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Salivary proteome profiling of oral squamous cell carcinoma in a Hungarian population

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The authors declare they have no conflict of interest.

Running title: Proteomic analysis of salivary biomarkers in OSCC

Abbreviations

OSCC – oral squamous cell carcinoma
ELISA – enzyme-linked immunosorbent assay
MS – mass spectrometry
UPLC – Ultra-Performance Liquid Chromatography

Keywords: OSCC, proteomic analysis, ELISA, biomarker

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Abstract

Oral squamous cell carcinoma (OSCC) is the 7th most common malignancy and the 9th most frequent cause of cancer death in Europe. Within Europe, Hungary has one of the highest rates of OSCC incidence and mortality. Thus, there is an urgent need to improve early detection. Saliva, as a readily available body fluid, became an increasingly important substance for the detection of biomarkers for many diseases. Different research groups have identified salivary biomarkers specific for OSCC for different countries. In this study, saliva samples of Hungarian OSCC patients were studied in order to discover disease- and perhaps region-specific biomarkers. LC/MS/MS analysis on a linear ion trap-Orbitrap mass spectrometer was used for qualitative and quantitative salivary protein profiling. More than 500 proteins were identified from saliva by shotgun proteomics. The up- and downregulated proteins in the saliva of patients with OSCC highlighted the importance of protein-protein interaction networks involving the immune system and proteolysis in disease development. Two potential biomarkers from our shotgun analysis and a third candidate reported earlier by a Taiwanese group were further examined by ELISA on a larger reference set of samples. Resistin, a biomarker reported in Taiwan but not validated in our study, highlights the necessity of application of standardized analysis methods in different ethnic or geographical populations in order to identify biomarkers with sufficient specificity and sensitivity.

Introduction

The oral cavity is the most frequent site of head and neck cancers, developing predominantly as oral cavity squamous cell carcinomas (OSCC) in the upper aerodigestive epithelium [1,2]. The three major recognized risk factors of OSCC are tobacco~~,~~ and alcohol-consumption and poor oral hygiene [3–5]. OSCC mortality rates reflect the different consumption patterns of alcohol and tobacco in European countries [6]. Annually more than 300,000 new patients are diagnosed with OSCC worldwide. The disease is associated with poor prognosis and high mortality mainly due to late diagnosis because of the lack of reliable early diagnostic markers [7]. Mortality rate from OSCC is about 10-fold higher for men than for women. However, female OSCC incidence increased dramatically in the last decade. In addition, a rising tendency was observed in younger patient cohorts [8]. In contrast to other European countries where the mortality rates of OSCC started to decline, unfavorable incidence and mortality figures remained exceedingly high in Hungary since the 1970s' representing a major public health challenge [9].

Development of cancer diagnostic tools with sufficiently high sensitivity and specificity is required to enable early detection of OSCC [10]. Recent treatment strategies of OSCC patients are based on traditional stage-predicting indices and histological grading [11]. Unfortunately, these predictors are relatively subjective and unreliable because tumors with same staging and grading may respond to therapy differently. Thus, improving the diagnostic methods is required. A potential way of improving our diagnostic tools is to perform in-depth salivary analyses to discover and to assess biochemical and immunological markers in the saliva for early oral cancer diagnosis [12-13]. Biomarkers identified in the last decades in biological fluids can be linked to carcinogenesis and may serve as prognostic factors and saliva is a new clinical biomarker source that can be easily collected by non-invasive means [14-18]. Since there is direct contact between saliva and the oral lesion(s) disease-related concentration changes of saliva ingredients may provide as good or better clues than serum samples [19]. More than 3700 salivary proteins have been identified by several research groups [20,21]. Many proteins were declared potential salivary biomarkers of OSCC in different countries [22–24]. In this

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study, we present a two-stage approach for the discovery of candidate OSCC-specific salivary biomarkers in the Hungarian population. LC/MS/MS analysis using ultra-Performance Liquid Chromatography (UPLC) coupled to a linear ion trap-Orbitrap hybrid tandem mass spectrometer was applied for qualitative and quantitative salivary protein profiling. Selected proteins, based on the shotgun analysis of a few randomly selected samples, were further investigated by ELISA on a reference set of samples.

Materials and Methods

Patients and saliva collection

Donor enrollment, sample collection and processing were conformed to the principles of the Helsinki Declaration. Ethical approval was obtained from the University of Debrecen Ethics Committee (No. 3385-2011) and all subjects provided written informed consent. Clinical examinations were performed by dental surgeons from the Faculty of Dentistry, University of Debrecen. Adult patients (>18 yrs) with histology-proven OSCC were recruited into the study. Saliva samples were collected before starting any anti-tumor therapy. Age-matched controls (MCTL) were consecutive patients and young controls (YCTL) were medical students admitted to the Faculty of Dentistry for regular dental checkup. Exclusion criteria included children (</= 18 yrs), pregnancy and breast feeding, diabetes mellitus, human papillomavirus infection, human immunodeficiency virus infection, autoimmune and immunodeficiency disorders and cancer other than OSCC.

Unstimulated saliva samples were collected from 43 donors between 9 a.m. and 11 a.m. at the Faculty of Dentistry, University of Debrecen (collection between 2013.05.09-2016.02.29.). The test set contained 3 randomly selected samples from patients with OSCC and controls for proteomic analysis, whereas the reference set contained samples from 20 patients with OSCC (mean age: 57 year), 6 young (mean age: 24,5 year) and 11 age-matched CTLs (mean age: 59 year) for biomarker verification. Saliva samples were kept on ice during collection and were filtered using 5 µm pore size Millipore SLSV025LS syringe filters (Merck, Billerica, MA, USA). The filtered saliva was aliquoted and immediately placed to -70°C until further use.

Sample preparation for mass spectrometry

Filtered saliva was dried in speed-vac and redissolved in 25 mM pH 8.5 ammonium bicarbonate buffer. Total protein concentration of salivary samples was measured using the Bradford method [25]. Following denaturation with 8 M urea, all samples were reduced with 10 mM dithiothreitol (Bio-Rad, Hercules, CA, USA) in ammonium bicarbonate buffer. Then samples were alkylated with 20 mM iodoacetamide (Bio-Rad, Hercules, CA, USA) in ammonium bicarbonate buffer and diluted with 25 mM ammonium bicarbonate (Sigma, St. Louis, MO, USA) in order to reduce the urea concentration to 1 M. Each sample was digested by MS grade modified trypsin (ABSciex, Framingham, MA, USA) in 1:25 enzyme to protein ratio (w/w) at 37°C, overnight. The digested samples were dried in speed-vac and redissolved in 0.1% formic acid. The digests were desalted on Pierce C18 Tips (Thermo Scientific, West Palm Beach, FL, USA) and the eluates were dried and stored at -70°C until mass spectrometry analysis.

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Mass spectrometry analysis

Tryptic digests representing 2 µg total protein were analyzed by LC-MS/MS using a Waters nanoAcquity UPLC on-line coupled to a linear ion trap-Orbitrap hybrid tandem mass spectrometer (Orbitrap Elite, Thermo Scientific) operating in positive ion mode. After trapping at 3% B (Waters Symmetry C18 180 µm x 20 mm column, 5 µm particle size, 100 Å pore size; flow rate: 10 µl/min), peptides were fractionated using a linear gradient of 3 to 40% B in 100 min (Waters BEH C18 75 µm x 250 mm column, 1.7 µm particle size, 300 Å pore size; solvent A: 0.1% formic acid/water, solvent B: 0.1% formic acid / 5% dimethyl sulfoxide / acetonitrile; flow rate: 400 nl/min). Data acquisition was carried out in a data-dependent fashion, the 10 most abundant, multiply charged ions were selected from each MS survey (m/z: 380-1600, resolution: 60000, acquired in profile mode) for MS/MS analyses. CID analyses were performed in the linear ion trap (normalized collision energy: 35). Dynamic exclusion was enabled (exclusion time: 30 s).

Protein identification

Peak lists generated from the MS/MS data by the 'PAVA' software [26] were searched against the human subset of the Uniprot database (downloaded 06/10/2014; 136245 target sequences concatenated with a randomized sequence for each entry) using the ProteinProspector search engine (v.5.10.9.). Search parameters: enzyme: trypsin with maximum 1 missed cleavage site; fixed modification: carbamidomethyl (Cys); variable modifications: acetylation (protein N-terminus), oxidation (Met), pyroglutamic acid formation (N-terminal Gln) allowing up to 2 variable modifications per peptide; mass accuracy: 5 ppm and 0.6 Da for precursor and fragment ions (both monoisotopic), respectively. The following acceptance criteria were applied: score>22 and 15, and E-value<0.01 and 0.05 for protein and peptide identifications, respectively. The false positive rates of the identified proteins and peptides were less than 1%. Relative abundance of individual proteins was estimated by spectral counting: the number of identifications per protein (PSMs) was normalized to the total number of identifications, then these relative spectral counts were compared across the different samples.

Functional analyses were performed in case of proteins with at least three unique peptide identifications. For the calculation of the OSCC/control ratio those proteins were considered which were identified with at least 3 unique peptides in at least two out of three samples in either the control or the OSCC group.

Validation of the candidate biomarkers using ELISA

All saliva samples from patients with OSCC and controls were analyzed in duplicate with quantitative ELISA. The ELISA kit for heparin cofactor 2 (#Cat. number: LS-F13221) was purchased from LifeSpan Biosciences (Seattle, WA, USA), for resistin (#Cat. number: KHP0051) from Thermo Fisher Scientific (West Palm Beach, FL, USA) and for complement c5 (#Cat. number: ab125963) from Abcam (Branford, Connecticut, USA). The concentration of the studied proteins in saliva was measured by sandwich enzyme-linked immunosorbent assay method according to the instruction provided by the vendor of each kit. Absorbance was measured at 450 nm and concentrations were calculated based on the recorded 7-point calibration curves.

First, the variation coefficient of the parallel measurements was calculated and those data having more than 25 CV % value were excluded from statistical analysis.

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Bioinformatics

The cluster analysis was carried out with Cluster 3.0 (http://cluster2.software.informer.com/) using the C Clustering Library version 1.52 and the heat map was created with Java TreeView version 1.1.6r4 [27].

The protein-protein interaction network of salivary proteins was generated using String version 10.5 [28,29] applying default settings and medium stringency. After the generation of networks the enriched GO terms provided by the software were also examined.

The statistical analysis of ELISA data was performed using the Mann–Whitney U test and the two sample T-test in order to compare the protein concentrations between groups. Those data were considered significantly different where the p value was $p < 0.05$.

Results and Discussions

Demographic and clinical characteristics of patients with OSCC

Among the included 17 patients 13 were males and 4 females between the age of 44-73 yrs. In six cases the tumor developed in the tongue (T), in 4 cases in the floor of the mouth (F) and in 3 cases it was detected in the gingival (G) region. In 4 cases the tumor development showed multiple localization, in 2 patients the tumor developed in the tongue and either in the floor of the mouth or the gingival region, while in another 2 patients the tumor development was detected in the tongue, in the floor of the mouth and also in the gingival region. Eight patients were discovered in early tumor development stage (stage I.: 5, stage II.: 3) and 9 patients were diagnosed with advanced tumors (stage III.: 4 and stage IV.: 5). There were six well differentiated (W), 7 moderately differentiated (M) and 4 poorly differentiated (P) OSCC samples (Table 1).

Shotgun proteomic analysis of saliva samples

Three randomly selected samples from patients with OSCC and matched controls respectively, were subjected to shotgun proteomics analysis. More than 500 proteins were identified from salivary samples. For protein quantification spectral counting was used and the ratios of OSCC:CTL protein quantities have been determined. Detailed information of the identified proteins is presented in Supplementary Table 1.

The proteins with at least 3 unique sequences and with at least 2 fold change value (OSCC/CTL ratio <0.5 or >2) were subjected to further examination. A cluster analysis was carried out and a heat map was generated to visualize the changes in protein amount in CTL and OSCC samples (Figure 1). Based on cluster analysis the protein levels can discriminate the OSCC group from the CTL group. Proteins were classified as salivary proteins or proteins being present in saliva under normal conditions and as acute phase proteins (Table 2). For protein classification the Uniprot and the Sys-BodyFluid databases were used, the latter contains more than 10,000 proteins of different body fluid proteomes [30]. In addition, some proteins were classified as salivary proteins based on literature data [21, 31–35].

2 proteins, the cytochrome c and mucin-7 were only present in the CTL samples and 6 proteins, the complement factor H (CFH) and C5 (C5), corticosteroid binding globulin (SERPINA6), heparin cofactor 2 (SERPIND1), apolipoprotein E (APOE) and serum paraoxonase/arylesterase 1 (PON1), were only present in the OSCC samples (Table 3).

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Functional analysis of salivary proteins

It was observed that the level of apolipoproteins, components of the complement system, proteinases, proteinase inhibitors, components of the coagulation cascade is upregulated indicating a change in proteolysis most probably associated with the interrelated coagulation cascade-complement activation processes. In the same time the level of proteins having role in metabolism and host defense was downregulated showing extensive cancer-related changes (Table 2). For a more detailed functional analysis of the differentially expressed proteins gene ontology (GO) analysis was performed; the Biological Process, Molecular Function and Cellular Localization according to GO (http://www.geneontology.org/) was examined. First, the network of differentially expressed proteins was generated using String version 10.5 [28,29] followed by GO enrichment analysis provided by String. The network of downregulated proteins contained 35 proteins (nodes) and 27 possible protein-protein interactions analyzed at medium stringency (Figure 2A). No biological function was enriched in the down-regulated proteins in this loosely connected network (Figure 2B), however 7 out of 35 downregulated proteins are metabolic enzymes participating mainly in carbohydrate metabolism and 10 proteins out of 35 have a role in defense. The upregulated 45 proteins show a highly interconnected protein-protein interaction network with 400 interactions analyzed at medium stringency (Figure 2C). The enriched functions indicate active regulatory mechanisms implicating the immune system, lipid metabolism, plasminogen activation, antioxidant activity and inhibition of enzymatic activities (Figure 2D). Regarding localization of up- or downregulated proteins, all are mainly extracellular proteins according to GO (Figure 2B and 2D), but a part of the upregulated proteins originate from lipid particles or platelet alpha granules indicating the presence of a possibly cancer-induced complex process involving systemic mechanisms.

In order to get more insights into the changes associated with OSCC a literature search was performed to see which proteins have been associated with oncogenesis. Most of the proteins were already associated with OSCC, and 32 proteins were identified to be present in saliva in this pathological condition.

Complement C4B (C4B), complement factor B (CFB), complement C3 and alpha-1-antitrypsin were shown to be associated with the risk of developing OSCC according to a targeted proteomics study [36]. The level of apolipoproteins A and E, PON1, inter-alpha-trypsin inhibitor heavy chain H1, H2 and H4, kininogen 1, protein AMBP, nucleobinding-2, SERPIND1 and SERPINA6 were found to be upregulated in OSCC in shotgun proteomics experiments carried out on saliva samples [23]. The presence of ApoE was related to the increased invasion potential of OSCC [37].

The alpha-1-acid glycoprotein, alpha-1B glycoprotein, alpha amylase, beta-2-glycoprotein, carbonic anhydrase 1, cystatin-SA, hemopexin, phosphoglycerate kinase and serum albumin were identified as potential salivary markers of OSCC [14,16,22].

Some of the proteins found to be differentially expressed in our study such as fibrinogen alpha, beta and gamma chains, haptoglobin, SERPINB5, retinol binding protein 4 and ceruloplasmin, were shown to be plasma markers of OSCC while the presence of integrin alpha-M and fibronectin FN1 was demonstrated in the OSCC tissue [12,38-43].

In case of 36 proteins no association with OSCC was found so far (Table 2). Angiotensinogen and plasminogen themselves were not found to be associated with OSCC but the plasminogen activator system was shown to be a predictive marker for early OSCC and by bioinformatics analysis the angiotensin converting enzymes were associated with malignant epithelial neoplasia characteristic for OSCC [44,45]. In case of 6 proteins not the

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protein from our list, but another protein from the same family was already demonstrated to be differentially expressed in OSCC (Table 2). In case of SERPINB5 there are contradictory data; in our study the level of SERPINB5 was found to be elevated in OSCC, however the SERPINB5 and different forms of SERPINS from clade B were found by other groups to be downregulated in OSCC on mRNA level and higher SERPINB5 levels were found to correlate with better prognosis of patients with oral cancer [46,47].

Plasma protease C1 inhibitor (SERPING1), antithrombin III and fibronectin were found to play a role in carcinogenesis but their implication in oral cancer, especially in OSCC has not been demonstrated yet [48,49]. The complement factor H was previously identified in lung adenocarcinoma and cutaneous squamous cell carcinoma, but not in OSCC [50,51] and Apo B100 was found in serum of patients with head and neck squamous cell carcinoma [52]. No data were found on the presence of complement C5 and mucin-7 in cancer, however other components of the complement system and other forms of mucins were all identified in different forms of cancer and in OSCC as well [36, 53].

As for the involvement of cytochrome c, it was shown that the HIF-1alpha-dependent suppression of hypoxia-induced apoptosis in OSCC happens through the inhibition of cytochrome c release [54].

Examination of the level of selected proteins by ELISA

Many of the studies published in the scientific literature are based on shotgun proteomic experiments. Only few of the proteins listed in Table 2 were verified or validated either using SRM-based targeted or antibody-based methods. Considering the proteins present only in OSCC based on our shotgun experiments, the data presented in the literature, and the availability of antibodies, SERPIND1 and C5 were selected for further studies. In order to test the utility of potential biomarkers identified in Asia for a European population, resistin reported to be a potential biomarker for OSCC in Taiwan [23] was also selected.

The concentrations of C5, SERPIND1 and resistin were examined in the saliva of patients with OSCC, age-matched and young controls using quantitative sandwich ELISA kits (Figure 3). In case of C5 the difference was significant but only when young controls and patients with OSCC or young controls and age-matched controls were compared indicating that the level of C5 was age-dependent or it was influenced by other factors. One such factor can be the inflammatory status related to poor oral hygiene often observed in the middle-aged and elderly population in Hungary [55]. This means that despite the differential expression of C5 in the OSCC group, the level of C5 does not discriminate between the target age-matched control and diseased group and hence it cannot be used as a biomarker for OSCC.

In case of resistin and SERPIND1 no significant differences were found between the groups. Resistin was not up- or downregulated according to our shotgun experiments and did not show significant differences in the ELISA experiments either. In case of SERPIND1 one possible explanation of the disagreement between the shotgun proteomics and ELISA data can be that the low number of samples (3 for each group) tested by shotgun proteomics and the high individual variation of the saliva samples collected from the patients may lead to false positive results. This outcome highlights the importance of validation of the shotgun proteomics data on larger patient cohorts to decrease the false positivity of biomarker identifications.. In a two-stage experimental approach, starting with a shotgun proteomics experiment, the level of resistin was found to be significantly higher in saliva samples of OSCC patients compared to controls. However, following ELISA assays showed that the median values in the OSCC group were only slightly elevated compared to the control group [23]. In the

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same study SERPIND1 was not validated but was shown to be upregulated in the saliva samples of patients with OSCC. In our study a similar experimental setup was applied; in the shotgun experiment the level of SERPIND1 was higher but the level of resistin did not change markedly in the OSCC group and the validation of SERPIND1 and resistin show that none of them turned to be useful potential biomarkers. The fact that resistin was identified as a biomarker for OSCC in Taiwan but not in Hungary gives further evidence for the importance of regional studies highlighted in our previous work [56].

Conclusions

Global analysis of salivary samples from patients with OSCC and controls contribute to the better understanding of the disease, including the interaction of tumor cells with their environment and the influence of cancer lesion on salivary protein ecology. Salivary proteins, characterizing patients with OSCC in this study highlighted the importance of networks involving the immune system and proteolysis in this disease. Six proteins were only detected in OSCC samples by proteomic analyses and two of them were further examined using ELISA assay but none of the proteins turned to be a potential biomarker in OSCC in our study group. The fact that resistin was shown to be a possible biomarker in Taiwan but not in our study highlights the importance of regional or population-tailored studies.

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Author contribution statement

IM, EC and CK designed the experiments, IM, JN performed stomatologic examination of patients, BM, ZD carried out the experiments, BM, ZD and EC evaluated the data, BM, ES, EC prepared the figures and tables, EC, BM, ZD wrote the manuscript, JT, KM, CK and IM reviewed the manuscript.

Supplementary information available.

Reference

1 Massano J, Regateiro FS, Januário G & Ferreira A (2006) Oral squamous cell carcinoma: Review of prognostic and predictive factors. *Oral Surgery, Oral Med. Oral Pathol. Oral Radiol. Endodontology* **102**, 67–76.

2 Argiris A, Karamouzis M V, Raben D & Ferris RL (2008) Head and neck cancer. *Lancet* **371**, 1695–1709.

3 Gandini S, Botteri E, Iodice S, Boniol M, Lowenfels AB, Maisonneuve P & Boyle P (2008) Tobacco smoking and cancer: A meta-analysis. *Int. J. Cancer* **122**, 155–164.

4 Bagnardi V, Blangiardo M, La Vecchia C & Corrao G (2001) A meta-analysis of alcohol drinking and cancer risk. *Br. J. Cancer* **85**, 1700–1705.

Accepted Article

5 Rosenquist K (2005) Risk factors in oral and oropharyngeal squamous cell carcinoma: a population-based case-control study in southern Sweden. *Swed. Dent. J. Suppl.*, 1–66.

6 Suba Z, Mihályi S, Takács D & Gyulai-Gaál S (2009) [Oral cancer: morbus Hungaricus in the 21st century]. *Fogorv. Sz.* **102**, 63–8.

7 Torre LA, Bray F, Siegel RL, Ferlay J, Lortet-Tieulent J & Jemal A (2015) Global cancer statistics, 2012. *CA. Cancer J. Clin.* **65**, 87–108.

8 Myers JN, Elkins T, Roberts D & Byers RM (2000) Squamous cell carcinoma of the tongue in young adults: Increasing incidence and factors that predict treatment outcomes☆☆☆. *Otolaryngol. - Head Neck Surg.* **122**, 44–51.

9 Garavello W, Bertuccio P, Levi F, Lucchini F, Bosetti C, Malvezzi M, Negri E & La Vecchia C (2010) The oral cancer epidemic in central and eastern Europe. *Int. J. Cancer* **127**, 160–171.

10 Santosh ABR, Jones T & Harvey J (2016) A review on oral cancer biomarkers: Understanding the past and learning from the present. *J. Cancer Res. Ther.* **12**, 486–92.

11 Sparano A, Weinstein G, Chalian A, Yodul M & Weber R (2004) Multivariate Predictors of Occult Neck Metastasis in Early Oral Tongue Cancer. *Otolaryngol. Neck Surg.* **131**, 472–476.

12 Shpitzer T, Hamzany Y, Bahar G, Feinmesser R, Savulescu D, Borovoi I, Gavish M & Nagler RM (2009) Salivary analysis of oral cancer biomarkers. *Br. J. Cancer* **101**, 1194–8.

13 Shpitzer T, Bahar G, Feinmesser R & Nagler RM (2007) A comprehensive salivary analysis for oral cancer diagnosis. *J. Cancer Res. Clin. Oncol.* **133**, 613–617.

14 Sannam Khan R, Khurshid Z, Akhbar S & Faraz Moin S (2016) Advances of Salivary Proteomics in Oral Squamous Cell Carcinoma (OSCC) Detection: An Update. *Proteomes* **4**, 41.

15 Shah FD, Begum R, Vajaria BN, Patel KR, Patel JB, Shukla SN & Patel PS (2011) A review on salivary genomics and proteomics biomarkers in oral cancer. *Indian J. Clin. Biochem.* **26**, 326–334.

16 Winck F V., Prado Ribeiro AC, Ramos Domingues R, Ling LY, Riaño-Pachón DM, Rivera C, Brandão TB, Gouvea AF, Santos-Silva AR, Coletta RD & Paes Leme AF (2015) Insights into immune responses in oral cancer through proteomic analysis of saliva and salivary extracellular vesicles. *Sci. Rep.* **5**, 16305.

17 Csősz É, Kalló G, Márkus B, Deák E, Csutak A & Tőzsér J (2017) Quantitative body fluid proteomics in medicine — A focus on minimal invasiveness. *J. Proteomics* **153**, 30–43.

18 Yoshizawa JM, Schafer CA, Schafer JJ, Farrell JJ, Paster BJ & Wong DTW (2013) Salivary Biomarkers: Toward Future Clinical and Diagnostic Utilities. *Clin. Microbiol. Rev.* **26**, 781–791.

19 Nagler R, Bahar G, Shpitzer T & Feinmesser R (2006) Concomitant Analysis of Salivary Tumor Markers--A New Diagnostic Tool for Oral Cancer. *Clin. Cancer Res.* **12**, 3979–3984.

20 Schulz BL, Cooper-White J & Punyadeera CK (2013) Saliva proteome research: current status and future outlook. *Crit. Rev. Biotechnol.* **33**, 246–259.

21 Grassl N, Kulak NA, Pichler G, Geyer PE, Jung J, Schubert S, Sinitcyn P, Cox J & Mann M (2016) Ultra-deep and quantitative saliva proteome reveals dynamics of the oral microbiome. *Genome Med.* **8**, 44.

22 Hu S, Arellano M, Boontheung P, Wang J, Zhou H, Jiang J, Elashoff D, Wei R, Loo JA & Wong DT (2008) Salivary proteomics for oral cancer biomarker discovery. *Clin. Cancer Res.* **14**, 6246–52.

23 Wu C-C, Chu H-W, Hsu C-W, Chang K-P & Liu H-P (2015) Saliva proteome profiling reveals potential salivary biomarkers for detection of oral cavity squamous cell carcinoma. *Proteomics* **15**, 3394–3404.

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24 Yu J-S, Chen Y-T, Chiang W-F, Hsiao Y-C, Chu LJ, See L-C, Wu C-S, Tu H-T, Chen H-W, Chen C-C, Liao W-C, Chang Y-T, Wu C-C, Lin C-Y, Liu S-Y, Chiou S-T, Chia S-L, Chang K-P, Chien C-Y, Chang S-W, Chang C-J, Young JD, Pao CC, Chang Y-S & Hartwell LH (2016) Saliva protein biomarkers to detect oral squamous cell carcinoma in a high-risk population in Taiwan. *Proc. Natl. Acad. Sci.* **113**, 11549–11554.

25 Bradford MM (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.* **72**, 248–54.

26 Guan S, Price JC, Prusiner SB, Ghaemmaghami S & Burlingame AL (2011) A Data Processing Pipeline for Mammalian Proteome Dynamics Studies Using Stable Isotope Metabolic Labeling. *Mol. Cell. Proteomics* **10**, M111.010728–M111.010728.

27 Alok J. Saldanha (2004) Java Treeview—extensible visualization of microarray data, *Bioinformatics* **20**, 3246–3248,

28 Szklarczyk D, Morris JH, Cook H, Kuhn M, Wyder S, Simonovic M, Santos A, Doncheva NT, Roth A, Bork P, Jensen LJ & von Mering C (2017) The STRING database in 2017: quality-controlled protein-protein association networks, made broadly accessible. *Nucleic Acids Res.* **45**, D362–D368.

29 Szklarczyk D, Franceschini A, Wyder S, Forslund K, Heller D, Huerta-Cepas J, Simonovic M, Roth A, Santos A, Tsafou KP, Kuhn M, Bork P, Jensen LJ & von Mering C (2015) STRING v10: protein-protein interaction networks, integrated over the tree of life. *Nucleic Acids Res.* **43**, D447–D452.

30 Li S-J, Peng M, Li H, Liu B-S, Wang C, Wu J-R, Li Y-X & Zeng R (2009) Sys-BodyFluid: a systematical database for human body fluid proteome research. *Nucleic Acids Res.* **37**, D907–D912.

31 Swaak AJ, Visch LL & Zonneveld A (1988) Diagnostic significance of salivary levels of beta 2-microglobulin in Sjögren's syndrome. *Clin. Rheumatol.* **7**, 28–34.

32 Guo T, Rudnick PA, Wang W, Lee CS, DeVoe DL & Balgley BM (2006) Characterization of the Human Salivary Proteome by Capillary Isoelectric Focusing/Nanoreversed-Phase Liquid Chromatography Coupled with ESI-Tandem MS. *J. Proteome Res.* **5**, 1469–1478.

33 Rossetti DV, Martelli C, Longhi R, Iavarone F, Castagnola M & Desiderio C (2013) Quantitative analysis of thymosin β_4 in whole saliva by capillary electrophoresis-mass spectrometry using multiple ions monitoring (CE-MIM-MS). *Electrophoresis* **34**, 2674–2682.

34 Morzel M, Chabanet C, Schwartz C, Lucchi G, Ducoroy P & Nicklaus S (2014) Salivary protein profiles are linked to bitter taste acceptance in infants. *Eur. J. Pediatr.* **173**, 575–582.

35 Alves DBM, Bingle L, Bingle CD, Lourenco SV, Silva AA, Pereira DL & Vargas PA (2017) BPI-fold (BPIF) containing/plunc protein expression in human fetal major and minor salivary glands. *Braz. Oral Res.* **31**.

36 Kawahara R, Bollinger JG, Rivera C, Ribeiro ACP, Brand?o TB, Leme AFP & MacCoss MJ (2016) A targeted proteomic strategy for the measurement of oral cancer candidate biomarkers in human saliva. *Proteomics* **16**, 159–173.

37 Jayakar SK, Loudig O, Brandwein-Gensler M, Kim RS, Ow TJ, Ustun B, Harris TM, Prystowsky MB, Childs G, Segall JE & Belbin TJ (2017) Apolipoprotein E Promotes Invasion in Oral Squamous Cell Carcinoma. *The American Journal of Pathology* **187**,2259-2272.

38 Chen Y, Azman SN, Kerishnan JP, Zain RB, Chen YN & Wong YL (2014) Identification of host-immune response protein candidates in the sera of human oral squamous cell carcinoma patients. *PLoS One.* **9**,

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e109012.

39 Jayadeep A, Raveendran Pillai K, Kannan S, Nalinakumari KR, Mathew B, Krishnan Nair M & Menon VP. (1997) Serum levels of copper, zinc, iron and ceruplasmin in oral leukoplakia and squamous cell carcinoma. *J Exp Clin Cancer Res*. **16**, 295-300.

40 Pro Tung CL, Lin ST, Chou HC, Chen YW, Lin HC, Tung CL, Huang KJ, Chen YJ, Lee YR & Chan HL. (2013) Proteomics-based identification of plasma biomarkers in oral squamous cell carcinoma. *J Pharm Biomed Anal.* **75**, 7-17.

41 Ohara T, Kawashiri S, Tanaka A, Noguchi N, Kitahara H, Okamune A, Kato K, Hase T, Nakaya H & Yoshizawa K. (2009) Integrin expression levels correlate with invasion, metastasis and prognosis of oral squamous cell carcinoma. *Pathol Oncol Res.* **36**, 429-36..

42 Chen Y, Azman SN, Kerishnan JP, Zain RB, Chen YN & Wong YL, (2014) Identification of host-immune response protein candidates in the sera of human oral squamous cell carcinoma patients. *PLoS One.*, **9**:e109012.

43 Yen CY, Huang CY, Hou MF, Yang YH, Chang CH, Huang HW, Chen CH & Chang HW. (2013) Evaluating the performance of fibronectin 1 (FN1), integrin α4β1 (ITGA4), syndecan-2 (SDC2), and glycoprotein CD44 as the potential biomarkers of oral squamous cell carcinoma (OSCC). *Biomarkers.*;**18**, 63-72.

44. Magnussen S, Rikardsen OG, Hadler-Olsen E, Uhlin-Hansen L, Steigen SE & Svineng G (2014) Urokinase Plasminogen Activator Receptor (uPAR) and Plasminogen Activator Inhibitor-1 (PAI-1) Are Potential Predictive Biomarkers in Early Stage Oral Squamous Cell Carcinomas (OSCC). *PLoS ONE* **9,** e101895.

45 de Carvalho Fraga CA, Farias LC, Jones KM, Batista de Paula AM & Guimaraes ALS (2017) Angiotensin-Converting Enzymes (ACE and ACE2) as Potential Targets for Malignant Epithelial Neoplasia: Review and Bioinformatics Analyses Focused in Oral Squamous Cell Carcinoma. *Protein Pept Lett* **24**, 784-792.

46 Shiiba M, Nomura H, Shinozuka K, Saito K, Kouzu Y, Kasamatsu A, Sakamoto Y, Murano A, Ono K, Ogawara K, Uzawa K & Tanzawa H (2010) Down-regulated expression of SERPIN genes located on chromosome 18q21 in oral squamous cell carcinomas. *Oncol Rep* **24**, 241-249.

47 Xia W, Lau YK, Hu MC, Li L, Johnston DA, Sheng S, El-Naggar A & Hung MC (2000) High tumoral maspin expression is associated with improved survival of patients with oral squamous cell carcinoma. *Oncogene* **19**, 2398-2403.

48 Kocsis J, Mészáros T & Madaras B (2011) High levels of acute phase proteins and soluble 70 kDa heat shock proteins are independent and additive risk factors for mortality in colorectal cancer. *Cell Stress & Chaperones*.;**16**, 49-55.

49 Daly M., O'Meara A. & Hallinan F. M. (1987), Identification and characterization of a new antithrombin III familial variant (AT Dublin) with possible increased frequency in children with cancer. *British Journal of Haematology*, **65**, 457–462.

50 Cui T, Chen Y, Knosel T, Yang L, Zoller K, Galler K, Berndt A, Mihlan M, Zipfel PF & Petersen I (2011) Human complement factor H is a novel diagnostic marker for lung adenocarcinoma. *Int J Oncol* **39**, 161-168.

51 Riihilä PM, Nissinen LM, Ala-aho R, Kallajoki M, Grénman R, Meri S, Peltonen S, Peltonen J & Kähäri V (2014) Complement Factor H: A Biomarker for Progression of Cutaneous Squamous Cell Carcinoma.

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Journal of Investigative Dermatology **134**, 498-506.

52 Li G, Da M, Zhang W, Wu H, Ye J, Chen J, Ma L, Gu N, Wu Y & Song X. (2016) Alteration of serum lipid profile and its prognostic value in head and neck squamous cell carcinoma. *J Oral Pathol Med*. **45**, 167-72.

53 Li, Ping, Li Ying Xiao & Hong Tan. (2015) "Muc-1 Promotes Migration and Invasion of Oral Squamous Cell Carcinoma Cells via PI3K-Akt Signaling." *International Journal of Clinical and Experimental Pathology* **8**, 10365–10374.

54 Sasabe E, Tatemoto Y, Li D, Yamamoto T & Osaki T (2005) Mechanism of HIF-1alpha-dependent suppression of hypoxia-induced apoptosis in squamous cell carcinoma cells. *Cancer Science* **96**, 394-402.

55 Bánóczy J & Rigó O (1991) Prevalence study of oral precancerous lesions within a complex screening system in Hungary. *Community Dent. Oral Epidemiol.* **19**, 265–7.

56 Csősz É, Lábiscsák P, Kalló G, Márkus B, Emri M, Szabó A, Tar I, Tőzsér J, Kiss C & Márton I (2017) Proteomics investigation of OSCC-specific salivary biomarkers in a Hungarian population highlights the importance of identification of population-tailored biomarkers. *PLoS One* **12**, e0177282

Figure legends

Figure 1. Cluster analysis and heat map of proteins identified in CTL and OSCC groups. The relative peptide count (%), characteristic for each sample is shown.

Figure 2. The protein-protein interaction network and functional classification of up- and downregulated proteins in OSCC The network of downregulated (A) and upregulated (C) proteins in OSCC displayed by String 10.4 using default settings and medium stringency. Each node represents a protein and the edges represent protein-protein interactions based on different levels of evidences collected by String. The enrichment table of GO terms calculated by String in case of downregulated (B) and upregulated (D) proteins is shown indicating the number of the proteins belonging to each term and the false discovery rate value calculated by String.

Figure 3. Examination of potential salivary biomarkers using ELISA assay The concentration of SERPIND1 (A), resistin (B) and complement C5 (C) proteins in the saliva samples collected from OSSC patients (OSCC), young controls (YCTL) and age-matched controls (MCTL). The p value is indicated, * refers to $p<0.05$.

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Table 1. Demographic and clinical characteristics of OSCC patients. In case of each patient the gender, age tumor localization, TNM classification, stage and stage of differentiation is given. M is for male, F for female. Regarding tumor localization, T is for tongue, G is for gingiva, F is for floor of the mouth. The W is for well differentiated, M is for moderately differentiated and P is for poorly differentiated tumors.

Patient code	Gender	Age (year)	Tumor localization	TNM classification	Tumor stage	Stage of differentiation
1	M	73	T	T2N1M0	III	W
2	F	69	G	T4N0M0	IV	W
3	F	67	F	T4N2M0	IV	W
4	M	52	T;G;F	T4N1M0	IV	M
5	M	57	T	T3N0M0	III	W
6	F	59	T	T1N0M0	I	W
7	M	67	F	T1N0M0	I	W
8	F	50	T	T2N0M0	II	M
9	M	52	T;G	T2N2M0	IV	M
10	M	48	T	T1N0M0	I	M
11	M	64	T	T2N0M0	II	P
12	M	44	G	T4N1M0	IV	M
13	M	44	T;F	T3N0M0	III	M
14	M	60	F	T2N0M0	II	M
15	M	49	T;G;F	T3N1M0	III	P
16	M	47	G	T1N0M0	I	P
17	M	64	F	T1N0M0	I	P

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Table 2. List of proteins with at least two fold change between OSCC and CTL groups. The Uniprot protein ID, the protein name and function is presented. The representative identification and quantification data, the number (#) of unique peptides, the sequence coverage (% Cov) and the OSCC/CTL ratio are given in each case. Classification indicating salivary (S) or acute phase (A) proteins is presented. The type of sample from patients with OSCC where the protein was identified is also listed. * indicates that not the protein itself, but another close family member of it was already found in OSCC. NI denotes proteins not identified in OSCC yet.

Protein ID	Protein Name	# unique peptide	% Cov	OSCC/ CTL ratio	Classification	Function	Type of OSCC sample
O60218	Aldo-keto reductase family 1 member B10	5	17	0,10	S	metabolic enzyme	saliva* [23]
P02763	Alpha-1-acid glycoprotein 1	8	37	3,14	AS	immune response, transport	saliva [16]
P01011	Alpha-1-antichymotrypsin	12	31	3,29	AS	protease inhibitor	NI
P01009	Alpha-1-antitrypsin	25	62	3,70	S	protease inhibitor	saliva [36]
P04217	Alpha-1B-glycoprotein	12	39	3,25	S	immune response	saliva [16]
P02765	Alpha-2-HS-glycoprotein	7	26	2,70	AS	protease inhibitor, immune response, transport	NI
P01023	Alpha-2-macroglobulin	54	51	2,16	S	protease inhibitor	NI
P04745	Alpha-amylase 1	42	83	0,21	S	metabolic enzyme	saliva [14]
P01019	Angiotensinogen	7	18	8,50	AS	renin-angiotensin system	NI
P01008	Antithrombin-III	7	22	2,08	AS	protease inhibitor, blood coagulation	NI
P02647	Apolipoprotein A-I	24	69	2,14	S	lipid metabolism	saliva [23]
P02652	Apolipoprotein A-II	7	67	3,85	S	lipid metabolism	saliva [23]
P06727	Apolipoprotein A-IV	5	16	3,55	S	lipid metabolism	saliva [23]
P04114	Apolipoprotein B-100	42	13	8,12	S	lipid metabolism	NI
P02649	Apolipoprotein E	4	18	only in OSCC	S	lipid metabolism	saliva [23]
P17213	Bactericidal permeability-increasing protein	4	12	0,24	S	immune response	NI
P02749	Beta-2-glycoprotein 1	12	44	3,02	S	lipid metabolism, blood coagulation	saliva [22]
P61769	Beta-2-microglobulin	5	57	0,46	S	immune	NI

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						response	
Q96DR5	BPI fold-containing family A member 2	11	41	0,49	S	immune response, defense	NI
Q14CN2	Calcium-activated chloride channel regulator 4	5	8	0,23	S	transport	NI
P27482	Calmodulin-like protein 3	6	64	0,37	S	metal binding, chaperone	NI
P27797	Calreticulin	4	19	0,37	S	chaperone	NI
P00915	Carbonic anhydrase 1	6	34	8,55	S	metabolic enzyme, acid-base balance	saliva [22]
P00450	Ceruloplasmin	27	37	3,65	AS	metal binding	blood [39]
O00299	Chloride intracellular channel protein 1	7	34	0,31	S	transport, cell cycle regulation	NI
P01024	Complement C3	84	61	2,77	AS	immune response	saliva [36]
P0C0L5	Complement C4-B	32	25	6,69	AS	immune response	saliva [36]
P01031	Complement C5	7	5	only in OSCC	AS	immune response	NI
B4E1Z4	Complement factor B	22	22	5,44	AS	immune response	saliva [36]
P08603	Complement factor H	21	22	only in OSCC	AS	immune response	NI
P05156	Complement factor I	3	7	6,42	AS	immune response	NI
P22528	Cornifin-B	6	79	0,45	S	cornification	NI
P08185	Corticosteroid-binding globulin	4	15	only in OSCC	AS	protease inhibitor	saliva [23]
P04080	Cystatin-B	6	86	0,39	S	protease inhibitor	NI
P01034	Cystatin-C	7	43	0,33	S	protease inhibitor	NI
P09228	Cystatin-SA	13	69	0,35	S	protease inhibitor	saliva [14]
P99999	Cytochrome c	4	32	0,00	A	electron transport chain, apoptosis	tissue [54]
Q02413	Desmoglein-1	8	12	0,40	S	desmosome component	NI
P61916	Epididymal secretory protein E1	4	33	0,30	S	lipid metabolism, cholesterol transport	NI
Q01469	Fatty acid-binding protein, epidermal	12	79	0,49	S	lipid metabolism	saliva* [23]
P02671	Fibrinogen alpha chain	11	13	2,67	AS	blood coagulation	blood [40]
P02675	Fibrinogen beta chain	20	49	2,91	AS	blood	blood [40]

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						coagulation	
P02679	Fibrinogen gamma chain	18	48	2,43	AS	blood coagulation	blood [40]
B7ZLE5	FN1 protein	24	17	5,73	S	cell adhesion	tissue [43]
P00738	Haptoglobin	29	67	2,61	AS	hem binding	blood [38]
P69905	Hemoglobin subunit alpha	11	92	3,37	S	oxygen transport	saliva* [23]
P68871	Hemoglobin subunit beta	17	94	4,41	S	oxygen transport	saliva* [23]
P02790	Hemopexin	20	52	2,41	AS	hem binding	saliva [16, 22]
P05546	Heparin cofactor 2	8	17	only in OSCC	A	blood coagulation	saliva [23]
Q9Y6R7	IgGFc-binding protein	52	17	0,49	S	immune response	NI
P11215	Integrin alpha-M	6	9	2,01	S	immune response	tissue [41]
P19827	Inter-alpha-trypsin inhibitor heavy chain H1	8	14	3,76	S	protease inhibitor	saliva [23]
P19823	Inter-alpha-trypsin inhibitor heavy chain H2	10	18	11,23	S	protease inhibitor	saliva [23]
Q14624	Inter-alpha-trypsin inhibitor heavy chain H4	13	22	5,33	S	protease inhibitor	saliva [23]
P02538	Keratin, type II cytoskeletal 6A	21	39	0,44	S	cytoskeleton	NI
P01042	Kininogen-1	11	18	2,89	S	protease inhibitor, blood coagulation	saliva [23]
P61626	Lysozyme C	7	54	0,47	S	host defense	NI
P40926	Malate dehydrogenase, mitochondrial	4	17	0,37	S	metabolic enzyme	NI
Q8TAX7	Mucin-7	4	12	0,00	S	host defense	NI
P80303	Nucleobindin-2	8	26	0,32	S	metal binding	saliva [23]
Q6UX06	Olfactomedin-4	7	20	0,47	S	cell adhesion	NI
P36871	Phosphoglucomutase-1	6	13	0,08	S	metabolic enzyme	NI
Q96G03	Phosphoglucomutase-2	5	11	0,40	S	metabolic enzyme	NI
P00558	Phosphoglycerate kinase 1	10	33	0,44	S	metabolic enzyme	saliva [22]
P36955	Pigment epithelium-derived factor	4	12	7,17	S	tumor development, angiogenesis	NI
P05155	Plasma protease C1 inhibitor	8	21	5,95	S	protease inhibitor, blood coagulation	NI
P00747	Plasminogen	9	17	5,11	AS	blood coagulation	NI

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P02760	Protein AMBP	6	23	5,41	S	protease inhibitor, host defense	saliva [23]
O60888	Protein CutA	3	33	0,43	S	metal binding, enzyme binding	NI
P02753	Retinol-binding protein 4	5	24	2,54	S	protease inhibitor, host defense	blood [42]
Q9NQ38	Serine protease inhibitor Kazal-type 5	11	13	0,32	S	lipid metabolism	NI
P36952	Serpin B5	4	14	2,63	S	tumor suppressor	blood [12]
P02768	Serum albumin	71	84	2,53	S	transport	saliva [22]
P27169	Serum paraoxonase/arylesterase 1	9	37	only in OSCC	A	detoxification	saliva [23]
P35326	Small proline-rich protein 2A	6	79	0,29	S	cornification	saliva* [23]
Q6UWP8	Suprabasin	12	33	0,05	S	cell proliferation	NI
P62328	Thymosin beta-4	5	64	0,42	S	actin binding, cell proliferation	NI
O60235	Transmembrane protease serine 11D	3	10	0,20	S	protease, host defense	NI
P68363	Tubulin alpha-1B chain	4	12	0,40	S	microtubule component	saliva* [23]
O75083	WD repeat-containing protein 1	7	19	0,13	S	cell migration	NI

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Table 3. Proteins identified only in the OSCC or CTL groups.

Protein ID[1]	Protein name	Gene name	Function	Presence	Reference to previous studies
P02649	Apolipoprotein E	APOE	lipid metabolism	only OSCC	identified in saliva of patients with OSCC [23]
P01031	Complement C5	C5	innate immune response, complement component	only OSCC	not identified in cancer yet
P08603	Complement factor H	CFH	innate immune response, complement component	only OSCC	identified in other forms of cancer but not in OSCC [50,51]
P08185	Corticosteroid-binding globulin	SERPINA6	protease inhibitor	only OSCC	identified in saliva of patients with OSCC [23]
P05546	Heparin cofactor 2	SERPIND1	blood coagulation	only OSCC	identified in saliva of patients with OSCC [23]
P27169	Serum paraoxonase/arylesterase 1	PON1	detoxification	only OSCC	identified in saliva of patients with OSCC [23]
P99999	Cytochrome c	CYCS	electron transport chain, its release from mitochondria initiates apoptosis	only Ctrl	its release was inhibited in OSCC [54]
Q8TAX7	Mucin 7	MUC7	antibacterial activity, host defense	only Ctrl	not identified in cancer yet

Based on [1]http://www.uniprot.org/.

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Figure 1.

Figure 2.

A.

B.

Pathway ID	Pathway description	Observed gene count	False discovery rate
I. Localization			
GO.0070062	Extracellular region part	24	3.56e-09
GO.0005576	Extracellular region	26	1.69e-09
GO.0031988	Extracellular exosome	25	1.11e-12
GO.0044421	Membrane-bounded vesicle	25	1.09e-10

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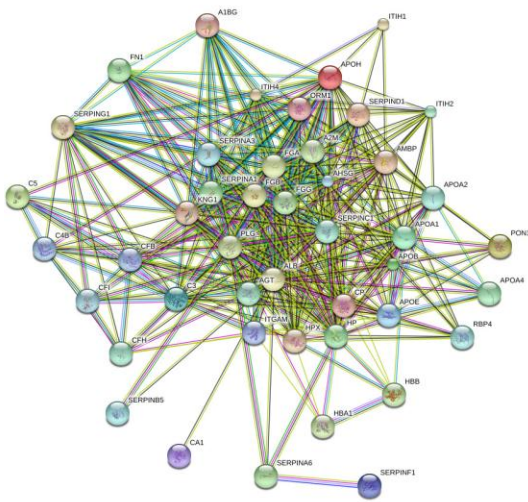
C.



D.

Pathway ID	Pathway description	Observed gene count	False discovery rate
I. Biological process			
GO.0002697	Regulation of immune effector process	10	9.91e-07
GO.0031639	Plasminogen activation	4	9.91e-07
GO.0034370	Triglyceride-rich lipoprotein particle remodeling	4	9.91e-07
GO.0034374	Low-density lipoprotein particle remodeling	4	9.91e-07
GO.0019216	Regulation of lipid metabolic process	8	9.53e-06
II. Molecular Function			
GO.0005319	Lipid transporter activity	6	9.06e-06
GO.0017127	Cholesterol transporter activity	5	7.23e-08
GO.0016209	Antioxidant activity	5	6.64e-05
GO.0004857	Enzyme inhibitor activity	19	4.76e-20
GO.0004867	Serine-type endopeptidase inhibitor activity	14	4.76e-20
III. Localization			
GO.0070062	Extracellular exosome	43	9.48e-34
GO.0034364	High-density lipoprotein particle	6	8.65e-10
GO.0005577	Fibrinogen complex	4	8.3e-08
GO.0005576	Extracellular region	43	7.68e-26
GO.0031091	Platelet alpha granule	10	6.88e-15

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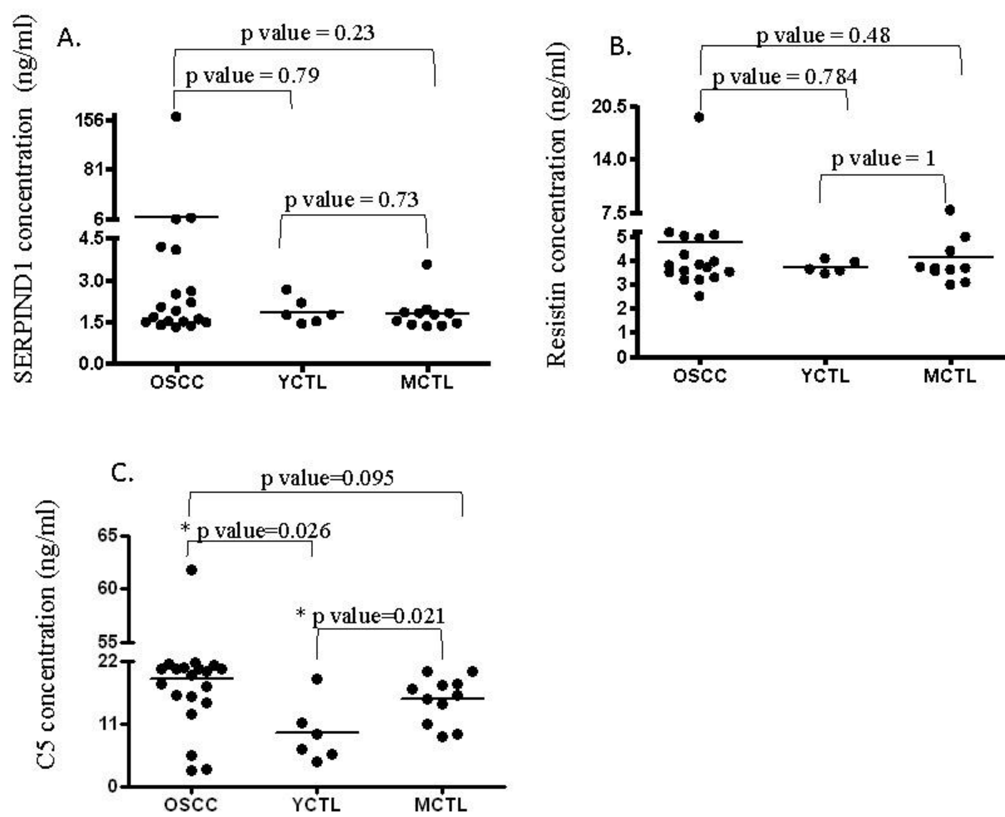
Figure 3.



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