Classification of brain tumors by Nanopore sequencing of cell-free DNA from cerebrospinal fluid

Ann-Kristin Afflerbach[1]
Christian Rohrandt[2], Björn Brändl[3], Marthe Sönksen[4], Jürgen Hench[5], Stephan Frank[5], Daniela Börnigen[6], Malik Alawi[6], Martin Mynarek[4], Beate Winkler[4], Franz Ricklefs[7], Michael Synowitz[8], Lasse Dührsen[7], Stefan Rutkowski[4], Annika K. Wefers[9], Franz-Josef Müller[3], Melanie Schoof[1] and Ulrich Schüller[1]
[1] Research Institute Children's Cancer Center Hamburg
[2] Institute for Communications Technologies and Embedded Systems, University of Applied Sciences Kiel
[3] Center for Integrative Psychiatry, University Hospital Schleswig-Holstein
[4] Department of Pediatric Hematology and Oncology, University Medical Center Hamburg-Eppendorf
[5] Department of Pathology, University Hospital Basel
[6] Bioinformatics Core, University Medical Center Hamburg-Eppendorf
[7] Department of Neurosurgery, University Medical Center Hamburg-Eppendorf
[8] Department of Neurosurgery, University Hospital Schleswig-Holstein, Campus Kiel
[9] Institute of Neuropathology, University Medical Center

Background & objectives

For exact molecular diagnosis of CNS tumors, tumor tissue obtained through surgery is usually needed. However, knowledge on the molecular tumor type beforehand might influence surgical strategies and reduce surgery-associated risks. Molecular analyses of liquid biopsies have therefore gained increasing interest in order to secure diagnosis before surgery or to replace surgery in rare instances. Moreover, the minimally invasive nature of liquid biopsies enables a tight and longitudinal tumor monitoring with potentially higher sensitivity than imaging approaches.

Our aim was to establish a novel diagnostic method for brain tumors, using cell-free (cfDNA) from cerebrospinal fluid (CSF) and Nanopore sequencing.

Methods

In this study, we applied Nanopore sequencing to 129 cfDNA samples from the CSF of brain tumor patients, with 70.5% coming from pediatric patients. We analyzed copy number variations (CNV) and methylation patterns with a recently published random forest classifier (NanoDx).

Results

Circulating tumor DNA (ctDNA) was detected and successfully classified the tumor in 45% of all technically successful samples, both in pre- and early post-surgery samples as well as in samples from >14 days post-surgery, often with clinically unclear residual tumor or disease relapse. In all samples containing detectable tumor DNA, CNV analysis was more frequently successful than methylation analysis with overall detection rates of 88% and 44%, respectively. CNV analysis revealed diagnostic alterations, such as C19MC amplifications in ETMR as well as Chr.1q gain and Chr.6q loss in PFA ependymoma, which are important prognostic markers. Methylation analysis also allowed the classification of tumors with balanced genomes, like craniopharyngiomas. Finally, we were able to perform longitudinal analyses and found aberrations in the CNV profiles that were private to the tumor relapse, highlighting the potential of liquid biopsies to detect potentially relevant changes of tumor biology.

Conclusion

Our results show that Nanopore sequencing is a promising approach to establish initial brain tumor diagnosis and to monitor disease courses by sequence and methylation analysis of lumbar punctures.