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Chromosomal aberrations identified in culture of squamous carcinomas are confirmed by fluorescence in situ hybridisation

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Abstract

***Aims*—Chromosomal aberrations in tumour cells are often not discernable by direct analysis. Although cell culture allows qualitative analysis of the karyotype, potential selection and evolution during growth in vitro may yield misleading data. To determine whether aberrations observed in vitro are representative of the original lesion, chromosomal aberrations found after prolonged growth in vitro of two squamous cell carcinomas of the head and neck (SSCHN) were evaluated with fluorescence in situ hybridisation (FISH) on the original tumour nuclei.**

***Methods*—Specific karyotypic aberrations identified in cultures of two squamous cell carcinomas were targets for FISH analysis on tumour sections. Chromosome painting mixtures were selected based on in vitro karyotypic data. FISH was performed on cultured interphase and metaphase cells, and on histological sections from the original tumours.**

***Results*—The 9cen and 17cen probes yielded FISH signals consistent with the aneusomies predicted for the respective chromosomes from the culture karyotypes. Whole chromosome 9 paint confirmed the prior existence in the tumours of i(9p) and i(9q), although only the latter hybridised with the 9cen probe. FISH data also supported in vivo representation of the diploid and tetraploid tumour subclones observed in cultures. In tumour HFH-SCC-8a, FISH results were generally concordant between cultured interphase and metaphase cells and the histological sections, and improved the interpretation of marker chromosomes identified in culture.**

***Conclusion*—The karyotypes obtained in these cases after prolonged passage in culture were consistent with the genetic alterations in the original tumours.**

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Keywords: fluorescence in situ hybridisation; squamous cell carcinoma; karyotypic aberrations; cell culture

Direct chromosome analysis from solid tumours is rarely satisfactory. Cell culture allows qualitative analysis of the karyotype, but potential selection and evolution during growth in vitro may yield misleading data. Therefore, it is important to show that karyotypic aberrations defined after in vitro growth are identical to those present originally. Fluorescence in situ hybridisation (FISH) using chromosome specific probes can be used to examine the chromosomes of cells in archival tissues.[1 2] Karyotypes from cultured tumours can direct the selection of appropriate FISH probes, and the application of FISH to paraffin wax embedded sections permits the analysis of cells in their histological context. Thus, metaphase and interphase cytogenetic studies provide complementary information about chromosomal aberrations in tumours.[1–5]

We used FISH on histological sections to ask whether the chromosomal aberrations found after prolonged growth in vitro of two squamous cell carcinomas of the head and neck (SSCHN) were also present in the original tumour nuclei. We also demonstrated that aneuploid subclones in the primary and passaged cultures corresponded to those of the tumour.

Materials and methods

SOURCE MATERIALS

Surgical samples from two SSCHN tumours were cultured for periods extending to 272 days (XP1) and 195 days (8a) in vitro, and harvests for chromosome analysis were obtained according to previously published methods.[6 7] Paraffin wax blocks of the same tumours were recut, and haematoxylin and eosin stained slides were used to guide the delineation of tumour and non-tumourous areas for FISH analysis.

The karyotype of HFH-SCC-XP-1 is: 45,XY,−4,t(5;7)(q11.2;p22),i(9p),i(9q)[6]/90, XXYYidem × 2[68]/44,XY,−4,i(9p),i (9q),der (11)t(10;11)(q21;p14),−21[19]/88,XXYYidem × [52]/44, XY, −4, i(9p), i(9q), der(14)t(13;14) q21;q32),−21[8]/88,XXYYidem × 2[43].[6]

The HFH-SCC-8a karyotype is: 80–86,X, −Y × 2,del(1)(p22p36.1) × 2,add(2)(q37),+del (2)(q11.2),dic(3;11)(p11;q12) × 2,add(4)p12) × 2,del(4)(p12p16) × 2,del(5)(q11.2q22) × 2,−6,del(6)(p11.2p25),−7[3],der(8)t(2;8)(q24; q24.3) × 2,der(8)t(8;9)(p11.2;q12) × 2,−9 × 2, del(10)(p12p15) × 2,−11 × 2,−12,−13 × 2,i (13q) × 2,add(14)(p11.2) × 2,add(15)(p11.2) [9], × 2[21],der(15)t(9;15)(?q11;p11.2) × 2, −16 × 2,del(17)(p11.1p13)[8], × 2[24],−19 × 2,−21,add(21)(p11.2),−22,+3–9mar[cp69].[7]

FISH ON CULTURED CELLS

FISH was performed on cultured interphase and metaphase cells using previously G banded slides, destained in methanol, and dehydrated in a 70%, 90%, and 100% ethanol series. Centromere specific satellite probes included DXZ1, DYZ3, D7Z1, D9Z1, D15Z1, D16Z2,

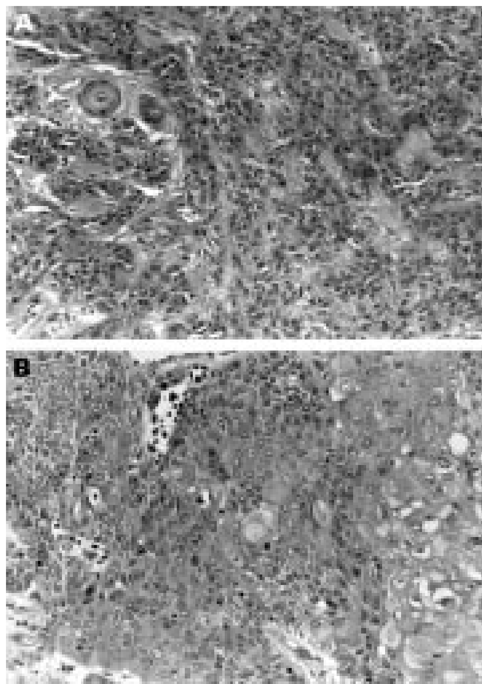

Figure 1 (A) Photomicrograph of an area of moderately well differentiated squamous cell carcinoma of the cheek (HFH-SCC-XP1 tumour). Haematoxylin and eosin stained; magnification, ×100. (B) Photomicrograph of a representative region of the HFH-SCC-8a tumour that was used for FISH analysis. This tumour was described as a moderately to poorly differentiated squamous cell carcinoma that originated from the floor of the mouth. Haematoxylin and eosin stained; magnification, ×100.

and D17Z1 (Oncor Inc, Gaithersburg, Maryland, USA). For single colour FISH, 30 ng of centromere specific satellite probe (3 µl) and 30 µl hybridisation buffer were used. The fluorescein labelled chromosome 9 paint probe (Oncor) was dispensed and pretreated according to the manufacturer's recommendations. Hybridisation, postwashing, amplification, and detection procedures were the same as those used with paraffin wax sections. For each centromere specific satellite probe, FISH signals were scored in 100 interphase and six or more tumour metaphases. Fifteen metaphases were scored for identification of chromosome 9 with whole chromosome 9 specific paint on HFH-SCC-XP-1.

PROBING TUMOUR SECTIONS

FISH on histological sections from the original tumours was performed using the "chromosome in situ kit" (S1372-TF) from Oncor. The hybridisation, signal detection, and amplification procedures were performed according to the manufacturer's published protocol, with minor modifications as described previously.[8] The centromeric probes listed above were biotin labelled and the resulting signals were designated as Xcen, 4cen, 7cen, 9cen, 15cen, 16cen, and 17cen. FISH signals were scored in areas of HFH-SCC-XP1 (fig 1A) and HFH-SCC-8a (fig 1B) that contained carcinoma. In some histological sections of HFH-SCC-XP-1 (not shown), normal epithelial cells were discernable at the periphery and these areas were also scored for comparison with the tumour. For each probe, 300–500 tumour nuclei were scored on 4 µm histological sections, on slides marked to localise tumour areas after comparison with corresponding haematoxylin and eosin stained sections.

Chromosome specific centromeric probes (Oncor) were chosen based on karyotypes of the cultured tumours. In HFH-SCC-XP-1, the hypodiploid subclones had one copy of chromosome 4, two derivatives of chromosome 9, consisting of i(9p) and i(9q), two structurally normal copies of chromosome 17, and one intact X chromosome. The corresponding hypotetraploid subclones displayed two copies each of chromosomes 4, i(9p), i(9q), and X, and four copies of chromosome 17. FISH probes specific for Xcen, 4cen, 9cen, and 17cen, and human chromosome 9 specific paint (Oncor) were used for analysis of this tumour. HFH-SCC-8a was hypotetraploid with two copies of X, 9, and 16, three or four

Table 1 FISH analysis of HFH-SCC-XP-1

	Cultured cells				Paraffin wax embedded sections: Normal epithelium		Paraffin wax embedded sections: Tumour epithelium	
	No of signals	No of interphases	Percentage	No of metaphases	No of nuclei	Percentage	No of nuclei	Percentage
X chromosome	0	26	26		7	7	70	14
	1	22	22		100	94	335	65
	2	50	50	3			97	19
	>2	2	2				1	0.2
	Total	100		3	107		513	
Chromosome 4	0	27	27		15	4	76	11
	1	37	37		40	11	405	59
	2	33	33	3	295	84	194	28
	3	3	3				4	0.6
	Total	100		3	350		689	
Chromosome 9	0	18	18				67	12
	1	34	34				408	76
	2	46	46	3			64	12
	>2	2	2				1	0.1
	Total	100		3			540	
Chromosome 17	0	8	8				89	16
	1	6	6				59	10
	2	11	11				411	72
	3	31	31	3			15	2.2
	4	40	40					
	5	4	4					
	Total	100		3			569	

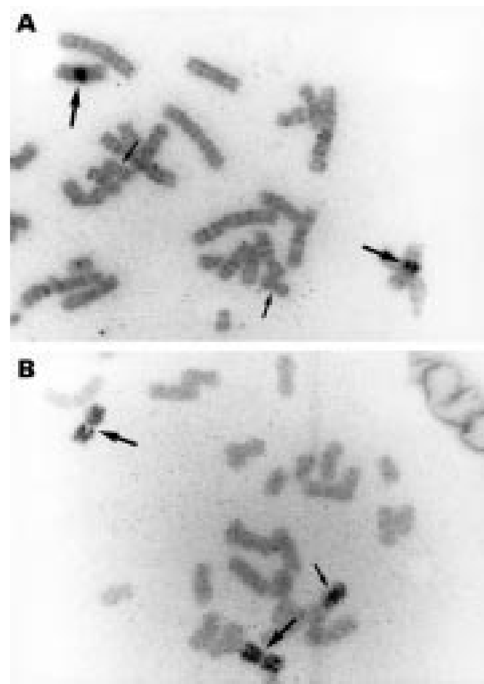

Figure 2 (A) FISH with centromere probe D9Z1 showing 9cen signals on the i(9q) chromosome only (large arrows). The i(9p) copies in this field (small arrows) did not give a hybridisation signal with the 9cen probe. (B) Chromosome 9 painting of HFH-SCC- XP-1. In this photograph from a previously G banded near tetraploid metaphase, both i(9q) chromosomes (large arrows) and one i(9p) chromosome (small arrow) are present and stained.

Table 2 FISH analysis of HFH-SCC-XP-8a

	Cultured cells				Paraffin wax embedded sections	
	No of signals	No of interphases	Percentage	No of metaphases	No of nuclei	Percentage
X chromosome	0	10	6		143	28
	1	15	10		126	24
	2	121	80	6	244	43
	>2	5	3		6	1
	Total	151		6	519	
Chromosome 7	0	9	7		78	15
	1	11	8		48	9
	2	17	13		45	9
	3	39	29		234	46
	4	52	39	4	94	18
	>4	6	4		19	4
	Total	134		4	518	
Chromosome 9	0	21	19		112	21
	1	40	36		149	28
	2	50	45	3	265	50
	>2					
	Total	111		3	528	
Chromosome 15	0	18	15		80	13
	1	15	13		76	13
	2	19	16		72	12
	3	30	33	3	280	46
	4	26	22		96	16
	Total	117		3	604	
Chromosome 16	0	25	18		58	11
	1	20	14		34	6
	2	30	21		99	18
	3	51	36	2	220	41
	4	15	11	1	90	17
	5				33	6
	Total	141		3	534	
Chromosome 17	0	9	6			18
	1	12	8		54	9
	2	21	14		78	13
	3	40	27	3	285	47
	4	52	35	2	63	10
	>4	12	8		9	2
	Total	146		5	599	

copies of chromosome 7 and the centromeric regions of 15, respectively, two copies of an intact chromosome 17, and one or two copies of a del(17)(p11.1p13). Accordingly, probes for chromosomes X, 7, 9, 15, 16, and 17 were used for FISH analysis.

Results

HFH-SCC-XP-1

HFH-SCC-XP-1 was a moderately well differentiated squamous cell carcinoma (SCC) with areas containing well developed keratin pearls (fig 1A). HFH-SCC-XP-1 cultures contained both diploid and tetraploid subclones.[6] Diploid range tumour cells from this patient were expected to have one Xcen signal, one 4cen signal, two 17cen signals, and two 9cen signals (one from each isochromosome); the tetraploid tumour cells were expected to have two Xcen and 4cen signals, and four 9cen and 17cen signals. FISH analysis was performed on cultured interphase and metaphase cells from passage 5 (224–260 days) of tumour culture. Results were consistent with the previously characterised near tetraploid tumour karyotype of this subclone,[6] with most cells having two Xcen and 4cen signals, and four signals for chromosome 17 (table 1). However, there were only two signals with the 9cen probe instead of the expected four. When the classic satellite 9 probe was hybridised to metaphase chromosomes, it bound to two copies of the i(9q) chromosome, but not to the i(9p) chromosomes (fig 2A). Chromosome 9 paint confirmed that both i(9p) and i(9q) originated from chromosome 9 (fig 2B). FISH results using the same probes on 4 μm tumour sections are summarised in table 1. Both the near diploid and near tetraploid populations[6] were observed. In tumour sections most tumour nuclei expressed one signal each for Xcen, 4cen, 9cen, and two 17cen signals, consistent with a near diploid tumour cell population. However, a minority of near tetraploid tumour nuclei (12–28%) contained two signals each for Xcen, 4cen, and 9cen. A small proportion (2.2%) contained at least three 17cen signals, although most had two or fewer (table 1).

HFH-SCC-8A

HFH-SCC-8a was a moderately to poorly differentiated SCC (fig 1B). The cultured tumour cells were near tetraploid. Based on the karyotype these cells were expected to display two Xcen, 9cen, and 16cen signals, and three or four 7cen, 15cen, and 17cen signals. FISH analysis was performed on cultured interphase and metaphase cells from passage 5 (224–260 days) of tumour culture. In these, the signal frequencies indicated that there were predominantly two copies of Xcen and 9cen, four copies of 7cen and 17cen, and three copies of 15cen and 16cen (table 2). FISH results on 4 μm sections were remarkably similar to those on cultured cells (table 2), with losses consistent with sectioning artifacts. The G banded metaphase spreads had shown 15cen signals on both copies of der(15)s and one or two copies of the add(15)(p11), confirming the interphase

data of three or four copies of 15cen in each nucleus. However, the original karyotypic interpretation of only two copies of chromosome 16 was modified, based on the observed three to four 16cen signals in the cultured interphase and metaphase cells and in tumour sections (table 2). Metaphase spreads hybridised to the 16cen probe contained two normal copies of 16 and one to two copies for each cell of a previously unidentified marker chromosome that included the centromeric domain of chromosome 16.

Discussion

FISH analysis demonstrated that genetic alterations observed in vitro on cultured metaphase and interphase cells were represented in the original tumour. The near diploid and near tetraploid populations observed in vitro within HFH-SCC-XP-1 tumour tissue were recognised in histological sections. The maintenance of subclones of differing ploidy in early tumour harvests and in passaged cultures was also demonstrated by DNA content flow cytometry analysis of HFH-SCC-XP-1.[9]

Somatic cells from the donor of HFH-SCC-XP-1 contained an inv(9) variant with a large heterochromatin block translocated from the long to the short arm and a smaller heterochromatin block translocated from the short to the long arm.[6] The isochromosomes of chromosome 9 in the tumour evidently originated from the normal chromosome 9; the i(9q) had large heterochromatin blocks, and the i(9p) had smaller blocks. The failure to demonstrate 9cen on the i(9p) chromosome suggests that classic satellite DNA was lost or greatly reduced during isochromosome formation, resulting in the single 9cen signal that was observed in near diploid tumour cells. When isochromosome formation leads to unequal distribution of the pericentromeric heterochromatin, the reduction in centromere specific satellite DNA should yield a weak or absent hybridisation signal. In such cases, chromosome painting or use of regional cosmid probes might be needed to verify the isochromosome formation. The near tetraploid clone of HFH-SCC-XP-1 showed fewer than the expected number of copies of 17cen. With increasing anaplasia, the diameter of an SCC nucleus increases, doubling in poorly differentiated tumours (MJ Worsham, PhD Thesis, 1993, Wayne State University School of Medicine, USA). As nuclear size increases, the detection of multiple signals in each cell is reduced because of disproportionate effects of nuclear sectioning.[2] This was seen to a lesser extent in the signal reductions between cultured and sectioned cells of HFH-SCC-8a. Alternatively, if tetraploidisation occurred after non-dysjunctional loss of chromosome 17 from a near-diploid tumour, the resultant population would then appear disomic for this chromosome. The observed tetraploidy of HFH-SCC-8a in vitro was confirmed in tumour sections. Discrepancies in copy number of chromosome 16 were resolved by FISH analysis of previously G banded tumour metaphases, and led to identification of a marker chromosome.

Questions are often raised about the value or relevance of cultured cell lines because selection pressures in vitro might cause the cell lines to become highly divergent from the original tumour. Our data show that cell cultures can serve as an accurate snapshot of the original tumour. Other studies have shown that SCC cell lines express cell surface antigens including HLA antigens, pemphigus and pemphigoid antigens, and cell surface integrins after months or years of serial passage.[9–13] The cultured cells retain distinctive morphology in vitro, and induce tumours in nude mice with many of the same characteristics as the patients' original tumours.[14 15] Similarly, other established cell lines have stable karyotypic profiles maintained after primary passage[6 16–18]; for example, the Philadelphia chromosome in B cell derived tumours,[19 20] the t(8;14) translocation in Burkitt's lymphoma cell lines,[21] and specific chromosomal aberrations in other SCCHN.[16]

In our report we show concordance of cytogenetic alterations between cultured cells and nuclei from the original tumour, indicating that cell lines from human cancers can serve as valuable resources for ongoing investigations. The culture karyotypes were validated as representative of genetic alterations that arose in vivo, and the combined analyses permitted a more accurate interpretation of the tumour karyotypes. FISH and culture karyotypes complemented each other in defining genetic markers in SCCHN, and for evaluation of tumour heterogeneity.

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