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Cellular DNA content and prognosis in surgically treated squamous carcinoma of the larynx

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There have been 26 previous series reported of ploidy in squamous cell carcinoma of the head and neck. Of these 12 do not discuss survival (Danes *et al.*, 1987; Ensley *et al.*, 1989; Feinmesser *et al.*, 1990; Franzen *et al.*, 1987*a*,*b*; Graessel-Pietrusky & Hornstein, 1982; Hemmer & Kreidler, 1990; Johnson *et al.*, 1985; Kaplan *et al.*, 1986; Kearsley *et al.*, 1990; Olinici & Caluser, 1987; Wilson *et al.*, 1988). Of the remaining 14 articles, four do not state the type of treatment the patient received (Boecking *et al.*, 1985; Chen, 1989; Lampe *et al.*, 1987; Oloffson *et al.*, 1986). In seven reports the patients were treated by a variety of combinations of radiotherapy, chemotherapy or surgery (Feichter *et al.*, 1987; Goldsmith *et al.*, 1986; Goldsmith *et al.*, 1987; Guo *et al.*, 1989; Holm, 1982; Sickle-Santanello *et al.*, 1986; Tytor *et al.*, 1987).

Tytor *et al.* (1989) reported that aneuploid tumours of the oral cavity were more likely to respond to preoperative radiotherapy. Kokal *et al.* (1988) found, in a series of 76 patients treated initially by surgery, that patients with diploid tumours fared better. They did not state whether their patients had postoperative radiotherapy, but the wording of their article suggests that they did. Furthermore the patients in their series had tumours at various different sites and it is well known that survival varies widely between different sites. However they allowed for this by multivariate analysis.

We have previously reported that patients with end-stage disease submitted to chemotherapy trials do better if they have an aneuploid tumour (Cooke *et al.*, 1990).

Thus there has been no report of a large number of patients with tumours at one site treated in a similar fashion. We report a relatively large series of patients with a tumour of one site (the larynx) of similar stages (stages III and IV), all submitted to surgery.

Patients

This report is based on 1,128 patients with a laryngeal tumour seen personally by one of us (PMS) between 1963 and 1990. These patients have been treated throughout by a uniform policy of radiotherapy for T_1–T_3N_0 tumours not causing stridor, and surgery for patients with palpable lymph node metastases, advanced tumours (T_4) and patients with stridor. Two hundred and ninety-eight patients with a previously untreated squamous carcinoma were treated initially by surgery. Unfortunately too little histological material was available from specimens of patients seen before 1978. Histological blocks containing enough material for flow cytometry were still available on 110 patients seen since 1978, and these form the basis of this report.

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Storage of the data and follow-up

The data on all these patients have been recorded prospectively, initially on cards, and for the last 10 years on a microprocessor. Data have been kept up to date by personal contact, and by information from general practitioners, the Mersey Regional Cancer Registry, and the National Health Service Register. Two patients (2%) have been lost to follow-up.

Staging

The TNM stage of all patients was classified by the UICC (1987) convention, with appropriate stage grouping.

General condition

The patients' performance status was recorded by the ECOG classification (Beahrs *et al.*, 1988).

Method

DNA measurement and classification

Thick sections from tumours were examined by flow cytometry, consecutive 5 μm sections being stained by haematoxlin and eosin to confirm the presence of tumour in all samples studied. Briefly, nuclei were extracted from formalin fixed paraffin embedded tissue by the method described by Hedley *et al.* (1983). Multiple 50 μm sections were dewaxed in xylene and rehydrated through 0.5% pepsin in 0.9% NaCl with a pH 1.5 for 30 min at 37°C. The digest was then centrifuged, washed and resuspended. After resuspension in 1 ml of phosphate buffered saline the digest was syringed 3–4 times to disaggregate nuclear clumps and then filtered through 40 μm nylon mesh. Nuclear concentrations were adjusted when necessary to give a final concentration of 10^6 nuclei per ml. DNA analysis was performed using a Profile-II flow cytometer (Coulter Corp. Hialeh, Florida, USA). Where possible fluorescence from 100,000 nuclei was recorded, a minimum of 10,000 being required to give interpretable histograms. Histograms were classified as aneuploid or diploid, and only those with a coefficient of variation of less than 8% being accepted. Tetraploid tumours, especially if they represent a small fraction of the whole section, may be difficult to detect as the G0 and G1 of these tumour cells have the same DNA content as normal cells in G2 and M. A 4c peak accounting for more than 15% of the whole cell population was designated aneuploid as it is unlikely that normal cells would have such a high G2/M peak.

Analysis of the data

Qualitative data are displayed in contingency tables, and analysed by χ^2. The relation between ploidy and host and tumour factors was analysed by weighted logistic regression.

Survival curves were drawn up by the life table method (Armitage, 1987). Differences between survival curves were analysed by multivariate regression analysis (Cox, 1972).

Results

Ploidy and host/tumour factors

The relation between ploidy and the various host and tumour factors is shown in Tables I and II. There was no statistically significant difference between the diploid and aneuploid tumours with respect to host factors. As regards tumour factors, diploid tumours had a higher proportion of Stage IV tumours, but not significant so, whereas aneuploid tumours were more likely to arise from the supraglottic area and be poorly differentiated.

Weighted logistic regression confirmed that neither sex ($z = 0.02$) nor age ($z = 0.56$) nor general condition ($z = 0.45$) were significantly associated with ploidy. However supraglottic tumours were significantly more likely to be aneuploid ($z = 2.11$, $P < 0.05$), even more so if they were poorly differentiated ($z = 2.47$, $P < 0.025$). However histological grade ($z = 0.43$) and stage ($z = 1.51$) were not independent indicators of ploidy.

Table I Ploidy and host factors

	Ploidy	
	Diploid	*Aneuploid*
Sex		
Men	45	37
Women	15	13
	$\chi^2_1 = 0.01$	
Age		
Mean (in years)	60.2	59.3
ECOG status		
O	43	34
I-IV	15	16
Not recorded	2	0
	$\chi^2_1 = 0.23$	

Table II Ploidy and tumour factors

	Diploid	*Aneuploid*
Site		
Supraglottic	27	38
Glottic	9	5
Subglottic	12	0
Transglottic	12	7
	$\chi^2_3 = 15.5$, $P < 0.01$	
Histology		
Well differentiated	13	3
Moderately differentiated	20	19
Poorly differentiated	25	27
Ungraded	2	1
	$\chi^2_2 = 5.63$, $P = 0.07$	
Stage grouping		
I	0	0
II	0	0
III	31	30
IV	29	20
	$\chi^2_1 = 0.46$, N.S.	

Survival

The 5 year adjusted survival was 50% for diploid tumours, and 48% for aneuploid tumours. A direct comparison of these two rates is not valid because of the differing incidence of sites, histological grade and stage group. However, Cox's multivariate regression showed that there was no statistically significant difference between survival rates for diploid and aneuploid tumours when these variable factors are taken into account ($z = 0.63$). Indeed there was no significant overall regression in this group of patients when they were analysed for all known prognostic factors ($\chi^2_4 = 10.89$, $P + 0.09$).

Node metastases

Thirty-nine patients later developed a lymph node metastasis: 26 were submitted to surgery, eight were untreated and five had palliative radiotherapy or chemotherapy. The incidence of histologically proven later lymph node recurrence was 31% for diploid tumours, and 45% for aneuploid tumours (at 3 years). However, Cox's regression analysis showed that ploidy was not a significant predictor of later node recurrence ($z = 1.00$).

Discussion

In brief this series shows that ploidy was significantly related to subsite within the larynx: supraglottic tumours were more likely to be aneuploid, particularly if they were poorly differentiated. However ploidy was not related to any other tumour factor (stage or histological grade) nor to host factors (age, sex and general condition). Secondly, we found that ploidy did not affect survival once confounding by the site effect referred to above was taken into account.

We found a higher incidence of nodal metastases in aneuploid tumours, but this was not significant. The difference could be due to the fact that aneuploid tumours are more likely to be supraglottic and the latter tumours are more likely to metastasise as the supraglottis has a well developed external lymphatic drainage.

Our findings are at odds with some investigators, but many published series have been small – the six series referred to in the introduction contained only 157 patients in all, whereas our series contained 110 patients. Furthermore the authors of the above series did not use multivariate methods to assess the relation of ploidy with host and tumour factors, and only one (Kokal *et al.*) used multivariate analysis of survival to allow for confounding. A meta analysis of all the reports of squamous carcinoma of the head and neck published to date shows that tumour DNA analysis has no prognostic significance in laryngeal cancer, though it probably does in mouth cancer (Stell, 1991). Finally, unlike all previous series, our is homogeneous with respect to site, stage of disease and treatment.

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