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Papers

Cell Kinetics of Pleomorphic Adenomas of the Parotid Gland

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The aim of the present study is to characterise the cell kinetics of pleomorphic adenoma of the parotid gland by assessing DNA content and proliferating cell nuclear antigen (PCNA) positivity. In 22 parotid adenomas, DNA content was measured by densitometry in histological serial sections stained with Feulgen's method and PCNA positivity was determined by immunohistochemistry with the monoclonal antibody PC10. To assess the proliferative activity, DNA index and PCNA index were evaluated. It was possible to distinguish two types of adenoma. In Group I there was a prevalence of diploid cells with a low PCNA index. Group II is represented by adenomas with a large percentage of triploid cells and a PCNA index significantly higher than that of Group I. Our findings suggest that the possibility of recurrence or malignant transformation depends on intrinsic biological properties of each adenoma. Copyright © 1996 Elsevier Science Ltd

Keywords: cell kinetics, DNA content, PCNA index, pleomorphic adenoma, parotid gland

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INTRODUCTION

The pleomorphic adenoma is the most common tumour of the parotid gland. It is a benign lesion characterised by histomorphologic heterogeneity, being made up of an epithelial component and a more or less abundant stroma which may be mucoid, myxoid or chondroid and is the product of the altered metabolism of the epithelial cells. On the basis of cell differentiation and amount and nature of the stroma, Seifert *et al.* [1] have proposed a subdivision of pleomorphic adenomas into four groups. However, irrespective of the histological aspect, local recurrence occurs in 10–12% of parotid adenomas and malignant transformation is not uncommon [2, 3].

Cytogenetic studies on this type of tumour have revealed different clonal chromosome anomalies and it has been hypothesised that these alterations may cause variations in proliferative activity [4–9].

So far, however, the cell kinetics of pleomorphic adenoma of the parotid gland have been poorly investigated [10, 11]. The aim of the present study, therefore, was to characterise the proliferative capacity of this tumour by assessing DNA content and labelling index with proliferating cell nuclear antigen (PCNA) of the epithelial component, and in particular to determine the percentage of cells in the S phase, which many studies have shown to be closely correlated to the biological behaviour and aggressiveness in various types of neoplasias [12–16].

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MATERIALS AND METHODS

Twenty-two parotid adenomas, selected from 1989 to 1991, were investigated. In 19 of these, histological examination showed the tissue polymorphism typical of pleomorphic adenoma. The other 3 cases were diagnosed as "carcinoma in pleomorphic adenoma" (malignant mixed tumour), on the basis of histological observation showing the coexistence of carcinoma and a component having the typical appearance of pleomorphic adenoma [17].

In all the adenomas (19 cases), and in the adenomatous areas adjacent to the carcinomas (3 cases), DNA content was measured by densitometry and PCNA positivity was determined by immunohistochemistry in serial sections. Since the aim of this study was to assess proliferative activity only in benign tumours, the carcinomas were not investigated.

Immunohistochemical staining for PCNA

Paraffin sections of 4-μm-thick from the same blocks used for histological diagnosis were air-dried overnight at room temperature and immunostained with the monoclonal antibody PC10 at dilution of 1:200, using an immunoperoxidase method (ABC complex) with light haematoxylin counterstaining. All immunostained sections were examined using a × 100

Table 1. Histology, DNA and PCNA index status for all cases

Case No.	Histologic type	DNA index	Mean value	PCNA index	Mean value
1	Pleomorphic adenoma	0.99		3.7	
2	Pleomorphic adenoma	1.07		14.2	
3	Pleomorphic adenoma	1.00		8.0	
4	Pleomorphic adenoma	1.00		8.2	
5	Pleomorphic adenoma	1.06		14.8	
6	Pleomorphic adenoma	1.05		11.3	
7	Pleomorphic adenoma	1.02	1.04	7.3	10.43
8	Pleomorphic adenoma	0.97		4.1	
9	Pleomorphic adenoma	1.03		12.4	
10	Pleomorphic adenoma	1.11		15.0	
11	Pleomorphic adenoma	1.01		6.8	
12	Pleomorphic adenoma	1.10		15.0	
13	Pleomorphic adenoma	1.12		14.8	
14	Pleomorphic adenoma	1.45		36.8	
15	Pleomorphic adenoma	1.47		35.4	
16	Pleomorphic adenoma	1.5		41.0	
17	Pleomorphic adenoma	1.62		42.7	
18	Pleomorphic adenoma	1.56	1.54	38.7	42.08
19	Pleomorphic adenoma	1.6		53.9	
20	Carcinoma in pleomorphic adenoma	1.65		50.9	
21	Carcinoma in pleomorphic adenoma	1.48		37.5	
22	Carcinoma in pleomorphic adenoma	1.52		41.8	

objective. A minimum of 1000 cells in random fields was counted in every case.

The PCNA index was defined as the number of cells with strong unequivocal nuclear staining, corresponding to cells in S phase, divided by the total number of cells counted, expressed as a percentage.

Nuclear cytometry (densitometry)

DNA content was measured with a Zeiss VIDAS image analyser. The integrated optical density of the parotid nuclei was estimated on 5-μm-thick histological sections and then correlated with the density of the control diploid nuclei (tissue lymphocytes). This correlation, defined as the DNA index, is equal to 1.00 when the tissue under investigation is made up of a cell population with a normal 2C DNA content (diploid), and equal to 1.5 when a marked percentage of 3C (triploid) cells is present (S phase).

For staining we used Feulgen's method, based on the interaction between Schiff's reagent and the aldehyde groups of the deoxyribose molecules, previously unmasked by acid hydrolysis (5 N HCl at 22°C for 60 min) which removes the purinic bases.

In each section we examined 200–250 adenoma epithelial cell nuclei and 50 lymphocytes as control. Nuclei that appeared to be overlapping or not clearly defined were excluded from the study.

Statistical analysis

The difference between the mean DNA and PCNA index values in the two tumour groups observed was calculated by variance analysis (F-test). The linear correlation between the two proliferative indices was determined using the Spearman rank test. The selected level of significance was $P<0.05$.

RESULTS

Table 1 shows the results of the cytometric and immunohistochemical analyses.

The histograms obtained with the image analyser show for each case different percentages of cells with 2C, 3C and 4C DNA content and, consequently, different DNA index values (range = 0.97–1.65; mean value = 1.24). Cells with above-tetraploidy values (>4C) were not found.

The PCNA index showed considerable variability, with values ranging from 3.7% to 53.9% (mean value = 23.38%).

Comparative evaluation of the results obtained by the two approaches revealed a close correspondence. The linear correlation between the two proliferative indices (DNA index and PCNA index) was significant, with a correlation coefficient $r=0.974$ ($P=0.001$) (Fig. 1). Moreover, it was possible to distinguish two types of adenoma on the basis of the results.

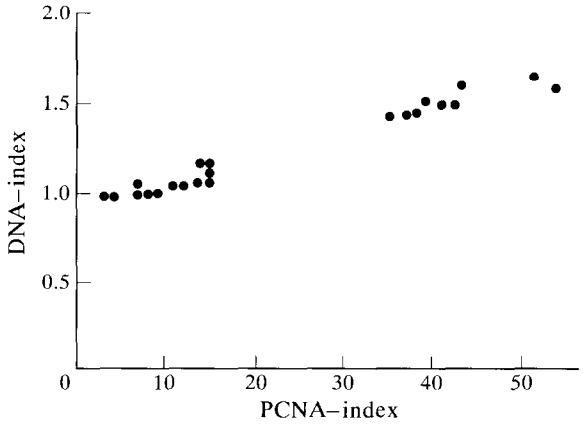

Fig. 1. Linear correlation between DNA index and PCNA index (Spearman's rank test).

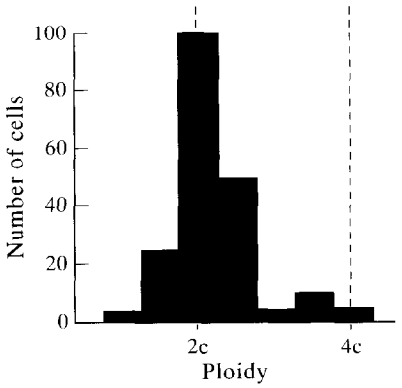

Fig. 2. Histogram showing representative DNA content in Group I adenomas (prevalence of 2C cells).

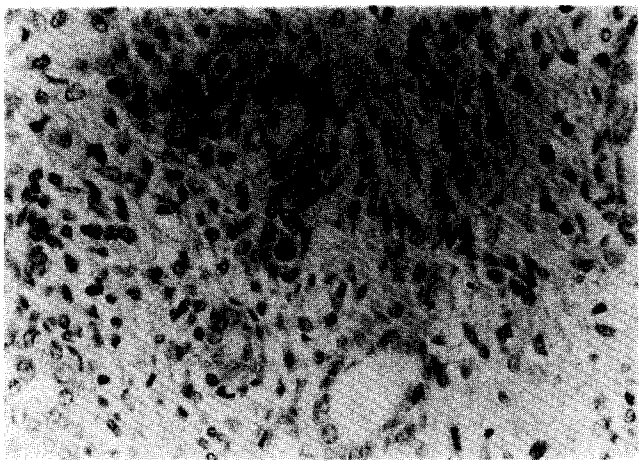

Fig. 3. Low PCNA index value in Group I adenoma section (× 500). Arrow shows a PCNA strong-positive nucleus (phase S-cells).

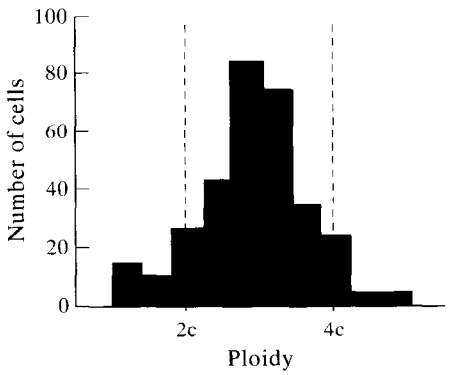

Fig. 4. Histogram showing representative DNA content in Group II adenomas (prevalence of 3C cells).

In Group I (cases 1–13) there was a prevalence of cells with a diploid DNA content (Fig. 2), a mean DNA index of 1.04 and a percentage of PCNA-positive cells ranging from 3.7 to 15% (mean value = 10.43%) (Fig. 3). Group II (cases 14–22) was made up of adenomas with a high proliferative activity, represented by a large percentage of DNA triploid cells (Fig. 4) and, therefore, a mean DNA-index value equal to 1.54 and a

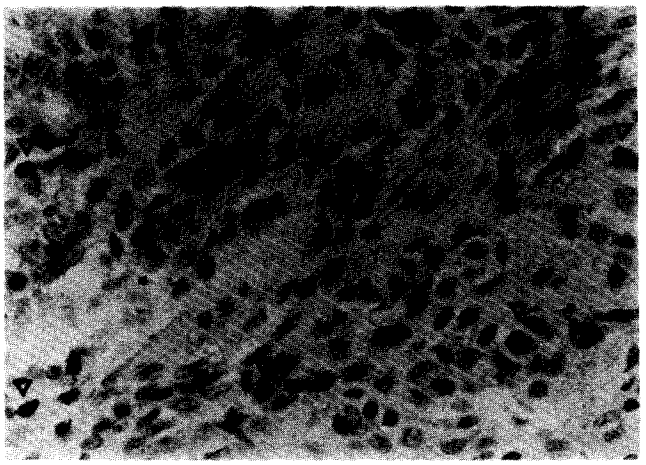

Fig. 5. High PCNA-index value in Group II adenoma section (× 500). Arrow shows PCNA strong-positive nuclei (phase S-cells).

PCNA index significantly higher than that of Group I, ranging from 35.4% to 53.9% (mean value = 42.08%) (Fig. 5).

The differences between the mean DNA and PCNA index values in the two tumour groups were statistically significant ($F = 196.81$; $P = 0.0001$).

DISCUSSION

In recent years, thanks to the availability of new biotechnologies, many studies have been performed in both benign and malignant tumours to assess the prognostic significance of such biological parameters as ploidy, cell growth factor, chromosome pattern and prolifertion antigens [12–16].

In particular, pleomorphic adenomas of the parotid gland have been the object of a number of karyotype studies, due to the fact that unlike other solid tumours, this type of tumour lends itself to cytogenetic analysis and provides analysable metaphases in a high percentage of cases. The results obtained by various authors have made it possible to identify both normal karyotypes and repetitive clonal chromosome anomalies. Bullerdiek *et al.* [6] have proposed the subdivision of these tumours into three groups on the basis of cytogenetic findings, as follows: (a) normal karyotype; (b) structural rearrangements involving chromosome 8, with a constant breakpoint on the long arm at band q12; and (c) structural rearrangements involving chromosome 12, with breakpoints at bands q14–15.

Mark *et al.* [8] have identified a further group of adenomas with altertions in the p21 region of chromosome 3. According to Mark *et al.* these alterations are secondary to a normal karyotype, resulting from a clonal evolution during tumour growth. Bullerdiek, on the contrary, hypothesises that the various subgroups are aetiologically distinct entities and that the different chromosome patterns are already present at initiation of the adenoma.

All authors agree, however, that the various karyotypes observed correspond to different biological characteristics and especially to a variability in proliferative capacity. However, no correlation has been found in any study between the presence or absence of specific chromosomal alterations and subsequent tumour recurrence or malignant transformation [18].

Nevertheless, as yet the variability in growth pattern of the

pleomorphic adenoma of the parotid gland has not been analysed thoroughly. Indeed, the few studies in the literature have evaluated only the differences of PCNA immunoreactivity between benign and malignant salivary gland tumours [10, 11].

On the basis of the findings of our study on DNA content and PCNA index, we were able to identify two groups of parotid pleomorphic adenomas which, although histologically superimposable, were distinguished by a different proliferative cell profile. In Group I the percentage of cells which, according to densitometric examination, showed a DNA content between 2C and 4C was significantly lower than that observed in Group II. These data are corroborated by the fact that strong PCNA positivity, which corresponds to the DNA replication phase, was also lower.

PCNA expression is closely linked to the cell cycle [14, 19, 20]. Starting from the late G1 phase it increases progressively to reach maximum intensity in the S phase. In the subsequent G2 and M phases, PCNA positivity decreases, reaching intermediate values between those of the G1 and M phases.

Thus Group II includes adenomas with a greater frequency of cells in the S phase, as shown both by the DNA content and by the percentage of PCNA-positive cells, that is, with a higher proliferative capacity. Though these tumours are histologically classified as benign lesions they are actually very aggressive and, therefore, prone both to recurrence and to malignant transformation.

This hypothesis is somewhat speculative and it is supported by the observation that all DNA and PCNA values of the adenomatous areas adjacent to carcinomas belonged to Group II.

CONCLUSION

Our study suggests that the possibility of recurrence, especially in the form of multiple foci or malignant tumour, may depend not only on the presence of incomplete surgical removal as is commonly believed, but also on the intrinsic biological properties of each pleomorphic adenoma.

Follow-up studies are now under way. Nevertheless, the cases reported were selected from 1989 to 1991 and pleomorphic adenoma of the parotid gland may recur, as is well known, after more than 10 years [2]. Consequently, it is not possible at present to establish a correlation between the results so far obtained and clinical outcome.

We hope that, in the near future, from these data it will be possible to determine whether the cell fraction in the proliferating phase is a useful biological marker and prognostic indicator for pleomorphic adenoma of the parotid gland, as it has been demonstrated to be in other forms of neoplasia.

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