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Minireview

Glycosphingolipid-dependent cross-talk between glycosynapses interfacing tumor cells with their host cells: essential basis to define tumor malignancy

Senitiroh Hakomori*[,a,b], Kazuko Handa[a]

[a] *Pacific Northwest Research Institute, 720 Broadway, Seattle, WA 98122-4327, USA*
[b] *Departments of Pathobiology and Microbiology, University of Washington, Seattle, WA 98195, USA*

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Abstract Status of tumor progression (either remaining in situ, or becoming invasive/metastatic) may be defined largely by subtle interactions ('cross-talk') in a microenvironment formed by interfacing tumor cell and host cell membrane domains (termed 'glycosynapses') involved in glycosylation-dependent cell adhesion and signaling. Functional roles of tumor-associated gangliosides, organized in glycosynapses of three types of tumor cell lines, are discussed. Gangliosides function as adhesion receptors or as 'sensors' that can be stimulated by antibodies, with consequent activation of signal transducers leading to enhanced motility and invasiveness.

Key words: Glycosylation-dependent cell adhesion; Tumor-associated ganglioside; Interfacing; Signal transduction; Glycosynapse; Microenvironment

1. Introduction

Invasive/metastatic properties of tumor cells are controlled by multiple factors with complex machinery, many of which operate at the surface membrane. Of particular importance is the high expression of aberrant glycosphingolipids (GSLs) in specific types of tumors, which are involved in tumor cell adhesion and signal transduction, and thus define stage of tumor progression and invasiveness [1,46]. Recent studies indicate that GSLs and transmembrane glycoproteins, including adhesion receptors and growth factor receptors, as well as cytoplasmic signal transducers (TDs), are not randomly distributed, but rather assembled non-covalently in multiple types of microdomains (e.g. for review [2–5]).

Some of these microdomains define glycosylation-dependent adhesion and signaling and are termed 'glycosynapses' [6]; they may provide a microenvironment where tumor cells interface with host cells [7]. Such interfacing membrane units may define the 'destiny' of tumor cells, e.g. whether to differentiate or to follow an apoptotic pathway; whether to proliferate or to modify original malignancy; whether to activate motility and proteolysis to invade through extracellular matrix into microvasculature, leading to distant metastasis [1,46].

In this review, we propose a conceptual scheme for cross-talk of tumor cells with host cells through their interfacing glycosynapses, to define destiny of tumor cells.

2. Self-assembly of GSLs in membrane: *cis* vs. *trans* interaction

GSLs in membrane (i) interact side-by-side within the same membrane to form clusters (*cis* interaction), or (ii) interact through their carbohydrate surfaces between two interfacing membranes (*trans* interaction). Many electron microscopy studies using labeled anti-GSL antibodies have revealed remarkable GSL clusters at the cell surface [8,9], or on liposome surface even in the absence of cholesterol [10]. GSLs are presumed to have greater ability to both donate and accept hydrogen bond through the hydroxyl group of sphingosine, acylamide group, or glycosyl residue, compared to glycerophospholipids that have only the ability to accept hydrogen bond (this possibility cannot yet be confirmed, since solid-phase nuclear magnetic resonance is not practically developed). Sphingomyelin may interact with cholesterol whereas it separates from glycerophospholipids in gel state, as indicated by spin-label ESR and IR spectroscopy [11,12].

On the other hand, *trans* interaction between GSLs provides the basis for GSL-dependent cell-to-cell adhesion; it takes place through specific complementary structures and is catalyzed in many cases by Ca^{2+}. There are steadily increasing examples of such complementary GSL pairs providing a basis for cell-to-cell interaction [13,14], even though the concept was greeted by considerable skepticism when it was initially proposed [15–17]. Recently, such carbohydrate-to-carbohydrate interactions have been elucidated on a quantitative basis, using surface plasmon resonance (SPR) spectroscopy [18–20], aggregation of gold glyconanoparticles [21], molecular force microscopy [22,23], and pressure-area (π-A) isotherms in GSL Langmuir monolayer [24].

*Corresponding author. Fax: (1)-206-726 1212.
E-mail address: hakomori@u.washington.edu (S. Hakomori).

Abbreviations: BM, basement membrane; EC, endothelial cell; EpC, epithelial cell; GEM, glycolipid-enriched microdomain; GSL, glycosphingolipid; PBMC, peripheral blood mononuclear cells; PL, proteolipid (hydrophobic protein soluble in chloroform-methanol); T1–T6, stages of tumors as defined in the figure legend and the text; TD, signal transducer

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Self-clustered GSLs in glycosynapse are often associated with TD proteins having long aliphatic chain (e.g. cSrc, Src family kinases, Ras, RhoA), or with other TDs (e.g. FAK, paxillin) [25]. Thus, GSL-clustered domains, previously termed 'GEM' (glycolipid-enriched microdomain) or 'GSD', are involved in GSL-dependent cell adhesion that induces activation of such TDs to initiate signals to modify cellular phenotype. Such domains are separable from caveolae by immunoprecipitation or immunoadsorption with antibodies to GSLs or caveolin, and are distinguishable by chemical composition [26], or by application of photoactivatable ganglioside that does not bind to caveolin [27]. Glycosynapses of B16 melanoma cells are highly enriched in GM3, cSrc, RhoA, and FAK, but have low level of cholesterol, and no caveolin. In contrast, caveolar membrane is highly enriched in cholesterol, caveolin, GlcCer, and Ras [26]. Caveolar membranes are not involved in GSL-dependent adhesion or GSL-induced signaling to modify cellular phenotype. Recently, we observed the presence in GEM or GSD of lipophilic tetraspanin proteolipids (PLs) such as CD9 [28] and MAL-like PLs (Handa, K. et al., unpublished data), which may stabilize GSL microdomains and modulate signal transduction.

3. GM3-dependent adhesion and signaling in melanoma B16 cell glycosynapse

Metastatic and invasive abilities of mouse melanoma B16 cell variants, in the order BL6 > F10 > F1 ≫ WA4, are closely correlated with level of GM3 surface expression [29], and also with degree of adhesion to cultured endothelial cells (ECs) (mouse SPE1; human umbilical vein ECs) in vitro [29,30]. Such adhesion is not selectin- or integrin/ICAM1-dependent; the process is presumed to be based on interaction of GM3 with LacCer (expressed highly on SPE1 or human umbilical vein ECs), or with Gg3Cer (assumed to be expressed on mouse lung ECs) [30]. BL6 or F10 cells bound strongly to Gg3Cer-coated plates, whereas F1 or WA4 did not [17]. This process was therefore termed 'GM3-dependent cell adhesion'. Haptotactic or phagokinetic motility of BL6 or F10 cells was strongly promoted on Gg3Cer-coated surface [17,30]. This mechanism is synergistic with integrin-dependent adhesion and motility, as demonstrated on plates co-coated with GSL (Gg3Cer or LacCer) and extracellular matrix protein (fibronectin or laminin) [31].

The signaling process at the interface between melanoma and EC glycosynapses is therefore of crucial importance in melanoma progression and metastasis. We have been able to examine signaling and phenotypic changes in melanoma cells, but not yet in ECs. Results indicated that GM3-dependent B16 cell adhesion induces tyrosine phosphorylation of cSrc and FAK, as well as enhancing GTP binding to RhoA and Ras [25,26]. The downstream signaling pathway in this case is not yet clear, but activation of MAPK has been observed, and is modulated by presence of PLs. RhoA activation may well induce motility enhancement, but this remains to be studied. What are the signaling and phenotypic changes that occur in ECs interfacing with B16 cells? It seems plausible that ECs are activated to express ICAM receptor that interacts with melanoma cell integrin [32], or alternatively retract to create larger intercellular spaces (e.g. [33]) through which the melanoma cells can pass.

4. Disialyl-GalNAc-Lc$_4$Cer (RM2 antigen) that promotes metastasis of renal cell carcinoma

Metastatic properties of renal cell carcinoma (RCC) are closely associated with presence of slow-migrating gangliosides [34–36]. Two classes of gangliosides, one with globo-series, the other with type 1 lacto-series core, have been identified (see structures below; each defined by mAb as indicated in parentheses) [37–40].

Globo-series gangliosides: MSGb5 (RM1) [37] NeuAcα3-Galβ3GalNAcβ3Galα4Galβ4GlcβCer; DSGb5 (5F3) [38] NeuAcα3Galβ3[NeuAcα6]GalNAcβ3Galα4Galβ4GlcβCer.

Type 1 lacto-series gangliosides: DSLc$_4$ (FH9) [39] NeuAcα3Galβ3[NeuAcα6]GlcNAcβ3Galβ4GlcβCer; DSGalNAcLc$_4$ (RM2) [40] GalNAcβ4[NeuAcα3]Galβ3[NeuAcα6]GlcNAcβ3-Galβ4GlcβCer.

All these gangliosides, cSrc, FAK, and RhoA are components of glycosynapse. RM2 antigen was shown to be associated with these TDs when GEM fraction was treated with RM2 antibody coupled to 'HiTrap' column. By electron microscopy using colloidal gold-coated RM2, large clusters of antigens can be seen at the surface of RCC line TOS1 cells [41].

The above three disialogangliosides in RCC were identified as being bound to siglec7, which is expressed in peripheral blood mononuclear cells (PBMC). TOS1 cell suspension, when incubated with blood cells, forms large clumps with PBMC. Binding of TOS1 cells to lung tissue sections [36] is presumably due to presence of resident PBMC, and other types of cells expressing siglec7 or other siglecs, in lung tissue. A plausible (though still hypothetical) scenario is that (i) RCC cells migrate into blood vessels and interact with siglec7-expressing hematopoietic cells; (ii) this results in large clumps of tumor cells with PBMC; (iii) these aggregates lead to microembolisms in microvasculature which is highly developed in lung, with consequent lung metastasis [42].

5. Monosialyl-Gb5 (MSGb5) in glycosynapse of MCF-7 cells: cell migration and invasiveness are induced through signaling from monosialyl-Gb5

A typical example of control of tumor cell invasiveness and motility by ganglioside was demonstrated recently in human mammary carcinoma MCF-7 variants 7/AZ and 7/6 [43]. Both variants are non-invasive into collagen gel layer. Treatment of 7/AZ cells with anti-MSGb5 mAb RM1 greatly increased their invasiveness into collagen gel layer and motility in wound migration assay, whereas the same RM1 treatment had no such effect on 7/6. This RM1 effect on 7/AZ was highly specific, i.e. mAbs directed to GM2, Gb3, or Gb5 had no effect. All GSLs in 7/AZ and 7/6 were found in low-density membrane fraction (GEM). Levels of TDs cSrc and FAK; adhesion receptor integrins α1, α2, α3, E-cadherin, and β-catenin; and O-linked mucins MUC1 and MUC4, were all similar between the two variants.

The only clear difference was found in level of CD9, which was approximately four times higher in 7/AZ than in 7/6 cells. CD9 was enriched in GEM fraction of 7/AZ, but virtually absent in GEM of 7/6. A crucial distinction was that RM1 induced tyrosine phosphorylation of cSrc and FAK in 7/AZ, but not 7/6, although chemical levels of cSrc and FAK were

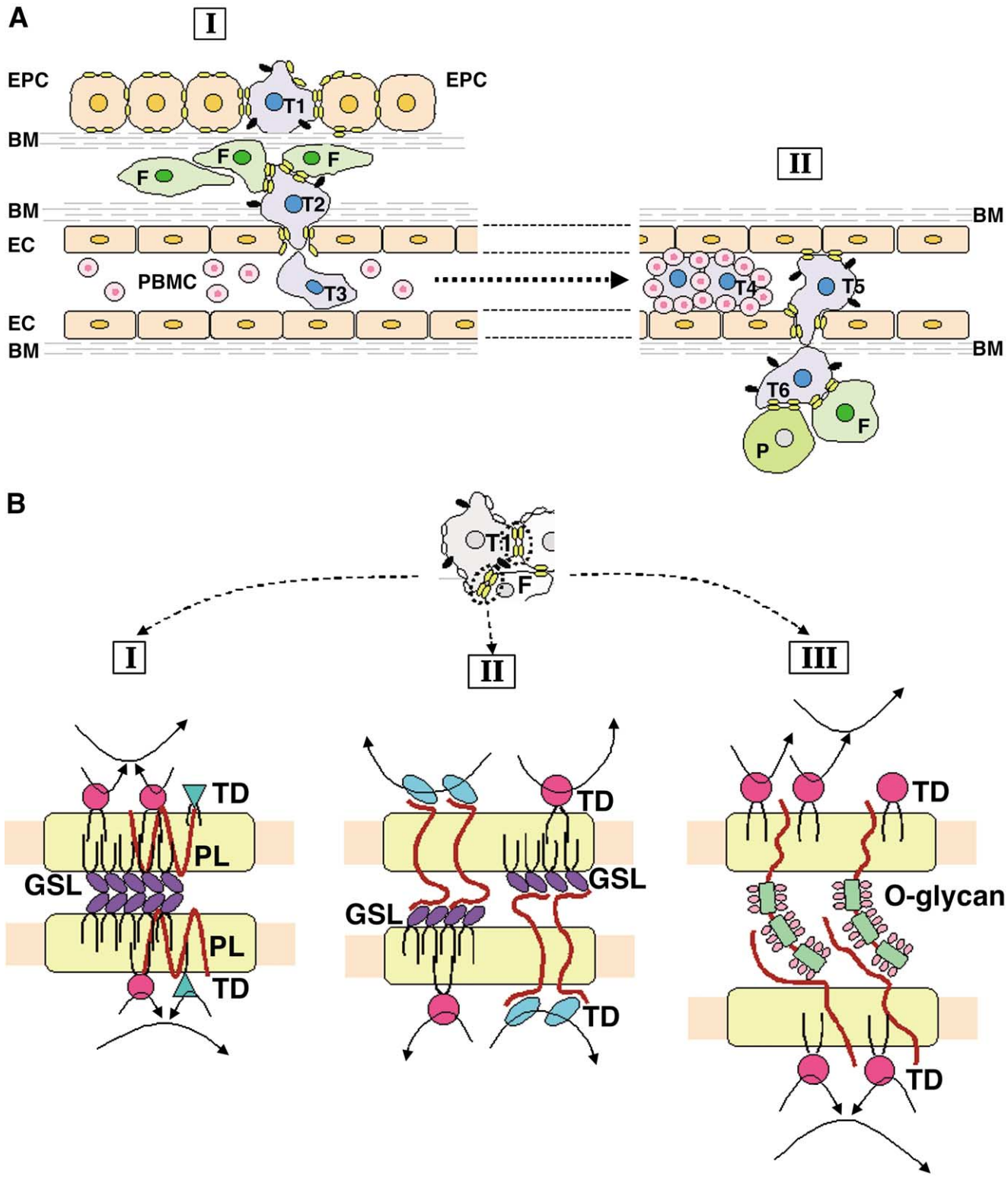

Fig. 1. Tumor progression through glycosylation-dependent cross-talk between glycosynapses interfacing tumor cells with host cells. A: Stages of tumor cell interaction (T1–T3) in I, and subsequent tumor cell adhesion to ECs in microvessels (T4–T6) in II. Each stage is explained in the text. Interfacing glycosynapses are indicated by yellow ovals, and integrins/growth factor receptors by black ovals. B: Three examples of interfacing glycosynapses. I: GSL to GSL. II: GSL to lectin. III: O-linked glycan to selectin or siglec. TDs and PLs are organized in glycosynapses (pale yellow rounded rectangle). Stimulation by binding of GSLs or O-glycans activates TDs to send signals as indicated. PLs may modulate signaling. Glycosynapse GSLs may be connected to TDs by interaction of longer fatty acyl residue (C22–24) of GSL with acyl chain of fatty acid or farnesyl group of TD. An alternative connection of GSLs to TDs could be mediated by interaction of PLs through their hydrophobic region with aliphatic chains of GSLs and TD, or interaction of GSL ceramide with hydrophobic domain of TD. Ligand binding to GSL or O-linked glycan may induce clustering of such groups in glycosynapse, which activates TDs. C: Growth factor receptors (GFR) and integrins (shown as black ovals in A) in glycosynapses which are not present in interfacing area. GFRs, integrins, and associated tetraspanins (TSP) are highly glycosylated, and surrounded by gangliosides (Gg). Their functions are controlled by glycosylation in each microdomain. For further details, see [6].

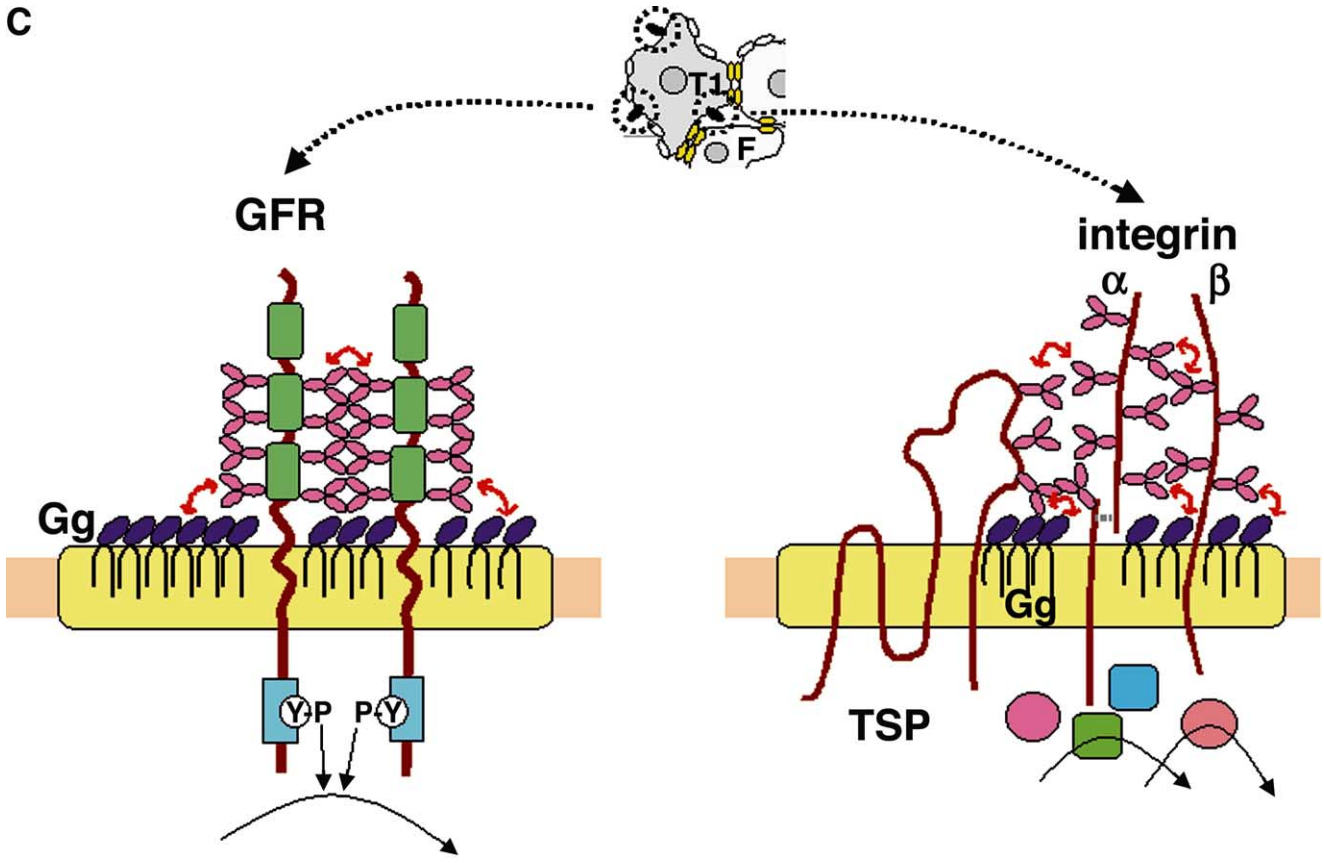

Fig. 1 (*Continued*).

identical. MSGb5 in glycosynapse of 7/AZ cells may be connected to cSrc or FAK through abundant presence of CD9, whereas MSGb5 in glycosynapse of 7/6 cells has no such connection, since CD9 is virtually absent in 7/6 glycosynapse. Thus, a crucial difference in organization of MSGb5 with cSrc and FAK in glycosynapse is suggested [44].

6. Proposed scheme for microenvironment of tumor cells interfacing with host cells, focused on glycosynaptic structure

Based on the data described in Sections 3–5, we hereby propose a scheme for functional roles of interfacing glycosynapses of tumor cells with host cells during tumor progression (see Fig. 1A, B).

The majority of human cancers are derived from epithelial cells (EpCs). When transformed (tumor) cells arise, their initial contact is with the 'parental' EpCs and surrounding fibroblasts (F), as well as extracellular matrix (ECM) components, particularly assembled in basement membrane (BM). In such microenvironment, interfacing glycosynapses of tumor cells with their host cells do not stimulate tumor cells, presumably because GSLs in the interfacing glycosynapses may not be aberrant in terms of quantity or quality (structure) (Fig. 1A,I, stage T1, i.e. in situ tumor cell). We presume that when GSL pattern becomes aberrant, interfacing glycosynapses are stimulated to induce signaling to activate tumor cell motility and proteolysis, and break down BM and ECM (stage T2). In this stage, integrin receptors complexed with tetraspanin and gangliosides [28], as well as matrix metalloproteases [45], probably associated within the same glycosynapse, play an essential role in tumor cell migration and penetration through BM and ECs. Interfacing of tumor cells with ECs through their glycosynapses may activate tumor cell motility as well as EC retraction and expression of selectin and/or ICAM, thereby facilitating tumor cell penetration into microvasculature (stage T3). When tumor cells penetrate into blood or lymphatic vessels, they migrate to a distant site to initiate metastasis (Fig. 1A,II). If tumor cells express high level of disialoganglioside in glycosynapse, they bind to blood cell siglec, leading to formation of tumor cell–blood cell aggregates and consequent microembolisms (stage T4). Some tumor cells highly expressing sialyl-Le^x, sialyl-Le^a, or their analogues are capable of binding to E-selectin (stage T5). Adhesion of tumor cells to ECs may take place through their interfacing glycosynapses, leading to activation of both tumor cells and ECs. Tumor cells are thereby translocated out of vessels to initiate metastatic deposits, and interface with parenchymatous cells (P) or fibroblasts (F) to stimulate tumor cell growth and motility (stage T6).

Examples of composition and possible organization of glycosynapse pairs interfacing tumor cells to host cells are depicted in Fig. 1B and its legend. In each case, glycosylation-mediated cross-talk takes place through mutual interaction of GSLs or O-linked glycans on interfacing glycosynapses. The exact mechanism by which GSLs are connected to TDs is unclear. One possibility is acyl chain interaction by the presence of longer fatty acids in GSLs; another is connection through PLs (see Fig. 1B, legend). In addition to glycosylation-mediated interaction between interfacing glycosynapses, tumor cell proliferation and motility are strongly controlled

by presence of growth factor receptors (GFR), and integrins complexed with tetraspanins (TSP) and gangliosides (Gg), which in many cases are included within glycosynapse or similar domain, but may not be interfacing (Fig. 1C).

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