Expanded Record of the AgNORs Reaction in the Investigation of the Parenchyma-Stroma Relationships in Breast Cancer

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Correlations between parenchymal and stromal AgNORs cells, on the one hand, and the morphometric parameters, HLA expression by the epithelium, and lymphocyte subpopulations, on the other, are analyzed in breast cancer. The relationships reflecting the interactions between parenchymal and stromal cells are evaluated in affected and intact tissues. The results obtained point at homeostatic stability of some structures in affected tissue.

Key Words: *nucleolar organizers; breast carcinoma; morphometry; parenchyma and stroma*

Recently, a number of new histological methods, including immunophenotyping of tumor and immunocompetent cells and AgNORs impregnation of nucleolar-forming sites (NFS) on chromosomes [4], have been used in oncomorphological studies. In line with traditional morphometric characteristics of parenchyma and stroma, quantitative data obtained by these methods provide additional information regarding the correlations reflecting cell-to-cell interactions [6] in parenchyma and stroma [3,5].

The aim of this study was to analyze the correlations between the NFS parameters and various quantitative characteristics of the parenchyma and stroma in breast carcinoma and intact mammary gland.

MATERIALS AND METHODS

Tumor tissue and sites distant from the tumor were examined in 20 patients with breast cancer. Absolute counts of epithelial and stromal cells, lymphocytes, plasmacytes, granulocytes, and mast cells were determined in hematoxylin-eosin-stained sections in 40 fields of view at magnification 400. Mitotic index was calculated per 1000 cells. The percent of parenchyma and stroma was calculated routinely using the Avtandilov ocular grid. The area of the vascular bed was determined by vascular lumens using ocular micrometer. For identification of NFS impregnation with AgNORs was performed by the conventional method [7]. Nucleoli, total NFS, and NFS dispersed over the nucleus were counted in 100 epithelial and stromal cells at magnification 900. Three types of tumor and normal cells differing in the NFS distribution have been described [8], which we designated type I (quiescent cells), type II (proliferating and regenerating cells), and type III (cells of highly malignant tumors). The percent of each cell type was calculated in the epithelium. NFS were counted in type I-III cells, nucleoli in type I-II cells, and NFS dispersed over the nucleus in type II-III cells. The percent of activated lymphocytes and plasma cells (by the number of NFS) was determined in lymphoplasmacyte infiltrates. Cells with 0-3 large Ag grains were regarded as inactive. Immunofluorescent tests were performed using cryostat sections and the following monoclonal antibodies: ICO-1 (HLA-DR), ICO-53 (HLA-I) (percent of HLA^+ epithelial cells was determined in the epithelium), ICO-20 (CD38), ICO-88 (CD30) (for detection of activated lymphocytes), ICO-90 (CD3 antigen of mature T cells), ICO-80 and ICO-87 (T-

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cell CD5 and CD7 antigens, respectively), ICO-31 (CD8 of T suppressors/killers), ICO-86 (CD4 of T helpers/inducers), ICO-91 (CD22), and ICO-30 to IgM μ-chain (B cells). The antibodies were produced at the Oncology Research Center, Russian Academy of Medical Sciences. The results of the immunofluorescent test were expressed as the percentage of positively reacting cells relative to the total number of cells of this type.

The results were processed by parametric and nonparametric tests using a STATGRAPHICS 2.6 software. Only statistically significant correlations are presented.

RESULTS

The tumor and intact epithelium of the mammary gland (MG) contain three cell types differing by the distribution of Ag granules [8]. Type II cells predominated in affected tissue, while the occurrence of type I quiescent cells was higher in unaffected tissue (Fig. 1).

The NFS parameters were associated with the most important morphometric characteristics of the parenchyma and stroma (Table 1). The number of NFS in type II cells positively correlated with the mitotic index of tumor cells. The number of NFS in stromal cells of unaffected MG positively correlated with plasma cell and granulocyte counts and the mitotic index of stromal cells.

It is noteworthy that the NFS parameters in the tumor and adjacent stroma (NFS, nucleoli, and NFS dispersed over the nucleus) positively correlated with each other. Presumably, the relationship between tumor parenchyma and stroma is realized during the initial genetically-determined stages of biosynthesis providing the function of chromosomal NFS. The morphogenetic significance of type II tumor cells may be the highest: the NFS parameters of these cells positively correlate with the corresponding NFS parameters in stromal cells bordering the tumor. Such a correlation was not established for stromal cells in unaffected MG; however, the numbers of nucleoli (i.e., NFS products) positively correlated in epithelial and stromal cells. A positive correlation was established between the numbers of NFS and nucleoli in affected and unaffected tissue, respectively.

The content of the quiescent (type I) epithelial cells both in affected and unaffected tissue negatively correlated with the total count of immunocompetent cells (Table 1). The count of cells with enhanced synthetic and proliferative potential (type II) in affected and unaffected tissue positively correlated with the count of immunocompetent cells. In addition, the NFS parameters of type II tumor cells

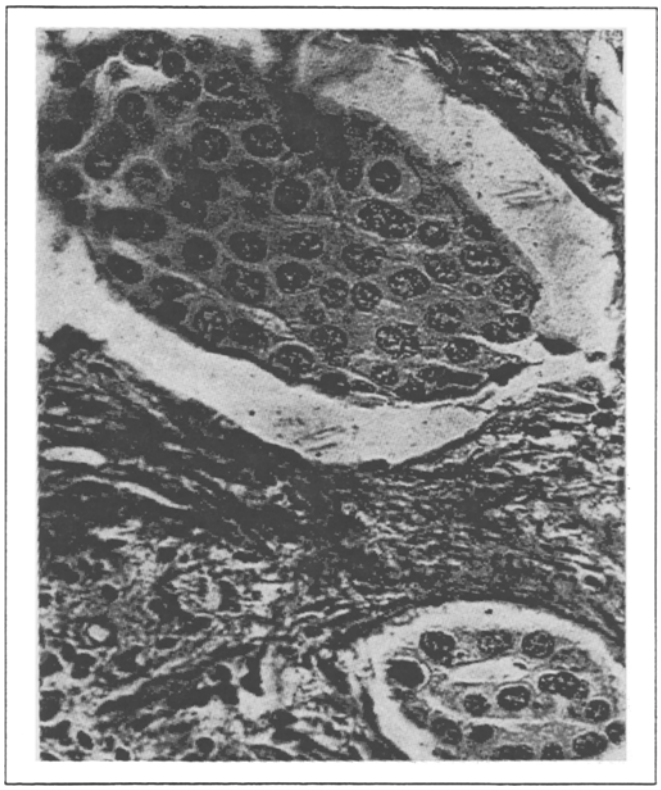

Fig. 1. Solid structures of breast carcinoma. Cells with Ag granules in the nucleoli and nuclei are seen. Type II distribution of NFS predominates. Impregnation with AgNORs. ×400.

positively correlated with the expression of HLA antigens on malignant cells. The analogs of highly malignant tumor cells (type III epithelial cells in unaffected tissue) positively correlated with immunocompetent cells and negatively correlated with HLA expression by unaffected epithelium. Interestingly, the number of NFS in tumor cells negatively correlated with HLA expression on these cells. The count of type III tumor cells did not correlate with the counts of immunocompetent cells or granulocytes.

In unaffected tissue, the count of each cell type correlated with those of lymphocytes expressing the activation antigen CD30 and of CD37-positive B lymphocytes which are known to influence the proliferation of the MG epithelium [1] (Table 1). The counts of types I and III tumor cells did not correlate with individual lymphocyte subpopulations, while the count of type II tumor cells both in tumor and unaffected MG correlated with that of B lymphocytes ($CD37^+$) and the total count of CD5-positive T cells.

The count and activity of lymphocytes in the tumor are increased [2]. In breast carcinoma, the percentage of activated lymphocytes (judging from the number of NFS) was higher than in unaffected tissue: 83.89% *vs.* 29.33% ($p<0.05$).

TABLE 1. Positive (+) and Negative (-) Correlations between the Parameters of Chromosomal Nucleoli-Forming Sites (NFS)

Parameter	Morphometric parameters		Individual lymphocyte subpopulations/ HLA expression by epithelial cells	
	unaffected tissue	tumor	unaffected tissue	tumor
Epithelium				
NFS	-	Lymphocytes, plasma cells (+)	None	None/HLA-I (-)
Nucleoli	-	Lymphocytes, plasma cells (-)	None	None
NFS dispersed over the nucleus	-	-	None	None
Type I cells	Plasma cells, granulocytes (-)	Lymphocytes, plasma cells (-)	CD37, CD30 (+)	None
Nucleoli	-	Epithelial cells (+)	*/none	*/none
NFS	-	Epithelial cells (-) Lymphocytes, plasma cells (+)	*/none	*/none
Type II cells	% of parenchyma (-) Plasma cells, granulocytes (+)	Tumor mitotic index (+) Lymphocytes, plasma cells (+)	CD37, CD30 (+)	CD37, CD5 (+)
Nucleoli	Stromal cells (-)	Epithelial vascular bed area (+)	*/none	*/none
NFS dispersed for nucleus	Lymphocytes (+)	Mitotic index of tumor (+) Plasma cells (+)	*/none	*/none
NFS	-	-	-	*/HLA-I (+)
Type III cells	Stroma mitotic index (+) Plasma cells, granulocytes (+)	Stromal vascular bed area (+)	CD37, CD30 (+)	None
NFS	-	-	*/HLA-I,II (-)	*/none
Activated cells (by the number of NFS):				
lymphocytes	Stroma mitotic index (+) Plasma cells, granulocytes (+), lymphocytes (-)	Mast cells (-)	B-lymphocytes (IgM, CD22, CD37) - (+) T-lymphocytes (CD4, CD3, CD8) - (+) Activated lymphocytes (CD30) - (+)	T (CD5) (-)
plasma cells	Plasma cells, granulocytes (+)	Plasma cells (-)	B-lymphocytes (IgM, CD22) (+) T (CD3, CD8) - (+) T (CD7, CD5) - (-) Activated lymphocytes: (CD30) (+) (CD38) (-)	None

Note. *Correlation analysis was not performed.

As Table 1 shows, in unaffected tissue, the count of activated lymphocytes positively correlates with that of plasma cells (blast transformation of B cells may predominate). A negative correlation between activated lymphocytes and their total count confirms that the majority of lymphocytes in unaffected tissue are not activated. Such correlations were not established for breast carcinoma. In unaffected MG, the counts of activated lymphocytes and plasma cells correlated with those of T and B cells. It should be emphasized that activated lymphocytes positively correlated with CD30-positive lymphocytes only in unaffected tissue. In breast carcinoma, the counts of activated lymphocytes and plasma cells did not correlate with individual lymphocyte subpopulations.

Thus, the present study revealed differences and similarities in correlations between the NFS parameters of epithelial cells in breast carcinoma and unaffected tissue. These similarities are confirmed by positive correlations between the NFS parameters of

tumor and native cells and between the number of type I and II epithelial cells in affected and unaffected tissue. These findings point at homeostatic stability of epithelial-mesenchymal structures in breast carcinoma, prompting the search for new approaches to its regulation.

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Distribution of Lectin Receptors on the Plasma Membranes of Brain Glioma Cells and Autologous Peripheral Blood Mononuclear Cells as a Function of the Degree of Anaplasia

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The content of the D-mannose-specific LCL-receptor that binds D-mannose-containing lymphokines (interleukin-1 and interleukin-2) is proportional to the degree of glioma anaplasia. This may reflect the mechanism whereby brain glioma utilizes lymphokines to proliferate and to escape immunologic surveillance.

Key Words: *gliomas; carbohydrate receptors to lectins; immunologic surveillance*

There is considerable evidence indicating that the structure of plasma membrane glycoproteins and glycolipids changes during cell growth, differentiation, and malignization [5,12]. A tendency toward the loss of N-acetylneuraminic acid and N-acetylglucosamine and an increase in the amount of D-galactose-specific receptors on the surface of tumor cells irrespective of tumor origin has been documented [5,12]. Receptors containing N-acetyllactosamine, mannose, and L-fucose were identified on metastatic tumors [5,12].

Glycoconjugates of cell membranes can be obtained with the use of lectins, nonimmune proteins capable of binding to simple and complex antigenic determinants [5]. It should be noted that the regulatory effects of lymphocytes are realized via lymphokines and carbohydrate-containing surface receptors [1]. It was found that the effect of lymphokines stimulating lymphocyte proliferation (IL-1 and IL-2) is determined by terminal D-mannose [9,14]. D-mannose is a constituent of the CD4 receptor of T helpers [11], while lymphocytes expressing the dis-

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