AUTORADIOGRAPHIC DETERMINATION OF PARAMETERS OF THE CELL CYCLE OF A HUMAN TESTICULAR TUMOR IN TISSUE CULTURE

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UDC 618.11-006.-018.15-092.4

Parameters of the cell cycle of a human testicular tumor in primary culture were studied by autoradiography. The cells were incubated in medium containing thymidine-H^3 for a long period. The duration of the mitotic cycle of the population as a whole and of its individual phases was: $T = 83.6$ h, $S = 5.35$ h, $G_2 = 18$ h, and $G_1 + M = 60.25$ h.

KEY WORDS: testicular tumor; monolayer culture; cell cycle.

As a result of the study of the duration of mitotic cycles and their individual phases in normal tissues and tumors the most effective plan for administration of antitumor preparations can be chosen [9].

Cells of primary cultures are known to preserve the histotypical and functional features of the original tissue [8]. As some workers have found [1, 5], the parameters of the mitotic cycle of tumor cells in culture are the same as in vivo. It is much easier, however, to study the duration of the cell cycle and its phases in tissue culture than in vivo.

This paper describes the results of an autoradiographic determination of the parameters of the cell cycle of a human testicular tumor in primary tissue culture.

EXPERIMENTAL METHOD

Material obtained at operations in the clinic of the Institute of Experimental and Clinical Oncology, Academy of Medical Sciences of the USSR, was used. To prepare a primary culture pieces of tumor (a teratoblastoma) measuring 1-2 mm^3 were fixed to narrow slides (0.8×8 cm) by means of the minimal quantity of mixture of chick embryonic extract and chick plasma and incubated at 37°C in tubes with medium No. 199 containing 20% bovine serum and 10% chick embryonic extract.

The cells of the primary culture grew on the slides as a monolayer of homogeneous fibroblast-like cells with branching processes of cytoplasm and with pale, vesicular, and highly polymorphic nuclei, differing mainly in size (Fig. 1). The cells were arranged on the slide mainly in one direction (radially relative to the fragment). The bundles of cells could interweave with increasing distance from the fragment and at the periphery of the zone of growth some cells lost their regular orientation and lay perpendicularly to the main direction of growth of the bundle. Some cells had vacuolated cytoplasm and nuclei with an irregular surface, on which "projections" could be seen.

To determine the parameters of the cell cycle, slides on which there were at least 1000 cells of the primary culture were chosen. The duration of DNA synthesis was determined from the increase in number of labeled cells in the monolayer during continuous incubation in medium with thymidine-H^3 in a dose of 0.3 μCi/ml for 48 h. The mean duration of the postsynthetic period was determined from the curve of

Department of Experimental Therapy, All-Union Research Institute of Antibiotics, and Department of Pathological Anatomy of Human Tumors, Institute of Experimental and Clinical Oncology, Academy of Medical Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR N. A. Kraevskii.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 79, No. 4, pp. 95-97, April, 1975. Original article submitted May 12, 1974.

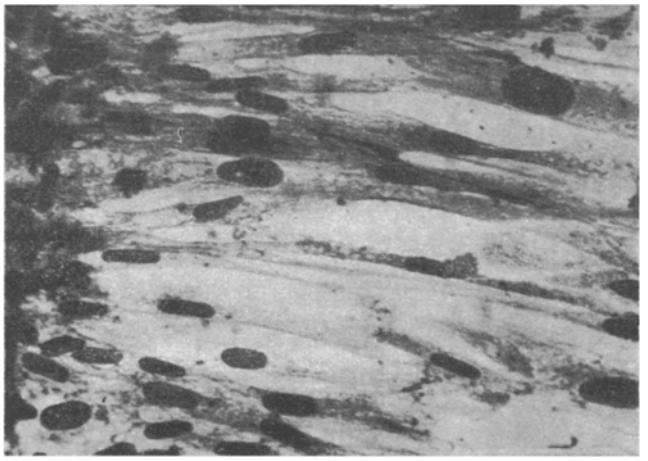

Fig. 1. Cells of human testicular tumor (16-day culture). Hematoxylin–eosin, 900×.

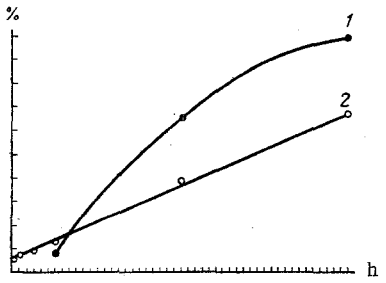

Fig. 2. Changes in number of labeled mitoses (1) and labeled cells (2) in tissue culture of human testicular tumor during continuous incubation for 48 h in medium with thymidine-H^3. Abscissa, time after injection of thymidine-H^3 (in h); ordinate, number of labeled mitoses and labeled cells (%).

labeled mitoses in the same preparations from the time when they reached the 50% level of labeled mitoses. The length of the cell cycle was found by calculation. The cells were fixed with acetic alcohol at different times after the beginning of incubation with the labeled nucleoside. Unincorporated thymidine-H^3 was removed from the cells by treatment with cold 5% perchloric acid for 30 min followed by washing in distilled water and treatment with alcohol and ether. The dried preparations were covered with type "R" liquid nuclear emulsion and exposed for 2-3 days at 4°C. The autoradiographs were stained with hematoxylin–eosin and counting carried out to determine the index of labeled cells (in 1000 cells on each slide), the mitotic index, and the index of labeled mitoses. Two or three slides with the culture were used at each period of the investigation.

EXPERIMENTAL RESULTS

A graph of the changes in the number of labeled cells and labeled mitoses in the human testicular tumor in tissue culture is shown in Fig. 2. The first labeled mitoses were found 6 h after incubation with thymidine-H^3 (9%). Their number 24 and 48 h after incubation was 66.6 and 100%, respectively. The mean duration of the postsynthetic (G_2) period, determined as the interval between addition of thymidine-H^3 and the appearance of 50% of labeled mitoses, was 18 h.

The index of labeled cells 15 min after addition of the thymidine-H^3 was 6.4%. Later during incubation the index of labeled cells increased as a linear function of time and reached 67.6% by the end of the second day.

The mean mitotic index was 4-5 ‰ at all periods of the investigation.

The duration of these experiments (48 h) was too short to allow the size of the proliferative pool of the culture to be determined, for the curve of labeled cells did not reach a plateau (Fig. 2). However, it can be assumed that the size of the proliferative pool was close to 100%. A culture on the 16th day after explantation was used in these experiments. The lag phase lasted up to 2 weeks. This suggests that the culture studied was in the logarithmic phase of growth.

Incubation of the testicular tumor cells in the constant presence of thymidine-H^3 enabled their transition from the presynthetic (G_1) period into the period of DNA synthesis (S) to be studied and the duration of the latter period (t_S) to be determined from the increase in the number of labeled cells, by the equation [3]:

$$t_s = \frac{S_{i_0} \cdot t}{S_{i_t} - S_{i_0}},$$

where S_{i_0} is the original index of labeled cells; S_{it} the index of labeled cells after time t. The duration of the period of DNA synthesis was found to be 5.35 h.

The duration of the mitotic cycle (T) was calculated by the equation [7]:

$$T = \frac{t_s}{S_{t_0}}.$$

The value of T was 83.6 h. Hence, the duration of the presynthetic period (G_1), allowing for the duration of mitosis (M), was 60.25 h.

A noteworthy feature of the culture used was the long duration of the cell cycle, due to the lengthened premitotic period (G_2) and the considerable duration of the presynthetic period (G_1). The duration of the G_1 period in human tumors is known to be relatively long. According to data in the literature, some human and animal tumors have a relatively long premitotic G_2 period [4, 10]. In the present experiments the mean duration of the G_2 period also was long (18 h). If the curve of labeled mitoses on the graph in Fig. 2 is extrapolated to the abscissa, the minimal value of the G_2 period can be seen to be approximately 4-4.5 h.

The results of this investigation indicate that the durations of the individual periods of the mitotic cycle of human tumor cells can be determined in a primary plasma culture. Such a possibility was demonstrated previously by the writers [2] for the Harding–Passy melanoma. Moreover, the results obtained in vitro were found to agree with estimates of the duration of the individual periods of the mitotic cycle of that tumor obtained in vivo by other workers [6].

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