CYTOKERATIN EXPRESSION IN NORMAL AND (PRE)MALIGNANT HEAD AND NECK EPITHELIA: AN OVERVIEW

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Intermediate filament proteins (IFPs) are important markers of tissue differentiation and have been receiving increasing interest, in particular, through their applicability in the characterization of malignant tumors. Cytokeratins (CK) are a family of IFPs that are typically specific for epithelial cells. They are expressed in certain combinations depending on the type of epithelium and the degree of differentiation. This review presents a critical analysis of the available data on CK expression in normal and (pre) neoplastic epithelia of the head and neck region. Special attention is paid to technical and cell biologic pitfalls, which can lead to false-negative or false-positive data. It appears that only a limited fraction of the reported data contributes substantially to our knowledge of IFP expression in head and neck cancer because of the use of ill-defined, often formalin-fixed and paraffin-embedded, tissue specimens, and the application of limited panels of monoclonal antibodies. It is concluded that the use of immunocytohistochemistry is promising for the differential diagnosis of head and neck tumors and contributes to our knowledge on their biologic behavior. However, documentations of more complete CK expression patterns of normal and (pre)malignant epithelium are required, together with their correlation to clinical parameters.

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For the management of cancer patients, there is a great demand for new diagnostic tools to improve the characterization of malignancies. New reagents, such as monoclonal antibodies or nucleic acid probes, are being developed for this purpose. The ultimate goals to be reached with such reagents include: (1) improving of tumor classification on the basis of the histopathologic diagnosis and the further differentiation of apparently identical tumors; (2) gaining more insight into the biologic behavior of a tumor in the individual patient; (3) improving methods to diagnose tumor tissue in fine-needle aspiration biopsy smears or other cytological preparations; and (4) detecting small neoplasias or microscopic metastases at an early stage.

In an attempt to better correlate the prognosis with the histopathology, extensive scoring methods have been introduced to increase the objectivity of tumor grading and to clarify the classification system. However, none of these methods has been found to have a better predictive value than the TNM classification.

In addition, more sophisticated techniques, such as immunohistochecmistry for a large variety of cell markers, flow cytometry, and DNA in situ hybridization, have been applied for the further characterization of tumor cells. Of all the markers used, intermediate filament proteins (IFPs), which form a major part of the cytoskeleton in eukaryotic cells, have been receiving in-
creasing interest during recent years.\textsuperscript{7} In this review, a critical analysis is presented of the data reported in the literature with respect to the expression patterns of IFPs in normal and (pre)neoplastic epithelia of the head and neck region. Special attention is paid to the cytokeratins (Cks), a family of IFPs specifically expressed in epithelial. Furthermore, the potential use of antibodies to IFPs as a new diagnostic tool is discussed.

**INTERMEDIATE FILAMENT PROTEINS**

Intermediate-sized filaments are major components of the mammalian cytoskeleton. Their proteinaceous constituents can be subdivided into six groups, each of which is relatively specific for certain tissues or cell types. The function of IFPs is still largely unknown, but it seems likely that they make a contribution to the physical strength and signal transduction in the cell.\textsuperscript{5,9} The largest subgroup of IFPs is formed by the Cks, which are generally specific for epithelia. They can be subdivided into type I (40–64 kDa molecular weight; acidic, including Ck nos. 9–20) and type II (52–68 kDa molecular weight; basic to neutral, including Ck nos. 1–8).\textsuperscript{10,11} Type III intermediate filaments\textsuperscript{12} consist of vimentin which is specific for mesenchymal cells, desmin which is specific for muscle tissue, glial fibrillary acidic protein (GFAP) which is expressed in glial cells and astrocytes, and peripherin\textsuperscript{13} which is specific for neuronal cells. Type IV are the neurofilament proteins expressed in nerve cells, type V the nuclear A- and B-type lamin proteins which constitute the nuclear lamina, and type VI IFPs comprise nestin, which is specific for CNS stem cells.\textsuperscript{14}

The particular type of IFP present has been used to help promote cell differentiation.

Coexpression of more than one type of IFP has been reported in normal adult and embryonal tissues and in tumors; in such cases, vimentin is the most commonly coexpressed IFP. Cks are coexpressed with vimentin in certain malignant epithelial and nonepithelial (mostly malignant) tumors, such as carcinomas of the head and neck,\textsuperscript{15,16} thyroid,\textsuperscript{17} lung,\textsuperscript{18} kidney,\textsuperscript{19} etc. Mesotheliomas,\textsuperscript{20} malignant lymphomas, melanomas, and spindle cell carcinomas\textsuperscript{21} may also coexpress Cks and vimentin. Normal epithelia, such as the nasal mucosa and enamel organ, occasionally show the coexpression of Cks and vimentin.\textsuperscript{22,23}

**CYTOKERATINS**

Types I and II, which make up the main class of IFPs in epithelial tissues, assemble into heteropolymers which require at least one type I (acidic) and one type II (basic) polypeptide.\textsuperscript{24–27} During development, the epithelial cells express a set of typical Cks for a particular program of differentiation.\textsuperscript{28} Cks are distributed in specific combinations depending on the type of epithelium in which they are expressed.\textsuperscript{10,29}

For example, stratified epithelia express Cks 5 and 14 in their basal cell layer.\textsuperscript{30,31} In noncornified stratified squamous epithelia Cks 4 and 13 are expressed in the suprabasal cell layers,\textsuperscript{32–34} whereas cornified epithelia show the specific expression of Cks 1, 2, 10, and 11. Corneal epithelium shows the exclusive expression of Cks 3 and 12.\textsuperscript{35}

Hyperproliferative epithelia and oral epithelia with a high cell turnover express Cks 6 and 16,\textsuperscript{36} although recent reports have indicated that these are more typical for the squamous type of differentiation.\textsuperscript{37} Simple epithelial cells (with a free luminal surface and contact with the basal lamina) express Cks 8 and 18 and, in most instances, also Cks 7\textsuperscript{38,39} and 19.\textsuperscript{40} Mixed epithelia express Cks 5, 14, and 17 in their basal cell layers, and Cks 8 and 18 in their luminal cells. Transitional epithelia of the urogenital tract express Cks 7, 8, 13, 18, 19, and 20.\textsuperscript{41,42}

In addition to these basic rules, Ck expression in a certain type of epithelium has been found to depend on various factors, such as the stage of embryonal development,\textsuperscript{43,44} inflammation,\textsuperscript{45–48} and environmental influences, such as vitamin A\textsuperscript{50,51} and (pre) malignant transformation.\textsuperscript{52}

**DETECTION METHODS**

IFPs can be analyzed by one- and two-dimenional gel electrophoretic techniques combined with immunoblotting and appropriate (monoclonal) antibodies. This procedure allows the complete separation of all 20 human Cks on the basis of their molecular weight and isoelectric pH, but can also serve to identify and quantitate any posttranslational modifications.\textsuperscript{10,52} However, a major drawback of this procedure is the difficulty in correlating the Ck profile to a specific cell type when dealing with a heterogeneous cell population, because in principle, the method uses whole tissue extracts. Furthermore, low lev-
els of Ck expression often cannot be detected by this laborious procedure.

Immunohistochemical procedures using specific monoclonal antibodies are more sensitive, as they allow the determination of the Ck expression patterns of individual cells in complex epithelia.

The first antibodies to be produced against IFPs were polyclonal antisera. These reagents recognize many epitopes (antigenic determinants) shared by different Ck polypeptides. More specific recognition of individual Cks can be achieved using monoclonal antibodies (MAbs).

Depending on the purpose of the examination, it is mandatory to choose between broad-range antibodies, which recognize epitopes common to many Cks, or narrow-range antibodies, which recognize one epitope that may not be shared among the Cks and may only be present in a single Ck polypeptide or Ck protein configuration.

To obtain optimal results and to avoid false-negative or false-positive data, it should be realized that IFP configuration and detectability can be strongly influenced by decalcification, fixation, and the embedding procedures used in routine histology. These procedures can easily change the epitopes, making them inaccessible to antibodies. For example, formalin fixation and subsequent paraffin embedding have been shown to diminish the immunocytochemical detectability of keratin and vimentin. These problems have been overcome by using frozen sections prepared from fresh tissues. During recent years, an increasing number of MAbs have become available which can be used in routinely prepared, formalin-fixed tissue samples embedded in paraffin. Examples of Ck staining with MAbs, which specifically stain basal cells and supra-basal cells in pseudostratified and stratified epithelium of the larynx, are shown in Figure 1.

THE APPLICATION OF INTERMEDIATE FILAMENT PROTEINS AS MARKERS FOR THE DISEASE DIAGNOSIS OF HEAD AND NECK TUMORS

The use of antibodies to IFPs, in particular Cks and vimentin, has been found to greatly assist in the identification of tumors which cannot be accurately identified using routine histopathologic procedures. These reagents have shown their value in solving differential diagnostic problems, such as differentiating between anaplastic nasopharynx carcinomas and sarcomas or non-Hodgkin lymphomas in the nasopharynx and/or neck. Distinguishing between true sarcomas (which predominantly express vimentin) and spindle cell carcinomas or carcinosarcomas (which express Cks and vimentin) is possible using MAbs. The differential diagnosis of salivary gland tumors, metastatic carcinomas, and neural tumors, can be achieved with antibodies against Cks, vimentin, neurofilaments, and GFAP.

Antibodies directed against Cks can also assist in providing a diagnosis in small amounts of tissue. MAbs against Cks are also useful for helping to resolve the problem of differentiating between reactive mesenchymal cells and individual tumor cells in postradiotherapy tissue. Light microscopic examination of small amounts of tissue obtained by various diagnostic procedures, such as fine-needle aspiration and bronchial brushings, is not always diagnostically conclusive. MAbs against Cks can refine the diagnosis in such cases. Besides these applications in oncology, IFPs have shown their value for studying benign lesions, such as psoriasis and cholesteatomas. Furthermore, the tissue specificity of IFPs can be employed for the characterization of unidentified cells and to observe functional changes in developing and adult tissues. Finally, IFPs can enhance the specificity of other techniques, such as flow cytometry and in situ hybridization.

CK EXPRESSION IN HEAD AND NECK TISSUES

Epithelia. During embryologic development, the epithelial lining of the head and neck region originates from the embryonic endoderm and ectoderm, which gives rise to a large variety of epithelia in the adult, including cornifying, non-cornifying, simple, and pseudostratified epithelia. The data reported on Ck expression in various head and neck epithelia are summarized in Tables 1 and 2. Only the studies that used either two-dimensional gel electrophoresis or well-defined, polypeptide-specific monoclonal Ck antibodies in immunohistochemical or Western blotting studies were used for the compilation of these tables. Without trying to be comprehensive, the expression patterns of Cks in head and neck tissues can be summarized as follows below.

Masticatory mucosa or cornifying stratified epithelium (CSE) covers the hard palate, the attached gingiva (alveolar mucosa), and the papillae of the tongue. The Ck profile of these epithelia show that cornification Cks 1, 2, 10, and 11, as well as hyperproliferation Cks 6 and 16.
are expressed in the suprabasal layers of this type of epithelium, whereas Cks 5, 14, 15, and 17 are present in the basal tissue layers.

Noncornifying stratified epithelium (NCSE) lining the oropharynx, nonattached gingiva (sulcular mucosa), the tongue, lingual epiglottis, vocal cords, surface palatine tonsil, and esophagus, generally lack cornification markers and show consistent expression of the stratification-related Cks 4 and 13 in addition to basal cell Cks 5, 14, 15, and 17. Simple epithelial markers, such as Cks 7, 8, 18, and 19, are expressed inconsistently in basal cell layers, whereas hyperproliferation Cks 6 and 16 are found irregularly in the suprabasal cell layers.

The posterior nasal cavity, paranasal sinuses, laryngeal ventricle, and bronchial epithelium are lined with respiratory or pseudostratified columnar epithelium (PSE), whereas a combination of respiratory and noncornifying stratified epithelium covers the nasopharynx and laryngeal epiglottis. In these tissues, simple cell Cks 7, 8, 18, and 19 and basal cell Cks 5, 14, 15, and 17 are expressed, but the expression of the stratification markers, such as Cks 4 and 13, is only observed occasionally.

The simple cell Cks 8 and 18 are expressed in the neuroendocrine Merkel cells, mostly located in the basal layers of the deep rete ridges of the epithelium.

Junctional epithelium, which forms the attachment site between the nonkeratinizing gingival epithelium and the tooth enamel, is a noncornifying stratified epithelium with a decreasing number of cell layers towards the enamel; it expresses Cks 4, 13, and 19.

In addition, expression of Cks 5 and 14, as well as a heterogeneous expression of Cks 6 and 16, has been described by Morgan et al.

**Salivary Glands.** Salivary glands can be divided into the major (parotid, submandibular, and sublingual) and minor salivary glands. These glands contain several epithelial structures, including...
Table 1. Cytokeratin expression in oral epithelia.

<table>
<thead>
<tr>
<th>Tissue localization</th>
<th>Epithelium</th>
<th>Cornification markers</th>
<th>Stratification markers</th>
<th>Basal cell markers</th>
<th>Simple cell markers</th>
<th>Hyperproliferation markers</th>
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<td>+</td>
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<td>(+)</td>
<td>+</td>
<td>+</td>
<td>Ouhayoun</td>
<td>2DGE + IHC</td>
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<td>+</td>
<td>+/-</td>
<td>+/-</td>
<td>+</td>
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<td>-</td>
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<td>IHC</td>
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<td>(+)</td>
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<td>Bosch</td>
<td>IHC + BLOT</td>
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<td>(+)</td>
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<td>-</td>
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<td>+</td>
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<td>+</td>
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<td>IHC</td>
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<td>Moll</td>
<td>IHC</td>
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Ck, cytokeratin; BLOT, immunoblotting; CSE, cornified stratified epithelium; 2DGE, two-dimensional gel electrophoresis; Epiglot, epiglottis; FOM, floor of mouth; IHC, immunohistochemistry; NCSE, noncornified stratified epithelium; PSE, pseudostratified epithelium; +, positive staining; +/-, heterogeneous/patchy staining; (+), trace of positive staining; -, negative staining.
Table 2. Cytokeratin expression in head and neck epithelia.

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<th>2</th>
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<td></td>
<td>Nagle 1985&lt;sup&gt;15&lt;/sup&gt; 2DGE + IHC</td>
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<td>Larynx</td>
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<td>Henzen&lt;sup&gt;15&lt;/sup&gt; Logmans 1988&lt;sup&gt;16&lt;/sup&gt;</td>
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</table>

Ck, cytokeratin; BLOT, immunoblotting; CIS, carcinoma in situ; 2DGE, two-dimensional gel electrophoresis; Epith, epithelium; GR, differentiation grade; IHC, immunohistochemistry; SCC C, squamous cell carcinoma cornified; SCC NC, squamous cell carcinoma noncornified; +, positive staining; +/-, heterogeneous/patchy staining; (+), trace of positive staining; -, negative staining; >, increased staining during malignant transformation/progression; <, decreased staining during malignant transformation/progression.
the secretory unit which consists of mucus- or serous-secreting acinar cells, surrounded by myoepithelium or basal cells. The intercalated and striated ducts consist of single-layered simple epithelium, and the excretory ducts consist of pseudostratified or stratified cuboidal epithelium.

Cks 5, 14, and 17 are expressed in the basal cells of the ducts and in the myoepithelium of acini. Ck 18 is expressed in the columnar and cuboidal luminal cells. Draeger et al. and Troyanovsky et al. described the expression of Ck 8 in the same areas, whereas the former authors observed the heterogeneous expression of Ck 13 in the large luminal cells. Ck 19 was found in the ductal system.

Enamel Organ. Kasper et al. described the Ck pattern of the human fetal and adult enamel organ. They observed the expression of the basal cell markers, Cks 5, 14, and 17, and of the simple cell markers, Cks 7, 8, and 19; Ck 18 was only expressed in trace amounts.

CYTOKERATIN EXPRESSION IN PRENEOPLASTIC LESIONS AND TUMORS OF THE HEAD AND NECK REGION

Generally, the cell type-specific pattern of IPF expression of various tumors is maintained during malignant growth and metastasis, although exceptions have been identified. Depending on the degree of differentiation, tumor cells may either express new subsets of IPFs (in particular, vimentin and Ck subsets) or even lose the expression of the original IPFs. For example, in lung cancer, Ck 13 is down-regulated as a result of the “dedifferentiation” of squamous cell carcinoma, whereas Ck 18 is expressed increasingly upon malignant progression. Van Eyken et al. showed the unexpected appearance of Ck 7 in hepatoblastoma.

Most malignancies in the head and neck area are squamous cell carcinomas derived from the epithelia which line the oral, nasopharyngeal, pharyngeal, or laryngeal cavities. Only scarce data are available on IPFs and Ck expression patterns in preneoplastic lesions and tumors of these head and neck epithelia. The results of recent studies are summarized in Table 2.

Prenoesplastic Lesions of the Head and Neck. The available data on preneoplastic lesions in oral epithelia suggest that Ck 19 expression in nonkeratinized oral epithelium increases with progression of the grade of dysplasia. If hyperkeratosis is present in these lesions, Ck 19 expression is nearly always absent. Dysplastic lesions of keratinized oral epithelia shows a patchy suprabasal Ck 19 expression, whereas this Ck type is normally found in the basal cell layers. Furthermore, carcinoma in situ of oral epithelia shows a patchy distribution of Ck 19. Lindberg and Rheinwald concluded that Ck 19 expression appears to increase during malignant transformation of oral epithelia. Murakami et al. described the expression of Ck 13 an increased expression of Ck 19 in simple hyperplasia of the pyriform sinus. According to these authors, the expression of Ck 19 increased and the expression of Ck 13 decreased with increasing grades of dysplasia. As a result, Ck 13 expression was absent, in carcinoma-in-situ, whereas Ck 19 expression was abundant.

Squamous Cell Carcinomas. Squamous cell carcinomas of the oral cavity normally express Cks 4, 5, 13, and 14, which are typical of the epithelia in this region. However, the data collected on squamous cell carcinomas from various epithelia of the head and neck area revealed an increased expression of simple cell markers (i.e., Cks 18 and 19) with increasing tumor grade. Morgan et al. showed a decreased expression of cornification markers (Cks 1, 10, and 11) and stratification markers (Cks 4 and 13) during malignant progression and increase of the tumor grade. A comprehensive study by Murakami et al. on the Ck profile of tumors from the pyriform sinus showed a decrease in Ck 4 and an increase in Ck 19 expression, correlating to the progression of malignancy. Moll et al. and Wild et al. reported Ck expression of the basal cell markers and supposed hyperproliferation markers in various head and neck squamous cell carcinomas.

Salivary Gland Tumors. Adenocarcinomas in the head and neck region are mostly derived from the ducts of the major and minor salivary glands. For the diagnosis of salivary gland tumors, IPF staining is mainly used for determining the epithelial nature of the spindle-shaped cells in the tumors.

Data on IPF coexpression in salivary gland tumors have been described by several authors. Two-, three-, and even fourfold co-expression of IPF, such as Ck, vimentin, glial fibrillary acidic protein, and desmin, have been observed in the normal tissue and tumors of the
salivary glands. The complexity of the IFP composition of these tissues and their tumors is also evident from the Ck staining patterns. Caselitz et al. demonstrated Ck 18 expression in pleomorphic adenoma, adenoid cystic carcinoma, and Whartin's tumor. Draeger et al. demonstrated the expression of Cks 7, 8, 14, 18, and 19 in the tubular and in the most unstructured solid and trabecular parts of pleomorphic adenomas. Cks 1, 2, 10, 13, and 14 are expressed in the epidermoid, partially keratinizing portions.

**Odontogenic Tumors.** The histogenesis of several odontogenic tumors has been a subject of considerable controversy. IFPs, predominantly Cks and vimentin, have been used to clarify the histogenesis and to gain more understanding of several odontogenic tumors, including calcifying epithelial odontogenic tumor, calcifying odontogenic cyst, granular cell ameloblastoma, and congenital gingival granular cell tumor.

However, at present only a limited number of tumors have been studied with a small panel of monoclonal antibodies. Therefore, no decision can be made on the usefulness of IFPs in the diagnosis of odontogenic tumors.

**Intermediate Filament Expression in Head and Neck Cancer Cell Lines.** Squamous cell carcinoma cell lines form useful models to observe the invasive potential and the therapeutic importance of chemotherapy and radiotherapy.

Four squamous cell carcinoma lines originating from tongue, larynx, and buccal carcinomas cultured in vitro were investigated by Rupniak et al. Two of these cell lines expressed typical Ck markers for keratinizing basal cells and hyperproliferative epithelium (ie, Cks 5, 6, 14, and 16). The other two contained Cks typical for simple epithelia (ie, Cks 8, 18, and/or 19). These data show that cell lines derived from squamous cell carcinomas of the oral cavity only partly retain the cytokeratin characteristics of the normal neoplasms. Croommans et al. investigated two cell lines derived from a well-differentiated squamous cell carcinoma from the floor of mouth, and from a moderately well-differentiated squamous cell carcinoma of the epiglottis, growing as xenografts in nude mice. Both expressed Cks 5, 7, 8, 10, 14, 18, and 19. One of the cell lines also expressed Cks 4 and 13. Only vimentin expression was observed in the other. These cell lines expressed a mixture of Cks typical for squamous and simple epithelia.

**DISCUSSION**

Monoclonal and polyclonal antibodies directed against IFP can be of great assistance in the characterization of tissue type differentiation. Therefore, they can be applied in the differential diagnosis of undifferentiated and anaplastic carcinomas, spindle cell tumors, micrometastases, and cells in fine-needle aspirations. Independent of the anatomic site, the Ck profiles correlate well with the histologic appearance and the degree of differentiation of the epithelium, even in carcinomas. The main purpose of this review was to provide a critical analysis of the literature on IFPs, in particular Ck expression patterns in head and neck epithelia and their application as markers for the diagnosis of (pre)malignant lesions.

Although several studies have been performed on this topic, only a fraction of the reported data add substantially to our knowledge of IFP expression in head and neck cancer. This is mainly due to the use of heterogeneous tissue specimens and the fact that only small panels of antibodies were applied. In addition, some of the applied methods were open to error. For example, if fixed tissues are used, possible fixation-induced alterations in the epitopes cannot be excluded.

When using MAb's it should be kept in mind that epitope masking is a common phenomenon. Examples of false-negative results, arising from epitope masking, have recently been described and may be overcome by staining with more than one MAb against a single keratin. Furthermore, sometimes no mention was made of the treatment protocols, such as radiotherapy or chemotherapy, and control specimens were frequently lacking. This might explain the great variation between some of the data reported by Murakami et al. and the other data shown in Table 3.

On the basis of the studies described above, the Ck expression pattern of the normal epithelial lining of the head and neck tract can be summarized as follows below (for references see “Epithelia”):

- **Cornifying stratified epithelium** of the attached gingiva and hard palate express cornification markers, stratification markers, basal cell markers, and (presumed) hyperproliferation markers.

- **Noncornifying stratified epithelia** of the oral cavity, pharynx, and larynx express stratification markers, basal cell markers, and hyperproliferation markers. In general, the normal strat-
### Table 3. Ck expression in preneoplastic lesions and tumors of the head and neck epithelia.

<table>
<thead>
<tr>
<th>Tissue localization</th>
<th>Epithelium</th>
<th>Commination markers</th>
<th>Stratification markers</th>
<th>Basal cell markers</th>
<th>Simple cell markers</th>
<th>Hyperproliferation markers</th>
<th>Others</th>
<th>Authors</th>
<th>Methods</th>
</tr>
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<tbody>
<tr>
<td>Oral cavity</td>
<td>Hyperplasia</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Lindberg 198910</td>
<td>IHC</td>
</tr>
<tr>
<td></td>
<td>Dysplasia</td>
<td>&gt;</td>
<td></td>
<td></td>
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<td></td>
<td>Morgan 19877,88</td>
<td>IHC</td>
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<tr>
<td></td>
<td>CIS</td>
<td>+</td>
<td></td>
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<td>Lindberg 198012</td>
<td>IHC</td>
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<td></td>
<td>SCC NC</td>
<td>+/-</td>
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<td></td>
<td>Lindberg 198601</td>
<td>IHC</td>
</tr>
<tr>
<td></td>
<td>SCC C Gr I</td>
<td>+/−</td>
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<td></td>
<td></td>
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<td></td>
<td>Lindberg 198601</td>
<td>IHC</td>
</tr>
<tr>
<td></td>
<td>SCC C Gr II</td>
<td>−</td>
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<td></td>
<td>Lindberg 198601</td>
<td>IHC</td>
</tr>
<tr>
<td></td>
<td>SCC Gr I</td>
<td>+/−</td>
<td>+/−</td>
<td>+/−</td>
<td>+/−</td>
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<td>IHC</td>
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<tr>
<td></td>
<td>SCC Gr II</td>
<td>(+)</td>
<td>(+)</td>
<td>−</td>
<td>−</td>
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<td></td>
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<td>Maxillary sinus</td>
<td>SCC</td>
<td>+</td>
<td>+</td>
<td>(+)</td>
<td>(+)</td>
<td></td>
<td></td>
<td>Moll 198210</td>
<td>2DGE + BLOT</td>
</tr>
<tr>
<td>Tongue</td>
<td>SCC</td>
<td>+</td>
<td>+</td>
<td>(+)</td>
<td>+</td>
<td></td>
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<td>2DGE + BLOT</td>
</tr>
<tr>
<td></td>
<td>SCC</td>
<td>−</td>
<td>−</td>
<td>−</td>
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<td></td>
<td></td>
<td>Ramaekers</td>
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<tr>
<td>Hypopharynx</td>
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<td>&gt;</td>
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<tr>
<td>Epiglotis</td>
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<td>(+)</td>
<td>+</td>
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<td>Hyperplasia</td>
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<td>+/-</td>
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<td>+</td>
<td></td>
<td></td>
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<td>IHC</td>
</tr>
<tr>
<td></td>
<td>Dysplasia</td>
<td>−</td>
<td>−</td>
<td>&lt;</td>
<td>−</td>
<td>&gt;</td>
<td>−</td>
<td>Murakami 199002</td>
<td>IHC</td>
</tr>
<tr>
<td></td>
<td>CIS</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>−</td>
<td>Murakami 199002</td>
<td>IHC</td>
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<tr>
<td></td>
<td>SCC Gr I</td>
<td>+</td>
<td>−</td>
<td>+/−</td>
<td>−</td>
<td></td>
<td>−</td>
<td>Murakami 199002</td>
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<tr>
<td></td>
<td>SCC Gr II</td>
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<td>−</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>+</td>
<td>Murakami 199002</td>
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<tr>
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<td>SCC</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>&gt;</td>
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<td>IHC</td>
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<tr>
<td>Head and neck epith</td>
<td>SCC</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>&gt;</td>
<td>Terry 1986104</td>
<td>IHC</td>
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</table>

Ck, cytokeratin; BLOT, immunoblotting; CSE, cornified stratified epithelium; 2DGE, two-dimensional gel electrophoresis; Epiglot, epiglottis; FOM, floor of mouth; IHC, immunohistochemistry; NCSE, noncornified stratified epithelium; PSE, pseudostratified epithelium; +, positive staining; +/-, heterogenous/patchy staining; (+), trace of positive staining; −, negative staining.
ified epithelia in the head and neck region are not hyperproliferative. Therefore, it is rather surprising that some of them express Cks 6 and 16, which have been described in the epidermis as hyperproliferation markers. There may be a correlation with their high turnover rate, although Wetzels et al. have recently shown that these Ck subtypes may show a strong correlation with squamous differentiation in malignancies.

*Pseudostratified epithelia* of the epiglottis, nasopharynx, and larynx express a Ck profile which reflects a combination of the profiles of stratified and simple epithelium.

From the small number of (pre)malignant lesions tested with a broad panel of antibodies, several trends can be observed.

When dividing Ck into the differentiation marker groups as described above, the Ck profiles of (pre)neoplastic squamous lesions and tumors generally suggest that

1. The expression of cornification or terminal differentiation markers decreases during malignant progression, invasion, and metastasis. The correlation with histologic (pre) cornification of the malignant epithelial cells, however, continues to exist.
2. The expression of stratification markers, especially Ck 13, in nonglandular epithelium decreases drastically during carcinogenesis and seems to correlate with the degree of differentiation of the preneoplastic lesion or carcinoma.
3. The “simple epithelial cell” markers are not specific for one-layered cubic epithelia. They are also expressed during early embryonic development and in undifferentiated tissues. This expression can be seen in dysplasia and squamous cell carcinomas and is correlated with an increase in tumor grade.

The trend of altered Ck expression during malignant progression is apparently independent of the site of the lesion in the head and neck region. These trends confirm comparable observations in epithelia at other sites of the body; the epithelia of the esophagus, skin, respiratory system, and urogenital tract show similar phenomena. A decrease in Ck 13 expression can be observed during malignant progression and transformation in skin and of Ck 10 in the esophagus. In transitional cell carcinomas, squamous cell carcinomas of the lung, (pre)malignant, and squamous cell carcinomas of the female genital tract, an increase in Ck 18 expression has been observed with tumor progression. These results are, however, slightly dependent on the MAb used.

Many questions still remain to be answered concerning the biologic background of Ck expression patterns in normal and malignant head and neck epithelia and possible relations with the biologic growth rate, metastatic potential, and response to different types of therapy. For example, as glandular cell type Ck markers are normally expressed in cuboidal and columnar epithelium and adenocarcinomas, it would be of importance to know whether squamous cell carcinomas, which express these “simple epithelial” markers, also show features of adenocarcinomas, comparable with those observed in lung squamous cell carcinomas. Another question that remains unanswered is whether or not increased Ck 19 expression is one of the early signs of malignant transformation.

A decrease in Ck 13 expression also correlates with an increasing degree of malignancy in several types of cancer. We wonder whether this change can be considered to be a sign of progression or malignant transformation.

Although Ck expression patterns are mainly related to light-microscopic observations and not to clinical parameters, recently correlated Ck 10 expression to the tumor stage of vulvar carcinomas. In tumors of the breast, a correlation has been observed between vimentin expression and poor prognosis. For the head and neck region such studies are not yet available, but future studies are expected to have clinical implications for the individual patient.

**CONCLUSIONS**

The use of IFP expression seems promising for making pathologic differential diagnoses of head and neck tumors, although there are still several technical and cell biologic pitfalls. Characterization of the Ck pattern of tumors is expected to increase our knowledge of the biologic behavior of tumors and the prognosis. Documentation on more complete Ck patterns in head and neck epithelia, premalignant lesions, and tumors are required together with correlations with clinical parameters. Further research will help to prove the true benefits of Ck characterization.
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