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Introduction

Our team has demonstrated that lymphatic exudate collected via surgical drains ("lymph") is a proximal biofluid rich in ctDNA and prognostic of recurrence in HPV-independent Head and Neck Cancer (HPV- HNSCC). Other analytes such as proteins, metabolites, and cells are also abundant in lymph. To understand the liquid tumor microenvironment (TME) immediately after tumor resection, we characterized lymph at 24 hrs in HNSCC. We performed a multi-omic survey, demonstrating that lymph is distinct from plasma and represents a largely unexplored source of biomarkers relevant to immune modulation and metastasis.

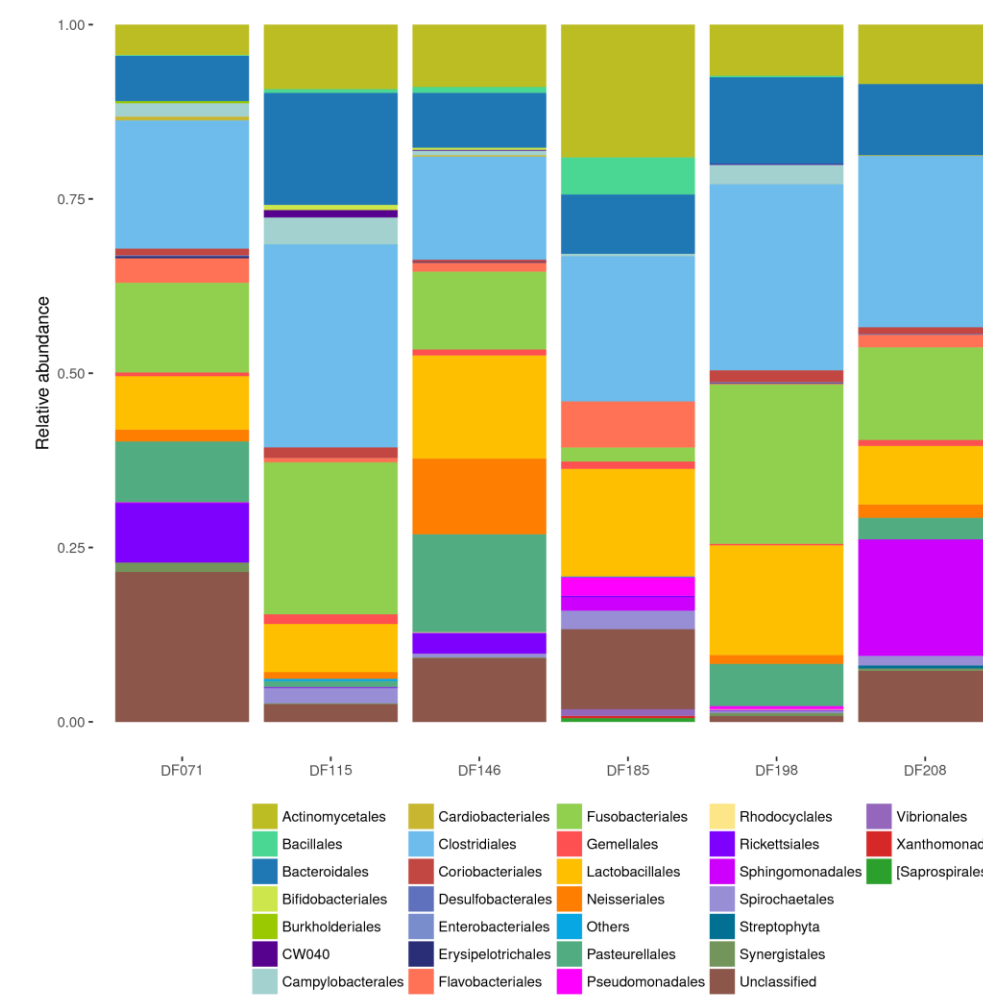
Methods and Materials



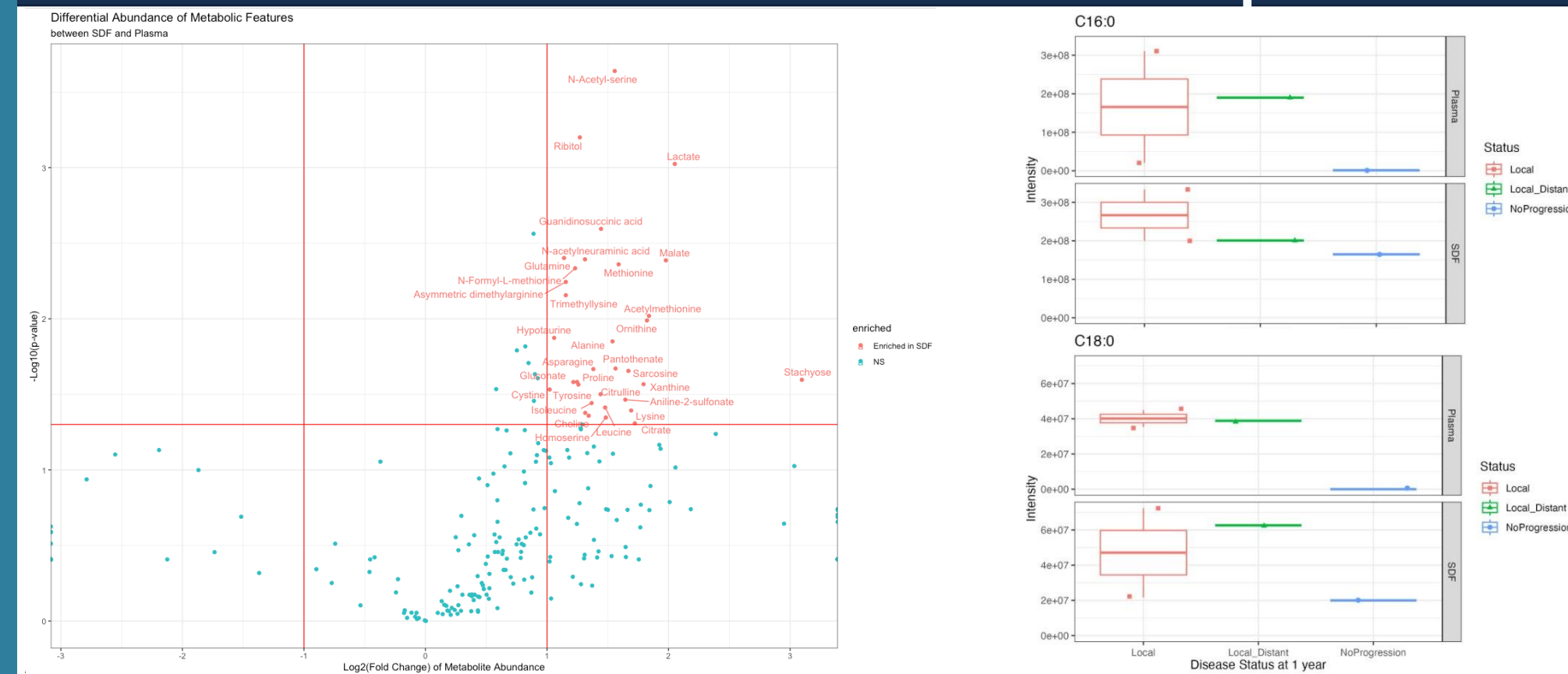
Metabolomics and proteomics were run on lymph supernatant and plasma. Proteomic screening was performed via Olink and normalized to internal standards for quantitative analysis. Metabolomic samples were assayed via LC-MS and normalized. 16s libraries were sequenced utilizing Illumina and analyzed in QIIME.

Microbiome of the Head and Neck Surgical Drain

- 16s sequencing on 6 patients indicated an array of bacterial genera present in post-surgical lymph with consistency across patients.
- Distribution consisted primarily of microbes native to the oral cavity, suggesting post-surgical lymph may reflect the native microbiome prior to surgery.
- Pathogenic blooms were observed in a subset of patients.

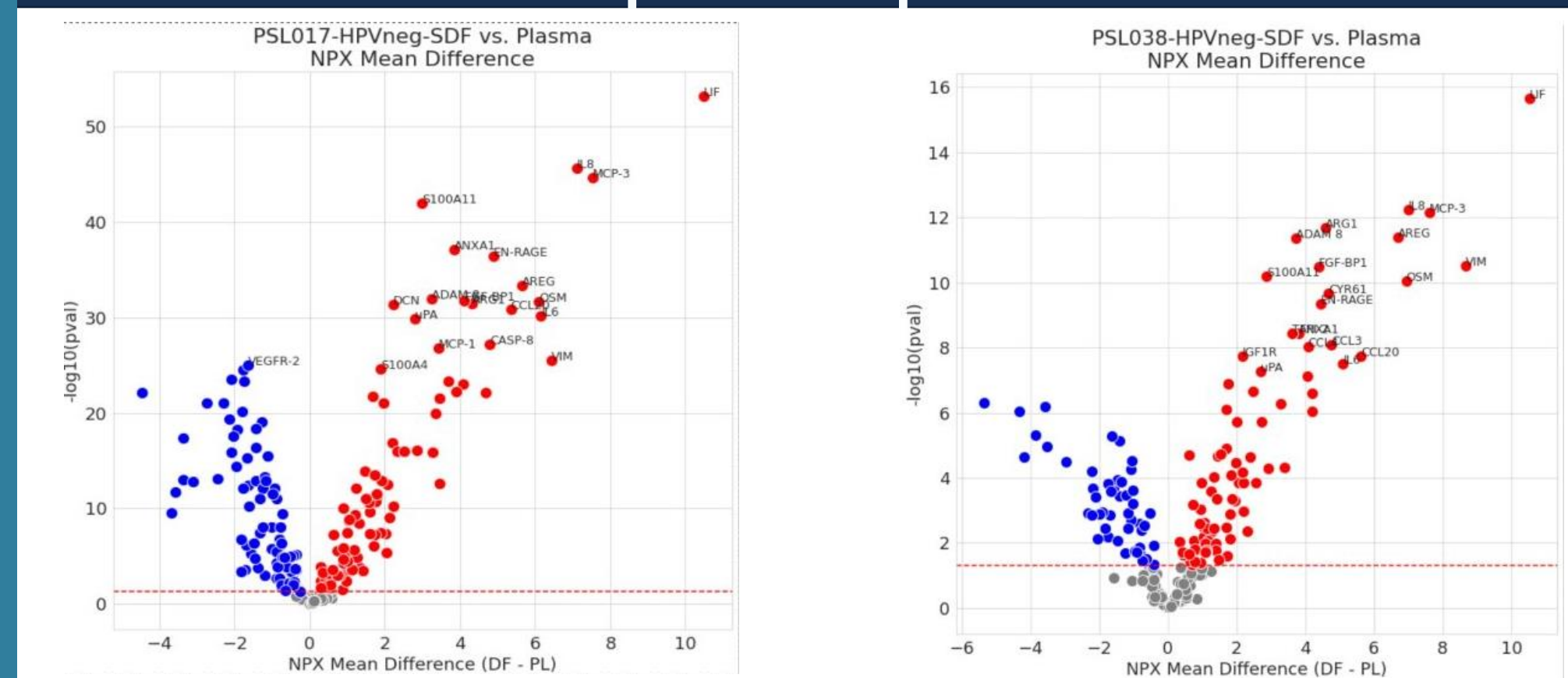


Post-surgical lymphatic exudate is more metabolite-rich than time-matched plasma



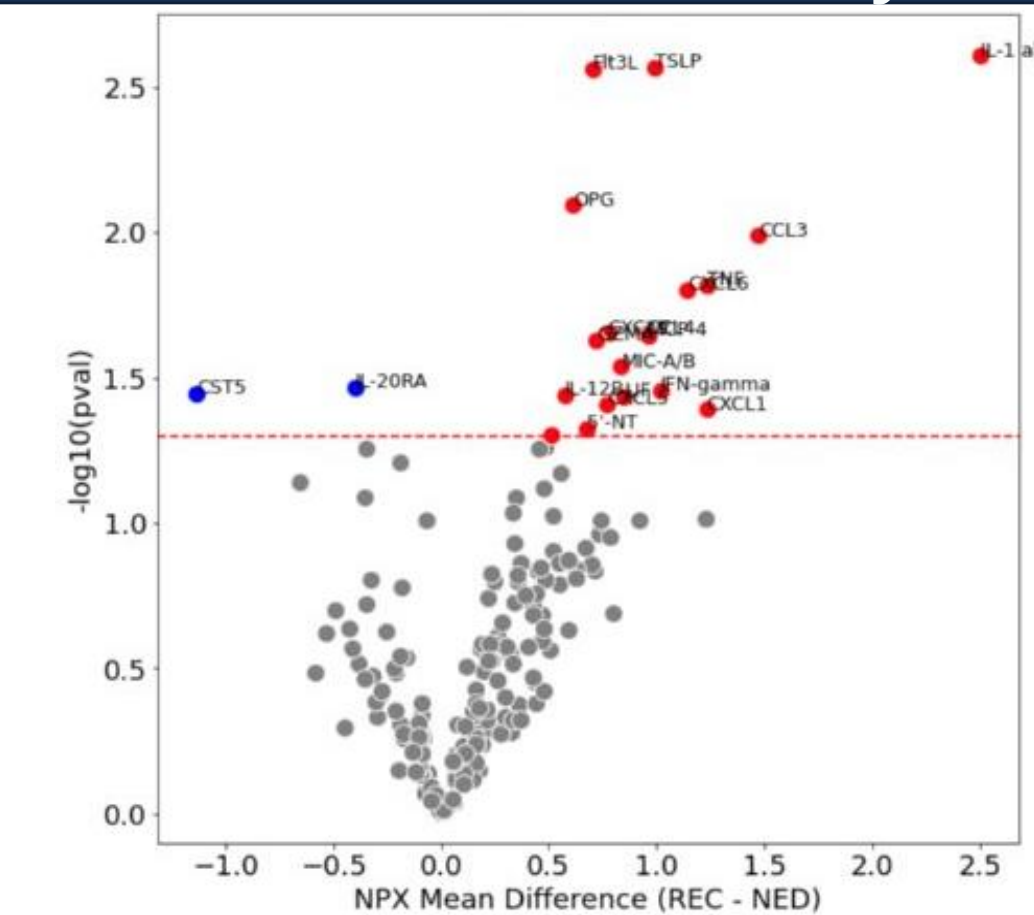
Metabolite profiling was performed on post-surgical lymph and time-matched plasma from 4 patients, including 3 who recurred within 1 year of surgery. The metabolite profile in lymph of all 4 patients was more diverse than plasma with higher metabolite abundance per mL. 13% of identified metabolites were >2-fold enriched in lymph. Lymph from recurred patients showed high levels of saturated fatty acids, reported to be associated with metastasis. Post-surgical lymph has a unique and largely uncharted metabolome to mine for biomarkers associated with recurrence.

Lymphatic exudate is enriched for proteins compared to plasma



Proteomes were compared between 24 hour post-op lymph and plasma from 44 HPV-HNSCC patients. This cohort consists of 25 recurred (REC) and 19 patients with no evidence of disease (NED) with >1 year of follow up. Lymph and plasma displayed highly non-overlapping protein repertoires. The mean absolute volcano score was 25.2, indicating a strong global difference between lymph and plasma. Lymph was significantly enriched in 91 proteins (p range 4.1e-47 to 0.037), many associated with inflammation.

Lymphatic exudate from recurred patients is enriched for inflammatory markers



REC lymph was also enriched in inflammatory markers compared to NED, including IL-1a, TNF, and TSLP (p = 0.002, 0.015, 0.003, respectively), which have been implicated in cancer metastasis, invasion, and angiogenesis. Lymph hits were largely non-overlapping with plasma, indicating the opportunity for novel biomarker discovery in this fluid.

Conclusions

- Lymphatic exudate is a rich proximal biofluid
- Microbial signatures are unique to each patient showing a variety of species native to the oropharynx
- 16s sequencing of surgical exudate could help screen for sepsis
- Lymphatic exudate is more metabolically active than blood or plasma and could serve as a rich source for biomarker discovery
- Proteomic signatures are divergent between patients that recurred and showed no evidence of disease after 1 year of follow up.
- Post surgical lymphatic exudate provides a novel window into the evolution of the tumor microenvironment and wound healing post surgery

Future Work

Continued study of the tumor microenvironment and development of novel recurrence biomarkers from postoperative lymphatic exudate

More Droplet Information

Please contact Wendy Winckler: wwinckler@dropletbiosci.com

Please visit posters:

#814 Spatial Protein Profiling and Multi-Modal Mapping of Tumor and Circulating Ecosystems

#2604 Postoperative lymphatic exudate as a proximal liquid biopsy source in muscle-invasive bladder cancer

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#7833 A novel extraction method for enrichment of circulating cell-free DNA

Thank you