Research Unit Radiation Cytogenetics, Helmholtz Zentrum München

Clinical Cooperation Group Personalized Radiotherapy in Head and Neck Cancer

MALDI MSI analysis enables a spatially resolved characterization of the proteome and metabolome in radiotherapy treated HNSCC

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Motivation

Head and neck squamous cell carcinoma (HNSCC) is known as a heterogeneous disease, mainly separated into two subgroups – human papilloma virus (HPV)-driven tumors and HPV-negative tumors that are caused by alcohol abuse and heavy smoking. There is a distinct inter-tumor and intra-tumor heterogeneity of both tumor entities, that affect therapy resistance of tumor cell populations as well as targeted therapies with drugs. As a consequence, there is a great need for an in-depth analysis of the molecular variances between HPV positive (HPV^+) and HPV negative (HPV^-) tumors.

For this purpose, we characterized a prospective cohort of HNSCC patients (n = 65) treated with surgery and/or radio(chemo)therapy with known HPV status for proteome and metabolome changes by matrix-assisted laser desorption ionization time of flight mass spectrometry imaging (MALDI TOF MSI). The resulting profiles of altered mass species were analyzed for their association with the HPV status and differences in heterogeneity between HPV positive and negative tumors.

For further integration of different omics levels, a proof-of-principle study has been performed on three HNSCC cases by combining MALDI-MSI analysis (metabolic changes) and next generation sequencing (genomic and transcriptomic changes) on the same tissue section. The successful combination of the different omics approaches allowed a comprehensive characterization of heterogeneity in HNSCC and thereby contributes to an improved identification of prognostic biomarkers and druggable targets.

Research question

Can specific molecular patterns be linked to the HPV status of HNSCC?

Molecular signatures
- Metabolomic patterns
- Proteomic patterns

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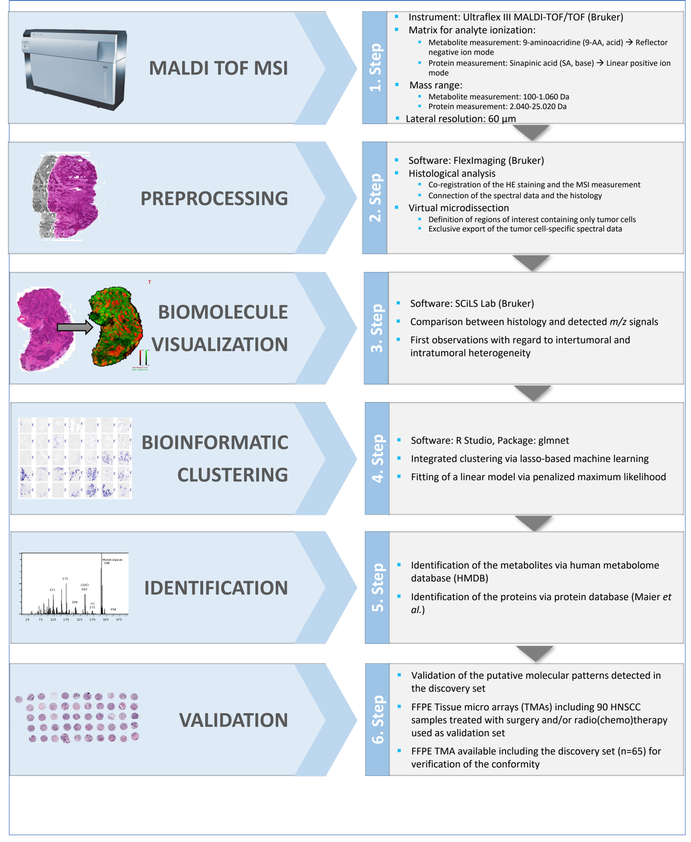
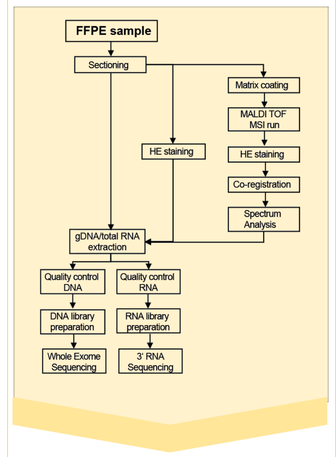
HPV status
- HPV positive
- HPV negative

Workflow

Metabolic HPV signature



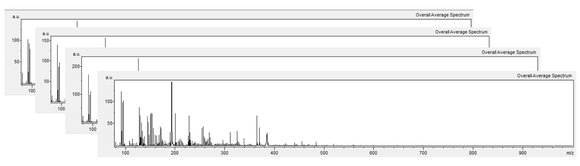
Multiomic analysis



PROOF-OF-PRINCIPLE
- The FFPE samples are sectioned, and processed in three different approaches
- Approach 1: Direct extraction of gDNA and total RNA following the sectioning (→ unprocessed = UP)
- Approach 2: Stained with hematoxylin and eosin following the sectioning, extraction afterwards (→ HE)
- Approach 3: MALDI MSI with an additional HE staining is performed to follow the regular procedure of an MSI experiment (→ MSI)
- The same tissues are than used for an extraction of the gDNA and total RNA
- Afterwards, the integrity of the nucleic acids is investigated, the libraries are prepared and the samples are sequenced respectively

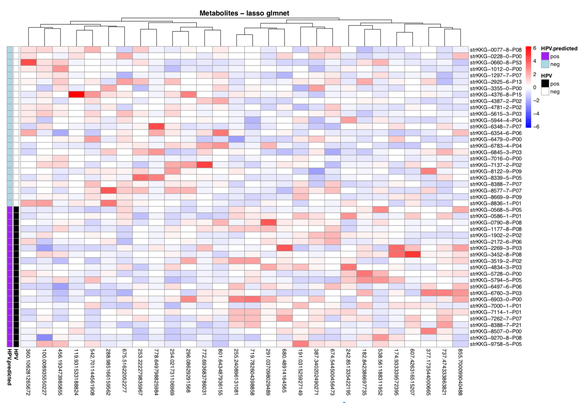
Multilevel analysis of HNSCC

28 metabolite signature predicts HPV status



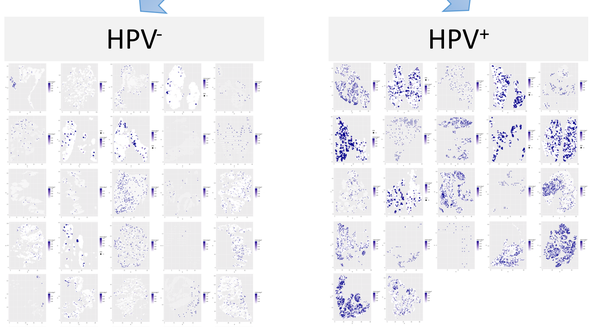
imzML export of the average spectra of each patient sample

lasso-based glmnet machine learning approach



28 metabolite HPV signature reveals 100 % correct prediction of the HPV status

HPV risk score calculation for each spectrum of single data points

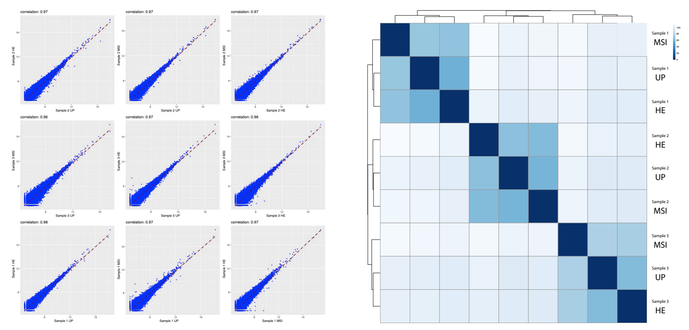


Significantly increased HPV risk scores in HPV^+ tumors in comparison to HPV^- tumors

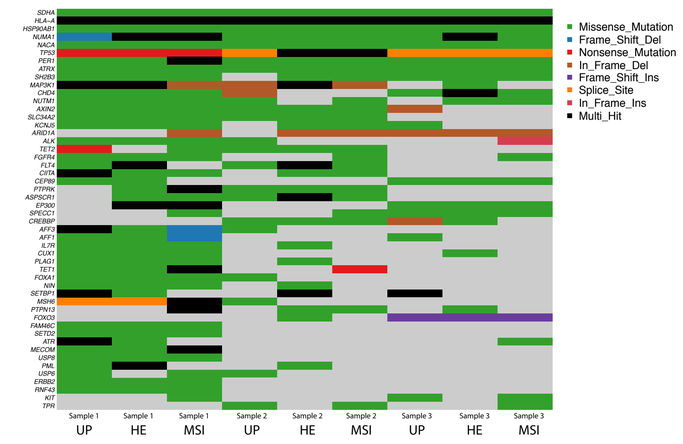
Multiomic analysis

MALDI MSI performance do not show significant influence on the exome or the transcriptome of HNSCC

HNSCC transcriptome profiles



HNSCC exome profiles



Further observations
- The amount of nucleic acid from a single tissue section of the MALDI MSI measurement is sufficient to perform genomic and transcriptomic analyzes
- This method combination can also be successfully performed on FFPE tissues

Future directions

- Which pathways are relevant for HPV^+ and HPV^- tumors?
- Which impact has the HPV status on the clinical outcome?
- Can therapy success be predicted by metabolic or proteomic signatures?
- How beneficial are HPV signature-based heterogeneity analyses?
- What degree of consistence do the different molecular levels show?

Poster QR code

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