

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

Project: Identification of Novel Glioblastoma Suppressor Genes on Chromosome 10q24-qter Using Microarray-based Genomic and Expression Profiling

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Introduction

Glioblastomas are the most common and most malignant primary brain tumors in human adult, accounting for about 50 % of all gliomas and 12-15 % of all intracranial tumors. Despite considerable progress in multimodality treatment, including neurosurgery, local radiation therapy, and chemotherapy, the prognosis of patients with glioblastoma is still poor, with a median survival time less than 1 year. Molecular genetic studies have identified a variety of chromosomal and genetic alterations in glioblastomas, with loss of chromosome 10 being present in up to 90 % of the cases. Chromosome 10 losses are far less common in lower grade astrocytic gliomas as compared to glioblastomas, suggesting that chromosome 10 carries glioblastoma-associated tumor suppressor genes (1). Previous studies have implicated the *PTEN* gene at 10q23 as an important tumor suppressor gene that is aberrant in affected in 25-40 % of primary glioblastomas (2). However, the absence of *PTEN* mutations in more than half of *de novo* glioblastomas and more than 90 % of secondary glioblastomas, i.e. glioblastomas that developed from a pre-existing lower grade glioma, clearly points to the presence of additional tumor suppressor genes on chromosome 10. By using loss of heterozygosity analyses, we and others have provided evidence for at least one additional glioblastoma-related tumor suppressor gene mapping to 10q24-qter, distal to *PTEN* (1, 2). Although several genes from this region, including *DBMT1* (3), *LG11* (4), *WDR11* (5), and *MX11* (6), have been proposed as glioblastoma suppressor gene, none of these candidates has been confirmed as being mutated in a significant fraction of tumors. Thus, the relevant glioblastoma suppressor gene(s) in 10q24-qter is/are still unknown.

Results/Project Status

In the first 3 months of our project, we have collected a core series of 100 primary gliomas with corresponding clinical and pathologic data. High molecular weight DNA and RNA have been extracted from these cases and are available for molecular analyses by the brain tumor network. At present, genomic and expression profiling using matrix CGH and oligonucleotide-based expression arrays is ongoing. The respective microarrays that are used for these experiments have been tailored to the specific questions concerning identification of glioblastoma-suppressor candidate genes on 10q24-qter.

With respect to the proposed project, we have already performed real time-RT PCR expression analysis of 15 genes located on 10q24-qter in a series of 34 *de novo* glioblastomas, 12 secondary glioblastomas, 11 anaplastic astrocytomas, 11 diffuse astrocytomas, and 5 glioma cell lines to identify novel candidate tumor suppressor genes that are downregulated in glioblastomas. The genes were either selected from the NCBI Human Genome Browser (www.ncbi.nlm.nih.gov) or from microarray expression profiling data of glioma cell lines treated with the demethylating agent 5-aza-2'-deoxycytidine and the histone deacetylase inhibitor trichostatin A. So far, we identified four (*ADD3*, *EMX2*, *OAT*, and *INPP5A*) genes that showed markedly reduced mRNA levels in substantial fractions of glioblastomas compared to diffuse astrocytomas and non-neoplastic brain tissue (Fig.1). Furthermore, treatment of glioma cell lines with 5-aza-2'-deoxycytidine and TSA

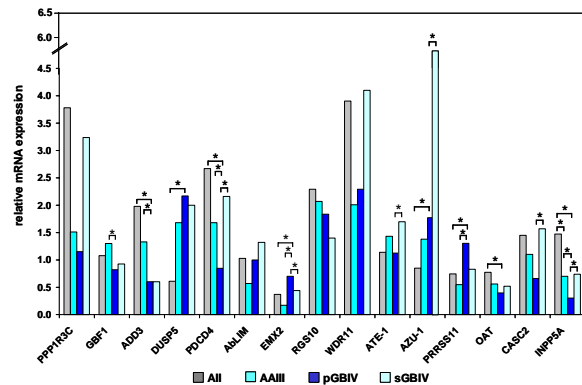


Fig 1: Graphical summary of real time-RT PCR-based expression analysis of 15 tumor suppressor gene candidates in 68 astrocytic gliomas (11 All, 11 AAll, 34 pGBMs, 12 sGBMs). The transcript level of each gene was normalized to *ARF1* (ADP ribosylation factor 1). Normal brain tissue samples from 7 different patients were used as reference tissue. Vertical bars represent the median expression level of each tumor group. Asterisks indicate significant (Student's *t*-test, $p \leq 0.05$) expression differences between the respective tumor groups.

resulted in increase expression of these genes, suggesting that their transcriptional downregulation may be due to promoter hypermethylation. Subsequently, we investigated the promoter regions of these four genes by sequencing of sodium-bisulfite modified DNA and identified methylated CpG sites in the *EMX2*-promoter sequence (Fig. 2) in a fraction of glioblastomas (7/20, 35 %) with reduced expression of the gene.

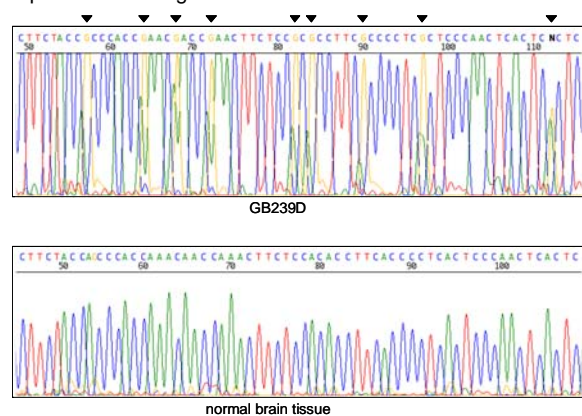


Fig 2: Demonstration of *EMX2* promoter methylation by sequencing of sodium-bisulfite modified DNA from a primary glioblastoma (GB239D, upper panel) and non-neoplastic brain tissue (lower panel). Note, methylation of all of the depicted CpG sites in tumor GB239D, whereas the DNA of the non-neoplastic brain tissue shows no methylated CpG residues. The mRNA expression level of *EMX2* in GB239D was 0.05 compared to non-neoplastic brain tissue. Shown are parts of the reverse sequencing. Arrowheads point to methylated CpG sites.

In contrast, promoter hypermethylation is not the cause of the reduced expression of *ADD3*, *OAT*, and *INPP5A*, although treatment of glioma cell lines with demethylating agent restored expression of these three genes. *EMX2* and *ADD3* were also investigated for mutation and homozygous deletion by SSCP/heteroduplex analysis and genomic duplex PCR, respectively, but both genes exhibited neither genetic nor chromosomal alterations.

The molecular mechanisms leading to the the decreased expression of *ADD3*, *INPP5A*, and *OAT* as well as the functional significance of these genes, including *EMX2*, in glioma pathogenesis have to be clarified.

Outlook

The project aims at the identification as well as molecular and functional characterization of one or more novel glioblastoma suppressor genes from 10q24-qter. This aim will be addressed by using different approaches, including the development and application of a region-specific genomic BAC-array for matrix CGH analysis, as well as the development and application of a region-specific cDNA array for mRNA-based expression profiling. Candidate tumor

suppressor genes identified by these approaches will be further characterized for mutations and/or epigenetic modifications in gliomas and subjected to functional analyses *in vitro*.

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