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

Project: Identification of an Astrocytoma Relevant Tumor Suppressor Gene on the Long Arm of Chromosome 22

Christian Hartmann and Andreas von Deimling - Department of Neuropathology, Charité, Universitätsmedizin Berlin – ch.hartmann@charite.de

Introduction

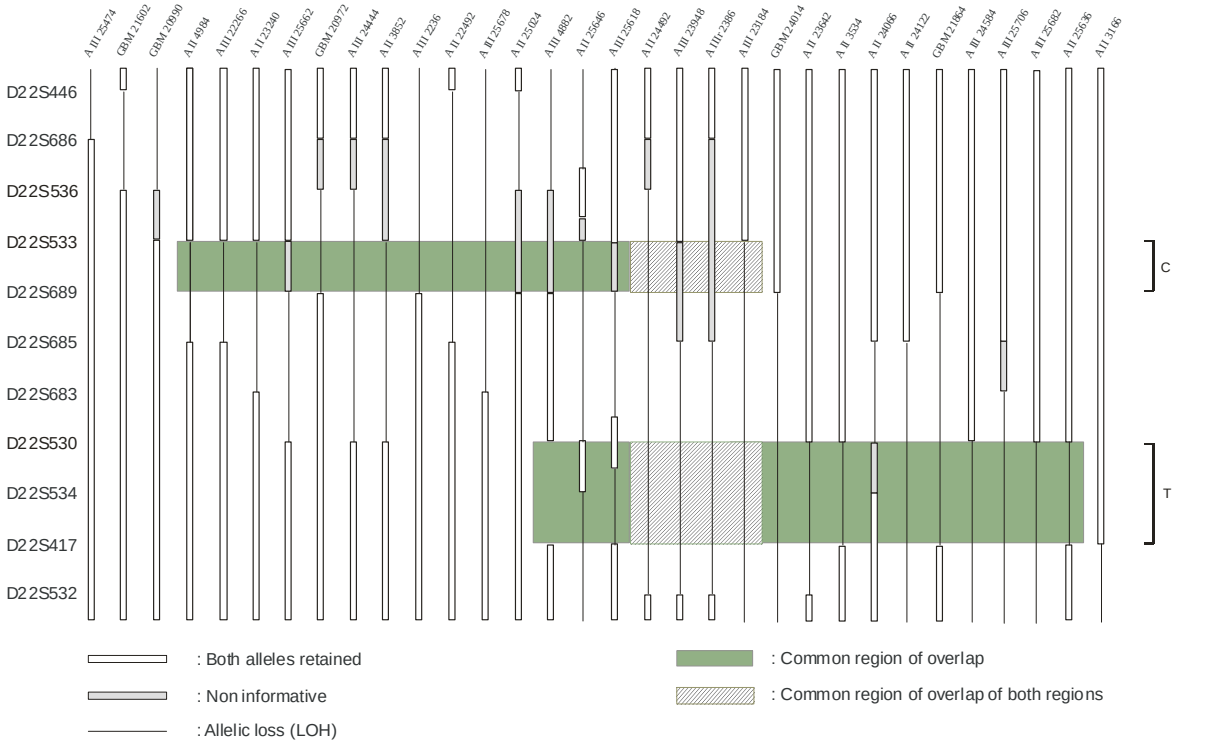
Astrocytomas are the most frequent primary brain tumors in adult patients. Various consistently arising genetic alterations have been established in these tumors. These include loss of heterozygosity (LOH) on the chromosomal arms 9p, 10p, 10q, 13q, 17p, 19q and 22q (1). In several instances, mutations in distinct tumor suppressor genes (TSG) match with chromosomal deletion of the corresponding allele such as CDKN2A/CDKN2B with LOH 9p, PTEN with LOH 10q, Rb with LOH 13q and TP53 with LOH 17p (2). However, for some regions with frequent LOH in gliomas such as 1p, 19q and 22q no candidate TSG could yet be elucidated. Among these regions, 22q seems to be of special interest in astrocytic gliomas. Up to 30% of astrocytomas have been demonstrated to carry LOH 22q (3) and a role of this alteration in progression to anaplasia has been suggested (3, 4). A standard approach for the identification of TSG in regions with frequent LOH relies on determining interstitial deletions in individual tumors and defining a minimal 'common region of overlap'. By fine mapping of 22q two common regions spanning 22q12.3-13.31 and 22q13.31-13.32 have been proposed in astrocytoma. The telomeric region was lost predominately in glioblastomas, the centromeric region as well in lower grade astrocytomas (3, 4). Chromosome 22 harbours TSG already shown to be involved in the genesis of brain tumors. The NF2 gene carries somatic mutations in many sporadic meningiomas (5). However, no NF2 mutations were found in astrocytomas (6). Mutations in hSNF5/INI1 were reported in different tumors, but no mutations were detected in astrocytomas (7).

To identify the expected astrocytoma TSG on 22q we applied different approaches: i.) relocating the already established candidate regions to the physical sequence of 22q based on the data of the human genome project, ii.) annotating all 22q genes regarding potential functions as tumor suppressors (8), iii.) focused fine mapping by microsatellite markers and matrix CGH arrays, iv.) expression profiling by cDNA and oligonucleotide microarrays and v.) sequence analysis by single strain conformation polymorphism (SSCP) and direct sequencing. Due to the observation that low grade and high grade astrocytomas exhibit different candidate regions we splitted or tumor samples for further analysis.

Material and Methods

For the microstallite marker based part of the study we obtained 153 astrocytic gliomas, consisting of 52 astrocytomas WHO grade II (A II), 70 anaplastic astrocytomas WHO grade III (A III), 31 glioblastoma WHO grade IV (GBM) and corresponding blood samples from patients treated at Charité, Universitätsmedizin Berlin and at University of Bonn Medical Center.

Fig 1: *Chromosome 22q mapping results. In this figure all 27 cases showing an interstitial deletion are included. A II: Astrocytoma WHO grade II, A III: Astrocytoma WHO grade III, GBM: Glioblastoma multiforme WHO grade IV; (C): centromeric deletion region, (T): telomeric deletion region, white bars:both allels retained, grey bars: non informative markers flanking deletions, single line bars: deletions.*



NGFN Nationales Genomforschungsnetz

GEFÖRDERT VOM Bundesministerium für Bildung und Forschung

The 22q GBM mapping project was based on a collection of 50 GBM treated at the Charité, Universitätsmedizin Berlin. A core series of 100 gliomas consisting of AII, AIII and GBM is established and will be utilized for the 22q project within the BTN based on samples from patients treated in Berlin and Düsseldorf.

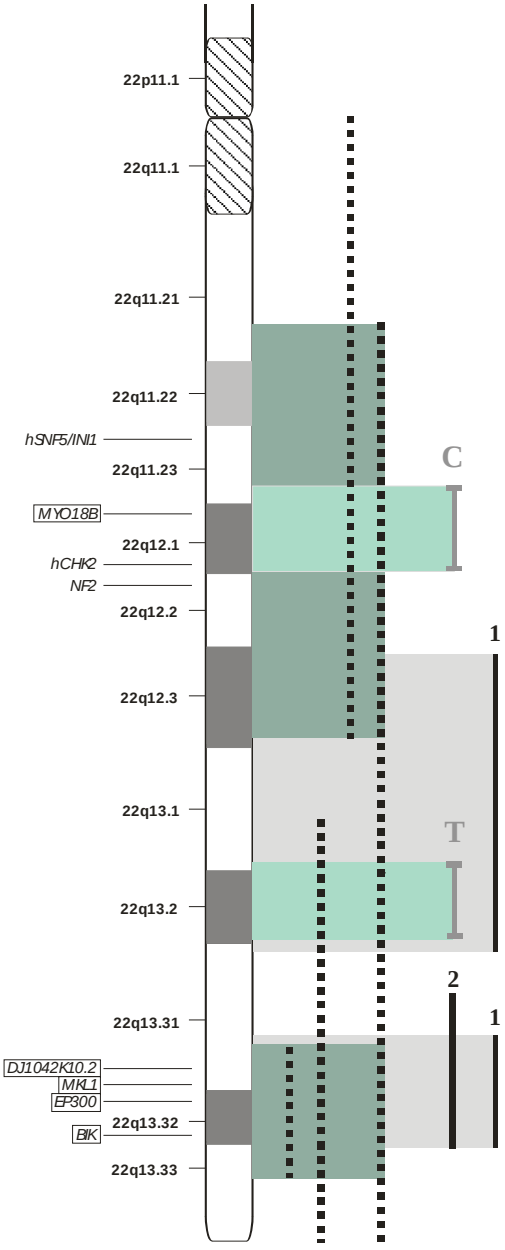


Fig 2: *Chromosomal location of genes (left) and gliomas candidate regions (right). Left side: Genes in boxes: genes analysed for mutations; genes without boxes: tumor suppressor genes on 22q not mutated in gliomas. Right side: bright green boxes: candidate regions based on own microsatellite marker results using astrocytomas of different grades (9); dark green boxes: candidate regions based on matrix CGH array analysis using GBM (10); grey boxes: candidate regions suggested by other studies using astrocytomas of different grades (3,4); C: centromeric candidate region; R: telomeric candidate region; punctuated lines: single cases with interstitial deletions based on matrix CGH results (10); continuous lines: candidate regions from 1: (3) and 2: (4).*

To identify LOH 22q 11 microsatellite markers were used. PCR products were separated on 4% or 8% denaturing acrylamide gels and visualized by silver staining. In collaboration with the Rudbeck Laboratory from Uppsala University, Sweden a 22q tiling path matrix CGH array was used to detected losses of chromosomal material. The arrays provided long-range coverage of 34.7 Mb and an average resolution of 75 kb. At present a matrix CGH array with nearby tiling path resolution is generated for the 22q project within the BTN.

PCR products from 142 genes within and flanking to the low grade astrocytoma candidate regions and 44 tumor related genes were spotted on glass slides and hybridized with 20 tumor cDNAs consiting of 10 astrocytomas with and 10 astrocytomas without LOH 22q. Currently an oligonuleotide-based expression array covering nearly all genes on 22q is in preparation for the 22q project within the BTN.

The genes DJ1042K10.2, MKL1, MYO18B, EP300 and BIK were selected for mutation screening genes based on annotation of 22q genes by criterions like identified mutations in other tumors (MYO18B ,EP300, BIK), chromosomal location inside the candidate regions (DJ1042K10, MKL1) and potential function as tumor suppressors (DJ1042K10, MKL1, MYO18B ,EP300, BIK). The coding sequence (DJ1042K10, MKL1) or sequences coding for functional domains (EP300, BIK) were analyzed by SSCP and direct sequencing. Candidate genes from the BTN 22q project will be analyzed by SSP, direct sequencing and hypermethylation studies.

Results/Project Status

F Using 11 microsatellite markers, we detected allelic loss on chromosomal arm 22q in 49/153 (32%) astrocytic gliomas. 32/49 LOH cases demonstrated only partial loss of chromosomal material (65%), whereas 17 cases (35%) presumably had lost the entire arm of chromosome 22q. The tumors with partial deletions could be employed for fine mapping analysis. Two distinct ‘common regions of overlap’ were identified (figure 1). A centromeric deletion region (C-region) between D22S533 and D22S689 with a size of 3 Mb was observed in 18 cases. A telomeric deletion region (T-region) between D22S530 and D22S417 with a size of 2.7 Mb was detected in 17 cases. Both regions were affected in 7 cases. In 3 cases (A II 25646, A III 4882, A III 25618) the C- and the T-deletion region were found to exhibit LOH and retained both allelic copies in between, thereby indicating 2 independent interstitial deletions. In the C-region spanning 3 Mb, 11 RefSeq genes and 11 mRNA based gene candidates were identified. In the T-region spanning 2.7 Mb, 39 RefSeq genes and 10 mRNA based gene candidates were found (9).

MYO18B, BIK, DJ1042K10.2, MKL1 and EP300 were analyzed by SSCP and sequencing. However, none of the genes showed aberrant bands in blood/tumor pairs, therefore indicating no mutation in these genes. Instead, several single nucleotide polymorphisms (SNP) were detected (9).

Twenty astrocytomas were expression profiled. By unsupervised hierarchical clustering different clusters were identified (fig. 2). No correlation between different clusters and LOH 22q versus non-LOH tumors was observed. Astrocytomas WHO grade II were clustered with a weak tendency in the middle part of the dendrogram. Four groups were defined: “complete LOH” (coLOH), “centromer LOH” (ceLOH), “telomere LOH” (teLOH) and “No Loss” (NL). Using supervised mean value analysis of the 4 groups coLOH, ceLOH, teLOH and NL, 4 genes (BCR, MCM5, MN1, X16865) were identified that were differentially expressed between the ceLOH group and the NL group. MN1 is located in, BCR centromer to and MCM5 telomere to the C-region, X16865 in the T-region. In raw data, MN1 revealed the highest quality, X16865 the lowest. No differentially expressed gene was identified in the teLOH group. At next, the 4 groups were analysed by the ANOVA test. Three

genes (MAPK12, KIAA1671, PMM1) were differentially expressed between the ceLOH group and the NL group (p=0.05). KIAA1671 is located close to the C-region, MAPK12 at the telomere of 22q and PMM1 in the T-region. The raw data of MAPK12 and PMM1 showed a higher quality level than the results for KIAA1671. Again, no gene of the teLOH group showed up. Removing the coLOH group and performing again an ANOVA test, the 2 genes MAPK12 and PMM1 were found to be differentially expressed in the ceLOH group (p=0.35; no genes with p=0.05).
Fifty GBMs were analyzed for chromosomal losses by matrix CGH array. Heterozygous deletions were detected in 28% of the tumors (14 of 50). The predominant pattern was monosomy 22 (10 of 50). In 4 cases partial deletions on chromosome 22 of different sizes were identified. One samples presented a large interstitial deletion (26.8 Mb) which encompassed the majority of 22q. In two samples we detected terminal deletions, one centromeric and one telomeric, 18.9 and 13.3 Mb in size, respectively. In one case we discovered a small tumor-specific deletion, 3.08 Mb in size. The distribution of overlapping deletions delimited two tumor suppressor gene loci (11.1 Mb respective 3.08 Mb) across the chromosome (10).
The BTN 22q project is based on a combination of the approaches using larger samples sizes. At present a core tumor collection is established and DNA and RNA is extracted. The matrix CGH array with nearby tiling path resolution is nearly finalized. The generation of all oligonucleotides for the expression array is completed; right now the oligonucleotides will be spotted on the chips.

Outlook

The major goal of the project is the identification and molecular characterisation of one or multiple tumor suppressor genes on the long arm of chromosome 22. Results from our earlier studies indicate two candidate regions for astrocytomas WHO grade II and anaplastic astrocytomas WHO grade III on the centromeric and middle region and a glioblastoma candidate region on the telomeric region of 22q (3, 9). In a small series of lower grade astrocytomas we already demonstrated the potential of expression profiling of chromosomal characterized tumors. In addition, we were able to demonstrate in GBM that matrix CGH arrays can be utilized for fine mapping with a much higher resolution than traditional microsatellite marker-based techniques allow (10). The 22q project of the BTN combines both approaches in a much larger series of astrocytic tumors: precise fine mapping by matrix CGH technology will separate all tumors in groups of astrocytomas with and without 22q losses and delineate tumor suppressor gene candidate regions. This information will be used to test nearly all genes on 22q for expression differences and to identify single candidate genes. These potential tumor suppressor genes will be genetically and epigenetically evaluated for alterations. Finally, the genes will be functionally characterized..

*Lit.: **1.** von Deimling, A., et al., Comprehensive allelotype and genetic anaysis of 466 human nervous system tumors. J Neuropathol Exp Neurol, 2000. 59(6): p. 544-58. **2.** Kleihues, P. and W.K. Cavenee, Pathology and genetics of tumours of the nervous system. 2nd edition ed. 2000, Lyon: IARC Press. 314. **3.** Ino, Y., et al., Common regions of deletion on chromosome 22q12.3-q13.1 and 22q13.2 in human astrocytomas appear related to malignancy grade. J Neuropathol Exp Neurol, 1999. 58(8): p. 881-5. **4.** Oskam, N.T., E.H. Bijleveld, and T.J. Hulsebos, A region of common deletion in 22q13.3 in human glioma associated with astrocytoma progression. Int J Cancer, 2000. 85(3): p. 336-9. **5.** Wellenreuther, R., et al., Analysis of the neurofibromatosis 2 gene reveals molecular variants of meningioma. Am J Pathol, 1995. 146(4): p. 827-32. **6.** Rubio, M.P., et al., Analysis of the neurofibromatosis 2 gene in human ependymomas and astrocytomas. Cancer Res, 1994. 54(1): p. 45-7. **7.** Weber, M., et al., Mutational analysis of INI1 in sporadic human brain tumors. Acta Neuropathol (Berl), 2001. 101(5): p. 479-82. **8.** Hartmann, C., et al., Transcript map of the 3.7-Mb D19S112-D19S246 candidate tumor suppressor region on the long arm of chromosome 19. Cancer Res, 2002. 62(14): p. 4100-8. **9.** Hartmann, C., et al., Fine mapping of chromosome 22q tumor suppressor gene candidate regions in astrocytoma. Int J Cancer, 2004. 108(6): p. 839-44. **10.** de Stahl, T.D., et al., Chromosome 22 tiling-path array-CGH analysis identifies germ-line- and tumor-specific aberrations in patients with glioblastoma multiforme. Genes Chromosomes Cancer, 2005. 44(2): p. 161-9.*