Virchows Arch (2001) 438:412–417
DOI 10.1007/s004280000365

CASE REPORT

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Clear-cell odontogenic carcinoma with pulmonary metastases resembling pulmonary meningothelial-like nodules

Received: 31 August 2000 / Accepted: 14 November 2000 / Published online: 7 February 2001

Abstract Clear-cell odontogenic carcinoma (CCOC) is a rare neoplasm with malignant potential and unknown cytogenetic alterations. We describe the case of a 43-year-old woman who presented with an unusual odontogenic epithelial tumor. Histologically, the tumor was composed of clear-cell areas and exhibited a squamous pattern with little nuclear pleomorphism similar to benign squamous odontogenic tumor. Multiple small pulmonary nodules occurring 3 years after primary surgical treatment histologically closely resembled benign minute pulmonary meningothelial-like nodules (MPMN) with clear-cell features. Comparative genomic hybridization (CGH) and immunohistochemistry, performed as diagnostic adjuncts, revealed in the odontogenic tumor and the pulmonary lesions a very similar pattern of chromosomal aberrations (loss of 9, gains of 14q, 19 and 20 in both, and additional loss of 6 in the odontogenic tumor) and the same pattern of expression (positive for cytokeratin 5, 6, 8, 19 and negative for cytokeratin 18, epithelial membrane antigen, and vimentin), differing from that of MPMN. These findings confirmed the final diagnosis of metastasizing CCOC with partial squamous differentiation, substantiated the unfavorable prognosis of the clear-cell component, and highlighted the diagnostic impact of CGH and immunohistochemistry for classification of these morphologically peculiar pulmonary CCOC metastases.

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Keywords Clear-cell odontogenic carcinoma · Squamous odontogenic tumor · Comparative genomic hybridization · Pulmonary meningothelial-like nodules · Cytogenetic alterations

Introduction

Clear-cell odontogenic carcinoma (CCOC) is a rare odontogenic tumor, first described by Hansen et al. [8] in 1985 as a clear-cell odontogenic tumor. This early description already indicates the locally aggressive potential of this neoplasm. In the same year, Waldron et al. [24] reported two cases of clear-cell ameloblastic (odontogenic) carcinomas, one of which metastasized. They noticed the possible association of clear-cell phenotype and metastatic potential of epithelial odontogenic tumors. In 1992 and 1989, one and three additional cases of CCOC, respectively, were described [2, 17]. Two of these cases, reported by Bang et al. [2], metastasized. Therefore, the authors proposed the term CCOC for these tumors, independent of the presence or absence of metastases. Among the 19 cases of CCOC described to date [1, 2, 6, 8, 10, 11, 12, 13, 14, 16, 18, 19, 23, 25], including the present one, seven have metastasized [2, 6, 12, 18, 23].

The malignant potential of odontogenic tumors with clear-cell features, comprising CCOC and ameloblastomas with clear cells, is now well accepted [11, 23, 25]. Up to now, there have been no definite morphologic or genetic criteria useful for predicting the occurrence of CCOC metastases. The current classification of odontogenic tumors by the World Health Organization [9] still designates clear-cell odontogenic tumors as benign neoplasms. We describe the radiographic, histologic, and genetic findings and the clinical behavior of a CCOC with both clear-cell differentiation and areas with squamous differentiation. This unusual combination of histopathologic features requires the diagnostic differentiation between a benign squamous odontogenic tumor (SOT) and a potentially malignant CCOC. Additionally, we report

on the unusual histologic features of pulmonary metastases of this CCOC that closely resembled those of pulmonary meningothelial-like nodules (MPMN). MPMN is a rare benign multiple pulmonary lesion which can be encountered as a paraneoplastic lesion of unclear histogenesis. The potential utility of comparative genomic hybridization (CGH) and immunohistochemistry for the differentiation of pulmonary metastases of CCOC and MPMN will be demonstrated.

Clinical history

In October 1996, a 39-year-old German woman was admitted to a clinic of oral and maxillofacial surgery. Radiological diagnostics revealed a partly well- and partly ill-defined radiolucent area in the region 31–43 of the mandible. Initial pathologic examination was consistent with an ameloblastoma. In November 1996, the patient was post-resected. Histopathologic examination now revealed a SOT. In February and October of 1998, she suffered recurrences that were treated by marginal mandibulectomy and then by partial resection of the right lateral mandible. The histologic diagnosis of the recurrent tumors was SOT.

In August 1999, the routine clinical control examination revealed multiple small disseminated pulmonary coin lesions in the computerized tomography of the thorax (Fig. 1), radiologically compatible with both atypical metastases and MPMN. A mini-thoracotomy was performed, and histopathology of the biopsy material from two lobes of the lung led to the diagnosis of multiple MPMN. Because it appeared doubtful that two extremely rare lesions would be present in the same patient, CGH analysis and further immunohistochemical investigations were carried out. A synopsis of the findings obtained by these techniques and by histopathology resulted in the revision of the diagnosis to metastasizing CCOC with partial squamous differentiation.

Materials and methods

For light microscopic examination, the surgical specimens excised from the jaw and lung were fixed in 10% formalin, routinely processed, and embedded in paraffin. Paraffin sections were stained with hematoxylin and eosin and periodic acid–Schiff (PAS) with and without prior diastase treatment.

Immunohistochemical staining for cytokeratin (clone KL1, Immunotech, Hamburg, Germany), the cytokeratin subtypes 5/6 (Boehringer, Mannheim, Germany), 8 (Boehringer), 18 (Boehringer), and 19 (Boehringer), epithelial membrane antigen (Dako, Glostrup, Denmark), vimentin (Dako), S-100 protein (Dako), chromogranin (Dako), and muscle-specific actin (Dako) was performed on representative sections of the lesions in the mandible and lungs using the alkaline phosphatase–anti-alkaline phosphatase (APAAP) method.

For CGH analysis, DNA was isolated separately from formaldehyde-fixed and paraffin-embedded tissue from the recurrence of the odontogenic tumor diagnosed in February 1998, the pulmonary lesions, and normal surrounding tissue by proteinase K digestion followed by spin column purification. CGH analysis was basically performed as described previously [4, 5]. Briefly, labeling of tumor DNA with biotin-16-dUTP (Roche, Mannheim, Germany) and of normal reference DNA with digoxigenin-11-dUTP (Roche) was carried out by means of standard nick translation. The denatured DNA probe containing 2 µg tumor DNA, 1.5 µg reference DNA, and 80 µg COT-1 DNA was hybridized for 3 days to a normal metaphase spread (15×15 mm cover glass area). The slides were then washed extensively, blocked with bovine serum albumin (BSA) solution, and subjected to detection with a mixture of fluorescein-conjugated avidin (Vector Laboratories, Burlingame, Calif.) and rhodamine-conjugated antidigoxigenin (Roche). The slides were washed and mounted in antifade solution (Vector Laboratories) containing 2.5 µg/ml 4,6-diamino-2-phenylindole (DAPI) counterstain.

Digital images of metaphase slides were obtained with a charge-coupled device (CCD) camera on a Zeiss Axioskop 2 epifluorescence microscope using a filter-set for DAPI, fluorescein isothiocyanate and rhodamine fluorescence. Images were analyzed with Quips CGH software (Applied Imaging, Santa Barbara, Calif.). The average green-to-red ratio for each chromosome was calculated for at least 12 metaphases in each case, and only definitely assigned chromosomes were included for analysis. The green-to-red ratio thresholds were set at 0.85 for losses and 1.15 for gains to indicate significant losses and gains of chromosomal material [21]. Centromeric regions were not considered in CGH results.

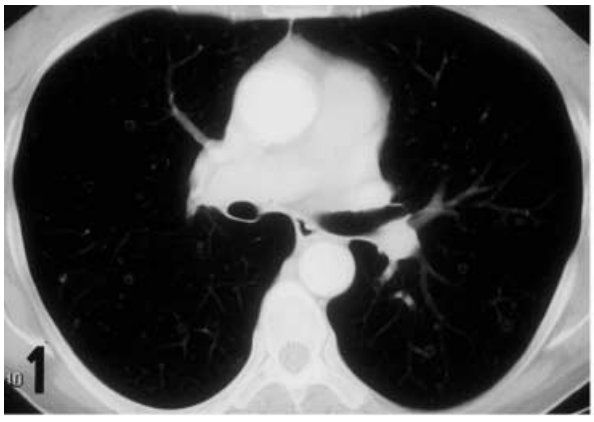
Pathologic findings

The microscopic examination of the primary CCOC revealed an epithelial tumor in the mandible consisting of round to elongated islands of cells separated by dense fibrous stroma. In some areas, the tumor cells were round to polygonal, had a clear, PAS-negative cytoplasm and slightly pleomorphic central nuclei (Fig. 2a). There was no palisading of cells in the periphery of the nests. The center of a few islands was necrotic. Other areas were composed of eosinophilic, PAS-negative cells with round to elongated oval, slightly pleomorphic nuclei. These cells were arranged in a squamous pattern without keratinization. There was one mitosis per ten high power fields. The recurrent tumors showed a preponderantly squamous pattern and had a minor clear-cell component (Fig. 2b).

The multiple pulmonary nodules were 1–3 mm large, histologically irregularly circumscribed, and consisted of confluent nests of clear cells expanding the alveolar septa (Fig. 2c, d). The alveolar epithelium of the affected alveolar septa was extensively preserved, giving an impression of a non-destructive lesion. The cells had a clear, PAS-negative cytoplasm and round, rather isomorphic nuclei without significant atypia.

The tumor tissue in the mandible and the lesions in the lung showed strong and extensive immunoreactivity for cytokeratin (cytokeratin 5, 6, 8, 19 and no immunoreactivity for cytokeratin 18), epithelial membrane antigen, vimentin, S-100 protein, chromogranin, and muscle-specific actin.

The molecular cytogenetic findings obtained using CGH analysis are shown in Fig. 3. For each of the altered chromosomes, represented by its ideogram, a CGH profile is shown, illustrating the ratio of labeled tumor DNA versus labeled normal reference DNA. The CGH findings in the investigated recurrence of the odontogenic tumor and the pulmonary lesions revealed a very similar pattern of chromosomal imbalancesm, including common gains for chromosomes 19 and 20 and the long arm of chromosome 14. There was an additional loss for 9 in the odontogenic tumor and a trend to loss of 9 in the pulmonary lesions. In addition, the odontogenic tumor showed a loss of chromosome 6 as a trend. This result was confirmed by a control experiment using DNA from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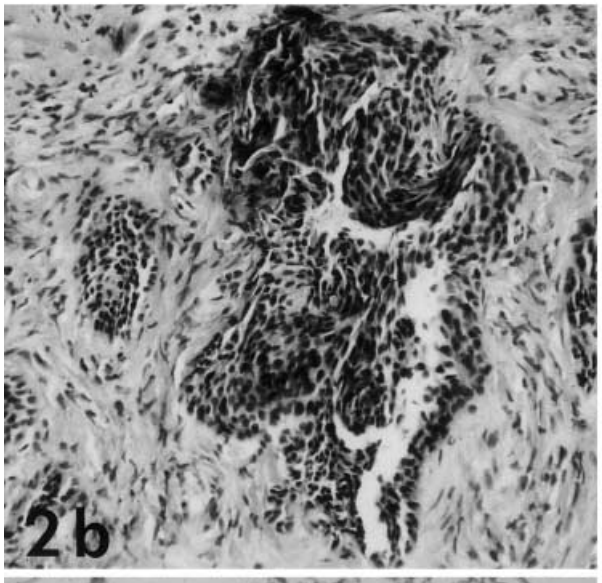
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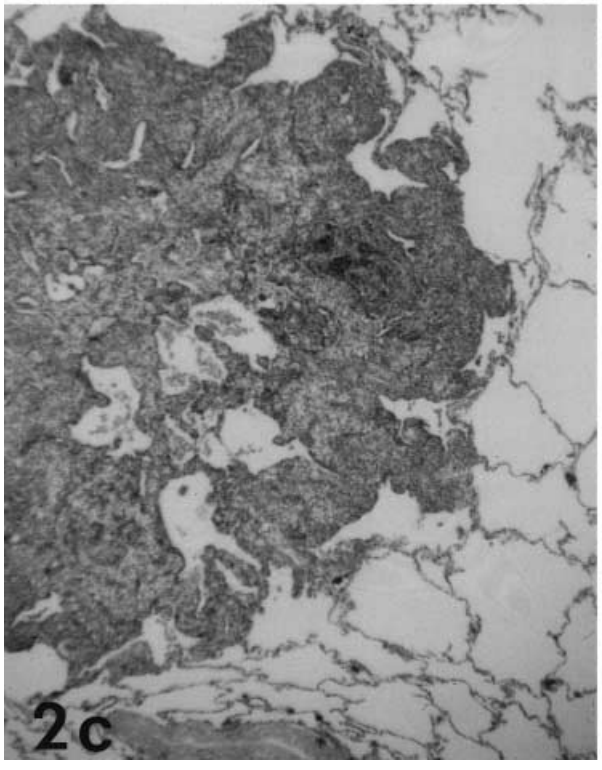
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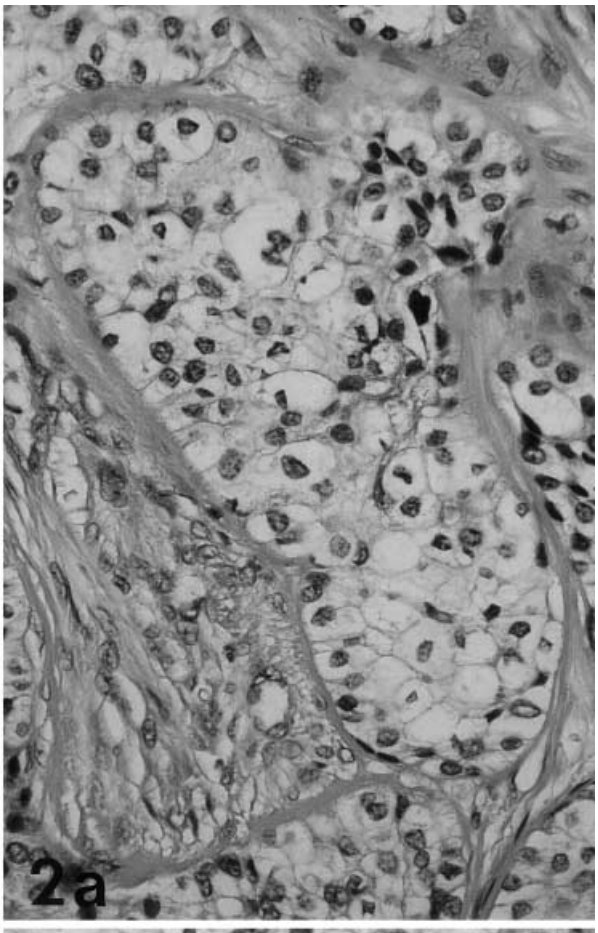
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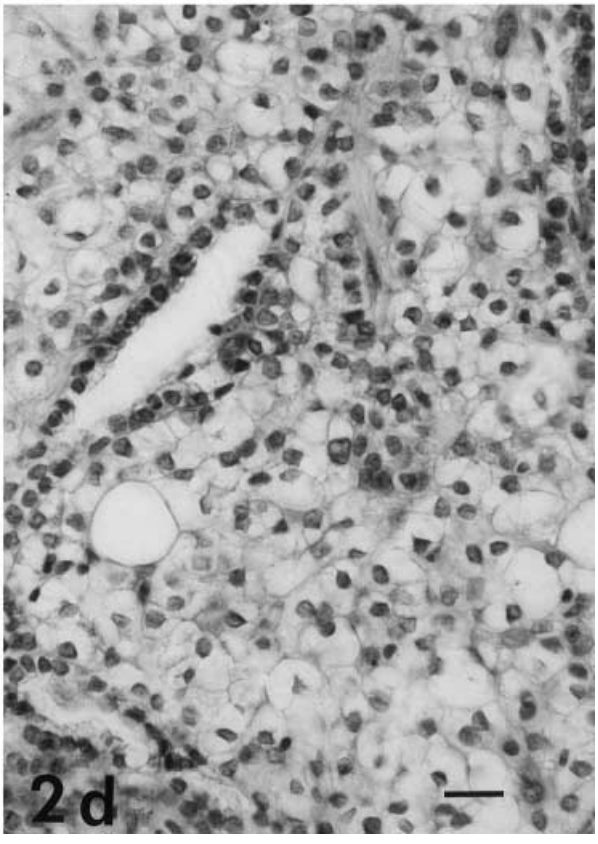
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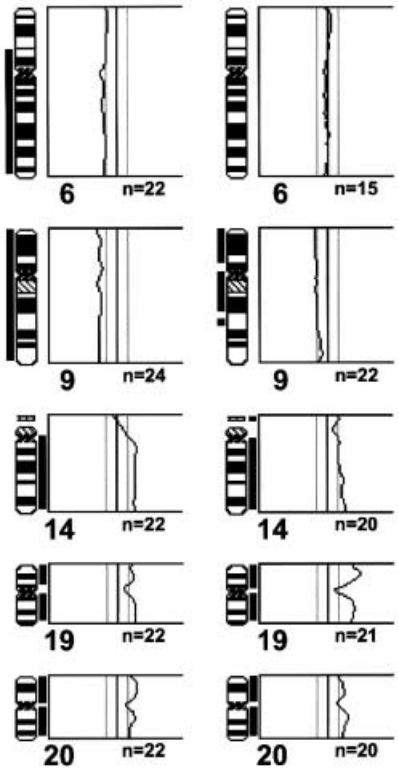

Fig. 3 Summary of altered comparative genomic hybridization (CGH) findings, including chromosomes 6, 9, 14, 19, and 20 for clear-cell odontogenic carcinoma (CCOC; *left panel*) and pulmonary CCOC metastases (*right panel*) *Bars* at the left and right sides of the ideograms display chromosomal imbalances surpassing threshold values of 0.85 for losses and 1.15 for gains, respectively. *n* number of chromosomes included in CGH analysis

surrounding normal lung tissue versus normal reference DNA for CGH, which demonstrated no chromosomal imbalances, especially for chromosomes 6, 9, 14, 19, and 20 (data not shown).

Discussion

The present CCOC is histologically unusual because it shows an admixture of clear-cell areas and squamous differentiation. A focal and slight tendency to squamous differentiation was recently reported for a single case of CCOC [23]. The very limited extent of additional squamous differentiation posed no difficulty in classifying this tumor as CCOC.

The differential diagnosis of the present CCOC included epithelial odontogenic tumors that may exhibit clear-cell features and/or squamous differentiation. Squamous cell differentiation in odontogenic tumors without frank atypia is a typical and diagnostic feature of SOT. This rare neoplasm may recur but has never been found to metastasize. Squamous differentiation is also a typical feature of the acanthomatous variant of ameloblastoma and has been detected in one case of CCOC after combined chemotherapy and immunotherapy, focally in four of ten reported CCOC recurrences and, to a very limited extent, in one primary CCOC [23]. The histologic spectrum of odontogenic tumors with clear cells comprises CCOC and ameloblastomas exhibiting a variable extent of clear-cell features. Exclusion of ameloblastoma in the present case was uncomplicated due to the lack of pronounced palisading of the peripheral cells in the sheets of tumor cells. However, the presence of extensive squamous differentiation in a solid epithelial odontogenic tumor with clear-cell features devoid of frank atypia, as presented here, may pose problems in histologic delineation of CCOC from SOT. The inherent diagnostic problem is that clear-cell features due to increased glycogen content is a well-known phenomenon in tumors with squamous differentiation in general and that the solid clear-cell areas without squamous differentiation in such a neoplasm may be mistaken as an unspecific finding without biological and diagnostic relevance. In the present case, the tumor was primarily classified as a benign SOT, exemplifying this diagnostic problem. Indeed, the occurrence of pulmonary metastases and the clear-cell features of these metastases underline the malignant potential of the clear-cell component of such a neoplasm, confirming the diagnosis of CCOC with partial squamous differentiation.

The diagnosis of CCOC should be based on the histopathological findings in the primary surgical specimen since recurrences may show a significant increase in or an additional component of tumor tissue with squamous differentiation, as demonstrated in the present case and in a previous report [25].

In the present case, the radiographic findings (rather well-defined radiolucent area in the anterior part of the mandible), the age of the patient (39 years at diagnosis), and the fact that she was female fit the general features of CCOC. Despite this, they are not very helpful in the diagnostic delineation from other epithelial odontogenic tumors with possible squamous or clear-cell differentiation.

The peculiar morphologic features of the pulmonary metastases without obvious destructive growth or significant atypia, closely resembling MPMN (Table 1), has not been described for CCOC. It is unknown whether these findings are general features of the early development of pulmonary CCOC metastases. The presence of multiple pulmonary metastases of similar size indicates a pronounced tendency to hematogenous spread and contrasts with the low histological grade of the primary tu-

◀ **Fig. 1** Chest computerizd tomography (CT) scan showing multiple disseminated ring-like lesions in both lungs

Fig. 2 Photomicrographs of clear-cell odontogenic carcinoma (CCOC). Primary tumor demonstrating islands of polygonal to round clear cells without palisading in a dense fibrous stroma (**a**). Recurrence, demonstrating irregular islands of squamous epithelium surrounded by fibrous tissue (**b**). Metastasis, showing an irregular configuration due to expansion along alveolar septa (**c**) and confluent nests of clear cells without significant atypia besides well-preserved alveolar epithelium, resembling minute pulmonary meningothelial-like nodules (**d**). **a**–**d** Hematoxylin and eosin; **a**, **d** ×600; **b** ×175; **c** ×30; *bar* 13 µm

Table 1 Clinicopathological features of minute pulmonary meningothelial-like nodules. *EMA* epithelial membrane antigen

Course of disease	No evidence of progression[a]
Macroscopic findings	Disseminated 1–3 mm large nodules, in the upper lobes more frequent than in others[a]
Microscopic findings	Irregularly configured, cellular nodules, round or spindle-shaped cells in nests, cytoplasm eosinophilic, vacuolated or clear, vesicular or finely granular chromatin, no mitoses, and fine capillary network between cell nests[a]
Immunohistochemical reactivity	Cytokeratin 0/14[b] 0/17[c] EMA 12/14 8/12 Vimentin 10/12 21/21 S-100 0/14 2/10 Actin 0/7 0/12

[a] Summary of findings according to Gaffey et al. [7]
[b] Number of lesions with positive immunohistochemical staining per number of lesions examined according to Gaffey et al. [7]

mor. Only two cases with pulmonary metastases of CCOC, excluding clear-cell ameloblastoma, have been described thus far [2, 18]. However, the metastases detected in these cases did not involve any diagnostic difficulties, possibly because of their size and marked induction of stroma.

To date, there are no reports on CGH findings for odontogenic tumors with clear-cell features or squamous differentiation (CCOC, SOT, variants of ameloblastoma). Future studies will clarify whether the pattern of chromosomal abnormalities described here is characteristic of CCOC and whether it predicts clinical behavior. Cytogenetic analysis of four previously reported cases of ameloblastomas demonstrated monosomy 22 in two of them [3, 20]. The pattern of chromosomal abnormalities detected in the recurrent CCOC investigated, (losses of 6 and 9, gains of 14q, 19, and 20), clearly differs from cytogenetic findings in ameloblastoma. Because there are transitions between CCOC and ameloblastoma that usually show very different clinical behavior (very low metastatic potential in ameloblastoma versus significant metastatic potential in CCOC), the apparent cytogenetic differences, if reproducible in future studies, may contribute to a genetically based delineation of ameloblastoma and CCOC in routine pathology.

It was not possible to histopathologically classify the pulmonary lesions as metastases solely on the basis of morphology because of their close resemblance to MPMN. Nevertheless, the similar chromosomal abnormalities in the pulmonary lesions and the odontogenic tumor clearly demonstrate the genetic relations between them. These findings strongly suggest that the pulmonary lesions are in fact metastases of the odontogenic tumor. Interestingly, the recurrent odontogenic tumor studied here had more cytogenetic alterations, i.e., loss of chromosome 6, than the pulmonary lesions. This finding suggests that the metastatic lesions and the recurrent odontogenic tumor investigated may have arisen from related, albeit different clones of the presumably genetically heterogeneous primary odontogenic tumor.

The close relationship between the odontogenic tumor and the pulmonary lesions was confirmed by the identical pattern of immunohistochemical findings (cytokeratin 5, 6, 8, 19-positivity; cytokeratin 18, epithelial membrane antigen, and vimentin-negativity) and the presence of clear-cell features evident in both of them. Growth of the pulmonary lesions, assessed using computerized tomography 6 months after primary detection, was consistent with the diagnosis of pulmonary metastases.

The immunohistochemical findings in the pulmonary lesions clearly differ from the pattern reported for MPMN [7, 15, 22], summarized in Table 1 and, therefore, support a variant histogenesis. However, the limited number of immunohistochemically studied cases of MPMN reported to date and the variability of the reported findings [7, 15, 22] substantiate the need for an additional technique that enables differentiation of MPMN from metastases. Genetic investigation of routinely processed tissue using CGH fulfills these requirements and is therefore a useful diagnostic adjunct for discrimination of MPMN from pulmonary metastases with similar histopathologic features.

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