CONTRIBUTION OF MOLECULAR CYTOGENETIC ANALYSES TO DIAGNOSIS AND TREATMENT OF MALIGNANT BRAIN TUMOURS.

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INTRODUCTION:

Diffuse gliomas are the most common primary tumors of the central nervous system affecting adults. It is a heterogeneous group of tumors with various histological subtypes that differ in response to treatment and in the prognosis of the disease. The most frequent tumors are derived from astrocytes and from oligodendrocytes.

The treatment of diffuse gliomas (surgery, radiotherapy and chemotherapy) is problematic due to their diffuse nature. Surgical intervention never succeeds in removing the tumor tissue completely. Optimal treatment modality was not found until now. This is why the disease relapses and progresses even in case of lower-grade tumors. 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.

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 tumor cells is one of the possibilities.

Specific chromosomal aberrations in different subtypes of diffuse gliomas:

	Type of diffuse glioma	WHO grade	Chromosomal aberrations
A	low-grade astrocytoma	II	trisomy 7, aneuploidy
A	anaplastic astrocytoma	III	deletion of *p53* gene, deletion of *p16* gene, deletion of *RB1* gene, aneuploidy
A	glioblastoma multiforme - primary	IV	**amplification of *EGFR* gene**, deletion of *p16* gene, deletion of *RB1* gene, deletion of *p53* gene (rare), **monosomy 10**, aneuploidy
A	glioblastoma multiforme - secondary	IV	deletion of *p53* gene, deletion of *p16* gene, deletion of *RB1* gene, **monosomy 10**, aneuploidy
O	low-grade oligodendroglioma	II	deletion of region 19q13.3, deletion of 1p36, aneuploidy
O	anaplastic oligodendroglioma	III	**deletion of 19q13.3 / deletion of 1p36**, aneuploidy (combined deletions are marker of good prognosis)

A – astrocytoma; O - oligodendroglioma

THE AIM OF THE STUDY:

- To perform detailed molecular cytogenetic analysis of the brain tumour cells in series of patients with histologicaly proved diffuse gliomas.
- To follow the frequency and representation of the most frequent chromosomal aberrations described so far in different glial subtypes, i.e.:
  - ✓deletions of tumour suppressor genes *p53*, *p16* and *RB1*
  - ✓deletions of chromosomal regions 1p36 and 19q13.3
  - ✓amplificaion of *EGFR* gene/trisomy of chromosome 7
  - ✓monosomy of chromosome 10
- To compare the results of the molecular cytogenetic analysis in all patients with morphological and clinical findings.

86 PATIENTS:

♂ 50 males ♀ 36 females

median age: 52 years (range 16-80 years)

Glial subtype	WHO grade	Number of patients
low-grade astrocytoma	I, II	20
anaplastic astrocytoma	III	11
glioblastoma multiforme	IV	36
oligodendroglioma	II	5
anaplastic oligodendroglioma	III	12
anaplastic oligoastrocytoma	III	2
in total		86

METHODS:

Sample Extraction and Processing

Tumour tissue samples taken during routine neurosurgical surgeries were resuspended in media and further processed using standard cytogenetic procedure (hypotonia, fixation). Standard microscopic preparations for I-FISH were prepared from fixed cell suspensions.

Molecular Cytogenetic Analysis

For detection of most frequent chromosomal changes in glial cells dual-colour interphase FISH with locus-specific and/or α-satellite DNA probes was carried out according to manufacturers' recommendations (list of DNA probes is in Table 2). Cells were counterstained with DAPI (4,6-diamidino-2-phenylindole). At least 200 interphase nuclei were analyzed per dual-colour hybridisation in fluorescence microscope OLYMPUS AX70 PROVIS. For documentation the images were captured by a sensitive CCD camera and the results were processed by specialized computer software (ISIS, MetaSystems™).

DNA probes for I-FISH:

Chromosomal aberrations	DNA probe mix
deletion of *p53*	LSI® *TP53* (17p13.1) / CEP® 17
deletion of *p16*	LSI® *p16* (9p21) / CEP® 9
deletion of *RB1*	LSI *RB1* (13q14) / LSI® 13q34
deletion of *1p36*	LSI® 1p36 / LSI® 1q25
deletion of *19q13.3*	LSI® 19q13 / LSI® 19p13
Amplification of *EGFR*	LSI® *EGFR* (7p12) / CEP® 7
Monosomy *10*	CEP® 10 / control CEP® probe

CEP - chromosome enumeration probe (centromeric, alpha satellite)
LSI - locus-specific probe

Abbott-Vysis™

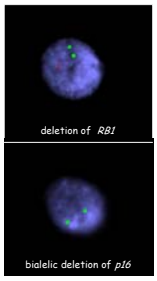
RESULTS:

original diagnosis according to morphology:

low-grade astrocytoma (WHO I, II)

20 patients

I-FISH findings	aberration	No. of cases	Conclusions according to I-FISH
	normal pattern	5	better prognosis
	deletion of *p16*	1	• anaplastic astrocytoma, worse prognosis
	deletion of *RB1*	1	• anaplastic astrocytoma, worse prognosis
	del(*RB1*), del(*p16*)	1	• anaplastic astrocytoma, worse prognosis
	polysomy	10	low-grade astrocytoma
	non-adequate tissue specimen	2	no prediction



original diagnosis according to morphology:

anaplastic astrocytoma (WHO III)

11 patients

I-FISH findings	aberration	No. of cases	Conclusions according to I-FISH
	deletion of *RB1*	1	anaplastic astrocytoma
	bialelic deletion of *p16*	4	anaplastic astrocytoma
	del(*RB1*), del(*p16*)	1	anaplastic astrocytoma
	polysomy	3	anaplastic astrocytoma, better prognosis
	non-adequate tissue specimen	2	no prediction

original diagnosis according to morphology:

glioblastoma multiforme (WHO IV)

36 patients

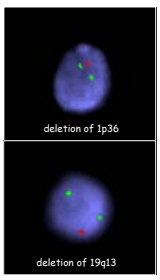
I-FISH findings	aberration	No. of cases	Conclusions according to I-FISH
	amplification of *EGFR*	3	glioblastoma multiforme - primary
	monosomy 10	9	glioblastoma multiforme - secondary
	amplification of EGFR, monosomy 10	11	glioblastoma multiforme - primary
	trisomy 7, monosomy 10	10	glioblastoma multiforme - secondary
	polysomy	2	glioblastoma multiforme
	non-adequate tissue specimen	1	no prediction

original diagnosis according to morphology:

oligodendroglioma (WHO II)

5 patients

I-FISH findings	aberration	No. of cases	Conclusions according to I-FISH
	combined deletion of 1p36/19q13	3	oligodendroglioma good prognosis
	combined deletion of 1p36/19q13, amplification of *EGFR*	1	• amplification of EGFR is a typical finding in primary glioblastoma multiforme → worse prognosis
	combined deletion of 1p36/19q13, del(RB1), monosomy 9, monosomy 10	1	• typical finding in glioblastoma multiforme → worse prognosis

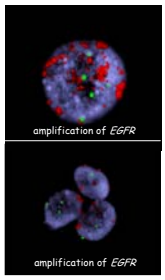


original diagnosis according to morphology:

anaplastic oligodendroglioma (WHO III)

12 patients

I-FISH findings	aberration	No. of cases	Conclusions according to I-FISH
	normal pattern	1	anaplastic oligodendroglioma worse prognosis
	combined deletion of 1p36/19q13	6	anaplastic oligodendroglioma good prognosis
	combined deletion of 1p36/19q13, polyploidy	3	anaplastic oligodendroglioma good prognosis
	combined deletion of 1p36/19q13, polysomy 7, monosomy 9, monosomy 10	1	• typical finding in glioblastoma multiforme → worse prognosis
	non-adequate tissue specimen	1	no prediction

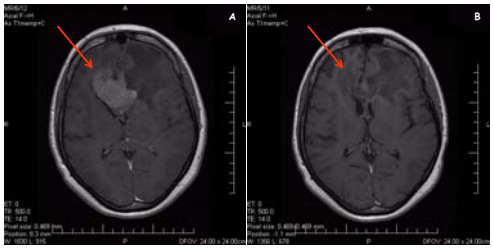


original diagnosis according to morphology:

anaplastic oligoastrocytoma (WHO III)

2 patients

I-FISH findings	aberration	No. of cases	Conclusions according to I-FISH
	deletion of 1p36, polyploidy	1	anaplastic oliastrocytoma worse prognosis
	combined deletion of 1p36/19q13	1	anaplastic oliastrocytoma good prognosis



Anaplastic oligodendroglioma before (A) and after (B) chemotherapy.

CONCLUSIONS:

- I-FISH analyses gave informative results in 80 samples (93%), in six other the results were uninformative due to non-adequate sampling of the tissue during surgery.
- The results of molecular-cytogenetic analyses were in accordance with histological and clinical findings and confirmed original diagnosis. In 57 patients out of 86 I-FISH contributed to establish more precise diagnosis and prognosis of the disease.
- The most important clinical significance had finding of combined deletion 1p36/19q13 in patients with oligodendroglial tumours, which is considered to be a predictor of good response to chemotherapy and fair prognosis. In all of them was chemotherapy recommended as primary treatment modality.

➡ **Molecular-cytogenetic analyses are suitable diagnostic methods to detect chromosomal aberrations in brain tumour cells:**

- ✓ **In patients with astrocytoma confirms histological diagnosis and contributes to more accurate prognosis**
- ✓ **In patients with oligodendroglioma is essential part of diagnostics and considerably influences treatment and prognosis.**

➡ **A systematic analysis of tumour cells using molecular cytogenetic methods advances in diagnosis, grading, classification and treatment of patients with brain tumours.**

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