# PROLIFERATION OF THE PARENCHYMA AND STROMA IN PRECANCEROUS HYPERPLASTIC NODULES IN THE MOUSE MAMMARY GLAND

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UDC 618.19-006.6-036.3-092.9

The index of labeled nuclei (ILN) was determined in the parenchyma and stroma of precancerous hyperplastic nodules in autoradiographs taken from C3H mice receiving single or multiple (up to 100) injections of thymidine-$H^3$. Several groups of nodules were found. A. Nodules in which ILN of the parenchyma and stroma were not significantly different from their levels in the corresponding components of normal acini. B. Nodules in which ILN was higher than normal in the parenchyma but not in the stroma. C. Nodules in which ILN of the parenchyma and stroma were significantly higher than in normal acini. The ability of the parenchymatus cells to proliferate intensively and the ability of the same cells to evoke a stromatogenic response can be regarded as different characteristics appearing independently in the course of carcinogenesis in the mammary gland.

The appearance of precancerous hyperplastic nodules in the mouse mammary gland (described below simply as nodules) is the earliest morphologically detectable phase in the development of tumors of this organ [2-4]. These nodules are therefore a convenient object in which to study tissue changes in the early phases of carcinogenesis. An important aspect of investigations of this type is the study of the kinetics of cell proliferation in these neoplasms.

Bresciani [2] found that the mean duration of the mitotic cycle in cells of hyperplastic nodules is shorter (33 h) than in the normal mammary gland (46 h). This increase in the intensity of tissue proliferation in the nodules is due mainly to shortening of the S and $G_1$ periods of the mitotic cycle.

The object of the present investigation was to study proliferation of the parenchyma and stroma in various nodules in situ.

## EXPERIMENTAL METHOD

Hyperplastic nodules of female C3H mice, with a high incidence of spontaneous mammary gland tumors at the age of 9-12 months, were investigated. Mice in which a tumor had already begun to appear in one mammary gland were used in the experiment. These mice received single or multiple injections of thymidine-$H^3$. The method of injection was similar to that described previously [1]. To detect the hyperplastic nodules, serial histological sections through mammary glands not affected by tumors were examined.

## EXPERIMENTAL RESULTS

The hyperplastic nodules in the sections consisted of large groups of alveoli of different diameters, lined by simple epithelium and separated from each other by a connective-tissue stroma, in which numerous

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. 10, pp. 75-77, October, 1971. Original article submitted April 8, 1971.

TABLE 1. ILN in Epithelial and Connective-Tissue Cells of the Mammary Gland during Tumor Development in Mice (M ± m)

| Characteristics of experiment | | | Mammary gland unaffected by tumor | | Hyperplastic nodule in mammary gland | |
|---|---|---|---|---|---|---|
| Time during which thymidine-$H^3$ injections were given (in h) | Time of sacrifice of mouse after last inject. of thymidine-$H^3$, h | Mouse No. | epithelium of acini | stroma | epithelium of acini | stroma |
| Single injection | 2 | 272[2] | 1,4±0,9 | 1,1±0,7 | (a) 1,9±0,7 | (a)0,8±0,6 |
| | 2 | 286 | 2,1±0,9 | 10,0±1,1 | (b) 2,7±0,8<br>2,1±0,8 | (b)1,1±0,7<br>7,0±3,2 |
| 24 | 2 | 348[1,3] | 3,2±0,5 | 2,1±0,6 | (I) 38,4±0,9<br>(II) 9,3±0,6 | 4,5±1,0 |
| | | 500 | 2,7±1,0 | 1,5±0,9 | 3,8±0,8 | 3,0±1,2 |
| | | 504 | 4,2±0,9 | 0,8±0,5 | 1,0±0,6 | 0,5±0,5 |
| 24 | 24 | 508[2] | 5,3±0,5 | 7,0±1,5 | (a) 37,0±2,0<br>(b) 50,1±3,5 | (a) 51,0±3,1<br>(b) 19,5±2,8 |
| | | 519 | 0,6±0,3 | 1,2±0,5 | 1,3±0,4 | 0,6±0,5 |
| 48 | 2 | 515 | 1,5±0,9 | 2,8±0,8 | 10,9±0,9 | 2,5±1,1 |
| 48 | 24 | 527[3] | 8,0±1,5 | 8,9±2,0 | (I) 4,5±0,8 | 8,4±2,3 |
| | | 536 | 2,5±0,8 | 3,3±1,0 | (II) 28,3±1,1 | 4,0±1,1 |
| | | 539 | 7,1±2,2 | 1,4±0,5 | 11,2±1,3<br>14,8±1,0 | 5,0±1,5 |
| 48 | 48 | 543 | 9,6±0,8 | 1,2±0,5 | 22,5±1,3 | 1,0±0,4 |
| 100 | 2 | 359[1] | 15,4±1,6 | 4,0±1,4 | 57,0±2,9 | 42,0±2,5 |
| | | 391[2] | 12,9±1,3 | 19,0±3,6 | (a) 88,0±1,1<br>(b)67,4±4,7 | (a) 36,0±3,4<br>(b) 41,3±2,5 |
| | | 402[2] | 18,6±1,4 | 11,2±1,6 | (a)63,4±1,0<br>(b)67,5±1,8 | (a) 29,2±2,3<br>(b) 21,8±2,1 |
| | | 413[2,3] | 6,3±0,5 | 3,6±0,9 | (a) 2,3±0,4<br>(b) 4,5±0,4<br>(c, I) 8,3±0,6<br>(II) 27,2±3,2 | (a) 3,6±0,8<br>(b) 6,2±1,1<br>(c) 22,4±1,7 |

[1]Intervals between repeated injections of thymidine-$H^3$ in mice Nos. 348 and 359 were 1 h, and for other mice 2 h.

[2]ILN was calculated for the parenchyma and stroma of several hyper plastic nodules (a, b, c).

[3]For some nodules ILN was calculated in different areas of the parenchyma of the same nodule (I, II) and in the zone of connective tissue adjacent to the nodule.

fibroblasts could be seen. The number of labeled cells as a percentage of the total number of cells of that type (the index of labeled nuclei, ILN) was calculated separately for the parenchyma and stroma in each nodule. The results of these calculations are summarized in Table 1. At least 200 cells were counted for each index.

The results in Table 1 show that the various hyperplastic nodules differed considerably in their intensity of proliferation. In some cases marked differences in ILN were found in different parts of the same nodule (Nos. 348, 413c). Depending on the character of proliferation in them, the nodules can be divided into three groups.

A. Nodules in which ILN for the parenchyma and stroma showed no significant difference from ILN of the corresponding components of the normal acini of the mammary glands (Nos. 413a, b, 519, 500). The

mammary gland cells can evidently acquire the ability to form nodules without any significant changes in the intensity of proliferation.

B. Nodules in which ILN of the parenchyma is significantly higher than in the normal mammary gland, but ILN for the stroma is indistinguishable from normal (Nos. 515, 536, 543). The parenchymatus cells of these nodules evidently were already capable of more intensive proliferation, but proliferation of the stroma had not yet begun to exceed its normal level.

C. Nodules in which ILN for the parenchyma and stroma were significantly higher than for the corresponding components of normal acini of the same mice. This subgroup included most of the nodules investigated. In all nodules except one (No. 508a) of this subgroup, ILN of the parenchyma was higher than for the stroma of the same nodules.

The greater intensity of proliferation of the parenchyma than of the stroma in the nodules of subgroups B and C may be significant as a factor limiting the size of the nodules. It can be postulated that tumors arise only from nodules of subgroup C, in which the cells have become capable of inducing intensive proliferation of fibroblasts, thus enabling a stroma to be formed for the actively growing parenchyma of the tumor [1].

The ability of parenchymatus cells to proliferate intensively and the ability of the same cells to induce a stromatogenic response can thus be regarded as important characteristics appearing independently in the course of carcinogenesis in the mammary gland. In other words, changes in these properties obey the rule of "independent progression of tumor characteristics" formulated by Foulds [5].

## LITERATURE CITED


1. N. N. Belyaeva and Yu. M. Vasil'ev, Byull. Éksperim. Biol. i Med., No. 7, 77 (1971).
2. F. Bresciani, in: Cellular Radiation Biology, Baltimore (1965), p. 547.
3. K. B. De Ome, L. J. Faulkin, Jr., and P. B. Blair, Cancer Res., 19, 515 (1959).
4. K. B. De Ome, P. B. Blair, and L. J. Faulkin, Jr., Acta Union Int. Canc., 17, 973 (1961).
5. L. Foulds, Cancer Res., 14, 327 (1954).