Chapter 11
Targeting Glycans for Immunotherapy of Human Cancers

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Abstract Surface carbohydrate-containing molecules, such as glycoproteins and glycolipids, have been shown to play crucial regulatory roles in the normal physiological process as well as in pathological conditions including tumor progression. Those glycans which are overexpressed on the surface of tumor cells, but not detected or only weakly expressed in some limited normal tissues, are designated as tumor-associated carbohydrate antigens (TACAs). These TACAs may serve as potential targets for immunotherapy. The biological functions of TACAs and therapeutic strategies against TACAs will be addressed in this review.

Keywords Cancer immunotherapy • GD2 • Globo H • Sialyl-Tn • GM2 • Immune checkpoints • Angiogenesis

11.1 Introduction

Glycosylation is an important posttranslational modification process to produce diverse glycans that are frequently attached to proteins and lipids. These glycoconjugates play a key role in cells, including receptor activation, cell adhesion, signal transduction, endocytosis, molecular trafficking, and clearance (Ohtsubo and Marth 2006). Altered glycosylation on glycoproteins and glycolipids is a prominent feature of cancer cells (Reis et al. 2010). These abnormal glycoconjugates are involved in tumor proliferation, invasion, angiogenesis, and metastasis. Patient with altered glycoconjugates in tumor tissue usually has poor prognosis (Miyake et al. 1992). Changes in glycosylation, including over-, under-,

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and neo-expression of sugar moieties, might result from the upregulation/downregulation of some glycosyltransferases and glycosidases. Increased N-glycosylation, such as 1,6-branched N-glycans, was observed in breast cancer and colon cancer (Dennis et al. 1987; Fernandes et al. 1991; Seelentag et al. 1998; Murata et al. 2004), which was mediated by GnT-V. On the other hand, O-glycosylation is often reduced resulting in the accumulation of core 1-based O-glycan during the tumorigenesis. The most common O-glycan epitopes were TF (Galβ1, 3GalNAc), Tn (GalNAc), Lewis^x, Lewis^a, and their sialylated counterparts (Springer 1984; Yuan et al. 1986; Itzkowitz et al. 1989; Itzkowitz et al. 1986; Tozawa et al. 2005). They were reported to enhance the intravasation of cancer cells, binding of circulating cancer cells to endothelium, and extravasation and colonization at the distant sites (Rosen and Bertozzi 1994; Borsig et al. 2001, 2002). For instance, increased expression of sialyl-Le^x (sLe^x) and sialyl-Le^a (sLe^a) was shown to assist the invasion and metastasis of tumor (Hoff et al. 1989; Kannagi 1997) and was associated with poor survival of patients (Makino et al. 2001). So far, a long list of TACAs has been identified, such as Tn, sialyl-Tn, TF, Lewis^y, sialyl Lewis^x, sialyl Lewis^a, Lewis^x, Globo H, stage-specific embryonic antigen-3 (SSEA-3), GD2, GD3, GM2, fucosyl GM1, Neu5Gc GM3, and polysialic acid. Some of these have been exploited as targets for immunotherapy of cancers.

Conventional treatments of cancer, including radiation, surgery, and chemotherapy, are not cancer-specific. Immunotherapy, on the other hand, provides a strategy to target specific cancer cells. Cancer immunotherapy can be categorized into active and passive immunotherapy. An active cancer immunotherapy is to activate the immune system of patients to attack cancer cells, which can trigger immunological memory. On the other hand, passive immunotherapy is to deliver tumor antigen-specific monoclonal antibodies to kill cancer cells through complement-dependent cytotoxicity (CDC) or antibody-dependent cell-mediated cytotoxicity (ADCC). Since the initial approval of anti-CD20 (rituximab) for the treatment of lymphoma in 1994, more than 10 monoclonal antibodies have been approved for passive immunotherapy of cancer and all of them target protein antigens. On the other hand, the first and only approved active immunotherapy is sipuleucel-T (*Provenge*, Dendreon), for the treatment of metastatic prostate cancer (Kantoff et al. 2010). Sipuleucel-T is an autologous cellular vaccine activated ex vivo by recombinant prostate acid phosphatase (PAP) fused to GM-CSF. Although a number of clinical trials of cancer immunotherapy targeting TACAs have been conducted over the past two decades, majority of the trials did not proceed beyond early phase I/II studies. Only three TACAs have reached clinical phase III development: sialyl-Tn, GM2, and GD2. Unfortunately, randomized phase III clinical trials of sialyl-Tn-KLH vaccine (Theratope) in metastatic breast cancer (Miles et al. 2011a) and GM2-KLH vaccine in melanoma (Eggermont et al. 2013) failed to demonstrate any benefit of the vaccine, although subsequent subgroup analysis did demonstrate survival benefit of Theratope in metastatic breast cancer patients on endocrine therapy (Ibrahim et al. 2013). On the other hand, passive immunotherapy with dinutuximab, a chimeric anti-GD2, has demonstrated a significant improvement in event-free survival and overall survival in patients with high-risk neuroblastoma

(Yu et al. 2010) which led to regulatory approval in the USA and Europe in 2015. Thus, GD2 is the first TACA proven to be an effective target antigen for cancer immunotherapy.

The approval of ipilimumab (anti-CTLA-4) for the treatment of melanoma in 2011 as the first monoclonal antibody targeting an immune checkpoint molecule (Hodi et al. 2010) heralded a new era of cancer immunotherapy. The success of ipilimumab was closely followed by the development of additional immune checkpoint inhibitors, including nivolumab (Robert et al. 2015) and pembrolizumab (Robert et al. 2014), which target PD-1 and ensure the emergence of many more immune checkpoint blockers on the horizon. Such breakthroughs beg the question whether TACAs may act as immune checkpoint molecules. Indeed, several cancer-associated gangliosides were shown to inhibit immune cell responses, including antigen processing and presentation (Peguet-Navarro et al. 2003), T-cell proliferation (Biswas et al. 2009; Chu and Sharom 1993; Morioka et al. 1991), and cytokine production, such as IFN-γ and IL-4 (Biswas et al. 2006; Irani et al. 1996). Purified gangliosides from cancer cells displayed immunosuppressive activities which aided cancers to escape from host immune surveillance (Ladisch et al. 1992; Wolfl et al. 2002), which were mediated by hampering the interaction of IL-2 with its receptor (Lu and Sharom 1996), inducing apoptotic cell death (Das et al. 2008) and deviation toward Th2 response (Crespo et al. 2006). Such ganglioside-induced T-cell dysfunction involved NF-kappa B inhibition (Uzzo et al. 1999) through degradation of RelA and p50 proteins (Thornton et al. 2004). In contrast, there are relatively few studies on the functions of Globo-series TACAs. The core structure of Globo-series glycosphingolipids is Galα1,4-Galβ1,4Glc-ceramide (Gb3), which is catalyzed by α1,4-galactosyltransferase (A4galt) through the transfer of a galactose to lactosylceramide (Kojima et al. 2000). Gb3 has been found in Burkitt lymphoma (Wiels et al. 1981) and germ cell-derived tumors (Murray et al. 1985) and also in a subpopulation of B cells in germinal centers (Klein et al. 1983) and on kidney proximal tubules and intestinal epithelial cells (Fujii et al. 2005). In addition, Gb3 could be induced on the surface of human monocytes by LPS (van Setten et al. 1996) or on endothelial cells by interleukin-1 and tumor necrosis factor-alpha (van de Kar et al. 1992). Besides, Gb3 could serve as a receptor on endothelial cells for verotoxins produced by *Escherichia coli* O157 (Jacewicz et al. 1986), which was further confirmed by increased sensitivity to LPS-induced lethal shock in A4galt knockout mice (Okuda et al. 2006) (Kondo et al. 2013). Intriguingly, in wild-type mice, injection of LPS did not increase the expression of Gb3 on the surface of endothelial cells although it induced the expression of A4galt RNA and globotetraosylceramide (Gb4), one of the Globo-series glycolipids generated by b1,3-N-acetylgalactosaminyltransferase (B3galnt1) through the transfer of galactosamine to Gb3, suggesting that Gb4, but not Gb3, might play a role in LPS-induced lethal shock. Indeed, administration of Gb4 increased the survival rate of mice injected with LPS. The protective effect of Gb4 on LPS-challenged mice was mediated by the binding of Gb4 to the complex of toll-like receptor-4 and myeloid differentiation factor 2 on the endothelia, thereby interfering with the binding of LPS to this complex (Kondo et al. 2013).

However, the effects of Globo-series glycolipids on immune cells have remained unclear until Tsai et al. reported the immunosuppressive activity of Globo H ceramide (Tsai et al. 2013). Globo H ceramides released from the surface of tumor cells were taken up by T and B lymphocytes, with ensuing inhibition of activation of lymphocytes. Tumor-infiltrating lymphocytes in close proximity to the Globo H-expressing tumor cells showed positive staining of anti-Globo H antibody by IHC, consistent with the in vitro observation of uptake of Globo H ceramide released from tumor cells by lymphocytes. Treatment of lymphoid cells with Globo H ceramide did not induce apoptosis nor expand regulatory T cells. The molecular mechanisms of Globo H ceramide induced immunosuppression involved upregulation of id3 and itch via upregulation of egr2/3, leading to diminished expression of Notch, which is crucial for T-cell activation (Palaga et al. 2003). These results provide the first evidence that Globo H ceramide acts as an immune checkpoint molecule to facilitate the escape of cancer cells from immune surveillance.

In addition to the function of TACAs as immune checkpoints, several gangliosides have been reported to exhibit angiogenic activities. Tumor cells with GM2 synthase/GM3 synthase deficiency formed avascular tumor on mice (Liu et al. 2014a), whereas upregulation of GM1, GM2, and GD1a enhanced blood vessel density in tumors (Manfredi et al. 1999). On the other hand, GM3 blocked the dimerization of vascular endothelial growth factor receptor 2 (VEGFR2) to inhibit the signaling transduced by VEGF (Chung et al. 2009). The effects of Globo-series TACA on angiogenesis were first addressed in a report showing angiogenic activity of Globo H ceramide (Cheng et al. 2014). Globo H ceramide induced tube formation of endothelial cell in vitro and angiogenesis in vivo. When Globo H-positive tumor cells were sorted into two subpopulations based on Globo H expression, the Globo H^{hi} tumor cells grew faster with greater vessel density than Globo H^{low} tumor cells in vivo. Consistent with this was the observation of higher vessel density in Globo H^{+} than Globo H^{-} breast cancer specimens. Mechanistic investigations linked the angiogenic effects of Globo H ceramide to its endocytosis and binding to TRAX, with consequent release of PLCβ1 from TRAX to trigger Ca^{2+} mobilization. This is the first globoside shown to display angiogenic activity, along with elucidation of its mechanisms. On the other hand, Globo H has been identified as one of the glycans that bind human RNase 1, facilitating the internalization of the RNase 1 which induced cell death. Blocking the interaction of Globo H and RNase 1 with anti-Globo H antibody partially rescued the cells from RNase-induced cell lysis (Eller et al. 2015). These findings suggested multifaceted roles of Globo H in tumor biology.

The findings of certain TACAs acting as immune checkpoint molecules and angiogenic factors further strengthen the scientific rationales for immunotherapy targeting TACAs. The following sections will address various strategies for developing TACA-targeted cancer immunotherapies.

11.2 Disialoganglioside (GD2)-Targeted Cancer Immunotherapies

Disialoganglioside (GD2), a b-series ganglioside, is a sialic acid-containing surface glycolipid that generated from precursor GM2 by GD3 synthase and GD2 synthase. It is expressed by neuroblastoma (>98 %), melanoma, glioma, small-cell lung cancer, sarcomas (Schulz et al. 1984; Cheung et al. 1987), breast cancer stem cell (Liang et al. 2013; Battula et al. 2012), as well as some normal neuroectodermal (Yanagisawa et al. 2011), and mesenchymal stem cells (MSCs) (Martinez et al. 2007; Jin et al. 2010). GD2 plays an important role in the proliferation and invasiveness of tumor cells (Yoshida et al. 2001; Shibuya et al. 2012). It could directly induce activation of the proto-oncogene c-Met to enhance proliferation of triple-negative breast cancer cells (Cazet et al. 2012). It upregulates integrin α2β1-mediated tyrosine phosphorylation of p125FAK, which enhances platelet adhesion to extracellular matrix collagen, thereby promoting metastasis of neuroblastoma cells (Chen et al. 2013). Furthermore, $GD2^+$ murine bone marrow MSCs (mBM-MSC) possessed not only much greater clonogenic and proliferative capabilities but also stronger differentiation potential to adipocytes and osteoblasts, as compared to unsorted mBM-MSCs (Xu et al. 2013). Moreover, in human osteosarcoma cell lines, a murine anti-GD2 antibody, mAb 14G2a, effectively inhibits cell invasiveness, MMP-2 activity, and cell viability (Liu et al. 2014b). On the other hand, in human tissues, only weak expression of GD2 is observed in neurons, skin melanocytes, and peripheral pain fibers (Svennerholm et al. 1994). Therefore, GD2 is an ideal glycan antigen target for immunotherapy. Three immunotherapeutic strategies have been developed so far, including GD2-specific monoclonal antibodies, GD2-specific chimeric antigen receptor T cells, and GD2 vaccines.

11.2.1 GD2-Specific Monoclonal Antibodies

11.2.1.1 3F8

3F8 is a murine IgG3 monoclonal antibody which binds to GD2-expressing tumor cells and mediates cytotoxicity by activating human complement system (Cheung et al. 1985). ^{131}I-labeled 3F8 has been used for neuroblastoma imaging (Miraldi et al. 1986) and shown to eradicate human NB xenografts (Cheung et al. 1986). A phase I clinical trial of 3F8 was conducted in 17 patients with relapsed or refractory neuroblastoma. Significant toxicities including neuropathic pain, tachycardia, hypotension, hypertension, fever, and urticaria were observed. Antitumor activities were noted in some patients, ranging from complete clinical remissions to mixed responses. All patients developed human anti-mouse antibodies (HAMA) to 3F8. A phase II study in 16 patients with stage 4 neuroblastoma showed clinical responses in bony lesions and marrow diseases (Cheung et al. 1998a). Subsequently, the effect

of 3F8 on minimal residual disease of stage 4 neuroblastoma was evaluated in 34 patients in first or subsequent response, and 13/34 patients remained progression-free for 53–143 months (Cheung et al. 2000). A series of sequential phase II studies in 139 patients showed an overall 5-year EFS of 62 % for stage 4 patients in first remission who received 3F8 + GM-CSF + cis-retinoic acid (Cheung et al. 2012) and a correlation of a better outcome for patients with the FCGR2A (R/R) genotype which favored the binding of the IgG3 antibody (Cheung et al. 2006). Moreover, a better survival correlated with a transient anti-mouse response or completion of 4 cycles of 3F8 treatment (Cheung et al. 1998b). Humanized 3F8 has been generated along with the generation of hu3F8/IL-2 and hu3F8/GM-CSF, which are undergoing phase I clinical trials in patients with high-risk neuroblastoma: hu3F8 (NCT01419834) and hu3F8/IL-2 (NCT01662804 and NCT01757626).

11.2.1.2 14G2a

MAb 14G2a was generated from murine 14.18 IgG3 anti-GD2 by class switch to IgG2a antibody (Mujoo et al. 1987, 1989). Three phase I trials of mAb 14G2a were conducted in patients with melanoma, neuroblastoma, and osteosarcoma. The dose of mAb 14G2a was escalated up to 500 mg/m^2/course, with significant dose and infusion rate-dependent toxicities, including pain, tachycardia, hypotension, hypertension, fever, hyponatremia, and urticaria (Uttenreuther-Fischer et al. 1995a; Murray et al. 1994; Saleh et al. 1992a). Pain was thought to be due to binding of antibody to peripheral nerve fibers expressing GD2 (Svennerholm et al. 1994). Clinical benefits were observed in some patients even in these early phase trials. To enhance the ADCC effect, 14G2a was combined with IL-2 and a maximum tolerated dose (MTD) of 14G2a plus IL-2 was 15 mg/m^2/day. Similar side effects were observed although IL-2 might have contributed to some of the toxicities, such as fever. One patient with neuroblastoma had a partial response (PR), and one patient with osteosarcoma had a complete response (CR) (Frost et al. 1997).

11.2.1.3 Ch14.18

A human-mouse chimeric anti-GD2 monoclonal antibody, ch14.18, was constructed by combining the variable regions of 14G2a and the constant regions of human IgG1-k (Gillies et al. 1989). MAb ch14.18 could activate complement system (Zeng et al. 2005) and mediate ADCC through neutrophils, natural killer (NK) cells, and lymphokine-activated killer (LAK) cells (Barker et al. 1991) with an efficiency 50–100 times greater than the murine mAb 14G2a (Mueller et al. 1990). Investigational New Drug (IND) application for ch14.18 was filed in 1989, marking the first IND application for mAb generated by recombinant DNA technology. Two phase I clinical trials of ch14.18 in relapsed/refractory neuroblastoma revealed similar toxicity profile as 14G2a (Yu et al. 1998; Handgretinger

et al. 1995). As expected, the half-life of ch14.18 was longer than 14G2a, with a beta t 1/2 of 66.6 ± 27.4 h for ch14.18 and 18.3 ± 11.8 h for 14G2a (Handgretinger et al. 1995; Uttenreuther-Fischer et al. 1995b). Among a total of 19 neuroblastoma patients, 2 CR and 3 PR were observed, although another phase I trial in 13 adult patients with metastatic melanoma showed no clinical responses (Saleh et al. 1992b). Based on the in vitro findings that GM-CSF not only raised the number of leukocytes but also enhanced their anti-GD2-mediated ADCC (Barker et al. 1991), a pilot study of ch14.18 + GM-CSF was conducted, which showed 5 CRs and 3 stable diseases (SDs) in 17 refractory/recurrent neuroblastoma (Yu et al. 1995). This was subsequently confirmed by a phase II Pediatric Oncology Group study showing 2 CR, 2 PR, and 1 mixed response in 32 neuroblastoma patients (Yu et al. 1997). In these early phase trials, most clinical responses occurred in patients with small disease burden, esp. bone marrow metastasis. Thus, anti-GD2 immunotherapy was subsequently developed to target neuroblastoma in the setting of minimal residual disease (MRD). The feasibility of administering ch14.18 in combination with GM-CSF, IL-2, and isotretinoin after high-dose chemotherapy and stem cell transplant period was demonstrated in 2 pilot phase I studies, and the maximum tolerated dose (MTD) of ch14.18 in combination with cytokines was 25 mg/m^2/d for 4 days (Gilman et al. 2009; Ozkaynak et al. 2000). These studies paved the way for the pivotal phase III randomized clinical trial of ch14.18 + IL-2/GM-CSF. Patients with high-risk neuroblastoma who achieved at least PR to induction therapy and received stem cell transplantation and posttransplant radiotherapy were randomly assigned, in a 1:1 ratio, to receive standard therapy with six cycles of isotretinoin or immunotherapy with six cycles of isotretinoin and five concomitant cycles of ch14.18 in combination with alternating GM-CSF and IL-2. Randomization was stopped early because interim analysis of 226 eligible patients revealed a significant 2-year overall survival (86 ± 4 % versus 75 ± 5 %, p = 0.02 without adjustment for interim analyses) and event-free survival (66 ± 5 % versus 46 ± 5 % at 2 years, p = 0.01) advantage for 113 patients receiving immunotherapy versus those 113 receiving standard therapy (ClinicalTrials.gov number NCT00026312) (Yu et al. 2010). This major breakthrough has now been considered as a standard treatment for high-risk neuroblastoma. It also marks the first successful immunotherapy to target a nonprotein antigen.

11.2.1.4 Hu14.18K322A

Ch14.18 was further humanized by CDR grafting of 14.18 V regions to generate humanized 14.18 (hu14.18) antibody (Metelitsa et al. 2002). Since anti-GD2-induced neuropathic pain is complement-dependent, a K322A mutation of the C region of IgG1 in hu14.18 was made to limit the ability of complement fixation of hu14.18. Preclinical studies in rats confirmed that hu14.18K322A elicited significantly less allodynia than ch14.18 while maintaining its ADCC activity (Sorkin et al. 2010). A phase I clinical trial of hu14.18K322A in 38 neuroblastoma showed

the MTD, and recommended phase II dose, of hu14.18K322A to be 60 mg/m^2 per day for 4 days (Navid et al. 2014). Adverse effects, predominately pain, were manageable and improved with subsequent courses. Median hu14.18K322A α (initial phase) and β (terminal phase) half-lives were 1.74 and 21.1 days, respectively. Objective responses (four complete responses; two partial responses) were noted in 6 of 31 patients evaluable for response by iodine-123 metaiodobenzylguanidine score. Several early phase trials in patients with GD2$^+$ tumors are in progress (ClinicalTrials.gov numbers NCT01576692 and NCT00743496).

11.2.1.5 Hu14.18-IL-2

Another strategy to enhance the antitumor efficacy of an antibody is to link the antibody with cytokine to generate immunocytokine fusion proteins that accumulate high cytokine concentrations in the tumor microenvironment and thereby stimulate cellular immune responses against cancer cell.

Hu14.18-IL-2 is a fusion protein of hu14.18 and IL-2 (Neal et al. 2004a, b). A phase I trial of hu14.18-IL-2 in recurrent/refractory neuroblastoma ($n = 27$) and melanoma ($n = 1$) patients showed the MTD to be 12 mg/m^2/day, with similar toxicities as anti-GD2 combined with IL-2. No measurable CRs or PRs to hu14.18-IL-2 were observed; however, evidence of antitumor activity was noted in three neuroblastoma patients (Osenga et al. 2006). A phase II study showed 5 CR in 23 neuroblastoma patients with evaluable disease only by MIBG and/or bone marrow histology, but no responses for patients with measurable disease (Shusterman et al. 2010). In this study, patients with KIR-ligand mismatch seemed to be associated with better clinical response (Delgado et al. 2010). Another phase I trial of hu14.18-IL-2 in adults with melanoma ($n = 33$) showed MTD to be 7.5 mg/m^2/day, with the dose-limiting toxicities of hypoxia, hypotension, and elevations of AST and ALT, which were reversible (King et al. 2004). Subsequently, a phase II study was conducted in metastatic melanoma patients ($n = 14$) who received hu14.18-IL-2 at 6 mg/m^2/day as 4-h intravenous infusions on days 1, 2, and 3 of each 28-day cycle. All patients received 2 cycles of treatment, and one patient had a PR (7.1 %, 1/14) and 4 patients had SD (28.5 %, 4/14). The toxicities were reversible, including grade 3 hypotension ($n = 2$) and grade 2 renal insufficiency with oliguria ($n = 1$). The accrual was held due to limited availability of hu14.18-IL-2 (Albertini et al. 2012).

11.2.1.6 Anti-O-Acetyl GD2 Monoclonal Antibody 8B6

Although the therapeutic efficacy of anti-GD2 has been well-documented, neuropathic pain can limit its application. O-acetyl GD2 is an analog of GD2 with an acetyl group linked to oxygen at the 9 position of NeuAc. An O-acetyl GD2-specific antibody 8B6 was shown to bind to neuroblastoma and other neuroectodermal tumors, but not peripheral pain fibers (Alvarez-Rueda et al. 2011). Thus, antibodies

against O-acetyl GD2 may have the advantage over anti-GD2 which is dose-limited by neuropathic pain. Indeed, a mouse-human chimeric antibody c.8B6 was reported to display potent antitumor activity without inducing allodynia in preclinical studies (Terme et al. 2014). Clinical trials of mAbc.8B6 are eagerly awaited.

11.2.1.7 Bispecific Antibody

Bispecific antibody which binds to two different types of antigen by combining fragments of two different monoclonal antibodies is an attractive alternative to immunocytokine. A GD2-targeting bispecific antibody 3F8 × CD3 (3F8BiAb) has been developed. It could redirect activated T cells to GD2-expressing murine neuroblastoma (Yankelevich et al. 2012). A phase I/II clinical trial of 3F8BiAb in children and young adults with neuroblastoma and osteosarcoma is under development (NCT02173093).

11.2.2 GD2 Chimeric Antigen Receptor

T lymphocytes can be engineered to express chimeric antigen receptors (CARs), which can bind to tumor antigens, leading to antitumor activity in an MHC-independent manner. CARs are generated by joining a single-chain variable fragment (scFv) of monoclonal antibody with the transmembrane and cytoplasmic portions of T-cell receptor (TCR) ζ-chain, via a flexible hinge region, to form a functional CAR (Savoldo and Dotti 2013). Louis et al. generated GD2-CAR-expressing T lymphocytes for the treatment of 19 patients with neuroblastoma. Persistence of GD2-CAR T lymphocytes beyond 6 weeks was associated with better clinical outcome, and three patients with active disease achieved complete remission. Thus, the GD2-CAR T lymphocytes might provide an alternative strategy for immunotherapy of neuroblastoma (Louis et al. 2011).

11.2.3 GD2-Specific Vaccines

11.2.3.1 GD2-KLH

The main challenge for developing carbohydrate vaccines is their poor immunogenicity. Chemical conjugation of glycans to a highly immunogenic protein scaffold, such as keyhole limpet hemocyanin (KLH), may enhance the immune responses to glycans. GD2-KLH is a synthetic GD2 conjugated to KLH. A phase I clinical trial of GD2-KLH using monophosphoryl lipid A (MPL-A) as an adjuvant in seven patients with recurrent or progressive gliomas showed no adverse effects. However, neither anti-GD2 antibody nor clinical response was observed (Becker et al. 2002).

Another phase I clinical trial of GD2-KLH using OPT-821 combined with oral beta-glucan as adjuvants was conducted in neuroblastoma. Anti-GD2 antibody was induced in 12 of 15 patients. Importantly, disappearance of MRD was observed in 6 of 10 patients (Kushner et al. 2014). A subsequent phase I study of combined GM2-KLH and GD2-KLH mixed with QS-21 adjuvant in 31 patients with melanoma or sarcoma showed successful induction of IgM/IgG anti-GM2 and anti-GD2 in 97 % and 73 % of patients, respectively (Chapman et al. 2000). These encouraging findings suggest that adjuvants may play an important role in glycan-based vaccine.

11.2.3.2 Anti-GD2 Idiotype Monoclonal Antibody 1A7

mAb1A7 is an anti-idiotype antibody mimicking GD2 antigen which was generated by immunizing mice with anti-GD2, mAb 14G2a (Saleh et al. 1993). Active immunotherapy with anti-idiotype antibody is anticipated to induce a gradual release of anti-GD2 via humoral antibody response, which may be beneath the threshold of anti-GD2-induced toxicities. In preclinical study, immunization of C57BL/6 mice and rabbits with mAb1A7 induced anti-GD2 antibodies of IgG isotype that recognized GD2 by ELISA and flow cytometry. These antisera specifically lysed GD2-positive target cells in an ADCC assay (Sen et al. 1998). Foon et al. initiated a clinical trial for anti-GD2 idiotype antibody (1A7) in patients with advanced melanoma. Patients ($n = 47$) received 1A7 (TriGem) at dose of 1, 2, 4, or 8 mg mixed with QS-21 (100 μg) weekly for 4 weeks and then monthly until disease progression. A majority of patients (40/47, 85.1 %) generated an anti-1A7 response. The isotypic specificity of the anti-1A7 antibody was predominantly IgG, with minimal IgM, and these antibodies reacted specifically with tumor cells expressing GD2 by flow cytometry. Immune sera from five patients tested displayed ADCC activity. Complete response lasting for 24 months was noted in one patient and stable disease (14+ to 37+ months) in 12 patients. Disease progression occurred in 32 patients (1–17 months) and 21 had died (1–16 months). The Kaplan-Meier-derived overall median survival was not reached. Toxicities were mild, including local reaction at the site of the injection, with mild fever and chills (Foon et al. 1998; Foon et al. 2000). In addition, a clinical trial of mAb1A7 as a GD2 vaccine was conducted in high-risk neuroblastoma patients ($n = 31$, 26 stage IV, 5 stage III) who achieved first or subsequent complete remission or very good partial remission (Yu et al. 2001). Patients received subcutaneous injection of 1A7 mixed with QS-21 as adjuvant every 2 weeks for 4 weeks and then monthly for 11 months thereafter and switched to 1A7 in aluminum hydroxide gel during the second year. After treatment, all patients had local reactions, four developed transient fever and chills, and one patient had serum sickness. All patients generated anti-1A7 antiserum, and immune sera from some patients displayed CDC and ADCC activities against neuroblastoma. At a median of 6.8 years from study entry, 76.1 % (16/21) patients who enrolled during first remission have no evidence of disease progression, whereas only one of ten patients who enrolled during second

or subsequent remission remains progression-free. Thus, active immunotherapy with anti-idiotypic antibody-based GD2 vaccine may offer therapeutic advantage over passive immunotherapy with reduced infusion-related toxicities.

11.3 Sialyl-Tn-Targeted Cancer Vaccine

Sialyl-Tn, Neu5Acα2,6-N-acetylgalactosamine (STn), is a carcinoma-associated carbohydrate determinant expressed on cancer-associated mucins, while it is weakly expressed in fetal and restricted normal adult tissues (Kjeldsen et al. 1988). Circulating STn has been detected in patients with gastrointestinal (Motoo et al. 1991) and ovarian (Kobayashi et al. 1992) malignancies. Expression of STn in colorectal carcinoma (Itzkowitz et al. 1990), gastric carcinoma (Ma et al. 1993), and breast cancer (Leivonen et al. 2001) correlates with poor prognosis and predicts a poor response to chemotherapy (Miles et al. 1994). In endometrial cancer, overexpression of STn correlated with overexpression of cyclooxygenase 2 (Ohno et al. 2006), which is linked to angiogenesis, tumor growth (Ohno et al. 2005a), and inhibition of the infiltration of CD8 T cell (Ohno et al. 2005b). STn has been reported to be involved in cell-cell aggregation, ECM adhesion, and migration and invasion of tumor cells, as shown in STn-overexpressing gastric cancer cells transfected with ST6Gal I transferase (Pinho et al. 2007). Moreover, STn on the tumor cells could interact with Siglec-15 expressed on tumor-associated macrophages to enhance the production of transforming growth factor-β through spleen tyrosine kinase (Syk) pathway (Takamiya et al. 2013). These findings suggest that STn may be a good candidate target for cancer immunotherapy.

A synthetic STn-keyhole limpet hemocyanin (KLH) vaccine (Theratope) was evaluated in clinical trials as an active specific immunotherapy in the treatment of advanced cancer. One of the first studies of Theratope was conducted by MacLean and colleagues in patients with metastatic breast cancer, ovarian cancer, and colon cancer (MacLean et al. 1996). They reported that 51 patients who produced anti-STn + mucin IgG titers higher than the median value survived longer than 46 patients who generated lower titers. Based on promising results of STn-KLH vaccine in early clinical trials, a phase III randomized trial was conducted in patients with metastatic breast cancer who had nonprogressive disease after first-line chemotherapy. A total of 1028 patients were randomly assigned to either STn-KLH plus Detox as adjuvant or KLH plus Detox (control group). The vaccine was well tolerated, with mild to moderate injection-site reactions and reversible flu-like symptoms. Specific IgG and IgM antibodies were detected at week 12. Unfortunately, there were no significant differences in the time to progression (TTP) and overall survival (OS) between STn-KLH vaccine group (3.4 and 23.1 months, respectively) and control group (3 and 22.3 months, respectively) (Miles et al. 2011b), although a post hoc analysis suggested benefit of concurrent endocrine therapy and STn-KLH vaccine for women with metastatic breast cancer

(Ibrahim et al. 2013). Several factors may have contributed to the lack of overall clinical efficacy of this vaccine. First, STn is not expressed uniformly in all breast cancer specimens. It ranges from low 20 % to high 80 % in various reports (Julien et al. 2012). In this phase III study, STn expression was not determined nor used as enrollment criteria, which might mask any benefit from the vaccine due to heterogeneity in STn expression among patients. Second, significant titers of anti-KLH IgM and IgG antibodies were observed in control group, which may have conferred some anticancer benefits. Nonetheless, lessons learned from this failed large randomized clinical trial may serve as stepping stones to the ultimate success by modifying the clinical design and patient selection.

11.4 GM2-Targeted Cancer Vaccines

While GM3 is the predominant ganglioside in normal melanocytes (Carubia et al. 1984), in malignant melanoma, activation of glycosylating enzymes leads to increased expression of GD3, GD2, GM2, and 9-O-acetyl GD3 (Tsuchida et al. 1987). GM2 is also expressed on metastatic prostate cancer specimens (Zhang et al. 1998) and adult T-cell leukemia (Suzuki et al. 1987). Antibodies against GM2 were able to induce apoptosis (Retter et al. 2005; Nakamura et al. 1999) or necrosis (Bjerkvig et al. 1991) of GM2-expressing cancer cell lines. Furthermore, GM2 was found to inhibit immunoglobulin production of human B cell lines through impeding the production of IL-10 and TNF-α (Kimata and Yoshida 1996). In addition, complex of GM2 and GM3 was shown to associate with cMet-CD82 to regulate hepatocyte growth factor-induced motility of HCV29 cells (Todeschini et al. 2008). These findings suggest that GM2 is an attractive target for immunotherapy.

In 1994, 122 patients with stage III melanoma ($N = 122$) were treated with unconjugated GM2 and bacillus Calmette-Guerin (BCG) or BGC alone. The OS and DFS were not statistically significant between patients treated with GM2/BCG and BCG, although DFS was greater in patients producing anti-GM2 antibody (Livingston et al. 1994a). Most anti-GM2 antibodies induced by GM2/BCG vaccine in patients were IgM, suggesting that BCG adjuvant in glycan vaccine could not efficiently trigger antibody isotype switch to GM2-specific IgG antibody, which is an important mediator of ADCC. Subsequently, potent carrier protein, KLH, and adjuvant, QS-21, were used to generate GM2-KLH/QS-21 vaccine which induced higher titers of IgM anti-GM2 antibody and more IgG anti-GM2 antibody responses than GM2/BCG vaccine (Helling et al. 1995). A phase I trial of GM2-KLH vaccine plus QS-21 as an adjuvant in 22 patients with AJCC stage III/IV melanoma showed the induction of IgM and IgG antibodies against GM2 in patients treated with 100 or 200 μg of QS-21 (Livingston et al. 1994b). This led to two randomized phase III trials. One was conducted in 880 patients with resected high-risk melanoma (AJCC stages IIB and III) comparing the therapeutic efficacy of GM2-KLH/QS-21 (GMK) vaccine with standard therapy, high-dose interferon alfa-2b (HDI) (Kirkwood

et al. 2001). The trial was closed after interim analysis showing inferiority of GMK compared with HDI, although patients with higher antibody responses to GM2 had a trend toward improved RFS and OS ($p = 0.068$ at day 29). Another phase III trial was conducted in 1314 patients with stage II melanoma to evaluate the efficacy and toxicity of GMK vaccine as compared to observation. Unfortunately, GM2-KLH/QS-21 failed to improve RFS, distant metastasis-free survival, and overall survival (Eggermont et al. 2013). In view of the impressive response of melanoma to immune checkpoint blockade therapy (Hodi et al. 2010), it is possible that clinical benefit of GM2 vaccine may become evident when combined with inhibitors of immune checkpoint.

11.5 Globo H-Targeted Cancer Vaccines

Globo H, a hexasaccharide (Fucα1 → 2Galβ1 → 3GalNAcβ1 → 3Galα1 → 4Galβ1 → 4Glcβ1), was initially identified as a ceramide-linked glycolipid in human breast cancer cell line MCF-7 (Kannagi et al. 1983) and subsequently found to be expressed on a variety of epithelial cancers including breast, colon, ovarian, gastric, pancreatic, lung, and prostate cancers (Zhang et al. 1997, 1998).

Examination of Globo H expression in breast cancer stem cells (BCSCs) by flow cytometry revealed Globo H expression in 61 % (25/41) of breast cancer specimens and in 20 % (8/40) of BCSC-enriched subpopulation ($CD44^+/CD24^-$). The expression of Globo H precursor, stage-specific embryonic antigen 3 (SSEA3), was 77.5 % (31/40) in breast cancer tissues and 62.5 % (25/40) in BCSCs. Like Globo H, SSEA3 expression in normal tissues was predominately at the secretory borders of epithelium, where access to the immune system is restricted. Immunization of mice with Globo H-KLH and alpha-GalCer induced antibodies reactive with Globo H and SSEA3, suggesting that a Globo H-based vaccine will target tumor cells expressing Globo H or SSEA3, including BCSCs (Chang et al. 2008).

The overexpression of Globo H in cancer with limited expression in normal tissues makes Globo H a potential target for cancer immunotherapy. The findings of Globo H ceramide as stem cell markers (Chang et al. 2008), immune checkpoint molecules (Tsai et al. 2013), and angiogenic factors (Cheng et al. 2014) provide further impetus for Globo H-targeted immunotherapy (Sabbatini et al. 2007). Two phase I clinical trials of Globo H-KLH/QS-21 vaccine were conducted in patients with relapsed prostate cancer ($n = 18$) (Slovin et al. 1999) and metastatic breast cancer ($n = 27$), respectively (Gilewski et al. 2001). The treatment schedules consisted of injection of Globo H-KLH at weeks 1, 2, 3, 7, and 19. In general, the vaccine was well tolerated, with only local reactions and occasional fever and chills. Humoral responses to Globo H-KLH vaccine were observed. In the trial of relapsed prostate cancer, the highest median IgM antibody titer was around 300 at the dose level of 10, 30, and 100 μg of Globo H-KLH, and peak response was observed at weeks 34, 3, and 9, respectively. For the dose level of 3 μg of Globo H-KLH, the peak titer was 150 at week 7. Interestingly, the production of Globo

H-specific IgG antibody showed a different pattern from IgM responses. There were two obvious peak titers at the dose level of 10 μg and 30 μg of Globo H-KLH. At 30 μg Globo H-KLH, the IgG antibody titer reached to the maximal titer of 160 at weeks 9 and 34. At 10 μg Globo H-KLH, the peak IgG antibody titer was 80 at week 3 and week 35. In patients treated with 100 μg or 3 μg of Globo H-KLH, the titers of Globo H-specific IgG was only 100 (around week 31) or less than 20 (week 26), respectively. In the trial of metastatic breast cancer, Globo H-specific IgM peaked around weeks 5–7. Antisera in several patients of both trials displayed CDC activity. Both trials demonstrated that the Globo H-KLH vaccine was safe and effective in inducing humoral antibody response with moderate Globo H-specific IgM antibody titers in most patients, but only minimal IgG antibody. Recently, a multinational randomized phase II/III clinical trial of Globo H-KLH vaccine vs. placebo in patients with metastatic breast cancer has completed accrual of 349 patients and is awaiting further follow-up for outcome analysis (NCT01516307). Another phase II clinical trial of this vaccine in ovarian cancer is ongoing. In addition, a new generation of Globo H vaccine consisting of Globo H conjugated to diphtheria toxin as a carrier protein was shown to elicit more desirable IgG anti-Globo H, when combined with a novel analog of NKT-stimulatory alpha-galactosylceramide (α-GalCer) as an adjuvant. The efficacy of this promising Globo H vaccine awaits clinical trial in the near future (Huang et al. 2013).

11.6 Lewis^y-Targeted Cancer Vaccine

In ovarian cancer, Lewis^y is overexpressed which promotes metastasis through epididymis protein 4 (Zhuang et al. 2013, 2014). The expression of Lewis^y antigen was considered as an independent, drug resistance-related risk factors (Gao et al. 2014). A clinical trial of Lewis^y pentasaccharide conjugated with KLH together with immunological adjuvant QS-21 in ovarian cancer patients ($n = 25$) showed that the majority of the patients (16/24) produced anti-Lewis^y antibodies with significant antitumor cell reactivity as assessed by CDC in some patients. The vaccine was well tolerated without any gastrointestinal, hematologic, renal, or hepatic toxicity (Sabbatini et al. 2000). Another phase II trial was conducted with a doxorubicin-conjugated chimeric variant of anti-Lewis^y monoclonal antibody, BMS-182248-01, in patients ($n = 15$) with advanced gastric carcinoma (Ajani et al. 2000). However, BMS-182248-01 vaccine appeared to be ineffective in patients with gastric carcinoma with 10 patients progressed on study.

11.7 Polysialic Acid-Targeted Cancer Vaccine

Polysialic acid (polySA), a carbohydrate polymer of negatively charged sialic acid attached to the neural cell adhesion molecule (NCAM), is overexpressed on the surface of various cancers including small-cell lung cancer (SCLC) (Tanaka et al. 2001), Wilms' tumor (Roth et al. 1988a, b), neuroblastoma (Gluer et al. 1998), and neuroectodermal tumors (Figarella-Branger et al. 1990). A clinical trial of polySA-KLH (30 μg) vaccine in small-cell lung cancer ($n = 13$) did not induce immune response, but N-propionylated (NP)-polySA (30 μg) developed high-titer anti-SA antibody along with peripheral neuropathy and ataxia in several patients (Krug et al. 2004). Another trial of lower dose of NP-polySA vaccine (10 μg) resulted in the induction of IgM antibodies against polySA antigen in all 18 patients, with self-limited grade 3 ataxia of unclear etiology in 1 of 18 patients (Krug et al. 2012).

11.8 Polyvalent Glycan Vaccine

A hexavalent vaccine, including GM2, Globo H, Lewis^y, glycosylated MUC-1-32mer, Tn, and TF in a clustered formation conjugated to KLH, mixed with QS-21 was administered in a phase II setting to 30 patients with relapsed prostate cancer. All 30 patients showed increased antibody titers to at least two of the six antigens, but these serologic responses were lower than those seen previously with the respective monovalent vaccines (Slovin et al. 2007). In another study, GPI-0100, a semisynthetic low toxicity saponin, was used as adjuvant at doses ranging between 100 and 5000 μg for a bivalent vaccine containing the Globo H and the mucin MUC2 conjugated to KLH with in groups of five prostate cancer patients who had no evidence of disease except for rising PSA levels. All doses of GPI-0100 were well tolerated with dose-dependent increases in antibody titers against Globo H and MUC2. At the 5000 μg dose level, toxicity remained minimal with only occasional grade II local toxicity at vaccination sites and occasional sporadic grade I elevations in ALT. Compared with a subsequent trial with the same bivalent vaccine plus QS-21 at the maximal tolerated dose of 100 μg, the 5000 μg dose of GPI-0100 induced comparable antibody titers (Slovin et al. 2005).

11.9 Conclusion

Tumor-associated carbohydrate antigens are attractive targets for cancer therapy. Glycan-targeted immunotherapy holds the promise to have less side effects and greater specificity compared to conventional cancer therapy. To date, passive immunotherapy with anti-GD2 antibody in patients with neuroblastoma is the

first successful glycan-targeted immunotherapy, which has documented that targeting TACA is a feasible strategy for cancer immunotherapy. On the other hand, carbohydrate-based vaccines for active immunotherapy have yet to be proven effective in phase III randomized trials, although encouraging results were noted in early clinical trials. New strategies are needed for enhancing the potency of carbohydrate-based cancer vaccine by improving the design of vaccine. Designs with better adjuvants that effectively boost IgG humoral and/or cellular immune response against TACAs are also critically needed.

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