Oral Oncol, Eur J Cancer, Vol. 28B, No. 1, pp. 17–21, 1992
Printed In Great Britain

0964–1955/92 $5.00 + 0.00

Multiple Cytogenetic Aberrations in Squamous Cell Carcinomas of the Head and Neck

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Chromosomal abnormalities in short term cultures have been investigated in 10 squamous cell carcinomas of the head and neck. Of these tumours, three demonstrated clonal chromosomal abnormalities, two showed random abnormalities and 5 patients' tumours had normal karyotypes. The 5 patients with aberrant karyotypes were all from previously treated tumours, of these, 4 patients had received radiotherapy and 1 surgery. On analysis of the three clonal tumours, two were found to be polyclonal, each with five separate clones. 116 breakpoints were demonstrated from the clonal data of these tumours, and all of the chromosomes were involved, apart from number 18. In this study we found three or more breakpoints at sites 1p36, 9q32 and 11q23. 1 of the patients investigated showed a clonal abnormality involving a breakpoint at the 11q13 site, with a further 2 patients having breakpoints at 1p22—sites previously reported to have marked clustering of cytogenetic abnormalities in oral cancer patients. Only further studies will demonstrate whether the breakpoints found are of clinical significance.
Oral Oncology, Eur J Cancer, Vol. 28B, No. 1, pp. 17–21, 1992.

INTRODUCTION

CYTOGENETIC STUDIES on solid tumours have contributed to the localisation of genetic alterations in a number of neoplasms [1], and have aided the understanding of molecular mechanisms at the level of oncogene amplification and the loss of tumour suppressor genes. Cytogenetic aberrations in squamous cell carcinoma of the head and neck cancer have shown that most chromosomes are affected, however a clustering of chromosomal breakpoints have been found at 1p22 and 11q13 [2]. The breakpoint region on chromosome 11 at 11q13 is of particular interest as six reports have indicated that *int*-2, *bcl*-1 and *hst*-1 oncogenes are amplified singly or together in this amplicon region [3].

A number of oncogenes have been shown to play a role in the development and progression of squamous cell carcinoma of the head and neck, for example, *erb*-1, *ras*, c-*myc* and the tumour suppressor gene p53. In certain cases these genes have been demonstrated to correlate with the patients clinical outcome [4–6].

In this paper we present chromosomal analysis of 10 squamous cell carcinomas originating in the head and neck. 8 of these patients received radiotherapy between 6 and 90 months before cytogenetic analysis was undertaken. This is the largest group of previously treated head and neck patients which has been cytogenetically analysed. The aim of this investigation is to further evaluate the importance of cytogenetic abnormalities in head and neck cancer and to assess whether patients who have been previously treated with irradiation demonstrated a different group of abnormalities compared with those previously reported in primary tumours.

MATERIALS AND METHODS

Samples for cytogenetic analysis were obtained from 14 patients with squamous cell carcinoma (SCC) of the mucosal surfaces of the upper aerodigestive tract. Tissues from the surgical specimens were also sent for routine pathological examination.

Fresh tumour tissue was finely minced and enzymatically disaggregated overnight in 0.08% collagenase (Sigma, type IV). Cultures were initiated in Leighton tubes in Hams F10 medium supplemented with 30% fetal calf and human AB serum, glutamine and antibiotics. Cultures were harvested as soon as enough proliferating cells were available i.e. between 6 and 21 days. Colcemid was added for 2 h, the cells were removed from the culture vessel following brief exposure to trypsin–EDTA, treated with hypotonic (0.075 mol/l KCl) for 30 min, then fixed gradually by adding a mixture of glacial acetic acid/methanol (1 : 3). Slides were incubated overnight at 60°C before GTG banding (G bands by trypsin using giemsa).

Where possible at least 20 metaphases were karyotyped from at least two separate cultures. Chromosomal aberrations were defined as clonal only if they were present in at least two metaphases with the same structural aberration or supernumerary chromosome, or when at least three cells with the same missing chromosome were detected [7, 8].

The following clinico-pathological parameters were available for all of the patients; TNM staging using the UICC convention, [9], site of tumour, details of previous treatment, histopathological differentiation, the pathology of lymph node metastasis and follow-up.

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Received 18 Dec. 1991; accepted 4 Feb. 1992.

RESULTS

Cultures were successfully established from fourteen tumours. Four of the tumour cultures failed to yield sufficient metaphases for cytogenetic analysis. From the remaining 10 tumours, 3 (patients 1–3) showed clonal abnormalities of varying complexity (8–21 days in culture), 2 (patients 4 and 5) had random abnormalities with no definitive clone identified (8–13 days in culture) and the remaining 5 (patients 6–10) had a normal karyotype (11–21 days in culture).

Clones from the three tumours demonstrating clonal abnormalities and the number of cells analysed from each clone are described in Table 1. Five abnormal clones were detected in the tumour from patient 1. Each clone was typified by a simple chromosomal change. Only clone II was found in more than one culture. Twelve cells from this tumour showed random abnormalities and 1 cell had a normal karyotype.

Five abnormal clones were also detected in the tumour from Patient 2 but in contrast to the former case they were complex apart from clone V. Four cells showed random abnormalities and there were no normal cells seen.

The tumour from patient 3 demonstrated a single clone in three cultures. This clone was complex and contained at least 16 aberrations. It was not possible to determine the exact nature of all the aberrations. One cell showed random abnormalities and no cells with a normal karyotype were found.

Table 1. *Clonal chromosomal abnormalities present in three tumours*

Tumour	Clone	Karyotype	No. of cells per clone
1	I	46, XX, t(1; 14)(p36; q11)	2
	II	46, XX, t(2; 4)(p21; q21), inv(3)(p21p25)	4
	III	46, XX, 21p+	2
	IV	46, XX, inv(7)(p12q36)	3
	V	46, XX, inv(9)(p12q32)	4
2	I	46, XY, −2, −14, t(3; 11)(q21; q23), der(6), t(6; ?)(p25; ?), t(6; ?)(?q24; ?), t(8; ?)(q11; ?), t(11; ?)(p13; ?), t(12; ?)(q24; ?), t(13; ?)(q32; ?), t(20; ?)(q13; ?), +M1, +M2.	6
	II	46, XY, +16, −5, −17, −17, t(2; 9)(q33; q34), inv(7)(p22q11), t(10; 14)(p15; q11), der(11)t(5; 11)(q31; q23), t(12; ?)(q22; ?), t(22; ?)(q13; ?), +M1, +M2, +M3.	4
	III	45, Y, −X, −5, der(X)t(5; 9; 22; X)(q15; q13; q11; q22), inv(1)(p13q44), t(1; 7)(q24; p15), del(6)(qter → p23:).	4
	IV	46, XY, t(1; ?)(p36; ?), t(6; ?)(q23; ?), inv(7)(p13q22), t(9; ?)(q32; ?), t(11; ?)(q21; ?), t(15; 16)(q25; q22).	4
	V	46, X, t(3; 15)(q29; q12), t(16; Y)(p13; q11).	2
3		46, −X, −15, −18, t(X; 11)(q24; q13), inv(6)(p23q24), t(7; ?)(q32; ?), t(9; ?)(q32;?), del(10)(pter → q24:), t(15; ?)(q25; ?), t(16; ?)(q22; ?), t(19; ?)(q11; ?), t(22; ?)(p11; ?), +r, +M1, +M2	11

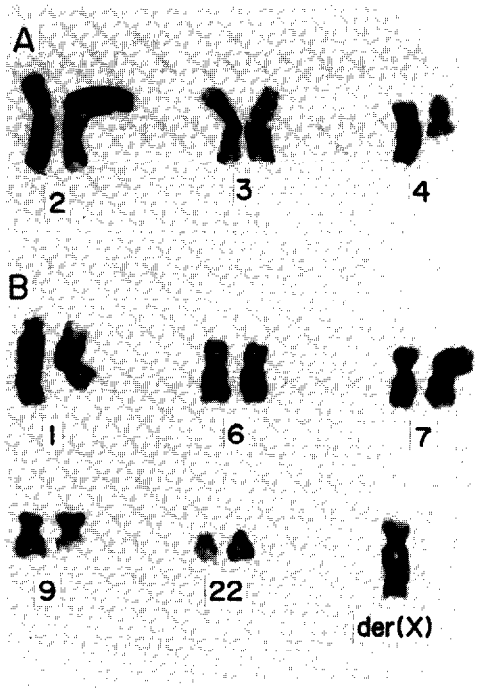

Fig. 1. Partial karyotypes demonstrating the structural rearrangements of 2 clones from tumours 1 and 2. A, Tumour 1: clone II: 46, XX, t(2; 4)(p21; q21), inv(3)(p21p25). B, Tumour 2: clone III: 45, Y, −X, −5, der(X)t(5; 9; 22; X)(q15; q13; q11; q22), inv(1)(p13q44), t(1; 7)(q24; p15), del(6)(qter → p23:).

Partial karyotypes illustrating clonal aberrations from tumours 1, 2 and 3 are shown in Figs 1 and 2. A composite figure showing all the chromosomal breakpoints corresponding to the structural breakpoints in the three clonal head and neck squamous carcinomas is shown in Fig. 3.

The clinicopathological details, previous treatment and follow-up are given in Table 2. 5 of the patients investigated (patients 2, 4, 5, 6, 9) had been previously treated with radiotherapy alone; 2 with both radiotherapy and surgery (patients

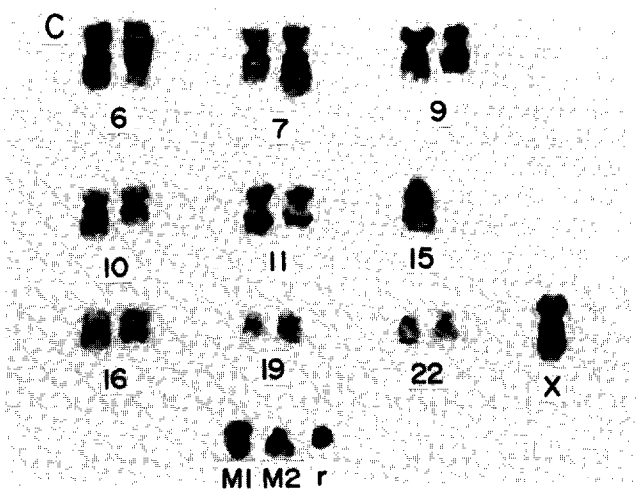

Fig. 2. Partial karyotype demonstrating the clonal structural rearrangements of tumour 3, (C): 46, −X, −15, −18, t(X; 11)(q24; q13), inv(6)(p23q24), t(7; ?)(q32; ?), t(9; ?)(q32; ?), del(10)(pter → q24:), t(15; ?)(q25; ?), t(16; ?)(q22; ?), t(19; ?)(q11; ?), t(22; ?)(p11; ?), +r, +M1, +M2.

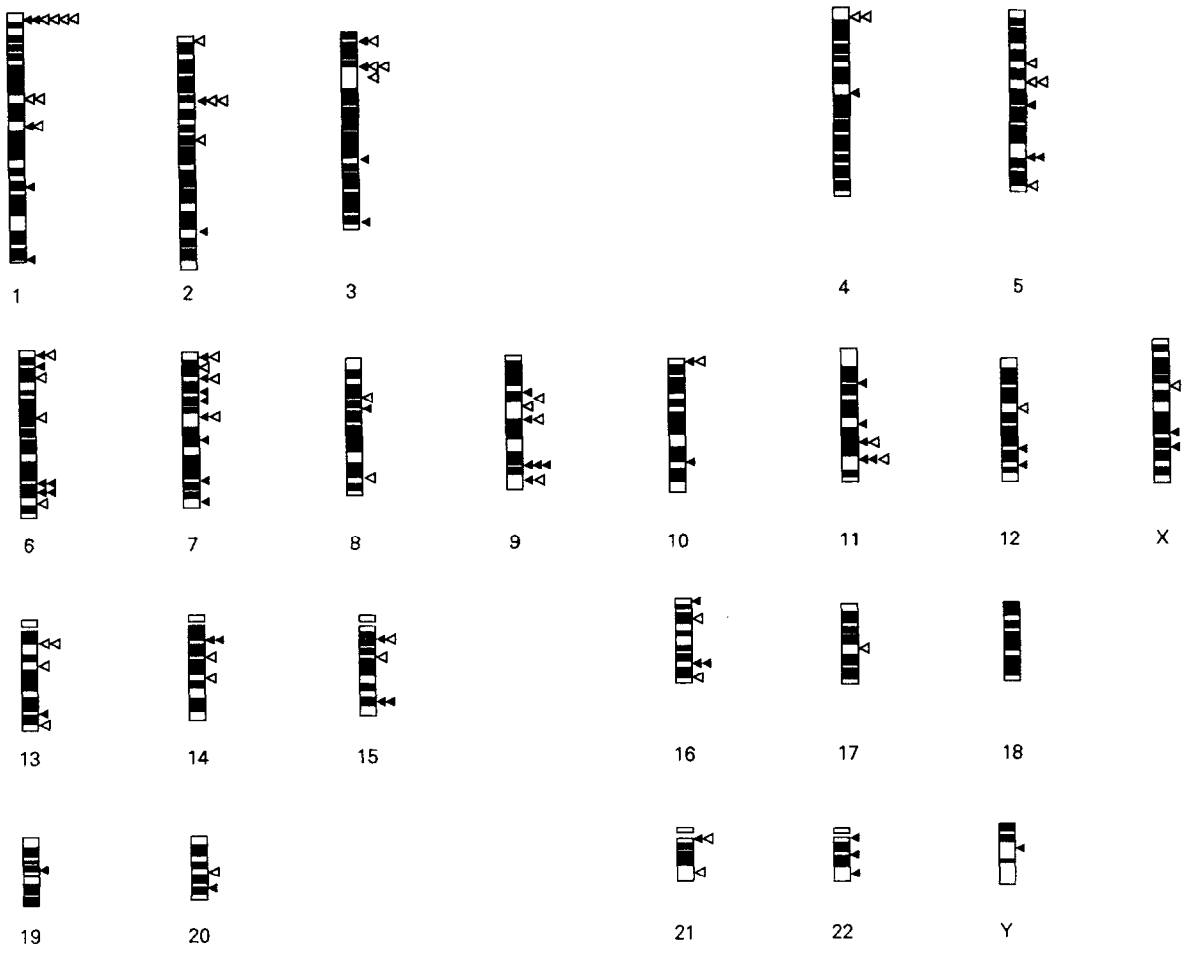

Fig. 3. Distribution of clonal and non-clonal breakpoints found in three tumours from patients with squamous cell carcinoma of the head and neck. Clonal breakpoints were represented by: (◄) and non-clonal by: (◅).

1, 7); 1 with surgery alone (patient 3), and the remaining 2 patients had no previous treatment (patients 8, 10).

DISCUSSION

We have cytogenetically analysed 10 squamous cell carcinomas from the head and neck and have shown that five had cytogenetic abnormalities. The five tumours with aberrant karyotypes were all from recurrent tumours, three of these tumours had clonal abnormalities and two had random abnormalities. Of these, 4 patients had received radiotherapy and 1 surgery. In the group of patients in which normal karyotypes were found, 3 had undergone radiotherapy and 2 had received no previous treatment (Table 2).

The analysis of the short term cultures consisted of a mixture of epithelial and mesenchymal cells, therefore it is not possible to distinguish whether the metaphases described in this paper belong to the stroma tissue of the tumour parenchyma. However this cautionary note has always to be considered when discussing any cytogenetic reports on solid tumour material.

The cytogenetic analysis of primary squamous cell carcinomas of the head and neck region provides a base line from which to assess which cytogenetic abnormalities may be important in the development of this disease [2, 10–16]. However, a complicated story has emerged from these initial reports indicating that oral squamous cell carcinomas possibly have a polyclonal origin, as a range of different clonal lines have been isolated from many of the tumours analysed [15]. It has been argued that chromosomal aberrations in neoplastic disorders may be of three types [17]; primary cytogenetic abnormalities which are essential to tumour invasion, secondary abnormalities which appear during the progression of the disease, and thirdly, a background level of cytogenetic noise, giving rise to non-consequential aberrations. The primary and secondary events have been correlated with particular chromosomes in a number of malignancies, whereas the "cytogenetic noise" is random throughout the genome.

In a study of 10 oral squamous cell carcinomas, Jin *et al.* [15] demonstrated 264 chromosome breaks using both clonal and non-clonal data. Recently the same group [2] undertook a comprehensive study describing 369 breakpoints in clonal rearrangements from 31 tumours. Many of the chromosome sites shown to have breakpoints in non-clonal tumours in their initial paper [15] were shown to be involved in clonal aberrations in the subsequent investigations with other tumours. Clustering of breakpoints were found at 11q13 and 1p22 in eight and nine tumours, respectively.

In the three clonal tumours identified in our study two were polyclonal, each having five separate clones. Analysis of the clonal and non-clonal karyotypes from these three tumours

Table 2. Clinicopathological parameters on the 10 squamous cell carcinomas of the head and neck

Patient no.	Karyo-type*	Primary† or previously treated	Previous‡ treatment	Site	TNM	Pathology§ (SCC)	pN‖	ECR¶	Time** interval to cytogenetic analysis	Fate
1	AC	PT	Local excision DXRT 1st and 2nd neck diss.	Oral	$T_xN_xM_0$	WD	1	0	7years 6 months	A
2	AC	PT	Radical DXRT	Oropharynx	$T_xN_xM_0$	MD	0	0	6 years 6 months	A
3	AC	PT	Surgery	Larynx	T_xN_xMo	MD	3	0	1 year 6 months	A
4	AR	PT	Radical DXRT	Hypopharynx	$T_xN_xM_0$	MD	0	0	6 months	D
5	AR	PT	Radical DXRT	Oral	$T_xN_xM_0$	WD	2	2	9 years 9 months	D
6	N	PT	Radical DXRT	Larynx	$T_xN_xM_0$	MD	0	0	11 months	A
7	N	PT	Previously Tr. elsewhere (surgery/DXRT)	Oropharynx	$T_xN_xM_0$	PD	3	0	9 months	A
8	N	P	—	Oral	$T_1N_0M_0$	MD	1	0	—	A
9	N	PT	Radical DXRT	Larynx	T_xN_xMo	PD	1	1	4 years	A
10	N	P	—	Hypopharynx	$T_3N_0M_0$	MD	2	2	—	D

*Karyotype—A = abnormal, C = clonal abnormalities, R = random abnormalities, N = normal karyotype.
†Primary squamous cell carcinoma.
‡Previously treated tumour. Previous treatment; surgery, (1st and 2nd neck diss.—first and second neck dissection), DXRT = radiotherapy.
§Histological differentiation: WD = well differentiated SCC, MD = moderately differentiated, PD = poorly differentiated.
‖Positive lymph nodes at pathology.
¶Extra capsular rupture of lymph nodes.
**Interval between first diagnosis of the disease and a specimen taken for karyotyping.

demonstrated 116 breakpoints. We have found a clustering of breakpoints in these tumours at sites 1p36, 9q32 and 11q23. Furthermore, 92 of the 116 breakpoints appear to correspond with those reported by Jin *et al.* [2, 15] and involve all of the chromosomes apart from number 18. In particular we have 1 patient (patient 3) with a clonal abnormality involving a breakpoint at the 11q13 site, and patients 2 and 3 each had random breakpoints at the 1p22 site. The amplicon region at 11q13 may represent a important genetic region in head and neck cancer as amplification of the *int*-2, *bcl*-1 and the *hst*-1 genes have been reported in many of these cancers [3].

In the present study tumours with cytogenetic aberrations were only found in patients who have been previously treated. However previous reports indicate that chromosomal aberrations are frequent in primary squamous cell carcinomas of the oral region. It is therefore of interest to speculate about the stability of cytogenetic aberrations throughout the progression of the disease and whether any particular chromosomal breakpoints persist in recurrences of the disease, many years later. Only when a large cytogenetic database of these tumours has been accumulated, will it be possible to determine which chromosomal breakpoints are important in the progression of squamous cell carcinoma of the head and neck. We are presently extending our database with both primary and previously treated tumours and in time it maybe possible to identify chromosomal regions containing specific genes which might play a role in the development of this disease.

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Acknowledgements—This work was supported by a grant from the North West Cancer Research Fund, UK. We would like to thank Mr AW Ferrie for preparing the figures for this paper.