Chromosomal Banding Patterns and in Vitro Transformation of Syrian Hamster Cells

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SUMMARY

Chromosome banding techniques were used to analyze the chromosomal constitution of hamster cells transformed by chemical carcinogens and fibrosarcomas obtained after injection of the transformed cell lines. Each transformed line is considered unique because each was derived from fetal material from a different pregnant animal. The chromosome modes of the transformed lines and tumors were generally near-diploid. Analysis of bands verified the occurrence of identical banding patterns in the marker chromosomes of transformed lines and tumor-derived cultures, thus providing unequivocal evidence that the fibrosarcomas were produced by the cells that were transformed in vitro and making it possible to recognize 10 marker chromosomes and their origin. Different carcinogens may produce transformations associated with the same specific marker, but not all transformed lines have the same markers even with the same chemical carcinogen. Some markers were found in the tumor-derived culture and not the transformed line or occurred in later passages of both the transformed line and tumor cultures but not in the early passage of these lines. In some transformed cell lines the chromosome number increased but did not involve a specific chromosome group or pair. The incidence of chromosomal deviation in cancer is species dependent and contributes to the characteristics of cancer. Chromosome alterations are responsible for the genotypic changes capable of autonomous growth and thus are considered reflections of secondary alterations. The question whether chromosome changes are the causal factors in carcinogenesis remains unanswered.

INTRODUCTION

The role of chromosomal changes in carcinogenesis has been debated since the time of Boveri (4) who suggested that a specific type or types of alterations in chromosome constitution were responsible for the new morphology (neoplastic) of the cell and the concomitant changes in biochemical behavior that lead to apparently unregulated growth and multiplication. Conflicting interpretations of chromosomal changes seen during in vitro transformation of cells induced by chemicals and in vivo experiments, as well as in studies on human cells, indicate the complexity of the problem and the frustration in attempting to develop inclusive theories.

Chromosome analysis by a conventional Colcemid technique of chemically or virally induced tumors or transformed lines of the Syrian hamster has resulted in the conclusion that no detectable universal changes exist and that the chromosome modes may range from hypodiploid through hypertetraploid (7, 10, 13, 31, 33). The chromosome alterations were considered minimal and of a random occurrence that was independent of the transformation (13). The use of banding techniques makes possible the identification of each pair of chromosomes of the Syrian hamster karyotype so that numerical changes in chromosome pairs and any uncommon banding patterns that occur in chromosomes in association with transformation may be accurately analyzed (34). In this study chromosomes of 9 transformed cell lines, each originally derived from a different animal, as well as a number of tumors obtained by injecting the transformed cells, are analyzed for numerical and structural changes in terms of their bands.

MATERIALS AND METHODS

Cell culture procedures and the transformation assays have been described (11, 13). Transformed cell lines were developed from isolated, carcinogen-treated, transformed colonies. Each cell line originated from embryos of different hamsters. When cells from the transformed lines were injected into hamsters, fibrosarcomas were produced at the site of injection (12, 13). The tumors were returned to culture and are referred to as tumor-derived cultures (t).

Cultures for chromosome study were grown in Falcon Petri dishes in Dulbecco's modification of Eagle's medium supplemented with 10% fetal bovine serum, in a humidified 10% CO₂ incubator. Chromosomes of tumor cells were examined from a 48-hr-old primary or 24-hr-old secondary culture. Cells were harvested after 4 hr of Colcemid incubation (0.08 μg/ml), treated with a 1:2 solution of medium and distilled water for 14 min, and fixed in methanol: glacial acetic acid (3:1). Slides were prepared by the air dry chromosome technique. The banding pattern of the hamster
chromosomes was established by 3 different techniques: modification of the acetic saline Giemsa technique (34) of Sumner et al. (43), the trypsin technique of Seabright (39), and the fluorescent technique of Caspersson et al. (5) as modified by Francke and Nesbitt (15). Well-spread metaphases with banding patterns were photographed. Karyotypes were prepared in accordance with the banding schematic idiom for Syrian hamster cells (34).

RESULTS

Nine different transformed cell lines obtained after treatment with BP, AFB, PS, or 4NQO and 6 tumor-derived cultures were examined. One cell line, AFB, and the corresponding tumor culture were examined at 2 different passage levels. The majority of the transformed cell lines (8 of 9) and the tumors (4 of 6) have near-diploid chromosome modes (Table 1). Where both the transformed line and corresponding tumor were examined, the stem-line was similar. The distribution of the markers in cell lines range from no marker, regardless of the banding technique used, to an as many as 4 markers (Table 1). The 21 pairs of autosomes and 2 sex chromosomes that constitute the normal hamster complement are clearly identifiable. In some transformed lines, no abnormal chromosomes were found and the modal chromosome number was 44. In these cases, the stem-line was near diploid and, although trisomy existed, the trisomy did not always involve a specific chromosome. For example, 4NQO17 has a major near-diploid mode and no chromosome marker but may have extra chromosomes belonging to B5 and C15 (Fig. 1); 4NQO17 also has a minor hypertetraploid mode, and in the cell shown (Fig. 2) the extra chromosomes belonged to Groups B5 and B10. Thus all chromosomes are identifiable as to specific pair.

On the basis of band analysis, the derivation of 10 marker chromosomes (p and q signify short and long arms, respectively) found thus far, is: M, t(A2q; q); M, t(D16q; D17q); M, t(C15p; F21); M, del(A1q); M, t(Aq; q); M, t(Xq; q); M, t(A1q; A2q); M, t(A4p; q); M, t(C15q; ?) (Figs. 3 and 4). In time, it will be possible completely to substantiate the derivation of the markers.

M, the most common marker in this series, is shown next to a pair of normal A2 chromosomes followed by the schematic representation of the M, and A2 (Fig. 3). The additional chromosomal material in the A2q arm required either an unequal translocation to produce a longer q arm or an insertion of a new piece of chromosome in the lower end of the q arm. Areas similar to the new area are found in Chromosomes A1, C15, and D17. In some cells, the presence of M, is accompanied by a loss of 1 A1, but in others such as in Fig. 5 both A1 chromosomes are present. The marker is never associated with a loss of D17 and, in fact, D17 often occurs as a trisomy. The most likely origin of the translocation in M, is the distal portion of the long arm of C15, which is always monosomic when M, or M, are present. Conversely, when a pair of C15's are present the marker is found. M, was found in 4 transformed cell lines and in 4 of the 6 tumors. This marker as well as the others occurred independently since each transformed cell line originated from a different animal. The incidence of M, was high; it was 80% in 1 cell line. Interestingly, M, appears in 4NQO16t, whereas 4NQO16 has no markers; BP29 had no markers, but 1 cell of BP29t had 1 M,. AFB, when examined at the 6th passage, and its corresponding tumor had only Marker M, at Passage 13; the transformed line and the corresponding tumor had M, and M, as well as other markers. M, also occurs in a nonrandom fashion, since it appears in 3 different transformed cell lines and in 2 different tumors. Two markers identified only in tumor 4NQO16t and in no transformed line are indicated by the letters a and b.

An example of a quinacrine mustard preparation from BP45 (Fig. 5) shows that, in addition to multiple markers, new chromosomes may be present such as the Di, a dicentric that is in part made up of a C12 and C13 as well as an unidentified chromosome piece. One chromosome of Pair B7, C12, C15, F21, and Pair C13 were missing. Some of these occurred as portions of new marker chromosomes. The total number of chromosomes in the D group has increased consistent with previous observations.

A karyotype (Fig. 6) with 44 chromosomes from 4NQO16t tumor exhibits trisomy for B7 and D17 and monosomy for A1, C15, and F21. Furthermore, in addition to the M, marker, 2 markers, M, and M, occurred only in Tumor 4NQO16t. The M, and M, markers result from translocations that have been added to the terminal portions of the A4p and C14q arms, respectively. The trans-

Table 1
Chromosome characteristics of transformed cell lines and tumor-derived cultures

<table>
<thead>
<tr>
<th>Cell line</th>
<th>Passage</th>
<th>Stem-line</th>
<th>Modal no.</th>
<th>Markers*</th>
</tr>
</thead>
<tbody>
<tr>
<td>BP45</td>
<td>13</td>
<td>43-46</td>
<td>42</td>
<td>1, 2, 3</td>
</tr>
<tr>
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<td>1</td>
<td>41-48</td>
<td>42</td>
<td>1, 2, 3</td>
</tr>
<tr>
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<td>44</td>
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<td>42-45</td>
<td>44</td>
<td>1*</td>
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<td>44</td>
<td>3</td>
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<td>44</td>
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</tr>
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<td>44</td>
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</tr>
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<td>43-46</td>
<td>44</td>
<td>1, 3, a, b</td>
</tr>
<tr>
<td>4NQO17</td>
<td>6</td>
<td>42-46</td>
<td>45</td>
<td>None</td>
</tr>
</tbody>
</table>

* Markers by the study of banding patterns in 20 karyotypes as well as in the metaphases.
* Tumor culture derived from corresponding transformed line; minimum of 50 metaphases used to obtain stem-line and modal number.
* Marker found in only 1 cell.

The abbreviations used are: BP, benzo(a)pyrene; AFB, aflatoxin B1; PS, propanesultone; 4NQO, 4-nitroquinolone N-oxide.

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location to the short arm of the A4 chromosome is stained uniformly dark.

The ability to analyze all the chromosomes on the basis of their banding patterns made possible the examination of a number of lines for changes in number of chromosomes constituting each identifiable pair (Table 2). The increase or a decrease in certain chromosomes is random. In fact, in PS66 and PS66t, which have tetraploid modes, occasionally one of the X chromosomes was missing. No significant increase, as indicated by the blocked number, was found among the transformed lines or tumors studied; however, in Tumors BP45t and 4NQO16t, trisomy for D17 was present in at least 16 of 20 karyotypes. Both tumors as well as the transformed line BP45 were monosomic for C15; the other C15 had been utilized in the formation of M1 and M3. It can be generalized that no increase or decrease in a specific chromosome number was associated with any of the transformations.

DISCUSSION

Chromosome banding techniques have permitted additional description of the role of chromosomes in carcinogenesis (26, 34). These newer techniques represent a powerful tool since, as demonstrated in this study, numerical changes in chromosome pairs and all chromosome markers become identifiable. Syrian hamster cells are mainly diploid in constitution with 1 to 2% in the tetraploid range (13). Carcinogen treatment results in transformed lines usually near-diploid but occasionally with tetraploid modes; injection of these cells into animals results in corresponding tumors with similar chromosome modes, karyotypes, and markers with identical banding patterns.

Some transformed lines have specific markers that can be cataloged according to the uncommon banding pattern of the chromosomes. The occurrence of the identical marker in the transformed cell line and the resulting fibrosarcoma are definite indications that the cells that underwent transformation were responsible for the tumor formation. Some markers, particularly M1 and M3, occurred as a result of treatment with different carcinogens; however, not all the transformed lines had the same markers even when transformed with the same chemical. Marker M1, which was observed most frequently in the transformed lines and tumor cell cultures, was also found in 1 case in a tumor but not in the parental transformed line. The presence of Marker M1 in a later passage of a transformed cell line and tumor as opposed to its absence at the 1st examination is an indication that changes at the chromosomal level are secondary. Therefore, although the specific chromosome change is considered to be nonrandom, it cannot be considered universal. The possibility that M1 is polymorphic for A2 was considered because the mechanism for the formation of this specific chromosomal translocation is not clear and because the arm ratio of A2 (2.5 to 2.8) may vary (24).

Reexamination of chromosomes of control cells prepared for bands has failed to show any deviation from the banding pattern previously reported (34). Marker 3, although not as common as M1, occurred repeatedly and thus can be considered nonrandom.
Marker M₂ found in the current study probably also occurred in other carcinogenesis studies prior to the advent of banding techniques (10, 13, 31, 33). Reports stating that E20 occurred as a trisomy in tumor cells (6, 8, 31, 33) may be in error since only by banding procedures can M₂ be shown to originate from C15 and F21. In studies on tumors from rats (23, 28, 36, 42) and Chinese hamsters (29), identical marker chromosomes were found by means of old techniques. However, reconciliation of older with current chromosome data is difficult. For example, a number of apparently similar chromosome aberrations found in human lymphoblastoid cell lines have been shown with the quinacrine fluorescent technique to be not identical (40).

Human primary tumors do not show a consistent chromosome pattern (1, 21, 25, 38) except for chronic granulocytic leukemia which possesses the unique Ph¹ chromosome in nearly every case (32). Recently, the quinacrine banding technique has been used to identify an addition of dully fluorescing material at the end of the long arm of 1 Chromosome 9 (37); the amount of added material is approximately equal to the amount missing from the Ph¹ chromosome. Thus the possible existence of a translocation would suggest that at the chromosomal level cancer may occur without any net change of genetic material.

Boveri's concepts of chromosomal imbalance, including the abnormal chromosome content of the cell as the underlying cause of neoplasia, continue to challenge investigators of cancer. In some hamster transformed cell lines, the chromosome number increase did not involve a specific chromosome group or pair. Gofman et al. (16, 27) using a semiautomatic chromosome analysis system expressed the imbalance as "abnormal ratio of chromosomes of particular type to chromosomes of another type, in contrast to the analogous ratio in the normal human diploid cell." Reevaluation of these results with the same approach failed to substantiate these findings because the average number of chromosomes of each type in a population of cells had been counted (3) rather than the absolute number of chromosomes in each individual cell. A similar computer method was used by Muldal et al. (30), who found in humans a significant gain in Group C and losses in Groups D and G. The finding of DG translocations has not been verified.

Variants of experimental tumor cells have been obtained by cell fusion (18) and by manipulation of cells at abnormal temperatures (20, 35). Rabinowitz and Sachs (35), as a result of studying "revertants" of polyoma virus-transformed line, have reported that the reversions could be associated with an increase in chromosome number from diploid to subtetraploid. The variants obtained from 1 transformed line which had been indirectly transformed by dimethylnitrosamine were also analyzed (20). Of the 5 variants analyzed, 4 had a major diploid mode whereas 1 had a major hypertetraploid mode. It was concluded that the higher number of chromosomes of the variants as opposed to the original line was responsible for "reversion." Harris states that in the hybrid cell from a malignant and nonmalignant cell, the nonmalignant contributes "some factor or factors, linked to specific chromosomes that can suppress the malignancy of the parental tumor cell or hold it in check" (18). Thus, both groups of investigators suggest that new chromosome combinations may lead to the suppression of cancer.

When 2 cells are fused, a new cell has been formed that has, in addition to the 2 sets of chromosomes, other characteristics of the nuclei and cytoplasm. For example, when human mouse hybrid cells were analyzed for rRNA synthesis, only the mouse type 28 S could be detected even in hybrids with up to 35 human chromosomes per cell; a plausible explanation is that the transcription of human ribosomal genes is repressed in hybrids (14). The changes in the degree of malignancy will have to be correlated with changes in properties of cell hybrids.

The chromosome evolution of tumors (22) and the finding that many primary cancers may consist mainly of cells with a diploid number of chromosomes (2, 9, 19) suggest that the observed chromosome changes are not necessarily associated with cancer. For example, in the case of chemical carcinogenesis utilizing in vitro systems, gene regulation may play an important role. Variants represent responses of cells to different environments, and the variability in the deviation from the diploid status indicates that a number of stem-lines are possible. Cells subjected to a new environment are perpetuated by genetic readjustment. Environmental changes will require different numbers of cell inoculum for tumor formation in adult animals but will not effect absolute reversal of the tumorigenic properties of the cells.

Normal development from a fertilized egg results in diverse cell types that constitute a variety of different organs in which no change in chromosomes is required. The growth of an entire carrot from 1 parenchymal cell (41) or a fully developed normal adult frog (17) from the nuclear transplantation from an adult intestinal cell to an enucleated frog ovum are evidence that changes in states of differentiation can occur without any chromosomal changes. The structural and numerical changes that occur after transformation depend upon the species and are part of the problem involving cell differentiation and the evolution of cell populations to autonomy.

REFERENCES

42. Sugiyama, T., and Brilliante, F. P. Cytogenetic Studies of Leukemia Induced by 6,8,12- and 7,8,12-Trimethylbenz(a)anthracene. J. Exptl. Med., 131: 331–344, 1970.
Fig. 1. Karyotype of a cell from a 4NQO17-transformed cell line. This line has no detectable structural modifications and all chromosomes have a normal banding pattern (trypsin preparations). The karyotype consists of 46 chromosomes; trisomy or B5 and C15 is present.

Fig. 2. A hypertetraploid cell from the same 4NQO17-transformed cell line. According to banding patterns, all chromosomes are identifiable as normal. There are 90 chromosomes, with the 2 extras belonging to Groups B5 and B10.

Fig. 3. Enlargement of M1, and a pair of normal A2 chromosomes (acetic saline Giemsa preparation) followed by schematic representation of Marker 1 and the A2 for comparison. This chromosome marker was obtained from a 4NQO16t cell, and it can be seen that, following the largest negative zone of the long arm, an additional 2 positive bands have been inserted.

Fig. 4. Representatives of all markers found subsequent to M1; M1 through M7, occurred in cell lines and/or tumor cultures, whereas M9 and M10 were found in only 1 tumor. The new chromosomes are either between their normal components or to the left of their normal analog.

Fig. 5. A karyotype of a subdiploid cell from BP45-transformed cell line (quinacrine mustard preparations). The new chromosomes are indicated by M1, M2, M3, and M4, as well as trisomy for D17. Missing are 1 B7, C12, C15, and a pair of C13 chromosomes.

Fig. 6. Karyotype of a cell from 4NQO16t culture with several markers as well as numerical changes in several different pairs of chromosomes (acetic saline Giemsa preparation). Groups B7 and D17 are trisomic and A1, C15 and F21 monosomic; M1 and M4 are present as well as M9 and B9, which were found only in this specific tumor line.
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M2
M4
M5
M7
Ma
Mb

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