Severe Chromosomal Aberrations in Pleural Mesotheliomas with Unusual Mesodermal Features

Comparative Genomic Hybridization Evidence for a Mesothelioma Subgroup

Michael Krismann, Klaus-Michael Müller,
Malgorzata Jaworska, and Georg Johnen

*From the Institute of Pathology, Professional Associations' Clinic
Bergmannsheil Bochum, University Clinic, Bochum, Germany*

Malignant mesotheliomas are tumors known for their extensive heterogeneity. Apart from the three classical patterns, predominantly epithelioid, sarcomatoid, and biphasic, some rare variants do exist. In some cases, one can find uncommon additional mesodermal tumor components. These tumors have previously been called “mesodermomas” and, like regular mesotheliomas, are usually associated with a previous asbestos exposure. We examined eight cases of mesodermomas by light microscopy, immunohistochemistry and comparative genomic hybridization (CGH). Besides biphasic and epithelioid areas, unusual epithelial, chondroid, osseous, or even angioblastic elements may be found to varying degrees. Immunohistochemical analysis shows similar staining results as with regular mesotheliomas. CGH reveals a high number of chromosomal imbalances (16.5 per case; range, 11–27). In 10 classical biphasic mesotheliomas that served as a control, defects of comparable number and severity could not be detected (8 per case; range, 2–16). The most frequent defects of mesodermomas (losses on 1p, 4pq, 9p, 13q, 14q, and gains on 1q and 15q), however, could also be found in mesotheliomas of the classical type. Thus, our results support the classification of the so-called mesodermomas as a separate tumor subgroup while maintaining the relationship to the classical mesotheliomas. Therefore, we propose to use the term mesodermoma for this subgroup. (*J Mol Diag* 2000, 2:209–216)

Every pathologist who is regularly confronted with mesotheliomas knows the broad histomorphological spectrum of these tumors. In the classical types of predominantly epithelioid, sarcomatoid, biphasic, or poorly differentiated histological appearance, the diagnosis is not very difficult to obtain if the panel of evaluated anti-

bodies shows unambiguous results. Nevertheless, a few unusual variants of mesotheliomas do exist, like the desmoplastic, lymphohistiocytic, small cell, or deciduoid peritoneal mesothelioma.¹ However, in some instances one may encounter histological structures that fit into neither the patterns of classical mesotheliomas nor those of the variants. These may be small cell solid areas, epithelial areas with features of squamous epithelium, or uncommon mesenchymal components like bone or cartilage formation. In that context, the term mesodermoma was introduced in 1981 by Donna and Betta for neoplasias that could be derived from undifferentiated pluripotent mesoderm.² It has never become popular; a recent Medline search request returned only three articles citing the term.^{2–4} Donna and Betta postulated that the mesothelium, which lines the serous membranes, was of mesodermal origin. The mesodermal epithelioid structure is supposed to persist during the postembryonic phase. Structures that can be derived from the mesoderm are connective tissue, cartilage, bone, muscles, cells of blood and lymphatics, vessel walls, kidneys, gonads, adrenal cortex, spleen, and serous membranes like the parietal and visceral pleura. Tumors derived from the mesoderm can therefore exhibit a broad spectrum of possible directions of differentiation. This could explain the myoblastic, angioblastic, lymphoblastic, chondroblastic, osteoblastic, fibroblastic, and uncommon epithelial differentiation of primary neoplasias of the pleura. Thus, so-called mesodermomas seem to be a variant type of mesotheliomas with additional characteristics of differentiation. Among more than 500 cases of mesotheliomas observed annually in the German Mesothelioma Registry, only one or two can be classified as mesodermomas. Because mesodermomas are not commonly known, they are rarely diagnosed. So far, no immunohistochemical or genetic characterization is available.

Our aim was to investigate if there are any morphological, immunohistochemical, or molecular clues to justify the clas-

Accepted for publication July 28, 2000.

Address reprint requests to Dr. Michael Krismann, Institute of Pathology, Professional Associations' Clinic Bergmannsheil Bochum, University Clinic, Bürkle-de-la-Camp-Platz 1, D-44789 Bochum, Germany. E-mail: patho-bhl@ruhr-uni-bochum.de.

Table 1. Patient Data

Case no.	Age	Sex	Asbestos exposure	Localization
M1	60	M	Yes	Pleura
M2	66	M	Yes	Pleura
M3	66	F	No	Pleura
M4	60	M	Yes	Pleura
M5	68	M	Yes	Pleura
M6	58	F	?	Pleura
M7	54	M	Yes	Pleura
M8	73	M	Yes	Pleura
B1	75	M	Yes	Pleura
B2	63	M	Yes	Pleura
B3	49	M	Yes	Pleura
B4	62	M	?	Pleura
B5	64	M	Yes	Pleura
B6	61	F	?	Petricardium
B7	75	M	Yes	Pleura
B8	68	M	Yes	Pleura
B9	56	M	Yes	Pleura
B10	58	M	?	Pleura

Mesodermoma group (M1–M8): Average patient age 63.1 years, six out of eight patients with a known history of asbestos exposure. Biphasic mesothelioma group (B1–B10): Average age 63.1 years, seven out of ten patients with history of asbestos exposure.

sification of mesotheliomas with uncommon mesodermal structures as a subgroup within the mesotheliomas.

Materials and Methods

We have investigated eight cases of pleural mesotheliomas with obvious uncommon mesenchymal or epithelial structures by immunohistochemistry and comparative genomic hybridization (CGH). The CGH is a well established method to screen tumor tissues for chromosomal imbalances.^{5,6} In comparison, we have analyzed a series of 10 biphasic mesotheliomas. Patient data of both tumor groups and the panel of antibodies are summarized in Tables 1 and 2.

Immunohistochemical Staining

For the diagnosis of mesotheliomas we used an antibody panel that has been proven to be reliable in more than a thousand mesotheliomas in the German Mesothelioma Registry. This panel is consistent with literature data.^{7–9} It consists of keratin AE1/AE3 (DAKO, Hamburg, Germany, 1:800, protease pretreatment), calretinin (SWant, Bellinzona, Switzerland, polyclonal, 1:3000, microwave pretreatment, citrate buffer, pH 6.0), human epithelial antigen

(BerEP4, DAKO), vimentin (DAKO, 1:4000, microwave pretreatment, citrate buffer, pH 8.0), and additionally Ki-67 (MIB-1, Dianova, Hamburg, Germany, 1:800, microwave pretreatment, EDTA buffer, pH 8.0). For differential diagnosis of predominantly sarcomatoid mesotheliomas, antibodies used in soft tissue pathology have to be applied. For this series, we have additionally tested smooth muscle actin (SMA, Immunotech, monoclonal). In one case, thyroglobulin (DAKO, monoclonal, 1:50) had to be applied for differential diagnosis. All immunohistochemical analyses were performed with a DAKO Techmate 500 immunostainer using the alkaline phosphatase-anti-alkaline phosphatase method. Moreover, each tumor specimen was previously tested histochemically for periodic acid-Schiff (PAS) with and without diastase pretreatment.

CGH

In all cases, we used paraffin-embedded tissues. Twenty sections of 10 μ m each were cut. The selected tissue was microdissected by using a scalpel. The genomic DNA was extracted and purified with the help of a commercially available kit (QIAamp Tissue Kit, Qiagen, Hilden, Germany). Similarly, reference DNA was isolated from normal human peripheral blood with a QIAamp Blood Kit (Qiagen). A slightly modified standard protocol for the hybridization was used,¹⁰ similar to that published by Petersen et al.¹¹ Labeling of reference and tumor DNA was performed by nick translation with digoxigenin-11-dUTP or biotin-16-dUTP (Roche, Mannheim, Germany), respectively. The probe length, checked by agarose gel electrophoresis, was between 300 and 3000 bp. Typically, 0.9 μ g labeled reference DNA and 0.9 μ g labeled tumor DNA were hybridized to normal human metaphase chromosomes (Vysis, Downers Grove, IL) for 3 days in the presence of 30 μ g human Cot-1 DNA (Roche) and 10 μ g herring sperm DNA (Promega, Madison, WI). Staining of the hybridized metaphase chromosomes was performed with 3.2 μ g/ml anti-digoxigenin-rhodamine (Roche) and 16 μ g/ml fluorescein-avidin (Vector Labs, Burlingame, CA). DAPI served as a counterstain for karyotyping. Each tumor sample was labeled, hybridized, and stained at least twice. The fluorescence images were recorded with a cooled 12-bit charged coupled device camera (SensiCam; PCO, Kelheim, Germany) on an AxioPhot fluorescence microscope (Zeiss, Jena, Germany). Images were processed and evaluated with the program Quips (Vysis) on a Macintosh G3 PowerPC (Apple, Cu-

Table 2. Immunohistochemical Results of Mesotheliomas with Uncommon Histoarchitectural Features

Antibody	Vendor	Epithelioid portions	Sarcomatoid portions
Keratin AE1/AE3	Camon	Strongly positive	Focally positive
Calretinin (polyclonal)	SWant	Strongly positive	Focally weak positive
HEA	DAKO	Negative	Negative
Vimentin	DAKO	Single cells positive	Focally positive
Thyroglobulin	DAKO	Negative	Negative
Actin HHF 35	DAKO	Negative; one case strongly positive	Focally positive
Ki-67 (MIB-1)	Dianova	Approx. 20–40%	Approx. 10–30%

The pattern is similar to that of the common types of malignant mesotheliomas.

Figure 1. Morphological findings with mesodermomas. **A:** Pleural mesodermoma, 60-year-old man with a known history of asbestos exposure (case M1). Epithelial follicular tumor area resembling thyroid gland tissue. Thyreoglobulin was not detectable immunohistochemically (H&E). **B:** Same case as presented in **A**. Chondroid area of the tumor that was included in the CGH analysis (H&E). Immunohistochemical results. **C:** Calretinin staining. Heterogeneous staining pattern with positive reaction in the large pleomorphic cells, but negative result in the small cell areas (case M8). **D:** Smooth muscle actin staining with positive reaction in the small cell portion of the tumor, the stromal cells being negative (case M8).

pertino, CA). Depending on the quality of a hybridization, 10 to 15 metaphases per case were analyzed and the results averaged. A 99% confidence interval was calculated and is depicted together with the average profile for each chromosome. The threshold values of the fluorescence ratio fluorescein/rhodamine were set to 0.80 and 1.20 for losses and gains, respectively. Losses are shown as vertical red bars on the left side of a chromosome ideogram; gains are shown as green bars on the right (Figure 2). As positive control, we used a tumor cell line with known defects (MPE 600, Vysis). Five samples from healthy tissues and a case of pleuritis of a patient without any known malignancies served as negative controls. In addition, we used reverse labeling of reference and tumor DNA (with fluorescein and rhodamine, respectively) to confirm the CGH results of the mesodermomas.

Results

Immunohistochemistry

The histomorphological heterogeneity of the studied malignant mesotheliomas with uncommon mesodermal histioarchitectural features (Figure 1, A and B) is reflected in varying immunohistochemical results (Table 2). Keratin shows a positive reaction with the solid pseudoglandular

and partially with the sarcomatoid areas. Vimentin is positive in fusiform cells as well as in a varying number of epithelioid cells. Calretinin is moderately to strongly positive in the epithelioid areas, but in some cases the pattern of expression is restricted to a few areas (Figure 1C). The spindle cell areas show only focally a positive reaction with calretinin, as it is found in common mesotheliomas. HEA is completely negative in all cases. Thyreoglobulin shows no positive immunoreactivity in case M1, which exhibits thyroid-like epithelial features (Figure 1A). Interestingly, one of the so-called mesodermomas shows a positive reaction with the SMA antibody not only in the sarcomatoid areas, but also in the epithelioid parts (Figure 1D). A positive actin stain in spindle cell areas can be observed in eight of the ten biphasic mesotheliomas of the control group, but in none of their epithelioid areas. The proliferative activity, as measured by expression of the Ki-67 antigen (MIB-1), is somewhat higher than in common types of mesotheliomas.

CGH

Detailed results of the CGH analyses are depicted in Tables 3 and 4, a comparison of both tumor groups is summarized in Table 5, and an overview is given in the two "superkaryograms" (superposition of all CGH profiles

Table 3. Mesodermomas: CGH Results in Detail

Case no.	Losses	Gains	Σ Losses	Σ Gains	Σ Defects
M1	1p21-31, 3q23-25, 4p15.3-qter, 5q15-22, 6q12-22, 8q21.2-22, 9p13-pter, 10q21, 13q21-22, 14q12-13, 15q13-14, 18p11.3, 18q12-qter, 21q11.2	1q21, 2q11.2-21, 2q35-qter, 3p24, 5q13, 5q31-qter, 7p, 7q, 11q12-13, 12p13, 12q13, 12q23-qter, 15q21-26	14	13	27
M2	1p13-21, 4p, 4q, 9p, 14, 15q11.2-14, 21, 22	1p33-35, 1q, 5p, 15q21-qter	8	4	12
M3a	1p, 3p14-pter, 3p12-qter, 4p13-14, 4q, 8p11.2-12, 8p22-pter, 9q21-pter, 9q33-qter, 10q22-24, 11p14-pter, 14q22-31, 15q13-14, 16q22-qter, 22	8q22-23, 11q21-22	15	2	17
M3b	1p, 3p, 3q, 4q, 8p22-pter, 9p, 10q23-24, 11p15, 14q23-24, 16q23-qter, 22q	—	11	0	11
M3c	1p, 3p, 3q, 4p13-14, 4q, 8p11.2-12, 8p22-pter, 9p, 9q, 10q22-24, 11p, 14q22-31, 15q13-14, 16q22-qter, 22	1q31-41, 7p, 7q, 11q12-13, 12q23-24.1	15	5	20
M4	1p13-21, 4, 9p, 9q13-21, 10p13-pter, 12q14-pter, 13, 14q11.2-24, 15q11.2-13, 18p, 18q	7q11.2, 11q12-13, 12q23-24.1, 15q21-qter	11	4	15
M5	1p21, 4q, 6q16-25, 8q23, 9p21-pter, 10q22-25, 11p12-14, 13q14-31, 14q, 15q13-14, X	1q, 2q34-qter, 6p, 7q11.2-pter, 8q13-pter, 9q, 11q23-24, 15q21-qter	11	8	19
M6	1p13-31, 2q32-34, 4q13-pter, 9p, 11p, 11q13-qter, 13q12-22, 14q11.2-21, 21q11.2, 22q, Xp	1p32-pter, 1q, 11q12, 12p12-pter, 15q22-23	11	5	16
M7a	1p12-31, 3q13.1-pter, 3q24-26.1, 4q, 6q12-22, 9p, 13q21-22, 14q11.2-12, 18q22-qter	1q21-22, 6p21.2-21.3, 15q22-23	9	3	12
M7b	1p13-31, 3p24-pter, 3q24-25, 4q, 6q13-23, 9p, 10p12-pter, 13q21-31, 14q11.2-12	1q21-22, 6p21.2-21.3, 15q22-23	9	3	12
M8	1p13-22, 3p14-21, 4, 5q13-21, 6q21-24, 7q32, 9p, 10p12-pter, 10q23-24, 11q23, 13, 17p, 18q	9q22-32, 15q	13	2	15

In case M3, the DNA was isolated from three different tumor areas (a: highly heterogenic; b: heterogenic with large epitheloid portion, high cellularity; c: desmoplastic, low cellularity). In case M7, the DNA was from two different areas of similar histology.

of each tumor group) in Figure 3. Reverse labeling confirmed all major and most of the other defects. Each of the eight cases of mesodermomas shows a remarkably high genetic instability. An example of a single CGH profile is given in Figure 2. Inverse labeling shows reproducible

results. We have found 16.5 defects per case on average, with a range between 11 and 27 defects per case. Losses were more common than gains (11.3 on average; range, 8–15). Gains of chromosomal material could be detected in 5.2 locations per case, with a range between

Table 4. Biphasic Mesotheliomas: CGH Results in Detail

Case no.	Losses	Gains	Σ Losses	Σ Gains	Σ Defects
B1	4p, 10p13-pter, 11q23-qter, 18p, 19p13.2-pter, 19q13.2-qter, 21	3p12-13, 3p22-24, 3q13.3-26.1, 7p11.2-15, 7q21, 7q31, 9q31-32, 13q21-32, 15q24-26	7	9	16
B2	4q27-34, 8q13-21.3, 9p12-21, 10q21-22, 22	6p, 11p, 21q	5	3	8
B3	1p13-21, 2q35-qter, 3p14-21, 6q16-qter, 8p12-pter, 9p12-21, 22q	1q21-32, 7, 8q22-qter, 15q22-qter	7	4	11
B4	1p13-31, 4p12-15.1, 4q13-32, 5q15-23, 9p13-21, 13q13-31, 14q13-24, 18q12-22, 21q11.2-22	—	9	0	9
B5	1p21-pter, 9p21-23, 9q, 22	1q22-qter, 3q21-24	4	2	6
B6	2q36-qter, 8p12-pter, 9p21-pter, 10q24-qter	7p14-pter, 7q11.2-31, 15q22-25	4	3	7
B7	9p13, 22	—	2	0	2
B8	4p12-15.1, 4q13-34, 6q16-22, 9p23-q22, 11q23-24, 22q	8q	6	1	7
B9	11q13-qter, 14q12-qter, 17p, 22	1q, 3p23-25, 7	4	3	7
B10	1p31-pter, 10p, 13q12-31, 14, 17p, 22	7q21-qter	6	1	7

Table 5. Comparison of the Most Common Chromosomal Defects in Mesodermomas and Biphasic Mesotheliomas

Mesodermomas		Recurrent losses		Biphasic mesotheliomas	
Chromosomal region	Frequency (%)	Chromosomal region	Frequency (%)	Chromosomal region	Frequency (%)
1p21 (1p13-21)	100 (75)	1p21, 1p31	30	1p21, 1p31	30
4q12-13 (4q)	100 (88)	4q27-32	30	4q27-32	30
9p21-pter (9p)	100 (75)	9p21 (9p13-21)	60 (50)	9p21 (9p13-21)	60 (50)
14q12 (14q11.2-24)	75 (50)	14q13-24	30	14q13-24	30
4p13-14 (4p12-15.3)	75 (63)	4p12-15.1	30	4p12-15.1	30
13q21-22	75	13q13-31	20	13q13-31	20
15q13	63	—	—	—	—
6q21-22	50	6q16-22	20	6q16-22	20
18q22-qter	50	18q12-22	10	18q12-22	10
3p14-21	38	3p14-21	10	3p14-21	10
3q24-25	38	—	—	—	—
10q23-24	38	10q24-qter	10	10q24-qter	10
21q11.2	38	21q11.2-22	20	21q11.2-22	20
22q	38	22q	70	22q	70
11q23	25	11q23-24	30	11q23-24	30

Mesodermomas		Recurrent gains		Biphasic mesotheliomas	
Chromosomal region	Frequency (%)	Chromosomal region	Frequency (%)	Chromosomal region	Frequency (%)
15q22-23 (15q21-26)	88 (63)	15q24-25	30	15q24-25	30
1q21 (1q)	63 (38)	1q22-32	30	1q22-32	30
7q11.2	38	7q21, 7q31 (7q)	50 (30)	7q21, 7q31 (7q)	50 (30)
11q12	38	—	—	—	—
7p	25	7p14-15	40	7p14-15	40

Minimal overlapping areas of recurrent losses and gains are listed in order of their frequency of occurrence in mesodermomas. In some cases larger areas and their frequencies are also given (in brackets) to emphasize the consensus regions between both tumor groups. To be listed, a defect had to occur in three or more cases of at least one of the two tumor groups.

zero and 13. Recurrent losses were found in all cases in regions of chromosome arms 1p, 4q, and 9p (100%). Further frequent losses were localized on sections of chromosomes 14q, 4p, 13q (75%), 15q (63%), 6q, and 18q (50%), as well as 3p, 3q, 10q, 21q, and 22q (38%). In all, we have found 14 different recurrent (ie, occurring in >30% of the tumors) losses of chromosomal material in the tumors selected for this study (Table 5). Recurrent gains of DNA material were also detected. Chromosome 15q was affected in 88% of the cases, followed by chromosomes 1q (63%), 7q, and 11q (38%). In all, four recurrent gains were found. Thus, an overall number of 18 different recurrent defects could be observed.

On the contrary, in the group of biphasic mesotheliomas (Table 4) that served as a control, we only found five defects that occurred in more than 30% of the cases. The most frequent defects were losses on 22q (70%) and 9p (60%) and different gains on chromosome 7 (40 to 50%). The average number of total defects per case was 8, with 5.4 losses and 2.6 gains per case. The locations of the chromosomal defects were similar in both groups (Table 5, Figure 3).

For our CGH analysis, we have excluded possible artifact regions, especially for gains, on chromosomes 1p33-pter, 9q34, 16, 17, 19, 20, and 22. (For discussion of artifact regions, see Larramendy et al.¹²)

Discussion

The immunohistochemical spectrum of the eight tested tumors with uncommon mesodermal histoarchitectural

features (so-called mesodermomas) supports their histogenetic relationship to the mesotheliomas. A positive calretinin stain is typical for mesotheliomas as well as mesodermomas. At the moment, a positive calretinin stain in combination with a negative histochemical PAS staining after diastase pretreatment, a staining positive for vimentin and negative for HEA is, based on the experiences at the German Mesothelioma Registry, the best differential diagnostic panel to distinguish between mesotheliomas and metastases of adenocarcinomas. Actin is commonly found in both biphasic mesotheliomas and mesodermomas. Antibodies to keratin and Ki-67 also showed similar results in both tumor groups. Thus, the two groups cannot be distinguished by immunohistochemical analysis alone.

In contrast, our CGH results support, based on the markedly high number of chromosomal aberrations (16.5 defects per case), the unique position of the mesodermomas within primary malignant neoplasias of the serous membranes. None of the mesodermomas had fewer than 11 defects, whereas only two of the biphasic mesotheliomas had 11 or more defects (average, 8 defects per case). On the other hand, the classification of the mesodermomas as part of the mesotheliomas is also documented by the CGH analysis, because several corresponding defects were demonstrated. The recurrent defects that we found both in mesotheliomas and mesodermomas were located on chromosomes 1p, 4p, 4q, 6q, 9p, 13q, 14q, and 22q (losses) as well as 1q, 7q, and 15q (gains). On the contrary, losses on chromosome 15q could be detected only in the mesodermoma group.

Figure 2. CGH results. Fluorescence ratio profile of case M3a, 66-year-old woman without known history of asbestos exposure. High number of genetic imbalances. Losses are represented as red bars on the left side of each chromosome ideogram, gains are represented as green bars on the right side. The reference DNA was from a male donor. Therefore, the X chromosome shows a gain and the Y chromosome a loss.

Whereas most defects showed a higher frequency in mesodermomas, losses on 22q and gains on chromosome 7 were more prominent in the biphasic mesotheliomas. In general, defects in mesodermomas appeared more pronounced and tended to affect whole chromosome arms. The consensus regions of the defects in the two tumor groups were similar, but not always identical. This might in part also reflect the lower number of total defects derived from the investigated biphasic mesotheliomas and could change somewhat with an increasing numbers of cases. We also compared our results to published CGH studies of mesotheliomas.^{6,13-17} The known defects that have been found in more than one CGH study on malignant mesotheliomas are also found in our series of mesodermomas, like losses on consensus chromosome regions 1p21-22, 4cen-p15.3, 4cen-q24, 4q33-qter, 6q16-22, 9p21-22, 10q23, 13q21-22, 14q21-24, and 22q, as well as gains on chromosome arms 1q and 15q (compare Table 5). A well defined loss on the upper parts of chromosome arm 15q (around 15q13) that only had been described once for cultured mesothelioma cell lines¹³ can be seen in five of the eight presented cases. Interestingly, we found a frequent combination of defects in the mesodermomas on 15q: the loss at 15q13 and an additional gain of material on the lower part of 15q (15q21-26) was found in four of the eight cases (50%). This gain on 15q was in all four cases a high-level amplification (ratio = 1.5), one of these with a ratio of 2.0 (case M2). Besides frequent losses on 15q13 and 18q22-qter, cultured mesothelioma cell lines also shared a higher total

number of genetic changes with the mesodermomas. However, we were not able to detect recurrent gains on 5p and losses on 8p that were found by two independent groups in the mesothelioma cell lines.^{13,16}

We also examined different areas of the same tumors. For example, in a heterogenic portion of mesodermoma case 3 (M3a, Table 3) the gains were located on different chromosomal regions than in a more epithelioid area (M3b) or a desmoplastic area of low cellularity (M3c), whereas most losses were similar. In another case (M7), hybridizations of different tumor areas of similar histology (M7a and M7b) showed a few differences in the localization of the losses but identical gains. This reflects the intratumor heterogeneity and reminds not to overestimate hybridization results obtained from small areas of large tumors in view of chromosomal alterations that may be involved in the molecular pathogenesis of tumors. However, the chromosomal position of the most frequent defects can serve as a guide for further genomic localization of candidate genes. In total, we found a relatively high degree of similarity of genetic defects within the collection of mesodermomas. The remarkable genetic patterns were also mirrored in the histological appearance.

In conclusion, nearly 20 years after Donna and Betta's more general definition of mesodermomas, we propose to use this term for the described mesothelioma subclass. We suggest using this term for four reasons. First, we have documented that the discussed tumors are sufficiently distinct to be regarded as a special subgroup of mesotheliomas. Second, the term mesodermoma reflects the embry-

Figure 3. Summary of CGH results. Defects are represented in superkaryograms by superposition of all CGH profiles of each tumor group. **A:** Chromosomal imbalances of eight mesodermomas. **B:** Chromosomal imbalances of 10 biphasic mesotheliomas. Losses are shown as vertical lines on the left side of a chromosome, gains are shown on the right side.

ological background and makes sense in view of the pathogenesis of these highly heterogenic tumors. Third, the rather bulky and impractical term "mesothelioma with uncommon mesodermal histoarchitectural features" should be

avoided. Fourth, the term "mesoblastoma," though it would also be appropriate for description, could be confused with the mesoblastic nephroma. This renal tumor was first reported in the field of pediatric pathology in 1967.¹⁸

In summary, we propose to diagnose a tumor as mesodermoma if (i) the histological features of a primary tumor of the serous membranes include uncommon patterns of mesodermal differentiation like myoblastic, chondroblastic, osteoblastic, or uncommon epithelial patterns; (ii) immunohistochemical analysis shows a positive result for keratins, vimentin, calretinin, and negative results for CEA and HEA (Ber-EP4) as well as a negative histochemical result for the PAS staining after diastase pretreatment; and (iii) if a markedly increased number of genetic imbalances by the use of molecular genetic methods like CGH can be demonstrated.

The presented analytical tools should improve diagnosis of these mesodermomas and help to further our knowledge about the development of malignant tumors of the serous membranes.

References

- Battifora H, McCaughey WTE: Tumors of the Serosal Membranes. Edited by Rosai J and Sobin LH. Washington, DC, Armed Forces Institute of Pathology, 1995, pp 41–52
- Donna A, Betta PG: Mesodermomas: a new embryological approach to primary tumours of coelomic surfaces. *Histopathology* 1982, 5:31–44
- Donna A, Betta PG, Bianchi V, Ribotta M, Bellingeri D, Robutti F, Marchesini A: A new insight into the histogenesis of "mesodermomas:" malignant mesotheliomas. *Histopathology* 1991, 19:239–244
- Donna A, Betta PG: Differentiation towards cartilage and bone in a primary tumour of pleura: further evidence in support of the concept of mesodermoma. *Histopathology* 1986, 10:101–108
- Hermesen MAJA, Meijer GA, Baak JPA, Joenje H, Waldbloomers JJM: Comparative genomic hybridization: a new tool in cancer pathology. *Hum Pathol* 1996, 27:342–349
- Knuutila S, Aalto Y, Autio K, Björkqvist AM, El-Rifai W, Hemmer S, Huhta T, Kettunen E, Kiuru-Kuhlefelt S, Larramendy ML, Lushnikova T, Monni O, Pere H, Tapper J, Tarkkanen M, Varis A, Wasenius VM, Wolf M, Zhu Y: DNA copy number losses in human neoplasms. *Am J Pathol* 1999, 155:683–694
- Ordóñez NG: Role of immunohistochemistry in differentiating epithelial mesothelioma from adenocarcinoma. *Am J Clin Pathol* 1999, 112:75–89
- Riera JR, Astengo-Osuna C, Longmate JA, Battifora H: The immunohistochemical diagnostic panel for epithelial mesothelioma: a reevaluation after heat-induced epitope retrieval. *Am J Surg Pathol* 1997, 21:1409–1419
- Wiethege T, Philips S, Müller KM: Calretinin als Marker zur Differentialdiagnose von Mesotheliomen und Adenokarzinomen. *Verh Dtsch Ges Pathol* 1997, 81:417 (abstr)
- Krismann M, Adams H, Jaworska M, Müller KM, Johnen G: Patterns of chromosomal imbalances in benign solitary fibrous tumours of the pleura. *Virchows Arch* 2000, 437:248–255
- Ried T, Petersen I, Holtgreve-Grez H, Speicher MR, Schroeck E, du Manoir S, Cremer T: Mapping of multiple DNA gains and losses in primary small cell lung carcinomas by comparative genomic hybridization. *Cancer Res* 1998, 54:1801–1806
- Larramendy ML, El-Rifai W, Knuutila S: Comparison of fluorescein isothiocyanate- and Texas red-conjugated nucleotides for direct labeling in comparative genomic hybridization. *Cytometry* 1998, 31:174–179
- Balsara BR, Bell DW, Sonoda G, de Rieuzo A, du Manoir S, Jhanwar SC, Testa JR: Comparative genomic hybridization and loss of heterozygosity analyses identify a common region of deletion at 15q11.1-15 in human malignant mesothelioma. *Cancer Res* 1999, 59:450–454
- Björkqvist AM, Tammilehto L, Nordling S, Nurminen M, Anttila S, Mattson K, Knuutila S: Comparison of DNA copy number changes in malignant mesothelioma, adenocarcinoma and large-cell anaplastic carcinoma of the lung. *Br J Cancer* 1998, 77:260–269
- Björkqvist AM, Tammilehto L, Anttila S, Mattson K, Knuutila S: Recurrent DNA copy number changes in 1q, 4q, 6q, 9p, 13q, 14q and 22q detected by comparative genomic hybridization in malignant mesothelioma. *Br J Cancer* 1997, 75:523–527
- Kivipensas P, Björkqvist AM, Karhu R, Pelin K, Linnainmaa K, Tammilehto L, Mattson K, Kallioniemi QP, Knuutila S: Gains and losses of DNA sequences in malignant mesothelioma by comparative genomic hybridization. *Cancer Genet Cytogenet* 1996, 89:7–13
- Knuutila S, Armengol G, Björkqvist AM, El-Rifai W, Larramendy ML, Monni O, Szymanska J: Comparative genomic hybridization study on pooled DNAs from tumors of one clinical-pathological entity. *Cancer Genet Cytogenet* 1998, 100:25–30
- Bolande R, Brough A, Izant RJ: Congenital mesoblastic nephroma of infancy: a report of eight cases and the relationship to Wilms' tumor. *Pediatrics* 1967, 40:272–278