

Magdalena Mayer¹, Andrzej Kulig², Jacek Sygut³, Adam Dzik⁴, Dorota Simon¹, Anna Latos-Bieleńska¹, Tomasz Ferenc⁵

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Molecular Cytogenetic Analysis of Chromosome Aberrations in Desmoid Tumors

¹Department of Medical Genetics, University of Medical Sciences in Poznań,

²Department of Clinical Pathomorphology, Polish Mother's Memorial Hospital, Łódź,

³Department of Tumor Pathology, Świętokrzyski Center of Oncology, Kielce,

⁴Department of General and Colorectal Surgery, Medical University, Łódź,

⁵Department of Biology and Genetics, Medical University, Łódź

To date there are only few reports concerning chromosomal changes in desmoid tumors. To extend the knowledge in this field we examined 19 samples from the patients diagnosed with desmoid tumors. In the present study formalin-fixed and paraffin-embedded desmoid tumors were analyzed using fluorescence *in situ* hybridization (FISH) with α -satellite probes for chromosomes X, Y, 8 and 20. Chromosomal abnormalities were found in 6 cases, both abdominal and extra-abdominal tumors. FISH studies revealed one case of trisomy 8 and trisomy 20. In four patients we have identified monosomy 20. Our findings confirm earlier reports concerning the diversity of chromosomal changes in desmoid tumors and might suggest that both groups of abdominal and extra-abdominal tumors are genuine neoplasms.

Introduction

Desmoid tumor is a benign neoplasm which rarely turns malignant and is non-metastasising. Desmoids demonstrate ability for local infiltration of surrounding tissues and are characterized by high risk of recurrence after surgical treatment [5, 18]. Aggressive fibromatoses may occur in extra-abdominal, abdominal and intra-abdominal location [20]. The etiology of desmoid tumor is uncertain. It can occur in sporadic forms or be a part of some familial neoplastic syndromes. Sporadic desmoid tumors have been estimated to occur in 2 to 5 persons per 1 million population per year. The female to male ratios of occurrence are from 2:1 to 5:1 [1, 5].

Cytogenetic analysis of desmoid tumors has shown the presence of many chromosomal aberrations, however trisomy of chromosome 8 and 20 has been described as the most frequently found in the karyotype [7, 11, 12]. In patients with Gardner syndrome and familial adenomatous polyposis (FAP) deletion of the 5q was reported [8, 21]. Chromosome Y deletion has been found in tumor cells from male patients [3]. Larramendy et al. reported gain of band 1q21 as the most frequent aberration found by a CGH (comparative genomic hybridization) method [12].

In this study we performed FISH analysis on 19 paraffin-embedded desmoid tissue sections in order to analyze chromosomes 8, 20, X and Y copy numbers.

Materials and Methods

Sample preparation and extraction of nuclei

The analyzed material was collected from the Polish Departments of tumor pathology. All the sections were examined by two pathologists using a conference microscope and were histopathologically classified, as recommended [20].

Postoperative desmoid tumors were fixed in 10% buffered formalin and embedded in paraffin blocks. From each paraffin block five, fifty-micron sections were deparaffinized by three changes of Xylene and dehydrated in 100% ethanol as described previously [10, 13].

The material was manually minced with a scalpel and incubated with 0.5% pepsin (pH 1.5) for 30-50 minutes for tissue disaggregation. With minor modifications, protocol

published by Liehr et al. [15] was used: the suspension was passed through a 70 μm nylon mesh, centrifuged (1600 rpm, 10 min) and the supernatant was discarded. Samples were washed three times with phosphate-buffered-saline (PBS) and spotted on slides. Slides were drying overnight in room temperature.

Slide preparation

Slides were incubated in pre-treatment solution (Q-biogeneTM) for 15-30 minutes, 45°C and then digested with Protein Digesting Enzyme solution (Q-biogeneTM) (concentration 0.25mg/ml, 30-60 minutes, 45°C). Finally slides were dehydrated in 70%, 80% and 90% ethanol series.

Hybridization and microscopy analysis

In situ hybridizations were performed using α -satellite probes, direct-labeled with Fluorescein for chromosome X (DXZ1) and chromosome 8 (D8Z2) or with Rhodamine for chromosome Y (DYZ3) and chromosome 20 (D20Z1). All probes were provided by Q-biogeneTM. Each probe

was used in different slide. Hybridizations were performed by two independent investigators, according to the procedure described in "Tissue Conversion Kit Manual" (Q-biogeneTM) with minor modifications. Slides and probes were codenaturated for 14 minutes in 75°C on a hot plate. Hybridization was carried out overnight in 37°C in a humidified chamber. Post-hybridization washing was performed in 0.5XSSC/0.1%SDS (20 sec, 55°C). After 5 minutes washing in 1XPBD, nuclei were counterstained with DAPI. Slides were analyzed using a Zeiss fluorescent microscope, images were taken with CytoVision (Version 3.52) software. The case was qualified as monosomic or trisomic if >5% of cells contained one or three separate hybridization signals. A chromosome X α -satellite probe (DXZ1) served as the hybridization control.

Results

FISH studies were performed on 19 paraffin-embedded specimens. Non-overlapping, intact, large, oval or spindle-shaped interphase nuclei were counted. In cases 14 and 15 we were not able to obtain hybridization signals from

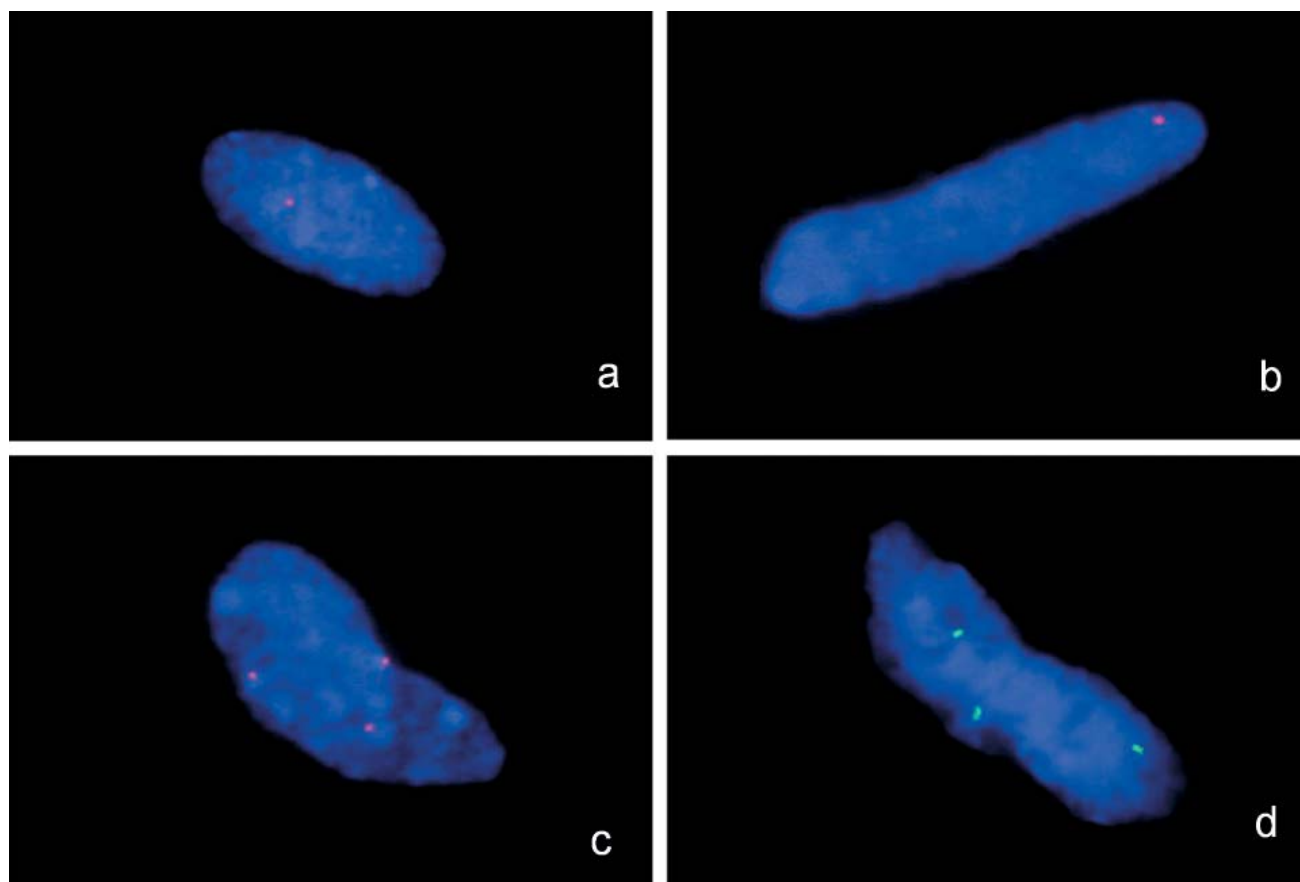


Fig. 1. FISH results on single cells from selected cases. Interphase nuclei from case 4, with one signal from chromosome 20 probe (a) Monosomy of chromosome 20 in case 10 (b). Presence of trisomy 20 in case 6 (c). Presence of trisomy 8 in case 17 (d).

TABLE 1

Summary of the clinical and cytogenetic data on 19 patients with desmoid tumors

Case no.	Age/ Sex	Location	FISH results			
			X	Y	8	20
1	29/F	Abdominal wall (P)	++ [196]	-	++ [98]	++ [96]
2	29/F	Abdominal (musculus rectus) (P)	++ [35]	-	++ [53]	++ [88]
3	54/F	Abdominal (musculus rectus) (P)	++ [200]	-	++ [192]	++ [98]
4	17/F	Abdominal (musculus rectus) (R)	++ [99]		++ [77]	+ [43]
5	66/M	Extra-abdominal (neck) (P)	+ [29]	+ [29]	++ [37]	++ [61]
6	28/F	Abdominal wall (P)	++ [58]	-	++ [76]	++ [90] +++ [22]
7	30/F	Extra-abdominal (thoracic wall) (P)	++ [200]	-	++ [180]	+ [60]
8	28/F	Abdominal wall (P)	++ [200]	-	++ [219]	++ [57]
9	29/F	Extra-abdominal (back) (P)	++ [66]	-	++ [69]	++ [65]
10	28/F	Abdominal wall (P)	++ [200]	-	++ [73]	+ [98] ++ [3]
11	26/F	Abdominal (musculus rectus) (P)	++ [175]	-	++ [143]	++ [141]
12	41/F	Abdominal (musculus rectus) (P)	++ [121]	-	++ [104]	++ [75]
13	31/F	Abdominal (musculus rectus) (P)	++ [88]	-	++ [83]	+ [83] ++ [2]
14	43/F	Extra-abdominal (arm) (P)	++ [25]	-	++ [24]	ND
15	33/F	Extra-abdominal (breech)	++ [200]	-	++ [89]	ND
16	23/F	Abdominal (musculus rectus) (P)	++ [128]	-	++ [96]	++ [82]
17	68/F	Extra-abdominal (thoracic wall) (P)	++ [86]	-	+ [3] ++ [40] +++ [21]	++ [68]
18	?/F	Abdominal wall (P)	++ [21]	-	++ [64]	++ [38]
19	30/M	Extra-abdominal (arm) (P)	+ [103]	+ [103]	++ [75]	++ [37]

F-female; M-male; P-primary lesion; R-recurrent lesion

- no signal detected

+ one signal detected

++ two signals detected

+++ three signals detected

ND – not determined

? - age unknown

Number of analyzed cells is represented in parentheses. Cases with chromosomal aberrations are highlighted.

all the probes used, because of the insufficient material to examine or due to technical problems.

The results of the clinical and molecular cytogenetic findings are presented in Table 1. Only one of the analyzed patients demonstrated recurrence (Case 4). In case 4 familial adenomatous polyposis has been diagnosed. One year after colectomy the mass of the size 6x3 cm was observed in the abdominal wall at the upper part of the postoperative scar. The mass was surgically removed together with the rectus muscle. After one year another mass of the size 7x8 cm was observed in patient's left hypogastrium, to the right of

postoperative scar. In the remaining patients the presence of primary lesions was ascertained.

Out of 19 analyzed cases, 17 were females, 2 were males. This was confirmed by the results of fluorescent *in situ* hybridization (FISH) with chromosome X and Y centromeric probes (Table 1). FISH studies demonstrated the presence of chromosomal abnormalities in six cases. Monosomy of chromosome 20 was present in four cases (case 4, 7, 10 and 13) (Fig. 1A, B). Extra copy of chromosome 20 was detected in one patient (case 6, Fig. 1C). Trisomy 8 was detected in one of the patients analyzed (case 17, Fig. 1D).

Discussion

We tried several methods to obtain appropriately digested desmoid tissue and clear, intact interphase nuclei. In our investigations, first digestion in pepsin solution, then passing the suspension through a mesh and later digestion with Protein Digesting Enzyme seemed to be the best procedure that allowed to obtain slides free from collagenous matrix, yielding efficient hybridization. In the present study we did not define the exact digestion time, because it was different for samples received from various centers of pathology.

Many clonal chromosomal aberrations have been observed in desmoid tumors [16], however trisomy 8 and trisomy 20 have been reported as the most frequent abnormalities found in karyotypic and FISH studies. Presence of the additional copy of chromosome 8 in a tumor tissue has been associated with an increased risk of recurrence and more aggressive clinical behavior [7, 9, 19]. However, there is a discrepancy between cytogenetic and molecular cytogenetic studies of desmoid tumors concerning chromosome 8. In many published cases, an additional copy of chromosome 8 was not detectable by karyotype analysis or it was found only in the minority of the analyzed cells, whereas it was observed at much higher frequency using a FISH method [4, 7]. This discrepancy has been explained by the presence of diploid cells (fibroblasts, vascular elements or skeletal muscle) in the tumor tissue that grow preferentially in short-term culture and obscure the trisomy 8 population cells [9]. Fletcher et al. observed that trisomy 8 was present in both primary and recurrent tumors. In the current study, trisomy 8 was detected in one primary lesion. Three signals for the chromosome 8 centromere-specific probe were present in 32.8 % of the counted cells. FISH studies revealed also one case of trisomy 20. An additional signal was observed in 22.2% of the analyzed cells. These findings confirmed the presence of chromosome 8 and 20 trisomies in desmoid tumors.

Loss of the one copy of chromosome 20 was observed in all of the analyzed cells in case 4 and 7, in 94% and 97,6% of the counted nuclei in case 10 and 13 respectively. Monosomy of chromosome 20 has not been described so far as a single chromosomal change in desmoid tumor. However Bridge et al. reported two cases, where monosomy 20 coexisted with other chromosomal abnormalities in a complex karyotype [3]. We cannot exclude the presence of other chromosomal changes in our monosomy 20 cases, which we were not able to detect using fluorescence *in situ* hybridization.

In earlier studies, CGH (comparative genomic hybridization) showed a relatively low frequency of

genomic changes in abdominally located desmoid tumors [2, 12]. Only one out of eight abdominal tumors had aberrations in the study by Larramendy et al. [12] and only two out of ten abdominal tumors showed chromosomal changes in the study by Brandal et al. [2]. Author concluded that abdominal desmoids might be karyotypically normal pseudoneoplastic tumors, or neoplastic tumors with small chromosomal aberrations not detectable by CGH. In our study, the frequency is much higher than that found previously. We found chromosomal aberrations in 4 out of 12 abdominal desmoid tumors. These results suggest that both abdominal and extra abdominal desmoids are genuine neoplastic tumors presenting with chromosomal changes, and that they might share a common or related mechanism of pathogenesis.

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Address for correspondence and reprint requests to:

Magdalena Mayer
Department of Medical Genetics
Poznań University of Medical Sciences
ul. Grunwaldzka 55 paw.15
60-352 Poznań
Phone: +48 61 8671216
Fax: +48 61 8675031
Email: mkanik@amp.edu.pl