CHRONOBIOLOGICAL ORGANIZATION OF REPRODUCTION OF EHRLICH'S ASCITES TUMOR CELLS

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KEY WORDS: Ehrlich's ascites tumor; mitotic index; radioactive index; desynchronization; chronobiological organization of cells; ploidy.

A previous study of a hyperdiploid strain of Ehrlich's ascites tumor (EAT) on the 5th-6th day of its growth with 12 h of daylight and 12 h of darkness showed that this tumor tissue is characterized by a distinct rhythm of the number of dividing cells, but by absence of rhythm of the number of DNA-synthesizing cells [3]. These data were evidence of desynchronization of DNA synthesis and cell division in the mitotic cycle, and indicated a disturbance of the chronobiological organization of cell reproduction in this strain of EAT.

In the present investigation rhythms of cell proliferation and DNA synthesis were studied in a hypotetraploid strain of EAT and compared with data obtained previously on the hyperdiploid strain of EAT.

EXPERIMENTAL METHOD

Experiments were carried out on 85 noninbred male albino mice weighing 18-20 g. The animals were kept in 12 h of daylight (from 4 a.m. to 4 p.m.) and 12 h of darkness, at a temperature of 18°C, and were given food ad libitum. After synchronization for 2 weeks the mice were inoculated with a hypotetraploid strain of EAT in a dose of 0.2 ml of undiluted ascites fluid, containing $8 \cdot 10^6$-$10 \cdot 10^6$ cells. The animals were killed on the 5th-6th day of growth of its tumor, every 3 h, with five to eight mice at each time of investigation, starting from noon on the 5th day. An injection of ^{3}H-thymidine, with specific radioactivity of 4.1 Ci/mmole, in a dose of 0.5 μCi/g body weight, was given to the mice 1 h before sacrifice. The extracted ascites fluid was treated by the method described previously [3], and autoradiographs were prepared. Altogether 3000-5000 cells from each animal were examined in the autoradiographs, and the mitotic index (MI) and radioactive index (RI) were calculated in promille. The numerical results were subjected to statistical analysis by the Fisher–Student test.

EXPERIMENTAL RESULTS

The results obtained by investigation of the hypotetraploid strain of EAT are given in Fig. 1. Maximal values of MI were observed at noon and 6 a.m. on the 5th day ($P < 0.05$) and minimal values at 3 p.m. and 9 a.m. on the 5th day. On the 6th day of tumor growth an increase in MI was observed at midnight ($P < 0.05$) and minimal values at 9 p.m. and noon on the 6th day. The period of oscillation was 18 h on both the 5th and the 6th days. The length of the active phase (AP) of the rhythm of mitosis on the 5th day was 7.5 h (Fig. 2). Mean values of MI for the 24-h period on the 5th and 6th days of EAT development were 15 and 13‰ respectively. The mean periodic value of MI differed only a little from the mean value for the 24-h period, namely 15 and 16‰. The absolute amplitudes of the oscillations was 23 and 8‰, their relative amplitude 7.0 and 2.0, the coefficients of synchronization 0.5 and 0.7, the pool of mitosis during the period of oscillations 187 and 206.5‰, and the fraction of mitoses in the active phase of the rhythm of MI was 67 and 66% on the 5th and 6th days respectively of tumor growth.

The number of DNA-synthesizing cells in the hypotetraploid strain during the 5th and 6th days of growth of EAT was 503 and 479‰ respectively. Fluctuations of RI during these days were not significant. Only a significant fall in RI toward noon on the 7th day of development of the hypotetraploid strain of EAT was observed (Fig. 1).

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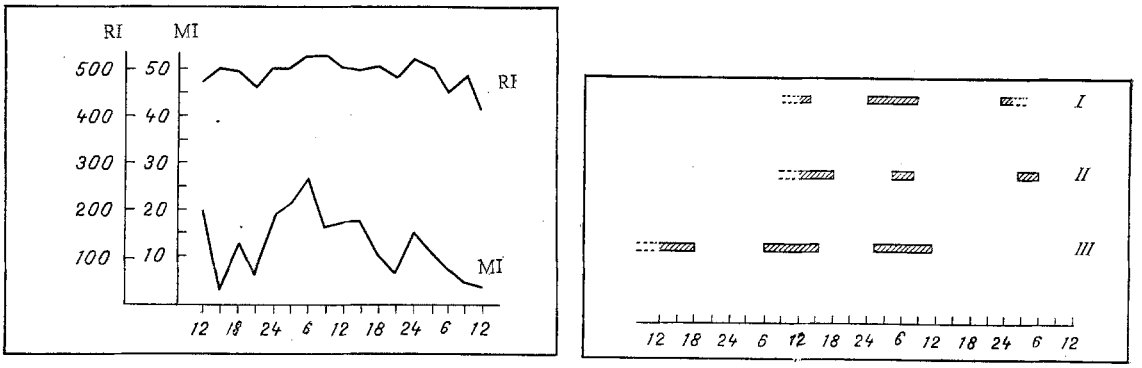

Fig. 1. Changes in number of dividing (MI) and number of DNA-synthesizing (RI) cells in hypotetraploid strain of EAT on 5th-6th day of tumor growth and light: darkness = 12 h: 12 h. Abscissa, clock time; ordinate, RI and MI (in %). Time given on 24 h clock.

Fig. 2. Phase diagram of biological rhythms of cell division in hypotetraploid (I) and hyperdiploid (II) strains of EAT on 5th-6th day of growth with light: darkness = 12 h: 12 h, and in hypotetraploid strain of EAT (III) on 4th-5th days of tumor growth under natural illumination [3]. Shaded rectangles indicate position of active phase of rhythms of mitosis during 24-h period. Time given on 24 h clock

This investigation thus revealed significant fluctuations in MI in the hypotetraploid strain of EAT on the 5th-6th day of its development under artificial illumination conditions and absence of fluctuations in the number of DNA-synthesizing cells. It can accordingly be concluded that the course of these processes in the mitotic cycle is not synchronized.

Similar results were obtained in a study of the hyperdiploid strain of EAT, which was undertaken simultaneously with the study of the hypotetraploid strain. The results also agree with those of a study of a hypotetraploid strain of Ehrlich's tumor under natural conditions of illumination [3].

For normal tissues the circadian rhythm of DNA-synthesizing cells is regarded as an intracyclic mechanism of the circadian rhythm of mitosis [1, 2, 4]. EAT is characterized by disturbance of the temporal organization of the cell reproduction system, expressed as nonsynchronization of processes of DNA synthesis and mitosis. This phenomenon was found to be typical for strains of EAT with different values of ploidy, and in experiments under different conditions of illumination. The results confirm the previous hypothesis [2] that the mechanisms of synchronization of cell populations before the S phase and before mitosis can function independently and, consequently, that rhythms of MI and of the number of DNA-synthesizing cells may be formed independently.

A phase diagram of the rhythm of MI in the hypotetraploid strain with light: darkness = 12 h: 12 h, in the hyperdiploid strain with the same illumination schedule, and in a hypotetraploid strain of EAT under natural conditions of illumination [2] is given in Fig. 2. The position of the active phase in the two strains of EAT coincides for time of day, and this could indicate that the level of ploidy has no effect on the character of the rhythm of cell proliferation in EAT. Incidentally, in all strains studied and with all conditions of illumination, the active phase of the rhythm of mitosis began sooner next day than on the previous day. As a result of this, the period of the rhythm of mitosis lasted less than 24 h (22.5, 21.0, and 18 h respectively). Although the conditions of illumination remained unchanged, a shift of the acrophase and of the active phase to the left along the time axis was observed, evidence that fluctuations of MI in EAT are to some degree independent of the rhythm of light and darkness.

The temporal organization of cell multiplication in EAT thus differs from the temporal organization of this process in normal tissues. The rhythm of mitosis in the tumor is linked with synchronization of cells in the G_2 phase. The characteristic of the rhythm of cell reproduction is essentially independent of the ploidy of the strain of EAT and of the conditions of illumination.

LITERATURE CITED

1. S. S. Laguchev and A. I. Pivovarova, Dokl. Akad. Nauk SSSR, 179, No. 2, 493 (1968).
2. Yu. A. Romanov, I. K. Rakhmatulina, and M. N. Zaikina, in: Biology of Cell Reproduction [in Russian], Moscow (1972), p. 7.
3. Yu. A. Romanov and V. A. Stepanenko, Byull. Éksp. Biol. Med., No. 2, 187 (1980).
4. J. J. Berger and C. W. Daniel, J. Exp. Zool., 224, 115 (1982).