Proliferative Activity of Tumor Cells as a Prognostic Factor in Breast Cancer

N. S. Gulyak, N. S. Sergeeva, E. N. Slavnova, V. I. Chissov, A. V. Babarina, V. P. Demidov, D. D. Pak, and S. K. Dzhubalieva

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The proliferative activity of 92 breast carcinomas was studied. Statistically significant correlations between the level of proliferative activity and degree of differentiation of tumor cells and nuclear grade were found. Statistically reliable differences in proliferative activity were revealed in the groups of patients under and over 50 years. These facts show that proliferative activity can be used as an individual factor for predicting the aggressiveness of the neoplastic process in patients with breast cancer.

Key Words: *breast cancer; proliferative activity; Ki-67; cellular differentiation*

The clinical aggressiveness of the neoplastic process in patients with breast cancer depends to a great extent upon the tumor's malignancy. A number of histological, cytological, and clinical signs of established prognostic significance are generally used for determining this parameter [7]. However, clinical experience indicates that the neoplastic process varies widely even in groups of patients where those parameters are similar [8]. Therefore, it is necessary to seek criteria for differentiating between clinically and morphologically similar tumors. The index of proliferating cells in the tumor, or the proliferative activity (PA), is one of the promising criteria [4,6]. In breast cancer, the mean longevity in patients with a high PA of the tumor has been shown to be reliably lower than in patients with a low PA [5]. Patients with a higher PA are most likely to have occult metastases. These facts show the significance of PA, at least for a group prognosis of aggressiveness of the neoplastic process in patients with breast cancer, and the necessity of studying how this parameter can be used for individual prognosis of the clinical aggressiveness of the neoplastic process.

The aim of the present study was to investigate the PA of breast carcinomas in comparison with a variety of clinical and morphological characteristics of the neoplastic process in order to determine the possible use of this parameter in specifying the biological aggressiveness of the neoplastic process.

P. A. Hertzen Research Oncological Institute, Moscow

MATERIALS AND METHODS

Surgical and biopsy specimens of primary breast cancer from 92 patients aged 26 to 74 years (mean age 48.5) were used. The majority of the patients (70, i.e. 75.3%) were at clinical stage II of the disease; stages I and III were less common: in 15 (16.1%) and 8 (8.6%) of patients, respectively. The presence of metastases was confirmed in 18 patients (19.4%).

The study was carried out on smears fixed in methanol for 5 to 10 min at -20°C. Cytological parameters of the tumor cells were studied on Pappenheim-stained smears [1]. Tumor cell differentiation was explored by method [2], counting shape and size of the tumor cells, nucleocytoplasmic ratio, secretory activity of the cells, their ability to form complexes, etc. Classification of tumor cell nuclei by method [3] was also used and took into account the state of the chromatin, number of nucleoli, shape and size of the nucleus, and its ra-

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tio to the cytoplasm. According to the latter classification the tumors were divided into three grades. Grade one describes tumors whose cells have nuclei of moderate size and round shape, with diffuse chromatin and few nucleoli. Grade three is assigned to tumors whose cell nuclei are of irregular shape and large size, with coarse fragmented chromatin, and multiple nucleoli. Grade two is for tumors whose cells have nuclei with intermediate parameters.

PA of the tumors was estimated by means of indirect immunofluorescent staining. Monoclonal antibodies Ki-67 (Dako) at dilution 1:25 were used as the first antibodies and IgG_2 conjugated with fluorescein isothiocyanate (Sigma), at dilution 1:75 was used as the second antibodies. Calculation of the index of proliferating cells was performed with an Opton Axioplan fluorescence microscope at magnification 400. Reaction in the tumor cells was taken to be positive if an intense specific luminescence was observed in the nuclei. At least 300 tumor cells were counted in each specimen. The index of proliferating cells was counted by calculating the ratio of positively stained cells and the total number of counted cells. The results were treated statistically using Student's t test.

RESULTS

The level of PA varied in breast cancer from 0 to 40%, averaging 9.6±8.7%. Notably, 16.3% of the tumors revealed extremely low levels of PA (≤1%). Levels markedly exceeding mean values (≥20%) were seen in 12% of all cases.

To find out whether PA can serve as an additional factor of aggressiveness of the neoplastic process, we compared the PA levels in groups of tumors with similar clinical and morphological prognostic factors. No statistically reliable differences in PA were revealed between groups of patients with different stages of the disease. The level of PA in patients with clinical stage I was 8.5±7.3%; with stage II 9.9±8.8%; and with stage III 9.7±10.4%. However, reliable differences in the PA levels were found in women of different age groups (Table 1). The mean PA was highest in patients under 50 years of age ($p<0.01$).

Comparison of the distribution of tumors according to tumor cell differentiation and nuclear grade showed that tumors with high cellular differentiation are typically characterized by nuclear grade one and tumors with poor cellular differentiation by grade three. But that was not true in some cases, most of them being tumors of mixed differentiation. We thus made a preliminary separate analysis of the PA levels in groups of tumors

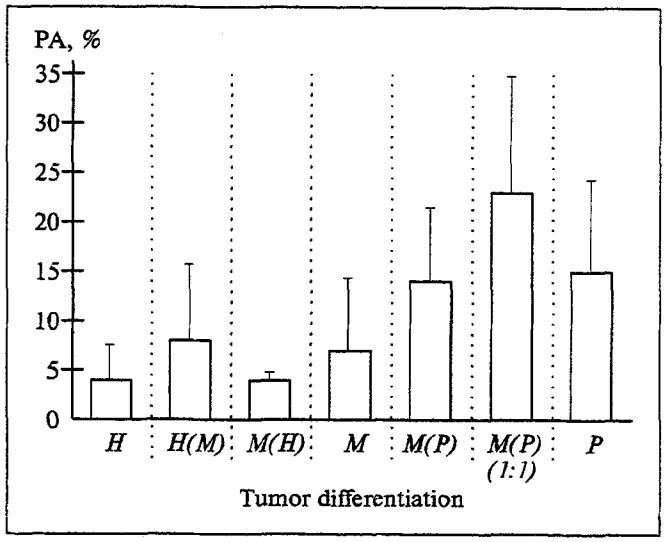

Fig. 1. Proliferative activity (PA) of breast carcinomas with different degrees of cellular differentiation. *H* = high differentiation, *M* = moderate differentiation, *P* = poor differentiation.

with different cellular differentiation and nuclear grades.

We found a correlation between the mean PA and degree of differentiation of the tumor cells (Fig. 1). The mean PA was found to be 3.8±3.6% in highly differentiated tumors, 7.5±6.2% in moderately differentiated tumors, and 14.9±9.1% in poorly differentiated tumors. An analogy was seen in the groups of tumors with different nuclear grades (Fig. 2).

The PA of grade one tumors was 4.5±4.3%; of grade two tumors, 6.5±6.5%; and of grade three tumors, 18.3±7.3%. Thus, since the group mean levels of PA correlate with such known factors of tumor aggressiveness as cellular differentiation and

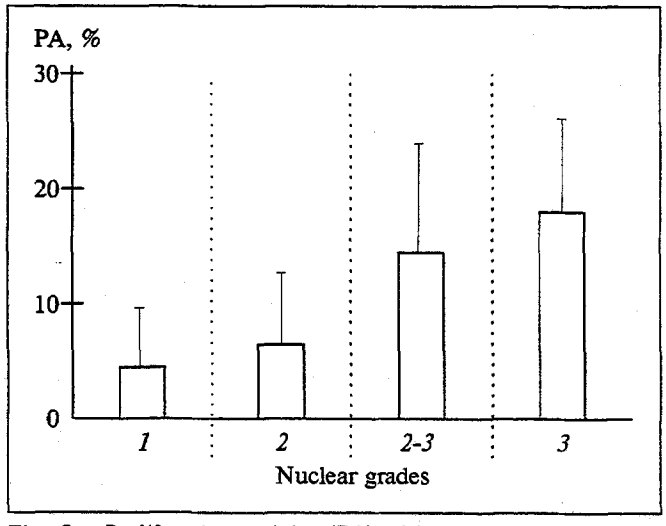

Fig. 2. Proliferative activity (PA) of breast carcinomas with different nuclear grades of tumor cells.

TABLE 1. Proliferative Activity (PA) of Breast Tumors in Patients of Different Age Groups ($M \pm m$)

Age, years	Number of cases	PA, %
0–40	22 (23.9%)	10.4±7.7
40–50	24 (26.1%)	11.5±10.6
50–60	27 (29.3%)	8.2±8.1
60–80	19 (20.7%)	8.6±7.5

nuclear grade, the PA of the tumor reflects the aggressiveness of the neoplastic process. On the other hand, since significant individual variations of PA are seen in each group of tumors with uniform cellular differentiation and nuclear grade (Figs. 1 and 2), we can consider PA to be and additional independent prognostic factor in all groups. It is worth noting that the mean values of proliferation activity in the groups of tumors with mixed differentiation (e.g., highly differentiated with moderately differentiated areas, moderately differentiated with poorly differentiated areas, etc.) proved to exceed expected rates significantly (Fig. 2). The mean PA of highly differentiated tumors with moderately differentiated areas was found to be 8.1±7.7%, which exceeds that of homogeneous moderately differentiated carcinomas. The mean PA of tumors with moderately and poorly differentiated cells in the approximate ratio of 1:1 was considerably greater than that of homogeneous poorly differentiated carcinomas: 23.3±11.3% and 14.9±9.1%, respectively (Fig. 2). The higher level of PA in carcinomas of mixed cellular differentiation might reflect a process of decline in tumor differentiation, which is accompanied by activation of the proliferative process and followed be an increase in biological aggressiveness of the tumor.

Thus, the above data attest that PA increases as tumor cellular differentiation declines and as nuclear grade increases. Significant individual variations in PA can be observed in each group of homogeneously differentiated tumors. The above data suggest that PA measured by means of Ki-67 monoclonal antibodies can serve, along with known prognostic factors, as a criterion for evaluating the aggressiveness of the neoplastic process within groups of tumors with homogeneous cellular differentiation.

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