Chromosome Abnormalities Associated with Salivary Gland Epithelial Cell Lines Transformed in Vitro and in Vivo with Evidence of a Role for Genetic Imbalance in Transformation

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ABSTRACT

Chromosomal abnormalities associated with five in vitro-transformed male mouse salivary gland epithelial cell lines were compared with those in three cell lines derived from in vivo-induced tumors. All cell lines were hypotetraploid. Structural chromosome abnormalities were found in all cell lines, but no consistent aberration was detected. Nevertheless, losses of chromosomes 1, 4, 7, 9, and 14 were observed in all of the in vitro-transformed cell lines. With the possible exception of chromosome 1, the same chromosome losses were noted in the in vivo-transformed cell lines. In addition, a consistent feature of both in vitro- and in vivo-transformed cell lines was the presence of double minute chromosomes and homogeneously staining regions. Where both of these chromosome types were present in the same cell line, they were mutually exclusive. The Y chromosome was absent in nearly all of the cell lines. These findings are consistent with the view that, in salivary gland epithelium, the malignant phenotype may result from a genetic imbalance caused by specific chromosome losses from tetraploid cells.

INTRODUCTION

Despite the biological importance of carcinomas, there have been surprisingly few detailed chromosome studies on epithelial tumor cells (see Ref. 6 for review). In the few studies that have been undertaken (17), epithelial tumor cells almost invariably show gross numerical and structural chromosome changes. These changes were observed, however, in already highly malignant cells, and it was therefore not possible to determine whether they were causal in the expression of the malignant phenotype or a later consequence.

Analysis of in vitro epithelial cell systems in which transformation can be followed through a series of defined stages (11) offers the opportunity to study chromosomal changes throughout the development of neoplasia. In particular, it is possible to analyze chromosomal changes immediately after the onset of tumorigenicity. One such system involves transformation of mouse salivary gland epithelium in vitro (14, 23, 24). In a previous report, transformation in this system was shown to involve tetraploid formation and chromosome loss (9). Prenuclear cells appeared to have lost chromosomes after becoming tetraploid but before the onset of tumorigenicity. This may imply that loss of a specific chromosome(s) is required to generate the malignant phenotype. Evidence was also presented which suggested that a similar mechanism may operate during transformation in vivo. In the present paper, a detailed karyotypic analysis has been made of early-passage cell lines transformed in vitro and in vivo. Consistent chromosome abnormalities were found between cell lines, and the significance of these changes is discussed.

MATERIALS AND METHODS

Cell Culture. All cell lines were maintained in Waymouths 752/1 medium (Gibco-Biocult, Paisley, Scotland) supplemented with 10% newborn calf serum (Gibco-Biocult). Cells were transferred and harvested using EDTA/pronase as described previously (23).

Chromosome Analysis. Cell cultures were treated with 1 μg vinblastine sulphate per ml (Sigma London Chemical Co. Ltd., Kingston, England) for 20 min, harvested, and placed in hypotonic solution (0.07 M KCl) for 10 min at 37°C and fixed in 3 changes of 3 parts of methanol to one part of acetic acid. Chromosome preparations were made using conventional air drying techniques. Chromosome banding was done by means of a modified trypsin-Giemsa technique as described previously (8).

All photographs were taken with a ×100 objective and high contrast Recordak Microfilm (Kodak, Hemel Hempstead, England) rated ASA 10.

RESULTS

The origin, ultrastructure, and tumorigenicity of the cell lines used in this study have been described elsewhere (14, 23). Five in vitro-transformed cell lines were analyzed, 4 of which arose in DMBA1-treated cultures and one of which arose in a DMSO-treated control culture (Table 1). Three cell lines derived from in vivo-induced carcinomas (23) were also analyzed. These tumors were induced using benzo(a)pyrene. All cell lines were analyzed soon after the demonstration of their tumorigenicity (passage 8 to 12) when the production of pure carcinomas confirmed their epithelial origin. The analysis was therefore on the parent cell line rather than a clone, the advantages of which have been discussed elsewhere (6). With one exception, CAE 553 (see later), all cell lines were hypotetraploid. This was confirmed by DNA quantitation analysis (9) in which no diploid or near-diploid population of cells could be detected in any of the cell lines. Chromosome counts from each cell line showed that the range was large. However, when present, duplication of chromosome markers in the higher ploidy cells demonstrated that they had arisen from endoreplication in the parent cells. This was confirmed in many of the cell lines.

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1 The abbreviations used are: DMBA, dimethylbenz(a)anthracene; DMSO, dimethylsulfoxide; HSR, homogeneously staining region; DM, double minute chromosome.
by the presence of diplochromosomes. Detailed Giemsa-band-
ing analysis of each cell line showed 2 features. (a) Slight differences existed between cells particularly in the dosage of individual chromosomes, and (b) within this apparently random variation consistent chromosome abnormalities could be de-
tected. The random variation between individual cells is prob-
ably due in part to the independent evolution of subclones with slightly different karyotypes and also in part to the artifactual loss of chromosomes during spreading.

From each cell line, 20 well-banded metaphases were ana-
lyzed, and the chromosome changes were recorded. From these results, modal karyotypes have been constructed (Figs. 1 to 8) which represent the consistent features of the cell lines. In addition to features invariably present in cells of a given cell line, features which were present in at least 80% of cells were also included. It is possible that in the remaining 20% of the cases false positives may have been scored as a result of chromosome misidentification. This is particularly the case with smaller mouse chromosomes which are sometimes indistin-
guished. This is particularly the case with the smaller mouse chromosomes which are sometimes indistin-
guishable and also with overlapping chromosomes. All karyo-
types were based on the standard presented previously (8), and the nomenclature used is that of Nesbitt and Francke (19).

Chromosomal Losses. Chromosome abnormalities con-
sisted mostly of losses of individual chromosomes. These losses are summarized for each cell line in Table 1. One copy of each chromosome group was lost unless otherwise stated. In all of the in vivo-transformed cell lines losses were found from Groups 1, 4, 7, 9, and 14 (Table 1). This was the case in DMBA-transformed cell lines and CSG 141 which arose in a DMSO-treated control culture. With the exception only of CSG 120/7, 2 copies from Group 4 and 14 were missing unless otherwise stated in parentheses.

Chromosome Rearrangements. With the exception of the HSR in CSG 120/7 (see below), no structural chromosome aberration was found consistently within any of the in vitro- or in vivo-transformed cell lines. However, structural aberrations were found in a percentage of cells in the majority of cell lines. These have presumably arisen after the onset of tumorigenicity, and where they were present in greater than 50% of cells they have been included at the foot of each karyotype in Figs. 1 to 8. Many of the marker chromosomes were small centric chromo-

A similar pattern was seen in CSG 120/7, but gains in Groups 5 and 10 were not seen in all cells. Chromosome gains were also observed in the in vivo-transformed cell lines where in CMT 106 chromosome 5 was again present in excess (Fig. 6). In CMT 114, chromosome gains were frequently found in Group 2.

Y Chromosome Losses. Loss of the Y chromosome was observed in the majority of the cell lines analyzed. In 2 in vitro-
derived cell lines, CSG 120/7 and CSG 122/17, the Y chro-

some was present in less than 20% of cells; usually there was only one copy, but on rare occasions there were 2. Y chromosome loss was also observed in the majority of in vivo-
derived cell lines (Table 1), and only CMT 106 retained the Y chromosome of which the majority of cells had 2 copies.

Chromosome Gains. Consistent patterns of chromosome loss were detected in all cell lines, but occasionally excesses of certain chromosomes were also present. Thus, in CSG 120/3 (Fig. 1), up to 7 copies of chromosome 5 per cell were noted, and the majority of cells contained 6 copies. Occasionally, excess copies of chromosome 10 were found in this cell line. A similar pattern was seen in CSG 120/7, but gains in Groups 5 and 10 were not seen in all cells. Chromosome gains were also observed in the in vivo-transformed cell lines where in CMT 106 chromosome 5 was again present in excess (Fig. 6). In CMT 114, chromosome gains were frequently found in Group 2.

Table 1

<table>
<thead>
<tr>
<th>Cell line</th>
<th>Carcinogen</th>
<th>Ploidy</th>
<th>Chromosome Losses</th>
<th>Y Loss</th>
<th>HSR's</th>
<th>DM's</th>
</tr>
</thead>
<tbody>
<tr>
<td>CSG 120/3</td>
<td>DMBA</td>
<td>69</td>
<td>1, 2, 4(2), 7, 9, 13, 14, 17</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>CSG 120/7</td>
<td>DMBA</td>
<td>72</td>
<td>1, 2, 4, 7, 9, 14</td>
<td>±</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>CSG 121</td>
<td>DMBA</td>
<td>65</td>
<td>1, 2, 4(2), 7, 9, 10, 14(2), 15, 17, 18, X</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>CSG 122/17</td>
<td>DMBA</td>
<td>70</td>
<td>1, 2, 4(2), 7, 9, 14, 16, 17</td>
<td>±</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>CSG 141</td>
<td>DMSO/acetone</td>
<td>67</td>
<td>1, 3, 4(2), 7, 9, 11, 14(2), 16(2), 19</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Cell line</th>
<th>Carcinogen</th>
<th>Ploidy</th>
<th>Chromosome Losses</th>
<th>Y Loss</th>
<th>HSR's</th>
<th>DM's</th>
</tr>
</thead>
<tbody>
<tr>
<td>CMT 106</td>
<td>BP^c</td>
<td>70</td>
<td>1, 2, 4, 7, 9, 13, 14(2), 15, X</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>CMT 114</td>
<td>BP</td>
<td>67</td>
<td>4(2), 7, 9(2), 13, 14(2), 15, 16, X</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>CAE 553</td>
<td>DMSO</td>
<td>115</td>
<td>See text.</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
</tbody>
</table>

^a One copy from each cell group quoted was missing unless otherwise stated in parentheses.
^b Cell lines in which the Y chromosome was present but in less than 20% of cells have been desig-
nated ±.
^c BP, benzo(a)pyrene.
cussed in detail elsewhere (7). In 3 cell lines, CSG 121, CSG
some is possibly involved. This is suggested in Fig. 10. In early
passage cells from CSG 121 . chromosome 18 has been trans
it was not possible to say unequivocally with which chromo
141, and CAE 553, the HSR was identical (Fig. 10). Although
located onto the distal tip of the HSR-containing chromosome.
expression of the malignant phenotype may result from ex

DISCUSSION

Many independent lines of evidence have suggested that
expression of the malignant phenotype may result from ex
cesses of certain chromosomes resulting in chromosome im
balance. Many reports have appeared in which a single chro
mosome trisomy or monosomy is the only karyotypic change
resulting in single chromosome gains (6, 10, 11). Imbalance
resulting in single chromosome gains (6, 10, 22) or losses (16). Excesses of parts of chromosomes may result from nonreciprocal translocations (2, 6). A third method of generating chromosome imbalance is through tetraploid
formation and subsequent chromosome loss. In all of these
instances, it is assumed that chromosomes carrying mutations
in genes involved in generating the malignant phenotype are in
excess over their normal homologs. In a previous report, DNA
content changes in single cells during in vitro transformation
were analyzed, and it was demonstrated that preneoplastic
cells had undergone tetraploid formation and chromosome loss
before the onset of tumorigenicity (9). Evidence was also
presented which suggested that a similar mechanism occurred in
vivo. It might be expected, therefore, that chromosome loss
in the first instance is random and that only when specific
losses occur is the malignant phenotype expressed. This hy
thesis is consistent with the observations in the salivary
gland cell lines. Each cell line has a variety of different chro
some losses, but amid the background of apparently ran
dom chromosome loss, copies of chromosomes 1, 4, 7, 9, and
14 are missing from all cell lines. With the possible exception
of chromosome 1, these losses were found in both in vitro- and
in vivo-transformed cell lines. Thus, genetic imbalance for one
or a combination of these chromosomes may be important in
the expression of the malignant phenotype in mouse salivary
gland epithelium. In addition to these losses, most of the
salivary gland cell lines had lost the Y chromosome; only CMT
106 retained at least one chromosome in all cells. Y chromo
some loss has been noted in human neoplasms (1, 3, 17), and
in a recent report (8) loss of the Y chromosome was frequently
observed in mouse bladder epithelial cell lines transformed in
vitro. The significance of Y chromosome loss from tumor cells
is not fully understood but in cultured cells may simply reflect
the genetic redundancy of the Y chromosome for in vitro
growth. With the exception of CSG 120/7, all salivary gland
cell lines showed a loss of 2 copies of either chromosome 4 or
14 or both. Loss of these chromosomes are possibly more
important than others, and it might be envisaged that the loss
of 2 copies of a single chromosome might enhance the imbal
ance responsible for the malignant phenotype. This was sug
sted to be the case by Muldal and Lajtha (18) where in
chronic myelogenous leukemia patients the onset of blastic
crisis was sometimes shown to be associated with the acquisi
tion of a second Philadelphia chromosome.

The analysis of chromosomes prepared directly from solid
tumors has been hampered by the difficulty in obtaining good
quality preparations. Even when this is possible, assessing the
importance of the observed changes is not always easy since
they reflect the progressive karyotypic evolution of subclones
of cells within the tumor. As such, the final karyotype may bear
little resemblance to that of the cells transformed initially.
Analysis of in vitro-transformed cells immediately after the
onset of tumorigenicity provides the opportunity to study chro
mosomal changes before the accumulation of nonspecific
changes. These systems, however, also have drawbacks since
cultured cells are known to undergo karyotypic evolution when
progressively subcultured and therefore the observed chro
mosome abnormalities may simply reflect an adaptation to in
vitro growth. The fact that all of the chromosome abnormalities
which were consistently seen in in vitro-transformed cell lines
were also observed in the in vivo-transformed cell lines argues
in favor of a possible role for these chromosomes in the
expression of the malignant phenotype rather than being cul
ture artifacts. The in vitro-transformed cell lines, however, were
established in culture from tumors (23) which may have under
gone some sort of adaptation. It is unlikely that these cell lines

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Table 2
Tentative identification of structural chromosome abnormalities observed in
salivary gland epithelial cell lines

<table>
<thead>
<tr>
<th>Cell line</th>
<th>Marker</th>
<th>Rearrangement</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>120/3</td>
<td>m1</td>
<td>del (1) (1F1)</td>
<td>Terminal deletion of chromosome 1.</td>
</tr>
<tr>
<td></td>
<td>m2</td>
<td>T (7-4)</td>
<td>Breakpoint in region F1</td>
</tr>
<tr>
<td></td>
<td>m3</td>
<td>? Proximal region of chromosome 13.</td>
<td></td>
</tr>
<tr>
<td>120/7</td>
<td>m1</td>
<td>T (7-4)</td>
<td>Breakpoint in region F1</td>
</tr>
<tr>
<td></td>
<td>m2</td>
<td>? Translocation of most of the X to proximal region of unidentified chromosome.</td>
<td></td>
</tr>
<tr>
<td>121</td>
<td>m1</td>
<td>T (7-X)</td>
<td>Breakpoint in the X at A2.</td>
</tr>
<tr>
<td></td>
<td>m2</td>
<td>T (8-8)</td>
<td>Breakpoints in region C3 and B2 causing duplication of region B3-C3.</td>
</tr>
<tr>
<td></td>
<td>m3</td>
<td>? Unidentified small chromosome.</td>
<td></td>
</tr>
<tr>
<td>122/17</td>
<td>m1</td>
<td>T (6-4)</td>
<td>Translocation between chromosome 6, breakpoint C2 and chromosome 4, breakpoint C2.</td>
</tr>
<tr>
<td>141</td>
<td>m1</td>
<td>T (1-5)</td>
<td>Translocation between chromosome 1, breakpoint somewhere in the D region and chromosome 5, breakpoint somewhere in the B region.</td>
</tr>
<tr>
<td></td>
<td>m2</td>
<td>? Unidentified small chromosome.</td>
<td></td>
</tr>
<tr>
<td>106</td>
<td>m1</td>
<td>T (15-8)</td>
<td>Translocation between chromosome 15 with breakpoint in the E region and chromosome 8 in the A4 region.</td>
</tr>
<tr>
<td></td>
<td>m2</td>
<td>T (X-7)</td>
<td>Translocation of all of the X to region F1 to an unidentified chromosome.</td>
</tr>
<tr>
<td></td>
<td>m3</td>
<td>? Unidentified small chromosomes.</td>
<td></td>
</tr>
<tr>
<td>CMT 114</td>
<td>m1</td>
<td>T (12-13)</td>
<td>Translocation of the distal part of chromosome 13, breakpoint in the B region and chromosome 12, breakpoint in D3.</td>
</tr>
<tr>
<td>CAE 553</td>
<td>m1</td>
<td>del (1) (3-5)</td>
<td>Deletion of region C3-C5 of chromosome 1.</td>
</tr>
<tr>
<td></td>
<td>m2</td>
<td>T (7-15)</td>
<td>Breakpoint in A2 of 15.</td>
</tr>
</tbody>
</table>

a Designation of markers, e.g., ml, for each cell line corresponds to the nomenclature used on Figs. 1 to 8.
b Breakpoint terminology according to Nesbitt and Francke (19).
would have acquired all of the changes observed in the in vitro-transformed cell lines during the adaptation period, particularly since the total DNA content in the initial tumor cells was virtually identical to that in the cell lines derived from them (9).

In an analysis of 9 epithelial cell lines derived in vitro from mouse bladder, it was demonstrated that, with the exception of Y chromosome loss, 3 other chromosomes either singly or in combination were invariably involved in karyotypic abnormalities (8). The aberrations were trisomy for chromosomes 6 and 15 and a rearrangement involving the translocation of an unidentified chromosome region to chromosome 3. There is no evidence that any of these chromosomes are consistently associated with transformation in salivary gland cells although they were occasionally lost or involved in chromosome rearrangements. It appears, therefore, that different chromosome abnormalities are associated with transformation of cells from different tissues. This might be explained in terms of the differentiated state of epithelial cells. It has been suggested (5) that in order to free programmed cells from their normal restraints and thus produce invasive cancer cells there may be an alteration in the pattern of gene expression in those cells. This alteration might be brought about by chromosome rearrangement or, as suggested above, as a result of genetic imbalance. Since each type of epithelial cell is determined by the particular set of genes it is expressing, it might be expected that the critical genes in the control of expression are on different chromosomes or at least different sites. Consequently, the particular abnormality required to generate aberrant gene expression, while being fairly consistent for a given cell type, differs depending on the origin of the cells. The apparent involvement of certain chromosomes with different tumors, e.g., chromosome 1 in humans (6), might simply mean that control genes on these chromosomes are expressed in many different cell types in addition to more exclusive ones. In the bladder epithelial cell lines, it was noted that the same chromosome aberrations were present in cell lines which arose in DMSO-treated controls and as a result of DMBA treatment. This was also the case in the salivary gland epithelial cell lines although only one arose from DMSO-treated controls. In addition, the same aberrations were also present in benzo(a)-pyrene-transformed cells in vivo. All of these observations suggest that the karyotypic changes associated with the various epithelial cell lines are not merely consequences of the carcinogen used to transform them.

Not only are the specific chromosome abnormalities associated with 2 in vitro epithelial cell transformation systems different, but the overall mechanism also appears to be different. Transformation in bladder cells is associated with minor karyotypic changes in diploid cells (8). Development of neoplasia in salivary gland cells involves tetraploid formation and chromosome loss (9). The near-diploid bladder cell lines were all derived from tissue from old (28- to 30-month) donors (21) whereas the salivary gland cell lines were derived from tissue from young (4- to 6-month) donors (14, 23). As such, the different mechanisms observed in the 2 systems may represent the different responses of old and young cells, possibly related to the relative mutability of those cells (8). Alternatively, the mechanism of transformation might depend on the differentiated state of the cells. In the bladder system, tetraploid formation is associated with terminal differentiation and thus may be excluded as a possible mechanism of transformation.

On the other hand, the salivary gland is an exclusively diploid tissue (9) in which tetraploid formation might allow the cells to escape normal cellular controls and proliferate, from which the process of chromosome loss is able to generate genetic imbalance and result in the malignant phenotype.

It is now generally accepted that DM's and HSR's represent genetic amplification in mammalian cells (4, 13). Both of these phenomena were observed in salivary gland cell lines induced both in vitro and in vivo. When DM’s and HSR’s were present in cells from the same cell lines, they were mutually exclusive. These findings are consistent with the view that DM’s possibly give rise to HSR’s by integration at homologous sites (7). A similar phenomenon appears to have occurred in a human adenocarcinoma of the ovary (20). In 2 of the cell lines, CSG 122/17 and CSG 120/7, the HSR was associated with chromosome 5 (7). In 3 other cell lines, another HSR was present which has tentatively been associated with the X chromosome. These observations might suggest that different genes are being amplified in the different cell lines although attempts to demonstrate amplification have been unsuccessful.2 It is possible, however, that the amplified genes are not being expressed. One in vivo-transformed cell line, CMT 106, was exceptional in that although DM’s were present during early stages of in vitro culture they soon disappeared. No HSR was evident in this cell line. These observations are consistent with those of Levan et al. (15) who showed that DM’s which are present in tumor cells in vivo are eliminated when put into culture.

Even though consistent chromosome changes were found to be associated with the in vitro- and in vivo-transformed epithelial cell lines, it was not possible to say which of these changes were important in the expression of the malignant phenotype and which were later consequences. Analysis of salivary gland cell lines is further complicated by the large number of apparently random changes occurring in tetraploid cells. Recently, it has become possible to obtain preneoplastic cell lines which with repeated subculture eventually produce carcinomas when injected into syngeneic hosts (23). It is hoped that an analysis of the karyotypes from pre- and post-tumorigenic cells will demonstrate chromosomal changes specifically associated with the acquisition of tumorigenicity.

ACKNOWLEDGMENTS

I thank Drs. L. M. Franks and C. B. Wigley for helpful criticism in preparing this manuscript and T. Barnes for technical assistance.

REFERENCES


2 J. K. Cowell, unpublished data.
J. K. Cowell

Fig. 1. Giemsa-banded karyotype of *in vitro*-transformed salivary gland epithelial cell line CSG 120/3.
Fig. 2. Giemsa-banded karyotype of *in vitro*-transformed salivary gland epithelial cell line CSG 120/7.
Fig. 3. Giemsa-banded karyotype of in vitro-transformed salivary gland epithelial cell line CSG 121.
Fig. 4. Giemsa-banded karyotype of in vitro-transformed salivary gland epithelial cell line CSG 122/17.
Fig. 5. Giemsa-banded karyotype of in vitro-transformed salivary gland epithelial cell line CSG 141.

Fig. 6. Giemsa-banded karyotype of in vivo-transformed salivary gland epithelial cell line CMT 106.
Fig. 7. Giemsa-banded karyotype of in vivo-transformed salivary gland epithelial cell line CMT 114.

Fig. 8. Giemsa-banded karyotype of in vivo-transformed salivary gland epithelial cell line CAE 553.
Fig. 9. Partial Giemsa-banded metaphase spreads showing DM's (arrows) from mouse salivary gland epithelial cell lines: A, CSG 122/17; and B, CSG 141.

Fig. 10. HSR's in salivary gland epithelial cell lines. In 2 cell lines (top), the HSR appears to be associated with chromosome 5. The possible association of an HSR with the X chromosome in 3 cell lines (bottom) is shown. In CSG 121, at passage 9 (121/9), chromosome 18 is translocated to the distal region of the HSR. This rearrangement is lost in later passage cells (121/64).
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