UKE Paper of the Month April 2021

Genome-wide methylation of glioblastoma cell-derived extracellular vesicle DNA allows tumor classification

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ABSTRACT:

Background: Genome-wide DNA methylation profiling has recently been developed into a tool that allows tumor classification in central nervous system tumors. Extracellular vesicles (EVs) are released by tumor cells and contain high molecular weight DNA, rendering EVs a potential biomarker source to identify tumor subgroups, stratify patients and monitor therapy by liquid biopsy. We investigated whether the DNA in glioblastoma cell-derived EVs reflects genome-wide tumor methylation and mutational profiles and allows non-invasive tumor subtype classification.

Methods: DNA was isolated from EVs secreted by glioblastoma cells as well as from matching cultured cells and tumors. EV-DNA was localized and quantified by direct stochastic optical reconstruction microscopy. Methylation and copy number profiling was performed using 850k arrays. Mutations were identified by targeted gene panel sequencing. Proteins were differentially quantified by mass spectrometric proteomics.

Results: Genome-wide methylation profiling of glioblastoma-derived EVs correctly identified the methylation class of the parental cells and original tumors, including the MGMT promoter methylation status. Tumor-specific mutations and copy number variations (CNV) were detected in EV-DNA with high accuracy. Different EV isolation techniques did not affect the methylation profiling and CNV results. DNA was present inside EVs and on the EV surface. Proteome analysis did not allow specific tumor identification or classification but identified tumor-associated proteins that could potentially be useful for enriching tumor-derived circulating EVs from biofluids.

Conclusions: This study provides proof of principle that EV-DNA reflects the genome-wide methylation, CNV and mutational status of glioblastoma cells and enables their molecular classification.

STATEMENT:

In this project we show for the first time that extracellular vesicle DNA enables non-invasive brain tumor classification and that glioma associated mutations and copy number variations are present in EV DNA. Additionally we provide potential glioma-associated EV proteins that may facilitate the enrichment of tumor-EVs. Our study represents an interdisciplinary collaborative effort of multiple departments, institutes and research cores within the UKE. Our innovative data will open new applications in the field of liquid biopsy and improve patient care follow up.

BACKGROUND:

This work was performed in the department of Neurosurgery (Director. Prof. Manfred Westphal) in the laboratory of brain tumor biology led by Prof. Katrin Lamszus. Dr. F.L. Ricklefs who got trained for 2 years in the group of Prof. Nino Chiocca (DFCI, Boston) initiated the research on extracellular vesicles in the context of malignant glioma in the lab (DFG 2616/3-1) and developed this project in collaboration with Dr. C.L. Maire who joined the lab in 2015 after her post doctoral training (DFCI, Boston). The presented study exemplifies the fruitful collaboration potential within the UKE (Tumorbiology, Neuropathology, Mass spec core, FACS core…) in the field of EV research with many additional applications.