Cytogenetic Profile of in Vitro Spontaneously Transformed and Chemical Tumor-derived Cell Lines Related to the Mouse Lung Alveologenic Carcinoma

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ABSTRACT

The karyotypes for a unique series of cloned untransformed and spontaneously and urethan-transformed mouse lung alveologenic carcinoma cell lines were compared by G-banding. All cell lines exhibited altered chromosome number accompanied by chromosomal aberrations. Both spontaneously and chemically transformed lines contained a higher proportion of cells carrying double minutes, short-arm chromosomes with no distinct banding pattern, and Robertsonian translocations between a variety of chromosomes. No common karyotypic abnormalities were observed among any of the transformed lines. These data suggest that enhanced genomic instability may lead to a critical subchromosomal molecular event(s) in generation of both spontaneously and chemically derived mouse lung alveologenic carcinoma.

INTRODUCTION

The mouse lung adenoma is an extremely common primary tumor of certain mouse strains and may arise spontaneously or in response to a wide variety of carcinogenic agents (1). The benign lesions may progress to alveologenic carcinoma (1–3). Several studies have been published which demonstrate varied mouse strain susceptibility to chemical carcinogens and which implicate as yet undetermined genetic loci (4, 5). DiPaolo performed early studies on the chromosomal constitution of the mouse lung adenoma (6) which, however, predated G-banding technologies. No detailed analysis of mouse lung adenoma-related karyotypic changes appears to have been published since that time.

We have previously described an in vitro model of spontaneously and chemically derived mouse lung alveologenic carcinoma (7–9). In this model untransformed clones underwent in vitro spontaneous transformation and were recloned to produce “sibling” stable untransformed and malignant secondary clones (8). Transformation was defined as the ability to generate invasive poorly differentiated carcinomas in immunosuppressed mice when implanted s.c. (9). These secondary clones may be expected to represent minimal deviation variants with respect to phenotypic changes between untransformed and malignant cells. The malignant spontaneous transformants, in addition, share many morphological and histopathological features with other clones derived directly from in vivo urethan-induced mouse lung tumors (7–9). These cell lines provide a useful opportunity for karyotypic analysis of both the spontaneously and the chemically derived tumorigenic cells related to mouse lung adenoma. This paper provides a G-banding analysis and comparison of these untransformed, in vitro spontaneously malignant and chemical tumor-derived malignant clones.
Karyotype Analysis. For each cell line, 50 well spread metaphase plates were examined and chromosome counting was performed. Well banded spreads were photographed and karyotyped. The nomenclature used for the mouse karyotype was according to the systems of Cowell and Nesbitt and Franke.

RESULTS

Chromosome Number. No dominant clonal karyotypes were observed within the cell lines. The majority of cells in the transformed cell lines had less chromosomes than their untransformed sibling counterparts (Table 1). In the untransformed clone, C,C,, most cells (i.e., 45%) had 61–70 chromosomes. The next most common chromosome count was 50–60 for 30% of clones the majority of cells, 62 and 66%, respectively, had 61–70 chromosomes/nucleus. A similar decrease was observed within the cell lines. The majority of cells in the malignant cell clone 48% of cells in the 51–60 range. The malignant clones NULB, and NULB, were derived from the untransformed cell lines. Here the untransformed cell lines had less chromosomes than their untransformed counterparts (Table 1). In the untransformed C,, cell line passage number at the time of examination.

Evaluation of the mean frequency of appearance of the individual chromosome number per cell (Table 3) has shown that chromosomes 15, 16, and 19 were present on average at 3.0, 4.8, and 4.3 copies/cell. The least frequent tetraploidy was 1.1, 1.1, and 1.1, respectively. Chromosomes 1, 9, 12, and 19 were overrepresented with mean frequency of 4.3, 4.3, 4.2, and 4.1, respectively. Chromosomes 11, 15, and X were on the average less common with the individual frequencies differed from one karyotype to another (data not shown).

The malignant clones NULB, and NULB, were derived from the chemical-tumor-derived cell strain NULI (11). In both clones the majority of cells, 62 and 66%, respectively, had 61–70 chromosomes with a modal number of 63. This score was considerably lower than that observed for the untransformed C,E, and B,D, but comparable with untransformed C,E,

Structural Aberrations. Further differences between the untransformed and the malignant cell lines were observed with respect to structural chromosomal aberrations. As seen from the summary in Table 2, transformed clones had a considerably higher proportion of cells carrying DM, short-arm chromosomes, and Robertsonian translocations. Despite the obvious difference in the proportion of cells carrying such aberrations the frequencies of these markers within any particular cell were similar in both normal and transformed cells. The C,C, and C,A clones had predominantly 2 copies of DMs and short-arm chromosomes per cell. One or two copies of these markers per cell were present in the B,D, and B,F, clones. The malignant C,E, cell line had 4–5 copies of DM and up to 10 small marker chromosomes in some cells.

Chemically transformed cell lines NULB, and NULB, also had a high proportion of cells manifesting DM, short chromosomes, and Robertsonian translocations, but the actual frequency of these per cell remained 1 or 2 copies.

Examination of the G-banded Karyotypes. Close study of the G-banded karyotypes of the C,A cell line revealed that 75% of the observed Robertsonian translocation occurred between chromosome 9 and 12 (Fig. 2). The resulting pseudometacentric chromosome was present as one copy in a cell. Ten% of the identified translocations involved chromosome 2 in association with a variety of other chromosomes or chromosomal fractions. Evaluation of the mean frequency of appearance of the individual chromosome number per cell (Table 3) has shown that chromosomes 1, 9, 12, and 19 were overrepresented with mean frequency of 4.3, 4.3, 4.2, and 4.1, respectively. Chromosomes 11, 15, and X were on the average less common with the individual frequencies of 1.3, 1.8, and 1.7/cell. All other chromosomes had an average frequency between 1.9 and 3.9.

Similar study of the C,E, cell line karyotypes showed that chromosomes 16, 17, and 14 appeared on an average of 4.9, 4.8, and 4.3 copies/cell. The least frequent tetraploidy was observed in chromosomes 3, 4, and 11 with the average frequency of 2.3, 2.4, and 2.7, respectively. G-band analysis did not reveal any other consistent structural abnormalities that could be defined as markers of this cell line.

A characteristic chromosome marker was discovered from the close examination of the karyotypes of the NULB, cell line. Here, an unusually long chromosome appeared in 80% of the examined cells (Fig. 3). Tentative identification of this chromosome suggests an insertion of additional chromosomal material of unknown origin in the 3G-3H1 region of chromosome 3. Study of the individual chromosome frequencies showed that chromosomes 15, 16, and 19 were present on average at 3.0,
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Fig. 2. Representative G-banded karyotype of the C11A2 cell line demonstrating characteristic marker chromosomes. m1, Robertsonian translocation t(9:12); m2, aberrant short arm chromosomes; m3, double minutes; M, unidentified aberrant chromosomes characteristic to this metaphase spread.

Table 3 Mean frequency of appearance of chromosomes 1–19 and X per cell

<table>
<thead>
<tr>
<th>Chromosome no.</th>
<th>C11A2 (82–100)</th>
<th>C11E5 (97–100)</th>
<th>NULB2 (80–90)</th>
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* Cell passage number at time of examination.

In the present study an attempt was made to detect specific chromosome changes that could be associated with the development of the malignant phenotype in closely related cloned untransformed and transformed mouse epithelial cell lines. A larger proportion of cells in the transformed cell lines carried structurally aberrant chromosomes in the form of double minutes, small marker chromosomes with no characteristic banding pattern, and other chromosomes of unknown identity. Similar chromosome markers were observed by Cowell and Wigley (14) during the examination of cells of the in vitro transformed mouse salivary gland epithelium. As seen from Table 1, these

DISCUSSION

Analysis of metaphase chromosomes of the B5SF1 cell lines revealed a marker not previously seen in any cell lines studied here (Fig. 4). Some 20% of the examined metaphases carried this structurally different chromosome. It appeared to represent a double centromeric chromosome with one of the centromeres in the acrocentric position and the other on various locations along the chromosome arms. From the microscopic examination and several photographs of the metaphase chromosomes it was noticed that the size of the chromosomes varied and therefore could conceivably represent different chromosomes or the same chromosome with structural rearrangements.

Table 3. Mean frequency of appearance of chromosomes 1–19 and X per cell.

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aberrations were also present in the nontransformed cell lines but in a lower proportion of the population. Since the sibling malignant and nonmalignant cloned pairs were of a similar passage number (i.e., the same age) during the examination the increase in the structural chromosome aberrations cannot be attributed to prolonged culturing effect alone but could be characteristic of the malignant karyotype.

It is not known what mechanism is involved in the generation of these markers or what role, if any, they may play in the evolution of the malignant phenotype. One possible explanation may be that these aberrations appear in a random manner during early passages and are preserved due to their imparting to the cell a proliferative advantage in vitro.

Alternatively, it is possible that these markers do not contribute to any advantageous character but arise randomly due to malfunctioning or absence of a mechanism which controls the integrity of the genome. The malignant cell clones would appear, in this event, to be particularly susceptible to these genomic aberrations. This conclusion supports the previously stated hypothesis (see Ref. 15) that genomic instability may play a key role in neoplastic transformation.

Since the three transformed cell lines (C^A5, C^8E0, B^5FS) were originally cloned from the same parent population, we examined these cells for a common alteration which might be involved in the process of malignant transformation in vitro. The t(9:12) translocation in the C^A5 cell line was identified immediately after cloning and persisted until the maximum analyzed passage number of 102. The presence of the t(9:12) translocation has been noted by other investigators in the B16 murine melanoma cell line (16, 17), but it did not appear in any other malignant clones examined here. This translocation does not therefore appear to represent a true marker of transformation. Similarly the pseudo-double centromeric chromosome observed in the B^5FS cell line cannot be considered as a marker of malignancy since it appeared only in this cell line. No common chromosomal markers were observed in any of the transformed cell lines. This is despite the previous observations (7–9) that the different spontaneously transformed and chemi-
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mosomes, strengthening the hypothesis that increased chromosomal instability is a feature of these transformed cells. The results indicate that the instability of the karyotype was common to both spontaneously transformed and chemical tumor-derived cell lines. Di Paolo (6), investigating spontaneously transformed and urethane-induced mouse lung adenomas, observed high levels of aneuploidy and a pseudo-metacentric chromosome (Robertsonian translocation); however, G-banding analyses were not possible at that time.

A characteristic marker (on chromosome 3) was prevalent in the NULB clone but was absent from karyotypes of NULB5. This marker is therefore unlikely to be associated with the onset of malignancy and is probably a random chromosome aberration.

In conclusion, chemical tumor-derived and spontaneously transformed cell clones related to mouse lung alveogenic carcinoma exhibited a range of chromosome markers (DM, short chromosomes). Despite the overall drop in the number of chromosomes in the malignant cells no evidence of a gene dosage effect was found. Overall genomic instability was the more consistent and common characteristic. Individual chromosome markers observed in C57B1, B6F1, and NULB were not considered fundamental to the generation of the malignant phenotype in this series of cell lines.

ACKNOWLEDGMENTS

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REFERENCES

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