

Recurrent cytogenetic aberrations in central neurocytomas and their biological relevance

Andrey Korshunov · Regina Sycheva ·
Andrey Golanov

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Abstract Central neurocytomas are rare central nervous system neoplasms. Since the first description, approximately 500 cases of these tumors have been published to date. Nevertheless, only a limited number of genetic studies on these tumors have been reported. Here we investigated 20 “typical” central neurocytomas using array-based comparative genomic hybridization (array-CGH) with the GenoSensor Array 300. The functional significance of detected chromosomal aberrations harboring potent candidate genes was also examined at the mRNA expression level. Each tumor examined displayed DNA copy-number aberrations (CNAs), and mean number of CNAs per tumor was 38.1 ± 7.1 (range 19–53). Frequent gains were mapped at 2p24.1–22.1, 10q23.3–26.3, 11q23–25, and 18q21.3–qter. Frequent losses were identified at 1pter–36.3, 1p34.3, 6q13–21, 12q23–qter, 17p13.3, 17q11–23, and 20pter–12.3. There were 10 gained and 23 lost single DNA clones affecting $\geq 40\%$ of samples tested. mRNA expression levels of 24 selected candidate genes harbored in these imbalanced clones were analyzed. *MYCN*, *PTEN*, and *OR5BFI* were strongly overexpressed, whereas *BINI*, *SNRPN*, and *HRAS* were found to be strongly underrepresented at the transcriptional

level. Thus these data support that *MYCN* oncogene gain/overexpression accompanied by reduced expression of *BINI* tumor suppressor may contribute to central neurocytoma tumorigenesis.

Keywords Central neurocytoma · Molecular genetics · Microarrays · Tumorigenesis

Introduction

Central neurocytomas are rare central nervous system (CNS) neoplasms that occur predominantly in young adults [11, 27]. The tumors are usually found in the lateral ventricles occupying the septum pellucidum region. Pathologically, these tumors demonstrate neuronal differentiation as suggested by electron microscopy and immunohistochemistry findings [11]. Central neurocytomas are generally considered benign and are associated with favorable prognosis. However, certain neurocytomas display histological “atypia” and possess increasing proliferative activity, resulting in poor clinical outcomes [25, 27]. The histogenesis of central neurocytoma is still unclear. It remains a matter of discussion whether these neoplasms arise from cells committed to neuronal differentiation, or from multipotent progenitor cells in the periventricular matrix [11, 27].

Since the first description of central neurocytoma by Hassoun in 1982, approximately 500 cases have been published to date [25, 27]. Nevertheless, a limited number of genetic studies on these tumors have been conducted. Conventional cytogenetic analyses revealed normal or near-diploid karyotypes, while a few studies discovered either a gain of chromosome 7 or loss of

A. Korshunov (✉) · R. Sycheva
Department of Neuropathology,
NN Burdenko Neurosurgical Institute,
Fadeeva Str. 5, Moscow 125047, Russia
e-mail: akorshunov@nsi.ru

A. Golanov
Department of Neurooncological Surgery,
NN Burdenko Neurosurgical Institute,
Fadeeva Str. 5, Moscow 125047, Russia

chromosome 17 copy-number [4, 9, 30]. No *TP53* mutation, *EGFR* and *MYCN* amplifications were found in central neurocytomas [6, 23, 31, 32]. Comparative genomic hybridization (CGH) analysis was used in ten samples and appreciable chromosomal alterations were revealed in six neurocytomas [32]. Gains at chromosomes 2p, 10q, 13q, and 18q were the more frequent genetic aberrations. Due to strong histological similarity between neurocytomas and oligodendrogliomas, analysis of the loss of heterozygosity (LOH) of 1p and 19q was also performed in these tumors. Tong et al. [31] observed interstitial deletions of 1p and 19q, although most of the informative markers were retained. Fujisawa et al. [6] were unable to find allelic losses on 1p and 19q in eight neurocytomas using FISH probes. Expression profiling of four central neurocytomas displayed their close genetic similarity to cerebellar liponeurocytomas and their marked differences relative to medulloblastoma transcriptional profiles [8]. Thus, the genetic background and molecular mechanisms underlying pathogenesis of central neurocytomas remain largely unknown, and consequently, the distinct nosologic position of these tumors is still uncertain.

In the present study we investigated the molecular pathogenesis of 20 central neurocytomas using a combined experimental design. Initially, we applied an array-based CGH method (or array-CGH) with the GenoSensor Array 300 to identify recurrent DNA copy-number imbalances in these tumors. The functional significance of detected chromosomal aberrations harboring potent candidate genes was further examined at the mRNA expression level.

Materials and methods

Patient population and pathological analysis

In total, 20 adult patients who were treated for newly diagnosed central neurocytomas at the Burdenko Neurosurgical Institute from January 1, 1995, to January 1, 2004 were included in this study. The mean age was 27 years (range 18–44 years) and there were 11 women and 9 men among them. In all cases the tumor was located in both lateral ventricles involving the septum pellucidum. All patients underwent open surgery with gross total (12 cases) and subtotal (8 cases) tumor resection. After the operation, all patients experienced a stable clinical course without signs of disease progression.

Histological diagnoses were performed according to the current World Health Organization histologic

classification [11]. All tumors were composed of sheets of uniform, round cells with perinuclear haloes intermingled with fine background neuropil and acellular fibrillary zones. Additionally, all samples displayed strong immunoreactivity for synaptophysin and Neu-N antibodies. Also, 11 tumors were examined with an electron microscope and “classic” neurocytoma patterns, such as dendrites, synapses and neurosecretory granules were found. There were no signs of histological atypia (mitotic activity, microvascular proliferation and necrosis) in these 20 neurocytomas. Immunohistochemistry with a Ki-67 (MIB1) antibody was performed on all samples and the Ki-67 labeling indices varied from 0.12 to 1.89% (mean $0.93 \pm 0.56\%$). Approval to link laboratory data to clinical data was obtained by the Institutional Review Board.

Sample processing

Samples from each tumor were collected at the time of initial operation, immediately frozen in liquid nitrogen, and stored at -80°C until nucleic acid isolation and analysis. To confirm the presence of a viable tumor (>90% of neoplastic cells), the cryosections of each manually dissected neurocytoma sample were stained with H&E and reviewed before DNA and RNA extraction. DNA was isolated according to a protocol that applies the DNeasy tissue kit (Qiagen, Hilden, Germany). Total RNA was extracted from 12 available tumor samples according to a protocol that applies TRIzol reagent and RNeasy midi spin columns (Qiagen). DNA and total RNA concentration and quality were determined by absorption spectrophotometry and their integrity and purity were analyzed on 1% agarose gel. Also, the integrity of each RNA sample was monitored with the Agilent 2100 Bioanalyzer (Agilent Technologies, Palo Alto, CA, USA).

DNA labeling, hybridization, and analysis of the GenoSensor Array 300

Pretreatment and analysis of the microarrays was performed as described previously [15]. Briefly, approximately 100 ng of DNA from tumor samples (test DNA) and sex-mismatched normal reference human DNA were labeled by a random priming reaction according to the protocols recommended by the manufacturer (Vysis, Inc., Abbott Laboratories S.A., Downers Grove, IL, USA). Aliquots of labeled DNA were mixed with the hybridization buffer and the hybridization mixture was then introduced to the genomic DNA microarray slides. We used the commercially available

GenoSensor Array 300 (Vysis, Inc.), which contained 287 genomic targets cloned from P1 and BAC libraries, and printed in triplicate. This microarray platform includes cancer amplicons/oncogenes, tumor suppressor genes, loci of insertions/deletions/duplications, telomeres and markers added to reduce gaps (for detailed information see <http://www.vysis.com>). The microarray slides were incubated for 72 h at 37°C, washed in formamide/standard saline citrate solutions, counterstained with DAPI and covered with coverslip glasses. The microarray slides were then analyzed using the GenoSensor Reader System (Vysis, Inc.) according to the manufacturer's instructions. Based on previous control experiments using the GenoSensor Array 300 [1, 15, 21], we determined losses, gains and amplifications of target DNA sequence copy-number by a green/red ratio <0.8, >1.2, and >2.0, respectively.

Fluorescence in situ hybridization (FISH)

Thirty-five paraffin-embedded samples of central neurocytomas (including all samples applied for microarray experiments) were examined by a two-color interphase FISH assay that was performed on 5 µm-thick sections. The following fluorochrome-labeled probes were applied (all produced by Vysis, Inc., besides 2p11–q11): centromere 2p11–q11 probe (RPZD, Berlin, Germany) and locus-specific 2p24(*MYCN*) probe, 10p11–q11/10q23(*PTEN*) dual-color probe set, and locus-specific 17p13(*LIS1*)/17q11(*RARA*) dual-color probe set. Pretreatment of slides, hybridization, posthybridization processing, and signal detection were performed as described previously [14].

Quantitative real-time polymerase chain reaction with genomic DNA (QRT-PCR)

QRT-PCR with genomic DNA was performed on a 7300 real-time PCR system (Applied Biosystems, Foster City, CA, USA) using TaqMan single exon-containing probes to *PTEN*, *HIC1*, *OR5B1*, and *ZNF439* (all pre-made probes from Applied Biosystems) and intron-containing TaqMan probe to *MYCN* obtained from the Institute of Molecular Biology (Moscow, Russia). All 20 DNA neurocytoma samples applied for array-CGH were examined, and PCR amplification was performed in duplicate (aliquots of 50 ng) for each sample. We quantified the tumor DNA by comparing the target locus to the reference *18S rRNA* probe. The relative target copy-number level was also normalized to normal human genomic DNA. Conditions for the quantitative PCR reaction and calculation of copy-number changes of target gene relative to the endogenous

control and calibrator were performed as described previously [22]. Based on these calculations, copy numbers < 1.58 were considered to be a loss of DNA targets whereas those >2.53 were considered to be gains.

Quantitative reverse transcription real-time polymerase chain reaction (QRT-RT-PCR)

Levels of mRNA for 24 selected candidate genes (see Tables 3, 4) were also measured using a real-time fluorescence detection method. Single-stranded cDNA was generated from total RNA using the High Capacity cDNA Archive Kit (Applied Biosystems). QRT-RT-PCR was performed in duplicate for each sample with the 7300 system according to the manufacturer's instructions, using TaqMan gene expression assays with pre-made gene primers (all Applied Biosystems). Amplification products were verified by agarose gel electrophoresis. To standardize the amount of sample cDNA, endogenous control amplicon (*GAPDH*) was used as a house-keeping gene. For tissue-specific normalization, a reference pool of human brain, cerebral cortex total RNA (Cat. No. 636561; BD Bioscience, Heidelberg, Germany) was applied as a calibrator tissue control. The relative quantification of each target was calculated using sequence detection SDS software (version 1.3.1.) according to a previously published algorithm based on threshold criteria of ± 2.0 [13].

Allelic discrimination testing using real-time polymerase chain reaction analysis of single nucleotide polymorphism (SNP) on 17p13.3 chromosomal region

Real-time PCR SNP analysis of 14 intragenic SNP markers spanning of 17p13.3 chromosome locus was performed using predeveloped TaqMan SNP genotyping assay reagents (see Table 5) according to the manufacturer's instructions. Order and allocation of the markers relative to 17p13.3 locus were determined according to the SNP Browser Software. Eight blood DNA samples, isolated from healthy individuals, were used a control. Additionally, we studied three DNA samples obtained from medulloblastomas with isochromosome 17q, which was detected by high-resolution array-CGH analysis [18].

Immunohistochemistry with antibody to LIS1 protein

Immunohistochemical analysis was performed on 5 µm-thick paraffin sections mounted on poly-L-lysine-coated slides. The sections were microwaved in antigen unmasked solution (BD Bioscience, San Jose, CA,

USA). Goat polyclonal antibody to LIS1 (N-19; sc 7577, 1:200; Santa Cruz Biotechnology, Santa Cruz, CA, USA) was applied and the sections were incubated overnight at 4°C. Immunostain visualization was achieved with standard streptavidin–biotin–peroxidase technique (ImmunoCruz™ Staining System; sc-2053; Santa Cruz Biotechnology). The slides were stained with 3,3'-diaminobenzidine, counterstained with hematoxylin, and mounted. For the negative control procedure, primary antibody was substituted according to commercially produced control reagents. For the positive control procedure, sections were obtained from normal non-tumoral human brain tissue.

Statistical analysis

Intraclass correlation analysis was used to assess the degree of linear associations between pairs of variables. The Chi-square test and Fishers' exact test were performed to analyze relationships between clinical variables, pathological features and genetic alterations. A significant correlation between two parameters was taken at the 95% confidence interval. Probability (*P*) values <0.05 were considered significant.

Results

Genomic imbalances in central neurocytomas detected by array-CGH

Each tumor examined displayed DNA copy-number aberrations (CNAs) and the mean number of CNAs per tumor was 38.1 ± 7.1 (range 19–53). The mean number of low-level gains was 14.7 ± 4.1 (range 7–26) and the mean number of copy-number losses was 20.4 ± 3.8 (range 11–31). There were no samples with high-level copy-number gains/amplifications in these 20 tumors. More frequent gains involving at least two neighboring clones were mapped at 2p24.1–22.1

(85%), 10q23.3–26.3 (70%), 11q23–25 (60%), and 18q21.3–qter (50%). Correspondingly, more frequent losses were identified in 1pter–36.3 (70%), 1p34.3 (60%), 6q13–21 (80%), 12q23–qter (60%), 17p13.3 (80%), 17q11–23 (70%), and 20pter–12.1 (60%). The frequently gained and lost single DNA clones affecting $\geq 40\%$ of samples tested are outlined in Tables 1 and 2, respectively.

Correlation analysis of CNAs by chromosomal location revealed a significant association between imbalances in neighboring DNA clones along the same chromosomes and an inverse correlation between loss of *BINI* locus and gain of *MYCN* ($r = -0.63$, $P = 0.0005$).

For validation of array-CGH findings, we compared the results of this study with data obtained by FISH (Fig. 1) and QRT-PCR analyses. There was a high level of concordance between the data from all these techniques (see Tables 1, 2). Also, results obtained by all these methods were correlated strongly for each individual case ($P < 0.05$).

We were unable to find any association between array-CGH data and known clinico–pathological variables, including patient age (<30 or >30 years), gender, and Ki-67 labeling index (<1 vs. >1%).

mRNA expression level for selected candidate genes detected by QRT-RT-PCR

To evaluate the potential effect of defined genomic CNAs for central neurocytoma molecular pathogenesis, we analyzed mRNA expression of 24 selected candidate genes harbored in imbalanced regions of genomic DNA (see Tables 3, 4). Compared with the human cerebral cortex total RNA, *MYCN*, *PTEN*, and *OR5BF1* genes were strongly overexpressed in all 12 samples tested, based on threshold ratio ± 2.0 . In contrast, *BINI* and *SNRPN* genes were found to be strongly underrepresented at the transcriptional level in all neurocytomas examined, whereas the *HRAS*

Table 1 Most frequently gained DNA clones ($\geq 40\%$ of samples examined)

Clone name	Cytogenetic location	Array-CGH (n = 20)	FISH ^a (n = 35)	QRT-PCR (n = 20)
<i>OR5BF1</i> /SHGC-18290	1q44	10 (50%)		9 (45%)
<i>MYCN</i>	2p24.1	14 (70%)	22 (63%)	13 (65%)
<i>MSH2</i> ; <i>KCNK12</i>	2p22.3–p22.1	10 (50%)		
<i>PTEN</i>	10q23.3	12 (60%)	20 (57%)	12 (60%)
<i>KAI1</i>	11p11.2	13 (65%)		
<i>MLL</i>	11q23	8 (40%)		
WI-6509	11q25	9 (45%)		
<i>IGH/D14S308</i>	14q32.33	12 (60%)		
D15S11	15q11–q13	10 (60%)		
<i>PACE4C</i>	15q26	9 (45%)		

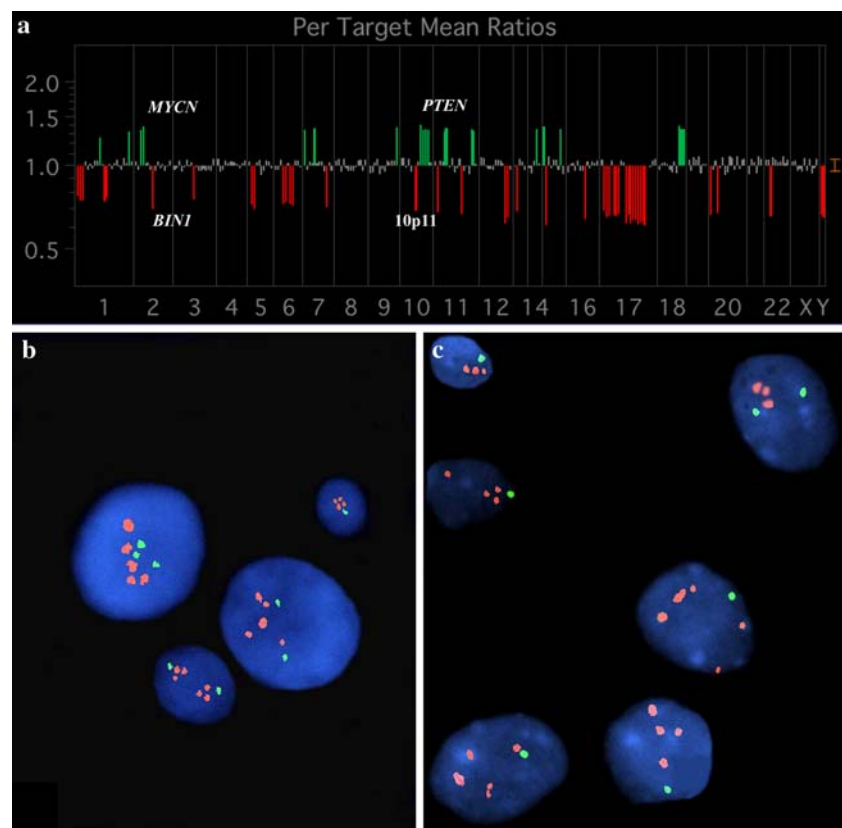
^a Including 20 samples applied for array-CGH and QRT-PCR analyses

Table 2 Most frequently lost DNA clones ($\geq 40\%$ of samples examined)

Clone name	Cytogenetic location	Array-CGH (n = 20)	FISH ^a (n = 35)	QRT-PCR (n = 20)
<i>CDC2L1</i>	1p36.3	10 (50%)		
<i>MYCL1</i>	1p34.3	9 (45%)		
<i>BINI</i>	2q14	12 (60%)		
D5S23	5p15.2	8 (40%)		
<i>APC</i>	5q21–q22	9 (45%)		
D6S414	6p12.1–p21.1	9 (45%)		
D6S268	6q16.3–q21	14 (70%)		
<i>SERPINE1</i>	7q21.3–q22	8 (40%)		
D10S167	10p11–q11	13 (65%)	22 (63%)	
<i>HRAS</i>	11p15.5	9 (45%)		
<i>DRIM; ARL1</i>	12q23	8 (40%)		
<i>RBI</i>	13q14	8 (40%)		
D13S237	13q34	9 (45%)		
<i>SNRPN</i>	15q12	12 (60%)		
<i>FRA16D</i>	16q23.2	9 (45%)		
<i>HIC1</i>	17p13.3	10 (50%)		14 (70%)
<i>LIS1(PAFAH1B)</i>	17p13.3	9 (45%)	15 (43%)	
<i>ERBB2(HER-2)</i>	17q11.2–q12	11 (55%)		
<i>BRCA1</i>	17q21	10 (50%)		
<i>RPS6KB1(STK14)</i>	17q23	10 (50%)		
<i>ZNF439/stSG42796</i>	19p13.2	10 (50%)		12 (60%)
20PTL18	20p ter	11 (55%)		
<i>JAG1</i>	20p12.1–p11.2	8 (40%)		

^a Including 20 samples applied for array-CGH and QRT-PCR analyses

Fig. 1 Array-CGH (a) validation by FISH (b, c). Array-based cytogenetic profile of central neurocytoma (female; 26 years) displays 44 copy-number aberrations including gain of *MYCN* (b; numerous red signals), loss of *BINI*, gain of *PTEN* (c; numerous red signals), and loss of 10p11–q11 (c; single green signals). In addition, numerous losses on both arms of chromosome 17 could be seen



gene was also down-regulated in 10 of 12 samples. For these six genes, the level of over- or underexpression tended to be greater in tumors with gain or loss of the corresponding DNA clones. However, these differ-

ences were not significant. No consistent gene dosage effects between DNA copy-number status and relative gene expression level for the remaining 18 respective candidates were observed.

Table 3 Expression level for 7 genes harbored in the frequently gained clones (12 samples)

Gene symbol	Gene location	TaqMan probe	mRNA expression level, mean ratio \pm SD (range)	Samples with TL > 2.0
<i>OR5BF1</i>	1q44	Hs01088105_s1	4.77 \pm 1.31 (2.19–7.81)	12 (100%)
<i>MYCN</i>	2p24.1	Hs00232074_m1	4.86 \pm 0.95 (2.88–8.31)	12 (100%)
<i>MSH2</i>	2p22.3–p22.1	Hs00179887_m1	0.59 \pm 0.53 (–0.12–0.85)	0
<i>PTEN</i>	10q23.3	Hs00829813_s1	5.16 \pm 1.19 (3.52–7.83)	12 (100%)
<i>KAI1</i>	11p11.2	Hs00174463_m1	0.69 \pm 0.92 (–1.02–1.55)	0
<i>MLL</i>	11q23	Hs00610538_m1	0.78 \pm 0.71 (0.16–1.72)	0
<i>PACE4</i>	15q26	Hs00159844_m1	0.31 \pm 0.52 (–0.31–0.77)	0

TL threshold level of mRNA expression

Table 4 Expression levels for 17 genes harbored in the frequently lost clones (12 samples)

Gene symbol	Gene location	TaqMan probe	mRNA expression level, mean ratio \pm SD (range)	Samples with TL < –2.0
<i>CDC2L1</i>	1p36.3	Hs00169250_m1	1.09 \pm 0.89 (–0.03–1.75)	0
<i>MYCL1</i>	1p34.3	Hs00420495_m1	–1.53 \pm 0.67 (–3.02 to –0.26)	5 (42%)
<i>BINI</i>	2q14	Hs00184913_m1	–3.51 \pm 0.88 (–5.11 to –2.32)	12 (100%)
<i>APC</i>	5q21–q22	Hs00181051_m1	0.73 \pm 0.65 (–0.61–1.39)	0
<i>SERPINE1</i>	7q21.3–q22	Hs00167155_m1	2.13 \pm 1.26 (0.21–4.61)	0
<i>HRAS</i>	11p15.5	Hs00610483_m1	–2.39 \pm 0.56 (–4.16 to –1.56)	10 (83%)
<i>DRIM</i>	12q23	Hs00205657_m1	0.77 \pm 0.63 (–0.12–1.79)	0
<i>RBI</i>	13q14	Hs00153108_m1	3.45 \pm 0.61 (1.49–4.28)	0
<i>SNRPN</i>	15q12	Hs00256087_m1	–4.93 \pm 1.51 (–7.77 to –3.03)	12 (100%)
<i>FRA16D</i>	16q23.2	Hs00249590_m1	0.85 \pm 0.67 (–0.53–1.19)	0
<i>HIC1</i>	17p13.3	Hs00359611_s1	1.07 \pm 0.82 (–0.33–1.75)	0
<i>LIS1</i>	17p13.3	Hs00181182_m1	0.74 \pm 0.58 (–0.43–1.17)	0
<i>ERBB2</i>	17q11.2–q12	Hs00170433_m1	1.13 \pm 0.71 (–0.33–1.72)	0
<i>BRCA1</i>	17q21	Hs00173237_m1	2.37 \pm 0.48 (0.86–3.32)	0
<i>RPS6KB1</i>	17q23	Hs00177357_m1	1.23 \pm 0.75 (0.02–1.98)	0
<i>ZNF439</i>	19p13.2	Hs00752163_s1	3.31 \pm 0.88 (1.46–4.98)	0
<i>JAG1</i>	20p12.1	Hs00164982_m1	1.69 \pm 0.67 (0.41–2.73)	0

TL threshold level of mRNA expression

Allelic discriminations analysis of 17p13.3 chromosomal region

In as much as 17p13.3 was found to be frequently deleted in central neurocytomas, we performed analysis of allelic status for 14 intragenic SNP markers spanning this region. All tumors showed at least one monoallelic loss on this chromosomal locus with a more frequent breakpoint involving 3' untranslated region of *LIS1* gene (Table 5, Fig. 2). Eight control blood DNA specimens disclosed diploid profile for all SNP markers tested, whereas three medulloblastoma specimens showed monoallelic losses for all probes examined.

Immunohistochemistry with LIS1 antibody

To analyze whether the frequently lost *LIS1* DNA clone is also down-regulated at the protein level, we performed an immunohistochemical analysis in 40 neurocytomas (including all 20 samples applied for array-CGH). We revealed strong cytoplasmic LIS1 protein reactivity in all tumors examined and all samples showed greater than 70% of immunostained cells.

Intercellular fibrillary zones and rosette-like structures were also immunopositive. The intensity of LIS1 staining was similar to that revealed in the human brain tissue applied as a positive control.

Discussion

To identify recurrent copy-number imbalances in 20 central neurocytomas, we used low-density DNA microarray assay with the GenoSensor 300 platform, which allows for the rapid investigation of multiple, cancer-associated abnormalities in chromosomal loci throughout the entire tumor genome [1, 15, 21]. During the current array-CGH screening, we found a set of frequently altered DNA clones that may include candidate genes for tumorigenesis of these infrequent CNS neoplasms. The results of microarray experiments were validated by FISH and QRT-PCR data analyses. Interestingly, neurocytomas, which are traditionally biologically benign neoplasms, contained significantly fewer CNAs per tumor than highly malignant glioblastomas, studied previously with the GenoSensor Array 300 [15].

Table 5 Allelic status of 14 intragenic SNP markers spanning 17p13.3 region

Gene name	Position	SNP probe ID	Location	Monoallelic losses ^a
<i>ABR</i>	0.85–1.03 Mb	C_12122386_1	Intron	13 (65)
<i>TUSC5</i>	1.12–1.15 Mb	C_15967479_1	UTR3'	6 (30)
<i>YWHAE</i>	1.19–1.25 Mb	C_1204773_10	Intron	9 (45)
<i>CRK</i>	1.27–1.31 Mb	C_1223186_10	Intron	10 (50)
<i>MYO1C</i>	1.31–1.34 Mb	C_1223175_1	Intron	13 (65)
<i>SERPINF2</i>	1.59–1.60 Mb	C_1387533_1	Intron	14 (70)
<i>SMYD4/SERPINF1</i>	1.61–1.68 Mb	C_12125626_10	Intron	12 (60)
<i>RPA1</i>	1.68–1.75 Mb	C_15957490_10	Intron	17 (85)
<i>OVCA2/DPHI</i>	1.88–1.89 Mb	C_8729828_10	UTR3'	9 (45)
<i>SRR/c17orf31</i>	1.91–2.19 Mb	C_1625593_10	Intron	13 (65)
<i>RUTCB1/FLJ10534</i>	2.19–2.23 Mb	C_11954781_10	Intron	11 (55)
<i>MNT</i>	2.23–2.26 Mb	C_3044268_10	Intron	12 (60)
<i>PAFAH1B1 (LIS1)</i>	2.44–2.55 Mb	C_9274341_10	Intron	13 (65)
<i>PAFAH1B1 (LIS1)</i>	2.44–2.55 Mb	C_2593428_10	UTR3'	19 (95)

Mb megabase pairs, UTR3'–3' untranslated region

^a Number (%) of tumors containing monoallelic losses of the SNP marker

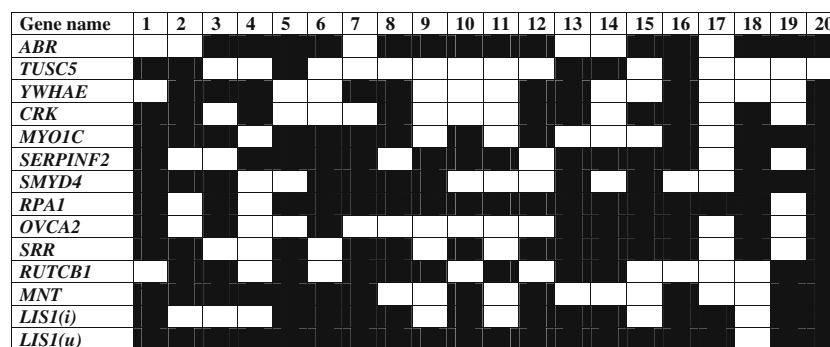


Fig. 2 Graphic summary of the results from the allelic discrimination analysis of 17p13.3 locus using 14 intragenic SNP markers. Case numbers are given on the *top of the panel* and the investigated SNP markers are given on the *left of the panel*. Order and allocation of the markers relative to 17p13.3 locus were deter-

mined according to the SNP Browser Software. *Filled squares* SNP loci with monoallelic loss (homozygosity), *open squares* heterozygous SNP loci with diploid (balanced) alleles; *LIS1(i)* intron SNP marker, *LIS1(u)* UTR3' SNP marker

Notably, some current array-CGH findings such as gains at 2p, 10q, and 18q are consistent with the data obtained previously by conventional CGH analysis [32]. Also, frequent deletions at both arms of chromosome 17 are in line with data obtained by Cerda-Nicolas et al. [4], who observed monosomy 17 in neurocytoma. The histopathological resemblance of neurocytomas with oligodendrogliomas prompted us to analyze 1p/19q status in these tumors. We found that small deletions including single DNA clones on 1p36.3 and 1p34.2 are frequent in neurocytomas, whereas array-CGH oligodendroglioma analyses usually disclosed stretched submegabase deletions at 1p [10, 19, 26]. Thus, our findings are in concordance with data of LOH analysis performed by Tong et al. [31], who found a few interstitial deletions on 1p in six of nine neurocytomas but most of the markers examined were retained. In contrast to oligodendrogliomas, we established deletions of clones from 19q13.3 in a single sample only.

A set of imbalanced DNA clones harbored a few genes that appeared to be promising candidates for the

molecular pathogenesis of neurocytomas. At first view, the frequent deletion of *LIS1* clone is a very attractive finding because this gene encodes a protein that regulates neuronal migration from the periventricular matrix. Loss of this locus is associated with cortical malformations (lissencephaly and subcortical band heterotopia) [17]. Nevertheless, expression analysis revealed appropriate mRNA levels for this gene accompanied by a strong immunoreactivity of LIS1 protein product in all examined cases. Similarly, other potent candidate genes showed no alterations at the transcriptional level. Moreover, a few genes from deleted DNA clones (*SERPINE1*, *RB1*, *BRCA1*, *ZNF439*) revealed strong mRNA expression in the majority of tested samples, suggesting dosage overcompensation mediated by the remaining allele. Probably, inactivation of the other closely linked genes included in these imbalanced DNA clones may be functionally significant for neurocytoma development. The results of allelic discrimination analysis with 14 intragenic SNP markers spanning 17p13.3 locus confirm this suggestion.

Nevertheless, six genes showed strong concordance between imbalanced DNA clones and relative mRNA expression levels, which strongly implicates their relevance in the molecular pathogenesis of central neurocytomas. A DNA clone at 2p24.1, which contains a *MYCN* proto-oncogene, was found to be gained in 70% of neurocytomas. Correspondingly, QRT-RT-PCR analysis revealed strong overexpression of *MYCN* mRNA in all samples tested. *MYCN* amplification/overexpression is frequently observed in neuroblastomas and medulloblastomas [11]. On the other hand, embryological studies showed that *MYCN* plays a significant role in neurogenesis, because its expression is essential for the rapid expansion of neural progenitor cells during CNS development and inhibition of neuronal differentiation [12]. Thus, *MYCN* may be a “driver oncogene” in neurocytoma pathogenesis and aid in renewal of the tumor cells population.

Gain of *MYCN* was inversely correlated with a *BINI* deletion and this gene was decreased at the transcriptional level in all samples tested. *BINI* (bridging integrator 1) encodes for multiple tissue-specific isoforms of the MYC-integrated adaptor protein implicated in tumor suppression and cell death processes in human malignancies [28]. *BINI* expression was reduced in various human tumors. Moreover, the mRNA level of its *MYCN*-integrating isoform was found to be down-regulated in neuroblastomas [28]. Therefore, *BINI* appears to be a promising candidate suppressor gene for neurocytoma tumorigenesis, whose structural and/or functional alterations can lead to development of these neoplasms. Distinct mechanisms underlying the remaining *BINI* allele inactivation (haploinsufficiency, point mutations, and promoter hypermethylation) should be elucidated in further studies.

The tumor suppressor gene *PTEN* plays an important role in neuronal development, because loss of its expression is associated with cerebellar dysplasia and other CNS migrational defects [16]. However, *PTEN* loss does not inhibit neuronal differentiation [16]. *PTEN* expression fluctuates in neurogenesis and seems to be up-regulated at or near of birth [16]. On the other hand, *PTEN* overexpression in the pheochromocytoma cell line PC12, inhibits neuronal differentiation and provokes down-regulation of nerve growth factor receptors [20]. Perhaps, strong *PTEN* overexpression in neurocytomas (in comparison to the normal human cortex) can inhibit further maturation of tumor cells thereby increasing the proliferating cell population. However, support for such an assertion will require additional analyses.

SNRPN encodes a small ribonuclear protein and deletion of this region is critical for development of Prader–Willi syndrome, which is associated with neurodevelopmental disorders [7]. Mice lacking genes responsible for Prader–Willi syndrome display a widespread deficit in neuronal migration, and the extension, arborization, and fasciculation of axons during of CNS development [24]. Deletions of *SNRPN* locus were discovered in glioblastomas [15] and gastric carcinomas [29] and methylation of this gene was established in germ-cell tumors [3]. The distinct role of frequent *SNRPN* inactivation for neurocytoma pathogenesis appears to be unclear, although, hypothetically, its tumor suppression function cannot be fully excluded in these tumors.

HRAS allelic deletion was found to be a common abnormality in various tumors and these findings suggest that a putative tumor suppressor closely linked to the *HRAS* locus on 11p15.5 is involved in human carcinogenesis. On the other hand, the Ras/MAPK signal transduction pathway regulates synaptic plasticity and survival of CNS neurons [5]. Moreover, *HRAS* protein is essential for differentiation and neurite outgrowth-promoting activity of human embryonic neural cells [2]. Perhaps, frequent *HRAS* down-regulation in neurocytomas may result in the inhibition of further differentiation in tumor cells.

OR5BF1 belongs to the human olfactory receptor family, which includes more than 900 members distributed throughout the human genome. These genes encode for an immense variety of membrane-bound G-protein-coupled receptors that can represent specific targets for a variety of human diseases including cancer. *OR5BF1* is strongly overexpressed in neurocytomas, and it is thought that it may function as a potent oncogene in these tumors. A detailed investigation of its function in neuronal tumors is warranted.

In summary, neurocytoma tumorigenesis appears to be associated with molecular mechanisms that activate the proliferation of tumor cells and, simultaneously, inhibit further neuronal maturation. Also, recurrent genetic alterations in central neurocytomas share some similarities with those defined in neuroblastomas including deletions on 1p36, gain/overexpression of *MYCN*, and the transcriptional inactivation of *BINI*. Thus, despite the striking histological and biological differences between neurocytoma and neuroblastoma, the current findings hint on a certain resemblance of pathobiological mechanisms responsible for the tumorigenesis of various neural-derived neoplasms. Further global microarray-based analyses of neurocytomas are necessary to identify new candi-

date genes and chromosomal regions having both pathogenetic and prognostic relevance for these enigmatic brain tumors.

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