Difference in proliferation-kinetics between tumor cells arrested in the brain and liver

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Summary. To elucidate the mechanisms of organ specificity of cancer metastasis, rat ascites hepatoma AH7974F cells were injected into rat carotid artery. Tumors were found in the liver, but rarely in the brain. Analysis of proliferation-kinetics of tritiated thymidine-labeled tumor cells arrested in the brain and liver made it clear that tumor cells arrested in the brain remained viable, but ceased dividing, while almost all of the tumor cells arrested in the liver divided.

The spread or metastasis of cancer often occurs to specific organ sites[2]. To explain this phenomenon Paget in 1889[3] proposed the 'seed and soil' hypothesis, which envisions that the unique properties of the cancer cell as well as the site of metastasis are collectively responsible for the organ specificity of metastasis. Although supporting data for Paget's hypothesis have been presented[4-9], some problems remain unsolved. One is the influence of the lodgement site on the proliferation-kinetics of tumor cells. Previously we reported[10] a difference in the proliferation-kinetics of rat ascites hepatoma AH7974 cells lodged in the brain and choroid plexus as observed with tritiated thymidine autoradiography. In the present paper, further studies were performed in another experimental system which was based upon a comparison of the brain and liver. The AH7974F tumor system was chosen for these studies, because it had been demonstrated that experimental tumor colonies were found in the liver but only rarely in the brain after the injection of tumor cells into the carotid artery[11], tail vein[12], or peritoneal cavity (i.p.)[12].

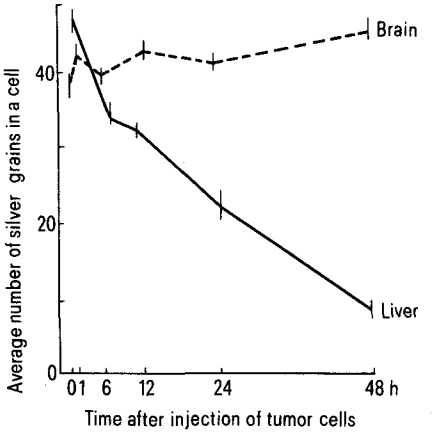
Materials and methods. The rat ascites hepatoma AH7974F, allogeneic to Donryu strain rat, was used for these studies. This tumor is a free-cell type strain that does not aggregate. The tumor cells were introduced into female Donryu strain rats weighing about 160 g. In the ascites the flash labeling index of AH7974F cells with ^{3}H-tymidine (^{3}H-TdR) was in the range of 60-70%. After removal of the ascites, tumor cells were washed by centrifugation at 1000 rpm for 1 min in a physiological saline solution. The tumor cells were resuspended at 1 or 2×10^7 cells/ml. The following experiments were performed:

Experiment 1. In order to ascertain the distribution pattern of tumor colonies in vivo 1×10^7 AH7974F cells were injected into the right internal carotid artery of 9 animals. All of the animals were autopsied at the time of death and the major organs were examined histologically.

Experiment 2. To examine the differences in labeling indexes among the AH7974F cells arrested in the brain or liver the following experiments were performed. For each experiment 1 ml of tumor cell suspension containing 2×10^7 cells was injected into the right internal carotid artery of 2-3 animals per group. Autoradiographs of 100 sections from the brain and 20 sections from the liver of every animal were obtained by dipping techniques using Sakura NR-M2. The exposure time was 11-14 days at 4 °C. Flash labeling of AH7974F cells in vivo was conducted as follows. 1 μCi of ^{3}H-TdR (New England Nuclear) per g of animal was administered i.p. at 1, 6, 12, 24, and 48 h after the injection of the tumor cells. 90 min after the administration of ^{3}H-TdR the animals were sacrificed with deep ether anesthesia. Continuous labeling was performed as follows: the 1st i.p. administration of ^{3}H-TdR (1 μCi per g animal) was made 10 min after the injection of tumor cells, and the successive administration of the same dose of ^{3}H-TdR was repeated at time intervals of 6-9 h thereafter. The animals were sacrificed with ether anesthesia 90 min after the last administration of ^{3}H-TdR.

Experiment 3. A silver-grain dilution test was performed as follows. 3 days after i.p. inoculation of AH7974F cells, ^{3}H-TdR (1 μCi per g animal) was administered i.p. and twice again at intervals of 6-9 h. 90 min after the final administration of ^{3}H-TdR, the tumor cells in the ascites were harvested and washed 3 times by centrifugation at 1000 rpm for 1 min in a physiological saline solution. By this procedure more than 99% of the tumor cells were labeled. The animals were sacrificed with ether anesthesia at 10 min (to determine the original grain number), 1, 6, 24, and 48 h after the injection of the tumor cells, and the number of silver-grains in the tumor cells determined.

Results and discussion. All of the animals receiving the AH7974F tumor cells died with tumors by 2 weeks after injection. Tumors were found in the brain of only 1 of 9 animals, while they were found in the livers of all animals. The labeling indexes of the AH7974F cells arrested in the brain were always less than in the liver. As shown in the table, flash labeling indexes of tumor cells in the brain ranged from 31 to 58%, but even with multiple administration of ^{3}H-TdR, the labeling indexes of tumor cells did not increase. In these same animals, the flash labeling indexes of tumor cells in the liver were ranged from 61 to 75% by 48 h post-injection, and continous labeling indexes of tumor cells reached 92% at 12 h post-injection (table). The results obtained from isotope dilution by counting of silver-grains corresponded to the labeling indexes. As shown in the figure, the average number of silver-grains per tumor cell in the brain did not decrease with time after the injection of tumor cells, while the average number of silver-grains in tumor cells in the liver was about ½-¼ of their original numbers at 24 and 48 h, respectively. By 48 h post-injection, the tumor cells in the brain were found in the blood vessels without forming distinct tumor nodules and they were deformed in shape to varying degrees. The tumor cells in the liver were found in the liver sinusoids without being deformed in shape before 12 h post-injection, and at 24 and 48 h some of them appeared to form tumor nodules



The average number of silver-grains in a tumor cell labeled with ^{3}H-TdR.

Labeling index of AH7974F cells with ^{3}H-TdR

	Time after injection (h)	Labeling index of tumor cells			
		Brain		Liver	
		Mean	SD (n)	Mean	SD (n)
Flash labeling	1*	47.5	3.9 (2)	64.5	3.2 (2)
	6	58.0	1.8 (3)	61.0	1.4 (3)
	12*	30.5	17.5 (2)	69.5	1.8 (2)
	24*	44.5	0.3 (3)	68.7	2.9 (3)
	48*	52.0	2.1 (2)	75.0	1.4 (3)
Continuous labeling	6*	60.6	2.4 (3)	75.3	2.1 (3)
	12*	48.0	0.4 (3)	91.6	1.4 (3)
	24*	47.3	6.0 (2)	89.5	1.8 (2)

* There was a statistical significance in the labeling indexes between tumor cells in the brain and liver ($p < 0.01$).

constituted by 2-3 tumor cells which compressed the hepatic cell strands.

The scarcity of tumor formation in the brain after the injection of AH7974F cells has been considered to be due to the low number of tumor cell emboli in the brain[11]. In the present experiments we have revealed the characteristics of the proliferation-kinetics of the tumor cells arrested in the brain. The results of flash-labeling indicated that the AH7974F cells arrested in the brain remained viable, and the results of continuous labeling and silver-grain dilution tests suggested that many of tumor cells in the brain did not divide, although they remained viable. On the other hand, it is apparent from the table and the figure, that almost all of the AH7974F cells arrested in the liver divided. Such differences in proliferation-kinetics among the tumor cells arrested at these sites was also reflected in the final formation of tumors. They rarely formed in the brain but frequently formed in the liver. These results appear to support the 'seed and soil' hypothesis of the mechanism of organ specificity of cancer metastasis.

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Abnormal development of cultured rat embryos in rat and human sera prepared after vitamin A ingestion[1]

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Summary. Human and rat sera were assayed for teratogenic activity using a whole rat embryo culture technique. Sera prepared from blood withdrawn 1-5 h after the ingestion of vitamin A capsules caused developmental retardation and craniofacial abnormalities. Control sera permitted normal growth and differentiation.

It has been suggested that relatively small increases in plasma vitamin A concentration - of exogenous and/or pathological origin - could adversely affect embryonic development without any noticeable effect on the mother[3]. This is based on indirect evidence from a study of the teratogenic effects of low doses of retinol (vitamin A alcohol) added to the medium in which rat embryos were cultured[4] and on the few cases of human malformations following maternal ingestion of large amounts of vitamin A[5].

The whole embryo culture technique[6] used to demonstrate the direct effect of vitamin A on embryogenesis has now been successfully applied to the assay of teratogenic activity of human[7] and primate[8] sera, using these sera supplemented with glucose as the culture medium for rat embryos. We have adopted this approach to assay human sera for teratogenic activity following the ingestion of commercially available vitamin A capsules. Having first established the time course of vitamin A uptake using rats we were able to obtain typical vitamin A-induced abnormalities in serum from human subjects who had taken an overdose of vitamin A.

Materials and methods. Vitamin A. 'Natural Vitamin A' capsules were purchased from a drugstore. Each capsule contained 25,000 USP units of vitamin A from fish liver oil, 625% the minimum daily adult requirement of vitamin A. The form of vitamin A was not specified.

Dosing and bleeding. The contents of 4 vitamin capsules (100,000 USP units in 1 ml fluid) were administered by gavage to each of 3 non-pregnant female CD rats (Charles River Breeding Laboratories, Inc., Wilmington, MA, USA). 3 control rats received a similar volume of pure safflower oil. All rats were allowed food and water ad libitum. 1 control and 1 experimental rat were bled for serum