ULTRASTRUCTURE OF THE PROTEOGLYCAN COMPONENT OF THE EXTRACELLULAR MATRIX IN THE INTACT MAMMARY GLAND AND IN BENIGN AND MALIGNANT BREAST TUMORS

V. F. Kondalenko and V. A. Golubeva

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Relations between cells and the extracellular matrix play an important role in processes of normal cell differentiation and proliferation, and also in neoplastic growth [1]. Proteoglycans (PG) are an essential part of the extracellular matrix. It has been postulated that qualitative and quantitative changes in PG can affect the behavior of tumor cells [7].

More attention has been paid to the quantitative and qualitative composition of PG, determined by biochemical methods, in various intact and tumor tissues than to the ultrastructure of this component of the extracellular matrix. It is evident that not only the chemical composition, but also the ultrastructural organization of the proteoglycan component and the morphology of its relations with cells and with other elements of the extracellular matrix are of the utmost interest for the elucidation of many tumor growth phenomena.

The aim of this investigation was an electron-microscopic study of PG in the intact mammary gland and its tumor with the aid of cationic dyes.

EXPERIMENTAL METHOD

The intact mammary gland and spontaneous mammary gland tumors of dogs were investigated. Considering that spontaneous mammary gland tumors appear in dogs mainly over 5-7 years of age, autopsy material taken not later than 15 min after death from dogs of the same age group with no evidence of tumors (four cases) was used as the control. Macroscopically unchanged regions of the gland, bordering on the tumor (operative material) also were investigated as the conventional control. During morphologic verification of neoplasms, the International Histologic Classification of Tumors of Domestic Animals, published by WHO in 1974, was taken for guidance. Altogether nine tumors were studied: five carcinomas (solid carcinoma of simple type – two, mucous carcinoma – two, papillary-cystic carcinoma – one) and four benign tumors (adenomas of complex type – two, benign mixed tumors – two). The following cationic dyes were used as reagents to detect PG: Alcian blue 8GS and safranin O. The known method of electron-microscopic demonstration of PG [3, 4] was adopted as the basis. Small pieces of tissue (1 mm^3) were fixed, in the case of staining with Alcian blue, in a 2.5-4% solution of glutaraldehyde with the addition of 1% of the dye in 0.1M phosphate buffer, pH 6.0-6.5, for 6-18 h at 4°C. The samples were then rinsed in the same number and fixed in a 1% solution of osmium tetroxide for 2 h at room temperature. Instead of glutaraldehyde, a 4% solution of paraformaldehyde can be used as the first fixative. The material was dehydrated in acetones and embedded in a mixture of Epon and Araldite. When safronin O was used for staining, the fixing mixture was made up beforehand, for when the dye is dissolved a precipitate is formed, and must be removed by filtration. A 2% solution of glutaraldehyde in 0.1M phosphate buffer, pH 7.4, with the addition of a 0.1% solution of safranin O was used as the fixative. The material was fixed for 6-18 h in a refrigerator, washed in the same buffer, and postfixed in 1% osmium tetroxide solution for 1 h at room temperature. Dehydration and embedding were carried out as in the case of Alcian blue. Ultrathin sections were stained with uranyl acetate and lead citrate.

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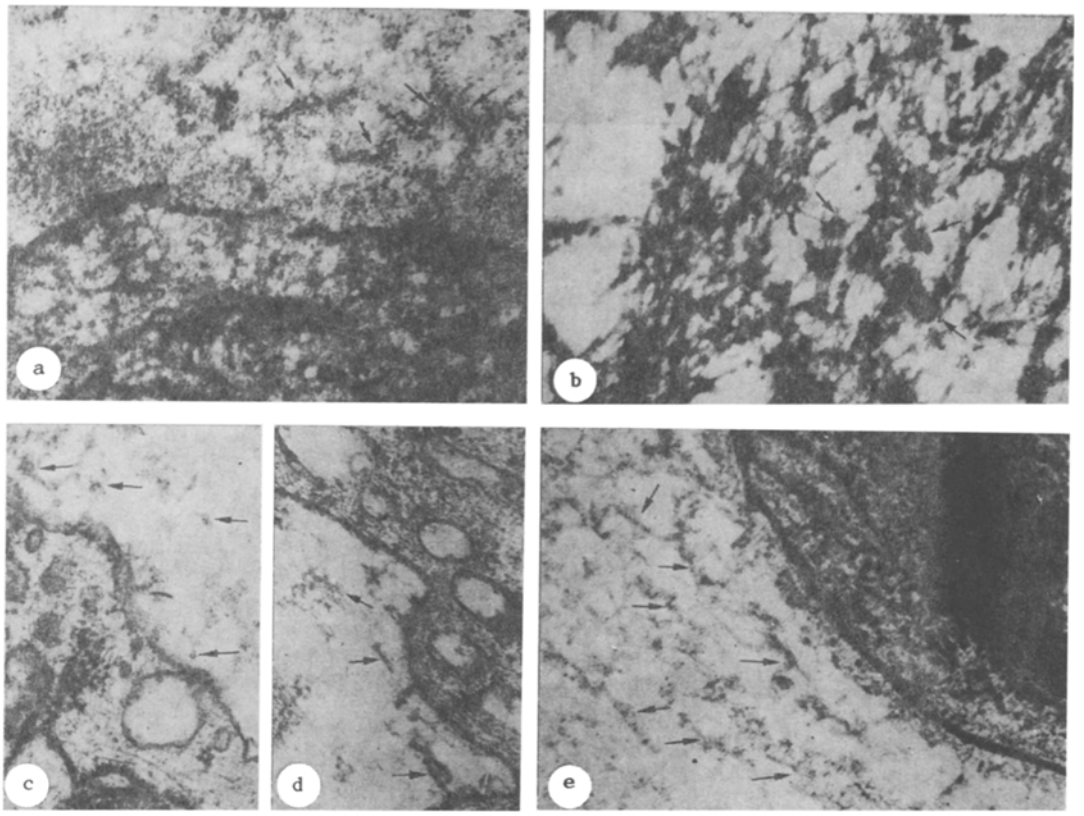

Fig. 1. PG of extracellular matrix of intact dog mammary gland: a) net-like structure formed by PG in pericellular matrix of secretory cell (arrows); b) large PG granules in extracellular matrix (arrows); c) small PG granules in pericellular space of fibroblast (arrows); d) PG granules in pericellular space of macrophage (arrows); e) delicate net-like structure of PG near adventitial cell of a blood capillary (arrows). a) Safranin, b-e) Alcian blue. Magnification: a) 120,000, b-d) 60,000, e) 90,000 ×.

EXPERIMENTAL RESULTS

In the control the extracellular matrix of the alveoli and ducts, lined with one or two layers of epithelial cells, was investigated. The secretory epithelium contained cisterns of the rough endoplasmic reticulum, lipid droplets, and also many free ribosomes in its cytoplasm. In the region of junctions between the epithelial cells expanded regions containing homogeneous masses of PG were frequently observed. Accumulation of PG also was noted in the basal regions of the plasma membrane of the epithelial cells. In the immediate vicinity of the basement membrane of the secretory epithelium PG granules of polygonal shape were observed to give off thin filaments, distributed radially, which connected with granules together and with collagen fibrils. As a result, a distinctive net-like structure was formed in the pericellular space of the secretory epithelium. Its nodes were formed by granules consisting of PG molecules, and the thin filaments between the nodes, according to some evidence, consisted of hyaluronic acid molecules [5]. The granules found evidently do not completely reflect the intravital structure of the PG molecules, for in the native state, according to data in the literature, they are shaped like filaments or bottle-brushes [5]. On staining with safranin O the granules and connecting threads were fused together into a single net-like structure (Fig. 1a). In the pericellular space of the myoepithelial cells the basement membrane was more marked and the PG network not so highly developed.

In regions more distant from the epithelium the structure of the PG component of the extracellular matrix was different from that in areas immediately adjacent to the cell. Granules were irregular in size, lay at different distances apart, and often were fused together. As a result, the empty spaces in the net differed in diameter (Fig. 1b).

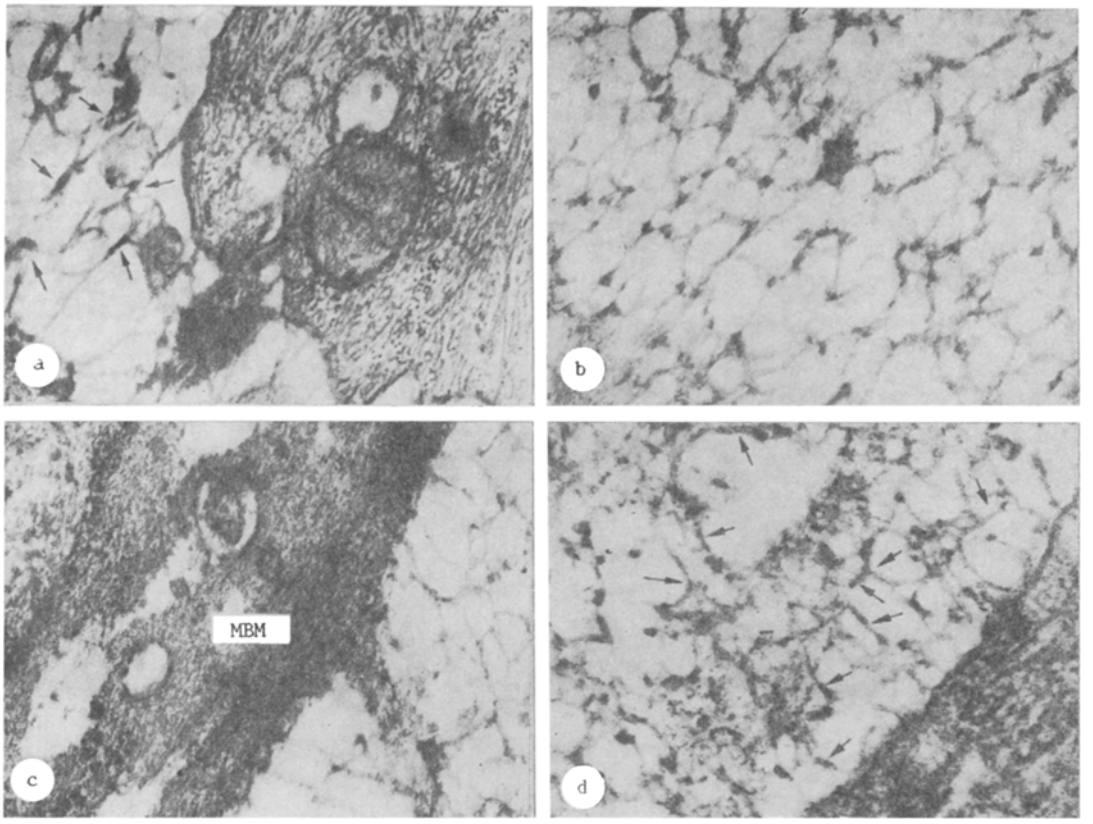

Fig. 2. PG in benign and malignant tumors of the dog mammary gland: a, b) mixed benign tumor: a) PG network in pericellular space (arrows), b) PG network in region of extracellular matrix away from the cells; c) simple solid carcinoma: accumulation of material of basement membrane type (MBM); d) mucous carcinoma: PG network in pericellular space (arrows). 80,000 ×.

Small PG granules near the fibroblasts were arranged in separate groups, and as a rule there were no connecting filaments (Fig. 1c). PG granules were just as sparsely distributed in the pericellular space of the macrophages (Fig. 1d).

The pericellular matrix in the basal part of the endothelium of the blood capillaries was organized in a characteristic manner. It was represented by a network of fine mesh, consisting of small PG granules and well developed connecting filaments. The matrix near the pericyte was similar in structure. The network of fine mesh was equally well defined near the adventitial cells of the capillaries (Fig. 1e).

In the adenomas the organization of the pericellular matrix was rather different from the control. PG granules in the matrix near the epithelium of secretory type were smaller and the connecting filaments were thinner. The basement membranes consisting of single myoepithelial cells was separated a little from the cell surface. In the space thus formed there were small PG granules.

Cells containing many fibrous structures in their cytoplasm were frequently found in the benign mixed mammary gland tumor. The basement membrane was absent in these cells and a wide-mesh network composed of large round or elongated proteoglycan granules with well-defined connecting filaments came close up to their surface (Fig. 2a). The extracellular matrix also had a similar ultrastructure at a distance from the cells (Fig. 2b).

Accumulation of homogeneous material resembling basement membranes was found in the pericellular space of the epithelium and in other structures of the extracellular matrix in simple solid carcinomas (Fig. 2c). These formations may perhaps contain type V collagen of which, according to data in the literature, there is 10 times as much in mammary gland carcinomas as normally [2]. A delicate proteoglycan network was seen between these formations.

The structure of the extracellular matrix in mucous and papillary-cystic carcinomas is heterogeneous. No signs of a basement membrane or proteoglycan network could be seen near the singly lying cancer cells. In more distant areas of the matrix large rod-shaped formations containing PG were infrequently found. Near the cells forming solid structures a PG network could be seen, together with laminar concentrations of material of average electron density (Fig. 2d).

Just as in benign mammary gland tumors, so also in malignant tumors the PG network characteristic of the capillary endothelium was still preserved, whereas the ultrastructure of the endothelial cells themselves showed considerable changes.

These results are evidence that the extracellular matrix of cells of the intact mammary glands, differing in origin and function, differs in its structure. PG occupy an important place in the submicroscopic organization of the matrix. Structures formed by them undergo changes in mammary gland tumors, and this is morphological confirmation of biochemical data in the literature relating to marked changes in the type and quantity of PG in tumors of epithelial and mesenchymal genesis [6, 8]. The data show that the differences in the character of changes in proteoglycan structures are found not only between benign and malignant tumors, but also within neoplasms of each of these groups.

LITERATURE CITED

1. Yu. M. Vasil'ev and I. M. Gel'fand, Interaction between Normal and Neoplastic Cells and the Environment [in Russian], Moscow (1981).
2. S. H. Barsky, C. N. Rao, G. R. Grotendorst, and L. A. Liotta, Am. J. Path., 108, 276 (1982).
3. O. Behnke and T. Zelander, J. Ultrastruct. Res., 31, 424 (1970).
4. K. Chen and T. N. Wight, J. Histochem. Cytochem., 32, 347 (1984).
5. G. K. Hascall, J. Ultrastruct. Res., 70, 369 (1980).
6. Y. Hatae, T. Atsuta, and A. Makita, Gann, 68, 59 (1977).
7. R. V. Jozzo, Hum. Path., 16, 2 (1984).
8. J. Takeuchi, M. Solue, E. Sanoe, et al., Cancer Res., 36, 2133 (1976).