Deciphering the methylation signature of circulating extracellular vesicle DNA and cell-free DNA for CNS tumor classification

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Background & objectives

Genome-wide methylation profiling has recently been developed into a tool that allows subtype tumor classification in central nervous system (CNS) tumors. We previously showed that extracellular vesicle (EV) DNA faithfully reflects the tumor methylation class, including information on the IDH mutation and MGMT promoter methylation status. Furthermore we showed that circulating plasma EVs are elevated in CNS tumor patients in comparison to non-tumor donors (HD) controls with tumor related protein profiles.

We now investigated, whether the methylation signatures of circulating DNA (both EV and cfNDA) can be used in liquid biopsy approaches for CNS tumor detection and classification.

Methods

We isolated DNA from circulating EVs (n=102), cfDNA (n=103) and tumor tissue DNA (n=701) of patients with glioblastoma (GBM), meningioma (MGN) and cerebral metastases (CM). Non-tumor Patients undergoing surgery were used as controls (HD, n= 27). EVs were classified by nanoparticle analysis, immunoblotting, imaging flow cytometry and electron microscopy.

Results

Isolated EV-DNA comprised many sorts of molecular weight (up tp >10Kb) in comparison to cfDNA (130-140bp). Healthy donors and tumor patients showed not differences in their DNA size profiles. We performed genome-wide methylation profiling by 850k Illumina EPIC arrays for all DNA analytes and tumor entities. Linear models and empirical Bayes methods identified significant differentially methylated CpGs (GBM vs. HD, MGN, vs HD, CM vs. HD), that revealed tumor specific signatures to detect and discriminate different CNS tumor entities. Visualization of differentially methylated CPGs by dimension reduction (PCA, t-SNE, Umap) verified tumor specific clusters. Classification of tumor entities was performed using a stochastic gradient decent classifier with ElasticNet regularization. To account for

heterogeneity in cellular composition, in-silico deconvolution of the normalized CpG intensities was performed using signature matrix consisting of cell-type and tissue specific CpG profiles. With the normalized CpG intensities and in-silico deconvolution results we were able to classify the three cancer types obtaining an accuracy of 83%, 77%, and 87%.

Conclusion

Our study shows that the methylation signature of circulating EV DNA and cfDNA can be used to separate healthy individuals from tumor patients and could potentially complement standard-of-care imaging to improve tumor detection, classification and surveillance.