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dia-PASEF Proteomic Analysis of HNSCC Tumor and Stroma Enriched Sections from FFPE Samples Prepared with Laser Capture Microdissection

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Introduction

Head and neck squamous cell carcinoma (HNSCC), an epithelial cancer is the most common type of head and neck cancer. HNSCC cells first invade the basement membrane of the native epithelium, and in >50% cases proceed to lymph node metastasis, which is associated with poor survival. Overall, the response to available treatments has been moderate. The genomic and transcriptomic landscape of HNSCC (The Cancer Genome Atlas) has been defined, but pinpointing the genetic aberrations linked to tumor phenotypes remains elusive. Here we performed deep proteome analysis of tumor and matched normal adjacent tissues (NATs) (Clinical Proteomic Tumor Analysis Consortium). The proteomic comparison of the cancer cells and its neighboring microenvironment may help identify novel targets for early detection, and intervention of HNSCC.

Methods

In this study, laser capture microdissection (LCM) was used to collect tumor and stroma enriched sections from formalin-fixed paraffin-embedded (FFPE) tissues. The samples were processed and digested with trypsin. dia-PASEF LC-MS/MS analysis was performed using the timsTOF HT mass spectrometer connected to a nanoElute 2 LC system via a CaptiveSpray 2 source. Each sample was analyzed in triplicate using a 32-minute gradient (500 ng peptide per injection, 40 min total run time on a 25 cm Aurora Ultimate 25cm x 75µm C18 column), resulting in a throughput of 24 samples per day. The dia-PASEF window scheme was calculated using the py_diAID tool developed by the Mann Lab (ref 1). Data analysis was performed using the directDIA+ workflow (Spectronaut 18 software) and the Uniprot-Human-reviewed database (20,383 protein entries).

Ref 1. https://github.com/MannLabs/pydiaid

Results

- Replicate injections of tumor and stroma sample showed excellent chromatographic reproducibility (Figure 1) with CVs under 10% at the protein level.

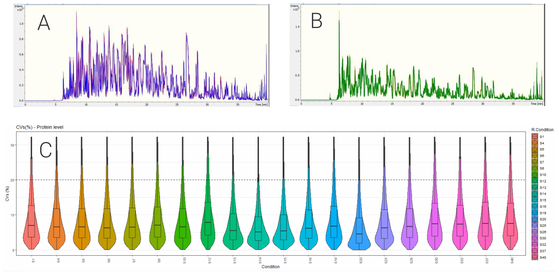

Fig. 1. Highly reproducible analysis. A and B show the overlap of three replicate injections of tumor and stroma samples. Median CVs of quantified protein groups were consistently under 10% (C).

- >8800 protein groups were identified in tumor tissue from over 106,000 peptides. Almost 7800 of the identified proteins were identified with at least two peptides.
- >8700 proteins were identified from the stroma from over 103,000 peptides

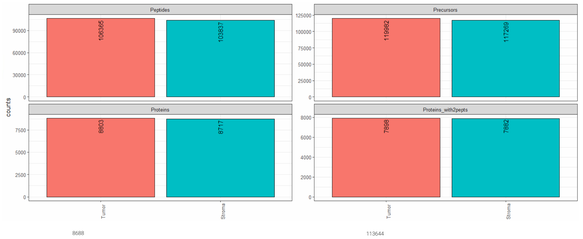

Fig. 2 More than 8800 proteins groups were identified in tumor samples and >8700 in stroma samples. >8600 proteins and >113,000 precursors were identified across all samples.

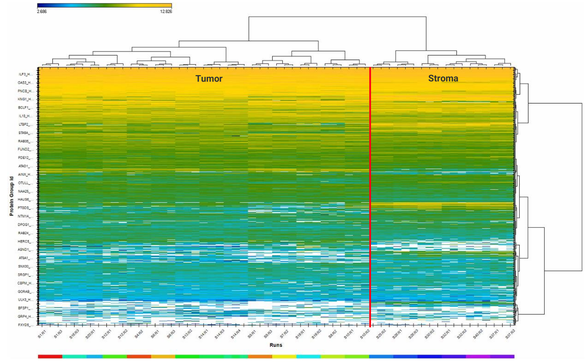

Fig. 3. All stroma samples clustered together and are clearly separated from the tumor group by hierarchical analysis.

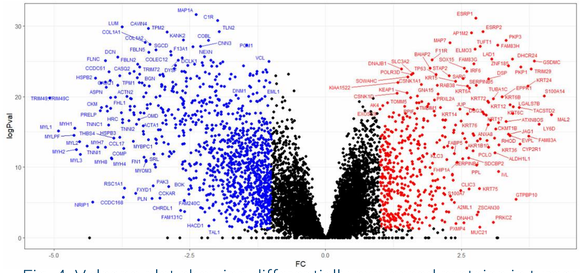

Fig. 4. Volcano plot showing differentially expressed proteins in tumor vs stroma samples.

GO Enrichment: Function

#term ID	term description	genes mapped	enrichment score	direction	FDR
GO:0031014	Troponin T binding	3	7.57764	stroma	0.00098
GO:0086083	Cell adhesive protein binding involved in bundle of His cell-Purkinje myocyte communication	5	5.4257	tumor	0.004
GO:0048407	Platelet-derived growth factor binding	9	5.11285	stroma	4.44E-05
GO:0032036	Myosin heavy chain binding	7	5.06376	stroma	0.00086
GO:0030280	Structural constituent of skin epidermis	24	4.88931	tumor	1.39E-06
GO:0086080	Protein binding involved in heterotypic cell-cell adhesion	7	4.79961	tumor	0.0022
GO:0097493	Structural molecule activity conferring elasticity	6	4.77105	stroma	0.0045
GO:0030021	Extracellular matrix structural constituent conferring compression resistance	8	4.72388	both ends	0.00019
GO:0051371	Muscle alpha-actinin binding	10	4.52576	stroma	0.0002
GO:0008307	Structural constituent of muscle	37	4.49102	stroma	1.84E-07
GO:0019215	Intermediate filament binding	10	4.47859	both ends	5.25E-05
GO:0031432	Titin binding	8	4.2018	stroma	0.0032
GO:0030020	Extracellular matrix structural constituent conferring tensile strength	19	3.97991	stroma	4.32E-06
GO:0051393	Alpha-actinin binding	14	3.97488	stroma	8.79E-05
GO:0005201	Extracellular matrix structural constituent	74	3.72914	stroma	1.61E-22
GO:0042805	Actinin binding	18	3.42854	stroma	0.00016
GO:0005518	Collagen binding	41	3.41049	stroma	5.78E-08
GO:0008201	Heparin binding	58	2.98938	stroma	8.20E-09
GO:0005539	Glycosaminoglycan binding	81	2.90727	stroma	9.70E-13
GO:0043394	Proteoglycan binding	20	2.77001	stroma	0.0022
GO:0001968	Fibronectin binding	20	2.41146	stroma	0.0093
GO:0043021	Ribonucleoprotein complex binding	24	2.23986	tumor	4.53E-05
GO:0048306	Calcium-dependent protein binding	29	2.18727	both ends	0.0031
GO:0004386	Helicase activity	31	2.06666	tumor	0.00016
GO:1901681	Sulfur compound binding	83	2.03762	stroma	6.06E-06

Fig. 5. GO functional and pathway enrichment analysis

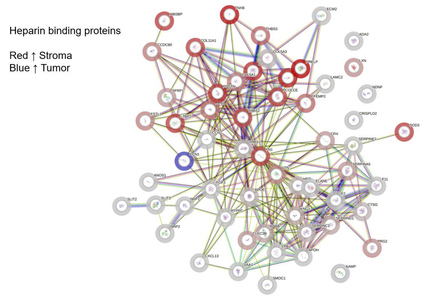

Fig. 6. Protein–protein interaction analysis using the STRING database (https://string-db.org/).

Summary

- > 8,800 protein groups and >100,000 peptides were identified in 32 minutes gradient time on the timsTOF HT.
- More than 8,600 protein groups were identified in all conditions (stroma and tumor).
- GO functional and pathway enrichment analysis of these proteins identified several functional groups relevant to stromal and tumor regions, e.g., higher abundance of growth factor binding, collagen binding, heparin binding proteins, and ECM structural constituents in the stromal region

Conclusion

- dia-PASEF acquisition on the timsTOF HT allows high throughput analysis of FFPE tissue samples with high depth of coverage.
- The methodology allows for comparative deep proteome analysis of tumor and its adjacent microenvironment in a scalable format.

Technology

Diego Assis and Matthew Willetts are current employees of Bruker Daltonics