Application of Monoclonal Antibodies to Intermediate Filament Proteins in Surgical Pathology of Head and Neck Tumours
An Overview

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Abstract. Intermediate filament proteins are distributed in a tissue specific manner throughout human tissues. Using monoclonal or polyclonal antibodies to cytokeratins, vimentin, desmin, neurofilament proteins or the glial fibrillary acidic protein, epithelial, mesenchymal, myogenic, nervous and glial tissues, respectively, can be distinguished by immunohistochemical techniques. Since tumour cells generally retain the intermediate filament proteins typical for their cells of origin, such antibodies can also be used to discriminate between different types of neoplasms, i.e. carcinoma, lymphoma, myosarcoma, etc. Furthermore, monoclonal antibodies to individual cytokeratin proteins can be used to distinguish between several types of epithelial tissues and different types of carcinomas. The application of such antibodies in the histopathology of head and neck tumours can be of great help in the characterization of tumours that cannot be identified on the basis of routine histological techniques.

Introduction

In surgical pathology most tumours to be examined can be typed by routine histological staining techniques applied to routine paraffin sections. However, a certain percentage of neoplasms may confront the histopathologist with serious diagnostic problems. Often, in such difficult cases it is not possible to decide whether the tumour is of epithelial or mesenchymal origin. For example, differential diagnostic problem cases may occur in tumours where malignant lymphoma has to be distinguished from metastatic anaplastic carcinoma. In the head and neck area the distinction between nasopharyngeal carcinomas and malignant lymphomas is often difficult. Furthermore, differentiation between melanomas on the one hand and metastatic carcinomas or Merkel cell tumours on the other hand may give rise to serious problems when only routine proce-
Table 1. Tissue and tumour specificity of intermediate filament proteins

<table>
<thead>
<tr>
<th>Type of IFP protein</th>
<th>Tissue type</th>
<th>Tumour type</th>
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<tbody>
<tr>
<td>Cytokeratins</td>
<td>epithelial tissues</td>
<td>carcinomas</td>
</tr>
<tr>
<td>Vimentin</td>
<td>mesenchymal tissues</td>
<td>lymphomas, sarcomas</td>
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<tr>
<td>Desmin</td>
<td>muscle tissues</td>
<td>myosarcomas</td>
</tr>
<tr>
<td>GFAP</td>
<td>astroglial cells</td>
<td>astrocytomas</td>
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<tr>
<td>Neurofilament proteins</td>
<td>nerve tissues</td>
<td>some neural tumours</td>
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dures are applied. Yet, an accurate diagnosis of a malignant tumour is of paramount importance in planning treatment and in estimating prognostic factors. It is, therefore, not surprising that immunohistochemistry has become a technique that is being applied in an increasing number of cases in tumour pathology. Especially in combination with well-defined monoclonal antibodies, this procedure complements the morphological studies and may facilitate the identification and classification of tumours.

Here I will concentrate on the application of antibodies to intermediate filament proteins (IFP) in the immunohistochemical characterization of head and neck tumours. These proteins form a cytoskeletal structure composed of 10-nm filaments in most mammalian cells and have been shown to be tissue specific to a certain extent [1]. Five different types of IFP have been distinguished so far, and the distribution of these different proteins has been shown to follow well-known histologic principles (table I). This tissue-specific distribution of IFP, in particular the keratins, allows one to use them as markers in histopathology.

Tissue Specificity of IFP and Their Application in Diagnostic Pathology

Monoclonal and polyclonal antibodies to the different types of IFP have been prepared by several groups working in the field [1–5] and many of them are commercially available now. Most of these antibodies react in a tissue-specific manner, meaning that in general antibodies to cytokeratin react only with epithelial tissues, antibodies to desmin only with muscle tissues, glial fibrillary acidic protein (GFAP) antibodies react only with glial cells and neurofilament antibodies react with neural cells. Antibodies to vimentin normally react only with cells and tissues of mesenchymal origin, but for this type of IFP some exceptions are known [6]. The different types of normal tissues denoted in table I can thus be distinguished by immunocytochemical methods, using specific (monoclonal) antibodies to IFP. Mesothelial cells have been suggested to co-express cytokeratins and vimentin [7], a phenomenon also seen in some other types of epithelial cells [8].

Antibodies to IFP have been tested elaborately for use in tissue characterization in the past few years and from the data obtained in these studies it is obvious that IFP can be used as powerful markers in tumour typing (table I) [2, 3, 5]. Most important, it has been shown that generally tumour cells retain their original IFP, with the exception of some types of neoplasms [9]. Therefore, carcinomas can be stained with antibodies to cytokeratins, while mesenchymal tumours such as lymphomas [10], soft tissue sarcomas [11], melano-
as [12], seminomas [13] and schwannomas, generally speaking, negative for cytokeratins, but positive for vimentin.

Tumours derived from striated or smooth muscle tissues, i.e. rhabdo- and leiomyosarcomas, can be differentiated from other soft tissue tumours because of their reactivity with desmin antibodies [14]. Moreover, astrocytomas are strongly positive for GFAP, while ganglioneuroblastomas and part of the neuroblastomas are positive with neurofilament antisera. However, some neuroendocrine neoplasms such as Merkel cell tumours [15] have been described to contain neurofilament proteins.

In contrast to their normal human tissue counterparts, certain neoplastic tissues may express different types of IFP. When tumour cells contain more than one IFP type, in most cases vimentin is one of them. In the case of epithelial tumours vimentin IFP have, for example, been found next to cytokeratin IFP in, for example, pleomorphic adenomas of the parotid gland [16, 17] and in adenoid cystic carcinomas of the salivary gland [18]. Co-expression of vimentin and desmin is observed in most if not all rhabdomyosarcomas [14] and in leiomyosarcomas. Co-expression of cytokeratins and neurofilament proteins has also been observed. This is the case in some neuroendocrine tumours, such as Merkel cell tumours [15]. The fact that some tumour types co-express different types of IFP can be of help in the diagnosis of such malignancies.

Cytokeratin Distribution in Normal and Malignant Epithelial Tissues

The cytokeratins are a family of IFP, which are characterized by a remarkable biochemical diversity, represented in human epithelial tissues by at least 19 different cytokeratin polypeptides ranging in molecular weight between 40 and 68 kdaltons [19]. Moll et al. [20] have published the catalogue of human cytokeratins and designated them 1–19, cytokeratin 1 being the polypeptide with the highest molecular weight and highest isoelectric pH and cytokeratin 19 being the polypeptide with the lowest molecular weight and a low isoelectric pH. These polypeptides are not expressed randomly throughout the epithelia but occur in cell-type-specific combinations [19, 20]. Also, the polypeptide composition of epithelial cytokeratin filaments varies with the state of differentiation. For example, the epithelia lining the human oral cavity exhibit different cytokeratin patterns when examined by biochemical and immunochemical techniques [21–24]. Also, the diverse types of carcinomas differ in their cytokeratin polypeptide content. The polypeptide patterns of epithelial tumours are either identical with the cytokeratin pattern present in the cell of origin or at least closely related to it [19, 20].

Several monoclonal antibodies to different cytokeratins have been prepared [4] and different reactivities of such antibodies with different subtypes of epithelial tissues and epithelial tumours have been noted [24, 25]. Next to broadly cross-reacting antibodies, which stain virtually all types of epithelial tissues, monoclonal cytokeratin antibodies, which cross-react with only one of the 19 cytokeratin polypeptides and which show a more tissue-specific staining pattern have been developed. So far, chain-specific monoclonal antibodies have been described or prepared for cytokeratins 3, 4, 7, 8, 10, 13, 18 and 19 [4]. Some of these antibodies have been used in tumour characterization and it has been shown that they can be used to sub-
divide groups of carcinomas. For example, monoclonal antibodies of cytokeratin 18 [26] recognize glandular epithelia but normally not stratified squamous epithelia, and can therefore distinguish adenocarcinomas from squamous cell carcinomas. An antibody to cytokeratin 10, on the other hand, stains only keratinizing cells, and can also recognize such cells in squamous cell carcinomas. Especially in cases of anaplastic carcinoma metastases these antibodies can be of great help for tumour diagnosis. Furthermore, such antibodies will be useful probes for the identification of subpopulations of tumour cells in heterogeneous neoplasms.

It may be obvious from the foregoing that IFP antibodies may also have important applications in cytopathology. In this area of tumour diagnosis the pathologist is often confronted with severe problems concerning cell recognition and characterization since the tumour cell preparations are mostly devoid of any morphologic information from which the histogenensis of the aspirated tumour can be deduced. Therefore, especially in this area of pathology tissue-specific probes are needed. It is our experience that especially increases for thin needle aspirates from palpable lymph nodes which are suspected for the presence of a tumour metastasis, application of cytokeratin antibodies may be of special importance and seems most promising.

**IFP Antibodies in Head and Neck Histopathology**

In the second part of this article the application of (monoclonal) antibodies directed against specific IFP in potential diagnostic problem cases in head and neck tumour pathology will be described.

**Nasopharyngeal Carcinoma vs. Lymphoma**

One of the most common problems in diagnostic histopathology may be the differentiation between (anaplastic) metastatic carcinoma and malignant non-Hodgkin's lymphoma [10]. Especially in the head and neck area tumours may be subjected to this question. Nasopharyngeal carcinoma and lymphoma can, however, easily be distinguished on basis of their IFP content, since the carcinomas contain cytokeratins and the lymphomas are only positive for vimentin (fig. 1a, b). Keratinizing areas within oral squamous cell carcinomas can be detected in frozen section material by monoclonal antibodies to cytokeratin 10, while monoclonal antibodies to cytokeratin 18 can recognize an adenocarcinomatous nature of an epithelial neoplasm. Application of a set of cytokeratin antibodies in combination with a vimentin antiserum has shown to be most helpful in cytological specimens of thin needle aspirates of palpable lymph nodes (fig. 1c–f). A cytokeratin-positive reaction is detected easily in the fluorescence microscope or in the immunoperoxidase technique, also in cases where only a few tumour cells are present in the

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**Fig. 1.** Immunoperoxidase staining reactions for intermediate filament proteins of a lymphoma in the tonsil (a, b) and a lymph node aspiration (c, d), as well as a fine needle aspiration of a lymph node containing a squamous cell carcinoma metastasis (e, f). Note the reaction of the lymphoma cells with the vimentin antibody (b, d) but not with the keratin antibody (a, e). On the contrary, the carcinoma cells are positive for keratin (e) and negative for vimentin (f). Only hematopoietic cells stain positive for the latter antibody.
Fig. 2. Indirect immunofluorescence technique showing different reactivity patterns of melanoma and Merkel cell carcinoma with antibodies to different intermediate filament proteins. a–c Melanoma (M) negative for cytokeratin (a) and desmin (c), but positive for vimentin (b). d, e Merkel cell tumour showing co-expression of cytokeratins (d) and neurofilament proteins (e). Note the typical dot-like appearance of the neurofilaments in these neuroendocrine skin tumours.

preparation. Such a positive reaction then very strongly indicates the presence of metastatic carcinoma cells in the lymph nodes examined. Application of the monoclonal cytokeratin antibodies mentioned above can give valuable clues as to the primary location of such a metastatic carcinoma. In fine needle aspirations of apparently normal lymph nodes cytokeratin-positive cells are generally not found.
Melanoma vs. Merkel Cell Tumour vs. Carcinoma

The diagnosis of melanoma may be problematic, especially in cases of amelanotic melanoma. This type of tumour may then be confused with carcinoma or even Merkel cell tumour (neuroendocrine carcinoma of the skin). If these questions in differential diagnosis arise, IFP antibodies can be a strong tool in reaching a final decision about the nature of the underlying neoplasm since melanomas have been shown to contain only imentin as their IFP (fig. 2a–c). Merkel cell tumours co-express cytokeratins and neurofilament proteins (fig. 2d, e), while carcinomas normally contain only cytokeratins. It is interesting to note here that Merkel cells present in human skin contain only cytokeratins and are negative for neurofilament antibodies [27]. Apparently, such neuroendocrine carcinomas obtain the additional neurofilament cytoskeleton upon malignant transformation.

Parotid Gland Tumours

In benign pleomorphic adenomas of the parotid gland we could, like other investigators [16, 17], detect several histologically and immunohistochemically distinguishable tumour cell compartments (table II). These included solid epithelial areas with cells co-expressing cytokeratins and vimentin (fig. 3a, b), epidermoid cells containing only cytokeratins, and myxoid tumour areas in which cells positive for either keratin, vimentin or both were found.

The ductal component of the tumours could be shown to consist of a basal cell compartment which co-expresses vimentin and cytokeratin (fig. 3a, b). This subpopulation of cells was, however, negative for monoclonal antibodies specifically directed against cytokeratin 18 (fig. 3c). On the contrary, the apical columnar cells lining the ducts are strongly positive for such antibodies (fig. 3c), but do not seem to contain vimentin (fig. 3b).

Interestingly, a small number of cells in the solid epithelial areas, in the myxoid tumour areas and in the chondroid areas contain GFAP (table II). The meaning of this peculiar finding remains unclear until now.

The typical staining pattern of pleomorphic adenomas makes it possible to distinguish them from other (metastatic) carcinomas such as oral squamous cell carcinomas or mucoepidermoid carcinomas, which are only positive for cytokeratin [17]. Although adenoid cystic carcinoma of the salivary glands is a tumour type that has also been shown to co-express cytokeratins and vimentin [18], the staining reaction of the cytokeratin 18 antibody is different from pleomor-
hic adenomas. IFP typing in *aspirates* from leiomorphic adenomas of the parotid gland has been found to be most helpful in confirming or establishing the cytologic diagnosis of such neoplasms. As in frozen sections, also in cytologic specimens of these tumours, several cell types can be detected which differ in their IFP pattern (fig. 3d–g). Amongst these are cells co-expressing cytokeratin and vimentin, but which are negative for cytokeratin 18 antibodies. Another cell type is positive for the polyclonal cytokeratin antisera as well as for cytokeratin 18, but negative for vimentin. As described above, these two combinations are typical for solid pleomorphic adenomas.

Malignant degeneration occurring in long-standing pleomorphic adenomas is a well-known phenomenon. Histologically, these tumors are usually characterized as adenocarcinomas or undifferentiated carcinomas.

In collaboration with Dr. W.M. Molenaar (Groningen) and Dr. P.J. Slootweg (Utrecht) 4 cases of malignant pleomorphic adenoma were studied for their interme-

diate filament pattern. In these malignant tumours the original benign pleomorphic adenoma was present as a sclerotic nodule with cells positive for cytokeratin, vimentin and GFAP. In 2 cases, malignant degeneration resulted in an adenocarcinoma with keratin-positive cells. In the 2 other cases, keratin-positive carcinomatous areas intermingled with vimentin and GFAP-positive chondroid cells. This IFP pattern supports the idea that malignant pleomorphic adenoma can occur as a carcinoma ex benign pleomorphic adenoma or as a malignant mixed tumour, a carcinosarcoma. An unusual case of a spindle cell carcinoma of the parotid gland was studied in collaboration with Dr. Molenaar. The patient presented with a tumour in the parotid gland and metastatic disease in the neck, but nevertheless survived for 17 years. Histologically, the spindle cell pattern led to an erroneous diagnosis of sarcoma. The epithelial character of the process was later established with help of cytokeratin antibodies, which gave a positive reaction in the metastatic tumour. Vimentin antibodies were consistently negative in tumour cells. The immunohistochemical findings were confirmed by ultrastructural examinations of the most recent lymph node specimen, which revealed many desmosomes and tonofilaments.

**Fig. 3.** Different cell compartments can be distinguished in pleomorphic adenomas of the parotid using polyclonal and monoclonal antibodies to intermediate filament proteins. a–c Frozen sections of a pleomorphic adenoma showing the ductal component and the solid epithelial areas stained with the polyclonal antiserum to cytokeratin (a), a monoclonal antibody to cytokeratin 18 (b), and an antibody to vimentin (c). d–g Corresponding staining reactions in a thin needle aspiration of a pleomorphic adenoma. d, g Polyclonal cytokeratin antiserum. e Monoclonal antibody to cytokeratin 18. f Monoclonal antibody to vimentin. d and e as well as f and g are double-label assays showing the same cells incubated with two different antibodies.

**Sarcomatoid (Spindle Cell) Carcinoma of the Upper Respiratory Tract**

It is generally believed that the spindle cell carcinoma of the larynx is a variant of the squamous cell carcinoma occurring in the upper respiratory tract, although it is sometimes hard to find histological or ultrastructural evidence for epithelial properties in the sarcomatoid areas. In an examination of such tumours from 13 patients, vimentin
Fig. 4. Spindle cell carcinoma of upper respiratory tract, stained intermediate filament proteins with the immunoperoxidase technique on paraffin sections. a Vimentin-positive reaction in a sarcomatoid area. b Cytokeratin positivity in the squamous cell carcinoma component. c, d Coexpression of vimentin (c) and cytokeratin (d) in interphase between carcinoma and spindle cell area.

Fig. 5. Frozen section of granular cell ameloblastoma showing different morphological entities and their specific immunofluorescence reactions when incubated with antiserum to cytokeratins.

tivity was found in 12 cases in the sarcomatoid areas (fig. 4a). Dysplastic epithelium squamous cell carcinoma components were positive for keratin (fig. 4b). In 10 cases strong indication existed for the co-expression of keratin and vimentin in a part of the that on histological grounds belong to sarcomatoid area or cells in the inter-

phase between carcinoma and spindle cell area (fig. 4c, d). It is obvious that large parts of the sarcomatoid areas of such tumours are negative for cytokeratin, however. Future studies will have to show whether metastases from such polyploid tumours consisting only of the spindle cell component may be mistakenly diagnosed as sarcoma.
Ameloblastoma/Oral Granular Cell Lesions

A study of IFP distribution in a granular cell ameloblastoma revealed that all tumour cells contained cytokeratins [27]. Figure 5 shows that a cytokeratin reaction could be observed in the several different epithelial components that can occur in such neoplasms.

The high peripheral columnar cells may exhibit only a weak uniform positive staining. Small stellate reticulum-like cells in the central parts of the tumour islands are very strongly cytokeratin reactive. The small round cells of some central parts of tumour islands were also positive for cytokeratin, but somewhat less than the stellate-like cells. Finally, the granular cells observed in this tumour show a rim of cytokeratin positivity which is of varying thickness and intensity. The study of this tumour with chain-specific monoclonal antibodies to cytokeratins indicated that this tumour is of a nonkeratinizing squamous epithelial nature [28]. The stromal components were only positive for vimentin. Five other oral granular cell lesions were examined for their IFP expression [29]. These included granular cell myoblastomas of the tongue and congenital gingival granular cell tumours. In both tumour types the malignant granular cell component was positive for vimentin. This indicates that, although granular cells in gingival tumours and in the ameloblastoma show histomorphological identity, this does not signify identity in histogenesis.

Muscle Cell Tumours vs. Malignant Fibrous Histiocytoma

Myogenic tumours, both rhabdomyosarcomas and leiomyosarcomas, in general retain desmin IFP in (part of) their tumour cells. Next to desmin these tumours contain also vimentin. It should be kept in mind, however, that desmin in especially leiomyosarcomas may become undetectable after formalin fixation and paraffin embedding. Therefore, it is recommended to use fresh frozen section material for IFP immunohistochemistry. Malignant fibrous histiocytoma contains only vimentin IFP, rendering a differential diagnosis of leiomyosarcoma and malignant fibrous histiocytoma possible on the basis of desmin reactivity of the former tumour type.

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