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CELL CYCLE AND PROLIFERATIVE POOL OF HUMAN TUMOR STRAINS TRANSPLANTED IN NUDE MICE

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To determine optimal schemes of treatment the cell cycle of many strains of mouse and rat tumors has been studied. However, the cell kinetics of strains of human tumors transplanted in nude mice, which are nowadays used to study methods of treatment of human tumors, has not yet been investigated.

This paper describes a study of the duration of periods of the cell cycle and the proliferative pool of human tumor strains obtained previously by serial transplantations in nude mice [3]: melanoma, Ewing's sarcoma, carcinoma of the kidney, and Wilms' tumor.

EXPERIMENTAL METHODS

Nude BALB/c mice bred by ourselves and aged 1-1.5 months, underwent subcutaneous transplantation of strains of human tumors in 0.5 ml Eagle's medium in the ratio of 1:3. When tumor nodes measuring 1000 mm^3 had formed the animals were given an intraperitoneal injection of ^{3}H-thymidine (1 μCi/g body weight, from "Izotop," specific activity 19.8 Ci/mole). To avoid differences due to circadian rhythms of mitosis in the tumors, the thymidine was injected always at the same time of day — between 11 a.m. and noon. Animals were killed 1 h later, and every 3 h thereafter for 36 h after injection of the labeled thymidine, 2-4 mice at a time for all transplanted strains. Altogether 130 nude mice were used.

Material was fixed in Carnoy's fluid and embedded in paraffin wax; sections were cut to a thickness of 5 μ and autoradiographs prepared. Light-sensitive type "M" emulsion (Photographic Chemical Research Institute), heated to 41°C, was applied to the sections in darkness. The sections were exposed to the emulsion for 5-6 weeks. They were then stained with hematoxylin and eosin. Cells were taken to be labeled if they had four granules or more. The number of labeled mitoses was counted in the superficial actively proliferating zones of tumor (not deeper than 300 μ), for it was only in that case that a curve of labeled mitoses with two distinct waves could be obtained. On the basis of the results of counting labeled mitoses graphs were plotted and the duration of periods of the cell cycle determined relative to the 50% level of values of the labeled mitosis curve [1]. To calculate the proliferative pool in the tumors the label saturation method [6] was used. ^{3}H-thymidine was injected into the mice seven times at intervals of 6 h and the animals were killed 1 h after the last injection. The proliferative pool (Pc) was determined as the number of cells per 1000 cells for the whole tumor, expressed in percent.

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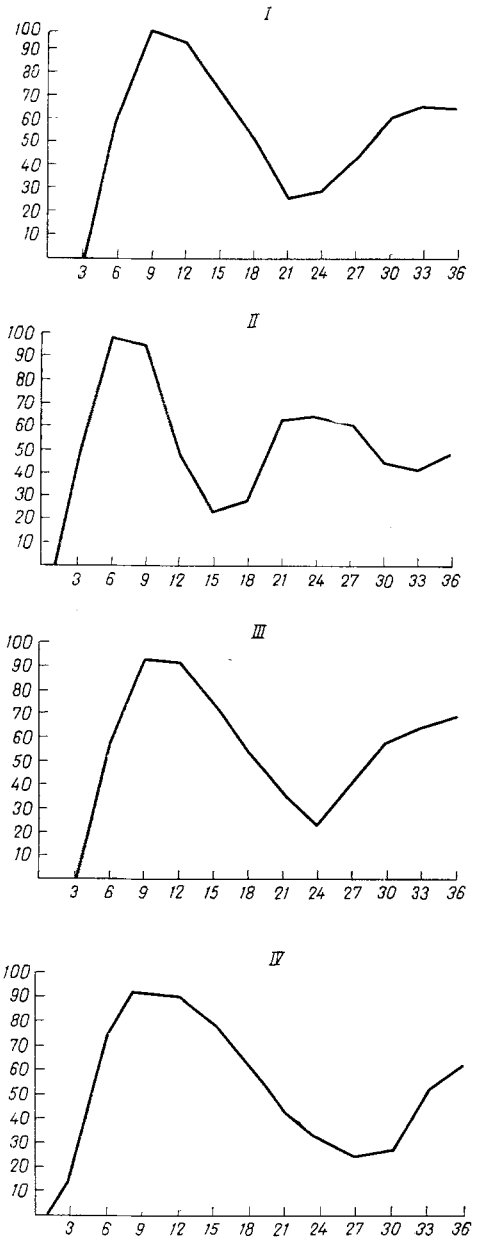

Fig. 1. Curve of labeled mitoses of human tumor strains transplanted in nude mice. I) Ewing's sarcoma; II) melanoma; III) Wilms' tumor; IV) carcinoma of kidney. Abscissa, time after injection of ^{3}H-thymidine (in h); ordinate, ratio of number of labeled mitoses to total number of mitoses (in percent).

TABLE 1. Autoradiographic Study of Cell Cybles and Proliferative Pool of Human Tumor Strains Transplanted in Nude Mice

Strain	Duration of cell cycle and its phases, h				Pc
	$G_1+\frac{1}{2}M$	S	$G_2+\frac{1}{2}M$	Tc	
Ewing's sarcoma	3,9	12,9	5,4	22,2	90,1
Melanoma	4,5	8,5	3,3	16,3	89,9
Wilms' tumor	4,1	13,1	5,6	22,8	71,7
Carcinoma of kidney	8,5	14,9	4,8	28,2	21,0

EXPERIMENTAL RESULTS

The curves of labeled mitoses of strains of melanoma, Ewing's sarcoma, kidney carcinoma, and Wilms' tumor are shown in Fig. 1. Data on the duration of the cell cycle and its phases obtained from these curves are given in Table 1.

The generation time of the Ewing's tumor strain (22.2 h) was a little longer than that of most sarcomas of mice and rats (13.5-16.5 h), but shorter than that of primary sarcoma of rats (40 h) [2, 7]. The increase in duration of the cell cycle of the Ewing's sarcoma was due to the relatively longer duration of the S and G_2 phases compared with that of most transplantable strains of this type of tumor in animals.

The generation time (16.3 h) of the melanoma strain transplanted in nude mice corresponded to Tc of transplantable mouse melanoma B16 (14.0 h) and hamster melanotic melanoma (17.5 h) [2, 5]. Meanwhile Tc of the primary tumor in man was 42-82.3 h [8, 9].

The melanoma strain which we obtained from tissue culture, when transplanted in nude mice, differed from primary human tumors in the sharply reduced Tc, on account of shortening of all phases of the cycle, and it corresponded in the parameters of the cycle practically completely to transplantable strains in laboratory animals. This was evidently due, first, to the characteristic selection of cells with a shortened life cycle, characteristic of all strains, during passage through nude mice and, second, to lengthening of the process of selection of these cells, for the melanoma strain was transplanted into mice from tissue culture after having already gone through many passages of its own.

The strains of Wilms' tumor and Ewing's sarcoma had equal generation times and phase ratios. The longer Tc of the kidney carcinoma was due to lengthening of the $G_1 + {}^1/_2M$ phase compared with the other strains [8, 5].

All the human tumor strains studied in these experiments had a high proliferative pool in the peripheral zones (about 100%). The percentage of proliferating cells decreased most sharply from the periphery toward the center of the tumor in the slowly growing kidney carcinoma strain, in which it was 21.0 for the whole tumor mass. Pc fell appreciably toward the center in the Wilms' tumor strain. In the strains of Ewing's sarcoma and melanoma the number of proliferating cells decreased only a little from periphery to center, with values of 90.1 and 89.9% respectively for the whole tumor. This high proliferative pool corresponds to transplantable tumors of animals and not to primary tumors of animals and man [4, 8, 9].

The results show that the parameters of the cell cycle of human tumor strains (melanoma, Ewing's sarcoma, carcinoma of the kidney, Wilms' tumor), transplanted in nude mice, correspond in values to those of tumor strains in experimental animals. Compared with primary tumors, the cell cycles of all strains studied were much shorter. However, each human tumor strain obtained had characteristic parameters of its cell kinetics, sufficient to explain the character of growth of these tumors and their sensitivity to various forms of treatment.

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