present in normal cells with biochemical analysis [16]. Then, he achieved a number of studies on the comprehensive aspects of cancer specific carbohydrates mainly with biochemical approaches [17].

Cancer-associated glycolipid antigens

The cancer-associated carbohydrate antigens might almost correspond to the "class 2" antigens defined by Old [7]. In a long pathway for searching cancer specific antigens [7], there have been many reports on carbohydrate antigens that are found only in cancer cells, but not in normal cells [17, 18]. They were named as cancer-associated carbohydrate antigens, and have been utilized for the diagnosis and treatment of various cancers [19, 20]. Generally, those carbohydrates are present on membrane proteins or lipids named mucins or ceramides, respectively. Some of those carbohydrate epitopes are carried in common on both proteins and ceramides. In particular, gangliosides, sialic acid-containing glycosphingolipids have been considered to be tumor markers of neuroectoderm-derived cancers such as malignant melanomas [9, 10, 21–23], neuroblastomas [24, 25], and gliomas [26–29]. They are also detected in osteosarcomas [30, 31], small cell lung carcinomas [32, 33], T-cell leukemias [34–37], and breast cancers [38, 39]. On the other hand, one of neutral glycosphingolipids, globotriaosylceramide (Gb3) was identified as a marker of Burkitt's lymphomas [40].

Since cDNAs of glycosyltransferases responsible for the synthesis of cancer-associated glycosphingolipids were cloned [41–45] and became available for the genetic engineering at the levels of cultured cells and experimental animals, a number of glyco-remodeling experiments have been performed. These efforts brought about big progress in understanding of their functional aspects [46]. In many cases, genetic engineering of glycosyltransferase genes

often resulted in marked changes in the phenotypes of cultured cells and animals [47]. Although the causing genes and resulting output are apparent, molecular mechanisms intervening between them are frequently obscure. This is because modulation of some glyco-gene generally affects all structures present along the synthetic pathways. Therefore, we need to access which specific glycosphingolipid species in the pathway is responsible for the resulting phenotypes.

Unique neutral glycolipids, globo-series glycolipids

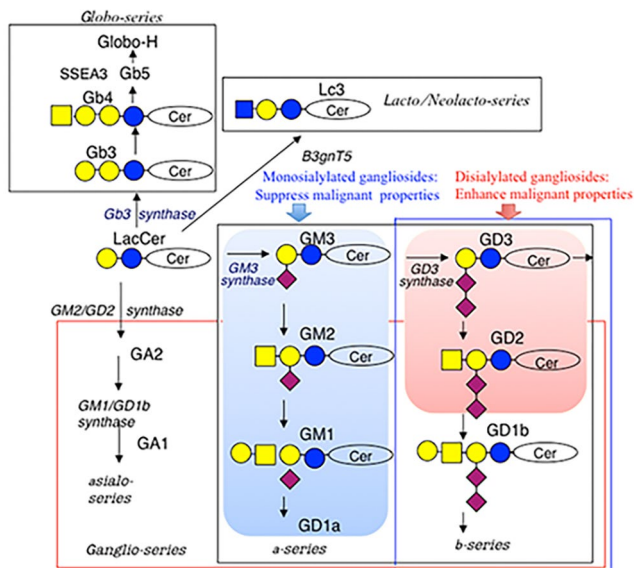
Globo-series glycolipids have been well known for a long time, but their real functions are recently getting to be disclosed.

Many glycosphingolipids except galactosylceramide and sulfatides are synthesized from glucosylceramide as a first glycolipid structure via lactosylceramide (Fig. 1). Among neutral glycosphingolipids, glycosphingolipids not containing ganglio-core, globo-series glycolipids have actually been known to exhibit unique distribution in the body, and have been expected to play important roles in some cell lineages (Fig. 2). In fact, globo-series glycolipids were enriched in human erythrocytes as shown by Yamakawa et al. [48, 49]. Globotetraosylceramide (Gb4) is highly enriched in any types of human erythrocytes except Pk and P blood group individuals [50, 51]. On the other hand, globo-series glycolipids with extended chains were reported to be embryonal stem cell (ES) markers [52], and they have been used as ES markers even now. In particular, SSEA3 (Gb5) is now utilized as an ES marker in regenerative medicine [53]. In the Oncology field, globotriaosylceramide (Gb3) was reported to be specific for Burkitt's lymphomas [40]. Roles of Gb3 in the apoptosis of immature B cells were reported [54], although the action mechanisms have not been well understood. Gb3 was also detected in metastatic tissues of colon cancers [55], breast cancers [56], and gastric cancers [57]. Monosialyl-Gb5 was reported to be a kidney cancer marker, while disialyl-Gb5 was reduced in cancer cells [58]. Recently, globo-H has been considered as a target of immune-therapy for breast cancers [59]. To clarify the actual *in vivo* functions of globo-series glycolipids, we isolated cDNA of Gb3 synthase [60], a key enzyme for the synthesis of all globo-series glycolipids, and analyzed roles of Gb3 and its derivatives in cultured cells [61] and in mice. The knockout mice of Gb3 synthase (*A4galt-1*) lacking all globo-series glycolipids exhibited no apparent abnormal phenotypes. As expected, *A4galt-1* KO mice showed complete resistance to pathogenic *E. coli* O157-derived verotoxins [62]. Using this KO mouse line, we identified TLR4/MT2 as an endogenous ligand molecule of Gb4 for the first

Table 1 Classes of cell surface antigens of human cancers defined by autologous typing

	Characteristics	Distribution
Class 1	Individually distinct (unique) autotumor antigens	Restricted to autologous tumor cells
Class 2	Shared tumor antigens	Present on autologous and some allogeneic tumor cells
Class 3	Normal cell surface antigens Heterologous serum components	Widely distributed on normal and tumor cells

Modified from ref. 7

**Fig. 1** Synthetic pathway of glycosphingolipids. Deleted structures in knockout mice are indicated by squares. Contrastive functions of monosialyl and disialyl structures are shown

time. Since TLR4/MT2 is well known receptor of lipopolysaccharides (LPSs), complex formation of Gb4 with TLR4/MT2 resulted in the disturbance of LPS toxicity on the surface of endothelial cells [63] (Fig. 2). This fact means that Gb4 could protect TLR4/MT2-mediated signals leading to NF- κ B activation and resultant inflammatory cytokine production such as TNF α and IL-6. These functions of Gb4 were also observed to protect septic shock in mice [63]. The complex formation seemed to occur in membrane lipid rafts based on the selective binding of TLR4/MT2 with Gb4 containing only saturated fatty acids.

Diverse effects of disialyl glycosphingolipids on cancer phenotypes

During function analyses of cancer-associated glycosphingolipids, it has been elucidated that disialylated

gangliosides, particularly disialylated structures with tandem mode often confer malignant phenotypes in normal cells, and/or enhancement of malignant properties of cancer cells [64] in various kinds of tumors (Figs. 1 and 3). For example, gangliosides GD3 and GD2 are involved in the enhancement of cancer phenotypes such as increased proliferation, invasion, cell adhesion to extracellular matrices, and cell motility. However, the effects of those gangliosides on the phenotypes are not necessarily same, and the modes of action also seem to be not identical among cancer lineages [65]. Depending on the molecular profiles expressed on the cell membrane and in the cytoplasm of the individual cells and tissues, the expression of cancer-associated gangliosides affects the features of membrane microdomains, glycolipid-enriched microdomain (GEM)/rafts [66, 67], and modulates the nature of individual cells [68].

In the case of melanoma cells, we reported that adaptor molecules such as p130Cas, paxillin, and focal adhesion kinase (FAK) were phosphorylated at tyrosine residue, and elucidated that they are actually involved in the increased cell proliferation and invasion under GD3 expression [69, 70]. Furthermore, during cell adhesion, integrins were also involved in the regulation of cell signaling via FAK and Src family kinase [71]. The tight linkage of integrins and FAK was also previously reported in small cell lung cancer (SCLC) cells. Anti-ganglioside GD2 monoclonal antibodies induced apoptosis of SCLC cells based on anoikis [33]. SCLC cells treated with anti-GD2 monoclonal antibodies showed reduced phosphorylation levels of FAK during the cell detachment from culture plates [72]. On the other hand, osteosarcoma cells showed increased cell invasion and motility under expression of GD3 and GD2 [30]. Interestingly, cell adhesion to extracellular matrix such as collagen 1 was strongly reduced in GD3/GD2-expressing cells, reflecting their high motility. This was contrastive with results of melanomas with high expression of GD3 [69]. Indeed, melanoma cells showed increased cell adhesion under GD3 expression [71].

Involvement of monosialyl glycosphingolipids in the suppression of cancer phenotypes

As for effects of monosialylated glycosphingolipids (or a-series gangliosides) on the cell phenotypes, similar suppressive effects on the cell signals were shown in various tumor systems (Figs. 1 and 3). First of all, a rat pheochromocytoma line, PC12, showed contrastive effects on the neural differentiation and NGF-TrkA signaling between GM1 expression [73] and GD3 expression [74]. In a mouse fibroblast cell line, SWISS-3T3, expression of GM1/GD1b

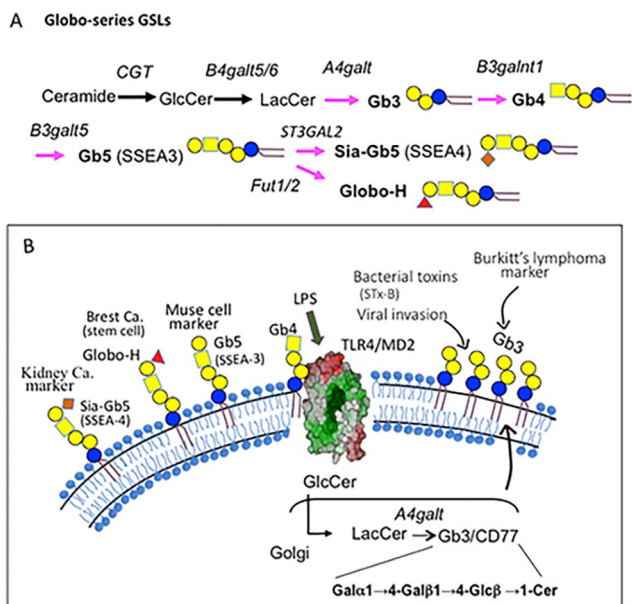


Fig. 2 Globo-series glycosphingolipids. A, Synthetic pathway of globo-series glycolipids via Gb3 with A4GALT. B, Main globo-series glycolipids with unique expression and/or functions reported so far

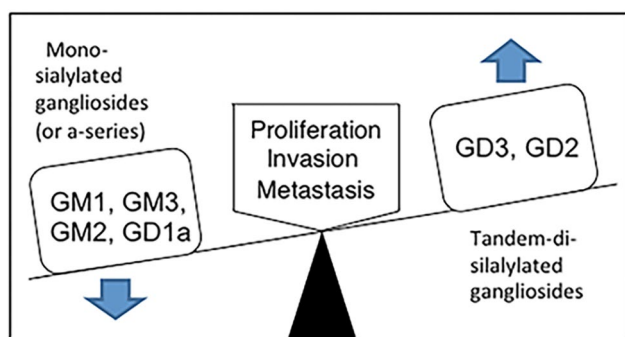


Fig. 3 Disialylated gangliosides enhance malignant properties, and monosialylated gangliosides suppress malignant properties of cancer cells

synthase resulted in the suppressed cell growth and platelet-derived growth factor (PDGF)-induced signals with changes of lipid rafts localization of PDGF receptor [75].

In a mouse Lewis lung cancer cell line, P29, transfection of GM2/GD2 synthase cDNA resulted in the neo-expression of GM2, leading to the reduced tumor phenotypes and suppressed activation levels of FAK [76]. By establishing high-metastatic Lewis lung cancer sublines, Zhang et al. found that high-metastatic sublines expressed reduced levels of GM1/GD1a compared with the low-metastatic parent line. Functional roles of GM1 in the suppression of metastasis were demonstrated using GM1-suppressed transfectants with RNAi-expression plasmid [77]. The reduced GM1 level resulted in the recruitment of MMP-9 and integrin molecules to GEM/rafts, leading to more aggressive

phenotypes. A virus-induced osteosarcoma study showed that GD1a also suppressed malignant properties [78], suggesting a-series gangliosides generally exert suppressive actions similarly with monosialylated gangliosides (Fig. 3).

As for malignant melanoma, a B16 mouse melanoma subline B78 was transfected with GM2/GD2 synthase cDNA, resulting in the reduced proliferation [79]. In human melanoma cells, GM1/GD1b synthase cDNA was introduced in SK-MEL-73 melanoma cell line, leading to the reduced cell growth and invasion activity. They also showed dispersed ganglioside distribution on the cell surface compared with the parent line that expressed mainly GD3 and GD2 [80]. Thus, not only GM2 and GM1, but GM3 also suppressed tumor cell mobility and malignancy [81].

All these findings observed in various cancer cells or normal cells indicated that the expression of monosialylated gangliosides (or a-series gangliosides) often lead cells to less malignant or milder phenotypes [64]. In particular, expression of GM1 results in the attenuation of malignant cell signals and malignant phenotypes as shown in Fig. 3. Suppressive function of GM1 on the signal activation was also observed in the isolated molecular clusters *in vitro* in addition to the phenotypic changes of cells [82]. Regulatory functions of the individual gangliosides were comprehensively compared using various glyco-remodeling melanoma lines originated from a subline of SK-MEL-28 [83]. These results were recently also recapitulated using genetically modified mice of ganglioside synthase genes [84, 85].

Membrane microdomains such as lipid rafts and GEM

In order to investigate molecular mechanisms of biological functions of glycosphingolipids, it seems critical to identify molecules that interact with and/or physically associate with glycosphingolipids. This is because glycosphingolipids are expressed on the outer layer of lipid bilayer membrane, lacking intra-cytoplasmic region [86].

Kai Simons proposed unique membrane microdomains, lipid rafts consisting of cholesterol, sphingomyelin, and GPI-anchored proteins [87]. This membrane microdomains are resistant to non-ionic detergent such as Triton X-100, and majority of glycosphingolipids are enriched in this lipid rafts. Hakomori defined this microdomain as glycolipid-enriched microdomain (GEM), and reported that GEM plays important roles as a platform of cell signaling [88, 89] (Fig. 4). For example, ganglioside GM3 exists in GEM/rafts, forming complexes with various tetraspanins [90]. Although mechanisms for molecular clustering in GEM/rafts are not completely understood, dynamic behaviors of raft-constituent molecules were demonstrated [91].

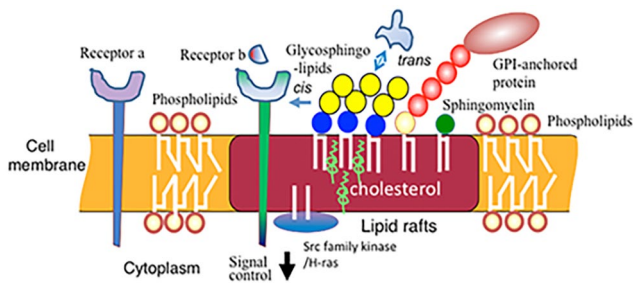


Fig. 4 Fundamental structure of lipid rafts. Glycosphingolipids regulate architecture and functions of lipid rafts, cell signaling and finally cell fates. Various molecules enriched in lipid rafts are shown. Membrane receptors sometimes shift their location (Receptor a/b) depending on the conditions of lipid rafts (mainly glycolipids)

Ultra-high-resolution imaging revealed that dimer formation of identical gangliosides might be a primary unit for the formation of GEM/rafts [92].

Analyses of intracellular localization of glycolipid-associated membrane molecules revealed that combination of extracellular stimulations and glycosphingolipids expressed on the membrane determine their location inside/outside of GEM/rafts (Fig. 5). In particular, differences in fine structures of sugar moiety seem to modulate the localization of membrane molecules inside/outside of GEM/rafts. They seem to be critical for the regulation of the composition of the microdomains [71, 93], leading to the determination of the quality and quantity of cell signaling and resulting cell fates [94] (Fig. 5).

Trials to identify interacting molecules with cancer-associated gangliosides

Importance of gangliosides in the regulation of membrane organization and function has been reported [95]. Molecular clustering of cancer-associated glycosphingolipids

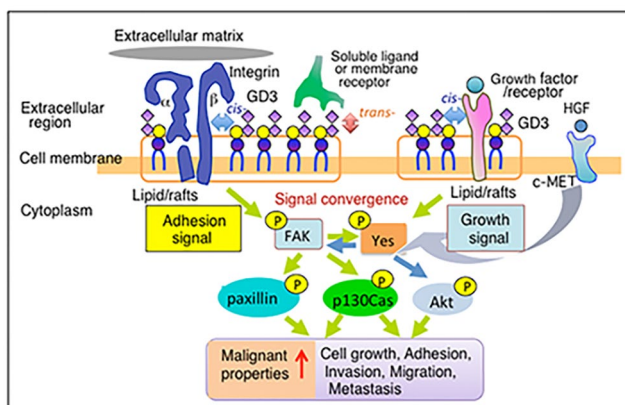


Fig. 5 Molecular complex formation at membrane lipid rafts, regulating cell signaling. An example of ganglioside GD3 in melanoma cells

and other molecules with physical association on the cell membrane (in a horizontal manner), and also with vertical connection has been elucidated so far. Enzyme-mediated activation of radical sources (EMARS), that was developed by Honke and Kotani [96], is a very efficient approach to identify neighboring molecules that exist around cancer-associated glycosphingolipids within 100~300 nm on the cell membrane (Fig. 6, upper). This approach has enabled us to raise candidate molecules that could be physically associated with the cancer-associated glycolipids in the vicinity on the membrane of living cells [97].

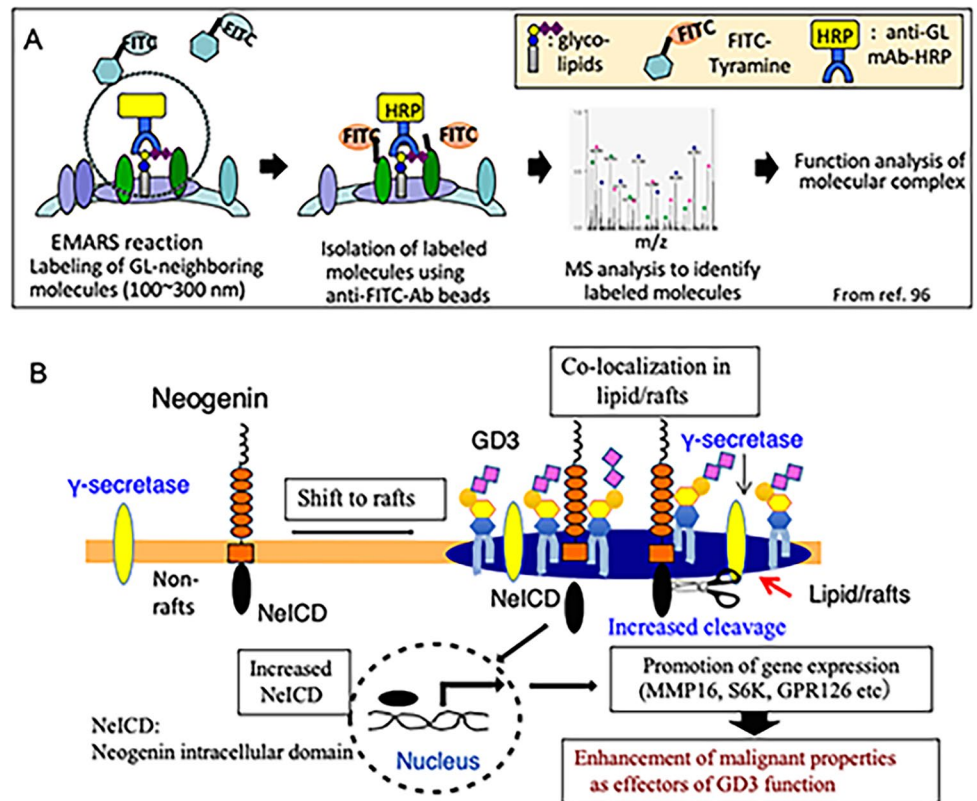
Among molecules that are detected both in lipid rafts and GD3-targeted EMARS-labeled groups in melanoma cells, only Neogenin was identified in GD3-positive cells [97]. Neogenin was found in lipid rafts of only GD3-expressing cells, but not of GD3-negative cells. GD3 expression resulted in the recruitment of Neogenin, and also of γ -secretase to lipid rafts, leading to the increased levels of intracytoplasmic domain (ICD) of Neogenin [98]. These results demonstrated the mechanisms by which cancer-associated GD3 enhances malignant phenotypes via promoting expression of various signaling molecule genes by the action of Neogenin ICD as a transcription factor [98] (Fig. 6, lower). In glioma cells, PDGF-R α was identified as a GD3-associated membrane molecule by EMARS-MS approach [99]. Cooperation of GD3 and PDGF-R α resulted in the activation of Yes kinase and subsequent activation of paxillin [99]. In SCLC cells, ASCT2 was identified as a GD2-associated molecule and localized in lipid rafts, leading to the activation of p70S6K1 and S6 etc. [100]. Actually, co-immunoprecipitation combined with immunoblotting revealed formation of molecular complexes consisting of the target glycosphingolipids and associated membrane molecules on the cell surface, and also intra-cytoplasmic proteins such as FAK, and Src-family kinases.

Thus, EMARS/MS approach has enabled us to clarify the molecular complex formation on the cell surface in a horizontal manner. It can, however, also provide us with triggering cue to clarify vertical molecular sequences beginning from cancer-associated glycosphingolipids.

Application of results of signal studies for the cancer treatments

As described previously, a number of trials have been performed to apply mouse and/or human monoclonal antibodies reactive with cancer-associated gangliosides [11, 14] for melanoma treatment. Anti-GD3 mAb, R24, was the first example to be administrated in patients with malignant melanomas [13]. Some cases showed excellent results. Human anti-ganglioside mAbs were also tried to treat melanoma

Fig. 6 EMARS/MS analysis to define glycolipid-associated molecules on the living membrane. A, A schema for EMARS/MS. B, an example of Neogenin defined by GD3-targeted EMARS



patients [101]. Anti-GD2 mAbs have been rigorously studied for their effects in the treatments of mouse melanomas [102], human neuroblastomas [103, 104], and human melanomas [105]. As for neutral glycolipids, globo H has been shown to be a crucial glycolipid in breast cancers, and vaccine therapy with globo H-KLH has been rigorously tried [104]. They showed good results in some cases, but not in all cases tried. Anti-GD2 mAbs showed significant effects in extending remission intervals of neuroblastoma cases when administered during maintenance period of the remission [103].

Generally, immune therapy of cancers toward cancer-associated glycosphingolipid antigens can be justified by the tumor-specific expression patterns [7], e.g. class 1 and class 2 group. Therefore, tumor-specific carbohydrate structures exclusively found on malignant cells alone have been required [7], although relatively high levels in malignant cells have been also accepted as shown in anti-CD20/CD19 therapy of B-cell malignancies [106].

To eradicate malignant tumors, various novel strategies have been constructed based on the expression and function analyses of cancer-associated glycosphingolipids, and been challenged. GD3 and GD2 play roles with individual cooperative membrane molecules, and specifically activate various signals that are essential for the malignant phenotypes of cancers. Therefore, any signaling molecules that are located under cancer-associated gangliosides to promote

malignant properties, can be targets for molecular therapy of cancers [107]. As fundamental studies for the substantial basis for anti-glyco-machinery therapy, anti-GD3 synthase study was tried by using siRNA and shRNAi expression plasmids of GD3 synthase gene in lung cancer cells [108]. Even cell apoptosis could be induced by the transfection of anti-GD3 synthase siRNA, and slower tumor growth was observed *in vivo* in severe combined immunodeficiency mice by introduction of the expression vector of shRNAi of GD3 synthase [108].

siRNA(s) mixed with atello-collagen was applied for the treatment of human malignant melanomas grafted on nu/nu mice [109]. Combined siRNAs against p130Cas and paxillin were more effective than single gene siRNA to reduce the tumor size in mice. Thus, novel cancer therapy targeting various molecules forming signaling domains of cancer-associated gangliosides will be tried in the near future [110, 111].

Future scope

Although antibody therapies towards cancer-associated glycolipids have been tried for various cancers such as malignant melanomas, neuroblastomas, and lung cancers, resultant effects are not very good as expected [112]. The reason for these unsatisfactory results seems to be due to

insufficient antibody effects to completely kill cancer cells, and loss of target glycolipid antigens on the cell surface leading to escape from antibody attack. Therefore, a number of trials to increase the efficiency of antibody therapies have been challenged [113]. In order to overcome these defects of antibody therapies, more efficient immunological effector cells have been expected. One of solutions for these difficulties might be immune therapy using chimeric antigen receptor (CAR)-expressing T cells or NK cells toward cancer-associated glycolipids [114].

Now, cancer-associated carbohydrate antigens are considered to be included in class 2 tumor antigens defined by Old [7]. Merit of class 2 antigens as targets of cancer immunotherapy can be summarized as follows; (1) Easy to construct therapeutic strategy because of common presence among similar cancers compared with class 1 individual antigens, i.e., neo-antigens. (2) It is possible to consider as targets of therapy depending on the relative abundance of antigens on cancers, while those antigens are expressed in particular sites, stage of life, or at lower levels in normal tissues. (3) Actually, many successful examples have been reported or on-going.

Acknowledgements We thank Ms S. Yamamoto, Y. Kitaura, T. Ito, and Y. Imao for excellent technical assistance. We also thank Ms M. Kojima for a nice secretarial help. This study was supported by Grants-in-Aids from the Ministry of Education, Culture, Sports and Technology of Japan (MEXT)(18H02628, 19K07393, 19K22518, 21K06828, 21H02699) and by JST-CREST (Grant Number: JPMJCR17H2).

Declarations

Conflict of interest The authors declare that they have no conflicts of interest.

References

- Old, L.J., Boyse, E.A.: Antigenic properties of experimental leukemias. i. serological studies in vitro with spontaneous and radiation-induced leukemias. *J. Natl. Cancer Inst.* **31**, 977–995 (1963)
- Old, L.J., Boyse, E.A., Stockert, E.: The G (Gross) leukemia antigen. *Cancer Res.* **25**, 813–819 (1965)
- Old, L.J., Stockert, E.: Immunogenetics of cell surface antigens of mouse leukemia. *Annu. Rev. Genet.* **11**, 127–160 (1977). <https://doi.org/10.1146/annurev.ge.11.120177.001015>
- Klein, G.: Tumor-specific transplantation antigens: G. H. A. Clowes memorial lecture. *Cancer Res.* **28**, 625–635 (1968)
- Klein, G., Sjogren, H.O., Klein, E., Hellstrom, K.E.: Demonstration of resistance against methylcholanthrene-induced sarcomas in the primary autochthonous host. *Cancer Res.* **20**, 1561–1572 (1960)
- Morton, D.L., Miller, G.F., Wood, D.A.: Demonstration of tumor-specific immunity against antigens unrelated to the mammary tumor virus in spontaneous mammary adenocarcinomas. *J. Natl. Cancer Inst.* **42**, 289–301 (1969)
- Old, L.J.: Cancer Immunology: the search for specificity – G.H.A. Clowes Memorial Lecture. *Cancer Res.* **41**, 361–375 (1981)
- Watanabe, T., Pukel, C.S., Takeyama, H., Lloyd, K.O., Shiku, H., Li, L.T., Travassos, L.R., Oettgen, H.F., Old, L.J.: Human melanoma antigen AH is an autoantigenic ganglioside related to GD2. *J. Exp. Med.* **156**, 1884–1889 (1982). <https://doi.org/10.1084/jem.156.6.1884>
- Dippold, W.G., Lloyd, K.O., Li, L.T., Ikeda, H., Oettgen, H.F., Old, L.J.: Cell surface antigens of human malignant melanoma: definition of six antigenic systems with mouse monoclonal antibodies. *Proc. Natl. Acad. Sci. U.S.A.* **77**, 6114–6118: (1980). <https://doi.org/10.1073/pnas.77.10.6114>
- Pukel, C.S., Lloyd, K.O., Travassos, L.R., Dippold, W.G., Oettgen, H.F., Old, L.J.: GD3, a prominent ganglioside of human melanoma. Detection and characterization by mouse monoclonal antibody. *J. Exp. Med.* **155**, 1133–1147 (1982). <https://doi.org/10.1084/jem.155.4.1133>
- Yamaguchi, H., Furukawa, K., Fortunato, S.R., Livingston, P.O., Lloyd, K.O., Oettgen, H.F., Old, L.J.: Cell-surface antigens of melanoma recognized by human monoclonal antibodies. *Proc. Natl. Acad. Sci. U.S.A.* **84**, 2416–2420: (1987). <https://doi.org/10.1073/pnas.84.8.2416>
- Schrump, D.S., Furukawa, K., Yamaguchi, H., Lloyd, K.O., Old, L.J.: Recognition of galactosylgloboside by monoclonal antibodies derived from patients with primary lung cancer. *Proc. Natl. Acad. Sci. U.S.A.* **85**, 4441–4445: (1988). <https://doi.org/10.1073/pnas.85.12.4441>
- Houghton, A.N., Mintzer, D., Cordon-Cardo, C., Welt, S., Fliegel, B., Vadhan, S., Carswell, E., Melamed, M.R., Oettgen, H.F., Old, L.J.: Mouse monoclonal IgG3 antibody detecting GD3 ganglioside: a phase I trial in patients with malignant melanoma. *Proc. Natl. Acad. Sci. U.S.A.* **82**, 1242–1246: (1985). <https://doi.org/10.1073/pnas.82.4.1242>
- Irie, R.F., Morton, D.L.: Regression of cutaneous metastatic melanoma by intralesional injection with human monoclonal antibody to ganglioside GD2. *Proc. Natl. Acad. Sci. U.S.A.* **83**, 8694–8698: (1986). <https://doi.org/10.1073/pnas.83.22.8694>
- Cahan, L.D., Irie, R.F., Singh, R., Cassidenti, A., Paulson, J.C.: Identification of a human neuroectodermal tumor antigen (OFA-I-2) as ganglioside GD2. *Proc. Natl. Acad. Sci. U.S.A.* **79**, 7629–7633: (1982). <https://doi.org/10.1073/pnas.79.24.7629>
- Gahmberg, C.G., Hakomori, S.: Surface carbohydrates of hamster fibroblasts. I. Chemical characterization of surface-labeled glycosphingolipids and aspecific ceramide tetrasaccharide for transformants. *J. Biol. Chem.* **250**, 2438–2446 (1975)
- Hakomori, S.: Tumor-associated glycolipid antigens, their metabolism and organization. *Chem. Phys. Lipids.* **42**, 209–233 (1986). [https://doi.org/10.1016/0009-3084\(86\)90054-x](https://doi.org/10.1016/0009-3084(86)90054-x)
- Lloyd, K.O.: Humoral immune responses to tumor-associated carbohydrate antigens. *Semin. Cancer Biol.* **2**, 421–431 (1991)
- Lloyd, K.O., Old, L.J.: Human monoclonal antibodies. *Cancer Res.* **49**, 3445–3451 (1989)
- Indelicato, R., Zulueta, A., Caretti, A., Trinchera, M.: Complementary Use of Carbohydrate Antigens Lewis a, Lewis b, and Sialyl-Lewis a (CA19.9 Epitope) in Gastrointestinal Cancers: Biological Rationale towards a Personalized Clinical Application. *Cancers.* **12**, 1509 (2020). <https://doi.org/10.3390/cancers12061509>
- Furukawa, K., Lloyd, K.O.: Gangliosides in melanoma. In: Ferrone, S. (ed.) *Human melanoma: from basic research to clinical application*. pp. 15–30. Springer, Heidelberg (1990)
- Portoukalian, J., Zwingelstein, G., Doré, J.F.: Lipid composition of human malignant melanoma tumors at various levels of malignant growth. *Eur. J. Biochem.* **94**, 19–23 (1979). <https://doi.org/10.1111/j.1432-1033.1979.tb12866.x>

23. Carubia, J.M., Yu, R.K., Macala, L.J., Kirkwood, J.M., Varga, J.M.: Gangliosides of normal and neoplastic human melanocytes. *Biochem. Biophys. Res. Commun.* **120**, 500–504 (1984). [https://doi.org/10.1016/0006-291x\(84\)91282-8](https://doi.org/10.1016/0006-291x(84)91282-8)
24. Saito, M., Yu, R.K., Cheung, N.K.: Ganglioside GD2 specificity of monoclonal antibodies to human neuroblastoma cell. *Biochem. Biophys. Res. Commun.* **127**, 1–7 (1985). [https://doi.org/10.1016/s0006-291x\(85\)80117-0](https://doi.org/10.1016/s0006-291x(85)80117-0)
25. Schultz, G., Cheresch, D.A., Varki, N.M., Yu, A., Staffileno, L.K., Reisfeld, R.A.: Detection of ganglioside GD2 in tumor tissues and sera of neuroblastoma patients. *Cancer Res.* **44**, 5914–5920 (1984)
26. Fredman, P., von Holst, H., Collins, V.P., Ammar, A., Dellheden, B., Wahren, B., Granholm, L., Svennerholm, L.: Potential ganglioside antigens associated with human gliomas. *Neuro. Res.* **8**, 123–126 (1986). <https://doi.org/10.1080/01616412.1986.11739744>
27. Wikstrand, C.J., Fredman, P., Svennerholm, L., Bigner, D.D.: Detection of glioma-associated gangliosides GM2, GD2, GD3, 3'-isoLM1 3',6'-isoLD1 in central nervous system tumors in vitro and in vivo using epitope-defined monoclonal antibodies. *Prog. Brain Res.* **101**, 213–223 (1994). [https://doi.org/10.1016/s0079-6123\(08\)61951-2](https://doi.org/10.1016/s0079-6123(08)61951-2)
28. Kawai, K., Takahashi, H., Watarai, S., Ishizu, H., Fukai, K., Tanabe, Y., Nose, S., Kuroda, S.: Occurrence of ganglioside GD3 in neoplastic astrocytes. An immunocytochemical study in humans. *Virchows Arch.* **434**, 201–205 (1999). <https://doi.org/10.1007/s004280050328>
29. Vukelić, Z., Kalanj-Bognar, S., Froesch, M., Bindila, L., Radić, B., Allen, M., Peter-Katalinić, J., Zamfir, A.D.: Human gliosarcoma-associated ganglioside composition is complex and distinctive as evidenced by high-performance mass spectrometric determination and structural characterization. *Glycobiology.* **17**, 504–515 (2007). <https://doi.org/10.1093/glycob/cwm012>
30. Shibuya, H., Hamamura, K., Hotta, H., Matsumoto, Y., Nishida, Y., Hattori, H., Furukawa, K., Ueda, M., Furukawa, K.: Enhancement of malignant properties of human osteosarcoma cells with disialyl gangliosides GD2/GD3. *Cancer Sci.* **103**, 1656–1664 (2012). <https://doi.org/10.1111/j.1349-7006.2012.02344.x>
31. Azuma, K., Tanaka, M., Uekita, T., Inoue, S., Yokota, J., Ouchi, Y., Sakai, R.: Tyrosine phosphorylation of paxillin affects the metastatic potential of human osteosarcoma. *Oncogene.* **24**, 4754–4764 (2005). <https://doi.org/10.1038/sj.onc.1208654>
32. Cheresch, D.A., Rosenberg, J., Mujoo, K., Hirschowitz, L., Reisfeld, R.A.: Biosynthesis and expression of the disialoganglioside GD2, a relevant target antigen on small cell lung carcinoma for monoclonal antibody-mediated cytotoxicity. *Cancer Res.* **46**, 5112–5118 (1986)
33. Yoshida, S., Fukumoto, S., Kawaguchi, H., Sato, S., Ueda, R., Furukawa, K.: Ganglioside G(D2) in small cell lung cancer cell lines: enhancement of cell proliferation and mediation of apoptosis. *Cancer Res.* **61**, 4244–4252 (2001)
34. Siddiqui, B., Buehler, J., DeGregorio, M.W., Macher, B.A.: Differential expression of ganglioside GD3 by human leukocytes and leukemia cells. *Cancer Res.* **44**, 5262–5265 (1984)
35. Merritt, W.D., Casper, J.T., Lauer, S.J., Reaman, G.H.: Expression of GD3 ganglioside in childhood T-cell lymphoblastic malignancies. *Cancer Res.* **47**, 1724–1730 (1987)
36. Furukawa, K., Akagi, T., Nagata, Y., Yamada, Y., Shimotohno, K., Cheung, N.K., Shiku, H., Furukawa, K.: GD2 ganglioside on human T-lymphotropic virus type I-infected T cells: possible activation of beta-1,4-N-acetylgalactosaminyltransferase gene by p40tax. *Proc. Natl. Acad. Sci. U.S.A.* **90**, 1972–1976 (1993). <https://doi.org/10.1073/pnas.90.5.1972>
37. Okada, M., Furukawa, K., Yamashiro, S., Yamada, Y., Haraguchi, M., Horibe, K., Kato, K., Tsuji, Y., Furukawa, K.: High expression of ganglioside alpha-2,8-sialyltransferase (GD3 synthase) gene in adult T-cell leukemia cells unrelated to the gene expression of human T-lymphotropic virus type I. *Cancer Res.* **56**, 2844–2848 (1996)
38. Cazet, A., Bobowski, M., Rombouts, Y., Lefebvre, J., Steenackers, A., Popa, I., Guérardel, Y., Le Bourhis, X., Tulasne, D., Delannoy, P.: The ganglioside G(D2) induces the constitutive activation of c-Met in MDA-MB-231 breast cancer cells expressing the G(D3) synthase. *Glycobiology.* **22**, 806–816 (2012). <https://doi.org/10.1093/glycob/cws049>
39. De Giorgi, U., Cohen, E.N., Gao, H., Mego, M., Lee, B.N., Lodhi, A., Cristofanilli, M., Lucci, A., Reuben, J.M.: Mesenchymal stem cells expressing GD2 and CD271 correlate with breast cancer-initiating cells in bone marrow. *Cancer Biol. Ther.* **11**, 812–815 (2011). <https://doi.org/10.4161/cbt.11.9.15178>
40. Wiels, J., Fellous, M., Tursz, T.: Monoclonal antibody against a Burkitt lymphoma-associated antigen. *Proc. Natl. Acad. Sci. U.S.A.* **78**, 6485–6488 (1981). <https://doi.org/10.1073/pnas.78.10.6485>
41. Nagata, Y., Yamashiro, S., Yodoi, J., Lloyd, K.O., Shiku, H., Furukawa, K.: Expression cloning of beta 1,4 N-acetylgalactosaminyltransferase cDNAs that determine the expression of GM2 and GD2 gangliosides. *J. Biol. Chem.* **267**, 12082–12089 (1992)
42. Haraguchi, M., Yamashiro, S., Yamamoto, A., Furukawa, K., Takamiya, K., Lloyd, K.O., Shiku, H., Furukawa, K.: Isolation of GD3 synthase gene by expression cloning of GM3 alpha-2,8-sialyltransferase cDNA using anti-GD2 monoclonal antibody. *Proc. Natl. Acad. Sci. U.S.A.* **91**, 10455–10459 (1994). <https://doi.org/10.1073/pnas.91.22.10455>
43. Nara, K., Watanabe, Y., Maruyama, K., Kasahara, K., Nagai, Y., Sanai, Y.: Expression cloning of a CMP-NeuAc:NeuAc alpha 2-3Gal beta 1-4Glc beta 1-1'Cer alpha 2,8-sialyltransferase (GD3 synthase) from human melanoma cells. *Proc. Natl. Acad. Sci. U.S.A.* **91**, 7952–7956 (1994). <https://doi.org/10.1073/pnas.91.17.7952>
44. Sasaki, K., Kurata, K., Kojima, N., Kurosawa, N., Ohta, S., Hanai, N., Tsuji, S., Nishi, T.: Expression cloning of a GM3-specific alpha-2,8-sialyltransferase (GD3synthase). *J. Biol. Chem.* **269**, 15950–15956 (1994)
45. Miyazaki, H., Fukumoto, S., Okada, M., Hasegawa, T., Furukawa, K.: Expression cloning of rat cDNA encoding UDP-galactose:GD2 beta1,3-galactosyl-transferase that determines the expression of GD1b/GM1/GA1. *J. Biol. Chem.* **272**, 24794–24799 (1997). <https://doi.org/10.1074/jbc.272.40.24794>
46. Furukawa, K., Tokuda, N., Okuda, T., Tajima, O., Furukawa, K.: Glycosphingolipids in engineered mice: insights into function. *Semin Cell. Dev. Biol.* **15**, 389–396 (2004). <https://doi.org/10.1016/j.semcdb.2004.03.006>
47. Stanley, P.: What Have We Learned from Glycosyltransferase Knockouts in Mice? *J. Mol. Biol.* **428**, 3166–3182 (2016). <https://doi.org/10.1016/j.jmb.2016.03.025>
48. Yamakawa, T., Iida, T.: Globotetraosylceramide (Gb4) Immunochemical study on the red blood cells. I. Globoside, as the agglutinin of the ABO system on erythrocytes. *Jpn J. Exp. Med.* **23**, 327–331 (1953)
49. Yamakawa, T., Yokoyama, S., Handa, N.: Chemistry of lipids of posthemolytic residue or stroma of erythrocytes. XI. Structure of globoside, the main mucolipid of human erythrocytes. *J. Biochem.* **53**, 28–36 (1963). <https://doi.org/10.1093/oxfordjournals.jbchem.a127654>
50. Furukawa, K., Iwamura, K., Uchikawa, M., Sojka, B.N., Wiels, J., Okajima, T., Urano, T., Furukawa, K.: Molecular basis for the p phenotype. Identification of distinct and multiple mutations in the alpha 1,4-galactosyltransferase gene in Swedish and Japanese individuals. *J. Biol. Chem.* **275**, 37752–37756 (2000). <https://doi.org/10.1074/jbc.C000625200>

51. Iwamura, K., Furukawa, K., Uchikawa, M., Sojka, B.N., Kojima, Y., Wiels, J., Shiku, H., Urano, T., Furukawa, K.: The blood group P1 synthase gene is identical to the Gb3/CD77 synthase gene. A clue to the solution of the P1/P2/p puzzle. *J. Biol. Chem.* **278**, 44429–44438 (2003). <https://doi.org/10.1074/jbc.M301609200>
52. Kannagi, R., Cochran, N.A., Ishigami, F., Hakomori, S., Andrews, P.W., Knowles, B.B., 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.* **2**, 2355–2361 (1983)
53. Wakao, S., Kushida, Y., Dezawa, M.: Basic Characteristics of Muse Cells. *Adv. Exp. Med. Biol.* **1103**, 13–41 (2018). https://doi.org/10.1007/978-4-431-56847-6_2
54. Mangeney, M., Richard, Y., Coulaud, D., Tursz, T., Wiels, J.: CD77: an antigen of germinal center B cells entering apoptosis. *Eur. J. Immunol.* **21**, 1131–1140 (1991). doi:<https://doi.org/10.1002/eji.1830210507>
55. Kovbasnjuk, O., Mourtazina, R., Baibakov, B., Wang, T., Elowsky, C., Choti, M.A., Kane, A., Donowitz, M.: The glycosphingolipid globotriaosylceramide in the metastatic transformation of colon cancer. *Proc. Natl. Acad. Sci. U. S. A.* **102**, 19087–19092: (2005) doi: <https://doi.org/10.1073/pnas.0506474102>
56. Stimmer, L., Dehay, S., Nemati, F., Massonnet, G., Richon, S., Decaudin, D., Klijanienko, J., Johannes, L.: Human breast cancer and lymph node metastases express Gb3 and can be targeted by STxB-vectorized chemotherapeutic compounds. *BMC Cancer.* **14**, 916 (2014). doi:<https://doi.org/10.1186/1471-2407-14-916>
57. Geyer, P.E., Maak, M., Nitsche, U., Perl, M., Novotny, A., Slotta-Huspenina, J., Dransart, E., Holtorf, A., Johannes, L., Janssen, K.P.: Gastric Adenocarcinomas Express the Glycosphingolipid Gb3/CD77: Targeting of Gastric Cancer Cells with Shiga Toxin B-Subunit. *Mol. Cancer Ther.* **15**, 1008–10017 (2016). doi:<https://doi.org/10.1158/1535-7163.MCT-15-0633>
58. Senda, M., Ito, A., Tsuchida, A., Hagiwara, T., Kaneda, T., Nakamura, Y., Kasama, K., Kiso, M., Yoshikawa, K., Katagiri, Y., Ono, Y., Ogiso, M., Urano, T., Furukawa, K., Oshima, S., Furukawa, K.: Identification and expression of a sialyltransferase responsible for the synthesis of disialylgalactosylglobo side in normal and malignant kidney cells: downregulation of ST6GalNAc VI in renal cancers. *Biochem. J.* **402**, 459–470 (2007). doi:<https://doi.org/10.1042/BJ20061118>
59. Chang, W.W., Lee, C.H., Lee, P., Lin, J., Hsu, C.W., Hung, J.T., Lin, J.J., Yu, J.C., Shao, L.E., Yu, J., Wong, C.H., Yu, A.L.: Expression of Globo H and SSEA3 in breast cancer stem cells and the involvement of fucosyl transferases 1 and 2 in Globo H synthesis. *Proc. Natl. Acad. Sci. U S A.* **105**, 11667–11672 (2008). doi:<https://doi.org/10.1073/pnas.0804979105>
60. Kojima, Y., Fukumoto, S., Furukawa, K., Okajima, T., Wiels, J., Yokoyama, K., Suzuki, Y., Urano, T., Ohta, M., Furukawa, K.: Molecular cloning of globotriaosylceramide/CD77 synthase, a glycosyltransferase that initiates the synthesis of globo series glyco-sphingolipids. *J. Biol. Chem.* **275**, 15152–15156 (2000). <https://doi.org/10.1074/jbc.M909620199>
61. Furukawa, K., Yokoyama, K., Sato, T., Wiels, J., Hirayama, Y., Ohta, M., Furukawa, K.: Gb3 and its derivatives in cultured cells Expression of the Gb3/CD77 synthase gene in megakaryoblastic leukemia cells: implication in the sensitivity to verotoxins. *J. Biol. Chem.* **277**, 11247–11254 (2002). <https://doi.org/10.1074/jbc.M109519200>
62. Okuda, T., Tokuda, N., Numata, S., Ito, M., Ohta, M., Kawamura, K., Wiels, J., Urano, T., Tajima, O., Furukawa, K., Furukawa, K.: Targeted disruption of Gb3/CD77 synthase gene resulted in the complete deletion of globo-series glycosphingolipids and loss of sensitivity to verotoxins. *J. Biol. Chem.* **281**(15), 10230–10235 (2006). <https://doi.org/10.1074/jbc.M600057200>
63. Kondo, Y., Ikeda, K., Tokuda, N., Nishitani, C., Ohto, U., Akashi-Takamura, S., Ito, Y., Uchikawa, M., Kuroki, Y., Taguchi, R., Miyake, K., Zhang, Q., Furukawa, K., Furukawa, K.: TLR4-MD-2 complex is negatively regulated by an endogenous ligand, globotetraosyl-ceramide. *Proc. Natl. Acad. Sci. U.S.A.* **110**, 4714–4719: (2013). <https://doi.org/10.1073/pnas.1218508110>
64. Furukawa, K., Ohkawa, Y., Yamauchi, Y., Hamamura, K., Ohmi, Y., Furukawa, K.: Fine tuning of cell signals by glycosylation. *J. Biochem.* **151**, 573–578 (2012). <https://doi.org/10.1093/jb/mvs043>
65. Furukawa, K., Hamamura, K., Ohkawa, Y., Ohmi, Y., Furukawa, K.: Disialyl ganglio-sides enhance tumor phenotypes with differential modalities. *Glycoconj. J.* **29**, 579–584 (2012). <https://doi.org/10.1007/s10719-012-9423-0>
66. Hakomori, S.: Cancer-associated glycosphingolipid antigens: their structure, organiza-tion, and function. *Acta Anat. (Basel).* **161**(1–4), 79–90 (1998). <https://doi.org/10.1159/000046451>
67. Hakomori, S., Yamamura, S., Handa, A.K.: Signal transduction through glyco(sphingo-lipids. Introduction and recent studies on glyco(sphingo)lipid-enriched micro-domains. *Ann. NY Acad. Sci.* **845**, 1–10 (1998). <https://doi.org/10.1111/j.1749-6632.1998.tb09657.x>
68. Hakomori, S.: Structure, organization, and function of glyco-sphingolipids in membrane. *Curr. Opin. Hematol.* **10**, 16–24 (2003). <https://doi.org/10.1097/00062752-200301000-00004>
69. Hamamura, K., Furukawa, K., Hayashi, T., Hattori, T., Nakano, J., Nakashima, H., Okuda, T., Mizutani, H., Hattori, H., Ueda, M., Urano, T., Lloyd, K.O., Furukawa, K.: Ganglioside GD3 promotes cell growth and invasion through p130Cas and paxillin in malignant melanoma cells. *Proc. Natl. Acad. Sci. U.S.A.* **102**, 11041–11046: (2005). <https://doi.org/10.1073/pnas.0503658102>
70. Hamamura, K., Tsuji, M., Ohkawa, Y., Nakashima, H., Miyazaki, S., Urano, T., Yamamoto, N., Ueda, M., Furukawa, K., Furukawa, K.: Focal adhesion kinase as well as p130Cas and paxillin is crucially involved in the enhanced malignant properties under expression of ganglioside GD3 in melanoma cells. *Biochim. Biophys. Acta.* **1780**(3), 513–519 (2008). <https://doi.org/10.1016/j.bbagen.2007.11.002>
71. Ohkawa, Y., Miyazaki, S., Hamamura, K., Kambe, M., Miyata, M., Tajima, O., Ohmi, Y., Yamauchi, Y., Furukawa, K., Furukawa, K.: Ganglioside GD3 enhances adhesion signals and augments malignant properties of melanoma cells by recruiting integrins to glycolipid-enriched microdomains. *J. Biol. Chem.* **285**, 27213–27223 (2010). <https://doi.org/10.1074/jbc.M109.087791>
72. Aixinjueluo, W., Furukawa, K., Zhang, Q., Hamamura, K., Tokuda, N., Yoshida, S., Ueda, R., Furukawa, K.: Mechanisms for the apoptosis of small cell lung cancer cells induced by anti-GD2 monoclonal antibodies: roles of anoikis. *J. Biol. Chem.* **280**, 29828–29836 (2005). <https://doi.org/10.1074/jbc.M414041200>
73. Nishio, M., Fukumoto, S., Furukawa, K., Ichimura, A., Miyazaki, H., Kusunoki, S., Urano, T., Furukawa, K.: Overexpressed GM1 suppresses nerve growth factor (NGF) signals by modulating the intracellular localization of NGF receptors and membrane fluidity in PC12 cells. *J. Biol. Chem.* **279**, 33368–33378 (2004). <https://doi.org/10.1074/jbc.M403816200>
74. Fukumoto, S., Mutoh, T., Hasegawa, T., Miyazaki, H., Okada, M., Goto, G., Furukawa, K., Urano, T.: GD3 synthase gene expression in PC12 cells results in the continuous activation of TrkA and ERK1/2 and enhanced proliferation. *J. Biol. Chem.* **275**, 5832–5838 (2000). <https://doi.org/10.1074/jbc.275.8.5832>
75. Mitsuda, T., Furukawa, K., Fukumoto, S., Miyazaki, H., Urano, T., Furukawa, K.: Overexpression of ganglioside GM1 results in the dispersion of platelet-derived growth factor receptor from glycolipid-enriched microdomains and in the suppression of cell growth signals. *J. Biol. Chem.* **277**, 11239–11246 (2002). <https://doi.org/10.1074/jbc.M107756200>

76. Chen, H.H., Fukumoto, S., Furukawa, K., Nakao, A., Akiyama, S., Urano, T., Furukawa, K.: Suppression of lung metastasis of mouse Lewis lung cancer P29 with transfection of the ganglioside GM2/GD2 synthase gene. *Int. J. Cancer*. **103**, 169–176 (2003). <https://doi.org/10.1002/ijc.10797>
77. Zhang, Q., Furukawa, K., Chen, H.H., Sakakibara, T., Urano, T., Furukawa, K.: Metastatic potential of mouse Lewis lung cancer cells is regulated via ganglioside GM1 by modulating the matrix metalloproteinase-9 localization in lipid rafts. *J. Biol. Chem.* **28**, 18145–18155 (2006). <https://doi.org/10.1074/jbc.M512566200>
78. Hyuga, S., Yamagata, S., Takatsu, Y., Hyuga, M., Nakaniishi, H., Furukawa, K., Yamagata, T.: Suppression by ganglioside GD1A of migration capability, adhesion to vitronectin and metastatic potential of highly metastatic FBJ-LL cells. *Int. J. Cancer*. **83**, 685–691 (1999). doi:[https://doi.org/10.1002/\(sici\)1097-0215\(19991126\)83:5%3C685::aid-ijc20%3E3.0.co;2-4](https://doi.org/10.1002/(sici)1097-0215(19991126)83:5%3C685::aid-ijc20%3E3.0.co;2-4)
79. Tsurifune, T., Ito, T., Li, X.J., Yamashiro, S., Okada, M., Kanematsu, T., Shiku, H., Furukawa, K.: Alteration of tumor phenotypes of B16 melanoma after genetic remodeling of the ganglioside profile. *Int. J. Oncol.* **17**, 159–165 (2000)
80. Dong, Y., Ikeda, K., Hamamura, K., Zhang, Q., Kondo, Y., Matsumoto, Y., Ohmi, Y., Yamauchi, Y., Furukawa, K., Taguchi, R., Furukawa, K.: GM1/GD1b/GA1 synthase expression results in the reduced cancer phenotypes with modulation of composition and raft-localization of gangliosides in a melanoma cell line. *Cancer Sci.* **101**, 2039–2047 (2010). <https://doi.org/10.1111/j.1349-7006.2010.01613.x>
81. Ono, M., Handa, K., Sonnino, S., Withers, D.A., Nagai, H., Hakomori, S.: GM3 ganglioside inhibits CD9-facilitated haptotactic cell motility: coexpression of GM3 and CD9 is essential in the downregulation of tumor cell motility and malignancy. *Biochemistry*. **40**, 6414–6421 (2001). doi:<https://doi.org/10.1021/bi0101998>
82. Hamamura, K., Tsuji, M., Hotta, H., Ohkawa, Y., Takahashi, M., Shibuya, H., Nakashima, H., Yamauchi, Y., Hashimoto, N., Hattori, H., Ueda, M., Furukawa, K., Furukawa, K.: Functional activation of Src family kinase yes protein is essential for the enhanced malignant properties of human melanoma cells expressing ganglioside GD3. *J. Biol. Chem.* **286**, 18526–18537 (2011). <https://doi.org/10.1074/jbc.M110.164798>
83. Ohmi, Y., Kambe, M., Ohkawa, Y., Hamamura, K., Tajima, O., Takeuchi, R., Furukawa, K., Furukawa, K.: Differential roles of gangliosides in malignant properties of melanomas. *PLoS One*. **13**, e0206881 (2018). <https://doi.org/10.1371/journal.pone.0206881>
84. Ohkawa, Y., Zhang, P., Momota, H., Kato, A., Hashimoto, N., Ohmi, Y., Bhuiyan, R.H., Farhana, Y., Natsume, A., Wakabayashi, T., Furukawa, K., Furukawa, K.: Lack of GD3 synthase (St8sia1) attenuates malignant properties of gliomas in genetically engineered mouse model. *Cancer Sci.* **112**, 3756–3768 (2021). <https://doi.org/10.1111/cas.15032>
85. Zhang, P., Ohkawa, Y., Yamamoto, S., Momota, H., Kato, A., Kaneko, K., Natsume, A., Yesmin, F., Ohmi, Y., Okajima, T., Wakabayashi, T., Furukawa, K., Furukawa, K.: St8sia1-deficiency in mice alters tumor environments of gliomas, leading to reduced disease severity. *Nagoya J. Med. Sci.* **83**, 535–549 (2021). <https://doi.org/10.18999/nagjms.83.3.535>
86. Groux-Degroote, S., Guérardel, Y., Delannoy, P.: Gangliosides: Structures, Biosynthesis, Analysis, and Roles in Cancer. *ChemBiochem*. **18**, 1146–1154 (2017). <https://doi.org/10.1002/cbic.201600705>
87. Simons, K., Ikonen, E.: Functional rafts in cell membranes. *Nature*. **387**, 569–572 (1997). <https://doi.org/10.1038/42408>
88. Hakomori, S.I.: Glycosynaptic microdomains controlling tumor cell phenotype through alteration of cell growth, adhesion, and motility. *FEBS Lett.* **584**, 1901–1906 (2010). <https://doi.org/10.1016/j.febslet.2009.10.065>
89. Simons, K., Toomre, D.: Lipid rafts and signal transduction. *Nat. Rev. Mol. Cell. Biol.* **1**, 31–39 (2000). <https://doi.org/10.1038/35036052>
90. Hakomori, S.I., Handa, K.: GM3 and cancer. *Glycoconj. J.* **32**, 1–8 (2015). <https://doi.org/10.1007/s10719-014-9572-4>
91. Simons, K., Gerl, M.J.: Revitalizing membrane rafts: new tools and insights. *Nat. Rev. Mol. Cell. Biol.* **11**, 688–699 (2010). <https://doi.org/10.1038/nrm2977>
92. Komura, N., Suzuki, K.G., Ando, H., Konishi, M., Koikeda, M., Imamura, A., Chadda, R., Fujiwara, T.K., Tsuboi, H., Sheng, R., Cho, W., Furukawa, K., Furukawa, K., Yamauchi, Y., Ishida, H., Kusumi, A., Kiso, M.: Raft-based interactions of gangliosides with a GPI-anchored receptor. *Nat. Chem. Biol.* **12**, 402–410 (2016). <https://doi.org/10.1038/nchembio.2059>
93. Yesmin, F., Bhuiyan, R.H., Ohmi, Y., Yamamoto, S., Kaneko, K., Ohkawa, Y., Zhang, P., Hamamura, K., Cheung, N.-K.V., Kotani, N., Honke, K., Okajima, T., Kambe, M., Tajima, O., Furukawa, K., Furukawa, K.: Ganglioside GD2 enhances malignant phenotypes of melanoma cells by co-operating with integrins. *Int. J. Mol. Sci.* **23**, 423 (2021). <https://doi.org/10.3390/ijms23010423>
94. Furukawa, K., Ohmi, Y., Kondo, Y., Ohkawa, Y., Hashimoto, N., Tajima, O., Furukawa, K.: The role of glycosphingolipids. Lessons from knockout mice. In: Silence, D. (ed.) *Lipid Rafts. Properties, controversies and roles in signal transduction*, pp. 1–20. Nova Publishers, New York (2014)
95. Sonnino, S., Prinetti, A.: Gangliosides as regulators of cell membrane organization and functions. *Adv. Exp. Med. Biol.* **688**, 165–184 (2010). doi:https://doi.org/10.1007/978-1-4419-6741-1_12
96. Kotani, N., Gu, J., Isaji, T., Uda, K., Taniguchi, N., Honke, K.: Biochemical visualization of cell surface molecular clustering in living cells. *Proc. Natl. Acad. Sci. U.S.A.* **105**, 7405–7409 (2008). <https://doi.org/10.1073/pnas.0710346105>
97. Hashimoto, N., Hamamura, K., Kotani, N., Furukawa, K., Kaneko, K., Honke, K., Furukawa, K.: Proteomic analysis of ganglioside-associated membrane molecules: substantial basis for molecular clustering. *Proteomics*. **12**, 3154–3163 (2012). <https://doi.org/10.1002/pmic.201200279>
98. Kaneko, K., Ohkawa, Y., Hashimoto, N., Ohmi, Y., Kotani, N., Honke, K., Ogawa, M., Okajima, T., Furukawa, K., Furukawa, K.: Neogenin, Defined as a GD3-associated Molecule by Enzyme-mediated Activation of Radical Sources, Confers Malignant Properties via Intracytoplasmic Domain in Melanoma Cells. *J. Biol. Chem.* **291**, 16630–16643 (2016). <https://doi.org/10.1074/jbc.M115.708834>
99. Ohkawa, Y., Momota, H., Kato, A., Hashimoto, N., Tsuda, Y., Kotani, N., Honke, K., Suzumura, A., Furukawa, K., Ohmi, Y., Natsume, A., Wakabayashi, T., Furukawa, K.: Ganglioside GD3 Enhances Invasiveness of Gliomas by Forming a Complex with Platelet-derived Growth Factor Receptor α and Yes Kinase. *J. Biol. Chem.* **290**, 16043–16058 (2015). <https://doi.org/10.1074/jbc.M114.635755>
100. Esaki, N., Ohkawa, Y., Hashimoto, N., Tsuda, Y., Ohmi, Y., Bhuiyan, R.H., Kotani, N., Honke, K., Enomoto, A., Takahashi, M., Furukawa, K., Furukawa, K.: ASC amino acid transporter 2, defined by enzyme-mediated activation of radical sources, enhances malignancy of GD2-positive small-cell lung cancer. *Cancer Sci.* **109**, 141–153 (2018)
101. Irie, R.F., Ollila, D.W., O'Day, S., Morton, D.L.: Phase I pilot clinical trial of human IgM monoclonal antibody to gangliosideGM3 in patients with metastatic melanoma. *Cancer Immunol. Immunother.* **53**, 110–117 (2004). <https://doi.org/10.1007/s00262-003-0436-1>
102. Becker, J.C., Varki, N., Gillies, S.D., Furukawa, K., Reisfeld, R.A.: An antibody-interleukin 2 fusion protein overcomes tumor

- heterogeneity by induction of a cellular immune response. *Proc. Natl. Acad. Sci. U S A.* **93**, 7826–7831 (1996). doi:<https://doi.org/10.1073/pnas.93.15.7826>
103. Kushner, B.H., Ostrovskaya, I., Cheung, I.Y., Kuk, D., Kramer, K., Modak, S., Yataghene, K., Cheung, N.K.: Prolonged progression-free survival after consolidating second or later remissions of neuroblastoma with Anti-G(D2) immunotherapy and isotretinoin: a prospective Phase II study. *Oncoimmunology.* **4**, e1016704 (2015). <https://doi.org/10.1080/2162402X.2015.1016704>
 104. Yu, J., Hung, J.T., Wang, S.H., Cheng, J.Y., Yu, A.L.: Targeting glyco-sphingolipids for cancer immunotherapy. *FEBS Lett.* **594**, 3602–3618 (2020). doi:<https://doi.org/10.1002/1873-3468.13917>
 105. Harel, W., Shau, H., Hadley, C.G., Morgan, A.C. Jr., Reisfeld, R.A., Cheresch, D.A., Mitchell, M.S.: Increased lysis of melanoma by in vivo-elicited human lymphokine-activated killer cells after addition of antiganglioside antibodies in vitro. *Cancer Res.* **50**, 6311–6315 (1990)
 106. McLaughlin, P., Grillo-López, A.J., Link, B.K., Levy, R., Czuczman, M.S., Williams, M.E., Heyman, M.R., Bence-Bruckler, I., White, C.A., Cabanillas, F., Jain, V., Ho, A.D., Lister, J., Wey, K., Shen, D., Dallaire, B.K.: Rituximab chimeric anti-CD20 monoclonal antibody therapy for relapsed indolent lymphoma: half of patients respond to a four-dose treatment program. *J. Clin. Oncol.* **16**, 2825–2833 (1998)
 107. Furukawa, K., Hamamura, K., Aixinjueluo, W., Furukawa, K.: Biosignals modulated by tumor-associated carbohydrate antigens: novel targets for cancer therapy. *Ann. NY Acad. Sci.* **1086**, 185–198 (2006). <https://doi.org/10.1196/annals.1377.017>
 108. Ko, K., Furukawa, K., Takahashi, T., Urano, T., Sanai, Y., Nagino, M., Nimura, Y., Furukawa, K.: Fundamental study of small interfering RNAs for ganglioside GD3 synthase gene as a therapeutic target of lung cancers. *Oncogene.* **25**, 6924–6935 (2006). <https://doi.org/10.1038/sj.onc.1209683>
 109. Makino, Y., Hamamura, K., Takei, Y., Buiyan, R.H., Ohkawa, Y., Ohmi, Y., Nakashima, H., Furukawa, K., Furukawa, K.: A therapeutic trial of human melanomas with combined small interfering RNAs targeting adaptor molecules p130Cas and paxillin activated under expression of ganglioside GD3. *Biochim. Biophys. Acta.* **1860**, 1753–1763 (2016). <https://doi.org/10.1016/j.bbagen.2016.04.005>
 110. Furukawa, K., Hamamura, K., Nakashima, H., Furukawa, K.: Molecules in the signaling pathway activated by gangliosides can be targets of therapeutics for malignant melanomas. *Proteomics.* **8**, 3312–3316 (2008). <https://doi.org/10.1002/pmic.200800228>
 111. Furukawa, K., Ohmi, Y., Ohkawa, Y., Bhuiyan, R.H., Zhang, P., Tajima, O., Hashimoto, N., Hamamura, K., Furukawa, K.: New era of research on cancer-associated glycosphingolipids. *Cancer Sci.* **110**, 1544–1551 (2019). <https://doi.org/10.1111/cas.14005>
 112. Brignole, C., Pagnan, G., Marimpietri, D., Cosimo, E., Allen, T.M., Ponzoni, M., Pastorino, F.: Targeted delivery system for antisense oligonucleotides: a novel experimental strategy for neuroblastoma treatment. *Cancer Lett.* **197**, 231–235 (2003). doi:[https://doi.org/10.1016/s0304-3835\(03\)00107-1](https://doi.org/10.1016/s0304-3835(03)00107-1)
 113. Prigione, I., Corrias, M.V., Airoidi, I., Raffaghello, L., Morandi, F., Bocca, P., Cocco, C., Ferrone, S., Pistoia, V.: Immunogenicity of human neuroblastoma. *Ann. N Y Acad. Sci.* **1028**, 69–80 (2004). doi:<https://doi.org/10.1196/annals.1322.008>
 114. Mount, C.W., Majzner, R.G., Sundaresh, S., Arnold, E.P., Kadapakkam, M., Haile, S., Labanieh, L., Hulleman, E., Woo, P.J., Rietberg, S.P., Vogel, H., Monje, M., Mackall, C.L.: Potent antitumor efficacy of anti-GD2 CAR T cells in H3-K27M⁺ diffuse midline gliomas. *Nat. Med.* **24**, 572–579 (2018). doi:<https://doi.org/10.1038/s41591-018-0006-x>

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.