2371

Contribution of GCF to the Saliva Proteome: Quantitative Proteomic Analysis

Andrew J. Creese[1], Melissa M. Grant[1], Helen J. Cooper[2], Michael Milward[1], Nicola Ling-Mountford[1], Upen Patel[3], Paul Weston[1], Marco de Jager[4], Iain L C. Chapple[1].

1. Periodontal research group, School of Dentistry, University of Birmingham, St Chads Queensway, Birmingham, B4 6NN, UK.
2. School of Biosciences, University of Birmingham, Birmingham, B15 2TT, UK.
3. Birmingham Dental School & Hospital, University of Birmingham, Edgbaston, Birmingham, B4 6NN, UK.
4. Clinical & Scientific Affairs, Philips Oral Healthcare, Snoqualmie, WA.

Overview

- Saliva is a complex biological fluid containing proteins derived from the salivary glands, gingival crevicular fluid (GCF), cell debris and bacteria. It is a rich source of biomarkers.
- Using bottom-up proteomics it is possible to identify and quantify thousands of peptides from hundreds of proteins from biological samples on a relatively short timescale.
- Here we ask: What is the contribution of GCF-derived proteins to the saliva proteome?

Introduction

- Saliva is a complex biological fluid with a large dynamic range. It is a rich source of protein biomarkers and collection is non-invasive. Currently the number of proteins in saliva which are derived from GCF is poorly defined.
- Tandem mass spectrometry is one of the most powerful tools for analysing complex protein mixtures. High mass accuracy and fast acquisition speed allow hundreds of peptides to be identified in less than an hour.
- Here we employ quantitative online data-dependent liquid chromatography LC-MS/MS analysis of saliva from healthy dentate and edentulous volunteers performed on a Thermo Fisher Scientific LTQ-Orbitrap Velos mass spectrometer.
- The results demonstrated that over 950 proteins were identified with 2 or more peptides. However, surprisingly none of the peptides were solely observed in the dentate sample.

Method

- Stimulated saliva was collected from 20 healthy volunteers: 10 dentate and 10 edentulous
- The samples were centrifuged for 10 minutes and the supernatant retained.
- 10 µL of each sample for dentate and edentulous was combined to give two 100 µL samples.
- The proteins in both samples were reduced and alkylated prior to overnight digestion with Lys-C and trypsin.
- The samples were labelled with two iTRAQ (ABSciex) isobaric tags from an 8-plex (115 and 118) and combined.
- The peptide mixture was fractionated by strong cation exchange (SCX) chromatography.
- SCX was performed with a Polysulfoethyl A column. Samples were eluted over a 60 minute salt gradient from 0-50% 500 mM KCl (pH 3). 16 fractions were collected.
- The fractions were loaded onto a 75 µm C18 reversed phase analytical column (LC Packings). Peptides were separated over a 30 minute gradient from 3.2 to 44% acetonitrile (0.1% formic acid).
- Samples were infused by use of an Advion Triversa Nanomate nanospray ionization source into a Thermo Fisher Scientific LTQ-Orbitrap Velos hybrid mass spectrometer.
- The mass spectrometer performed an initial high resolution survey scan, the three most intense multiply charged peptide ions were selected and fragmented by collision induced dissociation (CID) tandem mass spectrometry. The same three ions were then fragmented by higher energy collisional dissociation (HCD).
- Analysed peptides were placed on an exclusion list to avoid repeat analysis.
- Data were collected with Xcalibur 2.1 (Thermo Fisher Scientific) and analysed using the SEQUEST search algorithm and the IPI human database (v3.75) supplemented with known oral bacteria.
- The CID spectra were used to identify the peptides and the HCD spectra were used for quantification

Conclusions

- We have identified over 950 proteins from saliva with two or more peptides including 74 bacterial proteins (derived from *P.gingivalis* and *T.denticola*.)
- 97% of the proteins were identified in greater abundance from the healthy dentate saliva.
- After normalisation 35 proteins were identified with greater than 2 fold (log2) increase (dentate:edentulous). 29 proteins were identified with greater abundance in the edentulous.
- None of the proteins identified were only present in the dentate saliva. Several of the proteins identified were previously identified in GCF.

Results

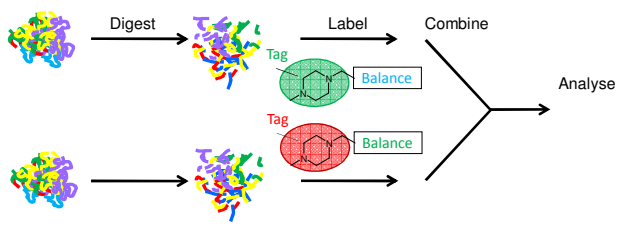

Figure 1: The analysis pathway for the saliva samples. Protein samples are digested, labelled with iTRAQ 115 and 118, combined and analysed with 2D LC-MS/MS

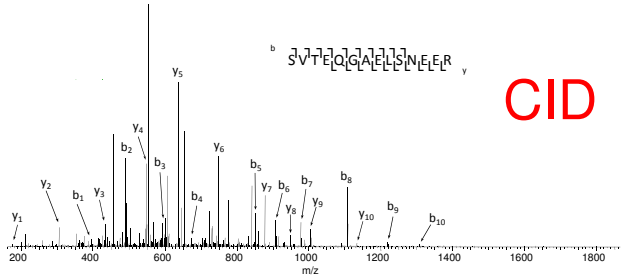

Figure 2: The collision induced dissociation (CID) mass spectrum of the triply charged peptide ion [iTRAQ-SVTEQGAELSNEER]$^{3+}$. Complete coverage is observed.

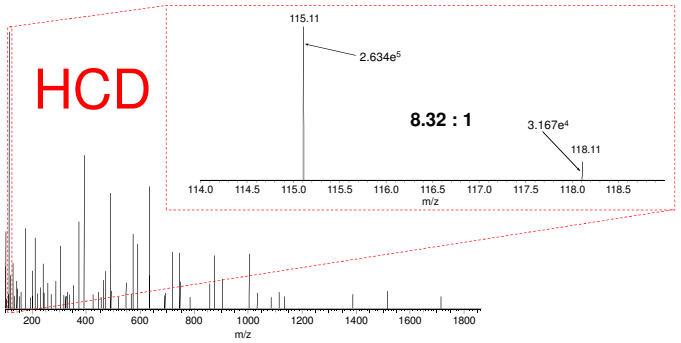

Figure 3: The Higher energy collisional dissociation (HCD) mass spectrum of the triply charged peptide ion [iTRAQ-SVTEQGAELSNEER]$^{3+}$. Inset is the mass region containing the iTRAQ labels.

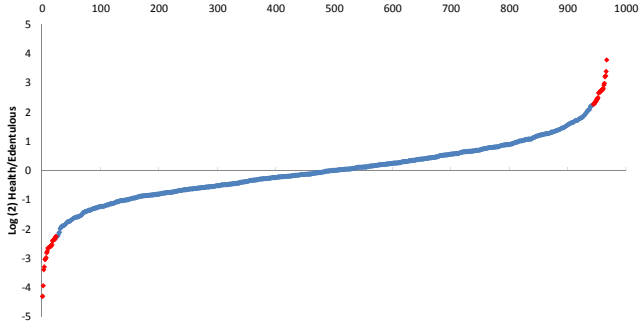

Figure 4: The normalized log(2) curve showing the ratios of proteins identified in dentate and edentulous saliva.

Proteomic analysis of dentate and edentulous saliva	
Number of CID events	97,659
Number of peptides identified	7,996
Number of human proteins identified	893
Number of bacterial proteins identified	74
Human proteins x2 upregulated (dentate/edentulous)	32
Bacterial proteins x2 upregulated (dentate/edentulous)	3

Table 1: The number of CID events, proteins and peptides identified from the LC-MS/MS analysis of dentate and edentulous saliva.

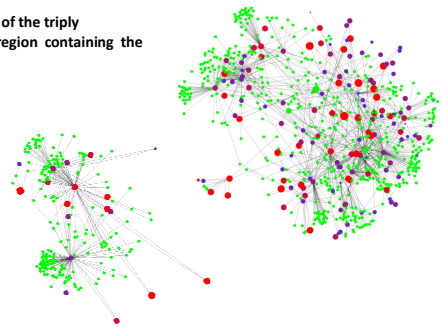

Figure 6: The protein-protein interaction maps for titin, top right (more abundant in edentulous) and neutrophil defensin 1 α, bottom left (more abundant in dentate saliva).

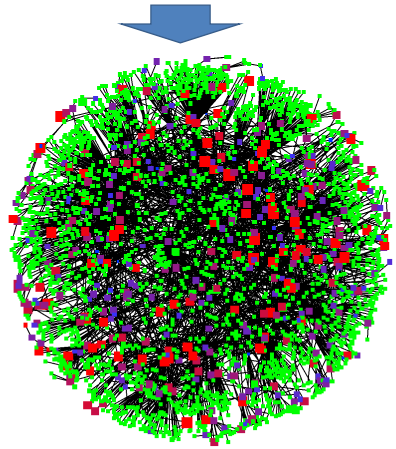

Figure 5: The protein-protein interaction map for the identified proteins.

With Thanks to Birmingham Science City Translational Medicine, Experimental Medicine Network of Excellence Project

Andrew Creese, Periodontal research group, School of Dentistry, University of Birmingham, St Chads Queensway, Birmingham, B4 6NN, UK. E-mail: a.creese.2@bham.ac.uk