Proteomic profiling of IDH-mutant gliomas enables prediction of chromosomal copy number variations

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Introduction

Recurrent chromosomal copy number variations (CNV) are hallmarks of different types of brain tumors. Status determination is an integral part of WHO classification. There is need for a better understanding of the consequences of gains or deletions involving whole chromosomal arms. A prominent example are IDH-mutant gliomas which are separated in two distinct types based on the deletion of chromosomal arms 1p and 19q. Oligodendrogliomas IDH-mutant are 1p/19q co-deleted while astrocytomas IDH-mutant are not. Therefore, determination of 1p/19q is important for prognosis and therapy.

Methods

35 FF (20 oligodendroglioma and 15 astrocytoma) samples were homogenized, lyzed with SDS and proteins were extracted with acetone precipitation. 1-1.5 mm punches were obtained from tumor regions of FFPE tissue (35 oligodendroglioma and 37 astrocytoma) und subjected to deparaffination and subsequent pressure lysis. All samples underwent tryptic digestion under pressure cycling in a Barocycler 2320EXT (Pressure Biosciences) and were analyzed using an Easy nLC 1200 (Thermo Fischer Scientific) coupled a high-resolution TIMS-QTOF (timsTOF Pro, Bruker Daltonics) with a CaptiveSpray ion source (Bruker Daltonics). The peptide mixtures (500 ng) were loaded onto a 50 cm home-packed reversed-phase pulled emitter column and separated using a linear gradient from 7.5 to 27.5% B (80/20/0.1% ACN/water/FA) within 60 min, followed by an increase to 37.5% B within 30 min and further to 55% within 10 min at a flow rate of 400 nl/min. LC-MS/MS data were acquired in PASEF mode of one TIMS MS scan followed by 10 PASEF MS/MS scans with 50 ms ramp time. Data analysis was carried out using MaxQuant version 1.6.17.0 (Jürgen Cox, Max Planck Institute of Biochemistry).

Results

134 and 214 significantly differentially regulated proteins (DRP), were identified from fresh frozen (FF) and formalin-fixed paraffin-embedded (FFPE) tumor tissue from astrocytoma and oligodendroglioma.
Data obtained from both tissue types shows good correlation and 54 commonly DRP.
Hierarchical clustering shows differentiation between both IDH-mutants in both tissue types and shows promising biomarker candidates.

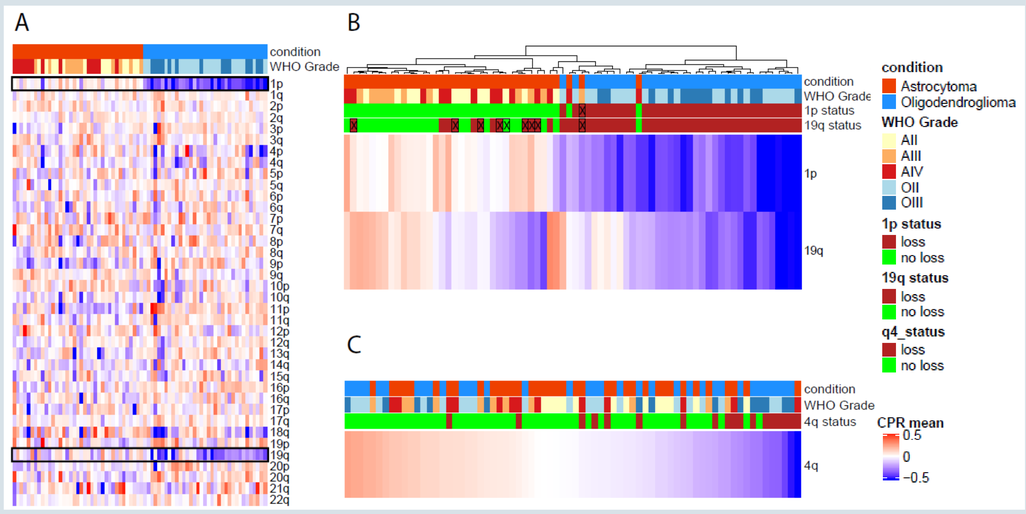

Figure 2: Analyses of chromosomal wide protein abundances in FFPE. A: Heatmap showing CPR-means across all chromosome arms, manually sorted by astrocytoma (red) and oligodendroglioma (blue); B: Hierarchical clustering using CPR-means of chromosome arms 1p and 19q. C: Hierarchical clustering of the FFPE tissue cohort using CPR-means of chromosome arm 4q. Ratios are sorted from high to low from left to right. Likelihood of a q4 loss status rises with lower CPRs and is independent from tumor diagnosis.

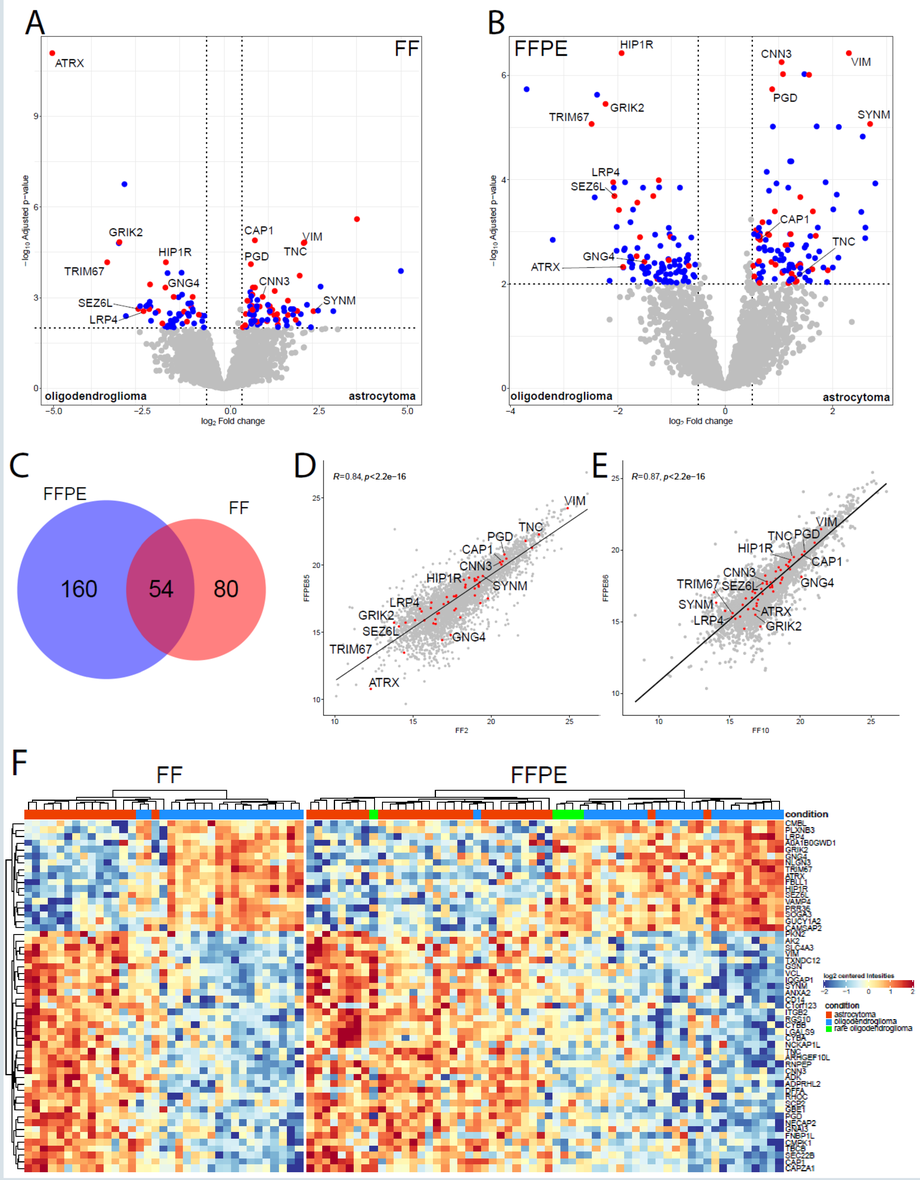

Figure 1: Differential protein analysis of astrocytoma and oligodendroglioma. Volcano plot of significantly DRP between astrocytoma and oligodendroglioma in FF (A) and FFPE (B) tissue. Blue dots represent proteins found exclusively in either, red dots represent proteins found in both FF and FFPE. C: Number of significantly DRP in FF and FFPE tissue. Pearson correlation analyses between a matched pairs of FF and FFPE of an astrocytoma (D) and oligodendroglioma (E). Hierarchical clustering of the FF and FFPE cohorts using the overlapping significantly DEP between astrocytoma and oligodendroglioma (F). Rare oligodendroglioma (condition green): 1p/19q co-deleted tumors with the highest brain tumor classifier score for "high grade astrocytoma, IDH-mutant".

Virtual copy number variation plots from the proteomic profile which we termed chromosomal protein ratio plots (CPRP) were generated and highly correlate with CNV plots from genome wide DNA methylation profiles.

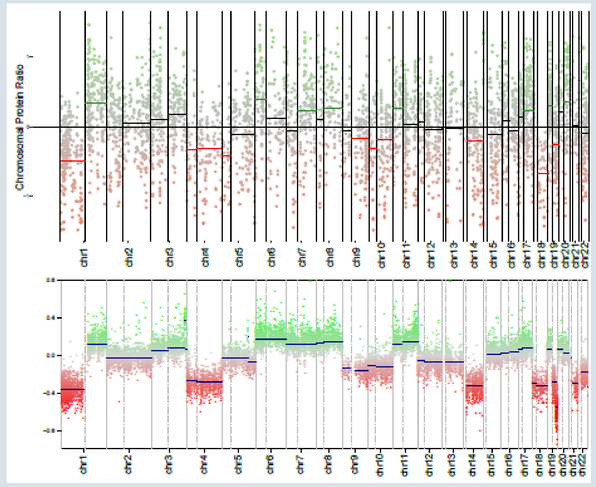

Figure 3: Segment plots from an oligodendroglioma (FFPE tissue): Top: Segment plot of CPRs derived from the proteomic data; bottom: CNV-Segment plot derived from DNA-methylation data. Chromosome arms are numerally ordered from left to right, chromosome arms p and q are divided by a dotted line for each chromosome. Horizontal lines represent CPR-means for proteins. Gains (green) and losses (red) exceeding the chromosome arm specific threshold are marked by lines.

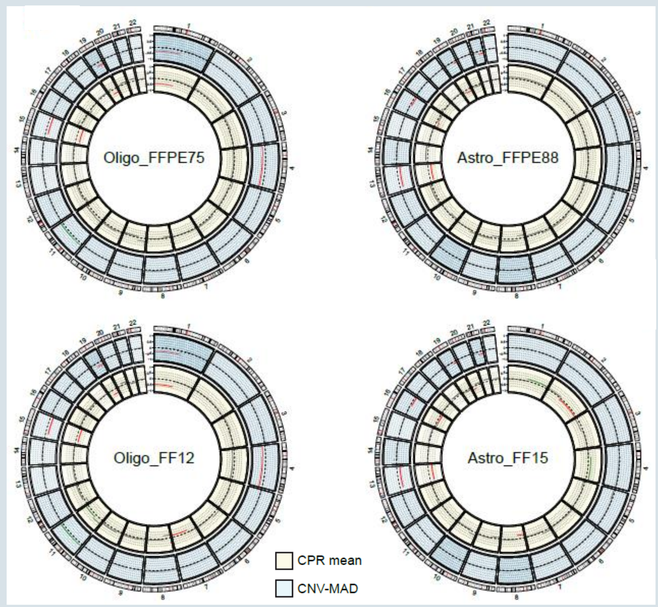

Figure 4: Chromosomal Protein Ratio Plot-CPRP. Circos-plots of FF/FFPE sample pairs of astrocytoma (right) and oligodendroglioma (left). Outer ring depicts chromosome orientation with their corresponding numbers, red lines mark the transition from p and q arms. Middle blue ring represents CNV-status. Inner yellow ring represents CPR-means. Gains and losses exceeding the chromosome arm specific thresholds on CNV and CPR-mean level are marked through green (gain) and red (loss) lines.

Conclusions

- **dda-PASEF based analysis FFPE tissue highly correlates with FF tissue allowing in depth differential proteomic profiling enabling the discovery of potential new biomarkers**
- **CPRP is a promising tool for the differentiation of tumors based on chromosomal copy number variation**

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