CK13 in craniopharyngioma versus related odontogenic neoplasms and human enamel organ

N.A. El-Sissy¹ and N.A. Rashad²

ABSTRACT The monoclonal antibody NCL-CK13 was studied in specimens of craniopharyngioma, ameloblastoma and calcifying odontogenic cyst neoplasms and the mandible and maxillae of normal human fetuses. There was a decrease in NCL-CK13 as the dental lamina developed, with a complete loss in the enamel organ. The neoplastic epithelia of the neoplasms revealed a clear phenotypic and immunohistochemical reactive relationship to the stratified embryonic mucosa, away from the enamel organ. This suggests that these neoplasms might have their histogenesis from early stage epithelium, the oral part of the dental lamina or its remnants.

Comparaison de l’immunoréactivité du craniopharyngiome et des tumeurs odontogéniques apparentées avec l’organe de l’œil humain

RESUME On a étudié l’anticorps monoclonal anticytokératine 13 dans des échantillons de tumeurs - craniopharyngiome, améloblastome et kyste odontogène calcifiant - et mandibules et maxillaires de fœtus humains normaux. Il y a eu une diminution de l’anticorps anticytokératine 13 au cours du développement de la lame dentaire, et absence totale dans l’organe de l’œil. L’épithélium néoplasique des tumeurs a révélé lien clair phénotypique et immunohistochimique avec les épithéliums stratifiés de la muqueuse embryonnaire située à distance de l’organe de l’œil. Cela suggère que ces tumeurs pourraient se développer à partir de l’épithélium à un stade précoce du développement, de la partie orale de la lame dentaire ou de ses reliquats.

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Introduction

The origins of craniopharyngioma (CP), ameloblastoma (AB) and calcifying odontogenic cyst (COC) neoplasms have been debated. The intracranial tumour CP is not of pituitary origin, although it is anatomically related to it and is thought to be derived from remnants of Rathke’s pouch [1,2]. Microscopically, CP (a benign, mainly childhood, tumour) exhibits features of AB. It also bears a striking resemblance to COC having ghost cell keratinization and calcific deposits [2]. Calcific deposits have been classified by the World Health Organization (WHO) as odontogenic tumours [3].

With these neoplasms all previous entities of the tumours may show cystic formation [4]. These tumours also exhibit the same biological behaviour; they grow slowly, infiltrate the surrounding tissues, tend to recur but do not metastasize [1,2].

The epithelium of the primitive stomodeum is the common origin of both the dentition and the anterior part of the pituitary gland [1,5]. Human odontogenic epithelia are derived from downgrowths of the oral epithelium overlying the dental ridges at 6–7 weeks of development: a stage when the oral epithelium is stratified but not mature [5]. The anterior part of the pituitary gland is known to be derived from Rathke’s pouch which evolves from the roof of the stomodeum at about 24 days gestation [6]. It follows that odontogenic tumours and some tumours of the pituitary gland may share that common origin [7].

Although there seems to be a morphologic relation between the three lesions as well as the normal developing odontogenic epithelium, their odontogenic origin has yet to be proved unequivocally [7,8].

While virtually all epithelial cells possess the potential to elaborate keratins, odontogenic epithelium in its fully differentiated state is not a keratinized tissue [4]. Human cytokeratins (CKs) are members of the family of intermediate filaments and form a major component of the cytoskeleton of epithelial cells. They consist of nearly 20 different polypeptides that are classified into two families based on their molecular mass and isoelectric point. They tend to be expressed as specific pairs of type I and type II subunits, both of which are essential for filament formation [9].

Recent progress in understanding the biology of keratins, together with the development of monoclonal antibodies to individual keratin proteins, provides the foundation for studying keratin expression in normal and pathological oral epithelia [10,11]. The expression of CKs is closely linked to epithelium differentiation, particularly during embryogenesis [10,12]. They are useful as epithelial marker proteins because of their high abundance, stability and antigenicity [13]. The pattern of CK expression within a particular epithelium varies with its anatomical location, developmental stage and state of differentiation. Epithelia can therefore be characterized by the specific pattern of polypeptides they express [9,11].

It is thought that studying the expression distribution of certain CK types specific for either the oral or dental epithelium might hold the key to solving questions about the origin of CP, AB and COC. Accordingly, this study was designed to examine cytokeratin 13, reported to be typical of stratified squamous epithelium and recognized as a marker of non-keratinizing epithelia [9].

A detailed assessment of the monoclonal expression of CK13 was required for a more critical comparison of CP, AB and COC lesions and the normal development of the human enamel organ. This might resolve questions about their possible histogenetic relationship.
Materials and methods

Case selection
Six mandibles and maxillae from normal human fetuses were fixed in 10% neutral buffered formalin and decalcified in 5% formic acid and 5 g sodium citrate. These specimens were obtained from legal abortions, between 12–15 weeks gestation. It has been suggested that final cell differentiation is initiated in gestational weeks 12–15 [1/4].

The study was conducted using 15 specimens of neoplasms, 4 CP cases, 6 intraossous AB cases, (4 in the mandible and 2 in the maxillae) and 5 COC cases, (all developed intraossously in the posterior region — 3 cases in the mandible and 2 cases in the maxilla). These cases were selected from the files of the Department of Oral Pathology, Faculty of Dentistry, Mansoura University and the Pathology Department, Faculty of Medicine, Alexandria University.

Preparation of specimens for light microscopic examination
All specimens had been previously fixed in 10% neutral buffered formalin and routinely processed before embedding in paraffin blocks, from which 4μ-thick serial sections were cut and prepared.

The sections were stained with haematoxylin and eosin (H&E) staining for histological examination and confirmation of the histological diagnosis. For immunohistochemical study the following processes were carried out.

• The paraffin sections were placed on sialinized glass slides for antigen retrieval using citrate buffer [1/5].
• A streptavidin-biotin immunohistochemical technique (ABC) [1/6] was used to detect the monoclonal mouse anti-human cytokeratin 13 (NCL-CK13) clone KS-1A3. This monoclonal antibody reacts with the acidic intermediate filament protein (54kD) identified as cytokeratin 13 (CK13).
• Counterstaining was performed using Meyer’s haematoxylin for one minute.
• For the negative controls the primary antibody was omitted and goat antiserum antibody was substituted.

Staining assessment
• Cells were considered immunochemically positive for NCL-CK13 when distinct, reddish-brown, intracytoplasmic staining was identified. Staining was verified by an arbitrary, semiquantitative method assessing the percentage of positive cells in certain areas and cell groups.
  - 100% of cells negative
  + < 50% of cells positive
  ++ >50% of cells positive
  +++ 100% of cells positive
• The staining intensity in NCL-CK13 positive cases was categorized as follows:
  • Strong: densely stained reaction visible at low magnification (objective 5×).
  • Weak: faintly stained cytoplasmic reaction only visible using a higher magnification.

Results

Histological findings
H&E-stained sections of the fetus mandibles and maxillae showed the normal appearance of dental lamina and the enamel organ at the late bell stage.

The H&E-stained sections of the four CP cases showed anastamosing epithelial
islands in glial tissue. These islands showed a peripheral palisaded layer of columnar cells and a centre of stellate cells. Cystic degeneration was an extremely common finding. Focal calcification was almost invariably present, alternating with areas of ghost cells, and often found in both the epithelium and the glial tissue component. Some fields showed well differentiated papillary squamous epithelium.

The six AB cases were five conventional and one unicystic case. In all cases the neoplastic epithelium showed prominent tall columnar basal cells with palisaded nuclei and loose stellate reticulum-like cells, arranged in follicular and/or plexiform patterns. Areas of squamous metaplasia were evident in some cases, as well as areas of cystic degeneration.

The five COC cases showed an irregular epithelial lining with a well defined basal layer of columnar cells, an overlying layer that was many cells thick and which resembled stellate reticulum, and masses of ghost cells. The foci of calcification were shown to be dispersed and/or among the ghost cells.

Immunochemical results
The embryonic oral epithelium showed a positive reaction with NCL-CK13 present in the embryonic oral epithelium and the dental lamina, except at its advancing tip which showed a negative reaction. With progressive development (bell stage), there was an obvious, abrupt, negative reaction for NCL-CK13 in the enamel organ and odontogenic part of the dental lamina, while the reaction remained evident in the oral epithelium and the superficial part of the dental lamina (Figures 1 and 2). Remnants of the dental lamina showed an intense positive reaction for NCL-CK13 (Figure 3).

Variations in staining intensity were verified among cells of different locations as shown in Tables 1 and 2.

In CP and COC type specimens, a dense immunoreaction for NCL-CK13 was frequently detected, mainly among suprabasal cells and groups of the central non-stellate cells (Figures 4, 5, 6 and 7). Some follicles also showed enclosed non-reactive non-stellate cells (Figure 7). Occasional areas of basal cells lining cystic spaces showed faint staining for NCL-CK13 (Figure 7).

<table>
<thead>
<tr>
<th>Table 1 NCL-CK13 distribution during odontogenesis</th>
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<tbody>
<tr>
<td><strong>Normal embryonic epithelium</strong></td>
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<tr>
<td><em>Fetal oral epithelium</em></td>
</tr>
<tr>
<td>Basal</td>
</tr>
<tr>
<td>Parabasal</td>
</tr>
<tr>
<td><em>Dental lamina</em></td>
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<tr>
<td>Superficial part (oral part)</td>
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<tr>
<td>Deeper part (which will be differentiated into enamel organ)</td>
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<tr>
<td>Remnants of dental lamina</td>
</tr>
</tbody>
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- = 100% of cells negative
+ = 50% of cells positive
++ = >50% of cells positive
+++ = 100% of cells positive
Table 2 NCL-CK13 distribution among CP, AB and COC neoplastic cells

<table>
<thead>
<tr>
<th>Tumours</th>
<th>Peripheral cells</th>
<th>Central cells</th>
<th>Ghost cells</th>
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<tbody>
<tr>
<td>CP</td>
<td>+++</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>AB</td>
<td>+++</td>
<td>+</td>
<td>None</td>
</tr>
<tr>
<td>COC</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

- = 100% of cells negative  
+ = 50% of cells positive  
++ = >50% of cells positive  
+++ = 100% of cells positive  
CP = craniopharyngioma  
AB = ameloblastoma  
COC = calcifying odontogenic cyst

Discussion

Our study monitored the expression of the monoclonal antibody NCL-CK13 in fetal oral and odontogenic epithelia and in certain tumours of possible related origins: CP, AB, and COC.

In normal oral embryonic mucosa, varying amounts of strong reactivity were evident among the suprabasal layers. The location of this staining might be attributable to the expression of CK13 (and possibly its pair). Similar distribution has previously been reported as typical of non-keratinized, stratified, squamous epithelia [9].

Occasional focal detection of reactive cells among the basal cell layer was observed, although this has not been previously reported. This might mean that the early expression of CK13 can take place among old basal cells. This explanation is supported by a report on CK13 mRNA expression in the basal cells as well as in the stratum corneum [17]. This could be further attributed to the increased inherent sensitivity of the molecular biology technique used in this study.

Expression of CK13 was evident in the oral part of the dental lamina, (superficial part), as well as in fetal oral epithelium. Interestingly, there was an abrupt change towards the deeper part of the dental lamina with a marked reduction in staining intensity for CK13 and no staining in the enamel organ from the cap stage to the bell stage. A similar distribution was reported by Gao et al. [18] and Heikinheimo et al. [17], who found that the enamel organ proper at cap stages to bell stages remained negative for all stratification-related CK markers (CKs 1, 4, 10, 13), at both the mRNA and polypeptide levels throughout the development span studied. This indicates that the CK content of enamel organ is less complex.
Figure 1  **Bell stage of developing human tooth development showing positive immunoreactivity in oral epithelium and the orodental part of the dental lamina.** Negative reaction is obvious towards the enamel organ (NCL-CK13 immune stain, ABC, counterstained with H&E × 100)

Figure 2  **Higher magnification clarifying the abrupt decrease immunoreactivity in the orodental junction of the dental lamina** (NCL-CK13 immune stain, ABC, counterstained with H&E × 400)
Figure 3: Cells of remnants of the dental lamina showing mostly positive immunoreactivity. (NCL-CK13 immune stain, ABC, counterstained with H&E × 200)

Figure 4: CP with intensely stained peripheral cells. Two groups of centrally located cells with obvious difference in immunoreactivity. Ghost cells are faintly stained among the glial tissue. (NCL-CK13 immune stain, ABC, counterstained with H&E × 100)

Figure 5: CP demonstrating faint staining reaction in ghost cells with focal strong reactivity in flattened keratinocytes surrounding ghost cells. (NCL-CK13 immune stain, ABC, counterstained with H&E × 200)
Figure 6 COC showing positive immunoreactivity of the peripheral cells and to some extent the cells surrounding the ghost cells (NCL-CK13 immune stain, ABC, counterstained with H&E × 200)

Figure 7 Positive reactivity seen among all neoplastic basal cells and variably in the suprabasal stellate cells of COC (NCL-CK13 immune stain, ABC, counterstained with H&E × 200)
Figure 8 Mixed AB (follicular and plexiform) revealing strong positive immunoreactivity among the basal cells. The central stellate cells are of lesser intensity (NCL-CK13 immune stain, ABC, counterstained with H&E × 200)

Figure 9 Follicular AB showing strong cytoplasmic positivity among the palisaded peripheral cells. The well-formed stellate cells (left) show faint membranous and cytoplasmic reaction. The less organized stellate cells reveal evident staining (right) (NCL-CK13 immune stain, ABC, counterstained with H&E × 400)
than that of the dental lamina and overlying oral surface epithelium.

The strong positive expression of CK13 frequently encountered among the epithelial remnants of the dental lamina in this study might indicate their possible derivation from the epithelium of the oral part of the dental lamina, with their phenotypic characterization expressing CK13 preserved and might be influenced by inflammation [19]. This means that at least some of the epithelial remnants of the dental lamina are of the oral part.

Further support for this interpretation is provided by previous studies based on other techniques. At the late bell stage, when starting to disintegrate and form epithelial rests, a centrally located CK4 mRNA expression has been found, together with immunostaining patterns similar to CK1, 4 and 13 maps. This finding indicates that terminal differentiation has occurred in the dental lamina [17].

As with the embryonic oral mucosa and the oral part of the dental lamina, the tumours investigated also revealed obvious positive immunoreactivity to the CK13 marker. This might mean a possible phenotypic origin for these lesions from the embryonic remnants of the oral mucosa and dental lamina. Equally, the negative immunoreactivity of the epithelial cells forming the dental part of the dental lamina and the enamel organs provides support for excluding these structures from the evolution of the lesions in question.

Our explanation could be supported by previous reports concluding that the columnar cells in AB have no counterpart in the developing tooth [7,18]. Moreover, it has been previously shown that the tumour cells of AB failed to synthesize enamel matrix protein [19,20].

The immunoreactivity of stellate reticulum-like cells for CK13 in the specimens analysed indicates that these types of cells retain squamous differentiation. Previous immunohistochemical studies also provide additional support for the suggestion of a closer relationship between these tumours and the oral part of the dental lamina [5,19].

Weak staining for CK13 was seen in the ghost cells of CP and COC with focal staining in the CP specimens and diffuse, faint staining in the COC. These staining patterns differ from those of previous studies. This could be attributed to the different clones employed [21,22]. The weak staining could indicate degenerative changes with residual amounts of CK13 preserved. Further support for this has been demonstrated by electron microscopy, with the ghost cells of COC exhibiting numerous tonofilaments [23] that were not apparent immunohistochemically [21,22].

Various theories about the nature of ghost cells have been proposed without any general agreement. Our results contradict those who consider ghost cells as representing squamous metaplasia [10], but accord with those who suggest that ghost cells are the product of coagulative necrosis of epithelial cells [24]. This might explain the lack of positive findings among ghost cells while the surrounding epithelial cells were clearly positive.

The morphological similarity between the tumours investigated (CP, AB and COC), which originate in different connective tissues, needs greater additional explanation than their being derived from the same cell phenotype. It could be that the activated immune cells produce a variety of soluble mediators which, in addition to regulation of immune processes, can also influence the non-immune cell population and, in particular, stimulate epithelial proliferation and alter keratinization [25].

Cytokeratin expression may be affected by environmental factors and, to some ex-
tent, by the influence of the underlying connective tissue strata [26].

It can be concluded that CP, AB and COC are derived from epithelial remnants of the dental lamina, particularly that of the oral part (nearer to the oral mucosa). This is supported by various reports on pathological growth lesions mimicking odontogenic epithelia as far from the dental apparatus as the adamantinoma of the long bones [27] and peripheral ameloblastoma [28], peripheral odontogenic tumours [8], and some cysts [29]. Others have reported that AB are derived from odontogenic epithelium, although they could not identify the part of the odontogenic epithelium from which this odontogenic tumour is derived [7,17]. Additionally, the recurrent tendency of this neoplasm may support this interpretation [2,3].

Conclusions

The following conclusions can be drawn.

• Just beyond the basal cells of oral mucosa, the epithelia of the dental lamina cease expression of CK13.

• The epithelia of CP, COC and AB reveal an obvious phenotypic relation to the stratified epithelia of embryonic oral mucosa with regard to CK13-type expression.

• Ghost cells in CP might represent abnormal keratinization and degenerated confluent or expanded tumour cells.

• Neither columnar nor stellate reticulum-like cells in the tumours studied have a counterpart in the epithelium of the enamel organ proper.

• The areas of early-stage tooth development implicated in contributing to the development of tumours are the oral part of the dental lamina and its remnants.

Recommendations

The use of recent advances in molecular biology techniques, such as polymerase chain reactions and in situ transcription of mRNAs are advocated for the types of tumours studied here.

Using these techniques, it may be possible to test the molecular pathogenesis of these lesions (or others) and discover if they are located in the appropriate expression of specific genes related to a specific class and/or classes of cytokeratin or other polypeptides. This information would gently assist patient care.

References


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The participation of national health systems in the generation of new knowledge provides dual benefits: it quickens the overall pace of advance, and it shortens the time it takes for results to be translated into practice. Hence the importance, in planning the financing and organization of health systems, of ensuring an adequate research and development base.