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The relationship between nuclear DNA content in salivary gland tumors and prognosis* **

Comparison of mucoepidermoid tumors and acinic cell tumors

K. Hamper[1], J. Caselitz[1], H. Arps[1], U. Askensten[2], G. Auer[2], and G. Seifert[1]

[1]Institute of Pathology, University Hospital Hamburg-Eppendorf, Hamburg, Federal Republic of Germany
[2]Department of Pathology, Karolinska Institute and Hospital, Stockholm, Sweden

Summary. Differences in prognosis between salivary gland mucoepidermoid tumors and acinic cell tumors were compared by means of conventional histopathological grading and nuclear DNA content which was assessed cytochemically by a scanning cytophotometric procedure. The mucoepidermoid tumors were found to show a stronger correlation between histopathological grading and prognosis than did the acinic cell tumors. By using DNA quantification, valuable additional information could be obtained for predicting the behavior of the mucoepidermoid tumors, whereas there was no correlation between DNA content and prognosis for the acinic cell tumors. Regarding the relatively "benign" clinical course of most mucoepidermoid tumors, the term "tumor" – as proposed by the World Health Organization's classification – seems appropriate. In contrast, the more severe clinical courses of the acinic cell tumors justify the use of the term "carcinoma" instead.

Key words: Salivary gland tumors – DNA assessment – Prognosis – Acinic cell tumor – Mucoepidermoid tumor

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** Dedicated to Jean "Papa" Chick of Meru, France, with affection

Offprint requests to: K. Hamper, Institute of Pathology, University of Hamburg, Martinistrasse 52, D-2000 Hamburg 20, Federal Republic of Germany

Introduction

Salivary gland tumors are relatively uncommon neoplasms that can cause substantial problems in predicting their clinical prognosis. Besides definitely benign tumors like the pleomorphic adenoma and definitely malignant tumors like the adenocarcinoma, there are certain distinct histological tumor entities, in which prognosis is not strictly bound to histology [30]. This tumor group consists of mucoepidermoid tumors and acinic cell tumors. The World Health Organization (WHO) lists these neoplasms in an intermediate group between "carcinomas" and "adenomas" in order to express their potentially malignant behavior [31]. This listing is enforced by the use of the terms "tumor" and not "carcinoma" in this classification. In Western Europe, the term acinic cell "carcinoma" is widely used, while mucoepidermoid "tumor" still is more common. In the literature of the last few decades, contradictory opinions have been given regarding the prognosis of the two groups, but data have accumulated indicating that these tumors more than occasionally show an unfavorable clinical course [1, 3–5, 7, 8, 10, 14, 16, 17, 19, 20, 23, 24, 27–29]. Until now, however, uncertainty still exists as to the prognostic classification of these tumors.

Cytophotometry is a laboratory study that has proven useful providing additional information as to the expectable clinical course of malignant tumors [2, 6, 25]. Cytochemical DNA assessment of tumor cell nuclei yields histograms that can be divided into

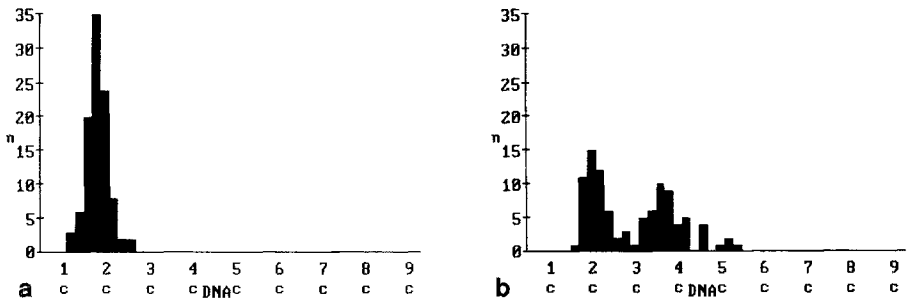

Fig. 1a, b. Examples of histograms. **a** "Diploid" histogram with peak in the 2c region. **b** "atypical" histogram with flattening of the 2c peak and a spread to the right

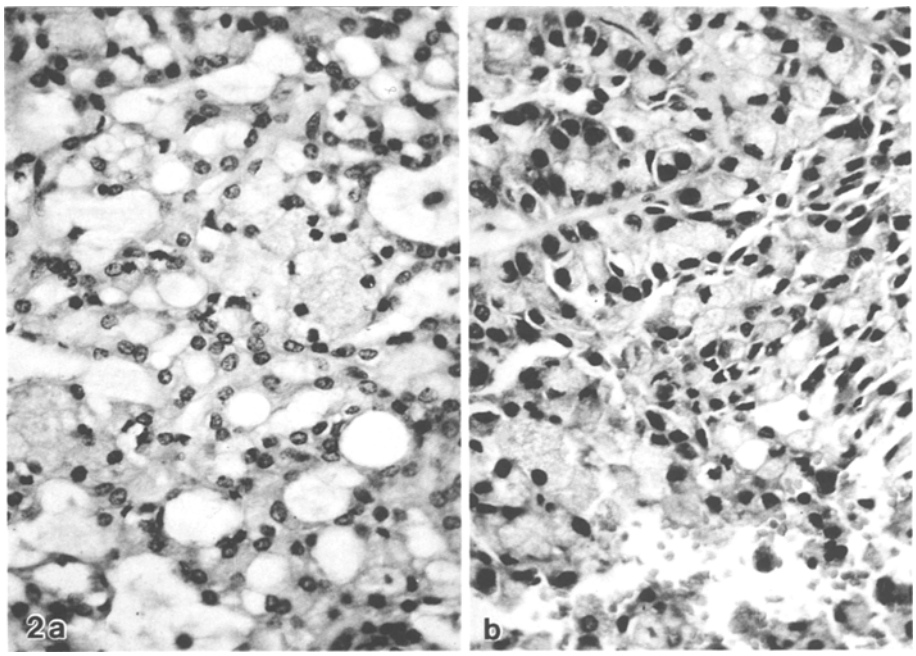

Fig. 2a, b. Acinic cell tumor. Hematoxylin and eosin stain, ×230. **a** Highly differentiated tumor, diploid histogram; death of patient due to tumor 56 months after primary diagnosis. **b** Poorly differentiated tumor, atypical histogram; no recurrence

"diploid", similar to the DNA distribution in normal non-neoplastic tissues, and "atypical" or "aneuploid" (Fig. 1). Diploid histograms can in general be related to a good prognosis, while prognosis of cases with an "atypical" histogram is usually poor.

Salivary gland tumors have only rarely been evaluated cytophotometrically [11–13, 15, 18, 21, 22]. Nevertheless, because of the possible therapeutical consequences, it is extremely desirable for the clinicians to obtain additional information on the prognosis of these tumors for the selection of an adequate therapy. In order to judge the value of cytophotometry in evaluating salivary gland tumors, it is important to compare all histograms with long-term clinical follow-ups. We have therefore used the case material of the Salivary Gland Registry at the Institute of Pathology, University of Hamburg, to establish prognostic criteria for salivary gland tumors of questionable biological behavior.

Material and methods

Out of more than 12,000 specimens of salivary gland disease collected from 1965 to 1987, 106 mucoepidermoid tumors and 55 acinic cell tumors from the years 1965–1980 were chosen for the present study. A histopathological grading was performed on each tumor as described by Seifert et al. [26]. The acinic cell tumors were graded into two groups (high differentiation and poor differentiation; Fig. 2), according to cellular anaplasia and the mitotic rate. In contrast, the mucoepidermoid tumors were graded into three groups (high differentiation = grade 1; intermediate differentiation = grade 2; poor differentiation = grade 3; Fig. 3), according to the ratio of solid/epidermoid to cytic/mucous stuctures. Each patient's postoperative course was assessed by questionnaires which were sent to those pathologists and clinicians who had contributed cases to the registry. The clinical course was separated into two groups: i.e., a "favorable" one with no tumor manifestation besides the primary neoplasm and an "unfavorable" one, with metastases, recurrences or deaths from tumor.

The method of cytophotometry is based upon the measurement of nuclear DNA content in histological sections which

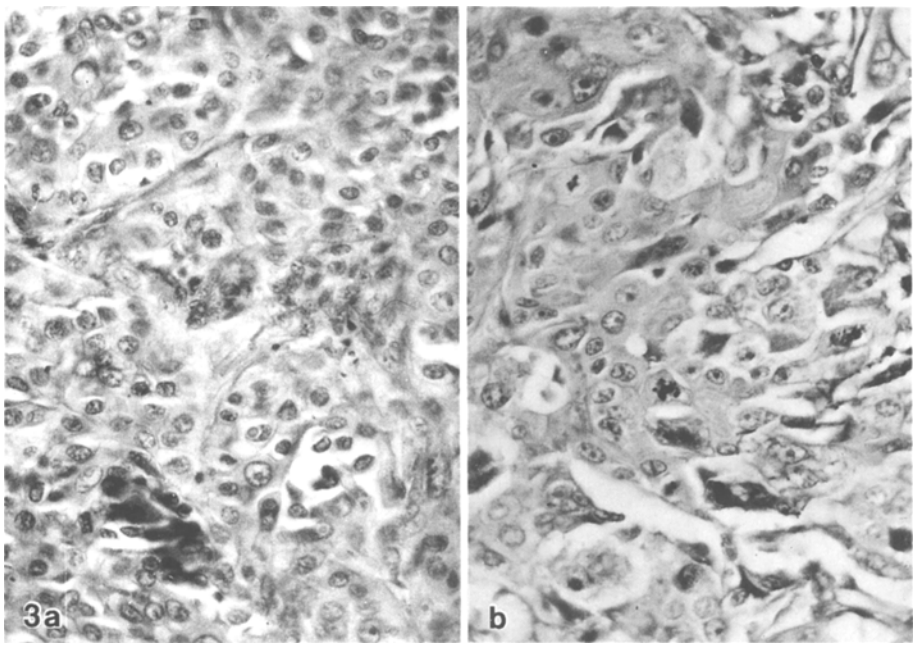

Fig. 3a, b. Mucoepidermoid tumor. PAS stain, × 230. **a** Poorly differentiated tumor, diploid histogram; no recurrence. **b** Poorly differentiated tumor, atypical histogram; death of patient due to tumor 15 months after primary diagnosis

have been stained by Schiff's reagent after acid hydrolysis. The measurement is performed under a microscope linked to a photometric device which allows measurement of single tumor cell nuclei. For cytophotometry, 8-μm-thick paraffin sections are cut and stained according to a method described previously [9]. In the present study, DNA assessments were performed in 46 cases of mucoepidermoid tumors and 35 cases of acinic cell tumors. The applied device was a "Leitz MPV Compact Cytophotometer" linked to a "Eurocos" Computer. One hundred tumor cell nuclei were measured in each case. The histograms produced by the computer program were interpreted graphically.

Table 1. Acinic cell tumors: tumor grade and clinical course

Tumor differentiation	High		Low		Total	
	n	%	*n*	%	*n*	%
Clinical course						
Favorable	13	(46.4)	2	(16.7)	15	(37.5)
Unfavorable	15	(53.6)	10	(83.3)	25	(62.5)
Total	28		12		40	

Table 2. Mucoepidermoid tumors: tumor grade and clinical course

Tumor grade	1		2		3		Total	
	n	%	*n*	%	*n*	%	*n*	%
Clinical course								
Favorable	24	(77.4)	9	(56.3)	9	(37.5)	42	(59.2)
Unfavorable	7	(22.6)	7	(43.7)	15	(62.5)	29	(40.8)
Total	31		16		24		71	

Results

The clinical courses of the patients are shown in Tables 1 and 2. Tumor differentiation showed a correlation to the clinical courses for the mucoepidermoid tumors (more cases with favorable prognose in the highly differentiated tumors and more cases with unfavorable prognoses in the poorly differentiated tumors.) This correlation occurred to a lesser degree for the acinic cell tumors (more cases with unfavorable prognoses in both the highly and poorly differentiated tumors). Tumor size or location were not related to prognosis.

Out of 35 histograms for acinic cell tumors, 34 were "diploid" and only 1 was "atypical" and no correlation to prognosis could be demonstrated. The only patient with an atypical histogram died of causes unrelated to his tumor, without evidence for any tumor recurrence 193 months after primary diagnosis. Two examples for acinic cell tumors are shown in Fig. 2.

In 46 mucoepidermoid tumors, 32 cases were "diploid" and 14 were "atypical". There was a highly significant statistical correlation ($P < 0.001$) with the clinical course (21 favorable versus 9 unfavorable courses in the diploid group; 12 unfavourable versus 1 favorable course in the atypical group). Two examples for mucoepidermoid tumors are shown in Fig. 3.

Discussion

The WHO classification of salivary gland tumors includes two tumor groups with biological behaviors dif-

Table 3. Mucoepidermoid tumors: prognostic accuracy of histological grading in combination with histogram (compare Table 2)

		Diploid		Atypical	
Grade 1	Favorable	12	(80.0%)	0	
	Unfavorable	3	(20.0%)	2	(100%)
Grade 2	Favorable	4	(57.2%)	0	
	Unfavorable	3	(42.8%)	2	(100%)
Grade 3	Favorable	5	(62.5%)	1	(11.1%)
	Unfavorable	3	(37.5%)	8	(88.9%)

ficult to predict: namely, acinic cell tumors and mucoepidermoid tumors. In the past, these tumors were generally considered to be benign or semi-malignant, but are relatively uncommon in clinical paractice [31]. Because of this, a clinical follow-up of larger collections of cases is difficult to perform. Although the histological diagnosis of these neoplasms should be of no problem to the experienced surgical pathologist, larger collections of these tumors are necessary in order to evaluate possible prognostic factors.

For mucoepidermoid tumors, our findings demonstrated that differentiation was of importance for prognosis. In all, 77.4% of the highly differentiated tumors showed a favorable clinical course, whereas 62.5% of the poorly differentiated tumors showed an unfavorable course. This was true, but to a lesser degree to the acinic cell tumors as well. Here, in both groups of differentiation, a preponderance of unfavorable courses was found (53.6% in the highly differentiated forms and 83.3% in the poorly differentiated forms).

As to the exceptions in prognosis, e.g., an unfavorable prognosis in some highly differentiated cases and a favorable prognosis in some poorly differentiated cases, it would be desirable to define other criteria that are related to prognosis. For acinic cell tumors, cytophotometry gives no additional information. Consequently, besides histological differentiation, other factors like clinical stage and facial nerve involvement (or palsy) have to be taken into account. In contrast, for mucoepidermoid tumors valuable additional information can be obtained by cytochemical DNA assessment. The higher level of accuracy in predicting the prognosis of these latter tumors by cytophotometry in combination with histological differentiation is shown in Table 3. The atypical histograms can especially identify those cases with an almost obligatory poor prognosis so that these can be managed by more aggressive treatment and clinical follow-up.

In conclusion, the prognosis of both acinic cell and mucoepidermoid tumor groups are difficult to evaluate. As mucoepidermoid tumors show a by far higher ratio of favorable to unfavorable courses (59.2% versus 40.8%) and only 12.7% of the patients in our collection actually died from their tumors, the term mucoepidermoid "tumor" as proposed by the WHO seems appropriate. In contrast, the term acinic cell "tumor" should be changed to acinic cell "carcinoma", as the ratio of favorable to unfavorable courses is by far smaller (37.5% versus 62.5%), and 30% of these patients finally died because of their tumors.

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