DNA SYNTHESIS IN THE STROMAL CELLS OF MOUSE MAMMARY GLAND TUMORS

N. N. Belyaeva and Yu. M. Vasil'ev

UDC 616-006-008.939.633.2

DNA synthesis in the stroma of the normal mammary gland and of spontaneous tumors of the mammary gland in line C3H mice was studied by autoradiography after single or repeated injections of tritiated thymidine. The intensity of proliferation of the fibroblasts surrounding the acini of the mammary gland was very low; the fibroblasts of the stroma in the interior of the tumor and in the "capsule" surrounding the tumor were actively proliferating cells.

A growing tumor consists of two interconnected components: the parenchyma and stroma. The kinetics of proliferation of the parenchyma of several tumors in man and experimental animals have been studied in many autoradiographic investigations [3, 4, 9, 13, 14, 18]. In particular, different aspects of the kinetics of proliferation of the parenchyma in spontaneous mammary gland tumors of mice have been fully described [4, 5, 8, 15-17].

The kinetics of proliferation of the stroma of various neoplasms, including mammary gland tumors, remains virtually unstudied. Nevertheless, there is considerable evidence to show that the character of tumor growth depends substantially on the relationships between parenchyma and stroma [1, 2, 20].

The objective of the present investigation was to study the parenchyma and stroma of mammary gland tumors in mice. It was hoped to answer the following questions: 1) does the intensity of proliferation of the stroma change during malignant transformation of the mammary gland epithelium, and 2) does the mass of the stroma in a tumor increase on account of proliferation of the connective-tissue cells inside the tumor itself, or on account of proliferation of fibroblasts surrounding the tumor?

EXPERIMENTAL METHOD

To answer these questions, the percentage of cells synthesizing DNA was determined in the stroma of the normal mammary gland and in 17 spontaneous mammary gland tumors in C3H mice. All the tumors histologically speaking were adenocarcinomas with acinar and solid areas of Dunn's types A and B [10].

Tritiated thymidine with specific activity 29 μCi/ml was injected as a single dose of 0.7 μCi/kg body weight. In the experiments in which thymidine-H^3 was injected repeatedly, the injections were given at intervals of 1-2 h for 1, 2, or 4 days by the method of Galand et al. [12], for Blenkinsopp [7] has shown that this method has none of the disadvantages of the method of continuous infusion of thymidine-H^3 [17]. The animals were sacrificed 2 h after the single injection of thymidine-H^3 or after the last injection when the isotope was given repeatedly. The test material was fixed with Carnoy's mixture, and serial sections were cut from the tumor and from unaffected areas of other pairs of mammary glands. The sections, 5 μ in thickness, were coated with type M emulsion and exposed for 3 weeks. After development of the emulsion, the

Department of Cytology and Histology, Faculty of Biology and Soil Science, Moscow University. 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 L. M. Shabad.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 72, No. 7, pp. 77-80, July, 1971. Original article submitted December 18, 1970.

TABLE 1. Index of Labeled Cells in Epithelial and Connective-Tissue Cells of the Mammary Gland in Mice with Tumors*

Duration of injection of thymidine-H^3	Mouse No.	Mammary gland unaffected by tumor		Tumor of mammary gland			
		epithelium of acini	stroma	parenchyma	connective tissue: inside tumor	connective tissue: inner zone of "capsule" of tumor	connective tissue: outer zone of "capsule" of tumor
Single injection	459	2,8±0,6	6,8±0,8	14,4±0,8	—	7,4±1,8	—
	471	3,4±0,8	0,8±0,5	18,9±1,2	—	12,0±1,5	—
	475	3,2±1,2	2,7±0,9	12,3±1,0	—	11,2±2,2	—
	438†	3,5±1,0	2,3±0,6	14,4±0,6	—	17,3±2,1	—
24 h	348	3,2±0,5	2,1±0,6	26,3±1,0	6,8±1,0	22,8±1,7	8,8±1,2
	500	2,7±1,0	1,5±0,9	25,1±0,6	—	6,4±1,1	—
	504‡	4,2±0,9	0,8±0,5	а) 4,2±0,6	—	9,2±2,0	—
				б) 67,0±1,5	—	88,4±2,3	—
48 h	369	8,8±1,0	4,1±0,6	62,0±0,9	61,0±3,5	65,0±2,4	67,0±3,3
	380	9,4±0,9	12,0±1,2	57,5±0,7	—	41,5±1,6	—
	511‡	4,2±1,4	5,2±1,3	а) 47,2±1,6	—	32,0±2,0	—
				б) 68,0±1,5	—	27,0±1,4	—
				в) 63,0±1,5	—	33,0±3,3	—
	515	1,5±0,9	2,8±0,8	33,8±0,7	—	30,0±3,2	—
	522	0,3±0,3	1,0±0,4				
100 h	359	15,4±1,6	4,0±1,4	79,0±0,9	80,0±2,8	59,2±1,6	59,2±1,6
	391	12,9±1,3	19,0±3,6	64,0±0,5	52,0±5,0	44,0±2,2	—
	402	18,6±1,4	11,2±1,6	71,8±0,5	—	77,2±1,7	—

*Mean values and their errors given. A minus sign denotes that ILC could not be calculated because insufficient cells of that type were present in the specimens.

†Mouse No. 438 was injected with physiological saline for 100 h at intervals of 2 h, and after the last injection a single dose of thymidine-H^3 was given. This animal was sacrificed 24 h later.

‡In mice Nos. 504 and 511, ILC was determined separately in several parts of the parenchyma (designated: a, b, c) and in the zone of connective tissue adjacent to each of these areas.

sections were stained with hematoxylin or with methyl green–pyronine. In each case the number of labeled cells was determined as a percentage of the total number of cells of that particular type, i.e., the index of labeled cells (ILC). ILC was determined for the parenchyma of different parts of the tumors and for the fibroblasts of the connective-tissue layers between areas of parenchyma within the same parts of the tumor (the "stroma inside the tumor"; Table 1). Many tumor nodules were surrounded by a wide zone of connective tissue in which several rows of fibroblasts could be seen lying parallel to the surface of the tumor nodules. When the ILC values were calculated for the "capsules" around the tumor, the specimen was moved parallel to the edge of the parenchyma so that the areas counted were at about the same distance from this edge. In this way two zones of the "capsule," each 190 μ wide (the width of the field of vision of the microscope, with magnification 10×90), were distinguished: an inner zone, directly adjacent to the edge of the parenchyma, and an outer zone, in contact externally with the preceding zone. The IMS was calculated for the epithelium of the acini and for fibroblasts located close to the epithelial ducts and acini in specimens of unaffected mammary glands of animals with tumors.

EXPERIMENTAL RESULTS

The values of ILS in the stroma of normal acini of the mammary gland, as well as in the parenchyma of these acini, after a single injection of thymidine-H^3 were low, of the order of 1-7% (Table 1). These results indicate that the intensity of proliferation of the fibroblasts around the resting acini of the mammary gland is very low. The slow increase in ILC with an increase in the number of thymidine-H^3 injections could indicate that these cells have a very long S-phase (of the order of several days), and that the proportion of cells in the S-phase during the experiment was very small.

ILC for the fibroblasts of the connective tissue surrounding the tumor nodules after a single injection of thymidine-H^3 varied from 7-17%. With an increase in the number of injections of thymidine-H^3 the value of ILC increased gradually to reach 80% after 2-4 days in some cases. These results indicate that the fibroblasts surrounding the tumor nodule are an actively proliferating population. ILC for the parenchyma and stroma varied considerably in different parts of the same tumor and also in different tumors fixed after equal numbers of injections, but at each given time the mean value of ILC for the parenchyma was slightly higher than for the stroma. It can accordingly be postulated that proliferation of the parenchyma in a given tumor takes place somewhat more intensively than proliferation of the stroma. However, the differences observed were small, and in general both the stroma and the parenchyma of the tumor consisted of actively proliferating cells.

After repeated injections of thymidine-H^3, ILC in the connective-tissue layers inside the tumor and in the connective tissue around it were equally high. These results confirm the view that intensive proliferation takes place in the connective tissue surrounding the tumor, in the structure called its "capsule." This tissue evidently participates actively in the formation of the tumor stroma, and it is therefore essential for growth of the parenchyma [1].

The values of ILC were equal in the zone of the "capsule" directly next to the parenchyma of the tumor and in the adjacent zone not in direct contact with tumor cells. These results suggest that the tumor cells stimulate not only proliferation of the fibroblasts in direct contact with them, but also proliferation of fibroblasts located some distance from the tumor cells. It is possible, therefore, that this stimulation is the result of secretion of certain humoral stimulating factors by the tumor cells into the connective tissue. This hypothesis is in good agreement with results indicating that stimulation of proliferation of some normal tissues is frequently observed in the zone surrounding a tumor [6, 11].

It has recently been shown that tumor cells of certain types in culture secrete into the medium a substance which stimulates DNA synthesis in normal fibroblasts [19].

LITERATURE CITED

1. Yu. M. Vasil'ev, Connective Tissue and Tumor Growth under Experimental Conditions [in Russian], Moscow (1961), p. 138.
2. A. A. Zavarzin, Outlines of Evolutionary Histology of the Blood and Connective Tissue [in Russian], No. 2, Moscow (1945), p. 35.
3. A. A. Zavarzin, DNA Synthesis and the Kinetics of Cell Populations in Ontogenesis of Mammals [in Russian], Leningrad (1967), p. 74.
4. O. S. Frankfurt, Tsitologiya, No. 3, 386 (1965).
5. O. S. Frankfurt, Tsitologiya, No. 3, 370 (1966).
6. T. S. Argyris, in: Advances in Biology of Skin, Vol. 7, New York (1966), p. 55.
7. W. K. Blenkinsopp, J. Cell Sci., 5, 575 (1969).
8. F. Bresciani, in: Cellular Radiation Biology, Baltimore (1965), p. 547.
9. E. H. Cooper, M. J. Peckham, R. F. Millard, et al., Europ. J. Cancer, 4, 287 (1968).
10. T. B. Dunn, in: Physiopathology of Cancer, London (1958), p. 38.
11. L. Foulds, Am. J. Cancer, 39, 1 (1940).
12. P. Galand, F. Rodesh, F. Leroy, et al., Exp. Cell Res., 48, 595 (1967).
13. F. J. Jerrold, Biophys. J., 8, 710 (1968).
14. O. H. Iversen, Europ. J. Cancer, 3, 389 (1967).
15. H. L. Mendelsohn, in: Cellular Radiation Biology, Baltimore (1965), p. 498.
16. H. L. Mendelsohn, Cancer Res., 29, 390 (1969).
17. H. L. Mendelsohn, Science, 135, 65 (1962).
18. J. Post and J. Hoffman, Exp. Cell Res., 57, 111 (1969).
19. H. Rubin, Science, 167, 1271 (1970).
20. D. Tarin, Brit. J. Cancer, 23, 417 (1969).