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Case report

Cystic meningioma with unusual histopathological features

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Summary. A parasagittal meningioma of an eighty year old female patient showed by light and electron microscopy cystic architecture (forme humide) as well as nuclear vacuoles (indentations) and cytoplasmic inclusions. The latter are the known pseudopsammoma bodies or hyaline inclusions as demonstrated by light and electron microscopy. Light microcopy on paraffin sections and cytological smear preparations revealed, in addition to the cells of endotheliomatous meningioma and those containing the inclusions a third type with small granular cytoplasmic content. Electron microscopy showed characteristic features of meningioma such as folded double membranes, desmosomes and filaments and thus gave evidence of the meningiomatous nature of the tumor.

By immunohistochemistry tumor cells in slightly focal distribution contained vimentin, whereas small clusters of cells with hyaline inclusions were strongly positive for cytokeratin. The dispersed cells of granular cytoplasmic content were positive for fibronectin. These findings, especially of the inclusion containing cytokeratin positive cell clusters may shed new light upon the concept of histogenesis and classification.

Key words: Cystic ("forme humide") meningioma – Hyaline inclusions (Pseudopsammoma bodies) – Immunohistochemistry: vimentin – Fibronectin – Cytokeratin

Introduction

Meningiomas are mainly benign tumours which are histologically uniform in their classical forms. There are variants, which pose the question of whether or not they belong to the meningioma group or a separate entity. Such variants include hemangiopericytic and angioblastic meningiomas, which are mostly similar to hemangiopericytoma and angioblastoma elsewhere in the body and are therefore better classified in this way (Mennel et al. 1988). In contrast, varieties such as lipo-, chondro- or osteoplastic meningiomas with otherwise typical cytology and architecture (Cushing and Eisenhardt 1938) are easily recognized as true meningiomas. Lately, cystic meningiomas, described as *forme humide* by Masson (1956) have been reviewed (Schober et al. 1988) and the occurrence of mast cells in this special form (Zülch 1956) was confirmed. Equally, nuclear vacuoles (Wolf and Orton 1933) were identified as invaginations (Gusek 1962; Robertson 1964) and cytoplasmic hyaline inclusions (Cushing and Eisenhardt 1938) were renamed pseudopsammoma bodies (Kepes 1961).

In recent years, immunohistochemistry has been used for the demonstration of the histogenetical derivation and differential diagnosis of tumours, including meningiomas. In meningiomas, the focal expression of vimentin and desmoplakin is recognized (Schwechheimer et al. 1984; Schwechheimer 1986). The occurrence of desmoplakin is consistent with the wealth of demosomes usually formed in such tumours, while vimentin corresponds to the content of intermediary filaments within the cytoplasm of such tumours. In contrast, there have been diverging reports as to the expression of S-100 protein, fibronectin and especially cytokeratin in meningiomas.

We present a case which shows an unusual expression of marker proteins and may help to answer some open questions regarding the histogenesis of, and differentiation in, meningiomas.

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Materials and methods

A 80 year old female complained of ataxia for two years. Dizziness and headache localized on the left side of the head were experienced shortly before admission. A brief episode of unconsciousness was the reason for neurological examination and admission. There were no conspicuous psychopathological alterations; neurological signs were discrete; there was slight central facial palsy and latent paresis of the left arm. Computed tomography revealed a right sided parasagittal tumour. Contrast enhancement was diffusely present. There was no midline shift, but distortion of the frontal ventricle was seen. This finding, typical for a meningioma, was later confirmed in nuclear magnetic resonance imaging. With this method, a moderate perifocal oedema, also visible in the CT-scan, was noted.

Following surgical resection, the tumour tissue was immediately processed for various methods. It was treated for pathological examination in the following way:

- Rapid smear preparations were performed for cytological examination using minute particles of tumour tissue given on glass slides and stained with Löffler's methylene blue. These preparations serve to perform rapid diagnosis; its reliability is at present between 70 and 90 percent (Mennel 1984; Mennel et al. 1989).
- Smear preparations were performed applying the same immunohistochemical markers as in paraffin sections (see below). Thus, additional information is available in doubtful cases, when only cytological preparations are available. Results with this technique have been reported for the Ki 67 antibody (Ostertag et al. 1987).

Finally, the NOR-silver staining technique was adapted for cytological smear preparations (Boldy et al. 1989).

Part of the specimen was frozen in liquid isopentan, which was precooled in liquid nitrogen and stored at $-80°$ C until further use.

- Frozen sections were processed for rapid diagnosis using routine stains. Frozen material was also the basis for biochemical and immunohistochemical demonstration of gangliosides (Bosslet et al. 1989).
- Conventional paraffin sections were stained with H&E, cresyl violet and some special stains including silver, PAS etc. These stains were used for histopathological diagnosis according to the WHO.

Table 1. Antibodies used in the reported investigations

Antibody against	Origin	Purchased from	Monoclonal/ polyclonal	Batch. No.
GFAP (glial fibrillary acid protein)	mouse	Dako	monoclonal	M 761
NF (Neurofilament protein)	mouse	Labsystem	monoclonal	M 762
Vimentin	mouse	Boehringer	monoclonal	814318
Cytokeratin	mouse	Dianova	monoclonal	CO2
S-100-protein	rabbit	Dako	polyclonal	Z 311
NSE (Neuron specific enolase)	rabbit	Dako	polyclonal	A 589
MBP (myelin basic protein)	rabbit	Dako	polyclonal	A 623
Fibronectin	rabbit	Dako	polyclonal	A 245

- Paraffin sections were used for the NOR-silver technique (Plate et al. 1988) and for immunohistochemical methods, which were performed in the same way as in smear preparations. The PAP-technique of Sternberger et al. (1970), rarely the APAAP-technique (Mason 1985) were applied. The commercially available antibodies were either monoclonal or polyclonal as is shown in Table 1.
- Part of the material was fixed in 5% glutaraldehyde and further processed by standard techniques for electron microscopy.
- Finally, tissue was cultured in-vitro for short term and, if successfull, permanent cultures. In-vitro grown cells were again analyzed with different morphological methods (Mennel et al. 1989).

Results

The usual histopathological techniques revealed a tumour which was distinctly lobulated. Lobules were defined by fine reticulin fibers. Within the lobules, the occurrence of multiple cysts or a reticular form for the tumour was conspicuous (Fig. 1). Whorl formation was not complete. Since the cytology of the tumour was otherwise characteristic, the architectural features of this peculiar neoplasm corresponded to what has been designed "forme humide" of meningiomas by Masson (1956). On the whole, the tumour had to be classified with the endotheliomatous (meningotheliomatous) variety of benign meningiomas, grade I WHO (Zülch 1979).

Several unusual formations were seen on the cytological level. There were intranuclear vacuoles, or rather cytoplasmic nuclear "indentations". These plug-like continuations of cytoplasm bulge into nuclei and appear like holes when sectionned in a transverse plane. In this case, the frequency of this phenomenon (which is not rare in meningiomas) is surprising (Fig. 1 a). Intracytoplasmatic inclusions were prominent in some cells. These inclusions had varying sizes ranging from that of a nucleus up to a psammoma body. These inclusions stained dark in cresyl violet preparations (Fig. 1 a), red in H&E (Fig. 1 b) and were strongly PAS positive. They correspond to hyaline inclusions or pseudopsammoma bodies (Kepes 1982). Granulated cells were scattered evenly through the tumour. In cresyl violet these cells were dark (Fig. 1 a), and in H&E brilliant red (Fig. 1 b). An intensive reaction had been noted with the PAS technique, while reaction for glycogen was negative. These cells were identified as mast cells by electron microscopy (see below).

In electron microscopy, the known features of endotheliomatous meningiomas and the ultrastructural correlates of the peculiar formations were found. The common features of menigothelioma-

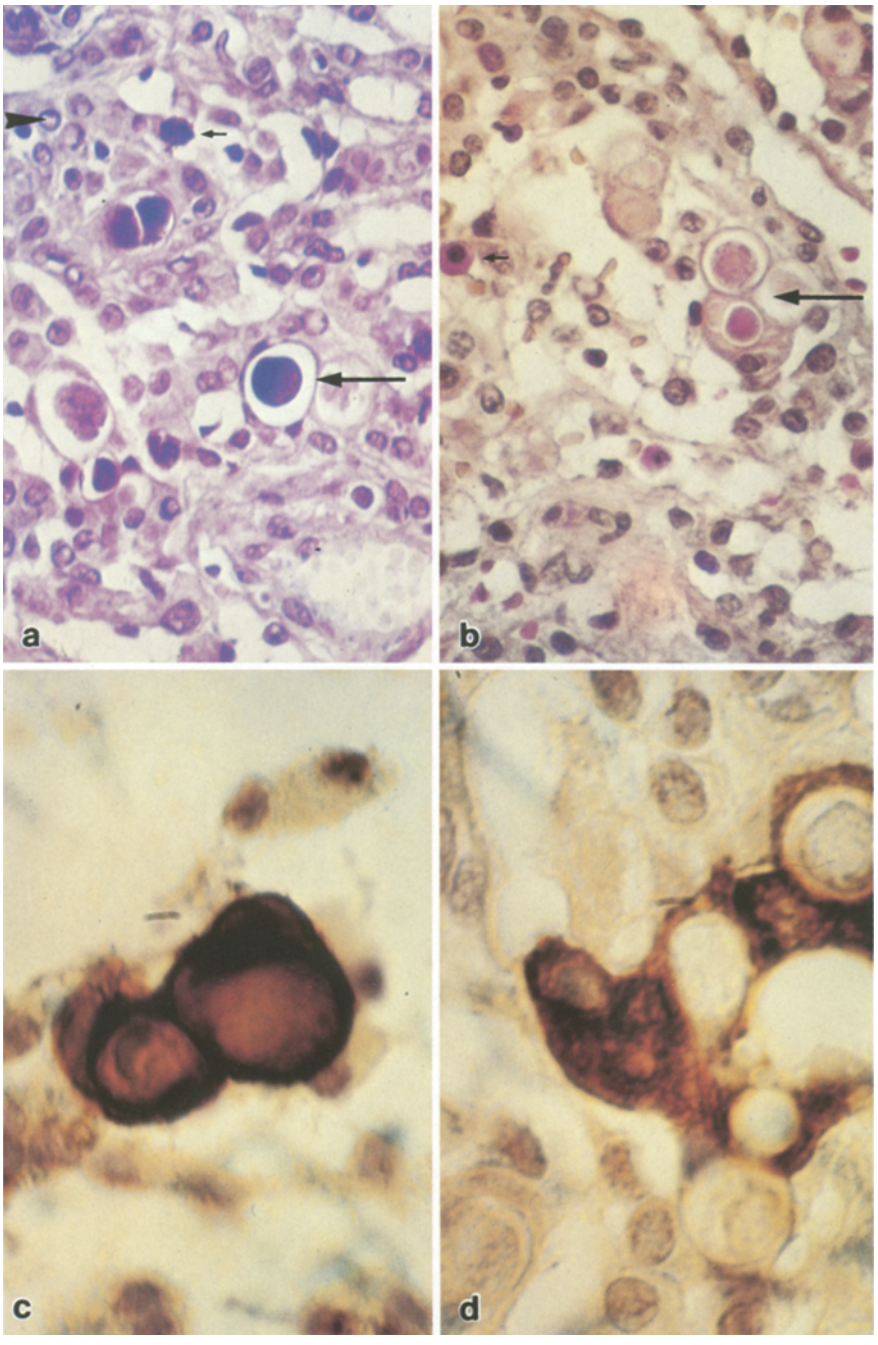

Fig. 1. a Cresyl violet stain of meningioma. In the *upper left* there is vacuolation in many nuclei (*arrow head*). Granulated cells are dark (*small arrow*), hyaline inclusions violet to dark (*large arrow*). **b** H&E: Mast cells show brilliant red staining (*small arrow*) whereas cytoplasmic inclusions stain light to strong red (*large arrow*). ×250. **c** Cluster of cytokeratin positive cells: The center of the cells in-vitro are lighter stained. **d** The same reaction in-vivo, where cytoplasmic inclusions are free from positive reaction. Both cytokeratin ×1000

tous meningiomas were evident as abundant folded double membranes, desmosomes and intermediate filaments. The latter formations were diffusely distributed within most cells; they were not very dense and inconspicuous. This corresponds to the unusual diffuse arrangement of vimentin expression in many tumour cells (see below). Both desmosomes and interdigitating membranes in this tumour had a special feature, due to the basic watery arrangement of tissue texture. Only few of the double membranes were adjacent over longer spaces; most were distended over an extension between two desmosomes, by empty spaces.

Thus, if thoroughly analysed, most of the apparent intracellular small vacuoles lie between cellular processes of the same or neighbouring cells, within the distance from one desmosome to the next. This basic principle of syncytial meningioma being transformed into microcystic and eventially oedematous "forme humide" has been previously

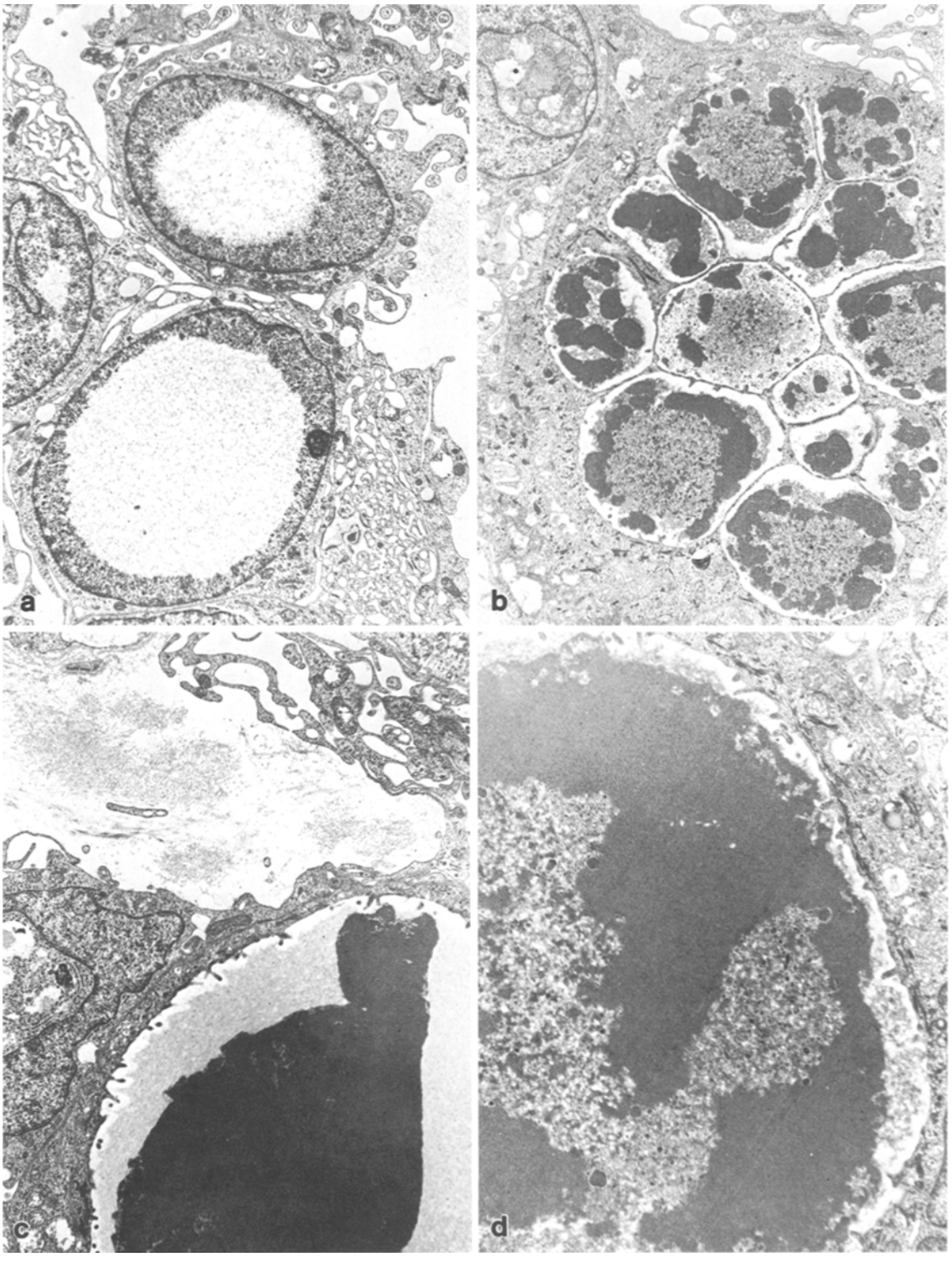

Fig. 2. Electron micrographs. **a** Intranuclar vacuoles without (*middle*) and with (*left margin*) membranous border. Intra-extracellular vacuolation is seen in the lower cell. Membrane foldings are visible in the cytoplasm on top. × 3000. **b** Subdivided cytoplasmic inclusion. Each compartment contains material of different density. *Upper left:* Intranuclear vacuole with cytoplasmic content. × 3000. **c** Pseudopsammoma body separated into finely granular and amorphous part. The "cyst lining" bears microvilli. × 4400. **d** Variegated density of content filling the cytoplasmic cyst. In some parts, microvilli are in close connection with the inner material. × 7000

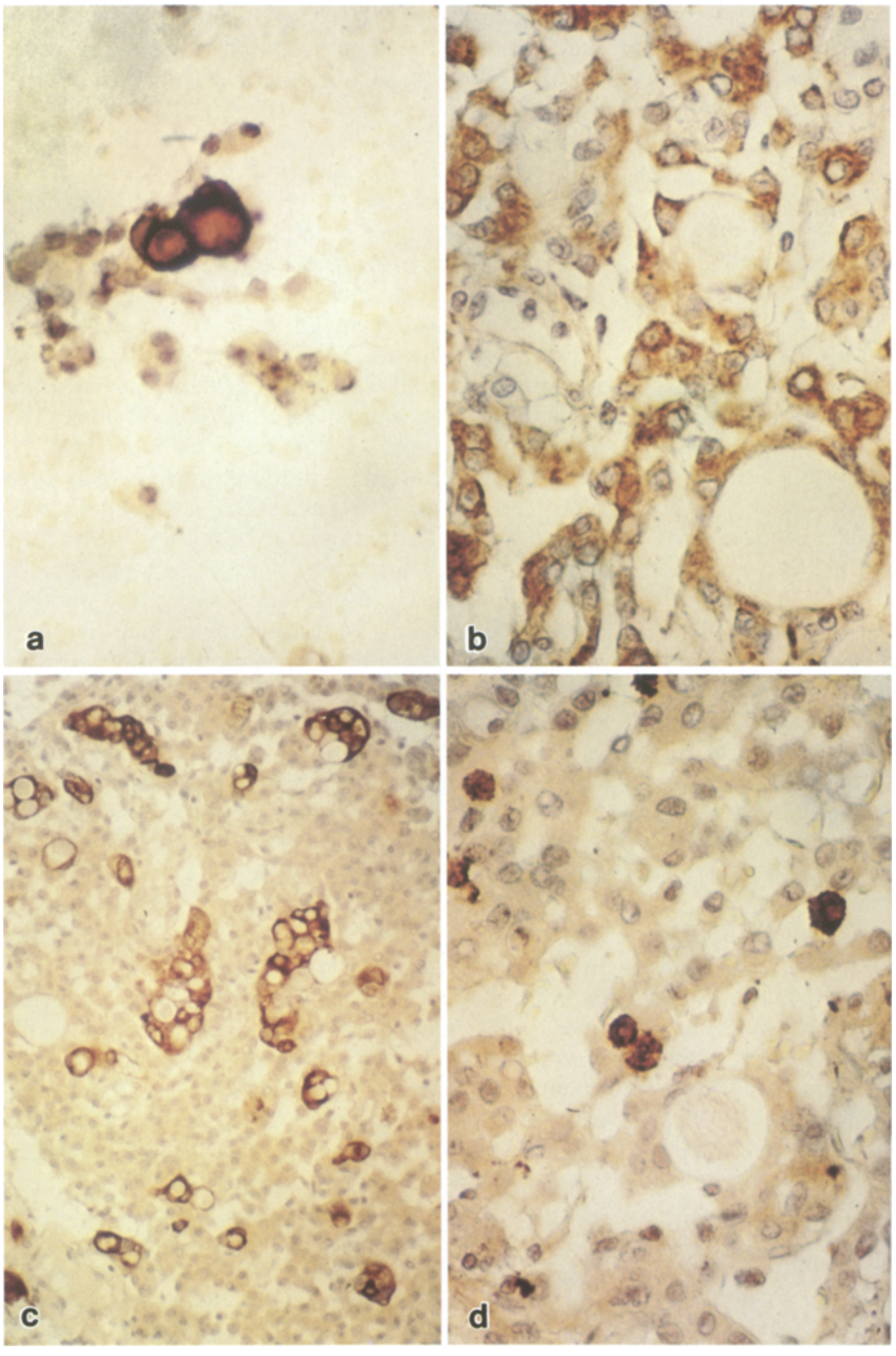

Fig. 3. Immunohistochemical reactions. **a** Reaction with cytokeratin in cytological smear preparations. × 500. **b** Pattern of vimentin expression in paraffin section. × 250. **c** Cytokeratin expression in section displays the cellular clusters formed by positive cells. × 125. **d** Reaction to fibronectin with visualization of scattered mast cells. × 250

demonstrated (Mennel et al. 1988). Many of the small cytoplasmic vacuoles in this case also can be identified in direct continuation of doubled membranes (Fig. 2a). Yet expansion of extracellular space, partly filled with granular (proteinaceous) material is also present. The intranuclear vacuoles are clearly depicted in the ultrastructural picture, with and without membranous limit, filled with the same finely granular or definitely cytoplasmic contents (Figs. 2a, b).

Hyaline inclusions consist of one (Figs. 2c, d) or multiple vacuoles (Fig. 2b), filled with material of partly granular, partly homogeneous, electron dense material. The lining of the cyst bears microvilli on its inner border (Figs. 2b–d). Both hyaline inclusions and nuclear vacuoles correspond, in

their different aspects, to what has been shown earlier by other authors and is summarized in the description of Kepes (1982).

The mast cells mentioned show the stuffed lamellar or amorphous cytoplasmic inclusions, characteristic of this cell type.

In none of the preparations was expression of either GFAP, NF-proteins, MBP or S-100 protein detected. Cells in paraffin sections and smear preparations contained vimentin, fibronectin and cytokeratin, yet in differing distribution. The reaction for cytokeratin and fibronectin was stronger and more distinct in cells of smear preparations (Fig. 3a), and particularly intense with anti-vimentin. In contrast, in paraffin sections, the intratumour distribution of cells expressing different markers was preserved (Figs. 3b–d).

The explanation of this variation becomes evident, if one compares cytokeratin expression in section (Fig. 1c) and smear preparations (Fig. 1d). In the paraffin stained section in hyalin inclusion containing, cytokeratin expressing cells, it is only the disk forming part of the cell which reacts, thus leaving the inclusion completely free, in smear preparations the whole cytoplasm reacts with the consequence that the vacuolar (unstained) part appears more translucent.

In paraffin sections, the distribution of vimentin positive cells was more diffuse than in other meningiomas of the endotheliomatous type, but the basic focal nature of cells expressing this type of intermediate filament was still visible (Fig. 3b). All cells containing hyalin inclusions/pseudopsammoma bodies showed marked cytokeratin expression (Figs. 3c; 1c, d). The most interesting result was that in paraffin sections these cells were grouped together in small clusters (Figs. 3c; 1c). Mast cells expressed fibronectin, obviously within their granules (Fig. 3d). These cells were evenly scattered through the tumour as visualised in paraffin sections.

Discussion

The different subtypes of meningiomas show several common features which makes it possible to consider them as an entity: at the cytological level these are the characteristic morphological pattern of the cells and the nuclei, histologically the tendency to form whorls and psammoma bodies and at the ultrastructural level the appearance of multiple folded double membranes, of desmosomes and intermediate filaments. Immunocytochemically meningiomas are characterized by the unique coexpression of desmoplakin and vimentin. Thus, it seems sensible to judge, on the presence of these characteristic features whether a given subtype should be summarised under the term meningioma.

It has long been discussed whether the hemangiopericytic and the angioblastic variants, which were introduced by the WHO as subtypes, are meningiomas proper. There is little doubt that rare metablastic variants (e.g. osteo-, chondro-, lipoplastic) are "true" meningiomas, as they all show the typical features in addition to the respective "blastic" structures. This is also true for meningiomas with vacuolized nuclei or pseudopsammoma bodies, or those which were introduced under the term "humid type" (forme humide) or "cystic".

The concept that all meningiomas have a common characteristic precursor cell with characteristic cytological features and the ability to differentiate into various directions, has gained wide acceptance. This view was strongly supported by immunocytochemical investigations: meningiomas show a focal expression of vimentin and desmoplakin (Schwechheimer et al. 1984) and, to a far lesser extent, of fibronectin (Kochi et al. 1983; Bellon et al. 1984). Cytokeratin as intermediate filament was described in only a minority of cases (Nagle et al. 1983). Our own investigations showed cytokeratin expression in 7 of 79 meningiomas (roughly 9 percent, Mennel et al. 1988).

The tumour presented here displays unusual but common features, such as vacuolated nuclei in addition to rare formations such as pseudopsammoma bodies and a "humid type" appearance. There was a strong expression of vimentin, fibronectin and cytokeratin in a characteristic predominance in certain cell types. Vimentin was localized within the typical meningioma cells and was dispersed more or less focally within the tumour. Fibronectin was positive in the scattered granular cells, which were identified as mast cells. The frequent occurrence of mast cells in cystic meningiomas was observed by Zülch (1956) and confirmed by Schober et al. (1988). Cytokeratin was positive only in cells containing pseudopsammoma bodies, which where lying side by side in small cluster. This formation of small foci of pseudopsammoma bodies producing and cytokeratin containing cells, can, in our opinion, be shown only in this distinct manner by the application of immunohistochemistry, using anti-cytokeratin antibodies. These cytokeratin positive, pseudopsammoma containing cells are most likely to be due to progressive differentiation. It was thought that pseudopsammoma bodies contain a secretion product and in our case cytokeratin expression

makes a epithelial differentiation likely. This would support the view that meningioma cells are capable of differentiation along different lines, including the expression of epithelial marker proteins. Thus, the intermediate epithelial-mesodermal-neuroectodermal position of meningiomas is further substantiated.

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