Salivary protein glycopatterns for natural regulation of oral microbiota

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Abstract

Background Little is known about the relationship between salivary protein glycopatterns and oral microbial communities in the patients of gastric cancer (GC) and type 2 diabetes mellitus (T2DM).

Methods In this study, the oral microbiome of healthy volunteers (HVs) and GC patients were detected using 16S rDNA sequencing. The Fucose-neoglycoproteins were synthesized and used to explore the effects of Fucose moiety against *Aggregatibacter segnis* (*A. segnis*). The sialoglycoproteins were isolated and used to assess the role of the Sia α 2-3/6Gal-linked glycans against *Candida albicans* (*C. albicans*).

Findings The results showed that five species (e.g. *A. segnis*, *Megasphaera micronuciformis*) were significantly increased ($p < 0.05$) while two species (*Streptococcus salivarius* and *Oral taxon 870*) were significantly decreased ($p < 0.01$) in the saliva of GC patients compared to HVs. The adhesion of *A. segnis* could be inhibited by 30 $\mu\text{g/mL}$ of fucose-neoglycoproteins. The growth and adhesion of *C. albicans* could be inhibited gradually by 50-400 $\mu\text{g/mL}$ sialoglycoproteins. And Sia α 2-3Gal moieties played a major role against the growth and adhesion of *C. albicans*.

Interpretation These findings provide a novel theory that dynamic communities of oral microbiota are regulated naturally by host salivary protein glycopatterns, having important implications for developing new carbohydrate drugs for oral and body health.

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Introduction

About 700 microbial species inhabit in the human oral cavity, of which 57% are officially named, 13% unnamed but cultivated, and others are not cultivated until now^[1]. The microbiota in the oral cavity plays an important role in maintaining oral health and even body health. The nososymbiocity of the polymicrobial communities involved in both dental caries and periodontitis is largely regulated by host factors, predominantly dietary sugars and inflammation^[2]. Recently, increasing evidence has shown that the oral microbiota is associated with human diseases, including diabetes, obesity, and cancers^[3-7]. The profiles of oral microbiota in gastric cancer (GC) patients has not been investigated, although some studies have involved the tongue coating microbiota community in GC patients^[8] and several oral pathogens in gastric precancerous lesions patients^[9]. Current studies have shown an increased probability of *Candida albicans* (*C. albicans*) infection in oral cavity of the diabetic patients^[10]. Importantly, our previous study has shown that the profile of Sia α 2-3Gal glycans was decreased in saliva of type 2 diabetes mellitus (T2DM) patients^[11]. However, whether the down-regulation of the expression level of the Sia α 2-3Gal structure related to the growth and adhesion of *C. albicans* in oral cavity remains unknown.

Glycosylation is a common and important post-translational modification of proteins, which directly affect glycoprotein structure, trafficking, recognition, and biological functions^[12-14]. Glycans attached to exogenous and secreted glycoproteins may act to block or modulate pathogen association to the epithelial surface. Such as Sia2-3/6Gal-linked glycans of the bovine milk proteins could as competitive substrates to disturb the influenza A viruses attachment to cell surface receptors^[15]. The special glycan structures such as α 1,4-GlcNAc-capped O-glycans could inhibit the growth of *H. pylori*^[16]. Our previous studies demonstrated that different types of diseases (e.g., GC, T2DM, hepatopathy, and breast disease) could induce various alterations in salivary protein glycopatterns (e.g., fucosylation in GC and sialylation in T2DM)^[17,18]. However, it is unclear whether the altered salivary glycopatterns are possible drivers leading to dysbiosis of oral microbial communities and ultimately dental caries and periodontitis.

To address this hypothesis, in this study, we compared the salivary microbiome of healthy volunteers (HV) and GC patients by 16S rDNA sequencing and screening the up-regulated abundance bacteria in GC. We synthesized the neoglycoproteins according to the altered salivary glycopatterns in GC patients, exploring the effects of neoglycoproteins to the oral bacterial growth or adhesion and then researching the underlining mechanisms. On the other hand, we isolated the proteins with Sia2-3Gal/Sia2-36Gal-linked glycans by serotonin-functionalized magnetic particle conjugates and assessed the role of the Sia2-3/6Gal-linked glycans by inhibiting and neutralizing the anti-fungal activity against *C. albicans* in vitro. This study aims to explore the relationship between salivary glycopatterns and oral microbiota, and to provide a novel theory that dynamic communities of oral microbiota regulated naturally by host salivary protein glycopatterns. This work contributes to reveal the role of special glycan moieties and oral microbiota in the genesis and development of several diseases.

Materials And Methods

Study population

The collection and use of human saliva samples for the research presented here were approved by the Ethical Committee of Northwest University (Xi'an, China), First Affiliated Hospital of Xi'an Jiaotong University (Xi'an, China). Written informed consent was received from participants for the collection of their saliva samples. This study was conducted in accordance with the ethical guidelines of the Declaration of Helsinki. After a standardized endoscopic procedure and histopathological evaluation, individuals who were diagnosed with GC (N=12) were enrolled in this study. The age- and sex-matched HVs (N=12) were free of any precancerous lesions, as confirmed by gastroscopy. All the participants were ≥ 40 years old, and no significant differences were observed between the two groups regarding demographic, socioeconomic, and lifestyle characteristics. Patients who received preoperative radiotherapy, chemotherapy, chemoradiotherapy or antibiotic therapy were excluded from the study. The clinical characteristics of the HVs and GC patients are summarized in Table S1.

Sequencing and Data analysis

Bacterial genomic DNA was extracted by a Bacterial DNA Isolation Kit D3350-01 (OMEGA, USA) following the manufacturer's instructions. The 16S rDNA V3-V4 regions were amplified by specific bacterial primers 341F-806R with barcodes 341F: CCTAYGGGRBGCASCAG, and 806R: GGACTACNNGGGTATCTAAT. All PCRs were carried out with Phusion High-Fidelity PCR Master Mix (New England Biolabs, USA). The PCR products were extracted and quantified, pooled in equimolar concentrations and sequenced using the Ion S5™ XL platform according to the manufacturer's recommendations. The methods for sequence screening, diversity analysis and taxonomy-based analysis were performed as described in previous reports^[19]. Differences between two arbitrary data sets were tested by the Wilcoxon test for each species using SPSS statistics 20.

Synthesis of neoglycoproteins

The synthesis of neoglycoprotein is divided into three steps (Fig S1A): the monosaccharides coupled with the linker (5-(4-hydroxyphenyl)pentanoic acid) to form glycan conjugates, and the carboxylates (-COOH) from glycan conjugates reacted to NHS in the presence of EDC, resulting in a semistable NHS, followed by reaction with amines (-NH₂) of BSA to form amide crosslinks. Compared with the band of identically processed BSA, the upward migration bands of neoglycoproteins are obviously observed in the SDS-PAGE results (Fig S1B).

First, 5 µmol of each monosaccharide (fucose, mannose, and galactose (Sigma-Aldrich, USA)) was mixed with 2.5 µmol of 5-(4-hydroxyphenyl) pentanoic acid, dissolved in N,N-dimethylformamide (DMF), decreased to a pH under 3.0 using sulfuric acid, and then incubated at 60 °C for 12 h under gentle shaking with an appropriate amount of silicon dioxide. Second, after chloroform extraction, the glycan complexes were mixed with 10 µmol EDC and 25 µmol NHS in activation buffer (0.1 M MES (2-[morpholino] ethanesulfonic acid), 0.5 M NaCl, pH 6.0) and incubated at room temperature for 4 h. Third, the products were added to 100 nmol BSA solution (BSA dissolved in PBS), and then the buffer pH was adjusted to 7.4 and incubated at room temperature for 4 h. The Quench reaction was performed by adding hydroxylamine to a final concentration of 10 mM. The synthesized neoglycoproteins were dialyzed against PBS overnight and concentrated using an Amicon ultra-4 30 kDa ultrafiltration unit (Millipore, USA). The neoglycoprotein solutions were collected, filtered through 0.22 µm filters and stored at -80 °C. The synthesized neoglycoproteins were analyzed by 10% SDS-PAGE and then stained directly with alkaline silver.

Bacterial strains and Cell culture

A. segnis were obtained from the American Type Culture Collection (ATCC) and cultured at 37°C with 5% CO₂ in ATCC 814 Medium supplemented with 5 µg/mL NAD (Solarbio, Beijing, China) and 30 µg/mL vancomycin (Solarbio, Beijing, China). *C. albicans* ATCC 10231 was obtained from the National Center for Medical Culture Collections (CMCC) and aerobically cultured at 37 °C in Sabouraud's dextrose broth supplemented with 50 mg/ml gentamicin (Solarbio, Beijing, China). CAL-27 and HOEC cells were obtained

from Otwo Biotech Inc. (Shenzhen, China), cultured in DMEM (HyClone, Waltham, MA, USA) supplemented with 10% FBS (Gibco, Grand Island, NY, USA) and maintained at 37 °C with 5% CO².

Real-Time PCR(qRT-PCR)

Total RNA was extracted using the Bacteria Total RNA Isolation Kit (Sangon Biotech, China) and converted into cDNA using the PrimeScript RT reagent Kit (TaKaRa, Japan). The cDNAs were then subjected to real-time quantitative polymerase chain reaction (qRT-PCR) analysis (ABI ViiA™ 7, Bio-Rad, USA) using gene-specific primers (Table S2) and TB Green Fast qPCR Mix (TaKaRa, Japan). The 16S rRNA gene was used as an endogenous control for normalizing the relative expression of target genes.

Adhesion assays

A. segnis or *C. albicans* were washed with fresh medium three times, centrifuged and diluted with fresh medium containing 100 µM FITC-d-Lys (Xiamen Bioluminor Bio-Technology, Xiamen, China). After 30 min of incubation at 37 °C, the cells were centrifuged, washed with fresh medium three times, and then resuspended in PBS. CAL-27 or HOEC cells (1×10⁵ cells) were inoculated into confocal culture dishes (JingAn Biotechnology, Shanghai, China) and cultured in complete medium. Cells were grown to 60-70% confluence and then synchronized by serum starvation overnight. One hundred microliters of serum-free DMEM with 5 µM DiD (AAT Bioquest, USA) was pipetted onto the dishes and gently agitated until all the cells were covered. After 30 min of incubation at 37 °C, the cells were washed three times with DMEM. *C. albicans* or FITC-labeled *A. segnis* (1×10⁶ cells/mL) was added to the dishes in 500 µL serum-free DMEM, which was allowed to infect HOEC or CAL-27 cells for 45 min for adhesion. The infected cells were washed three times with 1× PBS and immobilized by incubating with 0.2% Triton X-100 in 4% paraformaldehyde for 30 min at RT. After DAPI staining (Thermo Fisher Scientific, Waltham, USA), a laser scanning confocal microscope FV 1000 (Olympus, Tokyo, JPN) was used to acquire the images with the merged channels of FITC (Ex/Em(nm)=488/520), DiD (Ex/Em(nm)=650/670) and DAPI (Ex/Em (nm)=358/461). A fluorescence microscope (Olympus, Tokyo, JPN) was used to observe the adhesion of *C. albicans* to cells.

Lectin microarrays and Data Analysis

A lectin microarray was produced using 37 lectins with different binding preferences covering N- and O-linked glycans^[20]. The Cy3-labeled glycoproteins or bacteria were incubated on a lectin microarray at 37 °C for 3 h with gentle rotation in the dark. The slides were washed three times with PBST and PBS, centrifuged dry and scanned immediately with 70% photomultiplier tubes and 100% laser power settings using a Genepix 4000B confocal scanner (Axon Instruments, USA). The acquired images were analyzed at 532 nm for Cy3 detection by Genepix 3.0 software. Differences between two arbitrary data sets or multiple data sets were tested by Wilcoxon test or one-way ANOVA for each lectin signal using SPSS statistics 20.

Isolation of sialoglycoproteins from bovine milk

According to the protocol in the literature^[21]. In brief, serotonin-functionalized magnetic particle conjugates were thoroughly washed with binding buffer (20 mM Na₃PO₄, pH 6.0) and incubated sequentially with 1 mL of binding buffer containing 0.2 mL bovine milk with gentle shaking at room temperature for 3 h without light. The unbound proteins were removed from the conjugates with binding buffer until there were no detectable proteins. Finally, the sialoglycoproteins were eluted from the conjugates with eluting buffer (0.1 M NaHCO₃, 0.5 M NaCl, pH 8.3) and quantitated with the BCA method.

Destruction of Sia α 2-3/6Gal moieties and enzymolysis of Sia α 2-3 Gal moieties of the isolated sialoglycoproteins

The Sia α 2-3/6Gal structures of the isolated sialoglycoproteins were destroyed by treatment with 1 mM sodium periodate at 0 °C for 15 min, as described previously^[22]. Then, sodium periodate was removed by using an ultracentrifugal filter (Ultracel-3k, Millipore Corp., Billerica, MA). The Sia α 2-3 Gal moieties of the isolated sialoglycoproteins were digested by α 2-3 neuraminidase S (New England Biolabs, USA) according to the manufacturer's recommendations.

Results

Overall oral bacterial diversity of HV and GC cases

Among the 24 samples, an estimated 79 thousand raw sequence reads per sample with a median length of 423 base pairs was obtained. After quality trimming and chimera checking, 75 thousand clean sequence reads per sample remained, which were recovered for downstream analysis. The detailed sequencing data of each sample are shown in Table S3. In total, 789 unique species were identified in the oral cavity of healthy volunteers (HVs) and patients with GC; 525 species were common in the HVs and GC patients, while 128 and 136 species were observed in only the HVs and GC patients, respectively (Fig. 1A). The alterations in the microbial communities were evaluated between the HV and GC groups. The alpha-diversity analysis showed that there were no significant differences between the groups (Fig. 1B-E), while the beta-diversity analysis showed that the oral microbiota profiles of the two groups were different, and could be distinguished using PCA, PCoA and NMDS plots (Fig. 1F-H).

Significant variation of oral microbial community in GC patients versus matched HV

To further investigate the composition of oral microbial communities, the Wilcoxon test was used to compare the variance at each taxonomic level between the HV and GC groups (Table 1). The results showed that there were no significant differences at the phylum, class and order levels; however, the members of Carnobacteriaceae were increased in the GC patients compared with the HVs at the family level (Fig. 2A). The proportions of the genera *Alloprevotella* and *Megasphaera* were significantly increased whereas those of *Granulicatella*, *Bregeyella* and *TM7[G-6]* were decreased in the GC group (Fig. 2B). Five species (*Oral taxon 392*, *Oral taxon 308*, *Aggregatibacter segnis* (*A. segnis*), *Megasphaera micronuciformis* (*M. micronuciformis*), and *Oral taxon 396*) were significantly increased ($p < 0.05$) and 2 (*Streptococcus salivarius* (*S. salivarius*) and *Oral taxon 870*) were significantly decreased ($p < 0.01$) in the

GC patients compared with the HVs (Fig. 2C). Furthermore, the relative abundances of *A. segnis*, *M. micronuciformis* and *S. salivarius* were verified by PCR and qPCR, and their results were consistent with the results obtained by 16S rDNA sequencing (Fig. 2D-E).

Synthesis of the neoglycoproteins and the effect of neoglycoprotein on the adhesion of the *A. segnis*

Our previous studies demonstrated that the occurrence and development of GC could induce alterations in salivary protein glycopatterns, especially fucosylated glycoproteins, in the saliva of GC patients [23]. Here, a fucose-neoglycoprotein (Fuc-BSA) and other neoglycoproteins (Gal-BSA and Man-BSA) were synthesized (Fig. S1) and their roles in the proliferation and adhesion of *A. segnis* were evaluated. The results showed that the proliferation of *A. segnis* was not affected by 3-100 µg/mL Fuc-BSA, Gal-BSA, Man-BSA, their monosaccharides (Fuc, Gal, and Man) or BSA. In addition, 30 and 100 µg/mL Fuc-BSA significantly decreased the adhesion of *A. segnis* to CAL-27 cells (Fig. 3A); however, 3 and 10 µg/mL Fuc-BSA and 30 and 100 µg/mL Man-BSA and Gal-BSA did not affect this process (Fig. S2). A similar phenomenon was observed in HOEC cells, and 30 and 100 µg/mL Fuc-BSA also significantly decreased the adhesion of *A. segnis* to HOEC cells (Fig. 3B). These results demonstrate that the fucose moieties of proteins can disturb *A. segnis* attachment to the cell surface, which implies that they can prevent periodontal disease.

Fucose-neoglycoprotein altered the glycopatterns and related-glycosyltransferases of *A. segnis*

Among these seven bacteria, *A. segnis* and *M. micronuciformis* could be cultivated and showed increased abundance in the GC patients. *Haemophilus segnis* is first isolated in 1977[24] and reclassified into the *Aggregatibacter* genus in 2006[25]; it is a normal commensal in oral humans but is rarely reported as a pathogen. Infective endocarditis is the most well-known infection but is still rarely reported to be associated with *A. segnis*[26]. Periodontal disease, acute appendicitis, pancreatic abscess, and cholecystitis have also been associated with *A. segnis* on occasion[27,28]. Previous studies have focused mainly on case reports, and the true prevalence of this bacterium in human infections is probably underreported; the underlying mechanism associated with diseases is not yet explicitly known. To understand the related mechanisms, the glycopatterns of the outer membrane and total protein of *A. segnis* and *A. segnis* treated with 30 and 100 µg/mL Fuc-BSA were analyzed by lectin microarrays. There were 9 lectins (e.g., Jacalin, GSL-II, and SNA) that showed decreased expression levels of the glycopatterns (e.g., Galβ1-3GalNAcα-Ser/Thr, GalNAcα-Ser/Thr and Sia2-6Gal/GalNAc), while there were 3 lectins (PHA-E, LEL, and MAL-I) that showed extremely increased expression levels of the glycopatterns (Bisecting GlcNAc, high mannose-type N-glycans, and Galβ-1,4GlcNAc) on the outer membrane of *A. segnis* treated by Fuc-BSA (Fig. 4A). However, 4 lectins (PHA-E, PNA, LEL and GSL-I) showed increased expression levels of glycopatterns (bisecting GlcNAc, Galβ1-3GalNAcα-Ser/Thr, high mannose-type N-glycans, and αGalNAc/αGal) in the total protein of *A. segnis* treated with Fuc-BSA (Fig. 4B). These results implied that Fuc-BSA mainly affects glycopatterns on the outer membrane of *A. segnis*. Annotation of *A. segnis* in the carbohydrate-active enzymes database (CAZy) revealed that 24 putative glycosyltransferases (GTs) are distributed among 11 glycosyltransferase families. Of these, 8 GTs are

associated with lipopolysaccharide (LPS) biosynthesis, 7 GTs are associated with cell wall formation, and others are associated with glycosylation, glycogen synthase, and carbohydrate metabolism (Table S4) [29]. The qPCR results showed that 3 mRNAs of LPS-associated GTs (NCTC10977_00605 (Rfaq_1), NCTC10977_00729, and NCTC10977_00649 (WaaA)) were significantly decreased and that the mRNA of mannosyltransferase OCH1 was significantly increased in *A. segnis* treated with Fuc-BSA (Fig. 4C), which indicated that Fuc-BSA interfered with the biosynthesis of core oligosaccharides or O-antigenic polysaccharides of LPS. These results suggested that Fuc-BSA mainly altered the glycopatterns of the outer membrane of *A. segnis*.

Siaa2-3Gal structure inhibit the growth and adhesion of *C. albicans*

Candida albicans (*C. albicans*) is the most prevalent human fungal pathogen causing infections and oral diseases in T2DM patients [30,31]. Our previous work showed that the expression level of the SAa2-3 structure in the saliva of T2DM patients was significantly lower than that of healthy people, which was related to T2DM susceptibility to avian influenza virus [11]. Here, sialoglycoproteins from bovine milk were isolated and used to inhibit the growth and adhesion of *C. albicans* [21]. The results showed that their growth could be inhibited gradually by different concentrations (50, 100, 200, and 400 µg/mL) of sialoglycoproteins (Fig. 5A) and be inhibited entirely by 400 µg/mL sialoglycoproteins with an IC₅₀ of 142.8 µg/mL (Fig. 5B). To determine the roles of Siaa2-3/6Gal glycans, the desialoglycoproteins and des-α2,3 sialylated proteins were obtained from sialoglycoproteins treated with sodium periodate and α2-3 neuraminidase S, respectively. Then the obtained desialoglycoproteins, des-α2,3 sialylated proteins, sialic acid, and BSA were used to inhibit the growth of *C. albicans*, respectively. The results of lectin blot showed that Siaa2-3Gal (recognized by MAL-II) and Siaa2-6Gal (recognized by SNA) moieties were both decreased after sodium periodate treatment (Fig. 5C). The inhibition test results showed that both 400 µg/mL desialoglycoproteins and des-α2,3 sialylated proteins could not inhibit the growth of *C. albicans* while sialoglycoproteins could completely inhibit it at the same concentration, which implied that the Siaa2-3 moieties of sialoglycoproteins play a major role against *C. albicans* (Fig. 5D). Furthermore, 200 µg/mL sialoglycoproteins and desialoglycoproteins were used to blockade *C. albicans* to adhere to CAL-27 cells. Notably, sialoglycoproteins significantly inhibit the adhesion of *C. albicans* to CAL-27 cells; however, des-α2,3 sialylated proteins could not inhibit this process. These results also demonstrate that the Siaa2-3Gal moieties of sialoglycoproteins play a major role in inhibiting the adhesion of *C. albicans* to CAL-27 cells (Fig. 5E, F).

Discussion

Human oral cavity is the natural port of entry to both the gastrointestinal and respiratory tracts, harbours various indigenous microorganisms which interaction with host cells, microenvironment and each other, together to forms an equilibrium community normally. Maintenance of a balanced oral microbiome in symbiosis with the host is important for the promotion of health [1]. Currently, there are several processes that underlie the transition of a microbial community to a state of dysbiosis. In particular, diet can affect

the community composition, and overexposure to dietary carbohydrates and host factors promotes the production of extracellular polymeric substances and acidic metabolites, which cause the accumulation of acidogenic and aciduric microorganisms, thus driving the transition to a pathogenic biofilm community^[32-34]. Bacteria can obtain nutrients from glycoproteins in saliva and gingival crevicular fluid during fasting^[35]. Glycoproteins can be broken down into sugars and proteins and metabolized by bacteria that can gain energy to survive. During metabolism, sugars and proteins are further converted into acidic and basic small molecules, which can neutralize each other to maintain a neutral state in the mouth. If sugar consumption is low and infrequent, the microbial communities on teeth remain stable. Despite being able to produce acids that demineralize enamel, the episodic pH decrease can be readily neutralized by saliva, which restores and maintains the mineralization of enamel^[36]. However, once a community has transitioned to a dysbiotic state, the structural stability of functionally specialized components will allow the condition to persist for an extended period of time, promoting the development of oral diseases such as periodontitis and dental caries^[37-39]. However, our studies demonstrated that human disease could induce different alterations in salivary protein glycopatterns^[17]. The altered salivary protein glycopatterns (e.g., fucosylated or sialylated structures) induced by GC or T2DM were also drivers of dysbiosis of oral microbial communities and ultimately dental caries and periodontitis. The fucose-neoglycoproteins and Sia α 2-3Gal structure could inhibit the growth and adhesion of *A. segnis* and *C. albicans* from the oral cavity of GC and T2DM, respectively. These findings provide a novel theory that dynamic communities of oral microbiota are regulated naturally by host salivary protein glycopatterns, having important implications for developing new carbohydrate drugs for oral and body health.

Declarations

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Author contributions: JS, HY, ZL designed the experiments; JS, YW, XW, KZ performed experiments and analyzed the data; JS, XR, TC, and ZL wrote the paper. All authors read, revised and approved the final manuscript.

Competing interests: We declare no competing interests.

Supplementary materials:

Table S1 – S4

Fig. S1 – S2

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Figures

Figure 1

Comparison of the structures of the oral microbiota between the HV and GC groups. (A) Venn diagram illustrating the overlap of the species identified in the oral microbiota of the HV and GC groups, (B) OTU numbers, (C) Observed species, (D) Shannon index, (E) Simpson index, (F) PCA, (G) PCoA, (H) NMDS analysis.

Figure 2

Comparison of the relative abundance at the bacterial family (A), genus (B) and species (C) levels in the HV and GC groups. (D) Agarose gel electrophoresis of the PCR products and the box plot of the relative intensities. (E) The results of qPCR analysis of *A. segnis*, *M. micronuciformis* and *S. salivarius*.

Figure 3

The adhesion of *A. segnis* to CAL-27 (A) and HOEC (B) cells. *A. segnis* was added to cells with Fuc-BSA and allowed to infect for 90 min. Fuc-BSA (30 and 100 $\mu\text{g/mL}$) significantly decreased the adhesion of *A. segnis* to CAL-27 and HOEC cells. The images are acquired under the same conditions for the merged channels of FITC (Ex/Em(nm)=488/520), DiD (Ex/Em(nm)=650/670) and DAPI (Ex/Em (nm)=358/461).

Statistical significance analysis for FITC fluorescence intensity per cell was computed by one-way ANOVA (*: $p < 0.05$, **: $p < 0.01$, and ***: $p < 0.001$).

Figure 4

The glycopatterns of *A. segnis* altered by Fuc-BSA treatment. (A) Nine lectins showed decreased NFIs in the detection of outer membrane glycopatterns after Fuc-BSA treatment (30 and 100 $\mu\text{g/mL}$), while three lectins showed extremely increased NFIs. (B) Four lectins showed increased NFIs in the detection of total protein glycopatterns after Fuc-BSA treatment (30 and 100 $\mu\text{g/mL}$). (C) The mRNA levels of three LPS-related glycosyltransferases (*NCTC10977_00605*, *00729* and *0069*) decreased and another glycosyltransferase (mannosyltransferase *OCH1*) increased after Fuc-BSA treatment after Fuc-BSA treatment.

Figure 5

The growth and adhesion of *C. albicans* are affected by Sia2-3Gal moieties. (A) Silver nitrate staining and Lectin blotting. Proteins were visualized by Cy5 labelled MAL-II (left) and Cy5 labelled SNA (right). M: protein molecular weight markers, (B) Effects of different concentrations of sialoglycoproteins on the growth of *C. albicans*. (C) The IC₅₀ of sialoglycoproteins against *C. albicans*. (D) The effects of sialic acid, desialoglycoproteins and sialoglycoproteins without Siaα2-3Gal on the growth of *C. albicans*. Destruction of Siaα2-3 moieties significantly affected the inhibition activity of sialoglycoproteins against *C. albicans*, indicating that the Siaα2-3 moieties play a major role in the inhibition progress. (E) The effects of sialoglycoproteins and sialoglycoproteins without Siaα2-3Gal limitation on the adhesion of *C. albicans* to CAL-27 cells. (F) The adhesion rate of *C. albicans* to CAL-27 cells.

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