Immunocytochemical demonstration of intermediate filaments in a granular cell ameloblastoma

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The nature and location of intermediate filament proteins (IFP) may provide new insights into the origin and differentiation of neoplastic cells. An immunofluorescent study of these IFP in a case of a granular cell ameloblastoma revealed that all tumor cells contained the IFP keratin. Some granular cells, however, also contained the IFP vimentin, which is considered specific for mesenchymal tissues only. The implications of these observations are discussed. Study with monoclonal antibodies indicated the origin of the ameloblastoma from non-keratinized squamous epithelium. A comparison of the anti-keratin immunofluorescence pattern of the ameloblast-like cells in the present tumor with ameloblasts in the tooth germ revealed no similarities, indicating that despite some resemblance of the peripheral columnar cells to ameloblasts, these cells differ in other aspects.

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Ameloblastoma is a benign, but locally invasive, epithelial odontogenic tumor (Pindborg & Kramer 1971). Light microscopically, the tumor resembles the enamel of the developing tooth germ, since it exhibits a peripheral layer of cells, rather like ameloblasts or pre-ameloblasts, and a central area of cells similar to stellate reticulum. Ultrastructural studies have confirmed the odontogenic origin of the ameloblastomatous epithelium, in so far as the low columnar epithelial cells resemble normal outer enamel epithelium, while the centrally placed cells mimic the stellate reticulum cells of the normal enamel organ. The high columnar peripheral cells which, by light microscopy, resemble preameloblasts, have no ultrastructural counterpart in the tooth germ. At best, they resemble the ameloblast at a rather primitive stage of differentiation (Kim et al. 1979, Matthiessen et al. 1980). The granular cell ameloblastoma is a peculiar histological type about which there is controversy regarding the origin and nature of the composing granular cells (Hartman 1974). Ultrastructurally, it is demonstrated that these cells contain electron-dense granules that could represent lysosomes (Navarrete & Smith 1971, Tandler & Rossi 1977). This has
been substantiated by the demonstration of high levels of acid phosphatase activity within the granular cells (Mori 1970).

Another way of studying the nature and origin of tumors is the investigation of intermediate filament protein (IFP) types of the cytoskeleton. These filaments form an essential part of the cytoskeleton in eukaryotic cells and can be visualized by immunofluorescence and electron microscopy (Osborn et al. 1981). Several reports in the recent literature (Franke et al. 1981, Lazarides 1982) show that the polypeptide composition of intermediate filaments is tissue specific: Five filament subgroups can be distinguished. In vivo and in vitro epithelial, mesenchymal, myogenic, neuronal and glial cells can be identified and distinguished by use of specific antibodies to their specific intermediate filament proteins, i.e., keratin, vimentin, desmin, neurofilament protein and glial fibrillary acidic protein (GFAP), respectively. These intermediate filaments are retained when the cells become neoplastic (Ramaekers et al. 1981, 1982a, 1982b, 1982c, Gabbiani et al. 1981). Normally, tumor tissues do not seem to obtain additional IFP-cytoskeletons. IFP-antiserum, therefore, are useful for the immunohistochemical detection of the cell of origin and can be applied for differential diagnosis in surgical pathology (Almansiberger et al. 1981a, 1981b, 1982, Gabbiani et al. 1981, Battifora et al. 1980, Guserson et al. 1982, Schlegel et al. 1980, Casaliz et al. 1981, Bejui-Thiêuiolet et al. 1982, Löning et al. 1980).

Lesot et al. (1982) have studied the intermediate filaments in odontogenic tissues for the first time. This study of embryonic tooth germs of mouse molars revealed keratin in cells of the enamel organ and vimentin was observed in the dental papilla. With antibodies directed against keratin, pre-ameloblasts showed the same uniform strong staining as the other cells of the enamel organ. However, the staining pattern of differentiated and polarized ameloblasts was different: an intense fluorescence was restricted to the apical pole of these cells.

Studying the localisation and characterization of intermediate filaments in ameloblastoma by immunofluorescence may be helpful in clarifying some of the above mentioned uncertainties, especially those concerning the peripherally localized high columnar ameloblast-like cells and the granular cells. An effort in this direction is made in the present paper.

Case history

A 79-year-old woman was admitted to the maxillo-facial department of the Utrecht University Hospital in September 1982. At the end of 1978 the patient became aware of a painless swelling on the right lateral side of the face. In February 1979 she had consulted a doctor elsewhere. Roentgenograms revealed a multilocular osteolytic lesion, involving part of the mandible. Incisional biopsy showed the lesion to be an ameloblastoma of the granular cell type. Several conservative procedures in April 1979, December 1979, September 1980, April 1981, and February 1982 failed to regress the tumor. Examination in September 1982, showed a large swelling of the right side of the face involving the parotid-, temporal- and submandibular regions. CT-scan revealed a huge lesion which had caused considerable distortion of the mandible, the right maxilla and the zygomatic arch, extending into the temporal fossa. In October 1982, under general anaesthesia an en bloc resection of the whole lesion was performed without complications.

Material and methods

Tissue. Fresh tumor tissue was stored immediately after removal in liquid nitrogen for
immunohistochemical procedures. The remainder of the surgical specimen was fixed in buffered 4% formalin and fragments were prepared for light microscopy according to established methods. For frozen sections, the tumor was cut on a cryostat and the sections (4 μm thick) were fixed after drying in methanol at −20°C for 10 min and, thereafter, dipped in acetone. These sections were then processed for the indirect immunofluorescence technique as described before (Ramaekers et al. 1981, 1982b).

Antisera. The following antisera were used in this study:

1. A rabbit antiserum directed against human epidermal keratins isolated from foot callosities as described previously by Franke et al. (1979a) and Ramaekers et al. (1981).
2. A rabbit antiserum raised against vimentin isolated from calf lenses by preparative gel electrophoresis as described elsewhere (Ramaekers et al. 1981).
3. A rabbit antiserum directed against chicken-gizzard muscle desmin isolated as described previously (Ramaekers et al. 1982c).
4. An antiserum raised in a rabbit and directed against GFAP isolated as described by Dahl & Bignami (1976).

The specificity of these 4 sera can be summarized as follows: with the keratin antiserum we observed strong staining in all stratified squamous epithelia, in dermal appendages such as hair follicles, sebaceous glands and sweat glands, myoepithelial cells, epithelial cells of the urinary tract, the gastro-intestinal tract, the genital tracts, mesothelia, female breast lobuli and ducts, bile ducts and hepatocytes, pancreatic acini, and prostate; epithelial tumors, squamous-cell carcinomas and adenocarcinomas were also positive with this serum. Vimentin antibodies stained fibroblasts in connective tissue, endothelial cells in blood vessels, macrophages, cells of cartilage and bone, some vascular smooth-muscle cells, melanocytes, and other cells of mesenchymal origin, as well as some epithelioid cells in the eye, and Sertoli cells. Soft tissue tumors, lymphomas and other tumors of mesenchymal origin stained positive with the anti-vimentin antiserum. Desmin antibodies reacted exclusively with striated skeletal muscle, cardiac muscle and smooth muscle, and rhabdomyosarcoma cells. The anti-GFAP antiserum specifically reacted with astrocytes and astrocytomas.

Recently, monoclonal antibodies to different types of keratins have been developed. These antibodies can distinguish between subtypes of epithelial tissues and tumors derived therefrom. Their preparation and specificity has been described previously (Ramaekers et al. 1983a, 1983b): serum ROE 53 is specific for keratin in glandular epithelial tissues and adenocarcinomas; RKSE 60 reacts only with keratins in keratinizing stratified squamous epithelia and tumors derived therefrom. The last 2 sera were also applied in the present study.

Result

Light microscopy

The tumor showed the typical appearance of a follicular ameloblastoma. There was a pronounced cystic degeneration in the central parts of many epithelial islands, sometimes leaving behind only a rim of columnar basal cells with apically positioned nuclei. Moreover, there was an abundant formation of granular cells in the tumor. The cells themselves were large, round or elliptical in configuration with an eosinophilic granular cytoplasm, when viewed in sections stained
with hematoxylin and eosin. The nuclei of granular cells were generally pyknotic and, usually, eccentrically displaced or compressed against the cell wall. The granular cells replaced in whole or part the stellate reticulum. Frequently, the peripheral columnar or cuboidal ameloblast-like cells were partly replaced by granular cells; sometimes, there was an abrupt transition from columnar cells to granular cells. Some of the epithelial follicles consisted of granular cells with only a few ameloblastic cells remaining in the periphery. Fields, exclusively composed of granular cells were also present. A representative part of the tumor is shown in Figure 1. Within some extensive areas of stellate reticulum cells, fields of small, densely packed cells with compact nuclei, and an inconspicuous rim of cytoplasm were observed (Fig. 2). A similar morphology was seen in the biopsy specimens obtained in 1979 and 1981.

Immunohistochemistry

A. The epithelial component. Incubation with antibodies directed against keratin gave a positive reaction of the epithelial component of the tumor (Fig. 3). The high peripheral columnar cells exhibited a weak uniform positive staining. A very strong immunofluorescence was observed in the small stellate reticulum-like cells in the central parts of the tumor islands (Fig. 4). As can be seen (Fig. 4), many of these central cells show strong positive cytoplasmic processes. The granular cells also exhibit a positive, but, usually, less intense reaction with the antibodies to keratin (Fig. 5). Often there is only a small rim of varying thickness and intensity of keratin fluorescence in the periphery of granular cells, while the central part of the cytoplasm exhibits a very weak staining in a honeycomb pattern (Fig. 5). The small round cells of some
central parts of the tumor islands (resembling the cells from Fig. 2) were also positive to keratin, but somewhat less than the stellate-like cells (Fig. 6). Incubation with antibodies directed against vimentin gives mostly no reaction with the epithelial component of the tumor (Fig. 7). Occasionally, however, varying numbers of tumor cells in the central core of some of the tumor islands were positive for vimentin. Based on their cytological appearance, these were probably granular cells (Fig. 8). When anti-vimentin antiserum was absorbed with a purified vimentin preparation from bovine lens prior to the incubation, immunofluorescent staining diminishes (Fig. 9). This means that the positive staining with anti-vimentin antiserum of some tumor cells is not an aspecific reaction. No reaction was found in the tumor cells with antibodies to GFAP and desmin.

Incubation with monoclonal antibody RGE 53, which is specific for keratin in glandular epithelial tissues and adenocarcinomas gave no positive reaction. The same is true for the incubation with monoclonal antibody RKSE 60, which reacted only with keratins in the keratinizing stratified squamous epithelia.

B. The connective tissue stroma. The connective tissue of the tumor stroma showed no positive staining with antibodies to keratin or the monoclonal antibodies directed against keratin. A strong positive immunofluorescence was seen in these parts after incubation with antibodies to vimentin (Fig. 7). Also a strong vimentin-positive reaction was seen in the blood-vessel endothelium and some smooth-muscle cells. Antibodies directed against desmin gave a positive reaction only with smooth-muscle cells in the blood vessels of tumor stroma.
Figs. 4–6. Frozen sections of the ameloblastoma showing the different morphological entities with their specific immunofluorescent reactions when incubated with the anti-keratin antiserum, Fig. 4. ×250; Fig. 5. ×250; Fig. 6. ×50.

Fig. 7. Frozen section of the ameloblastoma incubated with the anti-vimentin antiserum. Note the strong reaction in the stroma that accompanies the tumor and in the blood vessels. No reaction is seen in the epithelial tumor cells ×150.

Fig. 3. Overview of some typical ameloblastoma tumor cells in a frozen section incubated with the anti-keratin antiserum. Note the strong reaction in reticulum stellate-like and granular cells, weak staining in columnar cells and no reaction in the stroma of the tumor ×100.
Discussion

The tumor described in this study was the granular cell variant of follicular ameloblastoma. Our histological findings are in conformity with the observations in foregoing studies of granular cell ameloblastoma (Hartman 1974, Tandler & Rossi 1977). Immunofluorescent study of the IFP cytoskeleton confirms the epithelial character of the ameloblastoma. All cells of the tumor islands revealed a positive staining with antibodies to the epithelial-specific keratin. Incubation with the monoclonal antibody RKSE 60, which is specific for keratins of keratinizing stratified squamous epithelia (Ramaekers et al. 1983a, 1983b), gave a negative reaction in all tumor cells. This suggests that the ameloblastoma is derived from cells closely related to non-keratinizing stratified squamous epithelium, which is in accord with the ultrastructural observations of Kim et al. (1979). These investigators found no ultrastructural characteristics of keratinizing epithelium, even in the squamous cells in the area of squamous metaplasia. When comparing the anti-keratin immunofluorescence of high peripheral columnar cells and the stellate reticulum-like cells to the observations on the normal mouse tooth germ of Lesot et al.
(1982), a similar strong uniform positive staining of the stellate reticulum-like cells of the tumor was observed. The IFP cytoskeleton of the stellate reticulum-like cells in an ameloblastoma seems, thus, to be comparable to that of the tooth germ. This is in accordance with the ultrastructural observation of several groups (Kim et al. 1979, Matthiessen et al. 1980). However, no apical polarization of keratin in the tall columnar peripheral cells of the ameloblastoma, as described by Lesot et al. (1982) for the differentiated and polarized ameloblasts of the mouse tooth germ, was observed in the present case. The tall columnar cells exhibited only weak immunofluorescence when incubated with antibodies to keratin and no intense fluorescence at the apical pole. It can be concluded that the tall columnar cells in the ameloblastoma have no counterpart in the tooth germ, neither with respect to the IFP cytoskeleton, nor ultrastructurally. The presence of a poorly developed keratin cytoskeleton in these peripheral columnar cells is perhaps in accordance with the undifferentiated state, as observed by several electron microscopic studies (Kim et al. 1979, Matthiessen et al. 1980). The immunofluorescent study of the IFP cytoskeleton also sheds some additional light on origin and nature of the granular cells in the ameloblastoma. There is now general agreement that the granular cells, like the other tumor cells, are of odontogenic epithelial origin and that transition between these cells and columnar or stellate cells is a notable histologic feature (Hartman 1974, Lucas 1976). Electron microscopy confirmed the epithelial origin of the granular cells (Navarrete & Smith 1971, Tandler & Rossi 1977). Ultrastructural study reveals that the cytoplasmic granules are pleomorphic electron-dense bodies resembling organelles usually described as lysosomes (Navarrete & Smith 1971, Tandler & Rossi 1977). Gold & Christ (1970) suggest that the cytoplasmic granules represent a metabolic phenomenon rather than a degenerative process. Tsukada et al. (1965) discussed the possibility that the granular cells in an ameloblastoma might represent an aging phenomenon. Hartman (1974) proposed that an aging or degenerative process appears unlikely, because the histologic pattern of the recurrent tumors was basically the same as was observed in the original tumors. Our observations support the findings of Hartman (1974), because all biopsies of our patient taken over 4 years, show the same histologic features. The number of granular cells did not increase during that time. Our study with antibodies directed against keratin confirms the epithelial character of the granular cells. As vimentin was also observed in the cytoskeleton of some tumor cells resembling granular cells, it seems that these cells either obtain an additional intermediate filament cytoskeleton or that some tumor cells are of mesenchymal origin. This phenomenon is not unique for the granular cells in an ameloblastoma, but has also been described in the pleomorphic adenoma of the salivary glands (Caselitz et al. 1981). These authors give 3 possible interpretations of this phenomenon. First, the tumor cells might originate from cells which are characterized by keratin and vimentin filaments. Our opinion is that this is unlikely, as the embryonic cells in the enamel organ of mice do not contain vimentin (Lesot et al. 1982). Secondly it may be possible that the tumor is a mixed cell type composed of both epithelial and mesenchymal components. In the case of the ameloblastoma there is, however, no such microscopic or ultrastructural evidence. The third possibility, which we consider to be most probable, is that the cells originate from cells which are characterized by keratin-type filaments, but which after neoplastic transformation, acquire the ability to produce vimentin filaments. In cell cultures many cells of epithelial origin acquire a vimentin system in
addition to the keratin system (Franke et al. 1979b, Ramackers et al. 1983c). At any rate, the presence of vimentin-positive cells in addition to cells containing keratin IFP, introduces new concepts with regard to the complex nature of the granular cell ameloblastoma. In our opinion the rather weak, but varying, immunofluorescence with antibodies directed to keratin and the possible presence of an additional intermediate filament cytoskeleton in the granular-like cells may indicate that these cells arise through dedifferentiation of the tall columnar and stellate reticulum-like cells, not through their degeneration. This implies that the granular cells in the granular cell ameloblastoma are not cells in an inactive endstage but, on the contrary, dedifferentiated tumor cells.

Future development of monoclonal antibodies to the keratin IFP of the various intraoral epithelia may provide a specific marker for odontogenic epithelium. Such a marker would be of great significance in differentiating between squamous-cell carcinoma and malignant odontogenic tumors.

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References


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