Review

Glycan changes: cancer metastasis and anti-cancer vaccines

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Complex carbohydrates, which are major components of the cell membrane, perform important functions in cell–cell and cell–extracellular matrix interactions, as well as in signal transduction. They comprise three kinds of biomolecules: glycoproteins, proteoglycans and glycosphingolipids. Recent studies have also shown that glycan changes in malignant cells take a variety of forms and mediate key pathophysiological events during the various stages of tumour progression. Glycosylation changes are universal hallmarks of malignant transformation and tumour progression in human cancer, which take place on the whole cells or some specific molecules. Accordingly, those changes make them prominent candidates for cancer biomarkers in the meantime. This review mainly focuses on the correlation between glycosylation and the metastasis potential of tumour cells from comprehensive aspects to further address the vital roles of glycans in oncogenesising. Moreover, utilizing these glycosylation changes to ward off tumour metastasis by means of anti-adhesion approach or devising anti-cancer vaccine is one of promising targets of future study.

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1. Introduction

Complex carbohydrates are termed as *glycans*, and they constitute the most complex group molecules in living organisms. Glycosylation produces an abundant, diverse and highly regulated repertoire of cellular glycans that are frequently attached to proteins and lipids. Glycans participate in many key biological processes including cell adhesion, molecular trafficking and clearance, receptor activation, signal transduction and endocytosis, all of which are of great interests to scientists (Ohtsubo and Marth 2006; Reis *et al.* 2010).

Glycans, presented in the form of glycoproteins, glycolipids, glycosaminoglycans or other glycoconjugates, have long been known to perform important functions in a variety of biological processes. They participate in molecular recognition activities such as cell migration and metastasis; host–pathogen interactions such as bacterial and viral infections; and initiation of immune response (Liang *et al.* 2008). In the cellular immune system, specific glycoforms are involved in the folding, quality control and assembly of peptide-loaded major histocompatibility complex (MHC) antigens and the T-cell receptor complex (Rudd *et al.* 2001).

2. Classification of carbohydrates

As for other macronutrients, the primary classification of dietary carbohydrates, as proposed at the Joint Food and

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Abbreviations used: CRD, carbohydrate recognition domain; DP, degree of polymerization; EMT, epithelial-mesenchymal transition; FAO, Food and Agriculture Organization; HCC, hepatocellular carcinoma; NGSH, *N*-(α-D-glucopyranoside) salicyloyl hydrazine; GSK, glycogen synthase kinase; GLP, glyco-lipopeptide; GSL, glycosphingolipids; GPI, glycosphosphatidylinositol; GPAA1,GPI anchor attachment protein 1; GSK, glycogen synthase kinase; HCC, hepatocellular carcinoma; AHSG, α2-HS glycoprotein; MHC, major histocompatibility complex; MMPs, matrix metalloproteinases; PECAM, platelet endothelial cell adhesion molecule; PSA, prostate-specific antigen; WHO, World Health Organization

Agriculture Organization (FAO)/World Health Organization (WHO) Expert Consultation on Carbohydrates in Human Nutrition convened at Rome in 1997 (FAO 1998), is by molecular size, as determined by the degree of polymerization (DP), the type of linkage (α or non-α) and the character of individual monomers (Cummings and Stephen 2007).

A chemical approach divides carbohydrates into three main groups, sugars (DP 1–2), oligosaccharides (short-chain carbohydrates) (DP 3–9) and polysaccharides (DP ≥10) (Cummings and Stephen 2007). Recent progress in life science has revealed that glycans exist in free state as well as conjugated state, like glycoproteins, proteoglycans, and glycolipids (Honda *et al.* 2003). A weighted q-gram method is also used for glycan structure classification (Li *et al.* 2010). Glycans are predominantly glycosidically N-linked to asparagine or O-linked to serine and threonine; these N- and O-linked glycans are often terminated with sialic acids, which are structurally diverse and widely distributed on the cell surface and secreted glycoproteins, where they function in cell–cell communication (Dennis *et al.* 1999).

3. Associated glycosylation in oncogenesis

Oncogenesis is a multi-step process with a wide variety of genetic or epigenetic changes in the malignant cells leading to six functional characteristics of cancer: persistent growth signals, evasion of apoptosis, insensitivity to anti-growth signals, unlimited replication potential, angiogenesis, invasion and metastasis (Yeung *et al.* 2008). Like normal cells during embryogenesis, tumour cells undergo activation and rapid growth, adhere to a variety of other cell types and cell matrices, and invade tissues. Embryonic development and cellular activation in vertebrates are typically accompanied by changes in cellular glycosylation profiles. Thus, it is not surprising that glycosylation changes are also universal features of malignant transformation and tumour progression (Varki *et al.* 2009). In addition, current studies have shown that several glacans on the tumour surface and host elements mediate key pathophysiological events during the various steps of tumour progression (Fuster and Esko 2005; Gilbert 2009).

Glycan changes in malignant cells take a variety of forms. Examples have been found of lack of expression or excessive expression of certain structures, the persistence of incomplete or truncated structures, the accumulation of precursors and, less commonly, the appearance of novel structures (Varki *et al.* 2009)**.** In this section, we mainly focus on the modification of glycans that are frequently altered on the tumour surface from several aspects to further address the important roles of glycans in cancer science.

3.1 *Correlation between N-glycan changes and cancer*

N-linked glycosylation is a prominent determinant of protein structure and function, which is a complex biosynthetic process that regulates maturation of proteins through the secretory pathway (Contessa *et al.* 2010; Waetzig *et al.* 2010). Branched *N*-glycans, such as bisecting GlcNAc, β1, 6 GlcNAc and core fucose (α-1,6-fucose), are enzymatic products of *N*-acetylglucosaminyltransferase III (GnT-III), *N*-acetylglucosaminyltransferase V (GnT-V) and α-1,6-fucosyltransferase (α1,6-FucT), respectively. These branched structures are highly associated with various biological functions of cell adhesion molecules, including cell adhesion and cancer metastasis (Zhao *et al.* 2008a).

A case in point is N-linked glycosylation on *E*-cadherin, which has been implicated in the modulation of cadherin-dependent tumour cell–tumour cell adhesion in association with tumour progression. Previously, the structural modifications of *N*-glycans of *E*-cadherin by GnT-III were shown to cause increased cell–cell adhesion, whereas structural modification of *N*-glycans on *N*-cadherin with increased branched structures by GnT-V resulted in decreased cell–cell adhesion, contributing to increased cellular motility and invasiveness (Pinho *et al.* 2009).

Oncogene activation stimulates increased expression of the Golgi enzymes that generate β1,6 GlcNAc-branched tetra-antennary *N*-glycans. These *N*-glycans, which are found on proteins including growth factor receptors and integrins, are shown to enhance growth signalling in motile tumour cells (Zavareh *et al.* 2008). It has also been reported that GnT-V activity and β1,6 GlcNAc-branched *N*-glycans levels are increased in highly metastatic tumour cell lines (Gu *et al.* 2009), both of which are increased in colon, hepatic and glial tumours (Wang *et al.* 2007). On the other hand, GnT-III contributes to suppress cancer metastasis in highly metastatic melanoma cells by reducing β1,6 GlcNAc branching in cell surface *N*-glycans and increasing bisected *N*-glycans, which results in an enhancement of cell–cell adhesion due to prolonged turnover of *E*-cadherin on the cell surface (Gu *et al.* 2009).

3.2 *Correlation between O-glycan changes and cancer*

O-Glycosylation is another type of glycosylation found in glycoproteins and consists of a glycan O-linked to a serine or a threonine residue. The frequency of O-glycosylation on glycoproteins is high, particularly on secreted or membrane-bound mucins, which are rich in serine and threonine (Reis *et al.* 2010). O-glycosylation pathways play a key role in biological activity of glycoproteins involved in the control of cell differentiation and the regulation of cell growth through apoptosis and proliferation pathways (Gallegos *et al.* 2010; Patsos *et al.* 2009). Altered O-glycosylation,

such as expression of the Tn and STn, have been implicated in various processes such as inflammatory responses, angiogenesis, autoimmunity and cancer (Ju *et al.* 2008; McCluskey *et al.* 2010).

O-Glycans play important roles in the attachment and invasion of cancer cells and their survival in the blood stream. *O*-Glycan chains of glycoproteins, as well as tissue and blood group antigens, including Lewis antigens, may be qualitatively and quantitatively altered, are often truncated and highly sialylated in cancer cells (Brockhausen 1999). For example, mucins are large, highly O-glycosylated proteins involved in the protection and control of signalling on epithelial surfaces. Mucins themselves exert profound tumour-promoting effects, partly mediated through interactions with their glycan moieties (Wu *et al.* 2009). In benign cells of breast, prostate, ovarian and pancreas, MUC1 is greatly glycosylated, limited to the apical side of the glands and minimally expressed. Transformation of these cells into malignant cells is associated with the over-expression of MUC1, loss of MUC1 expression polarity and dysregulation of *O*-glycans of MUC1 (Deguchi *et al.* 2010). In addition, cancer cells may contain membrane-bound mucin-like glycoproteins that have domains rich in *O*-glycans. The gene expression of these glycoproteins is cell-specific and often altered in cancer (Brockhausen 1999). Recently, there have been considerable insights into this field. O-glycosylated glycoproteins represent important elements in fibroadenoma development (Gallegos *et al.* 2010). Changes in mucin-type O-glycosylation are associated with somatic cell differentiation and cancer (Meyts *et al.* 2007). Other studies have demonstrated that up-regulated biosynthesis of complex O-glycosidically-linked glycans and galectin-3 favour breast cancer progression and brain metastasis (Mayoral *et al.* 2008).

3.3 *Correlation between sialic acid changes and cancer*

Sialic acids are typically found as terminal monosaccharides attached to cell surface glycoconjugates. They play essential roles in many physiological and pathological processes (Goswami *et al*. 2007). Altered expression of certain sialic acid types or their linkages can have prognostic significance in human cancer (Hedlund *et al*. 2008).

Elevation of sialic acid concentration in serum has been observed in various malignancies. Marth *et al.* (1988) have reported difference in sialic acid levels between benign and malignant tumours. Prostate-specific antigen (PSA) and acid phosphatase, which are also sialic-acid-containing glycoproteins normally present in circulation, increase in both benign and malignant prostatic growth (Goswami *et al.* 2007). Some reports demonstrated that sialic acid is over-expressed in colon cancer tissues and skin tumour metastases (Azab *et al.* 2008; Kazezoglu *et al.* 2007). The results of Wang *et al.* suggested that high level of α2,3-linked sialic acid residues are associated with metastatic potential of gastric cancer cells (Wang *et al.* 2009). Furthermore, α2,6-sialic acid is necessary for the cell surface residency of platelet endothelial cell adhesion molecule (PECAM), which performs an important function in cell adhesion, mechanical stress sensing, anti-apoptosis and angiogenesis (Kitazume *et al.* 2010). Consequently, enhanced expression of terminal 2-6-linked sias on cell surface N-linked glycans and of sialyl-Lewis^X on O-linked glycans often correlates with poor prognosis of many human malignancies (Varki and Varki 2007).

3.4 *Correlation between sialylated lewis structures and cancer*

In some solid tumours, incomplete synthesis of cell surface carbohydrates results in an increasing expression of ABH precursor structures such as Lewis^a, sialyl-Lewis^a and their isomers (Lewis^x and sLewis^x) (Takada *et al.* 1993; Dabelsteen 1996; Sozzani *et al.* 2008).

The antigenic epitope of sialyl-Lewis^a antigen has been used clinically as a tumour marker for pancreatic cancer, colorectal cancer and other selected malignancies (Nagao *et al.* 2007). The expression of sialyl-Lewis^a antigen is associated with tumour progression. Several lines of evidence suggest that sialyl-Lewis^a oligosaccharides are involved in the adhesion of several types of cancer cells to *E*-selectin presented on the surface of endothelial cells (Ugorski and Laskowska 2002).

On the other hand, Lewis^x is involved in selectin-mediated adhesion of cancer cells to the vascular endothelium and is thought to be closely associated with hematogenous metastasis and hence with the malignant behaviour of cancer cells (Takada *et al.* 1993; Sozzani *et al.* 2008). For example, colon cancer cells strongly express sialyl-Lewis^x, while they exhibit markedly decreased sialyl 6-sulfo Lewis^x expression compared with normal colonic epithelial cells. The sialyl-Lewis^x in cancer cells plays an important role in *E*-selectin-mediated cancer cell adhesion to vascular endothelial cells during the course of tumour angiogenesis and distant metastasis (Yusa *et al.* 2010).

3.5 *Correlation between selectin and cancer*

Selectins are expressed by leukocytes, endothelial cells and platelets, which interact with cell surface glycoconjugates and mediate tethering, rolling and adhesion of several types of cells. There are three subsets of selectins: *L*-selectin is constitutively expressed by leukocytes, *E*-selectin by activated endothelial cells and *P*-selectin by platelets and activated endothelial cells (Zarbock *et al.* 2007). It is known for a relatively long time that cancer cells, notably epithelial cancer cells, express high levels of sialylated fucosylated selectin ligands (Witz 2008).

L-Selectin links recruitment of inflammatory leukocytes to the sites of tumour cell emboli in microvasculature and their potential to facilitate metastasis (Laubli *et al.* 2006), while the endothelial adhesion molecule *E*-selectin is implicated in the metastatic spread of colorectal and breast cancer by facilitating adhesion of tumour cells to the endothelium (Zen *et al.* 2008; Hebbar *et al.* 2009). In addition, *P*-selectin plays a key role in mediating interaction between cancer cells, platelets and endothelial cells by interacting with its corresponding ligands. Alternatively, it has been reported that *P*-selectin binds several types of human cancer and human-cancer-derived cell lines, including colon cancer, lung cancer, breast cancer, malignant melanoma, gastric cancer, tongue squamous cancer and neuroblastoma (Mi *et al.* 2009). Numerous reports demonstrate that tumour cells can form multicellular complexes with platelets (via *P*-selectin) and leukocytes (via an *L*-selectin-dependent mechanism), which can then settle down in the microvasculature of distant organs and eventually extravasate and establish metastatic colonies (Thomas *et al.* 2009).

3.6 *Correlation between glycosphingolipids and cancer*

Glycosphingolipids (GSL) are lipid components of eukaryotic cell membranes that are important to the proper development of vertebrates, and are involved in multiple processes, including cell-type-specific adhesion, cell–cell interaction, embryogenesis, development and differentiation of neuronal cells and leukocytes, and tumour progression *in vivo* (Bektas and Spiegel 2003; Pizette *et al.* 2009; Varki *et al.* 2009).

GSL function in "defining oncogenesis and its reversion" is closely associated with hypoxia/epithelial–mesenchymal transition (EMT) process, which is considered to cause basic molecular changes associated with developmental processes and cancer progression (Igarashi and Kannagi 2010). Predominant expression of specific gangliosides, GD3, GM2 or GD2, has been observed on several types of tumour cells including melanoma, neuroblastoma, lymphoma and ovarian cancer cells (Bektas and Spiegel 2003; Hakomori 2008). Although GSL-GM3 is highly expressed in human bladder, benign, non-invasive tumour KK47 cells, the level of which is very low in highly malignant, invasive bladder cancer YTS1 cells (Hakomori 2010). Toshio Ariga *et al.* indicate that certain GSL antigens, especially SGGLs, GD3 and OAc-GD3, are expressed in neural tumour cells (Ariga *et al.* 2009).

3.7 *Correlation between expression of glycosylphosphatidylinositol-anchored proteins and cancer*

The glycosphosphatidylinositol (GPI) moiety is distributed in all eucaryotic membranes and serves to anchor the C-terminal end of a long list of surface proteins involved in signalling, cell–cell interactions and interactions with extracellular molecules in blood or connective tissue (Hoessli *et al.* 2007; Varki *et al.* 2009). Furthermore, GPI-anchored membrane cytokines have been shown to play an important role in host immune responses against tumour cells (Zhao *et al.* 2010).

Previous studies demonstrated amplification and over-expression of GPI transamidase subunits in head and neck squamous cell carcinoma (Jiang *et al.* 2007). Similarly, GPI-PLD mRNA expression is increased with tumour progression in human ovarian cancer cell lines, epithelial cells of the skin and highly malignant H-ras-transfected murine bladder carcinoma cells (He *et al.* 2002). Recently, some investigators have found that GPI-anchored CD55 and CD59 expressed in nearly all primary tumours and cancer cell lines examined. Some studies also indicated that GPI anchor attachment protein 1 (GPAA1) is up-regulated in HCC (hepatocellular carcinoma) (Jian-Hua *et al.* 2009). In addition, MMP25 (MT6-MMP) is one of the two GPI-anchored matrix metalloproteinases (MMPs) that has been suggested to increase mRNA expression in colon cancer (Sun *et al.* 2007; Radichev *et al.* 2010).

3.8 *Correlation between galectins and cancer*

Galectins are members of soluble lectins that bind β-galactoside-containing glycans and are defined by a conserved carbohydrate recognition domain (CRD) and a common structural fold. Among the various lectin types, galectins are probably the most conserved and ubiquitous family, with members identified in most animal taxa examined so far. As many as 15 galectins have been identified in mammals and proposed to mediate diverse biological processes involved in the regulation of innate and adaptive immune responses, such as cell activation, differentiation, cytokine secretion and apoptosis (Rabinovich *et al.* 2007; Le Mercier *et al.* 2010).

Immunohistochemical staining of galectin-1 in clinical cancer samples reveals that its expression is positively associated with the malignant progression of many tumour types, including glioma, prostate, colon, breast, cervical and oral squamous cells (Wu *et al.* 2009). Exogenous galectin-3 controls tumour cell adhesion and motility via FAK and PI3K activation and local F-actin reorganization through interaction with the $\alpha_5\beta_1$integrin, while galectin-8 can bind to$\alpha_3\beta_1$ and $\alpha_6\beta_1$ integrins to modulate tumour cell adhesion (Laderach *et al.* 2010). Numerous studies have proved that astrocytic tumours express high levels of galectin-3, which is also expressed on oligodendrocytes, endothelial cells, gastric cancer cells, colorectal cancer cells and macrophages/microglial cells in areas of solid tumour growth (Iacovazzi *et al.* 2010; Kim *et al.* 2010; Le Mercier *et al.* 2010).

Moreover, galectin-7 expression has been associated with tumour progression and found to be raised significantly in papillary carcinomas and human breast cancer (Demers *et al.* 2010).

4. The role of glycans in the diagnosis of cancer

The lack of specific and sensitive tumour markers for early detection of cancer is driving a search for new approaches to identify biomarkers (Peracaula *et al.* 2008). Glycoconjugate modification is a universal hallmark of cancer, which makes them important cancer biomarkers. Many of the current biomarkers used in clinics, in both tissue and serum assays, are based on these carbohydrate modifications (Reis *et al.* 2010). In spite of intensive research efforts, there are a very limited number of serum markers that are useful in terms of monitoring patient response to treatment or predicting relapse after cancer therapy (Dwek and Brooks 2004).

Alpha-fetoprotein (AFP) is a well-known glycoprotein produced in the developing embryo and fetus, and the level in a healthy adult is quite low. In particular, AFP is detected in patients with HCC; however, an elevation of AFP provides limited clinical information on HCC and is only suggestive of predicting HCC due to its low specificity (~50%)and the isoform AFP-L3 is particularly useful in early identification of aggressive tumours associated with HCC with specificity 96% (Kim *et al.* 2009).

At present, the familiar serological assays detect carbohydrate antigens such as SLe^a (CA19-9) and STn (CA72-4) or mucin glycoproteins such as MUC1 (CA15-3) and MUC16 (CA125) (Reis *et al.* 2010). Many studies have evaluated SLe^x levels for the diagnosis of cancer. SLe^x is a weak marker for some small-cell lung cancers (24% for all stages); however, this increases with progression, reaching 71% for late-stage cancer (Arnold *et al.* 2008; Huang and Chen 2010).

The glycan changes of two serum glycoproteins, PSA and pancreatic ribonuclease have been currently used as tumour markers for prostate and cancer pancreatic adenocarcinoma separately (Peracaula *et al.* 2008). The mAb F77 is defined as a unique prostate cancer marker as well (Zhang *et al.* 2010). Furthermore, Yi *et al.* (2009) and Rose *et al.* (2010) suggest that α2-HS glycoprotein (AHSG), anti-AHSG autoantibody and nonmetastatic B may be useful biomarkers for breast cancer screening and diagnosis. Alternatively, glycosylated MUC1 and MUC5AC has been perceived as potential effective tumour markers in gastric cancer diagnosis (Xu *et al.* 2009). Toshio Ariga *et al.* also indicate that certain GSL antigens, especially SGGLs, GD3,\ and OAc-GD3, may be considered as tumour-associated antigens that represent important biomarkers for neural tumours (Ariga *et al.* 2009).

5. The role of glycans in the therapy of cancer

It is widely known that the chemotherapy, radiotherapy and surgery are the three major treatments available for the treatment of cancer, and are deemed to be characterized by a low therapeutic index (Scatena *et al.* 2008). Improved analytical tools for the study of glycosylation and application of molecular techniques for the characterization of the genes encoding glycosyltransferases have, however, enabled the structural identification of some of the cancer-associated changes in glycosylation (Dwek and Brooks 2004).

A few recent reports indicate that chemical or genetic disruption of the N-glycosylation pathway in cancer cells decreases their malignant features. Therefore, targeting defective glycosylation pathways may be a novel approach for the treatment of malignancy and a strategy to prevent metastasis (Zavareh *et al.* 2008). N-Linked glycosylation inhibition is a novel therapeutic strategy for both gliomas and other malignant tumours (Contessa *et al.* 2010). The promising results also suggested that the new *N*-glycoside compound such as *N*-(α-D-glucopyranoside) salicyloyl hydrazine (NGSH) might be useful for cancer treatment (Zhao *et al.* 2008b). Meanwhile, the α-tocopherol *O*-glycosides can be considered as prodrugs in the prevention and treatment of colon cancer (Knas *et al.* 2009). Moreover, therapies based on the use of anti-ganglioside antibodies have been suggested in neuroblastoma (Bektas and Spiegel 2003; Lahiri and Futerman 2007).

Other researches have shown promising results: Garcea *et al.* have found glycogen synthase kinase (GSK) has potential as an important new target both in the treatment of resectable pancreatic cancer and in the palliation of inoperable tumours (Garcea *et al.* 2007). A long-acting rhIFN-α2b mutein, 4N-IFN, was found to have a 25-fold longer plasma half-life than the non-glycosylated one, which showed a markedly enhanced *in vivo* anti-tumour activity in human prostate carcinoma implanted in nude mice (Ceaglio *et al.* 2010). In addition, Tietze *et al.* have developed new glycosidic prodrugs to decrease side-effects (Tietze *et al.* 2008).

Up to now, such an approach has not eliminated tumours in patients without side-effects when a single drug is applied. Nevertheless, several agents that manipulate tumour metabolism may become a supportive therapy in combination with established cancer treatments (Sattler *et al.* 2010).

6. Glycan used in anti-cancer vaccines

The expression of glycosylation in malignant tumours cells is significantly altered when compared with normal cells. Aberrant glycosylation of glycoproteins and glycolipids of cancer cells, which correlates with poor survival rates, is being exploited for the development of immunotherapies

for cancer. In particular, advances in the knowledge of cooperation between the innate and adaptive system provide avenues for the rationale design of vaccine candidates (Buskas *et al.* 2009).

Fully synthetic anti-cancer vaccines targeting tumour-associated carbohydrates provide an attractive option for the treatment of cancer, which have major advantages as they can be designed to incorporate only those elements required for a desired immune response. It should also be mentioned that it is now accepted that glycopeptides can mediate classical MHC-mediated immune responses (Buskas *et al.* 2009). Besides, the use of synthetic glycopeptides as partial or full components of vaccines targeting these antigens is a favourable approach for the development of cancer immunotherapies because of their ability to be generated as homogenous formulations (Liakatos and Kunz 2007). As a result, tumour-associated glycan antigens have been viewed as the excellent for vaccine development and a number have proceeded into clinical trials (Oyelaran and Gildersleeve 2007).

A case in point is MUC1, a high-molecular-weight glycoprotein that is up-regulated in adenocarcinomas and hematological cancers. MUC1-based vaccines have quickly entered into human clinical trials and numerous clinical studies have demonstrated that MUC1 immunotherapy is beneficial and protects patients against recurrence for up to 8 years in early breast cancer patients (Tang *et al.* 2008). A recent reported synthetic vaccine consisting of a tumour-associated sialyl-TNMUC1 tandem-repeat glycopeptide and tetanus toxoid could induce strong and highly selective immune response (Kaiser *et al.* 2009). In addition, vaccines based on Tn, TF, sTn, Lewis^{Y}, polysialic acid and GloboH are currently in Phase I or Phase II clinical trials (Oyelaran and Gildersleeve 2007).

In Bettahi's study, they explored a self-adjuvant glyco-lipopeptide (GLP) as a platform for cancer vaccines (Bettahi *et al.* 2009). Latest advances in anti-breast-cancer peptide-based vaccine strategies with an emphasis on the self-adjuvant multivalent glycolipopeptide vaccine strategy has been developed in the laboratory and has shown promising results (Chentoufi *et al.* 2009). Fengshu Zhao *et al.* prepared a tumour vaccine expressing IL-21-GPI-anchored and GM-CSFs simultaneously and found it effective in anti-tumour immune therapy (Zhao *et al.* 2010).

Although still at an early stage, carbohydrate array technology has tremendous potential for accelerating the development of the glycan-related anti-cancer vaccine.

7. Discussion

Glycosylation can be seen in a range of human cancer cells, whereas aberrant glycosylation of N- or O-linked glycoproteins is closely related to tumour growth and metastasis, and therefore glycan analysis may be used for the early diagnosis, monitoring and therapy of a range of human cancers. Some cases in the clinical apply mentioned above also confirmed this new approach. Utilizing these glycosylations in the research on anti-tumour metastasis or anti-cancer vaccine is one of promising targets and breakthrough of future study. However, as the fundamental and precise structures of glycosylations are largely not understood, the discovery of cancer glycobiomarkers with high sensitivity and specificity that are useful for clinical diagnosis is a key problem that needs to be solved urgently. Moreover, some questions still need to be addressed, including the preparation and design of safe and effective cancer vaccines. Accordingly, large-scale clinical trials are necessary. Taken together, with the development of carbohydrate and peptide immunogens, application of mass spectrometry, high-performance liquid chromatography and spectroscopy would open up a new era for further research, which in turn will be of great significance for the prevention, diagnosis and treatment of cancer.

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