Malignant Transformation and Chromosome Change of Two Cell Lines from Syrian Hamster Embryonic Tissue *in vitro**

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After 10 months of continuous cultivation *in vitro*, two cell lines (HE3-2 and HE4-2 cell lines) of embryonic tissue origin from Syrian hamsters did not produce tumors upon subcutaneous implantation into the hosts. After 13 months, however, the similar implantation resulted in the development of rapidly growing transplantable fibrosarcomas. The tumor-producing capacity of HE3-2 cell line was higher than that of HE4-2 line. The chromosomal analysis of HE3-2 cell line after 10 months' culture *in vitro* showed hyperdiploid karyotypes ("B" karyotype) with modal value of 47, while that after 13 months showed the predominance of further altered karyotypes ("A" karyotype) with modal value of 45 to 46. On the other hand, the most population of HE4-2 cell line after 13 months *in vitro* contained "B" karyotypes and a minor part of it showed "A" karyotypes.

Most of the cell lines of normal tissue origin¹⁻¹¹ have been reported to undergo spontaneous malignant transformation, after long-term cultivation *in vitro*, and to acquire the capacity to produce tumors when implanted into the hosts. It becomes, therefore, apparent that the study on the shift of the various biological behavior of cells cultured *in vitro* is one of the ways to solve the problems of carcinogenesis. Two cell lines (HE3-2 and HE4-2 cell lines) were established *in vitro* from Syrian hamster embryos.¹² The shift of biological characteristics, such as cell morphology, growth rate, the ability to grow at low inoculum density, chromosomal constitution, and tumor-producing capacity was examined for 10 months. No distinctive morphological alterations were observed after the establishment. The cell lines acquired the ability to grow at low inoculum density. The populations shifted from the diploid range to the hyperdiploid. The cells implanted periodically into the hosts never developed tumors. However, the cells of two cell lines after 13 months *in vitro*, produced tumors upon subcutaneous implantation into the hosts. Chromosomal study performed at the

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* The second report of spontaneous malignant transformation *in vitro* of the cells from Syrian hamsters.

same period revealed that there were further shifts of the modal number and remarkable changes in the karyotype. This paper presents the tumor-producing capacity and the chromosomal features of these two cell lines.

MATERIALS AND METHODS

The previously described Syrian hamster cell lines¹² (HE 3-2 and HE4-2 cell lines) were maintained for 13 months *in vitro* and further observed. The growth medium is a modified Eagle's basal medium supplemented with 20 per cent bovine serum. Transfers of the cells were performed with 0.025% pronase solution in Hank's physiological fluid. An air-drying technique based upon that of Rothfels and Siminovitch¹³ was used for the preparation of the specimen for chromosomal analysis. Tests of the tumor-producing capacity were made by implanting subcutaneously the cultured cells into the 10- to 20-day-old hamsters. These animals were observed periodically for 4 months for the appearance of malignant growth. A more detailed description¹² on the materials and methods was presented in the paper which appeared previously.

RESULTS

Implantation

The 10- to 20-day-old hamsters were implanted subcutaneously at the side-abdominal region with cells from HE3-2 or HE4-2 cell lines and observed for 4 months for malignant growth. Gross observation was made once a week, and microscopic examination was made when the implanted hamsters were killed. When considerable progressive increase of the implanted cells was recognized and a malignant figure was found on the microscopic examination,

TABLE 1. *The tumor-producing capacity of HE3-2 and HE4-2 cell lines in Syrian hamsters*

Cell line	No. of cells implanted	No. of animals developing tumors/no. of animals implanted with cells	Average days of tumor appearance (0.5 cm in diameter)
HE3-2 cell line	1×10^6	6/6	15
	5×10^5	6/6	23
	2×10^4	10/10	31
	1.2×10^4	17/17	34
	6×10^3	13/13	60
	1.2×10^3	3/6	96
	6×10^2	1/10	136
	6×10^1	0/12	
	6×10^0	0/10	
HE4-2 cell line	2×10^6	8/8	32
	2×10^5	13/13	58
	2×10^4	5/13	91
	2×10^3	0/10	
	2×10^2	0/6	
	2×10^1	0/8	

the implant was decided as revealing the malignant growth. The diagnosis of tumor was further confirmed by serial transplantation to other hamsters. The resulting data are shown in Table 1.

HE3-2 cell line: The cells of HE3-2 cell line produced tumors in all hamsters at inoculum levels ranging from 6×10^3 to 1×10^6 cells per hamster. Implantation of 1.2×10^3 cells developed tumors in 3 of 6 hamsters inoculated, while that of 6×10^2 cells produced tumors in 1 of 10 hamsters. No tumor was produced in the hamsters implanted with 6×10 or less cells per hamster.

HE4-2 cell line: The cells of HE4-2 cell line produced tumors in all hamsters implanted with 2×10^5 to 2×10^6 cells per hamster, while at the inoculum levels of 2×10^4 cells the implants produced them in 5 of 13 hamsters. At the inoculum level of 2×10^3 cells or less per hamster, all implantations gave negative results.

Comparing HE3-2 cell line with HE4-2 cell line in the tumor producing capacity, cell numbers enough to produce tumors for all the implanted hosts were over 6×10^3 in the case of HE3-2 cell line, while those were over 2×10^5 in the case of HE4-2 cell line. The average days of tumor appearance in the case of HE3-2 cell line were significantly shorter than those observed with HE4-2 cell line. Thus it is quite apparent that the tumor-producing capacity of HE3-2 cell line is higher than that of HE4-2 cell line. From the results of histological examinations, it proved that tumors had characteristics of well-differentiated fibrosarcoma with elongated fusiform cells, infiltrating the surrounding connective and muscle tissues (Fig. 4).

Chromosomal study

The nomenclature and the criteria used in the construction of the karyotype followed those described by Moorhead *et al.*¹⁴

HE3-2 cell line: The chromosome number distribution of the cells is shown in Fig. 1. In a sample taken 10 months after the initial culture, its modal number was 47, but in a sample taken 13 months after the initial culture, approximately 50 per cent of the metaphases analyzed belonged to the hyperdiploid region ranging from 45 to 46 chromosomal number. Thus, the modal number shifted from 47 to the region of 45 to 46. The representative karyotypes are shown in Fig. 2. As compared with the normal female karyotype, the majority of karyotypes with 46 chromosomes has one X chromosome, one submetacentric between group #1 and #2, 2 chromosomes in group #1, 2 in #2, 6 in #3-4, 2 in #5, 8 in #6-7, 2 in #8-9, 2 in #10, 7 in #11-15, 8 in #16-19, 3 in #20 and 2 in #21. Most of the karyotypes with 44 and 45 chromosomes were similar to that with 46 chromosomes except for the chromosomal loss of group #6-7. Also 4 of 13 karyotypes with 47 chromosomes were similar to that with 46 chromosomes except as to the chromosomal increase of group #6-7. In this paper these karyotypes were temporarily designated "A" karyotype. As shown in Fig. 3, 9 of 13 karyotypes

Fig. 1. The chromosome number distribution of HE3-2 and HE4-2 cell lines (13 months *in vitro*).

with 47 chromosomes were similar to that of the normal female karyotypes except for having 5 chromosomes in group #8-9, 5 in #11-12 and 3 in #20. This karyotype was designated "B" karyotype.

HE4-2 cell line: In this population 52 per cent of metaphases analyzed possessed 47, while 22 per cent of those had 46 and 11 per cent had 45 chromosomes. Thus, the modal number of this cell line was 47 and similar to that of a sample taken 10 months after the initial culture. The karyotype analysis was made on 51 adequate metaphases with 47 chromosomes: 34 belonged to "B" karyotype, 4 revealed "A" karyotype and 13 revealed other various karyotypes. As mentioned above, most of the cell population of HE3-2 cell line

Fig. 2. The "A" karyotypes of HE 3-2 cell line.

A: chromosome number 46

B: chromosome number 45

C: chromosome number 44

possessed "A" karyotype, whereas most population of HE4-2 cell line possessed "B" karyotype.

DISCUSSION

The tumor-producing capacity of HE3-2 cell line was higher than that of HE 4-2 cell line. In the comparison of the two chromosomal constitutions, the cell

Fig. 3. The "B" karyotype of HE 4-2 cell line chromosome number 47.

Fig. 4. An example of the histologic pictures of tumors formed by cells of HE3-2 and HE 4-2 cell lines.

population of HE3-2 cell line contained a large proportion of cells with "A" karyotype, while that of HE4-2 cell line did a small part of those. It may be, therefore, considered that the cells with "A" karyotype is malignant and the cells

with "B" karyotype is not. The difference between the two cell lines in tumor-producing capacity is not substantial. It may rather consist merely in the proportion of karyotypes present. However, it cannot be concluded from the present experiment that the different malignant behavior of the two cell lines is associated with the difference in proportion of karyotypes present, unless a clonal analysis of the two cell lines is undertaken.

When examined 5 and 10 months after the initial culture, the stem cell karyotype of HE3-2 cell line was "B" karyotype and any tumor was not produced by means of the subcutaneous implantation into the hosts. However, 13 months after the initial culture, all implants produced tumors appearing at the site of implantation. Besides, it was found from the chromosomal study that there were further shifts of the modal number and remarkable changes in the chromosomal constitution. In general, is there any causal relationship between chromosome irregularity and malignant behavior? The malignant property of several heteroploid cell lines of normal tissue origin has been demonstrated by subcutaneous or intraocular transplantation of these cells or by testing their growth potentialities in the cheekpouch of hamsters. Levan and Biesele¹⁵ cultured *in vitro* embryonic mouse skin and followed the chromosome behavior throughout successive passages. It was found that the proportion of cells with the normal diploid chromosome number gradually decreased, and after the 16th passage the normal diploid cells disappeared entirely from the cell line. After the 21st passage the heteroploid cell population was transplanted and induced a subcutaneous tumor. Recently, Shelton *et al.*⁹ reported that normal tissue cells when kept in a millipore chamber for 23 months in the peritoneal cavity of mice, underwent heteroploid transformation and became malignant. On the other hand, however, several lines are known in which cells with a normal chromosome number are present in large numbers displaying malignant behavior. Especially, the known viral tumors¹⁶⁻²¹ have shown normal chromosome complement in the great majority of cases. Thus chromosome anomaly may not necessarily be associated with malignant behavior of cells. Hsu²² comments on the role of chromosomes in carcinogenesis as follows: "It seems highly possible that at least in certain instances, e.g., chemical carcinogens and the *in vitro* system, chromosomal constitution plays an important role in carcinogenesis. Two activities are the basic requirements: induction of mitosis through tissue trauma or otherwise and induction of chromosomal mutations. Without the burst of mitotic activity abnormal chromosomal constitution, even though induced during interphase, could not find the opportunity to enter the scene of competition and selection."

What induced a spontaneous malignant transformation in the cell lines of normal tissue origin after long-term cultivation *in vitro*? Although the nature and cause of its essential change are still unknown, several hypothesis have been

suggested up to the present time. Sanford²³ suggested four hypothesis as follows:

- 1) The removal of the tissue from the physiologic control of the animal.
- 2) The growth of the cells in a new environment and in an entirely heterogeneous culture medium.
- 3) The rapid rate of cell proliferation.
- 4) Some unidentified carcinogenic agency, such as chemical carcinogen, virus, or altered O₂ or CO₂ tension.

Goldblatt⁵ reported an effect of intermittent anaerobioses on the cells from rat myocardium, resulting in malignant transformation. Also there is the observation of Barski⁸ on the development of malignancy in the C57BL mouse lung tissue cells following treatments with trypsin. Recently, Schelton *et al.*⁹ reported from the study on the mouse connective tissue cells grown in diffusion chambers that it was possible to eliminate foreign serum protein and repeated stimulation of motosis as the factors of malignant transformation. Further, they stated: "It is more probable that the cause of the malignant transformation lies in the altered relationship between the tissue and its physiological environment."

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