Molecular Cytogenetic Stratification of Recurrent Oligodendrogliomas: Utility of Interphase Fluorescence in Situ Hybridization (I-FISH)

(brain tumours / oligodendroglioma / I-FISH / deletion of 1p36 and 19q13.3)

Z. ZEMANOVÁ[1], F. KRAMAR[2], L. BABICKÁ[1], Š. RANSDORFOVÁ[3], J., MELICHERČÍKOVÁ[3], P. HRABAL[4], P. KOZLER[2], K. MICHALOVÁ[1,3]

[1]Centre of Oncocytogenetics, Institute of Clinical Biochemistry and Laboratory Diagnostics, 1st Faculty of Medicine, Charles University, and General Teaching Hospital, Prague, Czech Republic
[2]Neurosurgical Department, Central Military Hospital, Prague, Czech Republic
[3]Institute of Haematology and Blood Transfusion, Prague, Czech Republic
[4]Department of Pathology and Anatomy, Central Military Hospital, Prague, Czech Republic

Abstract. In oligodendroglial brain tumours, losses of chromosomal material of the short arm of chromosome 1 and long arm of chromosome 19 have been shown to predict responsiveness to chemotherapy and prolonged patients' survival. Therefore, the correct diagnosis of these genetic alterations in tumours of oligodendroglial origin is particularly important. To detect deletions of 1p36 and/or 19q13.3 in oligodendroglial cells we used dual-colour I-FISH with locus-specific DNA probes. I-FISH was performed on isolated whole cell nuclei, prepared from fresh non-fixed tumour tissue samples resuspended in media and processed using a standard cytogenetic procedure, thus bypassing the problem of nuclear truncation. We examined 16 patients with histologically proved oligodendrogliomas (5x oligodendroglioma, 9x anaplastic oligodendroglioma, 2x anaplastic oligoastrocytoma). The results of molecular cytogenetic analyses were correlated with morphological and clinical findings. Molecular cytogenetic analyses were successful in 15 patients and, due to a non-adequate tissue specimen, were uninformative in one patient only. Combined deletions 1p36/19q13 were proved in 13 patients. However, in six of them additional genetic alterations typical for high-grade astrocytoma were found, which could have negative influence on the prognosis. One patient had isolated deletion of 1p36 and another had a normal genetic pattern without any chromosomal alterations. In summary, I-FISH on isolated cell nuclei is a powerful tool for detecting chromosomal aberrations in tumour cells. A systematic molecular cytogenetic analysis may advance diagnosis, prognostic stratification, and targeted treatment of patients with brain tumours.

Introduction

Diffuse gliomas are the most common primary tumours of the central nervous system affecting adults. It is a heterogeneous group of tumours with various histological subtypes that differ in response to treatment and in the prognosis of the disease (Smith et al., 2000). The most frequent tumours are derived from astrocytes and from oligodendrocytes. Traditionally, the classification and grading system of glial tumours is based on histopathological features, such as resemblance of astrocytic or oligodendrocytic cells, nuclear pleomorphism, mitotic activity, proliferation rate, necrosis, etc. The World Health Organization classification of gliomas recognizes three major histological types of tumours: astrocytomas, oligodendrogliomas, and oligoastrocytomas. Each type can be further divided into two or three malignancy grades (Coweli et al., 2004). Oligodendrogliomas are graded using a two-tiered system where well-differentiated tumours are considered to be Grade II and anaplastic variants are Grade III (Kelley et al., 2005).

The treatment of diffuse gliomas (surgery, radiotherapy and chemotherapy) is problematic due to their diffuse nature. Surgical intervention never succeeds in removing the tumour tissue completely. This is why the disease relapses and progresses even in case of lower-grade tumours (Godard et al., 2003). Differentiation of glial subtypes based solely on nuclear and cellular morphology is subjective and various subtypes sometimes cannot be distinguished even when using specific immunohistochemical markers. However, the correct diagnosis of tumours of oligodendroglial origin (including oligoastro-

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Corresponding author: Zuzana Zemanová, Centre of Oncocytogenetics, Institute of Clinical Biochemistry and Laboratory Diagnostics, 1st Faculty of Medicine, Charles University, and General Teaching Hospital, Prague, U Nemocnice 1, 128 08 Prague 2, Czech Republic, e-mail: zuze@vfn.cz

Abbreviations: CEP – chromosome enumeration probe, CGH – comparative genomic hybridization, EGFR – epidermal growth factor receptor, I-FISH – interphase fluorescence *in situ* hybridization, LSI – locus-specific.

cytomas) is particularly important because 60% to 70% of anaplastic oligodendrogliomas (AO) are sensitive to combined chemotherapy and their prognosis is significantly better than that for astrocytic tumours of corresponding grades (Coweli et al., 2004). Therefore, new diagnostic and prognostic indicators must be sought to enable stratification of the treatment and to help reduce morbidity and mortality of patients. Subclassification of patients according to specific chromosomal aberrations in tumour cells is one of the possibilities.

Cytogenetic and molecular studies of glial tumours performed during the last decade identified several recurrent, non-random genetic abnormalities associated with different subtypes of glial tumours. Specific gene mutations, loss of heterozygosity, deletions and/or amplifications of entire chromosomal regions were described in specific tumours including gliomas. Gliomas are characterized particularly by chromosomal deletions, inappropriately activated intracellular signalling pathways, and/or loss of the activity of tumour suppressor proteins. The most frequent specific alteration in oligodendroglial tumours, grades II and III, is the combined loss of chromosomal arms 1p and 19q, which could be found in 50–80% of patients. In astrocytic tumours of higher grades, other chromosomal aberrations associated with tumour progression could be found. The most frequent and most important alterations thought to influence the prognosis include 9p21 (*p16*, CDKN2A), 13q14 (*RB1*), 10q23 (*PTEN*) deletions and/or monosomy of the whole chromosome 10 as well as epidermal growth factor receptor (*EGFR*) amplification (Table 1).

Over the last few years, certain molecular cytogenetic and molecular genetic techniques have been developed and applied to the workup of gliomas. This has resulted in improved diagnostic and prognostic accuracy and has allowed the clinician to formulate a potentially more effective treatment strategy. In this study we used interphase fluorescence *in situ* hybridization (I-FISH) and/or comparative genomic hybridization (CGH) for detection of specific genetic alterations in oligodendroglial cells. I-FISH was performed on whole cell nuclei prepared from tumour cell suspensions by a standard cytogenetic procedure. This approach overcomes the problem of nuclear truncation, which is characteristic for analysis of sections prepared from frozen or formalin-fixed, paraffin-embedded tissue.

Our work was aimed at detailed molecular cytogenetic study in a group of patients with histologically proved oligodendrogliomas and/or oligoastrocytomas with focus on detection of the most frequent and prognostically most important chromosomal aberrations described in oligodendroglial tumours so far, i.e. deletions of chromosomal regions 1p36 and 19q13.3, and on comparison of the results of the molecular cytogenetic analysis in all patients with morphological and clinical findings.

Material and Methods

Patients

The study included 16 patients with histologically confirmed oligodendrogliomas and/or oligoastrocytomas (WHO grade II-III) admitted to the Department of Neurosurgery of the 1st Faculty of Medicine and Central Military Hospital in Prague between March 2004 and December 2005. All examinations were carried out on cells acquired from tumour tissue taken during a routinely performed neurosurgical procedure. All patients gave written informed consent to the proposed therapeutic procedure and to the provision of samples for research purposes.

Sample extraction and processing

Fresh non-fixed tumour tissue samples were resuspended in media (PBS, heparin) and further processed using standard cytogenetic procedure (hypotonia, fixation). Standard microscopic preparations for I-FISH were prepared from fixed cell suspensions. When a sufficient amount of a sample was available, part of the tumour tissue was used for isolation of DNA, which was further processed by CGH.

Table 1. Specific chromosomal aberrations in diferent subtypes of diffuse gliomas

type of diffuse glioma	subtype	WHO grade	chromosomal aberrations
astrocytoma	diffuse astrocytoma	II	deletion/mutation of *p53* gene, aneuploidy
	anaplastic astrocytoma	III	deletion of *p53* gene, deletion of *p16* gene, deletion of *RB1* gene, aneuploidy
	glioblastoma multiforme – primary	IV	**amplification of *EGFR* gene**, deletion of *p16* gene, deletion of *RB1* gene, **monosomy 10**, aneuploidy
	glioblastoma multiforme – secondary	IV	deletion of *p53* gene, deletion of *p16* gene, deletion of *RB1* gene, **monosomy 10**, aneuploidy
oligodendroglioma	low-grade oligodendroglioma	II	deletion of 19q13.3, deletion of 1p36, aneuploidy
	anaplastic oligodendroglioma	III	**deletion of 19q13.3 / deletion of 1p36**, aneuploidy (combined deletions are markers of good prognosis)

Molecular cytogenetic analyses

I-FISH: Dual-colour interphase FISH with locus-specific (LSI) and/or chromosome enumeration (CEP) DNA probes (Abbott Vysis™, Des Plaines, IL) was carried out according to manufacturers' recommendations for detection of the most frequent chromosomal changes in glial cells. All samples were analysed by the LSI 1p36/1q25 and LSI 19q13/19p13 dual-colour probe set for detection of the 1p36 deletion (spectrum green-labelled 1q25 control probe and spectrum orange-labelled 1p36 test probe) and the 19q13 deletion (spectrum green-labelled 19p13 control probe and spectrum orange-labelled 19q13 test probe). In some cases probes for detection of deletion of *RB1* and/or *p16* genes, amplification of the *EGFR* gene and/or monosomy of chromosome 10 were also used (used probes are listed in Table 2). Cells were counterstained with DAPI (4',6-diamidino-2-phenylindole). Slides were viewed using a fluorescent motorized Axioplan II microscope (Zeiss, Göttingen, Germany) with appropriate optical filters by two independent observers. In each case, hybridization signals of at least 200 tumour cell interphase nuclei were assessed. The ratio between the numbers of fluorescence signals of tested and control regions on chromosomes 1 and 19 was calculated. The image was captured by a sensitive CCD camera and the result was processed by specialized *ISIS* software (MetaSystems GmbH, Altlussheim, Germany) for documentation.

The cut-off value (i.e. the breakpoint for classification of the findings as positive or negative) was determined using control group samples obtained by brain tissue resection in patients who underwent epileptosurgery. In these samples, I-FISH analyses were performed using the same procedure and appropriate DNA probes. The average value and the standard deviation for the entire control group were determined. The breakpoint was defined as the average value +2 SD, which ensured the level of the diagnostic test specificity of 97.5% for amplifications and/or 95.0% for deletions, assuming normal error distribution.

CGH: CGH analysis was performed on the same material used for cytogenetic analysis according to the previously published procedure (Kallioniemi et al., 1992). Briefly, DNA was extracted using liquid nitrogen and a DNA isolation kit for mammalian blood (Roche Diagnostics GmbH, Manheim, Germany). Tumour DNA was labelled with a Spectrum Green dUTP by CGH Nick Translation Kit (Abbott Vysis™). Approximately 200–400 ng Spectrum Green tumour DNA and 200 ng Spectrum Red normal reference DNA (Abbott-Vysis) were ethanol-precipitated together with 10 μg Cot-1 DNA, centrifuged, resuspended in CGH hybridization buffer, denatured, and hybridized to normal metaphase slides for 3 days. After hybridization, the slides were washed for 2 min in 0.4x SSC/0.3% NP40 at 74ºC, and 30 s in 2x SSC/0.1% NP40 at ambient temperature. CGH analysis was performed using *ISIS* software (MetaSystems™). For each analysis, at least 15 metaphases were captured. Interpretation of CGH profiles was done according to the published criteria (Isola et al., 1994).

Results

Clinical data

We examined 16 patients with histologically proved oligodendroglial tumours (5x oligodendroglioma, WHO grade II; 9x anaplastic oligodendroglioma, WHO grade III; 2x anaplastic oligoastrocytoma, WHO grade III). There were six men and 10 women with mean age 43.5 years (ranging from 24 to 69 years). Clinical and treatment data of the patients are given in Table 3. Fourteen patients are alive, two women died – one of tumour progression and the other of non-oncological disease. Mean overall survival was 37.6 months (ranging from 4 to 135 months). Recurrence or tumour progression occurred in 10 cases (1–3 times). Mean time of progression-free survival (PFS) in our patient cohort was 24.2 months (median 12.5 months; ranging from 4 to 111 months). Six patients are alive at present, without any symptoms of progression and/or recurrence of the disease.

Table 2. DNA probes for I-FISH (Abbott Vysis)

Chromosome aberration	DNA probe mix	labelling
deletion of *p16 (CDKN2A)*	LSI® *p16* (9p21) / CEP® 9	SO /SG
deletion of *RB1*	LSI® *RB1* (13q14) / LSI® 13qter	
deletion of 1p36	LSI® 1p36 / LSI® 1q25	
deletion of 19q13.3	LSI® 19q13/ LSI® 19p13	
amplification of *EGFR*	LSI® *EGFR* / CEP® 7	
monosomy 10	CEP® 10 / control CEP® probe	

CEP – chromosome enumeration probe (centromeric, α satellite); LSI – locus-specific; SO – Spectrum Orange; SG – Spectrum Green

Table 3. Clinical data of patients

Pat. No.	Age at dg (years)	Sex	dg	WHO grade	Localization	KFS	Resection	Ki-67 index (%)	Necrosis	Chemotherapy	Radiotherapy	Reccurence or progression	PFS (mo)	OS (mo)
1	36	M	AO	III	F	100	total	3.0	no	PCV	no	yes	25	78+
2	54	F	AO	III	F	0	partial	nd	yes	temozolomide	no	yes	12	16
3	38	M	AO	III	F	100	total	20.0	no	PCV	no	yes	6	25+
4	38	F	AO	III	CC	100	biopsy	nd	yes	PCV	no	no	18+	18+
5	59	M	AO	III	PT	100	partial	nd	no	temozolomide	no	no	13+	13+
6	52	F	AO	III	PT	100	partial	nd	no	PCV	no	yes	10	21+
7	24	M	AO	III	F	100	total	5.0	no	PCV	yes	yes	30	58+
8	69	F	AO	III	F	90	total	nd	yes	PCV	no	no	5+	5+
9	62	F	AO	III	F	90	partial	nd	no	PCV	no	no	4+	4+
10	30	M	AO	III	F	100	partial	nd	yes	PCV	yes	yes	111	135+
11	24	F	AOA	III	F	100	total	nd	no	PCV	no	yes	54	63+
12	39	F	AOA	III	F	100	partial	10.0	yes	temozolomide	yes	no	9+	9+
13	56	F	O	II	F	0	partial	0.5	no	WS	no	yes	11	20
14	31	F	O	II	F	100	total	5.0	no	WS	no	yes	47	79+
15	54	F	O	II	F	100	biopsy	15.0	no	WS	no	yes	28	53+
16	30	M	O	II	F	100	total	0.5	no	WS	no	no	4+	4+

Diagnosis: O – oligodendroglioma, AO – anaplastic oligodendroglioma; AOA – anaplastic oligoastrocytoma
Localization: F – frontal lobe; CC – corpus callosum; PT - parietotemporal
Chemotherapy: PCV – procarbazine/lomustine/vincristine; WS – watch and scan
PFS – progression-free survival in months
OS – overall survival in months
nd – not done

Table 4. Molecular cytogenetic findings

Pat. No.	Deletion 1p36	Deletion 19q13.3	Polysomy 7 / *EGFR* amplification	Monosomy 9 / deletion CDKN2A	Deletion RB1	Monosomy 10	CGH
1	**yes**	**yes**	no	**yes**	no	no	nd
2	**yes**	**yes**	no	**yes**	no	**yes**	nd
3	**yes**	**yes**	**yes**	**yes**	no	no	nd
4	no	no	nd	nd	nd	nd	nd
5	no	no	nd	nd	nd	nd	nd
6	**yes**	**yes**	nd	nd	nd	nd	nd
7	**yes**	**yes**	**yes**	**yes**	**yes**	**yes**	nd
8	**yes**	**yes**	nd	nd	nd	nd	del(1p),del(5q),+7,-9,+11
9	**yes**	**yes**	**yes**	nd	nd	no	del(1p),-18,del(19q),del(22q)
10	**yes**	**yes**	nd	nd	nd	nd	nd
11	**yes**	**yes**	nd	nd	nd	nd	nd
12	**yes**	no	nd	nd	nd	nd	nd
13	**yes**	**yes**	**yes**	no	no	no	nd
14	**yes**	**yes**	no	nd	nd	no	del(1p),del(16q),del(19q),del(22q)
15	**yes**	**yes**	no	nd	nd	no	nd
16	**yes**	**yes**	no	nd	nd	no	nd

nd – not done

Data of I-FISH analysis

Molecular cytogenetic findings are given in Table 4. I-FISH results in most cases corresponded well with histological and clinical findings and confirmed original diagnosis. Molecular cytogenetic analyses were successful in 15 patients (94%) and were uninformative in one patient only due to a non-adequate tissue specimen (No. 4).

Combined deletions of 1p36 and 19q13.3 regions were proved in 13 patients (Figure 1a, 1b). Besides 1p36/19q13 deletions, additional genetic alterations typical for high-grade glioma were detected in six of

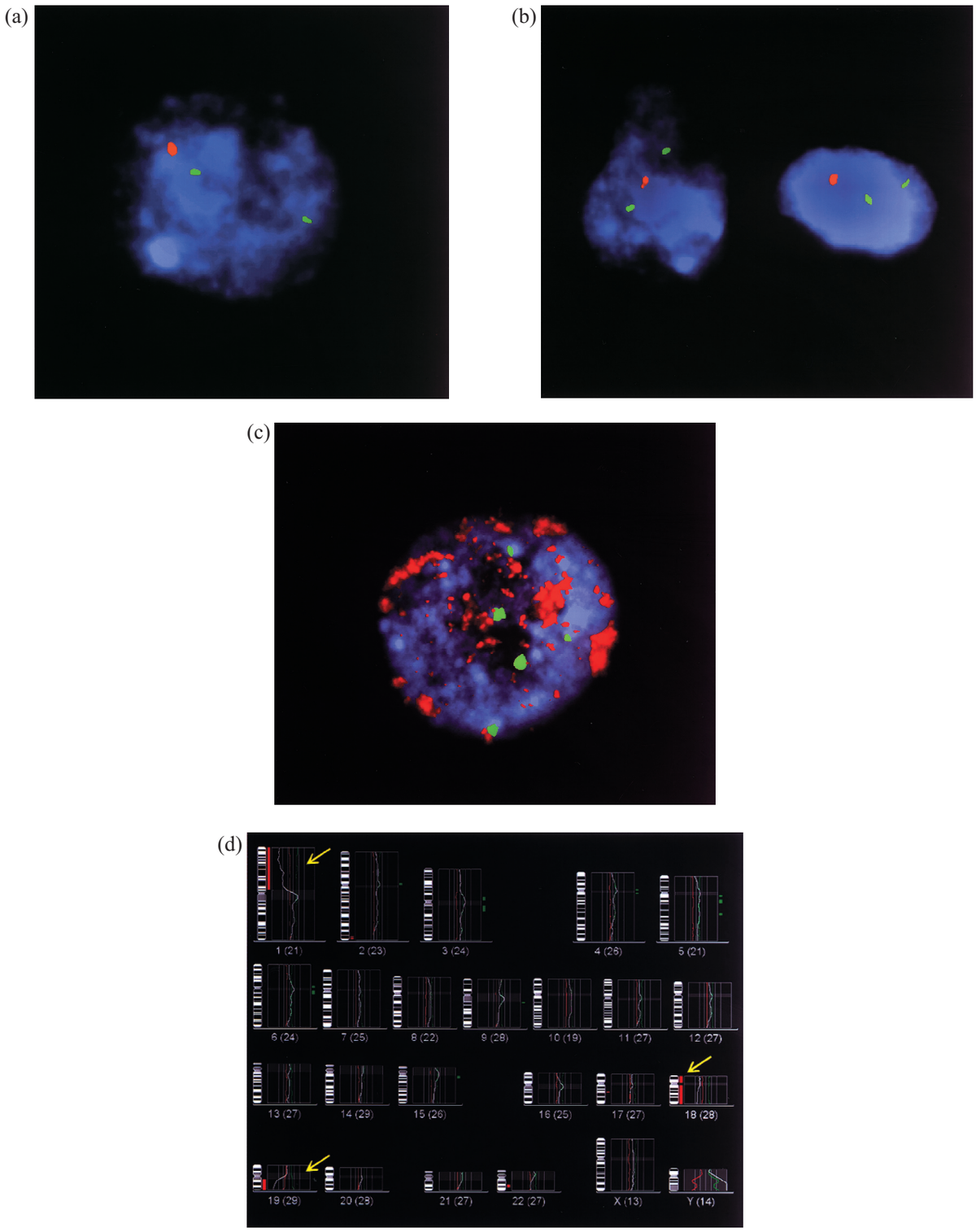

Fig. 1. **(a)** Non-dividing nuclei of brain tumour cells of patient No. 1 after hybridization with LSI 1p36 (red signal) / LSI 1q25 (green signal) Dual Color Probe Set (Abbott-Vysis™) showing deletion of the 1p36 region. **(b)** Abnormal cells of the same patient (No. 1) hybridized with LSI 19p13 (green signal) / LSI 19q13 (red signal) Dual Color Probe Set (Abbott-Vysis™). Deletion of the 19q13 region was proved. **(c)** LSI EGFR (red signal) / CEP 7 (green signal) Dual Color Probe (Abbott-Vysis™) hybridized to a nucleus indicating five copies of chromosome 7 and high amplification of the *EGFR* gene (patient No. 13). **(d)** CGH profile of patient No. 9, which shows loss of 1p, 19q and monosomy of the whole chromosome 18 [ish cgh dim(1)(p13pter),dim(18),dim(19)(q13qter)].

the patients. These findings could have a negative influence on the prognosis of the disease (*EGFR* amplification or polysomy of chromosome 7 in four cases (Figure 1c), monosomy 10 in two cases, monosomy 9 or deletion of *CDKN2A* in four cases, and deletion of the *RB1* gene in one case). Recurrence or tumour progression appeared in five of these cases. In one patient (No. 11), isolated deletion of 1p36 was detected. One tumour sample (No. 5) was classified as normal without any chromosomal alteration.

Data of CGH analysis

CGH analysis was done in three patients with oligodendroglioma. Combined deletion 1p/19q was confirmed in concordance with FISH analyses in patient No. 9 (Figure 1d) and patient No. 14. Patient No. 8 was positive for combined deletion of 1p36 and 19q13 according to FISH, contrary to CGH analysis, which did not prove deletion of the 19q region due to the resolution of this method. In addition, CGH revealed deletion of the terminal region of chromosome 5q, trisomy of chromosomes 7, 11, and monosomy of chromosome 9 in patient No. 8.

Discussion

Oligodendrogliomas are glial tumours in which the oligodendroglial cell is the predominant cell type. In contrast to other types of gliomas, oligodendroglioma is associated with generally good response to adjuvant therapy (Paleologos and Cairncross, 1999). Therefore, in patients with oligodendrogliomas chemotherapy is recommended as the primary treatment modality. The diagnosis of oligodendrogliomas, however, is frequently complicated by the fact that many tumours contain an astrocytic component. Furthermore, the pathology of these tumours is very complex and differentiation of glial subtypes based solely on morphology is subjective, and various subtypes cannot be distinguished even when using specific immunohistochemical markers. It is essential to distinguish oligodendrogliomas from astrocytomas because of the different therapeutic approaches to these tumours. Due to the limitation of histological classification and grading system, new prognostic factors must be found. Genetic alterations in gliomas seem to be one of these new independent prognostic markers (Belaud-Rotureau et al., 2006).

Cytogenetic and molecular studies have identified genetic aberrations during the tumorigenesis and progression of gliomas. The most characteristic are chromosomal deletions, inappropriately activated intracellular signalling pathways, and/or loss of the activity of tumour suppressor proteins. In high-grade gliomas, several specific deletions could be found: deletion of the 9p21 region (*p16*, *CDKN2A*), deletion of the 13q14 region (*RB1*), deletion of the 10q23 region (*PTEN*) and/or monosomy of the whole chromosome 10. Furthermore, a common finding in primary glioblastomas (grade IV) is the amplification of the gene for EGF transmembrane receptor (*EGFR*). All these findings are associated with tumour progression and rather poor prognosis of patients.

Interestingly, several studies based on karyotype analysis have shown that the deletion of the short arm of the chromosome 1 and/or of the long arm of the chromosome 19 are recurrently observed in gliomas, particularly in oligodendroglial tumours. These deletions generally involve nearly the whole chromosome arm (Belaud-Rotureau et al., 2006). In oligodendrogliomas, such finding is considered to be a marker of good response to chemotherapy and longer progression-free (PFS) and/or overall survival (OS).

Non-random losses of the genetic material from chromosome 1 (especially telomeric parts of short arms) were detected in a wide range of tumour diseases, from various types of solid tumours to leukaemias and myeloproliferative diseases, which gives evidence of the fundamental role of alterations of chromosome 1 in the pathogenesis of neoplasias. It is therefore expected that the 1p region contains one or more candidate tumour suppressor genes although they have not been precisely determined so far. In glioma, allelic losses in the 1p36 region most frequently occur between the genetic markers D1S1612 and D1S468 (Smith et al., 2000). The deletion of 1p36 is relatively rare in astrocytomas, but it is often found in anaplastic oligodendrogliomas, in which it is associated with higher sensitivity to chemotherapy and prolonged survival (Ino et al., 2001). On the contrary, deletions of 19q were described in all three glial subtypes, with minimum deleted regions defined in the locus 19q13.3 (D19S412-D19S596). In other malignancies, deletions of 19q are relatively rare, although they have been described, for example, in acute lymphocytic leukaemia, lung cancer or familial breast cancer. Therefore, it can be assumed that the 19q13.3 region includes a candidate tumour suppressor gene specific for gliomas (Smith et al., 1999). Concerning prognosis in gliomas, the finding of combined deletions of regions 1p36 and 19q13.3 is especially important for patients with anaplastic oligodendrogliomas and is a predictor of good response to chemotherapy and longer overall survival in patients treated with the PCV (proctarbazine, lomustine, vincristine) regimen (Smith et al., 2000; Ino et al., 2001). Combined 1p36/19q13.3 losses have been mostly observed in oligodendrogliomas (50–93 % cases), less frequently in oligoastrocytomas (9–58 % of cases) and very rarely in astrocytomas (<10 % of cases) (Fuller et al., 2003).

Over the last few years, certain molecular cytogenetic and molecular genetic techniques have been developed and applied to the workup of gliomas and, in some instances, they have become a routine part of the evaluation process. Besides polymerase-chain reaction

(PCR)-based strategies for detection of loss of heterozygozity (LOH), genetic alterations may potentially be detected by both basic molecular cytogenetic techniques FISH and/or CGH. I-FISH with specific DNA probes in paraffin-embedded tissues is a classical technique for analysis of chromosomal aberrations in brain tumour cells. However, one major limitation of this method is nuclear truncation in tissue sections, complicating evaluation and interpretation of hybridization results (Gelpi et al., 2003). To circumvent the problem of signal truncation, we analysed the 1p and 19q status in 16 samples of oligodendroglial neoplasms (4x oligodendrogliomas, grade II; 10x anaplastic oligodendrogliomas, grade III; 2x anaplastic oligoastrocytomas, grade III) using I-FISH on isolated whole tumour cell nuclei, prepared from fresh non-fixed tumour tissue samples, resuspended in media and processed using a standard cytogenetic procedure.

This approach yielded interpretable results in 15 cases out of 16 analysed (94 %). Molecular cytogenetic analysis was uninformative in one patient only due to a non-adequate tissue specimen (No. 4). Combined deletion of 1p36 and 19q13.3 regions was proved in 13 patients, eight with anaplastic oligodendroglioma, two with anaplastic oligoastrocytoma, and four with oligodendroglioma. Besides 1p36/19q13 deletions additional genetic alterations typical for high-grade astrocytoma were found in six of them. These findings could have negative influence on the prognosis of the disease. Recurrence or tumour progression appeared in five of these cases.

Deletion of the 9p21 region was e.g. proved in tumour tissue of patient No. 1. with original diagnosis of well-differentiated oligodendroglioma (grade II), in whom the tumour recurred three times and progressed to anaplastic oligodendroglioma (grade III). *CDKN2A*, a tumour suppressor gene on chromosome 9p21 encoding the *p16* protein, is homozygously deleted in a subset of previously reported anaplastic oligodendrogliomas and is considered a negative prognostic factor. The same 9p21 region is also similarly deleted in the majority of anaplastic astrocytomas and glioblastomas, suggesting that it is rather associated with high-grade gliomas in general than with a specific glial lineage. Analogously to our findings, the loss of *p16* has also been associated with progression from well-differentiated to anaplastic oligodendroglioma (Fallos et al., 2004).

In patient No. 13 with original diagnosis of oligodendroglioma, we found *EGFR* amplification, a typical finding in primary glioblastoma multiforme. This patient showed two tumour recurrences 11 and 8 months after previous surgery, respectively. Moreover, in three cases (Nos. 3, 7, 9) polysomy of chromosome 7 was proved, in which the *EGFR* gene is located.

In other two cases with anaplastic oligodendroglioma monosomy of chromosome 10 was detected. In this chromosome, tumour suppressor gene *PTEN* is located in the 10q23.3 region, which encodes dual lipid-protein tyrosin phosphatase (Li et al., 1997). Its deletion or inhibitory mutation occurs during progression in approximately 40% of high-grade gliomas, in particular primary and secondary glioblastomas.

10q deletions and EGFR amplifications are sufficiently rare in pure oligodendrogliomas to suggest the possibility of alternate diagnoses, such as glioblastoma or other astrocytic tumours. This glioblastoma variant displays considerable morphologic overlap with anaplastic oligodendrogliomas, including nuclear uniformity, clear perinuclear halos, a rich capillary network, and/or microcalcification. Identifying EGFR amplification in only one of our cases suggests that such a finding is even rarer in oligodendroglial tumours than 10q losses (Fallos et al., 2004).

We can conclude that I-FISH on isolated whole tumour cells nuclei is a sensitive method for detection and interpretation of specific chromosomal aberrations in brain tumour specimens. In patients with oligodendroglioma, it is the essential part of diagnostics and it considerably influences treatment and prognosis. Further studies will be necessary to identify the real prognostic significance of additional chromosomal aberrations in patients with oligodendroglial tumours and combined 1p36/19q13 deletions.

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