P04.07: Investigating Key Host, Microbial and Variant Peptides for Detection of Oral Cancer using Advanced Multi-omics Methods.

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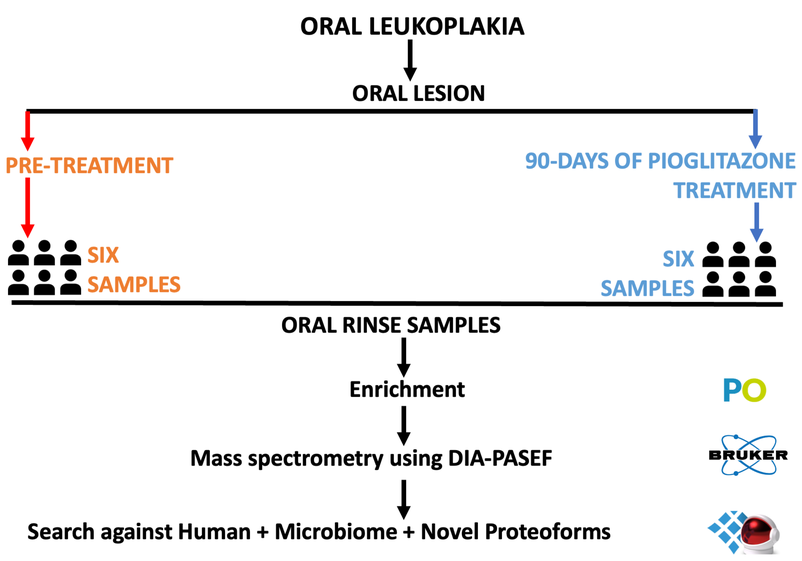
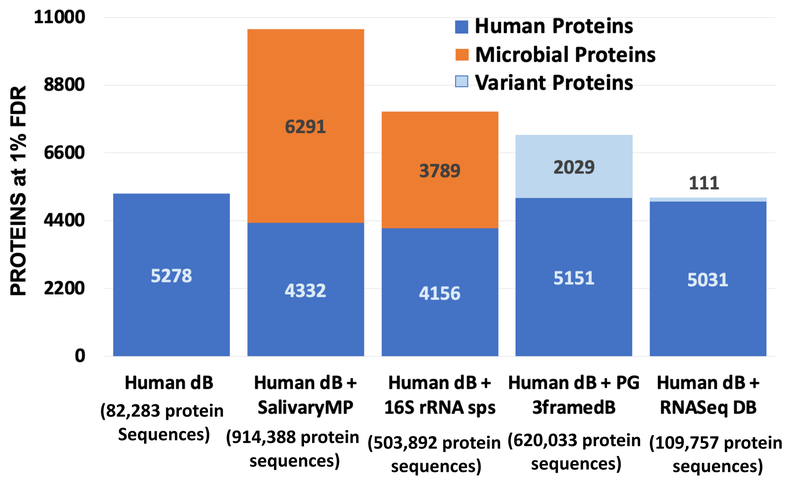
There is an unmet need for risk stratification biomarkers for precancerous oral leukoplakia patients. While host biomarkers are available for detection, there is a clear need to expand the biomarker panel to improve predictive performance. We have used the latest methods for enrichment of low-abundance proteins from non-invasively collected samples, sensitive mass spectrometry (MS) methods, and advanced bioinformatic analysis to delve deeper into host, variant and microbial proteome from precancerous patients.

METHODS: Oral rinse samples from six oral precancerous lesion patients (pre/post chemoprevention agent treatment) were processed using the PreOmics ENRICH kit to facilitate the detection of low abundance proteins. Digested proteins were analyzed by DIA-PASEF on a hybrid TIMS QTOF mass spectrometer (Bruker). 16S rRNA analysis and RNASeq analysis was used to generate a customized proteogenomics and metaproteomics database. The MS data was searched against the human proteome along with variant proteins and microbial proteins using Spectronaut (Biognosys). Bioinformatic and statistical analysis was performed to detect differentially expressed host proteins, microbial proteins and novel proteoforms.

EXPERIMENTAL WORKFLOW:

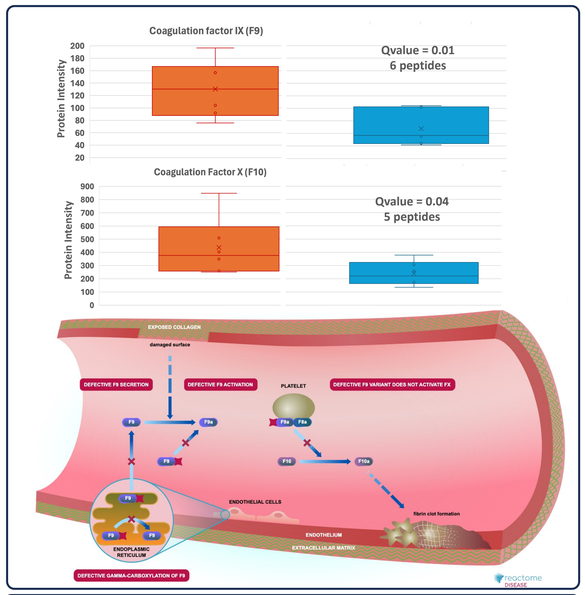


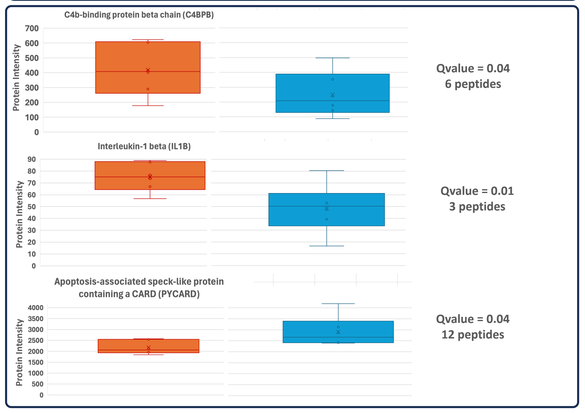
DATABASE SEARCH RESULTS:



HUMAN PROTEINS

- 34 HUMAN PROTEINS WITH HIGHER ABUNDANCE IN PRETREATMENT SAMPLES.
- 51 HUMAN PROTEINS WITH HIGHER ABUNDANCE IN TREATED SAMPLES.



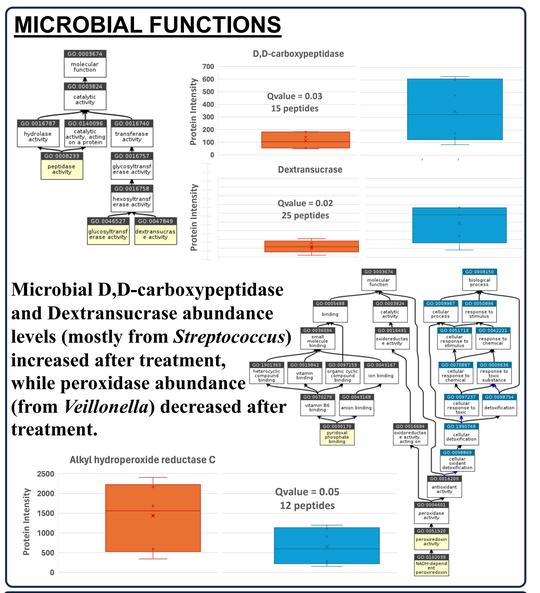


- UP-REGULATED CANDIDATE PEPTIDES: 80
- DOWN-REGULATED CANDIDATE PEPTIDES: 47

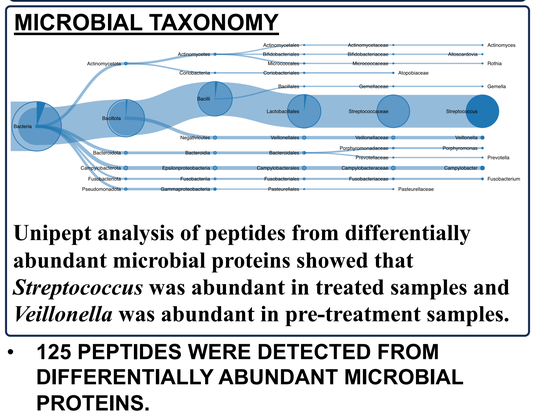
MICROBIAL PROTEINS

- 6 MICROBIAL PROTEINS WITH HIGHER ABUNDANCE IN PRETREATMENT SAMPLES.
- 16 MICROBIAL PROTEINS WITH HIGHER ABUNDANCE IN TREATED SAMPLES.

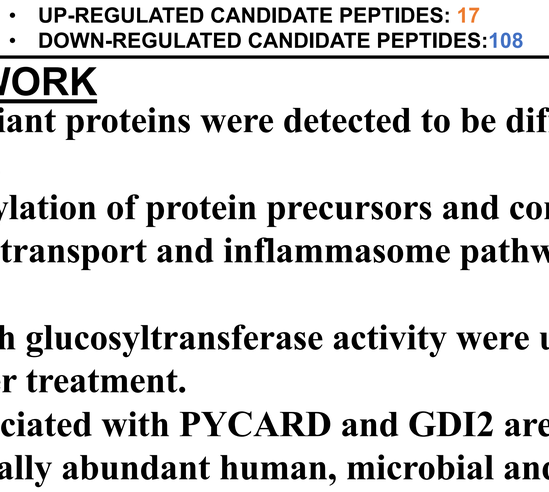
MICROBIAL FUNCTIONS



Microbial D,D-carboxypeptidase and Dextransucrase abundance levels (mostly from *Streptococcus*) increased after treatment, while peroxidase abundance (from *Veillonella*) decreased after treatment.



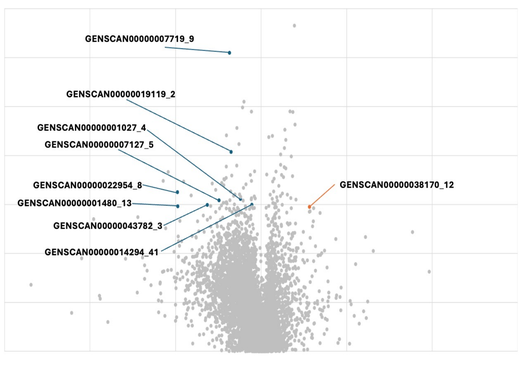
MICROBIAL TAXONOMY



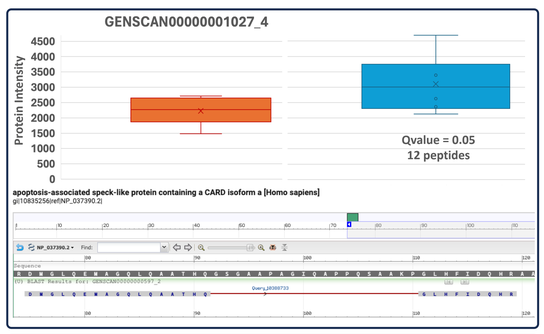
Unipept analysis of peptides from differentially abundant microbial proteins showed that *Streptococcus* was abundant in treated samples and *Veillonella* was abundant in pre-treatment samples.

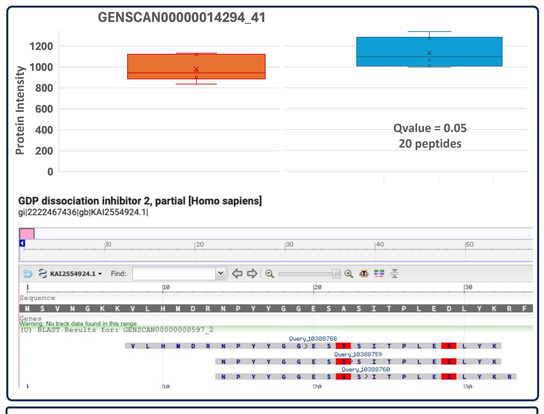
- 125 PEPTIDES WERE DETECTED FROM DIFFERENTIALLY ABUNDANT MICROBIAL PROTEINS.
- UP-REGULATED CANDIDATE PEPTIDES: 17
- DOWN-REGULATED CANDIDATE PEPTIDES:108

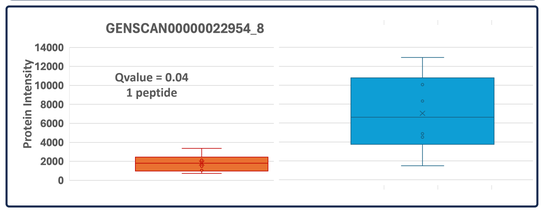
NOVEL PROTEOFORMS



- 6 VARIANT PROTEINS WITH HIGHER ABUNDANCE IN PRETREATMENT SAMPLES.
- 24 VARIANT PROTEINS WITH HIGHER ABUNDANCE IN TREATED SAMPLES.







- 80 PEPTIDES WERE DETECTED AS PEPTIDES CORRESPONDING TO NOVEL PROTEOFORMS.
- UP-REGULATED CANDIDATE PEPTIDES: 21
- DOWN-REGULATED CANDIDATE PEPTIDES: 59

CONCLUSIONS AND FUTURE WORK

- Several human, microbial and variant proteins were detected to be differentially abundant in pretreatment and treated samples.
- Pathways such as Gamma-carboxylation of protein precursors and complement cascade were upregulated and Vesicle-mediated transport and inflammasome pathways were downregulated after treatment.
- Microbial functions associated with glucosyltransferase activity were upregulated and oxidative stress functions were downregulated after treatment.
- Variant proteins and peptides associated with PYCARD and GDI2 are upregulated after treatment.
- Peptides associated with differentially abundant human, microbial and variant proteins will be used for targeted analysis.