DNA Aberrations in the Epithelial Cell Component of Adamantinoma of Long Bones

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Adamantinoma of long bones is a rare malignant tumor composed of cells with epithelial characteristics in various differentiation patterns surrounded by fibrous cells. Evidence as to whether this neoplasm should be designated as an epithelial bone tumor or a biphasic sarcoma with both epithelial and mesenchymal features is lacking. In this study the nature of the mesenchymal and epithelial components of adamantinoma was investigated by DNA flow cytometry, DNA image cytometry, p53 immunohistochemistry, and polymerase chain reaction-based loss of heterozygosity detection at the p53 locus. Specimens from 6 of 15 patients (40%) analyzed by flow cytometry had an aneuploid DNA index. Image cytometry analysis of Feulgen-stained paraffin sections of 6 aneuploid and 2 diploid tumors revealed that aneuploid nuclei were detected in cells with an epithelial phenotype only, whereas all fibrous cells were diploid. Immunohistochemistry for p53 on specimens from 25 patients revealed moderate or strong immunoreactivity in 12 tumors (48%) restricted to the epithelial cells. Loss of heterozygosity at the p53 locus could be confirmed in the epithelial component of an immunohistochemically p53-positive tumor. Additionally, sections of 7 lung metastases were studied histologically. Only keratin-positive epithelial cells, predominantly in the spindle cell pattern, were present in these metastases, whereas the osteofibrous tissue present in the primary tumors was not detected. These results suggest that either adamantinoma consists of a malignant epithelial part with a reactive osteofibrous stroma
or that the malignant epithelial cells develop next to a proliferating benign fibrous component. Additional analysis of common genetic abnormalities in the fibrous and epithelial cells of adamantinoma is therefore indicated. (Am J Pathol 1995, 147:1770–1779)

Primary malignant tumors of bone and soft tissue are normally classified as sarcomas because of the mesenchymal phenotype of the tumor cells. There are, however, tumors primarily originating in bone or soft tissue that show characteristics of epithelial cells, like the adamantinoma of long bones, as was already recognized by Fischer in the first description of the tumor in 1913.[1] Later, several reports claimed a vascular or endothelial derivation, mainly based on hematoxylin and eosin (H&E) morphology or electron microscopic characteristics.[2,3] The epithelial nature of the tumor cells is at the moment no longer a matter of discussion, on the basis of consistent immunohistochemical and ultrastructural studies.[4–8] These epithelial cells are present in various differentiation patterns and are usually surrounded by osteofibrous tissue in the center of the tumor. The two foremost hypotheses about development of adamantinoma claim either evolution from ectopic epithelial cell rests implanted in cortical bone during embryonal development or after trauma with a reactive production of osteofibrous stroma[5,9,10] or development from a multipotent stem cell with the ability to differentiate along epithelial and mesenchymal pathways. According to the latter, both the fibrous and epithelial parts are neoplastic elements of the tumor.[11–13]

In a recent study we characterized the epithelial component of adamantinoma using monoclonal antibodies to chain-specific keratins. This revealed a

basal epithelial cell phenotype, which is quite different from other bone and soft tissue neoplasms with epithelial characteristics, among them tumors with a biphasic phenotype.[14] In the present study we analyzed both components of adamantinoma using different markers for neoplastic growth: (1) aneuploidy by DNA flow cytometry (FCM) and image cytometry (ICM), (2) p53 protein accumulation by p53 immunohistochemistry, (3) loss of heterozygosity at the p53 locus by the polymerase chain reaction (PCR) after sorting of epithelial and fibrous cells, and (4) metastatic potential by histological review of the lung metastases in our series.

Materials and Methods

Tumor Material

Thirty-four cases of adamantinoma, diagnosed in the period 1953 to 1993, were obtained from the files of the University Hospital Leiden and from the Registry of the Netherlands Committee on Bone Tumors. Radiographic, histopathological, immunohistochemical, and clinical data of 32 patients were reported in detail earlier.[14-16] Two new cases were appended of which short case histories are presented below. The diagnosis of adamantinoma was based on clinical evaluation, radiology, histopathology (Figure 1), and keratin immunohistochemistry and was confirmed independently by members of the Netherlands Committee on Bone Tumors. The distinction of fibrous and epithelial cells was based on the typical histological organization patterns of the epithelial cell part and tumor architecture as described by Weiss and Dorfman[13] and Czerniak et al.[17] With respect to these subtypes, the tumor is referred to as classic adamantinoma or osteofibrous dysplasia (OFD)-like (or differentiated) adamantinoma. Moreover, keratin immunohistochemistry provided clear differentiation between the fibrous and epithelial cells, especially in tumors in which the OFD-like part dominated the lesion and epithelial cells could not be recognized on H&E sections. The amount of woven bone formation in the OFD-like component differed strongly among the adamantinomas in our series.

Paraffin-embedded tissue blocks were available from 25 patients, whereas snap-frozen tumor material for flow cytometry was available from three tumors. Slides from lung metastases, of which clinicopathological data were given elsewhere,[16] were analyzed by light microscopy (n = 7) and immunohistochemistry (n = 2) for the presence of the epithelial and the osteofibrous component as observed in the primary tumors.
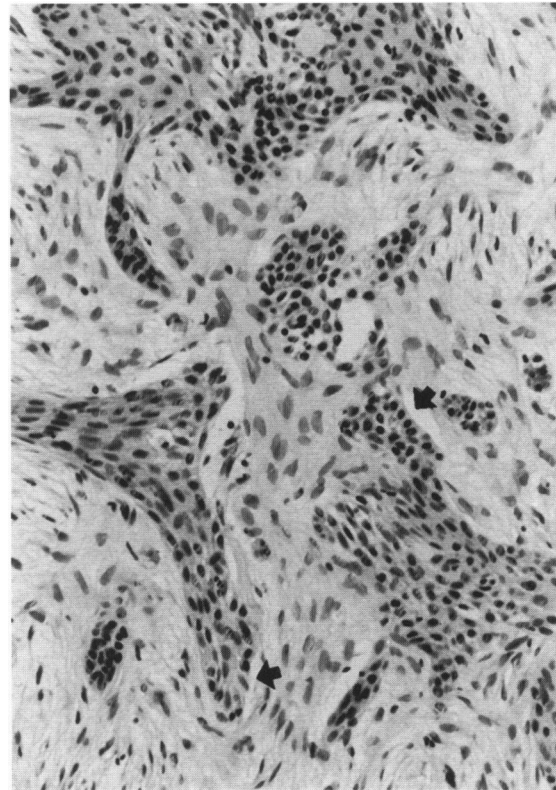

Figure 1. Light micrograph displaying the typical features of a basaloid subtype of adamantinoma of long bones. Nests of epithelial cells with peripheral pallisading at some places (arrows) are surrounded by a moderately cellular fibrous tissue (H&E; original magnification, X200).

Short Case Histories

Case 33

A 15-year-old girl presented with pain and swelling of her right knee. At radiographic examination, a small sharply demarcated osteolytic lesion was noted in the proximal anterior cortex of the right tibia. Radiographic diagnosis was considered to be a nonossifying fibroma and no additional treatment was applied. Six years later she returned with increased complaints. A biopsy revealed osteofibrous dysplasia. Immunohistochemistry did not show any keratin-positive cells. An en bloc resection was performed, and the cortical defect was filled with an inlay allograft. Immunohistochemistry on the resected specimen displayed small aggregates and isolated keratin-positive cells, and as such, a histological diagnosis of OFD-like adamantinoma was made. At latest follow-up, 19 months after resection, the patient was doing well without evidence of disease.

Case 34

An 11-year-old girl presented with a spontaneous painful swelling of her right tibia. Radiographic ex-

amination showed a sharply demarcated lobulated osteolytic lesion in the proximal anterior cortex without periosteal reaction. A clinico-radiological differential diagnosis of osteofibrous dysplasia or adamantinoma was considered. A biopsy was performed, and a diagnosis of fibrous dysplasia was made, as staining for keratins was negative. Six years later, the patient returned with increasing pain complaints. Magnetic resonance imaging showed a slightly increased lesion, with a minute defect in the anterior cortical border. A biopsy now revealed an OFD-like adamantinoma with clear keratin-positive cells. An *en bloc* resection was performed with an inlay allograft. At latest follow-up, 21 months later, the patient was doing well without evidence of disease.

Flow Cytometry and Cell Sorting

For univariate DNA FCM analysis, 29 specimens from 25 patients were available, consisting of 20 primary tumors, 7 local recurrences, and 2 lung metastases. Nuclei of paraffin tissue were isolated, according to the method of Hedley[18] with a prolonged pepsin digestion step of up to 2 hours instead of 30 minutes as minor modification, of the fresh-frozen tissue according to the Vindelov-method[19] with trout erythrocytes as an internal standard. FCM was performed on a FACScan flow cytometer (Becton Dickinson, Mountain View, CA). Single-parameter DNA histograms were evaluated according to accepted criteria.[20,21] In some specimens, interpretation of the histograms was hampered by the prolonged formalin fixation and formic acid decalcification, which influenced DNA stainability. Sorting of nuclei of the six aneuploid tumors was performed on a FACStar flow cytometer (Becton Dickinson) as described.[22] To minimize fraction to fraction contamination of diploid and aneuploid $G_{0,1}$ nuclei, narrow sorting windows were applied.

Image Cytometry

ICM on paraffin sections was used to measure DNA content in microscopically selected nuclei from the epithelial and fibrous component of six aneuploid and two diploid (classic) adamantinomas. Paraffin sections (2 μm) were stained according to the Feulgen method. A pilot study revealed that this was the optimal section thickness avoiding overlap of nuclei, which impedes proper analysis (not shown). Histological slides were analyzed with a Zeiss UEM light microscope equipped with a 25× Neofluar objective (N.A. = 0.60; Carl Zeiss, Oberkochen, Germany) and coupled to a digital image analyzer (IBAS with

AT-386 host computer, Kontron, Munich, Germany). As diploid control, nuclei of normal alveolar lung tissue in a lung metastasis of an adamantinoma were used. In every specimen, 100 fibrous cells and 100 epithelial nuclei were measured. The integrated optical densities (IODs) obtained when using ICM[23] cannot be used to calculate DNA indices but should be considered as the relative ratio of median values of IOD in epithelial and fibrous nuclei (the IOD ratio).

p53 Immunohistochemistry

Immunohistochemical detection of p53 protein accumulation was performed on paraffin sections of 29 specimens from primary tumors (21), local recurrences (6), or lung metastases (2) of 25 patients. After dewaxing, rehydrating, and endogenous peroxidase blocking, microwave antigen retrieval was performed with target unmasking fluid (TUF, Sanbio, Uden, The Netherlands).[24,25] Sections were incubated for 16 hours with monoclonal antibody DO7, diluted 1:200, or polyclonal rabbit antibody CM1, diluted 1:500 (both from Novocastra, Newcastle upon Tyne, UK). This was followed by a 30-minute incubation with horseradish-peroxidase-labeled rabbit anti-mouse immunoglobulins (1:100) for DO7 and a subsequent 30-minute incubation with labeled swine anti-rabbit immunoglobulins (1:50; both from Dakopatts, Glostrup, Denmark) for both DO7 and CM1. Visualization was carried out in a diaminobenzidine solution containing hydrogen peroxide. Ethyl green was used for counterstaining. As a negative control, slides were stained with phosphate-buffered saline instead of the primary antibodies and irrelevant mouse monoclonal antibody G250[26] was used.

Slides were scored as negative (−) when less than 1% of analyzed nuclei showed immunoreactivity, moderate 1+ for 1 to 10% of stained nuclei in epithelial or fibrous component, and strong 2+ for more than 10% of stained nuclei in either component.

Detection of Loss of Heterozygosity (LOH) at the p53 Locus

DNA extraction from the sorted nuclear suspensions, adjusted to 50 nuclei per μl, of six aneuploid tumors was performed by proteinase K incubation. PCR-based LOH detection for the microsatellite marker D17S513, chromosomal location 17p13, location of the p53 gene,[27] was performed as described.[22] Briefly, PCR was performed in 15-μl reactions containing 2 μl of purified template DNA; 200 μmol/L each dGTP, dTTP, dATP; 2.5 μmol/L dCTP; 0.75 μCi of

Table 1. *Results of Flow Cytometry, Image Cytometry, and p53 Immunohistochemistry on Adamantinoma of Long Bones*

Patient	Tumor	Subtype	DNA FCM DNA Index	DNA ICM (IOD ratio) Fibrous cells	DNA ICM (IOD ratio) Epithelial cells	p53 immunohistochemistry* DO7	p53 immunohistochemistry* CM1
1	Local recurrence	Squamous	1.00			2+	2+
3	Primary tumor	Squamous				−	−
6	Local recurrence	Spindle	1.27	1.00	1.37	2+	2+
9	Primary tumor	Mixed				2+	1+
10	Primary tumor	Basaloid	1.00	1.00	1.00	1+	1+
11	Local recurrence	Spindle				−	−
13	Primary tumor	OFD-like				−	−
	Local recurrence	OFD-like	1.00			−	−
	Lung metastasis	Spindle	1.00			−	−
16	Primary tumor	OFD-like				−	−
	Local recurrence	Spindle	1.00	1.00	1.03	1+	1+
17	Primary tumor	Spindle				1+	1+
	Lung metastasis†	Spindle	1.13		1.54	1+	1+
18	Primary tumor	Spindle	1.14	1.00	1.36	1+	1+
19	Primary tumor	Tubular	1.00			2+	2+
21	Primary tumor	Basaloid	1.13	1.00	1.34	2+	2+
22	Primary tumor	Tubular				−	−
23	Primary tumor	Tubular	1.00			−	−
24	Primary tumor	Tubular	1.19	1.00	1.32	2+	2+
25	Primary tumor	OFD-like				−	−
26	Local recurrence	OFD-like				−	−
27	Primary tumor 1	Tubular	2.86/2.84‡	1.00	2.32	−	−
	Primary tumor 2	OFD-like	1.00			−	−
28	Primary tumor	OFD-like				−	−
29	Primary tumor	Tubular				1+	1+
30	Primary tumor	OFD-like				−	−
31	Primary tumor	OFD-like	1.00			−	−
32	Primary tumor	Tubular				1+	−
33	Primary tumor	OFD-like	1.00/1.00‡			−	−
34	Primary tumor	OFD-like	1.00/1.00‡			−	−

*−, less than 1%; 1+, 1 to 10%; 2+, more than 10% of nuclei stained.
†The nuclei of alveolar cells of lung measured in this sample showed the same median value of IOD as the fibrous cells in the primary and recurrent adamantinomas; in this case the median IOD of the metastatic epithelial nuclei is related to the alveolar nuclei.
‡The second result was obtained with nuclei from snap-frozen material (Vindelov method). In patient 27, two tumor samples were measured: sample 1 from the center of the tumor, with abundant epithelium, and sample 2 from the periphery, lacking detectable epithelial cells.

[α-^{32}P]dCTP (3000 Ci/mmol, 10 μCi/μl); 3.0 pmol of each PCR primer; and 0.06 U of Super Taq (Sphaero Q, HT Biotechnology, Cambridge, UK). Amplification consisted of 33 cycles of 1 minute at 94°C, 2 minutes at 55°C, and 1 minute at 72°C, followed by a final extension of 6 minutes at 72°C. Denatured PCR products were subjected to electrophoresis on a 6.5% polyacrylamide gel containing 7 mol/L urea. Gels were dried and exposed to an x-ray film. LOH was scored by visual comparison of the intensity of alleles from DNA of the diploid and aneuploid nuclei, respectively. Moreover, intensity of shadow bands was also measured by densitometry (BioRad model 620).

Results

DNA Content Analysis by Flow Cytometry and Image Cytometry

Interpretable FCM DNA histograms were obtained from 17 of 29 specimens. The results are shown in

Table 1. Eleven specimens from 10 different patients had a diploid DNA index (DI = 1.00; Figure 2a). The other six specimens contained an aneuploid DNA content (Figure 2c). All aneuploid specimens were from classic adamantinomas with prominent epithelium, whereas aneuploidy was not detected in OFD-like adamantinomas. From patient 27, two paraffin embedded samples were measured. One specimen, having a DI of 2.86, originated from the center of the tumor with abundant epithelial cells in tubular and basaloid differentiation patterns, whereas the other was taken from the periphery, consisting of OFD-like tissue and lacking epithelial cells. This specimen had a DI of 1.00. Analysis of a fresh-frozen specimen from the same patient (from the center of the tumor), containing a trout erythrocyte intern control, showed a DI of 2.84 and confirmed that the left peak observed in the DNA histogram was diploid.

DNA ICM showed broad distributions of IODs, with nuclei from fibrous cells having the same median IOD as the alveolar nuclei from the lung metas-
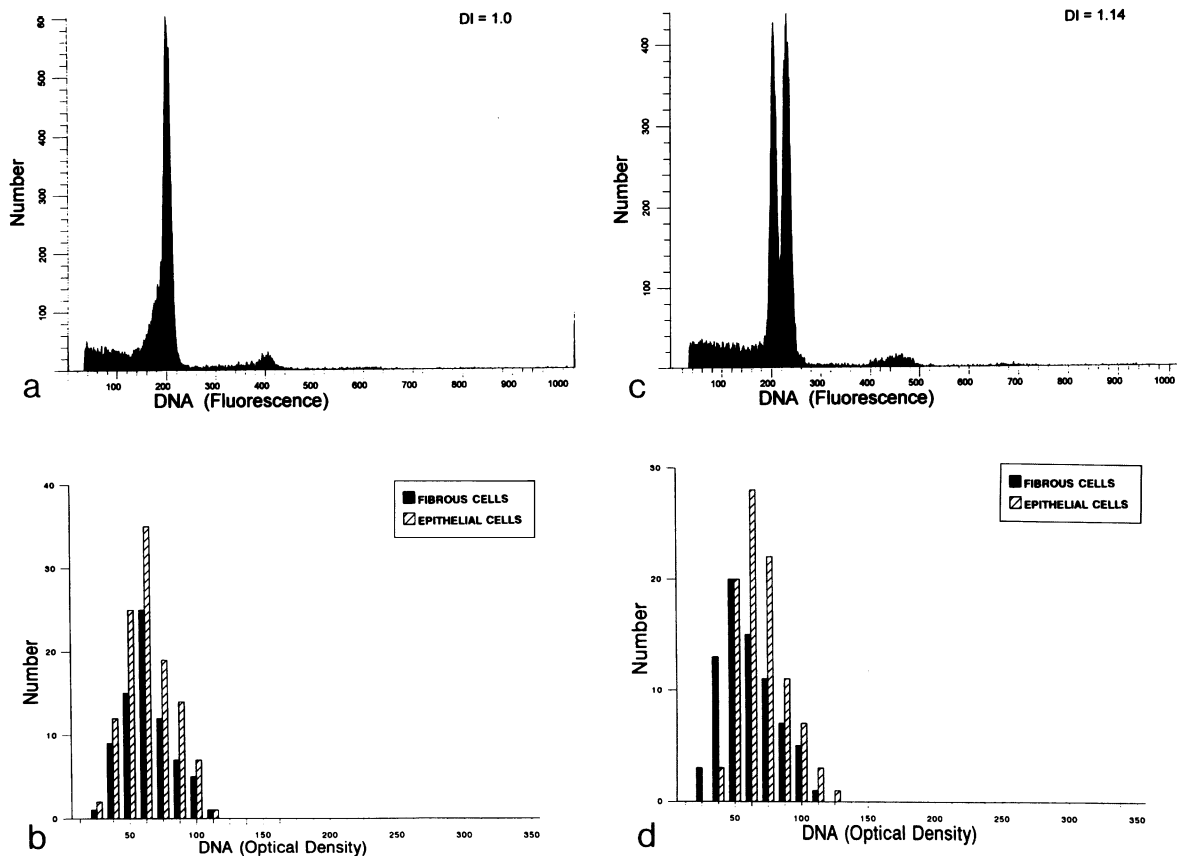
Figure 2. *Flow cytometry (a and c) and image cytometry (b and d) histograms of a diploid (left) and an aneuploid (right) adamantinoma. Although the IOD ratio of the fibrous and epithelial nuclei in ICM is not exactly the same as the DNA index calculated with FCM (Table 1), ICM showed that in FCM aneuploid tumors, the aneuploid component contained the epithelial cells, whereas the fibrous cells constituted the diploid peak.*

tasis, the control for diploid DNA content (Table 1). In the two diploid adamantinomas, the nuclei of the epithelial cells had the same IOD as the fibrous nuclei, with ratios of 1.00 and 1.03 (Figure 2b). In the six aneuploid tumors, the epithelial nuclei demonstrated a clear shift of IOD to the right compared with that of the fibrous component (Figure 2d). This indicates that the epithelial cells form the aneuploid part in adamantinomas with an abnormal DNA content.

p53 Immunohistochemistry

With antibody DO7, 6 tumor specimens (6 patients) showed strong immunoreactivity for p53 (>10% of analyzed nuclei) and 7 specimens (6 patients) moderate reactivity (1 to 10% of nuclei), whereas 16 specimens (14 patients) were negative (less than 1% of nuclei; Table 1 and Figure 3). With CM1, 5 tumor specimens (5 patients) were strongly positive, 7 moderately positive (6 patients) and 17 negative (15 patients). No cytoplasmic immunoreactivity was observed. Nuclear staining was intense in all cases except 1 (case 10), which showed weak staining

only. Of note, p53 protein accumulation was seen only in the epithelial cells of the tumor, all osteofibrous cells being negative. None of the adamantinomas with the OFD-like subtype showed immunoreactivity for p53. In 1 patient (case 16), the OFD-like primary tumor was negative, whereas the local recurrence of this tumor, with a classic epithelium-rich histology, showed moderate reactivity with both antibodies.

PCR Analysis of LOH at the p53 Locus

In Table 2, results of PCR analysis for LOH at the locus of the p53 gene with microsatellite marker D17S513 are recorded. By FACStar analysis the sample of patient 18 (DI = 1.14) showed too much overlap of the diploid and aneuploid population to be sorted properly, and this sample was therefore excluded. The sample of patient 24 did not yield a PCR product. The sample of patient 17 did not show heterozygosity for this marker. Of the remaining three samples, the tumors of patients 6 and 27 retained heterozygosity, whereas the tumor of patient 21
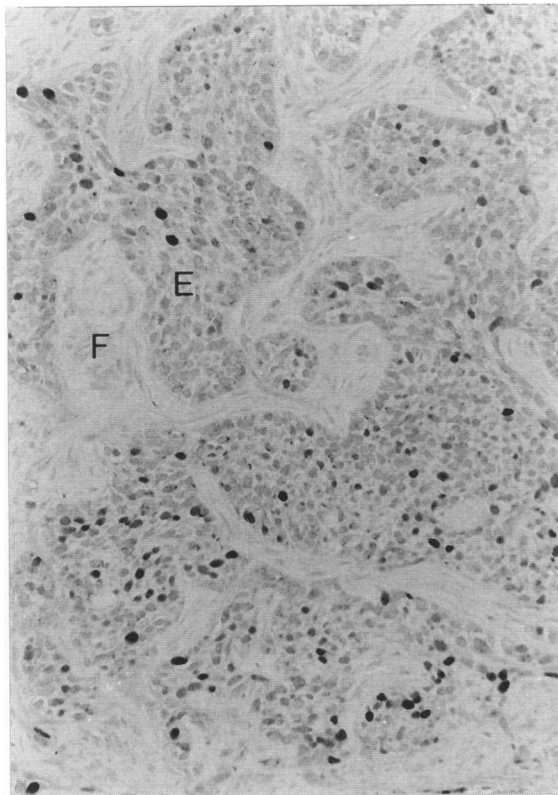
Figure 3. *Light micrograph of adamantinoma stained with p53 monoclonal antibody DO7. Immunoreactivity is found in the epithelial part (E) of the tumor only, whereas the fibrous part (F) remains unstained (light green counterstain; original magnification, ×100).*

Figure 4. *PCR on DNA isolated from flow-sorted nuclei using microsatellite marker D17S513 (chromosome 17p13, location of the p53 gene). D, diploid fraction; A, aneuploid fraction. For the origin of shadow bands, see Refs. 41 and 42. As a result of individual differences in amounts of tandem nucleotide repeats, the size of the observed alleles may diverge between individuals. Both patients 27 and 21 show heterozygosity for this marker. In case 27, both alleles are retained (arrowheads), whereas loss of the lower allele is detected in the aneuploid fraction (21A) of case 21 (arrows). Note that the shadow bands lying above and under the upper allele of 21A are equally intense, as was also measured by densitometry.*

showed LOH with a loss of the lower allele (Figure 4). Densitometry revealed equal intensity of the bands lying above and below the upper allele, which meant that the band lying below the upper allele consisted, for at least the major part, of shadow band.
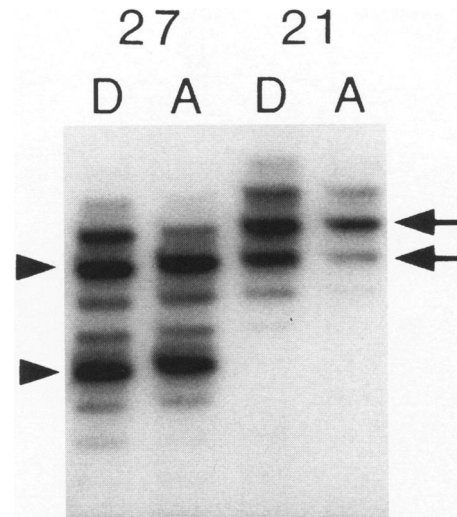
Analysis of Lung Metastases

The typical fibrous or osteofibrous component that is always found in primary tumors could not be identi-

Table 2. *PCR Analysis of LOH of the p53 Gene with Marker D17S513*

Patient	DNA index	p53 immuno-histochemistry	PCR informative*	LOH
6	1.27	2+	+	−
17	1.13	1+	−	
21	1.13	2+	+	+
24	1.19	2+	No product†	
27	2.84/2.86	−	+	−

*+, heterozygous for this marker.
†Probably a result of extensive decalcification or prolonged fixation. Of note, the specimens of patients 6 and 21 both show 2+ p53 immunoreactivity; patient 21 had LOH at the p53 locus, whereas in patient 6 heterozygosity was retained.

fied in the analyzed lung metastases (Figure 5). Additional review of the histological sections of two lung metastases on which keratin immunohistochemistry was performed[14,16] showed that, in addition to normal lung tissue, the metastatic tumor cells were all keratin positive and thus consisted of the epithelial component as seen in primary adamantinomas.

Relation of Ploidy Status and p53 Immunoreactivity to Follow-Up

For investigation of the relationship between p53 and ploidy status and rate of local recurrence and metastasis, clinical follow-up data of 24 (p53) and 15 (ploidy) patients were available[16] (Table 3). Of patients with p53-negative tumors, 4 of 14 (29%) developed local recurrences and 1 (7%) developed a metastasis. Of 10 patients with p53-positive tumors, 5 (50%) had a local recurrence, and 3 (30%) had a metastasis. Of 9 patients with a diploid tumor, 4 (44%) developed a local recurrence, and 2 (22%) developed a metastasis. Of the aneuploid tumors, 3 of 6 (50%) resulted in a recurrence, and 2 of 6 (33%) in a metastasis. A Fisher's exact test was performed, which showed that none of these differences reached a statistical significant level (all $P > 0.05$).

A further classification for histological subtype and initial treatment mode was made (Table 3, right part).
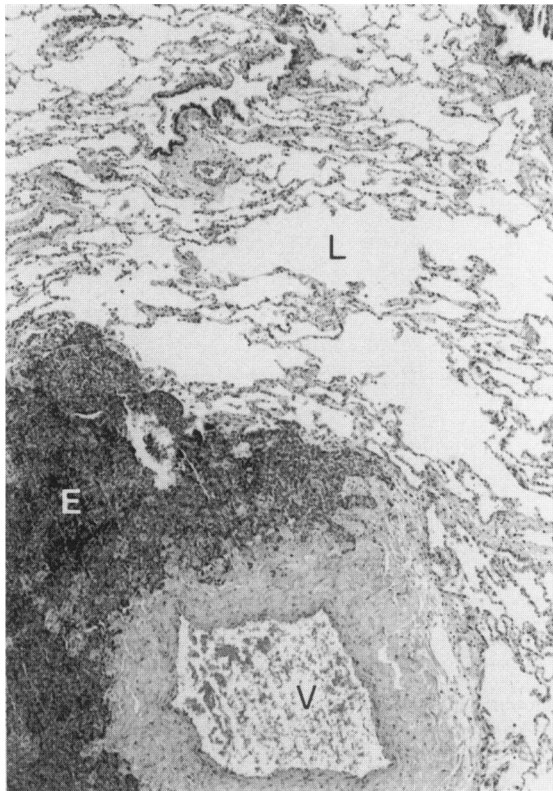
Figure 5. *Light micrograph showing a lung metastasis of ada-mantinoma. Nodular confluent fields of epithelial cells (E) in spindle cell pattern are found in between normal lung tissue (L) and a lung vein (V). Note the absence of the typical fibrous component as seen in Figure 1 (H&E; original magnification, ×50).*

The data were strongly influenced by subtype and treatment, as none of the OFD-like tumors was p53 positive or aneuploid, and all recurrences and metastases developed in patients that were treated by intralesional surgery. For that reason, no further statistical analysis for the relation between p53 and ploidy status and follow-up was considered.

Discussion

Recent studies on adamantinoma focus on its histogenesis and the potential relationship of this tumor to OFD. Cumulating evidence suggests that, indeed, osteofibrous dysplasia and adamantinoma are two related lesions at both ends of a continuous spectrum.[9,14,16,17,28,29] The present study addresses the histogenesis of adamantinoma by using a combination of histological, immunohistochemical, DNA cytometric, and molecular genetic techniques. Our most notable result was that, with these methods, all DNA alterations observed were restricted to the epithelial component of the tumor. Moreover, lung metastases did not contain the typical osteofibrous cells as seen in primary adamantinomas.

Formic acid decalcification or prolonged formalin fixation may cause DNA disintegration and influence DNA stainability, thereby reducing the number of cases suitable for analysis. These factors limited our 29 tumor specimens for FCM to 17 interpretable specimens of 15 patients. So far in the literature, FCM data are available of 4 adamantinomas, including 1 aneuploid tumor (DI = 2.4).[30,31] Although univariate DNA FCM showed the presence of aneuploid cell populations, this technique does not discriminate between epithelial and fibrous cells. For this purpose we used ICM on tissue sections. Formic acid decalcification appears to interfere minimally with the DNA Feulgen staining used in ICM.[32] In the 8 adamantinomas analyzed by ICM, the decalcified and nondecalcified specimens showed comparable DNA staining as measured by the median values of the IOD of the fibrous nuclei and the diploid control. Moreover, this technique clearly demonstrated that the aneuploid peaks found with FCM belonged to the epithelial component. ICM on 2-μm-sectioned nu-

Table 3.   *Relation of p53 Immunoreactivity and Ploidy Status to Frequency of Local Recurrence and Metastasis*

Primary tumor	Total number	Recurrence No	Recurrence Yes	Metastasis No	Metastasis Yes	Histological subtype OFD-like	Histological subtype Classic	Initial treatment Intralesional	Initial treatment Resection	Initial treatment Amputation
p53										
Negative	14	10	4	13	1	9 R = 3 M = 1	5 R = 1 M = 0	6 R = 4 M = 1	8 R = 0 M = 0	0
Positive	10	5	5	7	3	0	10 R = 5 M = 3	6 R = 5 M = 3	1 R = 0 M = 0	3 R = 0 M = 0
DNA index										
Diploid	9	5	4	7	2	5 R = 2 M = 1	4 R = 2 M = 1	5 R = 4 M = 2	4 R = 0 M = 0	0
Aneuploid	6	3	3	4	2	0	6 R = 3 M = 2	3 R = 3 M = 2	2 R = 0 M = 0	1 R = 0 M = 0

R, number of local recurrences in particular group; M, number of metastases in particular group. Patients with p53-positive tumors seem to show increased numbers of local recurrence (5 of 10 *versus* 4 of 14) and metastases (3 of 10 *versus* 1 of 14). These differences do not reach a statistical significant level (Fisher's exact test, all *P* > 0.05). Further classification for histological subtype and initial treatment mode (right part) shows that the clinical outcome is also strongly influenced by these factors, as none of the OFD-like tumors were p53 positive or aneuploid, and all recurrences and metastases occurred in patients that were treated by intralesional surgery.

clei, however, gives only a crude value for DNA content differences between the two cell populations, as is indicated by the broad distribution patterns of IOD (Figure 2, b and d) and the variations between DNA index measured with FCM and the IOD ratio calculated with ICM (Table 1). However, two other observations also indicate that the epithelial cells constitute the aneuploid peak in aneuploid adamantinomas. The first is the aneuploidy of the lung metastasis of patient 17, in which only normal alveolar lung cells and metastatic epithelial cells were present. The primary tumor of this patient was unfortunately not suitable for analysis. The second is the difference in FCM DNA index observed in two samples from patient 27, one originating from the central, epithelium-rich part and one from the peripheral part of the lesion, not containing any epithelium as shown with keratin immunohistochemistry. This comparison also demonstrated that aneuploidy was restricted to the sample containing epithelial cells.

Like many other tumors, adamantinomas showed high percentages of p53 protein accumulation, in our series 12 of 25 patients (48%) with antibody DO7, restricted to the epithelial component of the tumor. Immunohistochemical detection of mutant p53 with antibodies DO7 and CM1 in combination with microwave antigen retrieval has proven to be a reliable procedure.[24,25] Inadequate decalcification and fixation may, however, interfere with p53 immunoreactivity, resulting in false negative tumors. Additionally, it has been shown that in some tumors there may be substantial discrepancy between overexpression of the protein and molecular genetic alterations in the p53 gene.[24,33] For example, in this series, patient 21 showed p53 immunoreactivity combined with LOH at 17p13, but patient 6 retained heterozygosity, although this tumor was also immunohistochemically p53 positive (Table 2). This may be a result not only of a p53 gene mutation not detected by our LOH analysis but also of another genetic alteration that causes p53 protein accumulation,[33] for example, interaction of *mdm2* protein with p53.[34] Of further interest is the absence of p53 immunoreactivity in OFD-like adamantinomas. In patient 16 the primary OFD-like tumor showed no immunoreactivity whereas the local recurrence, a classic epithelium-rich adamantinoma, displayed moderate positivity. This finding, although substantiated in only one patient, may indicate that p53 mutation is a late event in development toward classic adamantinoma.

Additionally, patients with p53-positive tumors seemed to develop more recurrences and metastases (Table 3, left side). Statistical analysis on this small series of patients revealed no statistically significant differences. A putative association is biased by the surgical treatment mode, which proved to be a significant factor with respect to the rate of recurrence or metastasis.[16] All recurrences and metastases occurred in patients who were treated with an intralesional surgical procedure (Table 3, right side). It should be emphasized that the numbers of patients included in this series are too small to draw any relevant clinical conclusion with respect to a possible relationship between p53 and ploidy status and prognosis. For that purpose, a multinational study including far more patients will be required.

The data obtained in this study showed clear characteristics of neoplasia and malignancy in the epithelial component of adamantinoma, whereas in the fibrous part none of these features was found. This does not, however, exclude the possibility of the fibrous component being of a benign but neoplastic nature. For comparison, there is a certain analogy in theories about the histogenesis of adamantinoma, being a tumor with intermixed epithelial and fibrous elements, and the mixed Müllerian tumor (MMT) of the female genital tract. The MMT may be specified as a "collision" tumor (a mixture of two independent malignant cell populations), a "combination" tumor (with a common stem cell for both cell populations), or a "conversion" tumor (with metaplastic transformation of one neoplastic cell into the other).[35] The malignant nature of the epithelial part of adamantinoma has been confirmed in this study. With respect to the possibility of adamantinoma being a carcinosarcoma (or combination tumor), the gradual blending of epithelial and fibrous components, often observed in adamantinomas, gave rise to the idea that they might be both neoplastic (and possibly both malignant) elements of the tumor.[13] In this way the tumor could be compared with the biphasic synovial sarcoma. The finding of inconspicuous epithelial elements within the fibrous part in some tumors, which are not distinguishable by routine light microscopy, also sustains this hypothesis. However, three observations in this study contradict the concept of adamantinoma being a lesion with a malignant epithelial and mesenchymal component: (1) the consistent finding of a diploid fibrous component in aneuploid tumors and the absence of atypical mitoses in the fibrous part,[16] (2) the restriction of p53 mutation characteristics to the epithelial component, and (3) the absence of the typical osteofibrous cells in lung metastases. In contrast, MMTs show p53 immunoreactivity in the epithelial as well as in the mesenchymal part,[36] and metastases of biphasic synovial sarcomas as well as MMTs contain both epithelial and

mesenchymal components of their primary tumors.[37,38]

The conversion tumor theory may designate adamantinoma as a fibrocarcinoma, with a malignant epithelial and benign fibrous component. In the female genital tract, fibrocarcinomas are extremely rare, and it is still uncertain whether the fibrous component in these tumors is neoplastic or reactive.[37] On the basis of the data in this study it cannot be excluded that the metaplastic transformation of neoplastic osteofibrous cells as seen in OFD leads to the acquisition of epithelial characteristics of cells that then also obtain features of malignancy. This hypothesis implicates that OFD is not a reactive but rather a premalignant precursor lesion of adamantinoma.[16,39] With regard to the nature of the osteofibrous part of adamantinoma, the recently described clonal chromosomal abnormalities in two cases of OFD are of specific interest.[40] Supposing that the osteofibrous tissues in OFD and adamantinoma are related, this finding may point to a clonal and possibly neoplastic nature of the osteofibrous part of adamantinoma, which in this respect should denote the tumor as a fibrocarcinoma. Another explanation of the observed clonal chromosomal abnormalities in OFD[40] may be the clonal outgrowth and overgrowth of reactive osteofibrous cells, which obtained a growth advantage as a result of these abnormalities. This alternative designates adamantinoma as a kind of carcinoma with reactive osteofibrous tissue (with two independent cell populations). The growth of this tissue may be induced or enhanced by yet unidentified substances produced by the epithelial cells. A search for common genetic alterations in the fibrous and epithelial part of the tumor other than p53 mutations may offer a solution for this fascinating problem. The recently developed technique of PCR-based LOH analysis on DNA from flow-sorted cells from paraffin-embedded tissue[22] gives the opportunity to investigate both parts independently and can therefore be used for this purpose.

In conclusion, our results of DNA cytometry and p53 immunohistochemistry of both the epithelial and fibrous component of adamantinoma and the histological analysis of several lung metastases of this tumor provide additional indications that the epithelial part of adamantinoma is the fundamental malignant element. Because of the recent finding of clonal chromosomal aberrations in the fibrous cells of OFD,[40] which may be the same fibrous cells as those found in adamantinoma, additional studies with emphasis on common genetic abnormalities in the fibrous and epithelial parts of the tumor are indicated.

Acknowledgments

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