MITOTIC CYCLES OF HEPATOMA 22A CELLS

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UDC 616.36-006-018.15-092.9

The duration of the mitotic cycle on the 2nd, 5th, 8th, and 11th-12th day of development of the ascites variant and on the 10th and 15th days of development of the solid variant of hepatoma 22A was determined by an autoradiographic method in the cells of this tumor. The duration of the mitotic cycle of the cells of the ascites variant was found to increase considerably during the life of this strain (the duration of the S-period increased from 14 to 26-27 h and of the G_2-period from 2.2 to 13 h). The duration of the mitotic cycle in cells of the solid variant of hepatoma 22A was the same at both times of development of this tumor. Correlation was found between changes in the duration of the mitotic cycle during the life of the strain of tumor cells and its changes with an increase in the period after the first passage of that strain.
KEY WORDS: transplanted tumors; hepatoma 22A – periods of development; mitotic cycle.

Most workers consider that with an increase in the age of transplantable tumors the duration of the mitotic cycle increases considerably. The mitotic cycle of cells in the late stages of development of tumors may be several times longer than the mitotic cycle of the cells in the early stages of development. According to some observations [6, 8, 9, 11-13] this difference is the result of a uniform increase in the duration of all the periods of the mitotic cycle, but according to others [14] most of the increase is due to an increase in the duration of the G_1 -period. Meanwhile it is stated in the literature that the duration of the mitotic cycle in cells of the ascites forms of transplantable tumors does not always change during the life of the strain of tumor cells [10]. As regards the solid forms of experimental tumors the results are more consistent. They indicate that the duration of mitotic cycles in the cells of solid tumors is relatively constant throughout their individual development [7, 12].

The object of this investigation was to determine changes taking place in the mitotic cycle of cells of hepatoma 22A (its ascites and solid variants) during the individual development of this tumor. Previously the duration of the mitotic cycle of the cells of this tumor had been determined for only one time (the 5th day of development) of the ascites variant [2] and another time (the 10th day of development) for the solid variant [5].

EXPERIMENTAL METHOD

The solid form of hepatoma 22A obtained by Gel'shtein [3, 4] and the ascites form of the same hepatoma obtained by Vasil'ev [1] were used. Both variants of hepatoma 22A have been maintained by passage for several years as a group of tumor strains at the Institute of Experimental and Clinical Oncology, Academy of Medical Sciences of the USSR, from which they were obtained in 1972-1973. Male C3HA mice weighing 20-22 g were inoculated. Ascites fluid was injected intraperitoneally in a volume of 0.25 ml and a suspension of minced pieces of solid tumor in physiological saline were injected subcutaneously. Thymidine-H^3 was injected intraperitoneally in a dose of 0.3 μCi/g (specific activity 4.3 Ci/mmole). The animals were killed at intervals of 2 h over a period of 48 h (ascites form) and 16 h (solid form) after injection of the isotope (3-8 animals at each time). The experiments with the ascites form of hepatoma 22A were carried out on the 2nd, 5th, 8th, and 11th-12th day, and with the solid form on the 10th and 15th day of tumor development. Smears of ascites fluid and histological sections of the solid tumor were coated with type R

Laboratory of Chemical Factors for the Regulation of Cell Growth and Division, Institute of Biological and Medical Chemistry, Academy of Medical Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR V. N. Orekhovich.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 78, No. 8, pp. 97-99, August, 1974. Original article submitted October 4, 1973.

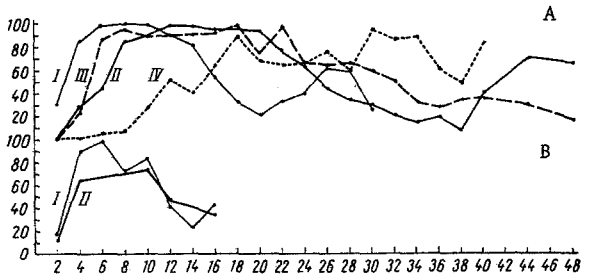

Fig. 1. Changes in percentage of labeled mitoses at different times of development of hepatoma 22A: A) ascites variant: I) 2nd, II) 5th, III) 8th, and IV) 11th-12th day of tumor development; B) solid variant: I) 10th, II) 15th day of tumor development. Abscissa, time, in h, after injection of thymidine-H^3; ordinate, percentage of labeled mitoses.

nuclear emulsion; the former were exposed for 4-6 days, the latter for 4-6 weeks. The percentage of labeled mitoses was determined in preparations developed and stained with Mayer's hematoxylin: in the ascites tumors on the 2nd, 5th, and 8th days of development by counting 100 mitoses and on the 11th-12th day of development by counting 25-50 mitoses, and in the solid tumors by counting 100 mitoses.

EXPERIMENTAL RESULTS

The results of the study of the number of labeled mitoses are shown in Fig. 1A for cells of the ascites form of hepatoma 22A at the different times of development. Despite the long duration of the experiment (48 h) no second wave of labeled mitoses was found for the tumors on the 8th and 11th-12th days of development; on the whole, therefore, only the durations of the S- and G-periods of the mitotic cycle could be compared.

It follows from Fig. 1 that on the second day of development of the ascites hepatoma 22A the mitotic cycle of the cells had its shortest duration, but with an increase in age of the tumor the duration of the cycle rose steadily. For example, the duration of the S-period of the mitotic cycle in the 2-day tumor was 14 h, in the 5-day tumor 19.8 h, and in the 8- and 11-12-day tumors about 26-27 h (in the last case the duration of the S-period could not be determined exactly), whereas the duration of the G_2-period increased from 2.2 h in the 2-day to 13 h in the 11-12-day tumor.

The curves of labeled mitoses shown in Fig. 1A also show that with an increase in the period of development a steadily increasing degree of desynchronization in the passage through the individual periods of the mitotic cycle by the ascites tumor cells was observed.

Curves of labeled mitoses for cells of the solid form of hepatoma 22A on the 10th and 15th days of development are given in Fig. 1B. The duration of the S- and G_2-periods of the mitotic cycle of the tumor cells did not in fact differ at these times and they were 8 and 3 h respectively.

The results obtained for hepatoma 22A confirm the observations of other workers who found a marked increase in the duration of the individual periods of the mitotic cycle in the course of individual development of ascites forms of transplantable tumors and the relative stability of the duration of the mitotic cycle in solid tumor cells.

According to an earlier [2] determination of the parameters of the mitotic cycle in cells of the ascites form of hepatoma 22A on the 5th day of development the duration of their S-period was 6-8 h and of their G_2-period 4 h. Meanwhile, for example [5], for cells of the solid variant of hepatoma 22A on the 10th day of development the duration of the S-period was 8.6 h, and for the G_2-period 2.4 h. In other words, the duration of the S and G_2-periods of the mitotic cycle for cells of both the ascites and solid variants of hepatoma 22A was about the same and resembled the duration of these periods in the hepatocytes of the regenerating mouse liver.

The duration of the mitotic cycle of hepatoma 22A cells was determined in the present investigation 8-10 years after the first passage of these tumors, i.e., after a considerable length of time. Results obtained by the present writers show that during this period the mitotic cycle of cells of the ascites form of

hepatoma 21 altered considerably: at identical times of development (the 5th day) the duration of the S-period, for example, more than doubled. At the same time, the mitotic cycle of cells of the solid variant of hepatoma 22A on the 10th day of development and over the same period of passage remained virtually unchanged, nor was any increase found in the duration of the S-period.

Comparison of the changes in the mitotic cycle throughout the life of a given strain of the tumor with its changes accompanying an increase in the duration of passage reveals the following relationship. In cells of the ascites form of hepatoma 22A the duration of the mitotic cycle increased during development of the tumor and it also showed a change of comparable magnitude in the course of repeated passages. Meanwhile the relative stability of the mitotic cycle of the strain of the solid form of hepatoma 22A corresponded with its stabilization during the same period of passage. In other words, correlation between changes in the duration of the mitotic cycle of a given strain of tumor cells and its changes during the period of passage of the tumor cells can be postulated. No indication of such a correlation could be found in the accessible literature. Presumably the conflicting data on the duration of the mitotic cycle of cells of the same ascites forms of transplantable tumors to be found in the literature can be explained by differences in the periods elapsing after their initial passage.

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