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Immunohistochemical Localisation of Cytokeratins in Craniopharyngioma

M. Kurosaki[1], **W. Saeger**[2], and **D. K. Lüdecke**[3]

[1] Division of Neurosurgery, Institute of Neurological Sciences, Tottori University School of Medicine, Yonago, Tottori, Japan
[2] Institute of Pathology, Marienkrankenhaus, Hamburg, Germany
[3] Department of Neurosurgery, University Hospital Eppendorf, Hamburg, Germany

Summary

Background. Although craniopharyngiomas have been examined in several microscopical studies to date, immunohistochemical analysis has not been sufficient.

Method. In addition to the routine haematoxylin and eosin staining, 38 cases of intra- and/or supra-sellar craniopharyngioma, including 34 adamatinomatous and 4 squamous papillary types, were studied using immunohistochemistry for expression of four types of cytokeratin.

Findings. Histological examination found epithelial cells in 26 of 38 (68.4%) cases. However, cytokeratins were demonstrated in 35 of 38 (92.1%) cases. The remaining 3 cases without demonstration of epithelial cell nests were supposed to be adamantinomatous craniopharyngiomas based on the findings in the stroma. In 31 of 34 adamantinomatous craniopharyngioma cases, the epithelium was detected by immunostaining for cytokeratins. The epithlieum expressed 56 kDa (KL-1) and 40 kDa (cytokeratin 19) cytokeratins with similar staining patterns and intensities. The staining intensity of 54 kDa cytokeratin (cytokeratin 7) was similar to that of the high molecular weight cytokeratin (keratin M-903). However, in many cases (15 of 27), immunoreactivity of cytokeratin 7 was not demonstrated in an outer palisaded basal layer. In all 4 squamous papillary craniopharyngiomas, moderate staining with cytokeratin 7 appeared in the superficial layer, whereas basal or mid-zone epithelial cells were negative for cytokeratin 7. The basal layer stained negatively for KL-1, as well as cytokeratin 7.

Interpretation. Immunostaining for cytokeratin is valuable in the investigation of craniopharyngioma, especially when specimens contain only a small or questionable part of epithelium. Most notably, KL-1 or cytokeratin 7 stainings are suitable for analyzing these tumours, with special reference to histological subtypes.

Keywords: Craniopharyngioma; cytokeratin; immunohistochemistry; transsphenoidal surgery.

Introduction

Craniopharyngiomas are thought to be derived from nests of squamous epithelial cells [20]. There have been several microscopical studies on this type of tumour [2–4, 11, 14, 18, 19]. The most important finding is the existence of squamous epithelium, but in some cases histological detection of the epithelium may be difficult as only small fragments can be obtained during surgery. There have been a few studies of craniopharyngiomas using immunostaining for anti-cytokeratin antibodies [4, 14, 18, 23, 25]. However, the specimens used in these immunohistochemical studies were mostly large enough to contain the epithelium.

In the present study, at first, we examined the existence of epithelium morphologically in 38 craniopharyngiomas at the light microscopic level. As a further step, different types of cytokeratin were demonstrated by the immunoperoxidase technique to elucidate whether the immunohistochemical study was valuable or, to determine which type of cytokeratin is most useful in the diagnosis of craniopharyngioma. In addition, we investigated the characteristic differences in these tumours, with special reference to histological subtypes.

Patients and Methods

Thirty eight cases of craniopharyngioma were studied. The tumour samples were obtained mostly by transsphenoidal surgery at the University Hospital Eppendorf, Hamburg, Germany between 1979 and 1999. Seventeen patients were female and 21 were male, and ranged in age from 12 to 55 years [mean: 25.3 $\pm$ 12.1 yrs standard deviation (SD)]. All tumours were diagnosed and classified histologically.

Expression of cytokeratins was investigated by immunohistochemistry on formalin-fixed, paraffin-embedded tissue sections using avidin-biotin-peroxidase complex (ABC) method. After deparaffinization, endogenous peroxidase was blocked with 3% hydrogen peroxide (H_2O_2) for 30 minutes at room temperature. The sections were treated with 5% non-immune goat serum for 30 minutes at room temperature, and were incubated with the different antibodies summarized in Table 1 for overnight at 4 °C. Subsequently, the sections were incubated with biotinylated goat anti-mouse immunoglobulin and ABC reagents (Vector Lab., Burlingame, CA) for 30 minutes

Table 1. *Applied Antibodies and Methods*

Antibody	Reagent	Source	Dilution	Pretreatment
Anti-KL1	cytokeratin 56 kD (monoclonal)	Immunotech S.A. Marseile, France	1:100	
Anti-Cytokeratin 7	cytokeratin 54 kD (monoclonal)	Dako Glostrup, Denmark	1:50	Microwave
Anti-Cytokeratin 19	cytokeratin 40 kD (monoclonal)	Dako Glostrup, Denmark	1:50	Microwave
Anti-Cytokeratin (high molecular weight) (Keratin-903)	cytokeratins 68, 58, 56.5 and 50 kD (monoclonal)	Enzo New York, USA	1:1500	Microwave

and were reacted with 3-3' diaminobenzidine (DAB)-H_2O_2. The sections were counterstained with haematoxylin and, analysed by two authors (MK, WS). In cases with questionable staining, we repeated this immunohistochemical method. The cytokeratin staining patterns of each specimen were examined under the light microscope and graded in a scale ranging from (−) (negative staining) to (+++) (strongly positive staining).

Results

Microscopic subtypes of craniopharyngioma included 34 cases of adamantinomatous type and 4 cases of squamous papillary type. Histological examination revealed epithelial cells in 26 of 38 (68.4%) cases. The epithelium had an outer basal layer, a mid-zone of stellate epithelial cells, and a superficial layer. Some specimens contained only a small part of these layers. In the remaining 12 cases, the findings in the stroma were helpful for diagnosis (Fig. 1a). Cholesterol clefts, calcifications, foreign body giant cells, lymphocytes, hemosiderin-containing macrophages were frequently observed in the stroma.

The results of the immunoreactivity for the different types of cytokeratin investigated in craniopharyngiomas are summarised in Table 2. Cytokeratins were demonstrated in 35 of 38 (92.1%) cases in a pattern that was diffusely cytoplasmic. The intensities and numbers of immunoreactive cells varied with cases.

In 31 of 34 adamantinomatous craniopharyngioma cases, the epithelium was detected by immunostaining for cytokeratins (Fig. 1b). These 31 cases revealed immunoreactivity for 56 kDa (KL-1) and 40 kDa (cytokeratin 19) cytokeratins ranging from 50% to almost 100% of the epithelial cells with similar staining patterns and intensities (Fig. 2). The staining intensity of the 54 kDa cytokeratin (cytokeratin 7) was similar to that of the high molecular weight cytokeratin (keratin M-903). However, the distribution of cytokeratin 7 and keratin M-903 differed among the epithelial cells. In the outer palisaded basal layer, cytokeratin 7 was not expressed in 15 cases, or stained weakly in 10 cases.

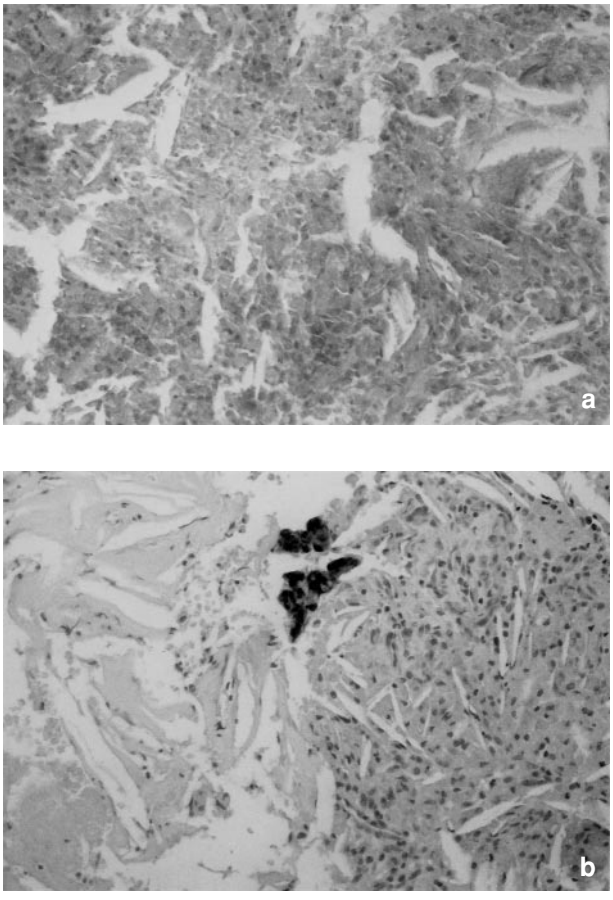

Fig. 1(a) This specimen includes a small part of epithelium, which was difficult to detect morphologically (Case 23). H&E Staining. Original magnification ×200. (b) Cytokeratin (KL-1) staining is confined to the epithelial cells. Counterstained with haematoxylin (Case 23). Original magnification ×200

Table 2. *Summary of Immunohistochemical Analysis for 4 Various Cytolkeratins in 38 Craniopharyngiomas*

Case no.	Age (years)	Sex	Epithelium H&E	KL-1			Cytokeratin 7			Cytokeratin 19			Keratin M-903		
				Basal	Mid	Super	Basal	Mid	Super	Basal	Mid	Super	Basal	Mid	Super
Adamantinomatous type															
1	13	F	yes	+++	++	+++	−	+	+	+++	+	+++	+	+	++
2	36	M	no	N.E	N.E	++	N.E	N.E	+	N.E	N.E	+++	N.E	N.E	+++
3	32	M	yes	+	+++	++	−	++	++	+++	+++	+++	+	++	++
4	28	F	no	N.E	+++	N.E	N.E	++	N.E	N.E	+++	N.E	N.E	−	N.E
5	19	M	yes	++	+++	N.E	−	++	N.E	+	+	N.E	+	+	N.E
6	12	M	yes	N.E	N.E	+	N.E	N.E	−	N.E	N.E	−	N.E	N.E	−
7	29	F	no	N.E	N.E	N.E	N.E	N.E	N.E	N.E	N.E	N.E	N.E	N.E	N.E
8	22	M	no	+++	+++	++	+	++	+	+++	+++	++	++	++	++
9	55	F	yes	−	+	+++	−	+	+	++	++	+++	−	−	++
10	29	M	yes	+++	+++	++	−	++	+	+	++	++	++	++	+
11	14	M	yes	+++	+++	+++	−	++	+	+++	+++	++	++	+	++
12	15	M	yes	++	++	++	+	++	+++	+++	+++	+++	+	+	+
13	27	M	no	++	+++	N.E	−	++	N.E	N.E	N.E	N.E	N.E	++	N.E
14	15	M	yes	N.E	N.E	N.E	N.E	N.E	N.E	+++	+++	+++	++	++	−
15	18	M	yes	+++	++	+++	−	+	++	+++	++	+++	−	−	−
16	17	M	yes	−	++	+++	−	−	+	+	+	+++	−	−	++
17	33	F	yes	++	+++	++	−	++	+	+++	+++	++	++	+++	+
18	24	F	no	+++	+++	++	−	++	N.E.	+++	+++	++	+	+	N.E
19	31	F	yes	+++	+++	++	+	++	++	+	++	+++	−	+	+
20	13	M	no	N.E	N.E	N.E	N.E	N.E	N.E	N.E	N.E	N.E	N.E	N.E	N.E
21	21	M	no	+++	+++	N.E	+	++	N.E	+++	+++	N.E	−	−	N.E
22	13	M	yes	++	+++	+++	−	++	++	+	++	+++	+	+	+
23	12	F	no	+++	++	N.E	−	++	N.E	++	++	N.E	−	−	N.E
24	21	M	no	+++	+++	N.E	+	++	N.E	+++	+++	N.E	+	+	N.E
25	47	F	yes	+++	+++	++	+	++	++	+++	+++	++	++	++	++
26	15	M	yes	++	++	++	+	++	+	++	++	++	+	+	−
27	13	F	yes	++	++	++	+	++	++	+++	+++	+++	++	++	++
28	22	F	no	+++	+++	N.E	+	+	N.E	+++	+++	N.E	−	−	N.E
29	34	M	yes	+++	++	+++	+	++	++	+++	+++	+++	+	−	+
30	16	F	yes	+	++	+++	−	++	++	+++	+++	+++	+	+	++
31	14	M	yes	++	++	++	++	++	++	++	++	++	++	++	++
32	18	F	no	N.E	N.E	N.E	N.E	N.E	N.E	N.E	N.E	N.E	N.E	N.E	N.E
33	36	M	yes	+	+++	++	−	+++	+	++	+++	+++	+	++	+++
34	18	M	yes	+++	+++	++	++	++	++	+++	+++	+++	++	++	+
Squamous papillary type															
35	43	F	yes	−	+	++	−	−	+	+	+++	+++	+	++	++
36	38	F	yes	−	++	+++	−	−	++	++	+++	+++	+	++	+++
37	45	F	yes	−	+	+++	−	−	++	++	++	+++	++	++	+++
38	53	F	yes	−	++	+++	−	−	++	+	++	+++	−	+	++

M Male; *F* Female; *yes* Epithelium was detected.; *no* Epithelium is not detected.; *basal* outer basal layer; *mid* mid-zone of stellate epithelial cells; *super* (keratinizing) superficial layer. Staining results were recorded by assessing the percentage of positive cells and intensity of staining. The intensity of immunostaining was graded as: − negative, + weakly positive, ++ moderately positive, +++ strongly positive. *N.E* No existence of the epithelial cells.

In all 4 squamous papillary craniopahryngiomas, moderate staining with cytokeratin 7 appeared specifically in the superficial layer, whereas basal or mid-zone epithelial cells were negative for cytokeratin 7 (Fig. 3a). The basal layer revealed negative immunoreactivity for KL-1, as well as cytokeratin 7. Both cytokeratin 19 and keratin M-903 were demonstrated in all layers of tumour tissue with a diffusely cytoplasmic pattern (Fig. 3b).

Discussion

Indeed craniopharyngiomas usually arise in the suprasellar area, but approximately one-third of all these tumours reported in the literature involve the pituitary fossa [1, 6, 12, 15]. A transsphenoidal operation is the available procedure for the intrasellar region [1, 6, 12, 15]. In some cases, however, subtotal tumour removal has been performed to avoid further damage to the

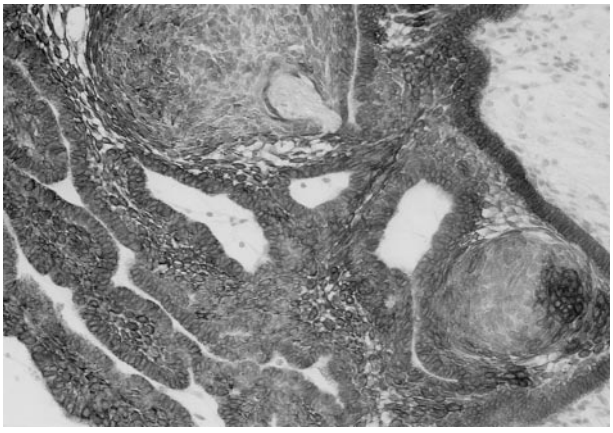

Fig. 2. Adamantinomatous craniopharyngioma of case 30. Immunoreactivity with anti-cytokeratin 19 is seen in the basal, intermediate, and keratinizing superficial layer with a diffuse cytoplasmic pattern. Counterstained with haematoxylin. Original magnification ×200

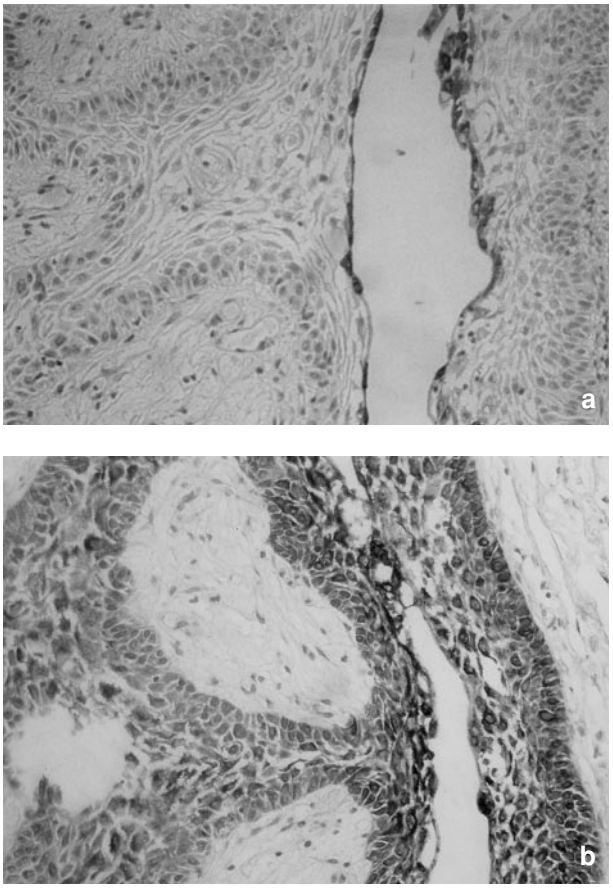

Fig. 3. Squamous papillary craniopharyngioma of case 38. Counterstained with haematoxylin. Original magnification ×200. (a) Immunoreactivity with anti-cytokeratin 7 is seen exclusively in the superficial layer. (b) Almost all epithelial cells are strongly positive for cytokeratin 19

pituitary gland [1, 12]. In the other cases, only small specimen may be obtained during surgery because of tumours with a mostly cystic component [19, 23]. Craniopharyngioma may be difficult to diagnose, especially when the epithelial structure is lacking. In the series of 131 craniopharyngiomas published by Szeifert *et al.* [23], 21% of the surgically removed samples did not contain enough material for correct histopathological classification.

In our series, we could not find the existence of epithelium histologically in 12 of 38 (31.6%) specimens using the routine haematoxylin and eosin staining. Most significantly, it was difficult to detect the small portion of epithelial cells in the granulation tissue of adamantinomatous craniopharyngioma (Fig. 1). However, cytokeratins were demonstrated in 9 out of these 12 cases. In such cases, the immunohistochemical identification of intracellular cytokeratins was, therefore, a valuable diagnostic tool in detecting the epithelium of craniopharyngioma. The remaining 3 cases were supposed to be adamantinomaotus craniopharyngiomas without demonstration of epithelial cell nests.

So far, 20 human cytokeratins have been catalogued by two-dimensional gel electrophoresis [16]. The detailed cytokeratin composition of the epithelium varies depending on the cell type, cellular growth environment, and changes during the course of differentiation and transformation [5, 7, 8, 13, 16–18, 21, 22, 24]. There have been a few immunohistochemical studies of craniopharyngiomas using anti-cytokeratin antibodies [4, 14, 18, 23, 25]. Our results showed that immunostaining of cytokeratins differed in each layer and the number or pattern of immunostained cells varied from one craniopharyngioma to another.

Even though two subtypes of craniopharyngioma have been recognised, these tumours frequently refute subclassification [9, 10]. Sixteen craniopharyngiomas were examined using immunohistochemistry for expression of simple-, stratified-, and skin-type cytokeratins by Uematsu *et al.* [25]. The authors emphasised that the pattern of expression was different between squamous papillary and adamantinomatous types. Our results confirmed this finding. In our series, the number of squamous papillary craniopharyngioma cases was small because many children's cases and intrasellar tumours were included. However, the distribution of KL-1 and cytokeratin 7 in the epithelium were definitely different in the two subtypes. Both KL-1 and cytokeratin 7 were demonstrated in all layers of

tumour tissue in the adamantinomatous type. On the contrary, in the squamous papillary type, cytokeratin 7 appeared specifically in the superficial layer and KL-1 revealed positive in the superficial and mid-zone epithelial cells with the exception of basal layer cells. These findings appeared to be due to a histogenetic difference between adamantinomatous and squamous papillary types of tumour. Furthermore, immunostaining for KL-1 or cytokeratin 7 can be used as a valuable method to distinguish the two types of craniopharyngioma.

Conclusions

Our observations suggest that the immunohistochemical identification of intracellular cytokeratins can be a valuable diagnostic tool in the surgical pathology of craniopharyngioma, especially when specimens contain only a small or questionable amount of epithelium. Specially, KL-1 or cytokeratin 7 staining is suitable for analysing these tumours, with special reference to histological subtypes.

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Correspondence: Masamichi Kurosaki, Division of Neurosurgery, Institute of Neurological Sciences, Tottori University School of Medicine, Yonago, Tottori, 683-8504 Japan.