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DNA ploidy and chromosome (FISH) pattern analysis of peripheral nerve sheath tumors

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Abstract. *Background and methods:* 44 peripheral nerve sheath tumors (PNST) (27 schwannomas, 9 neurofibromas and 8 malignant peripheral nerve sheath tumors (MPNST)) were analyzed to determine DNA ploidy pattern and to clarify the conflicting data in the literature concerning this topic (whether benign PNSTs are aneuploid or not). For further insight we analyzed 6 schwannomas, one atypical neurofibroma and five MPNSTs by fluorescence *in situ* hybridization (FISH) technique using centromeric chromosome probes (7, 17 and 18) and automatic image analysis station, Metafer 4. *Results:* Benign schwannomas (including the problematic variants as ancient, cellular, neuroblastoma like and multiplex schwannomas) could be characterized by euploid-polyploidisation and by their 4c peak height value which was usually more than 10% of total cell number measured. These characters were not found among neurofibromas and MPNST-s. FISH analysis revealed and confirmed that the 'normal' euploid–polyploid cells are mainly eusomic–polysomic containing two, four, eight or sixteen signals for each chromosomes examined, but in a small proportion aneusomy was found among tumor cells of benign schwannomas (average: 2.58; range 1.33–3.44). In contrast, the atypical neurofibroma displayed marked aneusomy (18.44%) but it contained normal eusomic and polysomic cells too. Two diploid MPNSTs proved to be clearly aneusomic with trisomy of chromosome 17 and monosomy of chromosome 18. *Conclusions:* All these data suggest that ploidy pattern determination combined with FISH analysis may be a very useful supplementary tool for making a right diagnosis (to differentiate benign versus malignant schwannomas in problematic variants) and to understand better the malignant transformation in PNSTs.

Keywords: Schwannoma, PNST, FISH, DNA cytometry, aneusomy

1. Introduction

PNST is a large fairly heterogeneous group of soft tissue tumors (STT) containing more than 20 entities. Although the most frequent tumors are conventional schwannomas and neurofibromas and they usually cause no differential diagnostic problems however some subtype of schwannomas such as ancient, cellular, neuroblastoma like and multiplex schwannomas do cause difficulties in distinguishing them from different malignant STTs [2,5,8,20]. Ancillary techniques are of great help to make a right diagnosis namely immunhistology and electron microscopy but conflicting data are available concerning DNA content determination of PNSTs. Some publishing groups have found consequently aneuploid DNA content among benign PNSTs [25,27–29] while others state that benign PNSTs are clearly diploid tumor (with euploid polyploidisation) [10,16,18,19,24,30,31] and some investigators are in between, because they have found nondiploid benign PNSTs in a very low proportion [5–7]. To clarify this issue we determined the DNA content of 36 benign PNSTs comparing them to 8 MPNSTs.

Cytogenetic and molecular genetic analyses of PNSTs have been made fairly extensively but concentrating usually on the well known 17q and 22q chromosome alteration in NF1 and NF2, as an initial step in the pathogenesis of neurofibromas and schwannomas [3,22,23,26,36]. Much less data are available concern-

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ing numerical alteration of chromosomes by FISH especially analyzing them on appropriate number interphase nuclei by automatic image analysis station [23]. We selected three chromosomes [7,17,18] for FISH analysis because gains and losses of these chromosomes are established by previous studies [9,22,32, 33]. Chromosome 22 should have been another candidate, but because of the very sensitive automatic image analysis and the possible cross-reacting centromeric probes of 5/19, 13/21 and 14/22, these chromosomes (including chromosome 22) were excluded from the study. We did not select any benign neurofibroma for FISH analysis, because they usually had no euploid–polyploidisation and they had no 4c peak height value $>10\%$ at all.

2. Materials and methods

Cases of 44 PNST were retrieved from the files of Department of Pathology, Semmelweis University, Budapest, Hungary. 27 benign schwannomas were selected, including 5 ancient, 2 cellular (pseudosarcomatous), 2 neuroblastoma like, 1 multiplex variant. The 9 neurofibroma cases contained one atypical-cellular subtype. Among the 8 MPNSTs two were malignant epithelioid schwannoma. All of benign tumors were sporadic cases. Only one of MPNSTs (No 40) had the clinical features of NF1. Clinical data are summarized in Table 1. Follow up has been available at least for 5 years. Histology, nuclear extraction, DNA measurements were performed in the Department of Pathology, Semmelweis University, Budapest while FISH analysis with automatic image analysis station, Metafer 4 was done in Institute für Pathologie, Bonn, Germany. All the processes, measurements and evaluation were coordinated and controlled by one pathologist (Dr. Sápi).

2.1. Light microscopy

The tissue blocks were fixed, processed and stained according to the standard hematoxylin and eosin (HE) histological protocol. Monoclonal antibodies were used to define the immunophenotype and to confirm the diagnosis in difficult cases (ancient, cellular, neuroblastoma like, etc., i.e. strong and diffuse S-100 positivity in any typ of schwannoma) (Fig. 1). S-100, α-smooth muscle actin (SMA), desmin, Ki-67, CD-99 were from Novocastra (UK). In all of the cases, the recommended dilutions were applied. As a developing system and substrate, Vectastain kit Novocastra (UK) and 3-amino-9-ethylcarbazole were used, respectively. For all of the immunoreactions, positive and negative slides were used to rule out the false results.

2.2. Image DNA analysis

We analyzed the nuclear DNA content by image DNA cytometry. Smears from FNAB were post-fixed in 4% buffered formalin but smears of nuclear extraction did not need this procedure. For nuclear extraction four sections (50-μm thick) were cut off the blocks and, following the conventional rehydration procedure, the slices were digested in 2 ml 0.5% pepsin (pH 1.5) solution at 37°C for 60 min. The digestion was checked microscopically, and the process was stopped with cold phosphate-buffered saline (PBS). Nuclear suspension was filtered and sedimented. The sediment was resuspended in 0.01% citrate buffer, and the aliquots were cytocentrifuged to glass slides. Hydrolyzis was carried out in 4 N HCl for 45 min at 28°C. Then we stained the samples by a stoichiometric method according to Feulgen using a Schiff reagent (Merck Darmstadt, Germany). Sample preparation, fixation and staining were performed according to the consensus report of the ESAP task force on the standardization of diagnostic DNA image cytometry [11].

Image-DNA-data processing was carried out using a regular microscope with an image-sensing scanner interfaced to a general purpose computer with the appropriate software (CYDOK®, Fa. Hilgers Königswinter, Germany). This system complies with the methodological conditions needed for precise DNA image cytometry. Details of the system concerning software corrections for shading and glare errors, for stability over time and densiometric linearity are described by Kindermann and Hilgers [13]. We used a $\times 40$ objective and an interference filter (565 ± 10 nm half value width for parafuchsine).

At least 100 diagnostic cells (but in many cases 200–300 cells) were analysed for each smears. The integrated optical density of Feulgen-stained reference cells (e.g. lymphocytes or granulocytes) was used as a known internal standard for the normal diploid content (2c) to rescale the IOD-values to c-values. The coefficient of variation (CV) of the reference cells was between 3% and 5%. These reference cells are non-tumor cells found in the samples. Having measured 30 reference cells and at least 100 diagnostic cells, the computer automatically generated image analysis histograms.

To define anueploidy, we used the stem line interpretation according to Böcking et al. [4]. This classical aneuploidy interpretation refers to the modal value (the most frequent value) of a cell population, i.e. the DNA

Table 1
Clinical data and DNA ploidy pattern of 44 peripheral nerve sheath tumors

	Diagnosis	Age, sex	Anatomic. loc.	DI	4c peak height value >10%	Euploid-polyploidisation
1	Ben. schwannoma	46 F	l. ankle	0.95	yes	yes
2	Ben. schwannoma	49 F	esophagus	1.05	yes	yes
3	Ben. schwannoma	34 M	l. thigh	0.95	no	yes
4	Ben. schwannoma	31 M	r. gluteal field	1.07	no	no
5	Ben. schwannoma	71 F	l. chest	0.95	no	yes
6	Ben. schwannoma	82 M	nasopharynx	0.92	no	yes
7	Ben. schwannoma	53 F	l. hip	0.93	no	yes
8	Ben. schwannoma	57 F	r. chest	0.92	no	no
9	Ben. schwannoma	42 M	r. forearm	0.98	yes	yes
10	Ben. schwannoma	52 F	r. arm	0.98	yes	yes
11	Ben. schwannoma	38 M	neck	0.99	yes	yes
12	Ben. schwannoma	59 M	r. inguinal field	0.97	yes	yes
13	Ben. schwannoma	61 F	neck	0.98	yes	yes
14	Ben. schwannoma	29 M	r. leg	0.96	yes	yes
15	Ben. schwannoma	61 F	l. arm	0.95	yes	yes
16	Ben. schwannoma	24 F	l. forearm	1.01	yes	yes
17	Ben. schwannoma	48 M	retroperitoneum	0.94	yes	yes
18	Ancient schwannoma	60 F	retroperitoneum	1.04	yes	yes
19	Ancient schwannoma	72 F	l. leg	0.98	yes	yes
20	Ancient schwannoma	42 M	l. thigh	0.96	yes	yes
21	Ancient schwannoma	34 F	back	1.01	yes	yes
22	Ancient schwannoma	71 F	retroperitoneum	1.00	yes	yes
23	Cellular schwannoma	58 F	r. chest	0.99	yes	yes
24	Cellular schwannoma	62 M	retroperitoneum	0.92	no	yes
25	Neuroblastoma like schwannoma	66 F	l. inguinal field	1.02	yes	yes
26	Neuroblastoma like schwannoma	42 M	l. sole	0.96	yes	yes
27	Multiplex schwannoma	55 F	l. arm	1.02	yes	yes
28	Neurofibroma	27 M	head	0.98	no	no
29	Pl. neurofibroma	31 F	l. sholder	0.99	no	yes
30	Neurofibroma	33 F	r. popliteal space	0.96	no	no
31	Neurofibroma	50 M	r. leg	0.98	no	no
32	Neurofibroma	79 F	neck	0.98	no	no
33	Neurofibroma	56 M	head	0.97	no	no
34	Neurofibroma	76 M	head	0.98	no	no
35	Neurofibroma	36 M	r. forearm	0.99	no	no
36	Cellular-atypical neurofibroma	80 F	r. forearm	1.01	no	yes
37	Superficial mal. epith. schwannoma	71 M	abdomen, skin	1.00	no	no
38	Deep mal. epith. schwannoma	65 F	mediastinum	1.27	no	no
39	MPNST	45 M	retroperitoneum	1.52	no	no
40	MPNST	51 M	r. arm	1.00	no	no
41	MPNST	25 F	r. arm	1.49	no	no
42	MPNST	19 M	retroperitoneum	1.21	no	no
43	MPNST	18	l. leg	1.58	no	no
44	MPNST	23	r. ankle-sole	0.97	yes	no

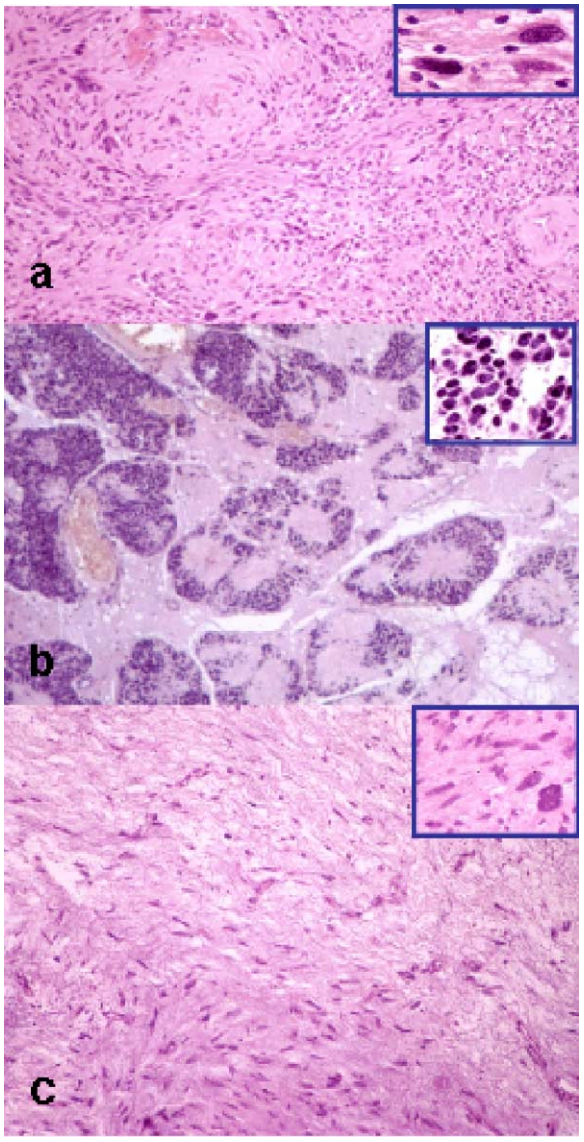

Fig. 1. (a) Ancient schwannoma with characteristic pleomorphic cells (insert) causing diagnostic difficulties. Case number: 18. (b) Neuroblastoma like schwannoma. Note the modified Verocay bodies as giant rosettes and the uniform small round tumor cells (insert). Case number: 25. (c) Atypical-cellular neurofibroma. This tumor had no capsule and displayed strong but focal S-100 positivity. Note the cellular area and the atypical tumor cells in insert. Case number: 36.

stem line. A stem line is considered DNA aneuploid if its value, registered in the Kolmogorov–Smirnow test, is significantly different ($p < 0.001$) from that of the diploid reference cells, and stem line ploidy is $<$1.8c or $>$2.2c.

We calculated a DNA index (DI) for each case. This index was determined from the ratio of the channel of the aneuploid peak divided by the channel of the normal/diploid peak, thus defining the site of a stem line. A DI of 1.0 corresponds to a stem line at 2.0 and a DI of 2.5 to a stem line of 5c. The 4c peak height value was determined too and this was divided by the total number of measured cells. The received value was indicated in percentage and was interpreted whether it was more or less than 10%.

2.3. Interphase cytogenetics

For interphase cytogenetic analysis, cell nuclei were isolated from the formol-paraffin blocks of the tumors or smears gained by fine needle aspiration biopsy (FNAB) were available. Four sections (50-μm thick) were cut off the blocks and, following the conventional rehydration procedure, the slices were digested in 2 ml 0.5% pepsin (pH 1.5) solution at 37°C for 60 min. The digestion was checked microscopically, and the process was stopped with cold phosphate-buffered saline (PBS). Nuclear suspension was filtered and sedimented. The sediment was resuspended in 0.01% citrate buffer, and the aliquots were cytocentrifuged to glass slides. Three FISH probes specific for the (peri)centromeric regions of chromosomes 7, 17, 18 were used (alpha 7 and 17 CEP Spectrum Green; alpha 18 CEP Spectrum Orange; Vysis Inc., USA). Probes were paired and cohybridized (double color FISH for chromosomes 17 and 18). In the course of double-color FISH, 5 μl hybridization mixture containing 10 μg probe/60% formamide in 2x sodium saline citrate (SSC) were propped onto the cell preparations and covered with plastic film. Samples and probes were denatured simultaneously at 82°C for 4 min followed by hybridization at 37°C in a wet chamber overnight. A post-hybridization wash was performed under the following conditions: three 5-min washes in 60% formamide/2x SSC (baker) at pH 7, 37°C, and three 5 min washes in 2x SSC at room temperature. The preparations were mounted with Vectashield mounting medium containing 0.02 μg/ml DAPI (4′-6′-diamidino-2-phenylindole) nuclear counterstain (Vector Lab, USA). In all of the cases, the recommended quantities and dilutions were used.

2.4. Automating image analysis of FISH signals

For the analysis of a large cell number the automatic image analysis system Metafer4-MetaCyte (MetaSystems, Germany) was used. The system was mounted to an Axioplan 2 motorized microscope (Carl Zeiss, Ger-

many), and included a high resolution CDD camera, an 8 slide motorized scanning stage (Maerzhaeuser, Germany) and a microcomputer system (Pentium IV, 1.8 GHz, Windows 2000 operating system). A predefined area on a slide was scanned without gaps, isolated cells (nuclei) were identified and images captured in three colors. As signals are randomly distributed across the 3-dimensional nuclei, extended focus images calculated from focus stacks were used for FISH spot analysis. For 300 cells per sample Metafer4-MetaCyte automatically detected the FISH signals.

3. Results

3.1. Image DNA analysis

DNA ploidy pattern of 44 PNSTs is summarized in Table 1. All of benign schwannomas were diploid and 25 of 27 (92.5%) showed characteristic euploid-polyploidisation. 4c peak value height was equal or more than 10% of measured cells in 20 cases of 27 (74.0%) (Fig. 2). All neurofibromas (9 cases), including the atypical one proved to be diploid, but only two of them displayed euploid-polyploidisation (22.9%) and none had equal or more than 10% in their 4c peak height value. 3 of 8 MPNSTs were diploid, the other 5 were clearly aneuploid. There was no euploid–polyploid case and only one of eight showed a higher than 10% 4c peak height value but these cells in the 4c region were in fact G2 phase cells rather than true tetraploid-tetrasomic cells proved by FISH analysis (see later in the FISH analysis section) (Fig. 3). S-phase fraction was not determined because the measured 100 tumor cells (concerning their number) are not sufficient to calculate it.

3.2. Interphase cytogenetic (FISH) analysis

In each cases (5 schwannomas, one ancient schwannoma, one atypical neurofibroma and five MPNSTs) 300 cells were analysed and the number of signals of chromosomes 7, 17 and 18 were counted respectively (Table 2). If the result of automatic signal interpretation was doubtful, relocalisation was done and signals were counted by visual control. Signal numbers were divided into two groups: eusomic–polysomic signals (signal numbers are 2, 4, 8, 16) and aneusomic signals (signal numbers are 1, 3, 5, 6, 7). Those cells, in which the signal interpretation was impossible after relocalisation, were omitted for evaluation. The average

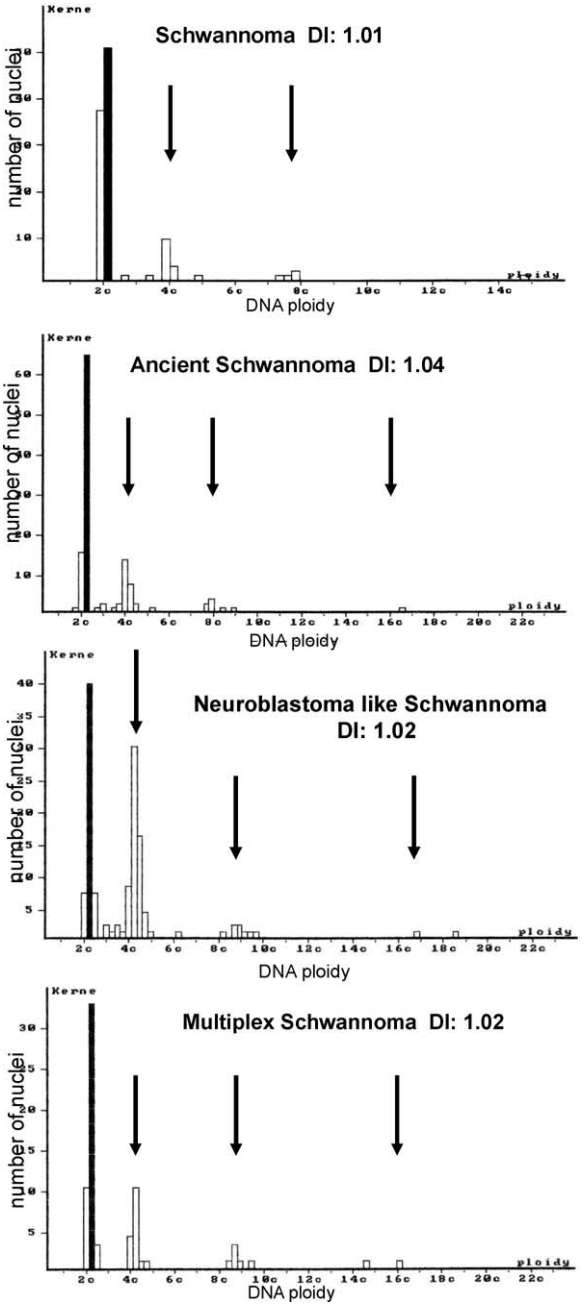

Fig. 2. Histograms of conventional, ancient, neuroblastoma like and multiplex schwannomas. Note the euploid polyploidisation indicated by arrows (4c, 8c, 16c values) and the 4c peak height values which are higher than 10%. Case numbers: 16, 18, 25, 27.

rate of inappropriate cells was 4.1%, range: 1.3–7.0%. Polysomy was observed in all of benign schwannomas, average rate was 13.9% which is in good harmony with the estimated rate of polyploidy measured by image cytometry. Polysomy rate was significantly higher in

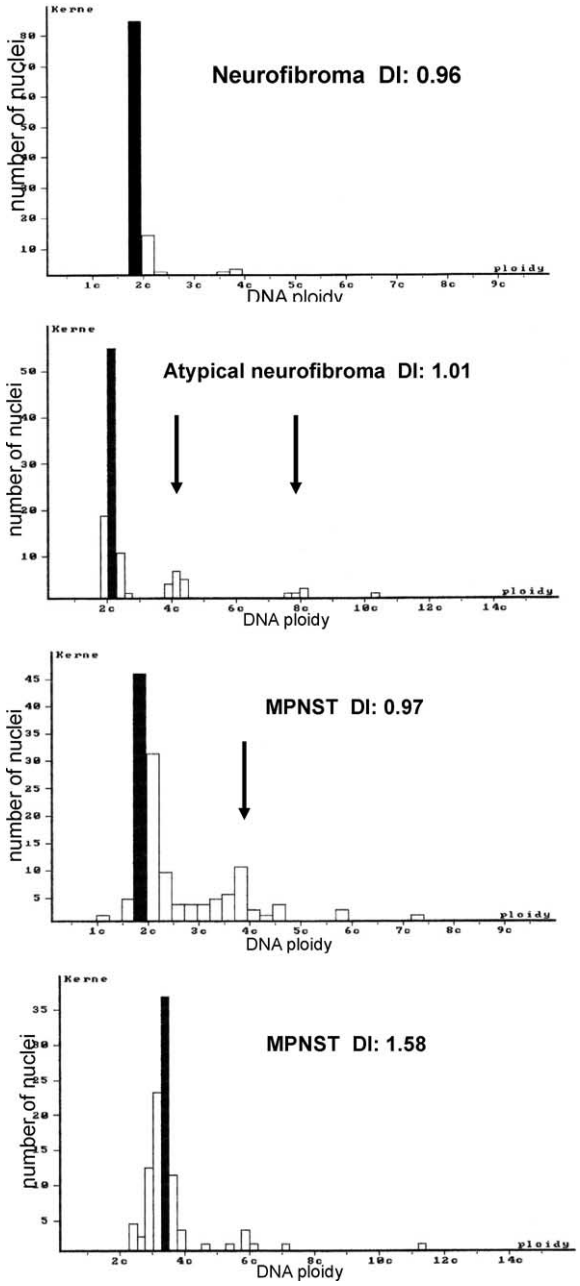

Fig. 3. Histograms of neurofibroma, atypical neurofibroma and two MPNSTs. There is no euploid polyploidisation except atypical neurofibroma. 4c peak height value was higher than 10% only in one MPNST (case number 55) but the cells here are G2 phase cells and not true tetraploid cells (see explanation in text). Case numbers: 30, 36, 44, 43.

ancient schwannoma (30.1%) which seems to be natural if we consider the high number of enlarged hyperchromatic, multilobated nuclei in this type of schwannoma (Fig. 4). Polysomy was observed in atypical neurofibroma (4.3%) too, but not in MPNST. The 0.7% tetrasomy in MPNST (case number: 44) compared with the higher than 10% 4c peak value in the same tumor, clearly shows that the cells in the 4c peak are G2 phase cells (containing double DNA material) and not real tetrasomic cells (because of the nondisjunction during mitotic process, the chromosomes in the G2 phase cells have only one centromer for each chromosome pair, so using centromeric DNA probes one can see the normal two signals for each chromosome). Very interestingly in a small proportion of aneusomy was detected in all benign schwannomas, the rate (number of aneusomic cells per total cell number) was 2.58%, range: 1.33–3.44%. In contrast, the atypical neurofibroma displayed marked aneusomy (18.44%) but in 4.30% polysomy was present too (Fig. 5). Important to recognize that the monosomy and the trisomy was in about equal number so the aneusomy was so-called "balanced" concerning the total DNA content, and in fact the DNA index was 1.01 in this case.

Two MPNSTs showed frank aneusomy for chromosome 17 (89.6%, No 44, trisomy) and for chromosome 18 (91.6%, No 40, monosomy) among the diploid MPNSTs.

In cases we did not have proper 300 cells (for technical reason), the evaluation was not performed (altogether 5 benign cases), though they showed very similar results comparing to the measured 6 benign schwannomas.

4. Discussion

DNA cytometry and FISH are complementary techniques such that DNA cytometry gives an "overview", whereas FISH gives more detailed information. Using both techniques in different tumor types leads to a better understanding of the chromosomal events that occurred during oncogenesis. Our aim was to map the ploidy and chromosome pattern of PNSTs and to see whether it is usable in the differential diagnostics, finally to try clarify the conflicting data concerning DNA ploidy in PNSTs. Our results confirm that benign schwannomas and neurofibromas are euploid tumors with polyploidisation, regardless what kind of subtypes are examined, which may be great help to distinguish them from different malignant STT in any circumstances (aspiration cytology and histology). Further character is the 4c peak height value which is usually more than 10% in schwannomas but not a characteristic feature in neurofibromas and in MPNST-s. This

Table 2
FISH signals in PNSTs

	Eusomic–polysomic signals				Aneusomic signals					IA
	2	4	8	16	1	3	5	6	7	
Schwannoma No 5										
Chr. 18	249	24	2	0	1	3	0	0	0	21
Chr. 17	242	29	2	0	2	4	0	0	0	21
Chr. 7	251	26	3	0	0	2	0	0	0	18
Schwannoma No 7										
Chr. 18	242	26	1	0	3	8	3	0	0	17
Chr. 17	255	22	1	0	1	4	0	0	0	17
Chr. 7	252	27	0	0	1	5	0	0	0	15
Schwannoma No 10										
Chr. 18	242	41	2	0	3	3	0	0	0	9
Chr. 17	239	40	2	0	2	7	0	1	0	9
Chr. 7	243	37	3	0	1	4	1	1	0	10
Schwannoma No 16										
Chr. 18	238	35	4	0	2	2	3	3	1	12
Chr. 17	236	37	3	0	2	2	4	2	2	12
Chr. 7	233	39	5	0	1	3	2	2	0	15
Schwannoma No 24										
Chr. 18	258	23	2	0	1	3	3	1	0	9
Chr. 17	259	22	2	0	0	4	2	2	0	9
Chr. 7	257	19	3	0	2	5	1	1	1	11
Anciant schwannoma No 18										
Chr. 18	188	82	8	2	1	2	2	0	0	15
Chr. 17	185	81	8	2	2	4	2	0	1	15
Chr. 7	190	78	9	1	2	4	2	1	0	13
Atypical neurofibroma No 36										
Chr. 18	217	12	1	0	14	29	4	4	1	18
Chr. 17	210	14	1	0	15	31	5	4	2	18
Chr. 7	211	11	0	0	26	29	1	1	0	21
MPNST No 44										
Chr. 18	277	4	0	0	1	3	0	0	0	15
Chr. 17	12	2	0	0	0	269	2	0	0	15
Chr. 7	282	1	0	0	0	1	0	0	0	16
MPNST No 43										
Chr. 18	11	2	0	0	0	281	0	0	0	6
Chr. 17	289	0	0	0	3	2	0	0	0	6
Chr. 7	15	243	0	0	0	16	18	0	0	8
MPNST No 41										
Chr. 18	1	48	0	0	0	17	205	9	7	13
Chr. 17	0	257	0	0	0	11	19	0	0	13
Chr. 7	7	11	0	0	0	270	0	0	0	12
MPNST No 40										
Chr. 18	21	0	0	0	275	0	0	0	0	4
Chr. 17	291	0	0	0	5	0	0	0	0	4
Chr. 7	289	0	0	0	7	0	0	0	0	4
MPNST No 37										
Chr. 18	272	0	0	0	9	12	0	0	0	7
Chr. 17	6	0	0	0	5	282	0	0	0	7
Chr. 7	276	0	0	0	7	11	0	0	0	6

IA: inappropriate for evaluation; Chr.: chromosome.

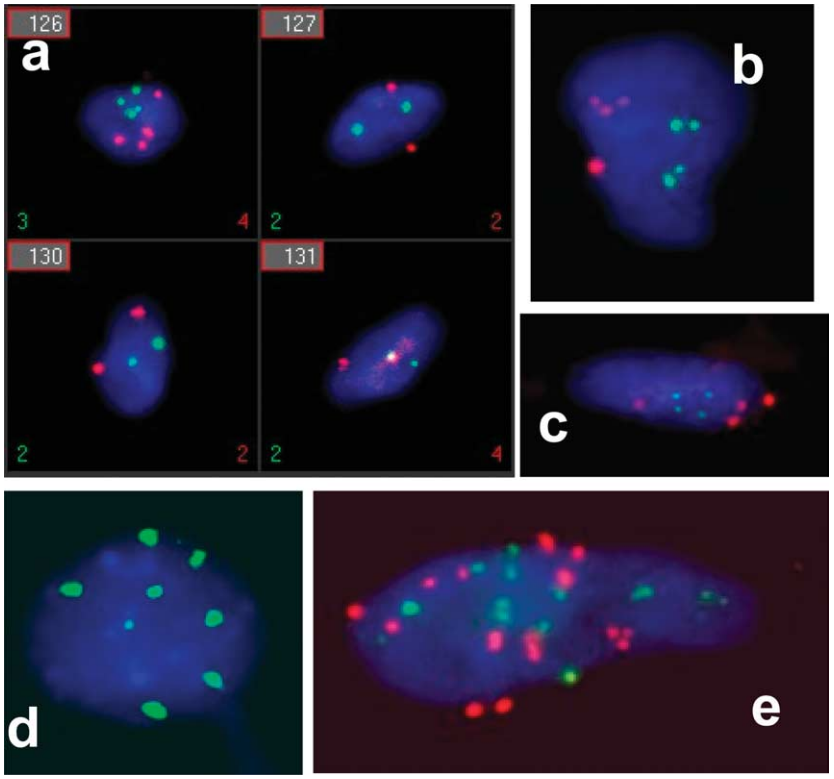

Fig. 4. FISH signals in ancient schwannoma displaying characteristic eusomic-polysomy. (a) Part of slideshow of Metafer 4 containing 3 disomic and one tetrasomic tumor cells. (b–c) Bizarre and 'normal' tumor cells with tetrasomy. (d) Octasomic tumor cells. (e) Enlarged tumor cell with 16 red and green signals. Some of red and green signals were out of focus when the picture was taken therefore they are not clearly visible. (a, b, c, e: Dual color FISH technique; red signals correspond to chromosome 18, green signals correspond to chromosome 17. (d): FISH technique; green signals correspond to chromosome 7.)

character may be of help to differentiate schwannoma from neurofibroma in special instances such as multiple schwannomas, pure Antoni B schwannoma, plexiform schwannoma and sinonasal schwannoma (lack of encapsulation) [2,12].

The high 4c peak height value may explain some interpretation problems by which previous authors found benign schwannomas as aneuploid or nondiploid tumors. For example, Agarwal et al. [1] found 4 of 4 schwannomas as nondiploid tumors but they used the following criteria: "Histograms that contained a tetraploid peak of more than 20% nuclei were considered to be nondiploid". Because they did not give the precise DNA index of these tumors it is easy to imagine that the tumors were in fact euploid–polyploid ones, with more than 20% 4c peak value. The situation is different with the articles of Salmon and coworkers [27]. They found real aneuploidy, but if we analyse their data in detail, they found only 2% true aneuploidy (triploid histogram $1.40 < \text{DI} < 1.60$), furthermore 2% tetraploidy and 15% hypertriploidy (near tetraploidy) among schwannomas (total number was 64). The 15% seems to be high, but they did not give the precise DNA index, so we did not know whether they were real aneuploid tumors or euploid–polyploid tumors with near tetraploid peaks. In the same article they found one of five neurofibroma (20%) to be hyperdiploid (no given DI!) as anueploid tumor. Very similar results were published by Salmon and coworkers in another article (1 tetraploid and 10 hypertriploid tumor out of 75 benign schwannomas, but no clear aneuploid one!) [29]. In fact, only Salmon and coworkers have published consistently high percentage of aneuploidy among benign PNSTs. Interestingly, flow cytometric studies give an account of diploid nature of benign PNST in majority or they found aneuploidy in a very low percentage among them [6,7,10,16,19]. A lot of image cytometric studies (including our data on different material than in this article) found that benign PNSTs are diploid tumors, some of these reports making mention of polyploidisation [30,31,34,35]. Based on our DNA ploidy results and considering the data

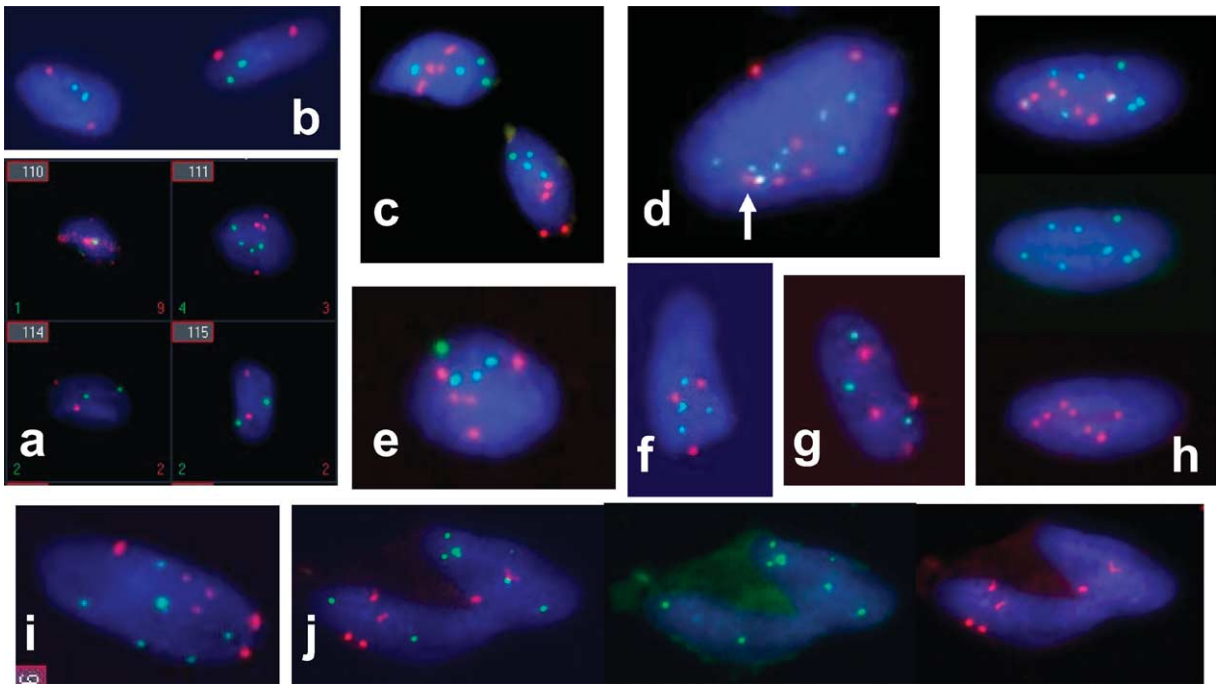

Fig. 5. FISH signals in atypical neurofibroma showing both eusomic-polysomy and aneusomy. (a) Part of slideshow of Metafer 4 containing two disomic and one tetrasomic tumor cells. One cell in the left upper corner proved to be inappropriate for evaluation. (b) Disomic tumor cells. (c) Tetrasomic tumor sells. (d) Octasomic tumor cell. Arrow indicates two red and one green signal. (e) Tetrasomy for chromosome 17 and pentasomy for chromosome 18. (f) Tetrasomy for chromosome 17 and trisomy for chromosome 18. (g) Tetrasomy for chromosome 18 and trisomy for chromosome 17. (h) Octasomy for chromosome 17 and heptasomy for chromosome 18. (i) Hexasomic tumor cell. (j) Splitting tumor cells; note the uneven distribution of chromosomes and the clear aneusomy. (Dual color FISH technique; red signals correspond to chromosome 18, green signals correspond to chromosome 17.)

found in the literature it seems that the vast majority of benign PNSTs are diploid tumors (schwannomas with polyploidisation) but in a small proportion aneuploid ones definitely exist. These anueploid benign PNSTs may contain a much higher ratio of unbalanced aneusomy comparing to that small percentage of balanced aneusomy that we found in benign schwannomas and to that higher percentage but balanced aneusomy found in atypical neurofibroma. However, the question is whether these aneuploid benign PNSTs are really true benign tumors? Of course, from morphological point of view they are, but from genetical point of view they are "dysplastic" soft tissue tumors and it is not a big surprise if they do not recur or give metastases after removal (as it happens with the dysplastic epithelial lesions). This question is especially interesting because a similar problem was raised recently by McMenamin and Fletcher [21], who described the epithelioid malignant change (EMC) in schwannomas and suggested that this represented a putative precursor lesion of epithelioid MPNST. It seems that "dysplastic-precursor" cells do exist both at a genetic and morphologic level.

So far we do not have clear explanation for the tendency of euploid-polyploidisation in some type of benign tumors and even in normal tissue (i.e. epithel of vesicula seminalis). Because the NF2 gene alteration is thought to be the initial step in the pathogenesis of schwannoma, perhaps this may partly be responsible for the euploid-polyploidisation process resulting in eusomic–polysomic cells. During this process any kind of further "damage" may results in theoretically more eusomic (polysomic) or aneusomic cells. The aneusomy can be balanced or unbalanced and may have a small or higher ratio. It seems that until the percentage of aneusomy is low and it is balanced, the tumor is absolutely benign as in our cases of schwannomas. If the percentage of balanced aneusomy increases, the tumor turns into "atypical" as in our atypical neurofibroma, having much higher chance to become true malignant tumor. Of course we are absolutely aware that the development of malignancy in MPNSTs is very complex (alteration in NF1 and p53, deletions of the INK4A

gene, abnormalities in p27 and so on) [14,15,17] but the polysomy–aneusomy pathway also may play role in this development. Perhaps this theory is supported by the fact that MPNSTs do not show characteristic cytogenetic alterations (there is no specific translocation or characteristic numerical alteration), but they display complex karyotypes with numerous structural and numerical changes, or if there is a sole numerical change this happens "by chance". In our case there were chromosome 17 trisomy and chromosome 18 monosomy but others reported chromosome 7 trisomy or chromose 22 monosomy as a single numerical change [22]. It is well known that at least three or more chromosomes gain is needed without chromosome losses to get an aneuploid (peridiploid) DNA index (DI $>$ 1.10).

The importance of FISH as a complementary technique appears from the fact that it is able to distinguish whether a tumor is really tetraploid or contains simply a high proportion of G2 phase cells. G2 phase cells, although they contain double DNA material, show up two signals for each chromosome, because of the nondisjunction (during mitotic process). This fact was clearly evident in case number 44 which had a 12% 4c peak height value but the percentage of tetrasomy for the chromosomes examined was only 0.7%.

Summing up: ploidy pattern determination combined with FISH analysis may be very useful supplementary tool for making a right diagnosis and to understand better the malignant transformation in PNSTs.

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