Glycoprotein B7-H3 overexpression and aberrant glycosylation in oral cancer and immune response

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Oral cancer is the 11th most-common cancer worldwide. An estimated 300,373 new cases and 145,353 deaths from oral cavity cancer (including lip cancer) occurred worldwide in 2012 (1). According to the data in GLOBOCAN 2012 published by the World Health Organization, oral cancer incidence and mortality in men in South and Central Asia is increasing, and it has become the second most common cancer in the region. Epidemiological studies reveal the strong association between oral cancer and the use of betel quid, alcohol, and cigarettes. Cancers of buccal mucosa, tongue, and gingiva constitute the majority of oral cancer in the Asian population, and that is related to the exposure to the carcinogen in these anatomical areas (2). It is estimated that approximately 90–95% of oral cancers are squamous cell carcinoma (OSCC) (3), and oral cancer has been shown to progress from hyperplasia, to mild-to-moderate dysplasia, severe dysplasia, and carcinoma in situ (4). Surgery is the standard treatment for OSCC, but the differences in primary sites and the complex anatomy of the head and neck give rise to intricate patterns of local invasion and regional spread. This distinction makes primary tumors difficult to eradicate once they have grown large enough to spread into adjacent tissues. Radiotherapy is an integral part of primary or adjuvant treatment, and chemotherapy is used as a combination therapy in advanced OSCC (5). Despite numerous prospective trials using various combination therapies to improve locoregional control, survival rates for advanced carcinoma-associated OSCC remain dismal (6). In contrast to many other cancers in which metastasis is the primary cause of death, local recurrence is the common cause of treatment failure and death in patients with OSCC (5). Therefore, locoregional control is a key therapeutic objective (7).

Glycosylation is an important biological process that occurs cotranslationally or posttranslationally on more than 50% of eukaryotic proteins and can affect protein folding, stability, solubility, and function. Aberrant glycosylation is often observed in pathological conditions such as inflammation and cancer metastasis. Altered terminal fucosylation and sialylation are believed to result from changes in expression and are associated with tumor malignancy (8). Atypical glycosylation of cell surface carbohydrates has been reported to be associated with malignant transformation of oral epithelium (9), and protein-bound sugar levels were higher in plasma and tissue samples of oral cancer patients (10). A series of studies about dysregulation of the N-glycosylation–regulating gene, DPAGT1, drives oral cancer cell disconnection by inhibiting adhesion of E-cadherin through Wnt signaling pathway were reported (11–13). It was also found that the expression of Lewis(α) on EGFR promotes migration of oral cancer cells (14).

To identify new markers as diagnostic and therapeutic targets for oral cancer, a sialylation probe was used to investigate the differential expression of glycoproteins in oral cancer and normal cells. Among the glycoproteins identified, receptor B7-H3 was selected for further study because it was more differentially expressed in cancer cells, B7-H3, also known as B7 homolog 3 or CD276 isoform 1, was discovered in 2001 (15) and is a 110-kDa, type I transmembrane glycoprotein with four Ig-like domains that contain a nearly exact tandem duplication of the IgV-IgC domain (4Ig-B7-H3). However, the potential binding partner of B7-H3 remains unclear (16), and the functional effect of B7-H3 on T cells is controversial (17). B7-H3 protein was also found on various cell types and organs. This broad expression pattern suggests more diverse immunological and probably nonimmunological functions of B7-H3, especially in peripheral tissues (18). Recently, B7-H3 has been significantly up-regulated in the tumor tissue of OSCC patients and correlated with increased tumor size and poor survival rate. Comparing the N-glycans of B7-H3 from Ca9-22 oral cancer cells and Smulow–Glickman (SG) normal cells, we also found that the glycans of B7-H3 from Ca9-22 contain the terminal α-galactose and are more diverse with higher fucosylation and better interaction with DC-SIGN [DC-specific intercellular adhesion molecule-3 (ICAM-3)–grabbing nonintegrin] and Langerin on immune cells than that from normal cells, suggesting that the glycans on B7-H3 may also play an important role in the disease.

Significance

Oral squamous cell carcinoma (OSCC) is characterized by high morbidity and mortality, and few therapeutic options. Here, we show that the expression of B7 Homolog 3 (B7-H3) is significantly up-regulated in the tumor tissue of OSCC patients and correlated with increased tumor size and poor survival rate. Comparing the N-glycans of B7-H3 from Ca9-22 oral cancer cells and Smulow–Glickman (SG) normal cells, we also found that the glycans of B7-H3 from Ca9-22 contain the terminal α-galactose and are more diverse with higher fucosylation and better interaction with immune cells. These findings indicate that glycoprotein B7-H3 is an important marker in oral cancer and its overexpression and aberrant glycosylation may provide a direction for the development of diagnosis and treatment of oral cancer.


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found in a variety of different human cancers, including prostate (19, 20), nonsmall cell lung (NSCLC) (21), gastric (22, 23), pancreatic (24), ovarian (25), colorectal (26), urothelial cell (27), clear cell renal cell (ccRCC) (28), and hypopharyngeal (29) cancers.

We are interested in understanding the expression pattern of B7-H3 in oral cancer and its possible underlying mechanisms. In this study, both the protein and the glycosylation profile of B7-H3 were investigated to explore their correlation with tumor progression with the expectation that the findings will provide more information to better understand oral cancer and improve therapies.

Results
Probing Glycosylation and Identification of B7-H3 in Oral Cancer Cell Lines. To delineate the molecular basis for aberrant glycosylation in the pathological processes of oral cancer, we investigated the differences in glycosylation between normal and cancer cells by using the sialylation probe reported (30). We compared normal oral epithelial cells (Smulow–Glickman; SG), which derived from adult human gingiva (31), and a representative oral cancer cell line (Ca9-22) by feeding cells with the peracetylated alkyln N-acetylmannosamine (ManNAcNe) to label sialylated proteins. Sialylated proteins incorporated with an alkyn group were then reacted with biotin-azide via Cu[I]-catalyzed click reaction and subjected to affinity enrichment via binding to the biotin tag. The enriched glycoproteins were treated sequentially with trypsin and PNGase F to release Asp-containing peptides for protein identification and glycosite mapping, respectively, through liquid chromatography-tandem mass spectrometry (LC-MS/MS) analysis (32). The results showed that NPTN (neuroplastin), PRNP (major prion protein), desmoglein-2 (DSG2), integrin β-1 (ITGB1), and CD276 (B7-H3) were detected only in Ca9-22 oral cancer cells in all three batches of samples (SI Appendix, Table S1). Of these overexpressed sialylated glycoproteins, B7-H3 was the most significant, although little is known about this glycoprotein in oral cancer. We thus decided to investigate the relationship between B7-H3 and oral cancer. Four normal oral epithelial cell lines and 13 OSCC cell lines from different anatomical areas were examined. It was found that B7-H3 was overexpressed in Ca9-22. However, although some cancer cell lines such as HSC-3, OEC-M1, SAS, and SCC-9 have slighter higher expression of B7-H3 than normal SG cells, the differences from the number of cell lines examined do not show statistical significance (SI Appendix, Fig. S1 A and B).

Correlation of B7-H3 Expression and Clinicopathological Parameters in OSCC Patients. To investigate the oncogenic potential of B7-H3 in oral cancer, we collected specimens from OSCC and oral epithelial dysplasia (OED) patients and healthy donors for immunohistochemical (IHC) staining of B7-H3. Fig. 1 shows representative IHC staining photomicrographs of normal oral mucosa, OED, and OSCC specimens. In general, normal oral epithelium was negative for B7-H3 or displayed a few B7-H3–positive cells in the basal cell layer of the epithelium (Fig. 1A). The positive B7-H3 staining on the cell surface was found predominantly in the lower one-third of the epithelium in severe oral epithelial dysplasia (OED) patients and healthy donors for immunohistochemical (IHC) staining of B7-H3. Fig. 1 shows representative IHC staining photomicrographs of normal oral mucosa, OED, and OSCC specimens. In general, normal oral epithelium was negative for B7-H3 or displayed a few B7-H3–positive cells in the basal cell layer of the epithelium (Fig. 1A). The positive B7-H3 staining on the cell surface was found predominantly in the lower one-third of the epithelium in severe OED specimens (Fig. 1B). B7-H3 staining was found strongly positive in the peripheral nonkeratinizing cells of OSCC tumor nests and negative in the central keratinized area (Fig. 1 C and D). These positively stained OSCC were basal cells of the epithelium and were viewed as cells with rapid proliferation rates. In addition, positive membrane B7-H3 staining was also observed in some endothelial cells and inflammatory cells in the OSCC stromal tissue. The mean B7-H3 labeling score increased significantly from normal oral mucosa (7.89 ± 14.4%) and OED (15.62 ± 8.8%) to OSCC samples (74.75 ± 47.2%, P < 0.001; SI Appendix, Table S2). The high labeling score of B7-H3 in OSCC was significantly associated with increased tumor sizes (P = 0.0001) and advanced clinical stages (P = 0.004). No significant association was found between the labeling scores of B7-H3 in OSCC and patient age, sex, location of cancer, lymph node involvement, metastasis, recurrence, differentiation of OSCC, alcohol consumption, betel quid chewing, or cigarette smoking (SI Appendix, Table S3). A Kaplan–Meier curve showed that the OSCC patients with the B7-H3 labeling scores ≥55% had a significantly poorer cumulative survival rate than those with the B7-H3 labeling scores <55% (log-rank test P = 0.005) (Fig. 1E).

Involvement of B7-H3 in Cell Proliferation and Tumor Formation. Next, we examined whether B7-H3 is involved in the proliferation of oral cancer cells and tumor formation in the animal study. By knockout and restoration strategies, we established three stable clones of B7-H3 knockdown Ca9-22 cells (shB7-H3-1 to -3) from two independent shRNA sequences. The knockdown efficiency in these three clones (91%, 94%, and 75%) was calculated based on the mRNA level of B7-H3 (Fig. 2A). The protein expression level was significantly reduced in B7-H3 knockdown cells (SI Appendix, Fig. S1C). The result of in vitro cell proliferation assay showed that B7-H3 knockdown clones had slower proliferation rates (Fig. 2B). To evaluate the effect of B7-H3 on tumor growth in vivo, the Ca9-22 shB7-H3 knockdown clones were injected s.c. into the flank region of NOD-SCID mice and examined for tumor growth. The results showed that knocking down B7-H3 dramatically suppressed the growth of Ca9-22 in vivo (Fig. 2C and D), and the
same result was also observed in orthotopic implantation (Fig. 2 E and F). Moreover, when B7-H3 was overexpressed in control clones to increase the expression of B7-H3 or in shB7H3-1 stable clones were measured by using a vernier caliper weekly. (C) Tumor growth curve. The mean ± SD (n = 3) from each group at each time point is shown. (D) Images of tumor specimens. a shows the gross view of isolated tumors. Strong membranous staining of B7-H3 in shCtrl groups (b) and almost no staining of B7-H3 in shB7H3-1 groups (c) were observed at the end point (day 56). (E and F) Knockdown of B7-H3 also reduced the volumes of tumor mass in the organ-specific environment, but metastasis was not found. Luciferase-transduced tumor cells were injected into buccal mucosa of NOD-SCID mice, and tumor growth and metastasis were detected by using the IVIS imaging system. (E) Tumor growth curve. (F) Bioluminescence imaging of mice at the end point (day 42). The mean ± SD (n = 3) from each group at each time point is shown. (G and H) Overexpression of B7-H3 in shCtrl groups (G) or restoration of B7-H3 in shB7H3-1 clones (H) enhanced tumor growth in mice. Data shows the mean ± SD (n = 5) from each group at each time point. P value was calculated by Student’s t test (*P < 0.05, **P < 0.01).

Glycoform Analysis of B7-H3 in Ca9-22 and SG. We chose Ca9-22 oral cancer cells (gingival carcinoma) and SG normal oral epithelial cells from gingiva to further analyze the glycosylation level of B7-H3 in different cells. Immunoblot results showed that B7-H3 was highly expressed in Ca9-22 cells compared with normal SG cells and other cell lines (SI Appendix, Fig. S1A). Moreover, B7-H3 protein from Ca9-22 cells showed lower mobility compared with that from SG cells. After treating with PNGase F to cleave the whole N-linked glycans, the mobility of B7-H3 from both cell lines became the same, and the size of protein was ∼58 kDa as expected (SI Appendix, Fig. S2A). This result indicated that N-glycosylation contributed to the differences in B7-H3 mobility in protein gel, and a higher glycosylation level of B7-H3 from Ca9-22 cells was observed. From these data,
we conclude that both the expression and glycosylation levels of B7-H3 were up-regulated in Ca9-22 oral cancer cell lines.

To dissect the B7-H3 glycoforms involved in tumor versus normal cells, site-specific glycome mapping and glycans sequencing of B7-H3 in Ca9-22 and SG cells were performed. To obtain higher protein amounts for the glycans profiling, full-length B7-H3 was overexpressed in both types of cells and purified for analysis. MS profiles showed that the glycans of B7-H3 from SG displayed a higher level of sialylation and B7-H3 from Ca9-22 had a higher level of fucosylation (SI Appendix, Fig. S2 A and C). The detailed glycosylation patterns on B7-H3 were subsequently analyzed by LC-MS/MS. Every individual B7-H3 glycopeptide derived from SG and Ca9-22 cells was compositionally assigned and quantified in a site-specific manner through matching with the calculated mass of both peptide fragments and glycans from the Consortium for Functional Glycomics carbohydrate databases, and confirming with the appearance of fragmented glycans in MS/MS spectra (Fig. 3A).

It has been verified that there are two sites of N-linked glycosylation in B7-H3 (Asn-322 and 407) (33). Our result showed that there are eight N-glycosylation sites (Asn positions 91, 104, 189, 215, 309, 322, 407, and 433) in B7-H3, as determined by Asn to Asp conversion after PNGase F treatment (SI Appendix, Table S4). Because B7-H3 contains a nearly exact tandem duplication of the IgV-IgC domain and there were four N-glycosylation sites located in each of the IgV-IgC domains, each pair of glycosylation sites, N91 and N309, N104 and N322, N189 and N407, and N215 and N433 were identified through identical peptide sequence. Within four pairs of N-glycosylation sites, one of them was identified to compose mostly hybrid type N-glycans (N104/N322) and three of them were attached mainly with complex N-glycan (N91/N309, N189/N407, and N215/N433) (Fig. 3B and SI Appendix, Fig. S3). The B7-H3 from SG cells contained mostly biantenary glycans, and the most abundant structure is BiS2F1 (42%). The B7-H3 from Ca9-22 cells contained a more diverse pattern of N-linked glycosylation, including complex type N-glycans (BiF1: 14%, BiH1F2: 13%, BiS1F1: 11%) and hybrid type N-glycans (Man3S1F1: 6.1%) (Fig. 3A and B).

The levels of sialylation and fucosylation on each pair of N-glycosites are shown in Fig. 3C. The B7-H3 in SG carries more sialic acid residues, whereas the B7-H3 in Ca9-22 carries more fucose residues, especially at positions N104/N322, and N189/N407 (Fig. 3C). In some other OSCC cell lines, higher fucosylation in B7-H3 was also detected by AAL (Aleuria aurantia lectin, preferentially binds to α1,2,3,4,6-linked fucoses) lectin blotting compared with normal cells (SI Appendix, Fig. S4), but the number of cell lines used is not large enough to show statistical significance. The result of glycan analysis showed a protein mobility shift of B7-H3 in SG cells after treatment with α2,3/6-sialidase, demonstrating that B7-H3 from SG cells had higher sialylation (SI Appendix, Fig. S2A). The higher expression level of B7H3 in Ca9-22 cancer cells (SI Appendix, Table S1) is obviously due to the higher expression of the protein instead of higher sialylation, because the sialylation level of B7-H3 is actually lower in Ca9-22 than in SG cells as revealed by the glycoform analysis.

Various kinds of lectins are expressed on immune cells and interact with different types of glycans. We compared the binding ability of human immune receptors with sB7-H3 derived from Ca9-22 cells to that derived from SG cells in the presence of calcium and magnesium. Among a panel of 44 recombinant innate immune receptor-Fc fusion proteins (34, 35), four gave clear and consistent positive signals that were significant in B7-H3 derived from Ca9-22. They are DC-specific intercellular adhesion molecule-3 (ICAM-3)–grabbing nonintegulin (DC-SIGN), mouse Kupffer cell receptor (mKCR), Langerin, and DC-SIGN–related protein (DC-SIGNR) according to the signal intensity (SI Appendix, Fig. S5). Based on the Consortium for the Functional Glycomics for glycan array analysis, DC-SIGN and Langerin bind to high mannose structures, fucosyl biantenary N-glycans, and fucose (Fuc)-containing antigens, such as B antigens, Lewis, or sialyl Lewis antigens, indicating their ability to recognize various glycans (36). This result provides further confirmation that B7-H3 derived from Ca9-22 carries more diverse N-glycan structures with higher fucosylation.

Identification of Terminal α-Galactose in B7-H3 N-Glycans. The results from glycoform analysis suggested that B7-H3 protein derived from Ca9-22 oral cancer cells had extra hexose(s) and a higher level of fucosylation on its N-glycans, such as the biantenary glycan structure with one hexose and two fucoses (BiH1F2). Based on the biosynthetic pathways of N-linked glycosylation, we propose that the main structure of BiH1F2 is composed of Man1−6-Man1−3 Manβ1−4GlcNAcβ1−4GlcNAcβ1−4Galβ1−4GlcNAc (N-acetyllactosamine) units, two fucoses, and an additional terminal α-galactose (α-Gal). The fucose residues can be at the core (α1−6 linked to the innermost GlcNAc residue) or terminal positions (e.g., α1−2 linked to Gal or α1−3 linked to GlcNAc residue).

![Fig. 3. Glycoforms of B7-H3 derived from SG and Ca9-22 cells are determined. (A) Glycoforms of B7-H3 glycopeptides derived from SG and Ca9-22. (B) Site-specific representative glycans of B7-H3. Abbreviations of glycans: Bi, biantenary; F, fucose; Man, high mannose; H, hexose; N, N-acetyllactosamine; S, sialic acid; Tetra, tetraantennary; Tri, triantennary. (C) Comparison of site-specific sialylation and fucosylation of B7-H3 derived from SG and Ca9-22 cells. Sialic acid index = [% with one sialic acid × 1] + [% with two sialic acid × 2] + [% with three sialic acid × 3])/100; Fucose index = [% with one fucose × 1] + [% with two fucose × 2] + [% with three fucose × 3])/100. Monosaccharide symbols used were as: red triangle, Fuc; yellow circle, Gal; blue square, GlcNAc; green circle, Man; purple diamond, Neu5Ac.](#)
To determine the linkage of terminal Gal, we treated Ca9-22-derived full-length B7-H3 with α-galactosidase, an exoglycosidase that recognizes terminal α-Gal. The result showed that after treating with α-galactosidase, all of the extra hexoses were cleaved (Fig. 4A). We then calculated the conversion rate of the extra hexose(s) categorized according to biantennary, triantennary, and tetraantennary glycan. After treatment with α-galactosidase, the percentages of BiH(1–3), TriH(2–3), and TetraH(2–3) increased from 22.4% to 34.8%, 0 to 13.4%, and 0 to 1.4%, respectively, which was in accordance with the percentages of BiH(1–2)F(1–3), TriH(1–2)F(2–3), and TetraH(1–2)F(2–3) (13.4%, 6.6%, and 2.1%, respectively) before enzyme treatment (Fig. 4B). This result indicated that the extra hexose(s) were terminal α-galactoses in complex type N-glycans in the Ca9-22 oral cancer cell-derived B7-H3. Moreover, the expression of B antigen [Galal(3)Galβ1–4GlcNAc-R] but not α-Gal antigen (Galalβ1–3Galβ1–4GlcNAc-R) was observed on the cell surface of Ca9-22 (SI Appendix, Fig. S6). Our result suggests that the terminal α-Gal structure detected in the complex type N-glycans of B7-H3 from Ca9-22 is B antigen.

**Discussion**

In the current study, we report that the expression of B7-H3 is significantly up-regulated in tumor tissues of OSCC patients and correlates with increased tumor size, advanced stage, and poor survival rate (SI Appendix, Table S3 and Fig. 1E). This finding is supported by a shRNA knockdown study with cell proliferation assay and in vivo animal studies (Fig. 2). According to these findings, we speculate that B7-H3 is a key factor in controlling tumor size in OSCC. This conclusion is consistent with recent studies in which B7-H3 overexpression was observed in various cancers, and up-regulation of B7-H3 in prostate, pancreatic, and colorectal cancers was reported to be correlated with increased tumor size (19, 24, 37). However, there are inconsistencies in some studies regarding the antitumor effect of B7-H3. Among these studies, abundant positive expression of B7-H3 in tumor tissues was observed in comparison with normal tissues (38, 39), but the statistical analysis revealed a better prognosis with higher expression of B7-H3 in tumor tissues. This discrepancy may be due to the lack of standardization of the positive B7-H3 expression levels in these studies. Moreover, B7-H3 is a member of the B7 family of immunoregulatory molecules that can be induced on T cells, B cells, and dendritic cells (DCs) by a variety of inflammatory cytokines (15). We detected the expression of B7-H3 on CD14+ and CD19+ cells but not on CD3+ cells derived from PBMCs of healthy donors. Although the expression of B7-H3 on immune cells could potentially complicate the analysis, we found that the expression of B7-H3 on intratumoral inflammatory cells in OSCC patients is scarce (2/72). Similar findings were also reported that B7-H3 protein was not detected on inflammatory cells in humans with tumors. In contrast, strong consistent staining of B7-H3 was observed on the tumor vasculature and also on the tumor cells themselves (40).

Aberrant glycosylation has been discovered in many kinds of cancer cells, but little is known about this phenomenon in oral cancer. Here, we found that B7-H3 was significantly expressed in Ca9-22 oral cancer cells (SI Appendix, Fig. S1A), and its glycan structures were different compared with normal cells. Based on our findings, the B7-H3 derived from Ca9-22 expressed α-galactosylation and more diverse N-glycan structures with higher fucosylation (Figs. 3 and 4). The terminal α-galactose (α-Gal) epitope can be found in the glycan structures of α-Gal antigen (Galalβ1–3Galβ1–4GlcNAc-R) or B antigen [Galalβ1–3(Fucβ1–2)Galβ1–4GlcNAc-R]; however, our glycoform analysis demonstrated that the terminal α-Gal on B7-H3 from Ca9-22 cells was fucosylated, suggesting the presence of B antigen. Flow cytometric analysis of oral cancer cell lines also revealed the appearance of B antigen but not α-Gal antigen on Ca9-22 and a subpopulation of OEC-M1 cells (SI Appendix, Fig. S6). Both Ca9-22 and OEC-M1 are gingival carcinoma and significant in the Asian population. According to the literature, besides erythrocytes, blood group antigen A, B, and H can be expressed in epithelial cells of many tissues including oral tissue, and their expression can be varied among degrees of differentiation, but decreased in tumor tissue of OSCC (41, 42). The loss of blood group antigens in OSCC specimens correlates significantly with both tumor grade and grade of malignancy (42). Because both Ca9-22 and OEC-M1 cancer cells are derived from gingiva, a type of keratinized stratified squamous epithelia, the existence of B antigen could be explained (43), but the functional role of B antigen in oral cancer cells requires further investigation.

B7-H3-specific receptor may exert an inhibitory rather than a stimulatory function in both T and NK cell-mediated responses. A number of recent studies have highlighted the role of the cross-talk between NK cells and dendritic cells in the early phases of innate immune responses, which shape the subsequent T-cell responses toward the TH1 phenotype (44). Dendritic cells are professional antigen presenting cells and serve as a bridge linking the innate immune system with adaptive immune response. An important family of antigen receptors on dendritic cells involved in recognition and uptake of glycan structures are the C-type lectin receptors such as DC-SIGN and DC-SIGNR. Apparently, the tumor cells evade the immune system by targeting a receptor that is able to transform proinflammatory signals into tolerogenic signals. DC-SIGN has therefore been proposed to be a homeostatic receptor that can be subverted by tumors through changes in their glycan phenotype (45), but the connection between tumor-associated B7-H3 and dendritic cells is not known. Our data revealed that DC-SIGN and DC-SIGNR interact with sB7-H3 derived from Ca9-22 (SI Appendix, Fig. S5A). Based on the glycoform analysis, the N-glycans of B7-H3 derived from Ca9-22 are more diverse and have a higher level of...
fucosylation (Fig. 3), which may support the recognition of lectin receptors on dendritic cells. Data revealed that binding of B7-H3 derived from Ca9-22 to dendritic cells is calcium-dependent (SI Appendix, Fig. S5B), suggesting that tumor-associated B7-H3 interacts with dendritic cells through carbohydrate-lectin receptor interaction.

In summary, this study demonstrated that glycoprotein B7-H3 is involved in the pathological process and tumor growth of OSCC, and the glycans of B7-H3 from Ca9-22 oral cancer cells contain terminal α-galactoses and more diverse N-glycan structures with higher fucosylation than that of SG normal cells. In addition, the presence of a carbohydrate–lectin receptor interaction between the tumor-associated B7-H3 from Ca9-22 cells and immune cells was detected. Overall, the overexpression of B7-H3 in oral cancer tissues and several oral cancer cell lines, and its aberrant glycosylation in Ca9-22 oral cancer cells compared with normal SG cells found in this study, may lead to further investigation and development of new diagnosis and treatment of this disease.

Materials and Methods

Cell Lines and Clinical Specimens. Normal SG cells and OSCC cell line of Ca9-22 (UCSF) were grown in MEM (GIBCO/Invitrogen) with 10% (vol/vol) FBS and 1% of units/mL penicillin and 100 μg/mL streptomycin. GC cells belong to normal human gingival epithelial cells. Clinical specimens used for H&E were obtained from National Taiwan University Hospital (NTUH, Taiwan) with informed consent and approval of institutional review board (NTUH Research Ethics Committee).