N-glycomic identification of novel soft tissue prognostic biomarkers for oral cancers

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INTRODUCTION:

The mortality rates of oral cancers have increased six-fold in the last 50 years [1]. As the number of malignant diseases proliferates, more than 377,000 oral squamous cell carcinoma (OSCC) (Figure 1A) new cases and consequently 170,000 deaths are diagnosed worldwide annually [1,2]. In addition, OSCC is an aggressive disease with a glycoproteomically unmapped disease progression and disheartingly low five-year survival rate [2]. Besides the most commonly known risk factors such as alcohol consumption, tobacco, poor oral hygiene, human papillomavirus (HPV) infection, long-term immunosuppressant therapies may also increase the risk and change the therapeutic response of secondary malignancies (FIGURE 1B) [1]. Alterations of protein *N*-glycosylation have a pivotal role in tumorigenesis and metastasis formation [3]. Thus, the aim of our study was to identify novel glycobiomarkers to predict more precise prognosis suggesting more efficient therapeutic alternatives for oral cancers.

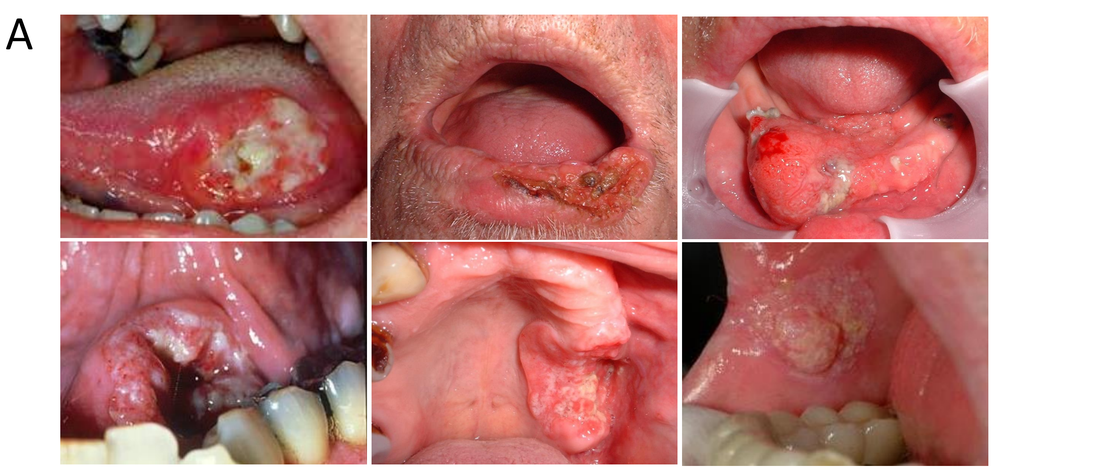


Figure 1. The most common type of oral cancers (A) and main risk factors (B) [1]

METHODS:

- Oral mucosal soft tissue samples were obtained by using incisional biopsy from five patients with OSCC, both from the malignant and the opposite healthy gingival sides, as well as from seven age-sex matched healthy controls with the appropriate Ethical Permissions and Informed Patient Consents (DE RKEB/IKEB: 6152-2022).
- The collected tissues were cut into 2 mg bits and placed into an automated tissue homogenizer (BeatBox Tissue kit 96x wells, PreOmics, Munich, Germany) and were properly homogenized in 100 µL of RIPA lysis buffer for 10 min at standard setting and a manual Potter system (VWR, Randor, PA).
- The obtained tissue homogenates were analyzed followed by N-glycan profiling of endoglycosidase released and fluorophore-labeled carbohydrates using capillary electrophoresis coupled with ultra-sensitive laser-induced fluorescent detection (CE-LIF, Beckman Coulter, Brea, CA) (Figure 2).

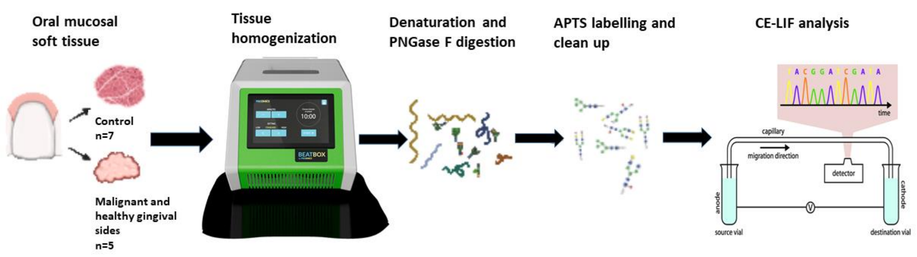


Figure 2: The glycoanalytical process workflow

RESULTS: The effectiveness of the high-throughput automated and manual sample preparation is compared in Figure 3 showing the same results in both cases. The elecropherograms of the control and the oral squamous cell carcinoma patients malignant (OSCC_T) and healthy (OSCC_C) gingival sides are compared in Figure 4. Thirty-nine peaks were identified by their GU value using publicly available databases (Table 1) in the control sample and used as the basis of significant differences. Exoglycosidase array based carbohydrate sequencing verified the identified structures (Figure 5). Ten out of the 39 identified *N*-glycan structures showed significant ($p<0.05$) differences between the malignant tissue samples of OSCC patients and the healthy controls. Comparing the healthy and the positive control oral mucosal samples two significantly different *N*-glycan structures have been revealed, while there were no differences between the *N*-glycan profiles of the malignant tumor and the positive control samples (Figure 4,6).

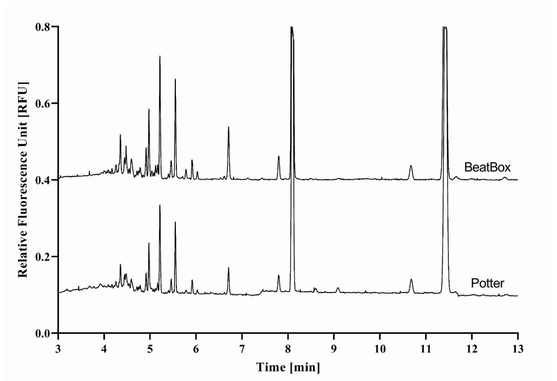


Figure 3: Comparison of the automated and manual sample preparation techniques

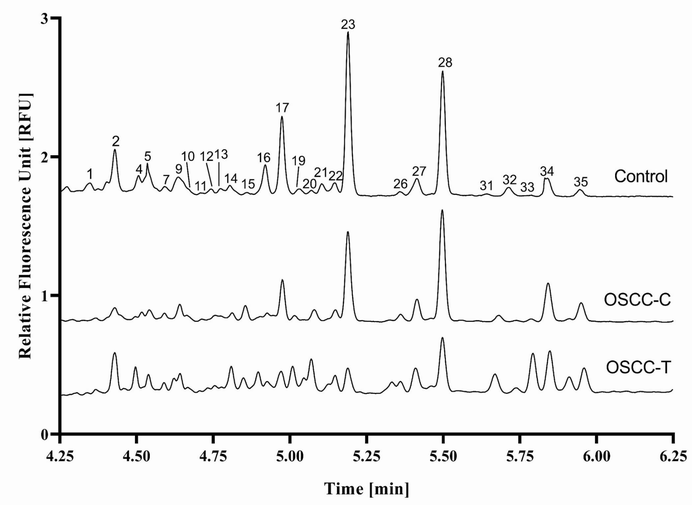


Figure 4: Elecropherograms of the control and the oral squamous cell carcinoma patients malignant (OSCC_T) and healthy (OSCC_C) gingival sides

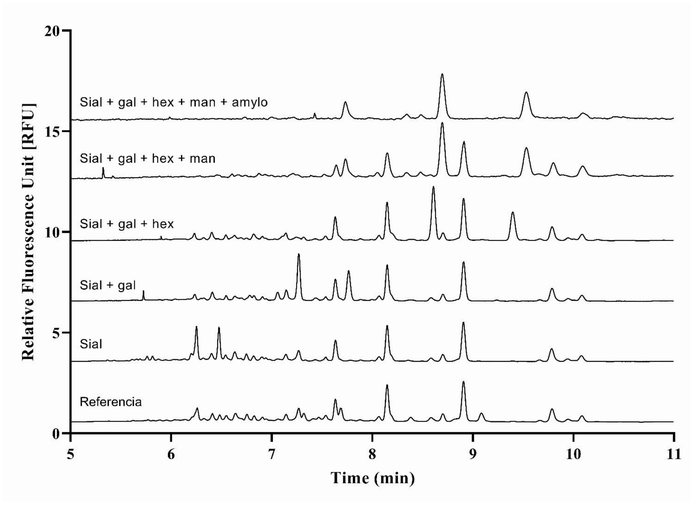
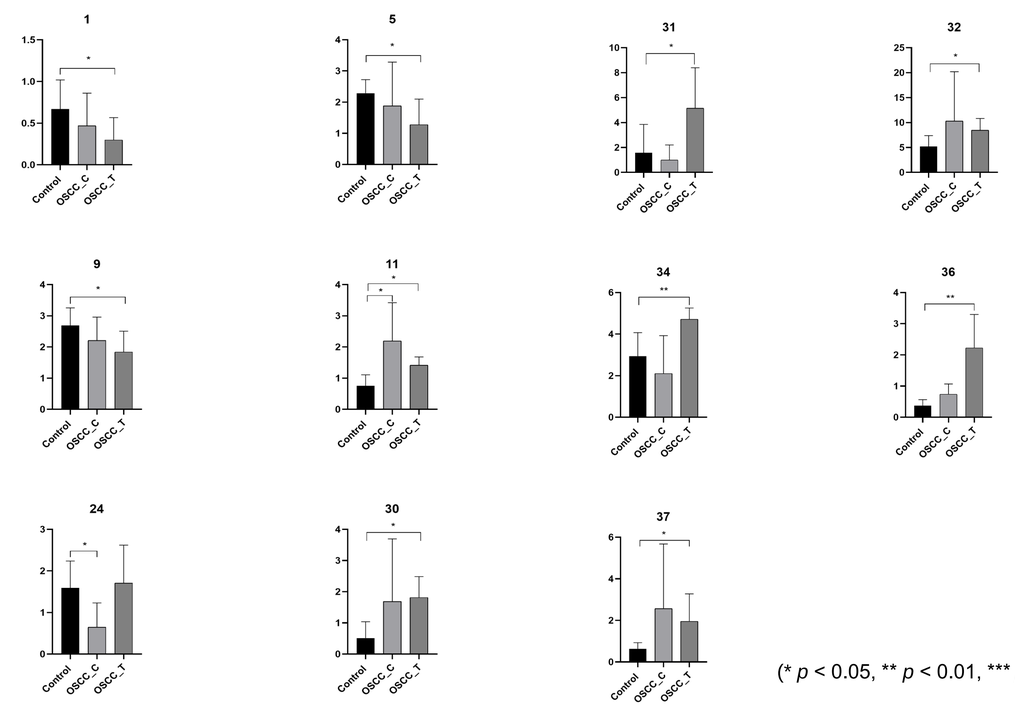


Figure 5: Exoglycosidase array based carbohydrate sequencing verified the identified structures



(* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$)

Figure 6: Significantly changed N-glycan structures in the three examined group

***OSCC_T* Oral squamous cell carcinoma patients malignant tumor sides**
***OSCC_C* Oral squamous cell carcinoma patients healthy gingival sides**

Table 1: Identified carbohydrate structures

Peak ID	Structures
1	A4G(4)4S(3)1
2	A2BG2
3	A2G(4)2
4	F(6)A2[3]G(4)1
5	F(6)A2[6]G(4)1
6	A3G(4)3S(3)1
7	A3G(4)3S(6)1
8	A4G(4)4S(6)2
9	A2G(4)2S(3)1
10	A2B
11	F(6)A2G(4)2S(6)1
12	Man-5
13	A3G(4)3S(3)2
14	A2G(4)2S(6)1
15	A4G(4)4S(3)3
16	A3G(4)3S(6)2
17	F(6)A2[3]G(4)1S(6)1
18	M3N2F
19	A3G(4)3S(3)3
20	A2G(4)2S(3)2
21	A2G(4)2S(6)2
22	M3N2
23	A4G(4)4S(6)4
24	A4G(4)4S(6)4
25	A4G(4)S(6)2(3)2
26	A4G(4)S(6)3S(3)1

CONCLUSION: The high-resolution CE-LIF-based glyocoanalytical method reported in this poster proved to be an efficient and sensitive workflow for glycobiomarker-based molecular diagnostics of oral malignant lesions, by using the automated tissue homogenizer. This will allow high-throughput screening of large patient cohorts to discover and validate novel cancer-related glycobiomarkers for accurate prognosis.

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