Network: **Brain Tumor Network (BTN) – Identification of Novel Diagnostic and Therapeutic Targets in Cranial Malignancies by Integrated Tumor Profiling**

Project: Genomic DNA Methylation Analysis of Gliomas

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Introduction

Altered DNA methylation patterns are one of the earliest and most consistent hallmarks of human cancers, including gliomas. The cancer-associated changes in DNA methylation can be summarized as follows: (1) Many tumors show locally restricted hypermethylation of tumor suppressor genes. Hypermethylation of promoter elements has been shown to be closely associated with repressive chromatin structures and transcriptional silencing. Thus, hypermethylation of tumor suppressor genes results in reduced expression of the corresponding gene products and might therefore play an important role in cellular transformation. (2) Most tumors show a decreased level of overall DNA methylation. Reduced genomic DNA methylation levels have been frequently linked to large-scale chromosome aberrations. Thus, low levels of genomic DNA methylation might contribute to tumorigenesis by promoting genomic instability. In the past, genomic DNA methylation levels have been determined by high performance liquid chromatography (HPLC). However, this method requires large amounts of intact genomic DNA and was therefore not particularly suited to the analysis of DNA from clinical samples. As a consequence, very little is known about overall changes in genomic DNA methylation levels during tumorigenesis.

Project Status

We have recently established a sensitive analytical method for the quantification of genomic DNA methylation levels from clinical samples (1). The procedure requires about 3 µg of isolated genomic DNA that is hydrolyzed to single nucleotides, derivatized with a fluorescent marker and then separated by capillary electrophoresis. Our method allows the analysis of large sample numbers and provides highly reproducible results. For example, the method has been used to analyze DNA methylation levels in a group of 83 chronic lymphocytic leukemia (CLL) patients. Our results revealed a statistically significant ($P<0.01$) association between high levels of genomic DNA methylation and high levels of VH homology, an established prognostic marker for CLL (2). These results provide an important validation of our methodology and also suggest that the genomic cytosine methylation level might be a useful biomarker for tumor classification. We have now started to determine the genomic DNA methylation level for every tumor in the central glioma collection (Fig. 1). The results indicated significant variability between individual tumors, which will provide the foundation for a more detailed data analysis.

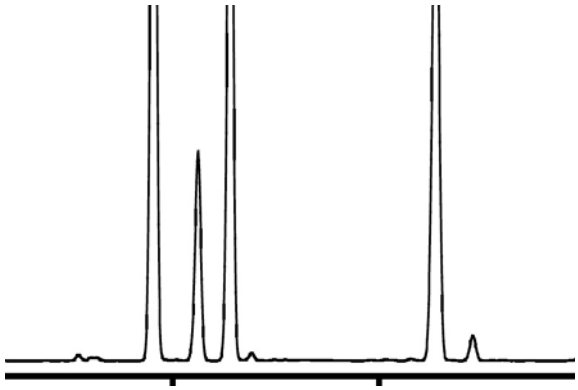

Fig 1: *Representative electropherogram of genomic DNA from a glioma sample with major peaks representing (from left to right) adenine, guanine, thymine, cytosine and 5-methylcytosine.*

Outlook

We will complete the methylation analysis for the entire glioma collection (n=100) and the results will be provided to the integrated tumor profiling. In addition, the methylation data will also be analyzed for associations with gene-specific DNA methylation patterns (in collaboration with Andreas Waha and Guido Reifenberger) and with clinicopathological parameters. The results will provide novel insights into the epigenetic changes related to gliomagenesis and further determine the value of the genomic DNA methylation level as a biomarker in cancer research.

Lit.: ***1.*** *Stach D et al. Capillary electrophoretic analysis of genomic DNA methylation levels. Nucleic Acids Res. 2003 Jan 15;31(2):E2.* ***2.*** *Lyko F et al. Quantitative analysis of DNA methylation in chronic lymphocytic leukemia patients. Electrophoresis. 2004 Jun;25(10-11):1530-5.*