Chromosomal Analysis of Tumors Derived From Mouse Cells After Neoplastic Conversion In Vitro in Various Serum-Supplemented Media ¹,²

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SUMMARY—In a previous study, cells from two different pools of C3Hf mouse embryo cells were cultured in chemically defined medium supplemented with different types of serum. The type of serum supplement influenced the chromosomal characteristics of the cells and also the time of their neoplastic conversion. In testing the cells for evidence of neoplastic conversion, we implanted a large inoculum of cells into syngeneic hosts. Of the implanted cells, only a portion might have been neoplastic. To define more clearly the chromosomal characteristics of the neoplastic components of the cell populations in vitro and to look for any common patterns of chromosomal constitution in the tumors, we examined the chromosomal characteristics of 14 tumors arising from the cell implants. A wide and sometimes characteristic distribution of chromosome numbers occurred in the tumor cells similar to that observed in each subline in vitro. These observations strongly suggest that most of the karyotypically diverse cells in culture were neoplastic and capable of continued progressive growth in vivo. No common pattern of chromosomal constitution in the different tumors could be detected. The occurrence of long acrocentrics and other asymmetric chromosomes in the tumor cells and their scarcity in the cells in vitro indicate that chromosomes of the tumor cells underwent more numerous breakages and that these occurred at random along their lengths rather than at or near the centromere, as observed in the cells in vitro.—J Nat Cancer Inst 43: 71-76, 1969.

THE RELATIONSHIP between chromosomal aberrations in cells during culture and their "spontaneous" neoplastic conversion is still unclear. At first it appeared that cells showing chromosomal instability during culture had a greater tendency to undergo neoplastic conversion in vitro (1-3).

In a more recent study, however, this relationship did not hold (⁴). Cells from two different pools of C3Hf mouse embryos were grown in chemically defined medium supplemented with 10% horse, fetal calf, calf, or bovine serum. Chro-

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mosomal characteristics were followed for the first 9–11 months in vitro, and at intervals, cells were tested in vivo for evidence of neoplastic conversion. As observed in the earlier studies (1–3), cells in fetal calf serum medium maintained euploidy longer than those in horse serum medium and exhibited fewer chromosomal alterations. Cells in calf and bovine serum medium showed chromosomal characteristics similar to those in fetal calf serum medium. Thus all bovine serums (fetal calf, calf, and adult) differed from horse serum in their effects on chromosome stability during the early months of culture. However, the cells of both lines on calf serum and one on bovine serum underwent neoplastic conversion at approximately the same time as cells on horse serum-supplemented medium.

In testing these cells grown on different serum supplements for evidence of neoplastic conversion, we implanted a large inoculum of several thousand or million cells into syngeneic hosts. Of the implanted cells, a variable proportion might be neoplastic and capable of continued progressive growth in the host. To define more clearly the chromosomal characteristics of the neoplastic components of the cell populations in vitro and to look for any common patterns of chromosomal constitution in the tumors, in the present study we examined the chromosomal characteristics of 14 tumors arising from the cell implants. Since the equine and bovine serums differed in their effects on chromosome stability during the early months of culture, it seemed also of interest to ascertain whether such differences persisted in the tumors arising from the tissue culture implants.

MATERIALS AND METHODS

The 14 tumors were derived from implants into C3Hf/HeN mice of cultured cells of the two lines described previously (4), line 5449 grown in NCTC 135 supplemented with 10% horse, calf, or bovine serum and line 5508 cultured in medium 135 with 10% horse serum. Intraocular tumors arising from cells implanted during the early months of culture in vitro could not be used for study, since these tended to ulcerate before enough sterile tissue could be explanted to culture. For this reason, all tumors studied were derived from the later intramuscular implants. By this time, the tissue culture cells in each type of culture medium had reached a chromosomal equilibrium both with respect to distribution of chromosome numbers and frequency of abnormalities (4). Consequently, the time elapsed between the last in vitro analyses and the implantation of cells in vivo should have little influence on the results obtained.

Since the various tumors were usually tough, collagenous, and not easily dissociated into individual cells, chromosome preparations of freshly excised tumor tissue, after Colcemid was administered to the animal (5) or the pieces were treated with Colcemid (6), were unsatisfactory. Even agitation of tumor tissue in 1.5 mg/ml solution of collagenase (7) in Dulbecco's saline with a magnetic stirrer failed to provide enough single, intact cells for study. Therefore, chromosome preparations from the tumor tissue were made after short in vitro culture of tumor pieces. From the experience of the senior author, 5–9 days of culture under the conditions used does not produce any radical change in the karyotype.

The tumors, as soon as palpable, were dissected and minced in NCTC 135 supplemented with the type serum to which the cells had been previously adapted in culture. The tumor mince was cultured for about 5–9 days under a sheet of perforated cellophane in T-30 flasks until the cells formed a confluent sheet. The cells were then removed from the flasks by gentle mopping and transferred to one T-30 flask. The following day, when the dense cell population was in log phase, the cells were prepared for chromosome analysis as described earlier (2).

OBSERVATIONS

Tumors From Line 5449 Cells Grown in Different Serum Supplements

Horse serum.—Chromosomes were studied in five tumors from cells of line 5449: two after 362, one after 419, and two after 440 days in vitro (text-fig. 1). Although the number of chromosomes in the various tumors ranged widely from 60 to above 160, the maximum frequency occurred in subtetraploid and/or hypertetraploid ranges in all five tumors. This distribution in chromosome numbers was similar to that in the tissue culture
cells of line 5449 when last studied at 341 days in vitro (4). A somewhat higher frequency of cells with more than 160 chromosomes was observed in certain tumors. These hyperoctoploid cells probably developed through doubling of chromosome number in the hypertetraploid cells. The average number of both minute and metacentric chromosomes per cell was similar in the tumors to that in the cells in vitro, except for one tumor in which the number of minutes was increased. One difference between the cells grown in vivo as compared with those in vitro was the regular appearance of large acrocentrics in all tumors.

Calf serum.—Chromosomes were studied in three tumors, two from cells after 419 and one from cells after 440 days in vitro. Most cells had 80–90 chromosomes (text-fig. 2), in contrast to those of the last analysis in vitro at 341 days where frequency distribution peaks lay between 64 and 76 (4). While the average number of minutes per cell in the tumors was usually higher than in the cells in vitro, the number of metacentric chromosomes was similar. All three tumors had some cells with long acrocentric chromosomes never observed in cells of this subline in vitro.

Bovine serum.—Chromosomes were studied in four tumors: one from cells after 362, two after 419, and one after 440 days in vitro. Chromosome ploidy in cells cultured in bovine serum medium had shifted less rapidly than in the other serum supplements, so that, even after 341 days in vitro, the line still possessed some near-diploid cells (4). Consistent with this trend in culture was the occurrence in two of four tumors of some cells with less than 60 chromosomes. While the chromosome numbers ranged widely, particularly in one tumor, most tumor cells possessed 66–78 chromosomes (text-fig. 3). In two of four tumors the average number of minute chromosomes per cell was higher than in the cultured cells when examined after 341 days in vitro. However, the average number of metacentrics had decreased in the tumors as compared to the cells in vitro. Again, the number of long acrocentrics was particularly high, ranging
from an average of 1–2.4 per cell in all four tumors.

Tumors From Line 5508 Cells Grown in Horse Serum Medium

Chromosomes were studied in two tumors, one from cells after 299 and one after 322 days in vitro (text-fig. 4). While most cells after 278 days in vitro had 60–82 chromosomes (4), in the majority of tumor cells the number of chromosomes varied from 118 to above 160. These observations indicate a doubling of chromosomes per cell probably during growth in vivo as tumors. The average number of minutes and metacentrics per cell was higher in the tumors than in the cells in vitro. The average number of long acrocentrics, particularly in one tumor, was very high.
DISCUSSION

An important finding of the present study was the wide and sometimes characteristic distribution of chromosome numbers in the tumor cells similar to that observed in each subline in vitro. These observations strongly suggest that most karyotypically diverse cells in culture were neoplastic and capable of continued progressive growth in vivo. Further, no common pattern of chromosomal constitution in the different tumors could be detected. Whereas the equine and bovine serum supplements produced different effects on chromosomal constitution during the first few months of culture, after the more prolonged growth in vitro and in vivo with accumulation of chromosomal aberrations, these differences became less distinct.

In the tumors under study, all attempts to disperse cells with trypsin or collagenase failed to give adequate chromosome preparations, and the analyses were, therefore, limited to tumor cells after short culture. The validity of results from such studies has sometimes been questioned on the basis of possible overgrowth of stromal cells or cell selection during culture (8-11). Nichols (12), Hellström et al. (13), and Koler and Lubs (14), on the other hand, found no differences between chromosome characteristics of tumors obtained directly and after short culture. Although direct chromosome analysis of the tumors in the present study was not possible, we found that the distribution of chromosome numbers in the tumors after a short culture of 5-9 days generally was similar to that in the precursor cells in vitro. Further, the complete absence of diploid cells clearly showed that tumor cells either outnumbered the stromal cells in the explanted fragments or were better adapted to survival and growth in vitro than the stromal cells.

The larger number of minute chromosomes in the tumor cells as compared with the cells in vitro probably resulted from chromosomal breakages. No micronuclei were ever observed. Since the tumor cells also possessed many large acrocentrics as well as other asymmetric chromosomes like rings and those with chromatid interchange, it appeared that, during a shift from in vitro to in vivo growth, chromosomal breaks occurred which resulted in new chromosomal balances and configurations on which selection operated during adaptation of cells to the different environment in vivo.

The high incidence of long acrocentrics and the regular appearance of asymmetric chromosomes in tumors in contrast to their scarcity in vitro, where they seemed to be of sporadic origin (4), suggested differences in the chromosome regions susceptible to breakage. From the rare occurrence of such chromosomes in cells in vitro, we concluded earlier (4) that the chromosome region susceptible to breakage in culture was at or near the centromere. For the origin of a long acrocentric chromosome, the points of breakage have to be distributed along the length of the chromosome. Further, the point of breakage in at least one chromosome must have been in the distal half, since all the large acrocentrics were 1.5 or more times the length of the largest member of the mouse karyotype. The high incidence of long acrocentrics and minutes in tumors suggested, therefore, that chromosomes during adaptation and growth of the cells in vivo
underwent more numerous random breakages along their lengths.

REFERENCES


