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Sequential Cytogenetic Alterations in Hamster Oral Keratinocytes During DMBA-Induced Oral Carcinogenesis

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Using the hamster cheek pouch oral cancer model, we have performed a comprehensive analysis of the cytogenetic changes in hamster oral keratinocytes during 7,12-dimethylbenz[*a*]anthracene (DMBA)-induced carcinogenesis. Tumour induction in the hamster cheek pouch required repeated application of the carcinogen for 14 weeks. We have found that this hamster oral cancer model to be suitable for cytogenetic studies. Unlike human oral cancers where chromosome breaks have been shown, this is only infrequently observed in DMBA-treated hamster oral keratinocytes. Of importance is the finding that at the beginning of the second week of DMBA treatment, there is a significant increase of karyotypes demonstrating tetraploid or near-tetraploidy. We propose that the significant increase in hamster oral keratinocytes exhibiting tetraploidy be further evaluated as a marker of premalignancy/malignancy.

Keywords: oral cancer, cytogenetics, animal model, hamster
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INTRODUCTION

SQUAMOUS CELL carcinomas account for over 90% of human oral cancers [1]. The molecular mechanism leading to the development of oral cancer is not understood. It is currently believed that the inappropriate activation and/or inactivation of a number of cellular genes (oncogenes and tumour suppressor genes) can result in the development of epidermoid carcinomas [2]. These alterations at the genetic level can be manifested as lesions evident at the cytogenetic level. There is much interest in the study of the chromosomal make-up of solid tumours with the ultimate goal towards the identification of genes involved in neoplastic transformation. Cytogenetic lesions such as breaks, deletions, translocations, and others in various cancers have proven to have clinical usefulness [3]. Consistent cytogenetic changes in tumours can be viewed as part of the necessary genetic perturbations for the development of malignancy.

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A major gap in the current understanding of the importance of cytogenetic changes in carcinogenesis is the lack of detailed and comprehensive pathology. Towards this goal, we have chosen to use the well-established hamster cheek pouch carcinoma model to elucidate the pattern of sequential cytogenetic changes during oral carcinoma induction by the chemical carcinogen 7,12-dimethylbenz[*a*]anthracene (DMBA) [4]. This animal oral cancer model has been shown to exhibit similarities to human oral cancer development at the clinical, histological, biochemical, as well as molecular levels [5–8]. Furthermore, cytogenetics for the Syrian hamster is well established. The first G-band karyotype and idiogram for arranging Syrian hamster chromosomes was originally proposed by Lehman *et al.* [9]. Pavia *et al.* [10] precisely measured the Syrian hamster karyotypes to determine total lengths and arm ratio and Li *et al.* [11] proposed an idiogram for metaphase and late prophase chromosomes and a nomenclature system for identifying individual bands of Syrian hamster chromosome. In addition the Syrian hamster has been used to document cytogenetic changes associated with the process of carcinogenesis [12]. Thus there is a wealth of information on the cytogenetics of the Syrian hamster. In this report, we characterised the sequential cytogenetic changes in the hamster oral keratinocytes during the DMBA-tumour induction process. The results showed that the increase of the number of tetraploid and near-tetraploid chromosomes of hamster oral keratinocytes can be used as a marker for DMBA-induced hamster oral carcinogenesis.

MATERIALS AND METHODS

DMBA-induced hamster cheek pouch tumours

Epidermoid carcinomas were induced in the cheek pouches of Syrian hamsters according to the protocol of Shklar [4]. This is a 14-week tumour induction protocol with the DMBA-treated hamster oral epithelium developing typical histopathological lesions (hyperplasia, dysplasia and carcinoma). The chemical carcinogen was a 0.5% solution of 7,12-dimethylbenzanthracene (0.5 g/100 ml) (DMBA, D-3254, Sigma Chemical Co.) dissolved in mineral oil (U.S.P.). The left buccal pouch of the hamsters was painted three times weekly with either 0.5% DMBA in mineral oil or mineral oil only, using a no. 4 soft sable brush. The body weight of the hamsters was recorded every week. Buccal pouches of hamsters were grossly examined at weekly intervals for epithelial changes and tumour development from week 2 to week 14. For the DMBA-treatment group, five hamsters were killed every 2 weeks (0, 2, 4, 6, 8, 10, 12 and 14) to produce oral keratinocytes cultures for cytogenetic analysis.

For the control group treated with mineral oil, five hamsters were killed to obtain oral keratinocyte cultures for cytogenetic analysis at weeks 10, 12 and 14. We have performed preliminary experiments and showed that there are no significant cytogenetic changes in the mineral oil treatment group prior to week 10.

Dissociation and culture of hamster oral keratinocytes

The procedure used for the dissociation, isolation and culture of hamster oral keratinocytes is based on the method of Polverini and Slot [13]. Briefly, buccal pouches of hamsters were excised aseptically, the hamster buccal pouch epithelium was detached enzymatically with a mixture of collagenase (type II, 1 mg/ml) (Worthington, Freehold, New Jersey, U.S.A.) and dispase (grade II, 2.4 mg/ml) (Boehringer Mannheim Biochemical, Indianapolis, Indiana, U.S.A.) for 1 h at 37°C. Single cell suspensions were prepared from the dissociated hamster oral epithelium using trypsin (0.5 mg/ml) (Gibco/BRL) and DNase (grade I, 0.06 mg/ml) (Calbiochem-Behring Corp, San Diego, California, U.S.A.). The resultant cell suspension was reconstituted into DMEM medium containing 20% heat-inactivated fetal bovine serum, 0.4 μg/ml of hydrocortisone (Collaborative Biomedical Products, Bedford, Massachusetts, U.S.A.), 10 ng/ml of epidermal growth factor (Collaborative Biomedical Products), 100 units/ml of penicillin, and 100 μg/ml of streptomycin (Gibco/BRL). Cells were then incubated at 37°C and 5% CO_2. Culture media were changed once every 3 days.

Karyotype analysis of hamster oral keratinocytes

Chromosomal analysis of the hamster oral keratinocytes harvested at different time points of DMBA or mineral oil treatments were carried out using short-term cultures according to previously described techniques [14, 15]. Cultured hamster oral keratinocytes, upon reaching 70–80% confluence, were treated with Colcemid (1.0 μg/ml) (Gibco/BRL) for 4 h. The keratinocytes were then detached from the flasks with trypsin-EDTA (Gibco/BRL). The resultant cells were resuspended in 0.075 M KCl for 10 min, at which time three–four drops fresh fixative (methanol:acetic acid at 3:1) was added. The cells were then washed three times with fresh fixative, dropped onto the surface of clean, wet slides and then air-dried. Slide were stained with 25% Wright's stain for 60–100 s. At each of the experimental time points, metaphase cells were scored from 100 well-spread metaphases for the experimental group. For the control group, 50 metaphases from weeks 10, 12 and 14 were examined.

Statistical analysis

Statistical analysis were performed using Student's *t*-test and the critical ratio analysis for the comparison of two proportional variables [16].

RESULTS

Using the tumour induction protocol of Shklar [4], epithelial alterations were consistently produced in the DMBA-treated hamster cheek pouches. Epithelial hyperplasia was seen by the 3rd week, dysplasia by the 6th week, carcinoma *in situ* by the 8th week, and epidermoid carcinoma by the beginning of the 10th week.

Sequential karyotypic analysis of hamster oral keratinocytes during DMBA tumour induction

Hamster oral keratinocyte cultures harvested at different time points of DMBA-treatment were analysed for cytogenetic alterations. One hundred metaphase spreads were examined from the five hamsters at each time point. The Syrian hamster's normal diploid number is 44 chromosomes (21 pairs of autosomes and two sex chromosomes). Table 1 summarises the karyotypes and cytogenetic changes in DMBA-treated hamster oral keratinocytes at 0, 2, 4, 6, 8, 10, 12 and 14 weeks. Karyotypic analysis of control hamster oral keratinocytes from pouches treated with mineral oil are summarised in Table 2. For the control studies, only 50 metaphases were examined from the five hamsters at weeks 10, 12 and 14. Figure 1 is a representative karyotype (44, XY) of a normal hamster keratinocyte.

Chromosome ploidy analysis

Chemical carcinogens are known to cause chromosomal numerical changes [12]. Examination of the karyotypes in Table 1 revealed that with progressive DMBA treatment, there is an accompanying increase in tetraploidy or near-tetraploidy, with a corresponding decrease in diploid or near-diploid karyotypes. We compared the two proportions between diploid (and near-diploid) and tetraploid (and near tetraploid) groups using the critical ratio analysis [16]. These results are shown in Table 3 for the DMBA treatment group, and in Table 4 for the mineral oil treatment group.

DMBA treatment of hamster oral keratinocytes increased the number of tetraploid (and near-tetraploid) karyotypes in all the groups examined (compared to week 0). The differences are statistically significant as early as week 2 ($P<0.05$), and remained significant from week 2 to 10 ($P<0.05$). For hamster oral keratinocytes examined after the 12th and 14th weeks of DMBA treatment, the differences are highly significant ($P<0.01$). Mineral oil treatment on the other hand, did not result in statistical differences between the proportions of diploid and tetraploid karyotypes at weeks 10, 12 or 14 ($P>0.05$). Figure 2 is the karyotype of a hamster oral keratinocyte treated with DMBA for 10 weeks exhibiting tetraploidy (88, XXYY).

Table 1. Summary of karyotypes in hamster oral keratinocytes with progressive DMBA treatment. *100 *metaphases were examined at each time point*

Week	Karyotypes	No. of cells*
0	42, Y, −4, −X	1
	43, XY, −7	1
	43, XY, −11	1
	43, XY, −16	1
	43, XY, −18	1
	43, XY, −19	1
	43, XY, −21	1
	44, XY	**85**
	44, XY, b2qc2	1
	44, XY, del(1) (qa1→qter)	1
	87, XXYY, del(4) (qb1→qter)	1
	87, XXXY, −1, −2, −6, +15, +X	1
	87, XXYY, −16	1
	88, XXYY, +15, −19	1
	88, XXYY	2
2	42, XY, −2, −21	1
	43, XY, −5	2
	43, XY, −16	1
	44, XY	**75**
	44, XY, del(13) (pa1→pter)	1
	44, del(X) (qa6→qter), Y	1
	45, XY, +6	1
	45, XY, del(2) (pa1→pter), +M1	1
	45, XXY	1
	81, XY, −2, −7, −10, −11, −21, −X, −Y	1
	82, XY, −1, −2, −5, −14, −X, −Y	1
	83, XXYY, −3, −7, −8, −13, −17	1
	84, X, del(X) (qa1→qter), YY, −2, −7, −18, −19	1
	84, XXYY, −5, −11, −16, −21	1
	84, X, del(X) (qa4→qter), YY, −1, b2qc3, −9, −10, −20, +M2	1
	85, XXYY, −2, −5, −12	1
	86, XXYY, −16, −17	1
	86, XXYY, −16, −21	1
	87, XXYY, −5	1
	87, XXYY, −7	1
	88, XXYY	3
	88, XXYY, b8pa2	1
	88, XX, 2del(Y) (qa5→qter)	1
4	43, XY, −8	1
	43, XY, −10	1
	43, XY, −14	1
	43, XY, −17	1
	43, X, −Y	2
	44, XY	**76**
	42, XY, −14, −20	1
	44, XY, del(3) (pa1→pter)	1
	44, XY, del(10) (qa2→qter) (qa2→pter)	1
	44, X, bYqa5	1
	70, XXYY, −1, −1, −2, −5, −7, −7, −10, −11, −12, −13, −16, −16, −17, −17, −18, −19, −19, −20, −20, +M3	1
	72, XXYY, −1, −2, −2, −7, −7, −10, −12, −13, −13, −15, −17, −17, −18, −18, −19, −21	1
	77, XXYY, −1, −2, −2, −4, −4, −5, −7, −7, −8, −9, −17, −21, +M3	1
	83, XXYY, −1, −5, −16, −19, −20	1
	85, XXY, −13, −16, −Y	1
	86, XXYY, −7, −8	1
	86, XXYY, −7, −9, −11, +M4	1
	87, XXYY, −4	1
	87, XXYY, −15	1
	87, XXYY, −16	1
	88, XXYY	3
	90, XXYYY, +14	1

Table 1. Continued

Week	Karyotypes	No. of cells*
6	42, XY, −1, −21	1
	42, XY, −2, −5	1
	43, XY, −4	1
	43, XY, −5	2
	43, XY, −6	1
	43, XY, −12	1
	43, XY, −14	1
	44, XY	**78**
	81, XXYY, −1, −2, −13, −14, −16, −19, −19	1
	82, XXYY, −4, −8, −12, −14, −19, −20	1
	83, XXYY, −7, −10, −12, −14, −16	1
	83, XXYY, −2, −4, −9, −16, −18	1
	86, XXYY, −11, −13	2
	87, XXYY, −7	1
	87, XXYY, −13, del(3) (pa1→pter)	1
	88, XXYY	3
	88, XX, 2del(Y) (qa3→qter)	1
	88, X, del(X) (qa4→qter), YY, +5, −12, −14, +16	1
	88, XXXXYY, −10, −12	1
8	43, XY, −1	1
	43, XY, −5	1
	43, XY, −9	1
	43, XY, −10	2
	43, XY, −11	2
	43, XY, −12	1
	43, XY, −13	1
	43, XY, −17	2
	43, XY, −10, −20, +t(15q; 15q)	1
	43, X, −Y	1
	43, XY, −4, −14, del(5) (pa2→pter), +M5	1
	44, XY	**70**
	44, XY, del(19) (pa12→qter)	1
	45, XY, +t(11q; 11q)	1
	75, XXYY, −1, −1, −3, −5, −6, −8, −9, −9, −12, −13, −16, −16, −17	1
	86, XXYY, −2, −2	1
	86, XXYY, −7, −12	1
	87, XXYY, −21	1
	89, XXYY, +18	1
	88, XXYY	9
10	43, XY, −14	1
	43, XY, −14, bXqa9	1
	43, XY, −20	1
	43, XY, −21	2
	43, X, −Y	1
	44, XY	**56**
	45, XY, +8	1
	45, XY, +20	1
	45, XYY	1
	68, XY, −2, −3, −5, −5, −7, −9, −11, −15, −15, −17, −18, −18, −19, −19, −20, −20, −21, −X, −Y	1
	70, XX, −1, −2, −3, −4, −5, −6, −6, +9, −11, −11, −13, −13, −14, −15, −16, −17, −18, −18, −19, −20, −20, −Y, −Y	1
	76, XXYY, −1, −2, −6, −7, −8, −9, −11, −12, −13, −15, −18, −20	1
	80, XX, −1, −1, −3, −4, −8, −9, −14, −20, −Y, −Y	1
	81, XYY, −4, −10, −17, −20, −20, −20, −X	1
	82, XXYY, −2, −5, −10, −18, −19, −Y	1
	82, XXYY, −3, −5, −9, −10, −13, −13	1
	83, XYY, +8, −12, −13, −17, −17, −17, −X	1
	83, XXYY, −17, −18, −18, −19, −21	1
	83, XXYY, −7, −8, −15, −18, −19	1
	84, XXYY, −1, −10, −10, −21	1
	84, XXYY, +1, +1, +1, +2, −3, +4, −5, −6, −6, −8, −9, +10, +10, −11, −11, −11, −13, −15, −18, +21	1

Table 1. *Continued*

Week	Karyotypes	No. of cells*
	85, XXYY, −2, +9, −11, −14, −19	1
	85, XXYY, −1, −2, −21	1
	85, XXYY, −4, −4, −11	1
	85, YY, −17, −X, −X	1
	86, XXYY, −2, −7	1
	86, XXYY, −2, −3	1
	86, XXYY, −11, −19	1
	87, XXYY, +7, −9, −16	1
	87, XXYY, −12	1
	87, XXYY, −13	1
	87, XXYY, −8, −17, +18	1
	87, XXYY, −3, del(7) (qa1→qter)del(11) (qa1→qter)	1
	87, XXYY, −Y	1
	88, XXYY, +2, −17	1
	88, XXYY, del(16) (qb1→qter)	1
	88, XXYY, −11, +14	1
	88, XXYY, −9, −11, −11, +15, +M6	1
	88, XXYY	6
12	42, XY, −2, −6	1
	43, XY, −8	2
	43, XY, −14	1
	43, XY, −20	1
	43, XY, −21	2
	43, X, −Y	3
	44, XY	**62**
	44, XY, del(1) (qb2→qter)	1
	44, XY, del(11) (pa1→pter)	1
	44, del(X) (qa7→qter), Y	2
	44, X, del(Y) (qa4→qter)	1
	45, XY, +8	1
	45, XY, +8, del(2) (qc1→qter)	1
	45, XXY	1
	45, XYY	2
	64, XYY, −2, −3, −3, −4, −5, −9, −9, −10, −10, −11, −11, −12, −12, −13, −13, −14, −14, −14, −16, −18, −20, −20, −21, −X	1
	80, XXY, −7, −7, −12, −13, −15, −20, −21, −Y, del(2) (pa3→pter)	1
	84, XXYY, −2, −10, −19, −21	1
	84, XXYY, −3, −7, −8, −21	1
	85, XXYY, −4, −10, −10, −14, +18	1
	85, XXYY, −8, −17, −21, del(1) (qa1→qter)	1
	85, XXYY, −2, −6, −8, −11, +15	1
	86, XYY, −21, −X	1
	86, XXYY, +1, −4, −7, −8	1
	87, XXYY, −3, −7, −21, +10, +14	1
	87, XXYY, −4	1
	87, XXYY, −7, −7, −17, −18, +21, 2del(X) (qa5→qter)	1
	87, XXYY, −21, del(12) (pa1→pter)	1
	88, XXYY, −8, −9, −12, −16, +20, +M7	1
	88, XXYY, −21, +15	1
	88, XXYY, +4, −21	1
	88, XX, 2del(Y) (qa1→qter)	1
	88, XXYY	1
	89, XXYY, −10, −11, +17, +18, +M8	1
14	43, XY, −20	1
	43, XY, −21	1
	44, XY	**75**
	44, XY, del(9) (pa3→pter)	1
	44, del(X) (qa1→qter), Y	1
	44, XY, −6, +20	1
	44, XY, b1qc4	1
	45, XY, +18	1
	83, XXYY, −2, −3, −4, −6, −14	1
	84, YY, −4, −4, −X, −X	1

Table 1. Continued

Week	Karyotypes	No. of cells*
	86, XXYY, −16, −17	1
	87, XXYY, −9, −13, +12	1
	87, XXYY, −21	1
	88, XXYY	5
	88, XXYY, +7, −13, del(1) (qb3→qter)	1
	88, XXYY, +3, −15	1
	88, XXYY, −11, +15	1
	89, XXYY, +1, −7, +14, −16, +20	1
	89, XXYY, +3, −18, +M9	1
	89, XXYY, +15	1
	91, XXYYY, +1, +1, −4, −4, +5, +18, +Y	1

*Table 2. Summary of karyotypes in hamster oral keratinocytes with mineral oil for 10, 12 and 14 weeks. *50 metaphases were examined*

Week	Karyotypes	No. of cells*
10	43, X, −13	1
	44, XY	**45**
	44, XY, del(3) (Pal→Pter)	1
	44, XY, b(10)qa4	1
	88, XXYY	1
	88, XXYY, +5, −17, −19, −20, +21, +Mar.	1
12	43, XY, −13	1
	43, XY, −16	1
	43, XY, −18	1
	44, XY	**41**
	44, XY, b(11)qb4	1
	45, XY, +11	1
	87, XXYY, −7	1
	88, XXYY	2
	89, XXYY, +5, −7, +14	1
14	41, del(X) (qa6→qter), Y, −3, −9, −21	1
	43, XY, −11	1
	44, del(X) (qa6→qter), Y	1
	44, XY	**43**
	45, XY, +11	1
	45, XXY	1
	88, XXYY	2

Chromosome loss/gain in hamster oral keratinocytes during DMBA-induced carcinogenesis

Tables 5 and 6 summarise the observed specific chromosome lost in hamster oral keratinocytes in the DMBA treatment and mineral oil treatment groups, respectively. The observations were further grouped according to the ploidy of the karyotypes (diploid/near-diploid vs. tetraploid/near-tetraploid). For the DMBA-treated hamster oral keratinocytes, with the exception of week 0, there were greater numbers of chromosome lost in the tetraploid group than the diploid group (Table 5). The total number of chromosome lost in the tetraploid group was 375, compared to 56 in the diploid group. Considering that the majority of the karyotypes in the DMBA treatment groups are diploid and near-diploid (65–94%), these results suggest a mechanism for the preferential loss of chromosomes from the DMBA-treated hamster oral keratinocytes exhibiting a tetraploid karyotype. In the diploid group, all chromosome except 1, 3, 15 were affected, while in the tetraploid group all of chromosomes had been observed to be affected. The frequency of specific chromosome loss ranged from 1.8% (1/56) to 12.5% (7/56) in the diploid group and 2.4% (9/375) to 7.2% (27/375) in the tetraploid group. Table 6 shows the specific chromosome loss in each of the 50 karyotypes observed in the mineral oil treatment group at weeks 10, 12 and 14. Comparing with the DMBA treatment group, which we documented 100 karyotypes, the frequency of

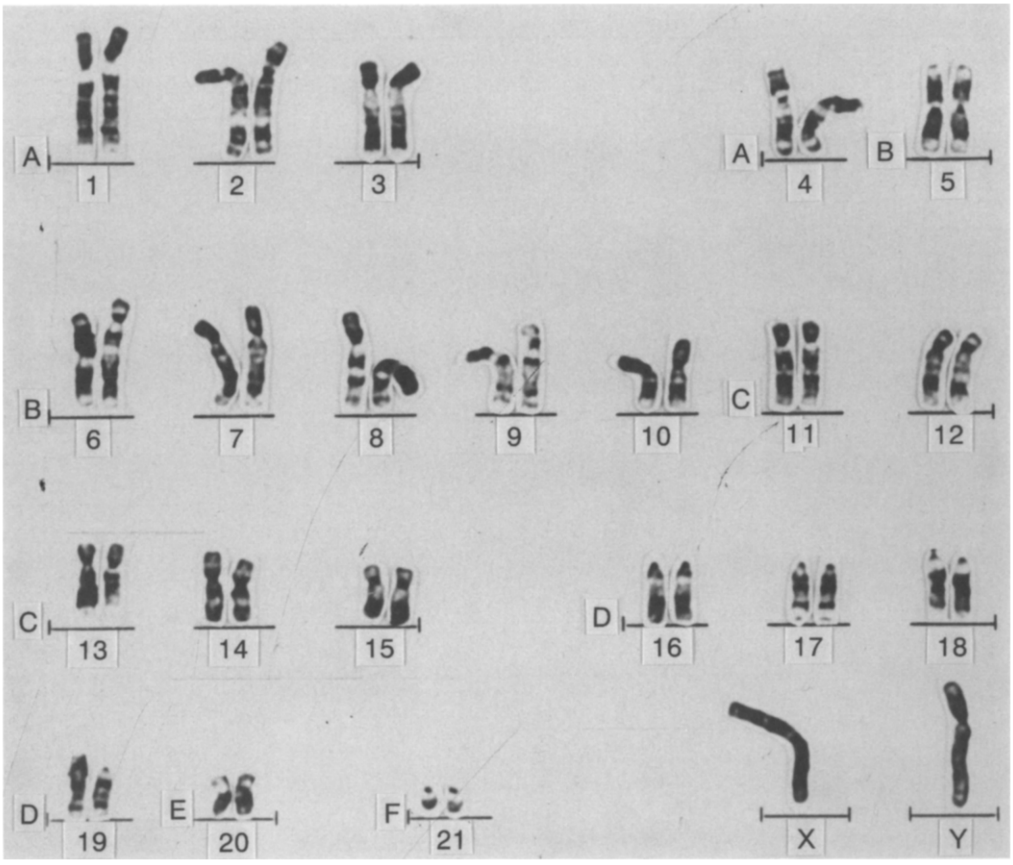

Fig. 1. Karyotype of normal hamster oral keratinocyte: 44, XY.

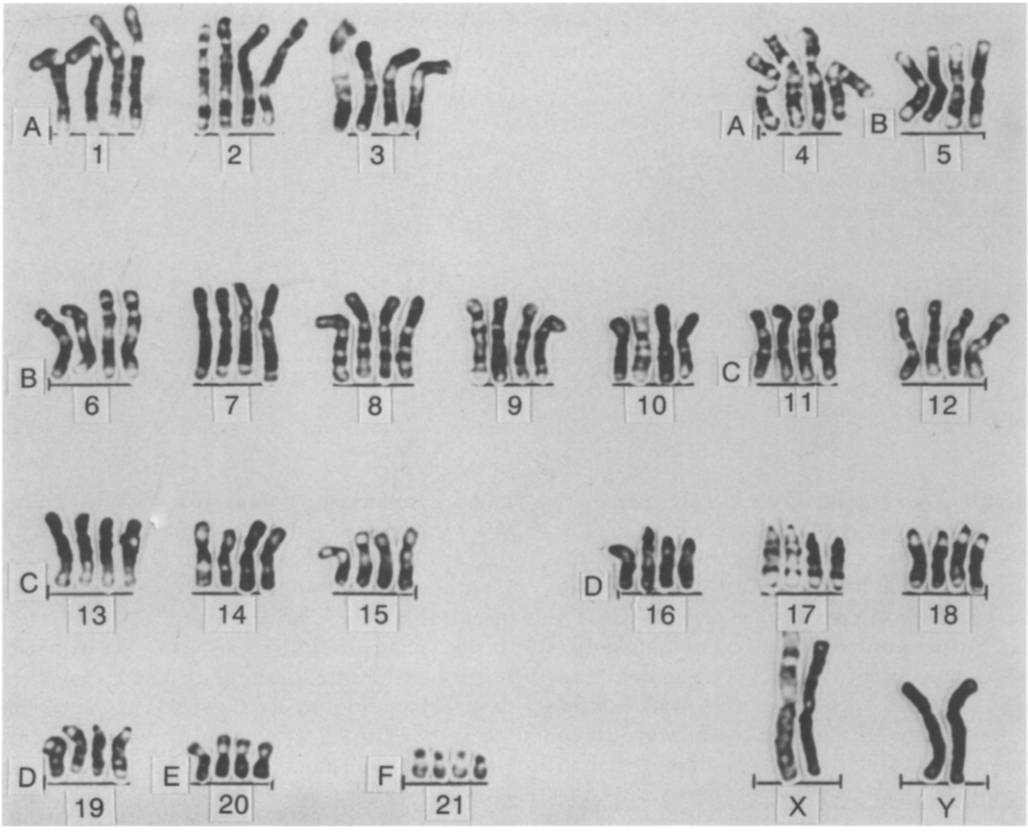

Fig. 2. Karyotype of hamster oral keratinocyte treated with DMBA for 10 weeks demonstrating tetraploid: 88, XXYY.

Table 3. Analysis of ploidy distribution in hamster oral keratinocytes at different weeks of DMBA treatment

Weeks after DMBA painted	Ploidy distribution: 2N±n (no. of 44, XY) / 4N±n (no. of 88, XXYY)	Cells no.	P*
0	94 (85) / 6 (2)	100	
2	84 (75) / 16 (3)	100	<0.05
4	86 (76) / 4 (3)	100	<0.05
6	86 (78) / 14 (3)	100	<0.05
8	86 (70) / 14 (9)	100	<0.05
10	65 (56) / 35 (6)	100	<0.05
12	82 (62) / 18 (1)	100	<0.01
14	83 (75) / 17 (5)	100	<0.01

P*: Comparsion of two proportions between diploid (near-diploid) group and tetraploid (near-tetraploid) group using the critical ratio of difference in proportions for the comparison of two proportions in independent samples [16].

Table 4. Analysis of ploidy distribution in hamster oral keratinocytes treated with mineral oil

Weeks of mineral oil treatment	Ploidy distribution: 2N±n (no. of 44, XY) / 4N±n (no. of 88, XXYY)	Cells no.	P*
0	94 (85) / 6 (2)	100	
10	48 (85) / 2 (1)	50	>0.05
12	46 (41) / 4 (2)	50	>0.05
14	48 (43) / 2 (2)	50	>0.05

P*: Comparision of two proportions between diploid (near-diploid) group and tetraploid (near-tetraploid) group using the critical ratio of difference in proportions for the comparison of two proportions in independent samples [16].

chromosome loss were similar for the diploid groups. In the tetraploid groups, the numbers of chromosome loss were 149/49/24 in the DMBA-treatment group, while for the mineral oil group the numbers were 3/2/0. Thus the number of chromosome loss in the DMBA-tetraploid group was much higher than the mineral oil-tetraploid group at weeks 10, 12 and 14.

Tables 7 and 8 summarise the observed specific chromosome gain in the DMBA-treated and mineral oil-treated

Table 5. Chromosome loss in hamster oral keratinocytes during progressive treatment with DMBA (results from 100 metaphases per 2 weeks)

	Diploid/weeks									Tetraploid/weeks								
Cx	0	2	4	6	8	10	12	14	Td	0	2	4	6	8	10	12	14	Ttr
1				1	1				2	1	2	5	1	2	6			17
2		1		1			1		3	1	4	5	2	2	8	2	2	26
3									0		1			1	7	2	1	12
4	1			1	1				3			3	2		5	3	5	18
5		1		2	1				4		4	3		1	7			15
6				1			1	1	3	1				1	5	1	1	9
7	1								1		4	8	2	1	4	7	1	27
8			1				1		2		1	2	1	1	5	5		15
9					1				1		1	2	1	2	8	1	1	16
10			1		2				3		2	2	2		6	4		16
11	1				1				2		2	2	1		13	2	2	22
12				1	1				2		1	2	3	2	3	2		13
13					1				1		1	4	3	1	9	1	2	21
14			2	1	1	2	1		7		1		3		3	1	1	9
15									0			2			6	1	1	10
16	1	1							2	1	3	5	4	2	2	1	2	20
17			1		1			1	3		2	5		1	9	2	1	20
18	1								1		1	3	1		10	1	1	17
19	1								1	1	1	3	3		8	1		17
20			1		1	1	1	1	5		1	2	1		9	1		14
21	1	1		1		1	1	1	6		3	2		1	4	9	1	21
X	1								1		2				5	1	2	10
Y			1		1		1		3		2				7	1		10
T	8	4	7	9	13	4	7	4	56	5	39	60	30	18	149	49	24	375

Cx: Chromosome number. Td: Total chromosomes loss in diploid metaphases. Ttr: Total chromosomes loss in tetraploid metaphases. T: Total chromosomes loss per 2 weeks.

Table 6. Chromosome loss in hamster oral keratinocytes treated with mineral oil for 10, 12 and 14 weeks (results from 50 metaphases per 2 weeks)

	Diploid/weeks				Tetraploid/weeks			
Cx	10	12	14	Td	10	12	14	Ttr
1				0				0
2				0				0
3			1	1				0
4				0				0
5				0				0
6				0				0
7				0		2		2
8				0				0
9			1	1				0
10				0				0
11			1	1				0
12				0				0
13	1	1		2				0
14				0				0
15				0				0
16		1		1				0
17				0	1			1
18		1		1				0
19				0	1			1
20				0	1			1
21			1	1				0
X				0				0
Y				0				0
T	1	3	4	8	3	2	0	5

Cx: Chromosome number. Td: Total chromosomes loss in diploid metaphases. Ttr: Total chromosomes loss in tetraploid metaphases. T: Total chromosomes loss per 2 weeks.

hamster oral keratinocytes, respectively. For the DMBA-treated hamster oral keratinocytes with a diploid/near diploid karyotype, gains of chromosomes 6, 8, 18, 20, X and Y were observed (Table 7). For those DMBA-treated hamster oral keratinocytes exhibiting a tetraploid/near-tetraploid karyotype, with the exception of chromosomes 6, 11, 13, 17 and 19, gain in all other chromosomes had been observed (Table 7). The total number of chromosomes gained in the diploid group was 10 vs. 48 in the tetraploid group. Table 8 shows the corresponding data for the mineral oil-treated hamster oral keratinocytes. In the diploid group, only chromosome 11 was observed to be gained; while in the tetraploid group chromosomes 5, 14, and 21 were seen to be gained. The frequencies of total chromosomes gained were similar in the diploid groups between the DMBA (2/4/2) and mineral oil (0/1/1) treatment, In the tetraploid group, the frequency of chromosome gain is higher in the DMBA (16/9/13) than the mineral oil (2/2/0) group.

Chromosome structural aberrations

We examined karyotypes of hamster oral keratinocytes for evidence of chromosome structural aberrations in terms of breaks, deletions and translocations. The results are summarised in Tables 9 and 10 for the DMBA and mineral oil treatment groups, respectively. In the DMBA-treated hamster oral keratinocytes, deletions accounted for the majority of the structural aberrations detected (34/42, 81%) (Table 9). Of these observed deletions, chromosomes X and Y accounted for 41% (14/34). Mineral oil-treated hamster oral keratinocytes, on the other hand exhibited a much lower frequency of structural aberrations. The numbers of breaks and translocations were similar to that of the DMBA-treated groups for 10, 12 and 14 weeks. Frequencies of deletions, however, were higher in the DMBA-treatment group. Figure 3 is the karyotype of a DMBA-treated hamster oral keratinocyte exhibiting 88, XXY del(Y) ($qa_1 \rightarrow qter$).

DISCUSSION

Using the well-established DMBA-induced hamster oral cancer model, we have performed an extensive cytogenetic analysis of the karyotypic changes in the hamster oral keratinocytes during the malignant transformation process. We have found the following three consistent alterations. First, by week 10 of DMBA treatment and thereafter, there were increases in the frequency of chromosome loss and chromosome gain in hamster oral keratinocytes exhibiting tetraploid/near tetraploid karyotypes. Second, also by week 10 of DMBA treatment and thereafter, the frequency of chromosomal deletions was increased. Third, the number of hamster oral keratinocytes exhibiting tetraploid/near-tetraploid increased significantly at the beginning of the second week of DMBA treatment.

Jin *et al.* examined the karyotypes of 12 human oral cancers and found a series of chromosomal breakpoints [17, 18]. Two other studies have attempted to determine cytogenetic changes in human head and neck cancers. Jin *et al.* have found that in 15 human head and neck cancers, there were frequent clusterings of chromosomal break points at 1p22 and 11q13 [19]. Since the cellular oncogenes *bcl*-1, *int*-2, and *hst*-1 were mapped to 11q13, while n-*ras* has been mapped to 1p22, these authors proposed that activation of these oncogenes located in these bands may proceed by cytogenetic mechanisms. More recently, Owens *et al.* have examined 10 human head and neck squamous cell carcinomas and found that there were three or more breakpoints at 1p36, 9q32 and 11q23 [20]. Thus from the existing literature on human oral cancer cytogenetics, chromosomal breaks seemed to be the major alterations observed. Our present study using the hamster oral cancer model suggest that chromosomal breaks in the DMBA treatment groups does not differ from that of mineral oil controls (Tables 9 and 10). The differences between the human and hamster studies could be due to: (1) the hamster oral cancer model permits a comprehensive analysis (100 metaphases from five hamsters at each time point of the DMBA group), (2) possible species differences between human and hamster, (3) DMBA does not cause chromosome break in the hamster oral cancer model. It is possible that carcinogens other than DMBA can cause chromosomal breaks similar to those observed in human oral cancers.

DiPaolo and Popescu have documented the cytogenetic changes in Syrian hamster embryo cells exposed to chemical carcinogens [12]. These authors conclude that the observed chromosomal alterations are largely random. Our results from the hamster cheek pouch carcinoma model supported this conclusion with the following exceptions. First, although we cannot detect specific chromosome loss (monosomy) or chromosome gain (trisomy) in the DMBA-treated hamster oral keratinocytes, we have noted that the frequency of

Table 7. Chromosome gain in hamster oral keratinocytes during progressive treatment with DMBA (results from 100 metaphases per 2 weeks)

	Diploid/weeks									Tetraploid/weeks								
Cx	0	2	4	6	8	10	12	14	Td	0	2	4	6	8	10	12	14	Ttr
1									0						3	1	2	6
2									0						2			2
3									0								2	2
4									0						1	1		2
5									0				1				1	2
6		1							1									0
7									0						1		1	2
8						1	2		3						1			1
9									0						2			2
10									0						2	1		3
11									0									0
12									0								1	1
13									0									0
14									0			1			1	1	1	4
15									0	2					1	2	2	7
16									0				1					1
17									0									0
18								1	1					1	1	1	1	4
19									0									0
20						1		1	2							1	1	2
21									0							1		1
X		1					1		2	1			2					3
Y							1		1			1					1	2
T	0	2	0	0	0	2	4	2	10	3	0	2	4	1	16	9	13	48

Cx: Chromosome number. Td: Total chromosomes gain in diploid metaphases. Ttr: Total chromosomes gain in tetraploid metaphases. T: Total chromosomes gain per 2 weeks.

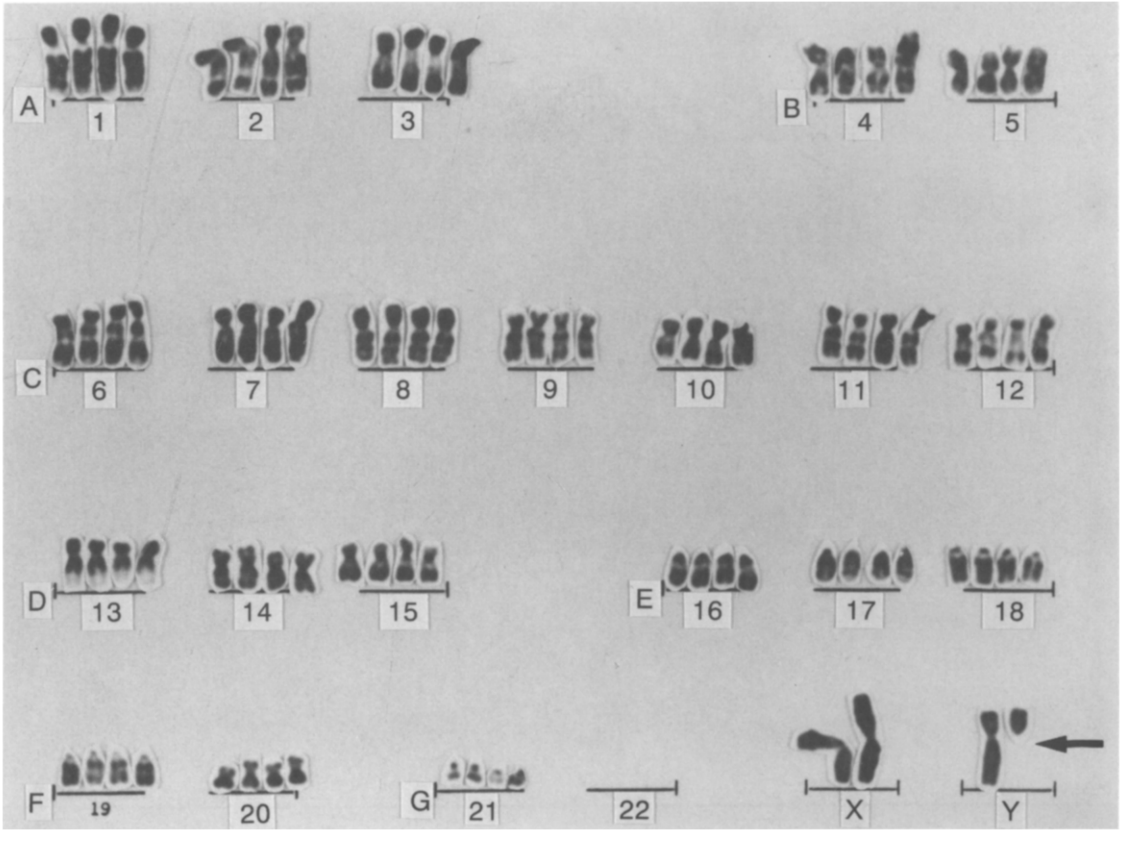

Fig. 3. Karyotype of a DMBA-treated hamster oral keratinocyte exhibiting tetraploidy and Y chromosome deletion: 88, XXY del(Y) ($qa_1 \rightarrow qter$).

Table 8. Chromosome gain in hamster oral keratinocytes treated with mineral oil for 10, 12 and 14 weeks (results from 50 metaphases per 2 weeks)

Cx	10	12	14	Td	10	12	14	Ttr
1				0				0
2				0				0
3				0				0
4				0				0
5				0	1	1		2
6				0				0
7				0				0
8				0				0
9				0				0
10				0				0
11		1	1	2				0
12				0				0
13				0				0
14				0		1		1
15				0				0
16				0				0
17				0				0
18				0				0
19				0				0
20				0				0
21				0	1			1
X				0				0
Y				0				0
T*	0	1	1	2	2	2	0	4

Cx: Chromosome number. Td: Total chromosomes gain in diploid metaphases. Ttr: Total chromosomes gain in tetraploid metaphases. T: Total chromosomes gain per 2 weeks.

Table 9. Structural aberrations of chromosomes in hamster oral keratinocytes upon progressive DMBA-treatment (results from 50 metaphases per 2 weeks)

	Break/weeks									Deletion/weeks									Translocation/weeks									
Cx	0	2	4	6	8	10	12	14	T_b	0	2	4	6	8	10	12	14	T_l	0	2	4	6	8	10	12	14	T_t	T*
1								1	1	1						2	1	4									0	5
2	1	1							2		1					2		3									0	5
3									0			1	1					2									0	2
4									0	1								1									0	1
5									0					1				1									0	1
6									0									0									0	0
7									0						1			1									0	1
8		1							1									0									0	1
9									0								1	1									0	1
10									0			1						1									0	1
11									0						1	1		2					1				1	3
12									0							1		1									0	1
13									0		1							1									0	1
14									0									0									0	0
15									0									0					1				1	1
16									0						1			1									0	1
17									0									0									0	0
18									0									0									0	0
19									0					1				1									0	1
20									0									0									0	0
21									0									0									0	0
X						1			1		2		1			3	1	7									0	8
Y			1						1		2		2			3		7									0	8
T†	1	2	1	0	0	1	0	1	6	2	6	2	4	2	3	12	3	34	0	0	0	0	2	0	0	0	2	42

Cx: Chromosome number. T_b: Total chromosome break count in each chromosome pair. T_l: Total chromosome deletion count in each chromosome pair. T_t: Total chromosome translocation count in each chromosome pair. T*: Total structural aberrations ($=T_b+T_l+T_t$). T†: Total structural aberrations per 2 weeks.

Table 10. *Structural aberrations of chromosomes in hamster oral keratinocytes treated with mineral oil for* 10, 12 *and* 14 *weeks (results from* 50 *metaphases per* 2 *weeks)*

	Break/weeks				Deletion/weeks				Translocation/weeks				
Cx	10	12	14	T_b	10	12	14	T_l	10	12	14	T_t	T*
1				0				0				0	0
2				0				0				0	0
3				0	1			1				0	1
4				0				0				0	0
5				0				0				0	0
6				0				0				0	0
7				0				0				0	0
8				0				0				0	0
9				0				0				0	0
10	1			1				0				0	1
11		1		1				0				0	1
12				0				0				0	0
13				0				0				0	0
14				0				0				0	0
15				0				0				0	0
16				0				0				0	0
17				0				0				0	0
18				0				0				0	0
19				0				0				0	0
20				0				0				0	0
21				0				0				0	0
X				0			2	2				0	2
Y				0				0				0	0
T†	1	1	0	2	1	0	2	3	0	0	0	0	5

Cx: Chromosome number. T_b: Total chromosome break count in each chromosome pair. T_l: Total chromosome deletion count in each chromosome pair. T_t: Total chromosome translocation count in each chromosome pair. T*: Total structural aberrations ($=T_b+T_l+T_t$). T†: Total structural aberrations per 2 weeks.

chromosome loss and chromosome gain of tetraploid and near-tetraploid chromosome metaphases consistently increased by week 10 in the DMBA treatment group. By this time of DMBA treatment, evidence of severe dysplasia (carcinoma *in situ*) could be seen. It is possible that the increase of chromosome loss or gain at this time be related to the onset of clinical malignancy. Second, chromosome deletions, especially the deletions on the X and Y chromosomes are notable structural aberrations. Thirdly, beginning the second week of DMBA treatment, there is a consistent and significant increase of tetraploid/near-tetraploid karyotypes in the DMBA-treated groups. We propose that this tetraploid and/or near tetraploid karyotype be further evaluated as a possible marker for premalignant/malignant progression in oral mucosal tissues.

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