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Pathology in Focus

Salivary neoplasms of the palate: a flow cytometric and clinicopathological analysis

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Abstract
In order to test the clinical and prognostic significance of flow cytometrically assessed DNA content in minor salivary gland tumours we evaluated 75 neoplasms of the palate, 55 of which were carcinomas. Benign neoplasms were exclusively DNA diploid with low S-phase fractions while 22 per cent of malignant tumours manifested a DNA aneuploidy and 23.5 per cent high S-phase fractions (>5 per cent). Significant statistical correlations between DNA content and tumour size, histological grade, lymph node metastasis and lethality were observed. Our findings suggest a potentially important role for flow-cytometry in the evaluation of these neoplasms.

Key words: Flow cytometry; DNA; Salivary gland neoplasms; Palate; Adenocarcinoma, terminal duct; Carcinoma, adenoid cystic

Introduction

Evaluations of the DNA content (ploidy and S-phase fractions) of salivary gland neoplasms, by either cytophotometrical or flow cytometric means, are few in number and most often have been carried out at major salivary gland sites, or on a specific histological class of these neoplasms (Eneroth and Zetterberg, 1973; Kino *et al.*, 1973; Eneroth and Zetterberg, 1974; Hamper *et al.*, 1989; El-Naggar *et al.*, 1990; Hamper *et al.*, 1990; Franzén *et al.*, 1991). There is little data available concerning the prognostic significance of DNA content in minor salivary gland neoplasms (Eneroth and Zetterberg, 1973).

In this report, we present the findings of a flow-cytometric study of 75 salivary gland neoplasms of the palate, including 55 carcinomas, and correlate these with conventional clinicopathological parameters. To our knowledge, there is no earlier comparable study.

Materials and methods

Eighty palatal salivary gland neoplasms with adequate tissue blocks from an equal number of patients treated at the University of Texas, M.D. Anderson Cancer Center (UTMDACC), from 1960 to 1990 formed the test material for this study.

Demographic data, tumour size, treatment, recurrences, metastases, and the last known patient status were derived by review of patient records.

Each of the palatal neoplasms was reviewed by two pathologists independently, then classified, and the malignant ones were histologically graded. Adenoid cystic carcinomas were graded according to the system of Szanto *et al.* (1984) into three grades. The three-tiered system of Batsakis and Luna (1990) was used for grading the mucoepidermoid carcinomas. Terminal duct (polymorphous low-grade) adenocarcinomas were divided into two groups (Batsakis and El-Naggar, 1991; Slootweg and Muller, 1987) according to absence (I) or presence (II) of a papillary component.

The DNA content and S-phase fractions were derived by flow-cytometry as shown below.

Flow-cytometry

At least one paraffin block of a representative area of each tumour was selected for flow cytometry. In 25 neoplasms, flow cytometric analysis was performed with two or three blocks. In four neoplasms extra blocks from recurrences or a metastasis were also used.

Nuclear suspensions of the neoplasms were prepared using a modified version (McLemore *et al.*, 1990) of the method of Hedley *et al.* (1983). Specimens were analysed on an EPICS Profile I (EPICS Division, Coulter Electronics, Hialeah, FL., USA) equipped with an argon ion laser operating at 488 nm, with a 610 long-pass filter and 488 band pass. Flow rates were adjusted to count approximately 75 nuclei per second. Peak *versus* integral signals were employed to gate out doublets.

Cytospin slides were prepared from a one million nuclei per ml concentration (unexposed to RNAse) and stained with Wright-Giemsa stain. The slides were reviewed to confirm the presence of tumour nuclei as well as to monitor the quality of processing. In each sample, at least 10 000 nuclei were cytometrically evaluated. Coulter cytologic software (version 2.2) was used for histogram analysis. All tumours analysed by the cytologic software used the same debris subtraction model.

The first G0/G1 population was used to denote the diploid stem line. A tumour was considered aneuploid when a distinct second G0/G1 peak, accounting for at least 10 per cent of the analysed cells, (or any number of such separate peaks) was present. The mean DNA indices (DI) were calculated by dividing the peak channel number of the aneuploid population by the peak

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TABLE I
RECURRENCES AND METASTASES OF SALIVARY GLAND TUMOURS OF PALATE

Histological type	No. of neoplasms	Incidence of recurrence (%)	Incidence of metastasis (%)
Adenoid cystic carcinoma	24	13 (54)	10 (42)
Terminal duct adeno-carcinoma	22	7 (32)	4 (18)
Mucoepidermoid carcinoma	11	3 (27)	2 (18)
Pleomophic adenoma	12	1 (8.3)	0 (0)
Carcinosarcoma	1	1 (100)	1 (100)

channel number of the diploid stem line. The coefficients of variation (CV) of the diploid stem lines ranged from 2.7 to 7.6 with a mean of 5.1. In the analysis of the cell cycle, a cut-off of five was considered to separate high from low S-phase. This was based on the analysis of 20 normal salivary gland tissues where the S + G2M phases of the cell cycle ranged from one to four per cent with a mean of 2.9 per cent.

The flow-cytometric data were compared with classical clinicopathological features by the Fisher exact and chi-square tests. Survival analyses were performed by the method of Kaplan and Meier (1958).

Results

Clinically and pathologically derived data

There were 45 females and 35 males in the series. Sixty (75 per cent) were white, 17 (21 per cent) were black and three (4 per cent) were Hispanic people. Median age at the time of diagnosis was 54 years (range: 15–87 years). Sixty-two patients received all of their treatment at UTMDACC. Eighteen were referred to our institution for the management of either recurrent (12) or residual (six) neoplasms. The primary treatment for all patients was surgical removal. Thirty-one patients also received post-operative radiotherapy.

The size of the palate neoplasms primarily treated at UTMDACC was less than 2 cm (T1) in 32 patients; 2–4 cm (T2) in 13 patients, and larger than 4 cm (T3) in 17 patients. In all, 44 (55 per cent) of the neoplasms were either T1 or T2 at the time of therapeutic intervention. Five patients manifested metastases at regional lymph nodes at the time of clinical presentation. Two of these were associated with recurrences at the primary site.

There were 60 malignant and 20 benign neoplasms. The benign salivary gland neoplasms were classified as: pleomorphic adenoma (12), canalicular adenoma (three) and myoepitheliomas (five). Three of the myoepitheliomas were plasmacytoid and two were epitheliod variants. Adenoid cystic and terminal duct adenocarcinomas were nearly equally represented i.e. 24 and 22 respectively. Eight of the adenoid cystic carcinomas were grade I, nine were grade II and 7, grade III. Eighteen of the terminal duct adenocarcinomas were grade I and four were grade II. Eleven carcinomas were mucoepidermoid in type; three were grade I, five were grade II and three were grade III. The two carcinomas which were expleomorphic adenoma were intralesional and non-invasive. There was one true malignant mixed tumour (carcinosarcoma).

Follow-up periods ranged from one to 22 years (mean of 10.7 years). Thirty-two patients with malignant neoplasms were followed-up for more than 10 years. Twenty-five patients experienced local recurrences before or after treatment in our institution. The recurrent neoplasms were adenoid cystic carcinomas (13), terminal duct adenocarcinomas (seven), mucoepidermoid carcinomas (three), and one each of carcinosarcoma and pleomorphic adenoma. Regional lymph node metastases occurred in 17 patients of which 10 had adenoid cystic, four had terminal duct and two had mucoepidermoid carcinomas. One had a carcinosarcoma. Metastasis at cervical lymph nodes also occurred in the patient with the carcinosarcoma (Table I).

Flow-cytometric data

Interpretable cytometric histograms could be obtained from

TABLE II
RELATIONSHIP OF FLOW CYTOMETRIC FINDINGS TO CLINICAL AND PATHOLOGICAL FEATURES IN 55 MALIGNANT SALIVARY GLAND NEOPLASMS OF THE PALATE

Clinical pathological features	Case no.	Aneuploidy (%)	*p*-Value	Mean DI	Mean S-phase (%)	*p*-Value
Sex						
Male	25	4 (16)	0.5	1.04	3.5	0.8
Female	30	8 (26)		1.10	3.5	
Age						
15–49 years	16	1 (6)	0.14	1.0	2.6	0.20
50–87 years	39	11 (28)		1.10	3.4	
T-category						
1	18	1 (5)	0.01	1.0	2.6	0.05
2	18	2 (18)		1.03	3.2	
3	18	6 (54)		1.2	5.3	
4	15	2 (13)		1.03	3.6	
Lymph Node Metastases						
Yes	17	8 (47)	0.002	1.16	5.0	0.04
No	36	3 (8)		1.03	2.8	
Histology*						
Adenoid cystic	22	4 (18)	0.27	1.06	3.8	0.15
Terminal duct	22	5 (22)		1.03	2.3	
Mucoepidermoid	8	1 (12)		1.08	4.2	
Grade						
I	30	3 (10)	0.04	1.05	2.4	0.03
II	14	4 (28)		1.05	3.0	
III	11	5 (45)		1.20	6.3	
Patient status						
Alive, NED	23	2 (8)		1.04	2.8	
DOC	13	1 (13)		1.0	2.6	
DOD or with recurrence	17	8 (47)	0.005	1.16	5.1	0.05

*Ca ex-pleomorphic adenoma and carcinosarcoma were excluded due to their low number.
NED: No evidence of disease; DOD: Died of disease; DOC: Died of other causes.

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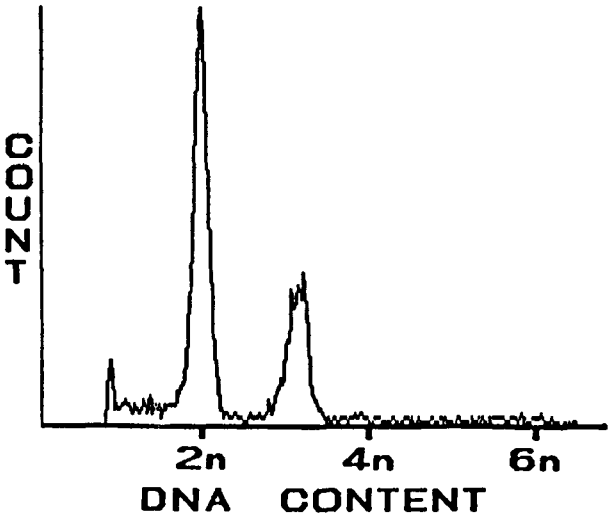

FIG. 1

A single parameter histogram of the DNA content of an adenoid cystic carcinoma showing an aneuploid stem line with a DI of 1.6.

55 of the 60 malignant neoplasms. Twelve had an aneuploid DNA content with a mean DI of 1.3 (range: 1.1–1.9). None of the 20 benign neoplasms were aneuploid (mean DI of 1.0).

The mean S-phase fraction for benign neoplasms was 2.9 per cent (range 1–6 per cent) while that for the malignant neoplasms was 3.3 per cent (range 1–13 per cent). Carcinomas with a diploid DNA content had a mean S-phase of 3.5 per cent. Carcinomas with an aneuploid DNA content had a mean S-phase of 5.5 per cent.

There were no significant correlations between DNA aneuploidy and sex (p = 0.5) or age (p = 0.14) of patients or with the histological type (p = 0.27) of carcinomas (Table II). Eighteen per cent of adenoid cystic carcinomas were aneuploid while 22 per cent of terminal duct adenocarcinomas and 12 per cent of mucoepidermoid carcinomas manifested aneuploid DNA contents. The DNA index (DI) of DNA aneuploid adenoid cystic carcinomas ranged from 1.1 to 1.8 with a mean of 1.36 (Figure 1), while those of terminal duct carcinomas ranged from 1.1 to 1.2 with a mean of 1.12 (Figure 2).

DNA aneuploidy correlated with the histological grade of the carcinomas: 10 per cent grade I; 28 per cent grade II; and 45 per cent grade III carcinomas were aneuploid (p = 0.04). A significant correlation also existed between the frequency of DNA aneuploidy and tumour size; T1 neoplasms had a five per cent incidence of abnormal DNA content while T2 and T3 carcinomas had an 18 and 54 per cent incidence respectively (p<0.01).

DNA aneuploidy occurred in 47 per cent of the carcinomas that produced metastases as opposed to an eight per cent aneuploidy in carcinomas without metastases (p = 0.002). Patients with recurrent or life consuming carcinomas had malignancies with abnormal DNA content in 38 per cent of the cohort. Only 10 per cent of patients who were alive and without evidence of recurrence had DNA aneuploid carcinomas (p = 0.005).

No statistical correlation was observed between high and low S-phase fractions and patients' sex (p = 0.8), age (p = 0.2) or tumour category (p = 0.15). A statistical correlation between S-phase fraction and tumour size (p = 0.05), histological grade (p = 0.03), regional lymph node metastasis (p = 0.04), and patient's survival (p<0.05) was observed (Figure 3).

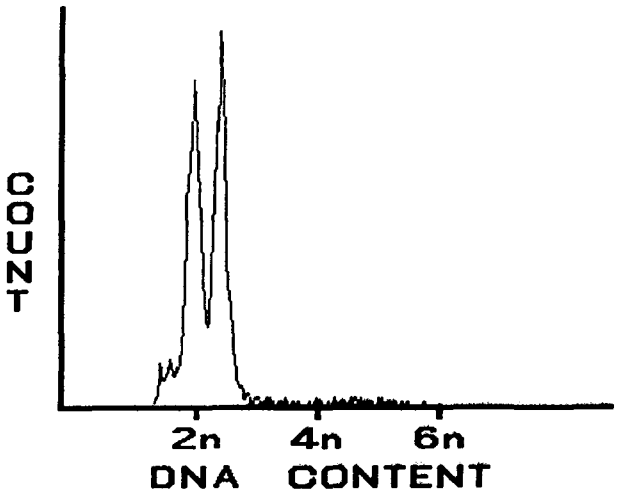

FIG. 2

A single parameter histogram of the DNA content of a terminal duct carcinoma with a near-diploid aneuploid stem line and a DI of 1.12.

Discussion

Over the past two decades Spiro and co-workers (1973; 1991) have studied minor salivary gland carcinomas in order to determine the most significant factors influencing survival. In 1973, they emphasized that histological findings, anatomical site and tumour extent were the most important prognostic indicators. Their 1991 study confirmed the overriding importance of clinical stage. It was also concluded that histological diagnosis, by itself, was of limited value and that histological grade significantly influenced survival only for mucoepidermoid and adenocarcinomas. Despite this generalization a subset of neoplasms, albeit small, within each stage and histological grade appears to defy such contention and continues to pose clinical challenges. New markers to identify these patients are needed.

Our data tend to support the additive prognostic information obtainable by flow cytometric DNA content analysis of minor salivary gland neoplasms. By restricting our evaluations to salivary gland neoplasms of the palate, we have eliminated any bias which might attend site of origin. Figure 3, depicting the cumulative survival (Kaplan–Meier) curves of the aneuploid and diploid carcinomas of the palate, is graphic evidence of the influence of ploidy. The correlation of DNA ploidy and S-phase fractions with size and histological grade of the carcinomas also suggests an interdependence and appears to agree with Spiro *et al.* (1991). Such an impression, however, should be validated by multifactorial regression analyses in a larger series.

Our study also allows a comparison between the histogen-

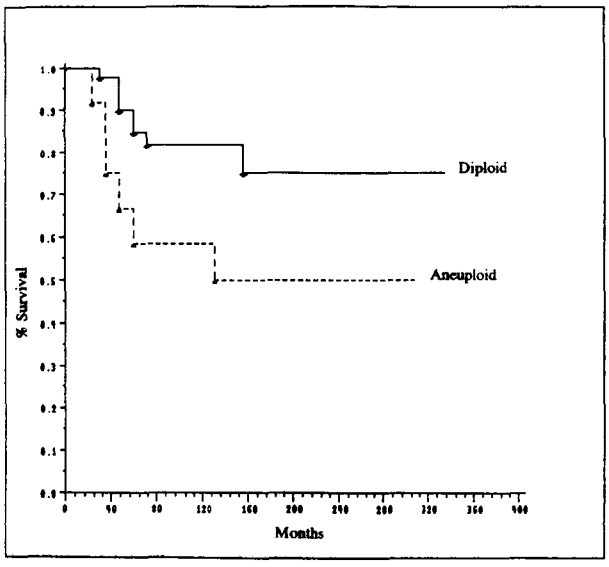

FIG. 3

Kaplan–Meier survival curve of patients with DNA diploid and aneuploid palatal salivary gland carcinomas.

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etically related adenoid cystic and terminal duct adenocarcinomas, each with a comparable number of cases. The incidence of aneuploidy and high S-phase fractions was very similar for each class of carcinoma. Four adenoid cystic carcinomas had an aneuploid DNA content and five had high S-phase fractions while five terminal duct carcinomas were DNA aneuploid and two had a high S-phase fraction. A significant statistical correlation between DNA aneuploidy and high S-phase fraction and the biological aggressiveness of these neoplasms was observed. Thirteen of the adenoid cystic carcinomas recurred; ten manifested metastases and 11 killed their hosts. Only one patient with terminal duct adenocarcinoma died of the neoplasm although seven carcinomas recurred and four metastasized, over the course of the follow-up period. The tendency to recurrence with a low mortality rate is typical of the terminal duct adenocarcinoma (Batsakis and El-Naggar, 1991). An analysis of the flow cytometric histograms of the aneuploid carcinomas in each group presents a partial answer to the differences in biological behaviour. The aneuploid adenoid cystic carcinomas have higher DNA indices while terminal duct adenocarcinomas have near-diploid DNA indices. Only one of the aneuploid terminal duct carcinomas caused death. An association between near-diploid DNA aneuploidy and a relatively benign clinical course has been previously observed in certain malignant neoplasms (Kimura *et al.*, 1992). This lends further credence to our findings in terminal duct carcinoma.

Summary

Our study indicates a significant statistical correlation between DNA content and certain traditional tumour parameters and biological course. The exclusive influence of DNA flow cytometry in the assessment of the biological behaviour of these neoplasms, however, must await studies of a larger group of patients.

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