Significance of altered glycosyltransferase expression levels in oral cancer

Mallikarjun Goni[1,2], Anil Kumar Desai[3], Niranjan Kumar[3], Veda Hegde[4], Palaksha Kanive Javaregowda[1] and D. B. M. Virupakshaiah[5,*]

[1]SDM Research Institute for Biomedical Sciences, A Constituent Unit of Shri Dharmasthala Manjunatheshwara University, 5th Floor, Manjushree Building, Sattur, Dharwad 580 009, India
[2]Department of Biotechnology, Basaveshwar Engineering College, Vidyagiri, Bagalkot 587 102, India
[3]SDM Craniofacial Surgery and Research Centre, A Constituent unit of Shri Dharmasthala Manjunatheshwara University, Sattur, Dharwad 580 009, India
[4]Department of Oral Pathology, SDM College of Dental Sciences and Hospital, A Constituent Unit of Shri Dharmasthala Manjunatheshwara University, Sattur, Dharwad 580 009, India
[5]Department of Microbiology, Davangere University, Shivagangotri, Davangere 577 002, India

We evaluate the relative quantification of mRNA expression levels of the gene encoding an enzyme that catalyses initial step in glycosylation of dolichol-linked oligosaccharide such as dolichyl-phosphate N-acetylglucosaminephosphotransferase 1 (DPAGT1). Furthermore, beta-1,4-mannosyl-glycoprotein 4-beta-*N*-acetylglucosaminyltransferase (MGAT3) and alpha-1,6-mannosylglycoprotein 6-beta-*N*-acetylglucosaminyltransferase (MGAT5) mediated *N*-glycosylation, selected α2, 3, α2, 6 sialyltransferases families and sialidase by using the Real-Time Quantitative Reverse Transcription PCR (qRT-PCR). Thirty human oral cancer tissue samples and twelve control samples were examined. Results indicated significant upregulation of ST6 *N*-acetylgalactosaminide alpha-2,6-sialyltransferase 1 (ST6GALNAC1) in oral cancer compared to controls. Additionally, the expressions of *DPAGT1*, *MGAT3*, *MGAT5*, ST3 beta-galactoside alpha-2,3-sialyltransferase 1 (*ST3GAL1*), ST6 beta-galactoside alpha-2,6-sialyltransferase 1 (*ST6-GAL1*), and *ST6GALNAC1* were considerably higher in the tumour in tongue lesion compared to the buccal cavity. Hence, our experiment demonstrates significance of glycosyltransferases in the progression of oral cancer.

Keywords: Aberrant glycosylation, invasion, metastasis, oral cancer, sialyltransferase.

ORAL cancer has a high incidence and fatality rate; it is the most frequent cancer in the world and one of the most aggressive epithelial malignancies. It has a five-year survival rate of around 43%, and most patients are not diagnosed until the tumour has progressed. As a result, it's critical to research effective diagnostic markers and innovate new treatment options for people with oral cancer at various stages[1–3]. A study of glycosylation-related molecular pathways that lead to the development of oral cancer is critical for better management of the disease. Protein glycosylation is a common post-translational modification that affects their function, stability, trafficking and turnover. Cancer is characterized by abnormal glycosylation, and altered protein glycosylation is a common manifestation in tumourigenesis, invasion, angiogenesis and metastasis[4,5]. The enzymes sialyltransferases (ST) and sialidase regulate sialylation, and aberrant sialylation has been associated with malignant progression and metastasis to distant lymph nodes. Besides, increased sialylation of glycans and the presence of tumour-associated carbohydrate antigens result from overexpression and/or altered expression of ST, which is considered the major tumour causing mechanism regulated by Ras and c-Myc proto-oncogenes[6,7].

The gene *DPAGT1*, a key player in the *N*-glycosylation metabolic pathway, has been linked to E-cadherin hyperglycosylation, which reduces intercellular adhesion in oral cancer. Further, collagen triple helix repeat containing 1 (CTHRC1) is activated as a result of dysregulation of canonical Wnt signalling as well as DPAGT1-dependent *N*-glycosylation, which induce cancer cell migration and metastasis[2,3,8]. While the genes *MGAT3* is involved in bisecting the GlcNAc structure and acts as a metastasis suppressor, *MGAT5* regulates 1,6-GlcNAc branching structures associated with increased malignancy and metastasis[9,10]. The dysfunction of E-cadherin has also been identified as the key driver of cancer epithelial cell migration. However, there is a lack of experimental evidence to support E-cadherin glycosylation and its role in tumour growth. According to some studies, wild-type E-cadherin regulates *MGAT3* gene transcription, which results in increased *N*-acetylglucosaminyltransferase III (GnT-III) expression. However, it was discovered that GnT-III and *N*-acetylglucosaminyltransferase III (GnT-V) compete for the modification of E-cadherin *N*-glycans[11,12]. Sialylation is the process of adding sialic acid to the terminal position of glycoprotein and glycolipid glycan chains. One of the most common glycosylation alterations found in cancer is aberrant sialylation and desialylation, which are strongly

*For correspondence. (e-mail: virudbm@gmail.com)

managed by two enzymes: ST (responsible for sialylation) and sialidase remove the terminal sialic acid on glycans[13]. The expression of *ST3GAL1*, ST3 beta-galactoside alpha-2,3-sialyltransferase 6 (*ST3GAL6*), *ST6GAL1*, *ST6GALNAC1* and neuraminidase 3 (*NEU3*) mRNA were also found to be significantly associated with lymph-node involvement, advanced cancer stage, and perivascular and perineural invasion, implying their role in cancer progression[14,15]. Therefore, the present study looks at the selected relative mRNA expression levels of glycosyltransferase in the tissues of oral cancer patients and healthy controls. Also, to find the association between glycosyltransferase expression levels and oral cancer subjects' clinicopathological data.

Materials and method

Patients and tissue specimen

The Institutional Ethics Committee approval for this study was obtained from SDM College of Medical Sciences and Hospital, Dharwad, with reference number SDMIEC : 0119 : 2018. Patients who agreed to participate in the study signed a written informed consent form. This study included 42 subjects between the years 2018 and 2020. This includes 30 cases of histopathologically proven oral cancer patients based on clinicopathological criteria and 12 healthy individuals as a control.

Tissues from lesions of oral squamous cell carcinoma were obtained from patients who underwent surgical excision and radical neck dissection at the Craniofacial Unit of SDM College of Medical Sciences and Hospital, Dharwad. Patients who had come for removal of impacted tooth molars constituted the control group. Normal tissues adjacent to impacted molars were collected as control samples and used as calibrator samples after gene expression levels of selected glycosyltransferase. All the tissue samples were collected in a sterile container having 1X phosphate-buffered saline and transported to a processing laboratory under ice-cold conditions. Tissue samples were subjected to RNA extraction within 2 h of collection.

Total RNA extraction from tissue specimen

Total RNA was extracted according to the manufacturer's guidelines using TRIzol reagent (Thermo Scientific). In brief, 50 mg of oral cancer tissue sample was minced in liquid nitrogen, and the resultant powder was lysed with 1 ml TRIzol reagent. Then, 0.2 ml chloroform was added and centrifuged at 12,000 × *g* for 15 min at 4°C. Followed by centrifugation, the aqueous phase containing RNA was collected. Later, RNA was precipitated using equal amounts of isopropanol and incubated at –20°C overnight, then centrifuged at 12,000 × *g* for 15 min at 4°C. The recovered RNA pellet was first washed in chilled absolute ethanol and then with 75% chilled ethanol, and later air–dried at 37°C before being resuspended in 50 µl of diethyl pyrocarbonate (DEPC)-treated sterile water (Sigma). Before using extracted RNA in experiments, the RNA samples were treated with DNase (Ambion DNA-free kit, Cat: AM1906) and purified according to the manufacturer's protocol to avoid genomic DNA contamination. The quality and quantity of pure RNA were determined using a biospectrophotometer (Eppendorf Biospectrometer Kinetic). The RNA integrity was checked prior to cDNA synthesis using a denaturing agarose gel electrophoresis procedure. The presence of sharp bands of 18S and 28S rRNA after electrophoresis demonstrated the presence of intact eukaryotic RNA.

Reverse transcription

RevertAid First Strand cDNA Synthesis Kit (Thermo Scientific, Cat: #K1622) was used to obtain first-strand cDNA. In brief, 500 ng of template RNA was combined with 1 µl of oligo (dT)18 primer. The volume was adjusted to 12 µl with nuclease-free water and the mixture was gently mixed, small spindown, and it was incubated at 65°C for 5 min. The vial was chilled on ice, spun down, and returned to the ice. In addition, 4 µl of 5X reaction buffer, 1 µl of RiboLockRNase inhibitor, 2 µl of 10 mM dNTP mix and 1 µl of RevertAid M-MuLV reverse transcriptase (200 U/l) were added to the vial. The process was then terminated by heating the mixture to 70°C for 5 min after incubating it for 60 min at 42°C. The resultant synthesized 20 µl cDNA was diluted 1 : 5 with nuclease-free water before being used for Real Time Quantitative Reverse Transcription PCR (qRT-PCR) experiments.

Relative quantification of mRNA expression

The expression study was performed in QIAGEN RotorGenQ, 6 Flex equipment, and the TAKARA TB Green, Premix Ex *Taq* Kit was used to perform qRT-PCR in a 20 µl reaction volume. Each reaction was made up of 10 µl of TB Green Premix (2X), 1 µl of forward and reverse primers with 10 pM concentration each and 1 µl of template, and the final volume was adjusted to 20 µl with nuclease-free water. Table 1 lists the oligonucleotide primer pairs used in PCR that were constructed using the cDNA target gene sequences. Initial denaturation at 95°C for 30 sec, then 40 cycles of denaturation at 95°C for 5 sec, annealing at 60°C for 30 sec and extension at 72°C for 30 sec were the PCR conditions. The qRT-PCR experiments were done in triplicate, and data were collected during the 'annealing' stage. The cycle threshold (Ct) for the genes was calculated using the Rotor-Gene software analysis module. Melting curve analysis was used to examine the specificities of all assays, and agarose gel electrophoresis was used to confirm the amplicon of PCR products of predicted

Table 1. Oligonucleotide primers and PCR product size of cDNA and hypoxanthine phosphoribosyltransferase 1 (*HPRT1*)

Gene	Forward primer	Reverse primer	Product size (bp)
DPAGT1	5′-TTGGAAGGTGATTGTCGGGA-3′	5′-GCTGAAGTGTCCCAAGATGC-3′	180
MGAT3	5′-TGTGCCTCATCTCCTTCCTG-3′	5′-AGTGGGAGTAGAGTGGGGTA-3′	183
MGAT5	5′-TGCTCCGAGTCCTTGATTCA-3′	5′-AACCACAAACCCCAGAAAGC-3′	167
ST6GAL1	5′-CCCCAGAAGAGATTCAGCCA-3′	5′-TCTTCTCATAGAGCAGCGGG-3′	195
ST6GALNAC1	5′-GCCTCAAACAAAGCCTTCCA-3′	5′-CTGGGTTTGTGTGTTGAGGG-3′	163
ST3GAL1	5′-ATGATCCTGGTGCCCTTCAA-3	5′-TGCAGCCAGTTGTCAAAGAC-3′	173
ST3GAL6	5′-CTTTTCTGTGTGCGGCTGAT-3′	5′-TCCTCCATTACCAACCACCA-3′	191
NEU3	5′-GTTGAGGATTGGGCAGTTGG-3′	5′-TCTGTTGACGCTCTGTGACA-3′	167
HPRT1	5′-AGGACTGAACGTCTTGCTCG-3′	5′-TCCAGCAGGTCAGCAAAGAA-3′	107

Table 2. Demographic and clinicopathological details of oral cancer patients

Details	Oral cancer patients ($N = 30$)	Details	Oral cancer patients ($N = 30$)
Mean age (years)	51.23	Clinical stage	
Age range (years)	25–45	Stage I	7 (23.33%)
Sex		Stage III	9 (30%)
Male	26 (86.66%)	Stage IV	14 (46.66%)
Female	4 (13.33%)	Site of the lesion	
Habits		Buccal mucosa	25 (83.33%)
Tobacco chewing	20 (66.66%)	Oral tongue	5 (16.66%)
Tobacco chewing + smoking	5 (16.66%)	Duration of the lesion	
Tobacco chewing + smoking + alcohol consumption	3 (10%)	1–2 months	7 (23.33%)
No habits	2 (6.66%)	3–4 months	17 (56.66%)
Duration of habits		5–6 months	6 (20%)
1–15 years	18 (60%)	Tumour differentiation	
More than 15 years	12 (40%)	Moderate	7 (23.33%)
Size of the tumour		Well differentiated	23 (76.66%)
T1	8 (26.66%)	Histopathology	
T2	9 (30%)	Squamous cell carcinoma	30 (100%)
T4a	13 (43.33%)	Perineural invasion	
Nodal status		Positive	9 (30%)
N1	23 (86.66%)	Negative	21 (70%)
N2a	1 (3.33%)	Perivascular invasion	
N2b	2 (6.66%)	Positive	6 (20%)
N0	4 (13.33%)	Negative	24 (80%)

lengths. With *HPRT1* as the normalizer gene and control tissue expressions as calibrator samples, the $2^{-\Delta\Delta Ct}$ method was applied to determine relative gene expression levels[16].

Statistical analysis

To compare the mRNA expression levels of glycosyltransferases in controls and oral malignancies, the data were represented as scatter plots and compared using the *t*-test in GraphPad Prism 7. The Kruskal–Wallis and Wilcoxon rank-sum tests were used to compare glycosyltransferase mRNA expression levels to demographic and clinicopathological variables using the Statistical Package for the Social Sciences (SPSS; ver. 20.0). Using R programming, the Pearson correlation coefficient test was used to identify relationships between glycosyltransferase mRNA expression levels.

Results

The study enrolled 30 patients with oral cancer and 12 healthy controls between 2018 and 2020. Table 2 lists the baseline demographic and clinicopathological characteristics of the oral cancer patients enrolled in the study.

The average age of the participants was 51.23 years, with a range of 45 years, twenty-six males and four females, in age and sex distribution. In terms of habits, 20 patients chewed tobacco, five smoked and chewed tobacco and three chewed tobacco, smoked and consumed alcohol. Two subjects, on the other hand, did not have any habits. The buccal mucosa was determined to be the most common location of occurrence of the tumour (with 25 cases), followed by the tongue (five cases). When the tumour staging was examined, seven patients were classified as stage I, nine as stage III, and 14 as stage IV. According to the histological

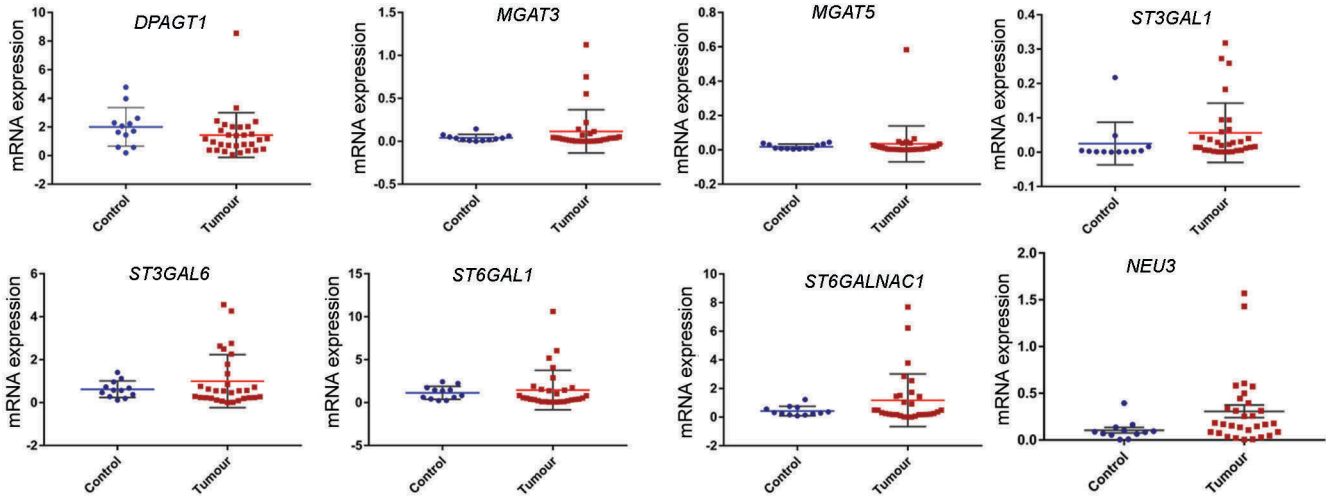

Figure 1. qRT–PCR was used to validate the expression of glycosyltransferases gene in healthy and oral cancer cases. Scatter plots depicting the mean of log fold change normalized to an endogenous control, as well as the standard error calculated using Graph Pad Prism's *t*-test.

differentiation of the tumours, 23 were well-differentiated and 7 were moderately differentiated.

Figure 1 summarizes the fold change in glycosyltransferase genes in tumours and controls specimens. The mean ± SE value of *ST6GALNAC1* gene expression relative to *HPRT1* gene expression was 1.17 ± 0.33, which was greater than the control 0.42 ± 0.09 ($P = 0.0377$).

Table 3 summarizes the relationship between glycosyltransferase gene expression levels and demographic and clinicopathological parameters of oral cancer. The oral cancer samples were separated into groups based on age, gender, habits, lesion site, tumour stage, histological cell differentiation, and perineural and perivascular invasion, among other factors. Our experimental results described that the expression levels of glycosyltransferases genes were unrelated to age, lifestyle, tumour stage, histologic differentiation and perineural invasion. Whereas, the tumour in tongue lesions, *DPAGT1*, *MGAT3*, *MGAT5*, *ST3GAL1*, *ST6GAL1* and *ST6GALNAC1* expressions were elevated with statistical significance compared to the buccal cavity ($P = 0.018$, $P = 0.037$, $P = 0.003$, $P = 0.037$, $P = 0.032$, $P = 0.055$). In terms of perivascular invasion, *ST6GALNAC1* expression was high in negative invasion ($P = 0.07$).

Figure 2 depicts the findings of a correlation matrix analysis performed on oral cancer samples to determine the relationship between glycosyltransferase gene expression levels. This revealed a link between *DPAGT1* expression levels and *MGAT5* expression levels and *ST6GALNAC1* ($r = 0.52$, $P = 0.003$ and $r = 0.76$, $P = 0.000$ respectively). Whereas *MGAT3* expression levels correlated with *MGAT5*, *ST6GAL1* and *ST6GALNAC1* ($r = 0.67$, $P = 0.000$, $r = 0.51$, $P = 0.004$ and $r = 0.61$, $P = 0.000$ respectively). Furthermore, *MGAT5* expression levels correlated with *ST6GAL1*, *ST6GALNAC1* and *NEU3* ($r = 0.57$, $P = 0.001$, $r = 0.77$, $P = 0.000$ and $r = 0.59$, $P = 0.001$ respectively). Also, *ST3GAL1* expression levels correlated with *ST6GAL1* and *NEU3* ($r = 0.75$, $P = 0.000$ and $r = 0.51$, $P = 0.001$ respectively), *ST3GAL6* expression levels correlated with *ST6GAL1* and *ST6GALNAC1* ($r = 0.75$, $P = 0.000$ and $r = 0.63$, $P = 0.000$ respectively).

Discussion

Oral carcinogenesis is a multistep process that involves several molecular events in the course of initiation, promotion and progression of the disease. The altered glycosylation of cell-membrane proteins is critically important in several stages of the carcinogenesis process and has been associated with all hallmarks of all cancers, including oral cancer[17]. Furthermore, the prevalence of oral cancer varies by country/region and has been associated with tobacco-derived carcinogens, excessive alcohol intake, or both[18]. Also, some laboratory studies examined the glycosylation alterations in cell-surface glycoproteins that occur during all stages of malignant transformation, particularly in oral cancer[14,17,19]. As a result, in the present study, we discovered that the expressions of *DPAGT1*, *MGAT3*, *MGAT5*, *ST3GAL1*, *ST6GAL1*, and *ST6GALNAC1* were much greater in the tongue tumour than in the buccal cavity.

Overexpression of *ST6GALNAC1* improved the ability of colorectal cancer cells to form spheres besides resistance to chemotherapy treatments by increasing the expression of sialyl-Tn (STn) antigen, which is performed by the cancer stem cell (CSC) marker CD44 (ref. 20). Furthermore, overexpression of *ST6GALNAC1* increased STn antigen production while inhibiting cell adhesion in prostate cancer cells[21]. *ST6GALNAC1* expression increased cell proliferation, improved cancer cell migratory activity, altered adhesion to target matrices or cells, and/or decreased apoptotic activity in gastric cancer cell lines and a mouse model[22]. We discovered that *ST6GALNAC1* gene expression was higher in oral cancer than in controls compared to

Table 3. Glycosyltransferase gene expression levels in oral cancer tissue according to demographic and clinicopathological factors[a]

Characteristics		*DPAGT1*	*MGAT3*	*MGAT5*	*ST3GAL1*	*ST3GAL6*	*ST6GAL1*	*ST6GALNAC1*	*NEU3*
Age[b] (*n* = 30)									
Below 40 years	6	0.33 ± 1.62	0.66 ± 0.73	1.31 ± 1.40	3.40 ± 1.87	0.77 ± 0.70	0.49 ± 0.41	1.12 ± 0.63	1.33 ± 1.88
41–50 years	10	0.25 ± 1.64	0.90 ± 1.15	0.98 ± 1.08	2.52 ± 2.00	1.18 ± 1.57	0.75 ± 0.89	1.09 ± 1.04	0.90 ± 1.10
51–60 years	7	0.45 ± 0.31	0.48 ± 0.38	0.59 ± 0.45	2.10 ± 2.24	1.14 ± 1.00	0.71 ± 0.72	1.00 ± 0.59	0.40 ± 0.49
61–70 years	7	0.74 ± 0.90	0.97 ± 0.55	1.60 ± 1.38	3.19 ± 2.41	1.06 ± 0.64	1.00 ± 0.58	1.47 ± 1.50	0.48 ± 0.24
P-value		0.260	0.504	0.530	0.541	0.760	0.426	0.945	0.721
Sex (*n* = 30)									
Male	26	0.33 ± 0.21	0.78 ± 0.80	0.97 ± 1.06	0.70 ± 0.70	1.04 ± 0.77	2.92 ± 2.10	0.98 ± 1.08	0.84 ± 1.14
Female	4	1.04 ± 1.16	0.72 ± 0.82	2.03 ± 1.30	1.06 ± 0.63	1.93 ± 1.90	1.78 ± 0.95	1.57 ± 1.06	0.33 ± 1.26
P-value		0.100	1.00	0.059	0.272	0.329	0.393	0.200	0.669
Habits[b] (*n* = 30)									
Tobacco chewing	20	0.48 ± 0.57	0.60 ± 0.55	0.97 ± 1.02	2.86 ± 1.94	0.91 ± 0.63	0.68 ± 0.59	1.14 ± 1.01	0.50 ± 0.58
Tobacco chewing + smoking	5	0.29 ± 0.23	0.86 ± 0.49	1.13 ± 0.49	1.53 ± 2.27	0.94 ± 1.04	0.68 ± 0.60	1.25 ± 0.59	1.49 ± 1.96
Tobacco chewing + smoking + alcohol consumption	3	0.31 ± 0.11	1.62 ± 1.87	1.62 ± 1.87	3.04 ± 1.37	1.13 ± 0.74	0.79 ± 0.65	0.82 ± 0.69	0.84 ± 0.82
No habits	2	0.38 ± 0.37	0.95 ± 1.32	0.95 ± 1.32	4.54 ± 3.88	2.80 ± 3.77	1.48 ± 1.94	1.69 ± 2.36	1.63 ± 2.28
P-value		0.882	0.882	0.687	0.203	0.914	0.989	0.831	0.518
Site of the lesion[c] (*n* = 30)									
Buccal	25	0.39 ± 0.21	0.67 ± 0.79	0.79 ± 0.88	2.41 ± 1.87	0.88 ± 0.69	0.61 ± 0.58	0.93 ± 0.61	0.73 ± 1.06
Tongue	5	0.98 ± 1.00	1.30 ± 0.69	2.71 ± 0.89	4.54 ± 2.37	1.99 ± 2.07	1.43 ± 0.86	2.31 ± 1.69	0.99 ± 1.28
P-value		0.018	0.037	0.003	0.037	0.278	0.032	0.055	0.452
Clinical stage[b] (*n* = 30)									
Stage I	7	0.26 ± 0.18	0.48 ± 0.57	1.02 ± 0.97	2.61 ± 1.87	0.66 ± 0.54	0.68 ± 0.72	0.92 ± 0.70	0.57 ± 0.82
Stage III	9	0.38 ± 0.21	0.79 ± 0.37	0.93 ± 0.91	2.47 ± 2.42	1.12 ± 0.83	0.89 ± 0.61	0.91 ± 0.54	0.75 ± 0.56
Stage IV	14	0.54 ± 0.67	0.91 ± 1.06	1.27 ± 1.36	3.04 ± 2.06	1.22 ± 1.39	0.69 ± 0.76	1.44 ± 1.26	0.89 ± 1.44
P-value		0.371	0.431	0.791	0.606	0.466	0.536	0.599	0.545
Histology[c] (*n* = 30)									
Moderately differentiated	7	0.32 ± 0.23	1.07 ± 1.30	1.10 ± 1.15	2.57 ± 2.15	0.69 ± 0.62	0.66 ± 0.36	1.16 ± 0.58	1.26 ± 1.70
Well differentiated	23	0.46 ± 0.54	0.68 ± 0.58	1.10 ± 1.15	2.83 ± 2.10	1.18 ± 1.17	0.77 ± 0.77	1.16 ± 1.09	0.63 ± 0.81
P-value		0.667	0.477	0.641	0.677	0.292	0.825	0.508	0.364
Perineural invasion[c] (*n* = 30)									
Positive	9	0.33 ± 0.21	0.88 ± 1.21	1.41 ± 1.06	2.62 ± 1.56	0.73 ± 0.71	0.72 ± 0.65	1.08 ± 0.78	1.01 ± 1.61
Negative	21	0.47 ± 0.56	0.73 ± 0.57	1.10 ± 1.19	2.83 ± 2.29	1.20 ± 1.19	0.76 ± 0.72	1.20 ± 1.08	0.66 ± 0.72
P-value		0.769	0.734	0.603	0.946	0.309	0.839	0.769	0.91
Perivascular invasion[c] (*n* = 30)									
Positive	6	0.31 ± 0.21	0.30 ± 0.39	0.40 ± 0.36	2.60 ± 1.73	0.82 ± 0.79	0.69 ± 0.86	0.57 ± 0.58	0.37 ± 0.37
Negative	24	0.45 ± 0.53	0.89 ± 0.83	1.29 ± 1.19	2.81 ± 2.18	1.12 ± 1.14	0.76 ± 0.67	1.31 ± 1.02	0.87 ± 1.18
P-value		0.604	0.430	0.108	0.959	0.604	0.5	0.07	0.378

[a]The data is represented as mean ± SE. [b]Kruskal–Wallis analysis was used to analyse the data. [c]Wilcoxon rank–sum test was used to analyse the data.

HPRT1 expression in the present study. As a result, *ST6GALNAC1* mRNA expression levels in individuals with oral cancer may reveal an important role in the development of the disease.

Invasion and metastasis are considered the critical components of cancer growth. Inflammatory mediators, including cytokines and chemokines, can enhance tumour dissemination. Perineural and perivascular invasion, defined as the presence of cancer cells in the perineurium, is a rare and often overlooked avenue of cancer progression. Biologically, the perineural and perivascular invasion has yet to be thoroughly characterized. However, a new theory connects reciprocal signalling interactions with the acquired ability of tumour cells to counter signals within the peripheral nerve, which promote invasion via nerve Siglec-4a and tumour cell MUC1 (refs 23, 24). *ST6GALNAC1* expression was associated with perivascular invasion in this study, although the pathophysiology of *ST6GALNAC1* expression linked to perivascular invasion needs to be studied further.

Certain studies reported that dysregulation of the *N*-glycosylation-regulating gene *DPAGT1* affects the integrity of *N*-glycan structures in the Golgi body. Increased expression of *MGAT5* is linked to the upregulation of DPAGT1 mRNA. As a result, the signalling pathways regulated by DPAGT1 may have an impact on MGAT5 (refs 2, 8, 25). *DPAGT1* gene expression was shown to be linked to *MGAT5* and *ST6GALNAC1* expression. ST6GALNAC1 is an enzyme that produces the sTn antigen by adding *N*-acetylneuraminic acid to an *O*-linked *N*-acetylgalactosamine on peptides or proteins (also known as Tn) with a 2,6 linkage[26]. Furthermore, increased expression of

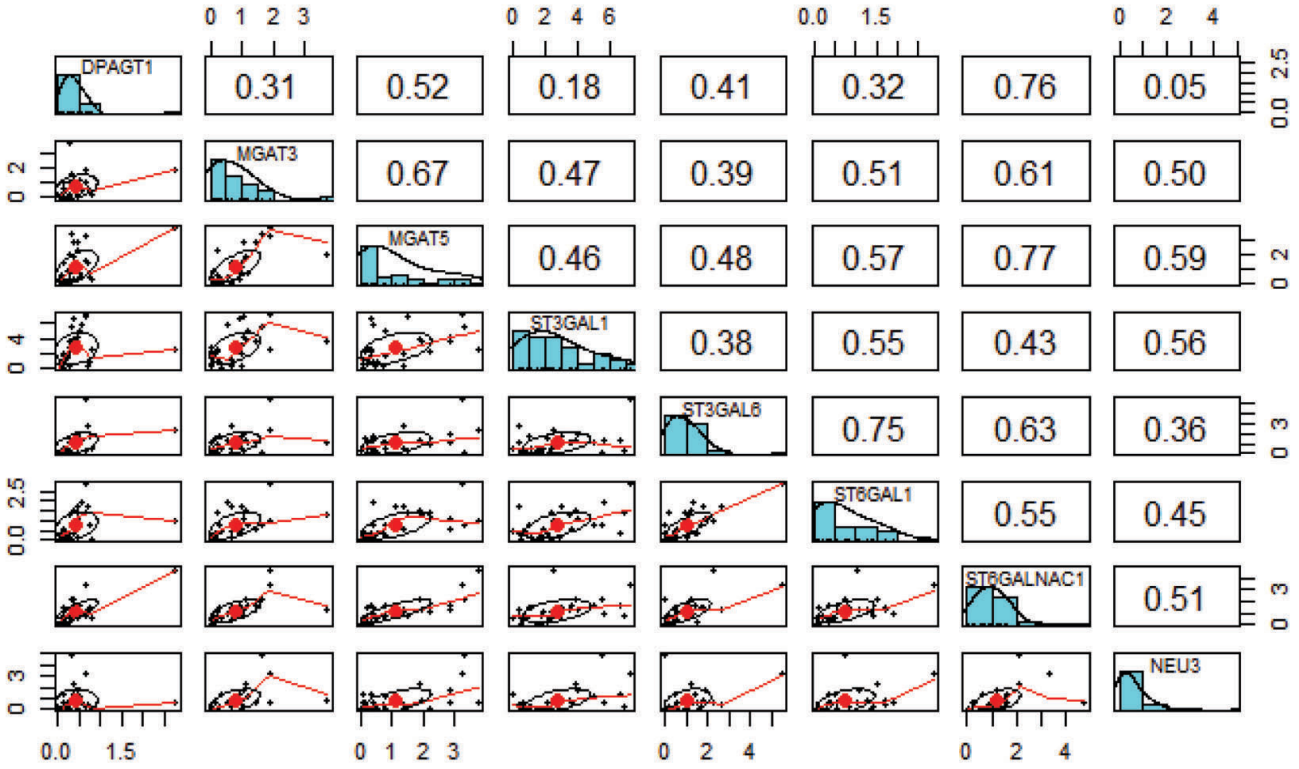

Figure 2. Depiction of glycosyltransferases gene expression levels in oral cancer specimens was analysed using a correlation matrix.

cancer cells and the use of *MGAT5* have long been recognized as key component in the production of cancer-associated glycans. Several studies have shown that increased *MGAT5* expression and the resulting massive tri/tetra antennary *N*-glycans, which lead to the formation of cancer-associated glycans, can alter cancer cell pathogenicity[9,27]. Thus, we found that *MGAT5* expression levels were associated with *ST6GAL1*, *ST6GALNAC1* and *NEU3*. In tumourigenesis, the process of sialylation in cancer cells compared to normal cell sialylation or fucosylation, overexpression of *ST3GAL1* was observed along with *ST6GAL1* and *ST6GALNAC1* to produce sialyl Tn and sialyl T antigens, which are involved in the aberrant *O*-linked glycosylation that occurs in breast cancer[28,29]. As a result, *ST3GAL1* expression has been correlated with *ST6GAL1* and *ST6GALNAC1* in this study.

Oral cancer glycobiology is an important but understudied arena. In the post-gene era, post-translational modifications of proteins are a major challenge, and glycosylation is at the centre of the problem. The findings of our pilot project, which focused on the early stages of glycosylation and sialylation changes in oral cancer, are reported in this study. In conclusion, we discovered that oral cancer specimens had higher levels of *ST6GALNAC1* expression than control tissues. Furthermore, expression levels were linked to oral cancer histological differentiation and development, and mRNA expression levels were correlated among selected glycosyltransferases.

Conclusion

In conclusion, we report a distinct pattern of altered sialyltransferase and sialidase in oral cancer patients. When comparing oral cancer tissue samples to healthy controls, molecular profiling of altered sialylation revealed a significant increase in *STGALNAC1* expression levels. In addition, the expression of *DPAGT1*, *MGAT3*, *MGAT5*, *ST3GAL1*, *ST6GAL1* and *ST6GALNAC1* were considerably higher in the tumour of tongue lesion compared to the buccal cavity, according to the study. An increase in selected glycosyltransferase expression levels as noted in squamous cell carcinoma of the tongue provides further impetus to identify the role of aberrant glycosylation in the tumourigenesis and metastasis of the oral cavity. However, the cohort numbers are small, which is a limitation of this study. Hence, we provide only preliminary data here.

Ethics approval and consent to participate: The study was cleared by the Institutional Ethics Committee for using clinical samples and patient data (SDMIEC : 0119 : 2018), and all participants provided written informed approval before the study.

Conflict of interest: The authors declare that they have no competing interests.

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Received 8 September 2021; revised accepted 11 April 2022

doi: 10.18520/cs/v123/i1/52-58