ONCOLOGY

Proteoglycans and Human Breast Cancer

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Comparative analysis of proteoglycans in the control and tumor tissue of human mammary gland revealed disorders in the biosynthesis of dermatan sulfate proteoglycans in tumor cells. Using the methods of reverse transcription-PCR and Western blotting for the analysis of decorin expression we showed that these disorders were paralleled by reduced expression of the core protein and changes in the structure of proteoglycan carbohydrate chains and could be a cause of malignant degeneration of mammary gland cells.

Key Words: *proteoglycans; breast cancer; dermatan sulfate; chondroitin sulfate; decorin*

More and more proofs of the important role of proteoglycans (PG) in the regulation of cell proliferation appear in recent years [1-3]. Proteoglycans are characterized by tissue-specific inhibitory effects on division of mouse cells *in vivo*. Proteoglycans isolated from intact tissue exhibit dose-dependent cytostatic and cytopathogenic effects on the primary culture of mouse spontaneous mammary adenocarcinoma cells. PG preparations isolated from the liver of old (24 months) rats or from transplanted mouse hepatoma exhibited no antimitotic activity in mouse organs and in primary cell culture. It is known that each tissue has a specific PG set changing with age and in different diseases, including carcinogenesis. Due to high negative charge of carbohydrate chains PG interact with different regulatory molecules (growth factors, extracellular matrix components). Disorders in the expression and/or posttranslation modification of PG molecule can be a cause of malignant transformation of cells. Therefore, the study of changes in the PG composition and possible causes of these changes in carcinogenesis is an important task.

We analyzed PG composition in normal and tumor tissue of human mammary gland.

MATERIALS AND METHODS

Human mammary tumor tissue and normal tissue adjacent to this tumor (control) resected during surgical intervention at the Municipal Clinical Hospital No. 1, Novosibirsk, were analyzed. Tissue specimens were stored at -70°C. Proteoglycans were isolated from tissue samples excised in 17 patients. PG carbohydrate chains were identified by treating PG samples with chondroitin-AS-liase (0.005 U/μl) in 25 mM Tris-HCl buffer (pH 7.4; the enzyme was added to samples in 1:10 ratio or for 17-20 h at 37°C) or with nitrous acid by incubating PG samples with 5% sodium nitrite and 33% acetic acid in 1:1:1 ratio for 40 min at 25°C.

The results of treatment were analyzed by electrophoresis (6.5 V/cm, 40 min, 4°C) in 1% agarose gel in 50 mM barium acetate (pH 5.0). The content of PG applied onto each row was standardized by the weight of tissue collected for PG isolation. Gly-

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cosaminoglycan mixture consisting of heparan sulfates (1 μg/μl), dermatan sulfates (1 μg/μl), and AS chondroitin sulfates (1 μg/μl) served as the reference sample. After electrophoresis the gel was stained with 0.1% toluidine blue in 1% acetic acid. RNA was isolated using Trizol reagent (Invitrogen). Reverse transcription was carried out using Oligo(dT)-primers and M-MLV reverse transcriptase (Promega).

A fragment of decorin gene was amplified by multiplex PCR. Glyceraldehyde phosphate dehydrogenase gene (*GAPDH*) served as the control. The PCR products were analyzed by electrophoresis in 0.9% agarose gel in TAE buffer (pH 8.0) with 1 μg/ml ethidium bromide (6 V/cm, 20 min).

Tissue homogenate for Western blotting was prepared in 50 mM Tris-HCl buffer (pH 7.5) with 150 mM NaCl, 1% IGEPAL, and protease inhibitor mixture (Roche Diagnostics, 1 tablet per 10 ml buffer. The buffer was added to tissue in 1:20 ratio. The homogenates were incubated with chondroitin-AS-liase (0.002 U/μg total homogenate protein) for 1 h at 37°C. The total protein concentration in the homogenate was measured after Bradford. The proteins were separated by step PAAG electrophoresis (2 h, 6 V/cm). A total of 10 μg protein was applied onto each row. Proteins were transferred onto PVDF membrane at a current of 3 mA/cm^2 gel in a buffer containing 25 mM Tris and 192 mM glycine (pH 8.3) for 2 h. Nonspecific adsorption was blocked with 5% dry milk in PBS with 0.1 Twin-20 for 1 h. The membrane was incubated for 1 h with mouse monoclonal antibodies to human decorin core protein (R&D Systems), then for 1 h with second antibodies conjugated with horseradish peroxidase (Amersham Biosciences). Antibody dilution 1:1000 in PBS was used. Western blotts were exposed with X-ray films and developed using an ECL kit (Amersham Biosciences).

RESULTS

Analysis of summary PG from control and tumor samples of human mammary gland showed that PG composition drastically changed after malignant transformation. Normal tissue contained heparan sulfate PG and dermatan sulfate PG (DSPG), while tumor tissue always contained chondroitin sulfate PG (CSPG) fraction, while DSPG fraction decreased or in some cases completely disappeared after malignant transformation (Fig. 1, rows *2*, *4*). Additional identification of PG carbohydrate chains with specific enzyme chondroitin-AS-liase showed that electrophoretic profile of PG in the control tissue virtually did not change, while in PG samples from

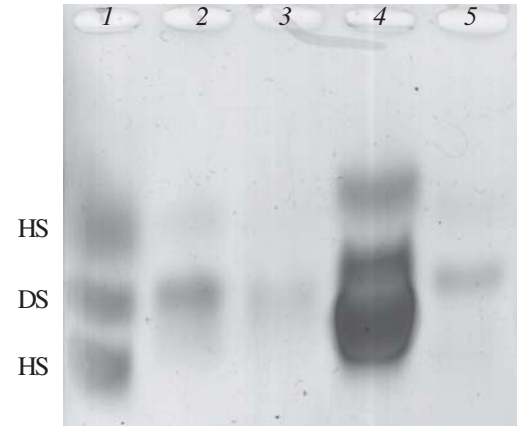

Fig. 1. PG from control and tumor samples of human mammary gland. Electrophoresis in 1% agarose gel in 50 mM barium acetate (pH 5.0). *1*) standard mixture of glycosaminoglycans: HS (heparan sulfates), DS (dermatan sulfates), CS (chondroitin sulfates-AS); *2*) control tissue; *3*) control tissue after chondroitin-AS-liase treatment; *4*) tumor tissue; *5*) tumor tissue after treatment with chondroitin-AS-liase.

tumor tissue the enzyme digested an appreciable part of DSPG carbohydrate chain and completely removed the lower electrophoretic fraction. This means that carbohydrate chains in PG from tumor tissue are primarily presented by chondroitin sulfate-AS (Fig. 1, rows *3*, *5*). PG specimens from control and tumor tissue were also treated with nitrous acid selectively destroying heparan sulfate-PG carbohydrate chains. No visible changes in this PG fraction isolated from tumor tissue were detected.

Since CSPG are precursors in DSPG biosynthesis, the decrease in the content of mature DSPG and appearance of CSPG fraction in tumor tissue can indicate disorders at a final stage of DSPG biosynthesis. Disorders in PG biosynthesis can be linked with not only carbohydrate chains of the molecule, but also changes in its protein core. We checked this hypothesis by reverse transcription-multiplex

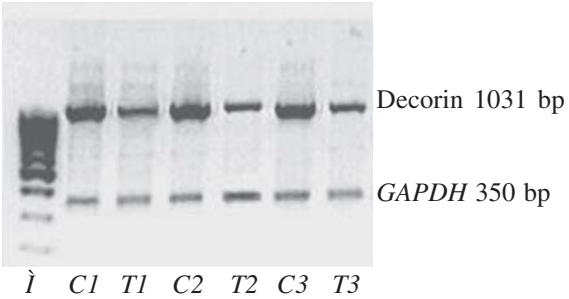

Fig. 2. Products of reverse transcription-multiplex PCR with primers for decorin and *GAPDH* genes. Electrophoresis in 0.9% agarose gel in TAE buffer (pH 8.0) with 1 μg/ml ethidium bromide. *M*: marker DNA; *C1-C3*: control tissue from different patients; *T1-T3*: tumor tissue from the same patients.

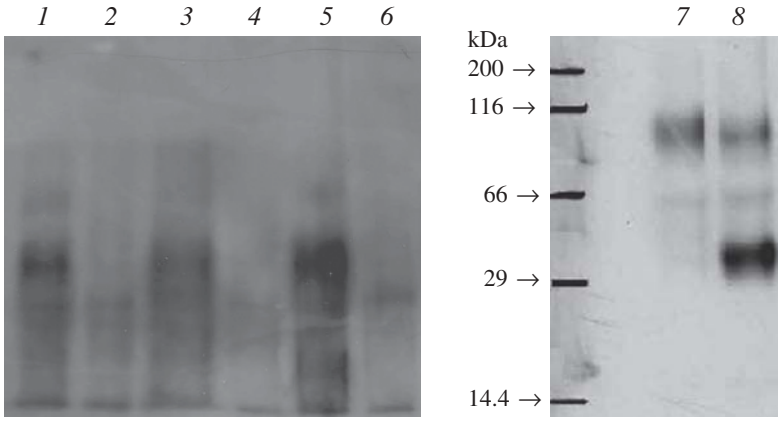

Fig. 3. Western blotting with antibodies to decorin core protein. *1*, *3*, *5*) control tissue from different patients; *2*, *4*, *6*) tumor tissue from the same patients; *7*, *8*) tumor tissue before and after chondroitin-AS-liase treatment, respectively.

PCR and Western blotting. Decorin, the main DSPG in mammalian tissues, was selected as a representative of DSPG. The expression of decorin gene in control and tumor mammary tissues was evaluated by reverse transcription-multiplex PCR with primers for decorin and *GAPDH* genes. It was found that the expression of decorin gene significantly decreased in tumor tissue compared to the control (Fig. 2). Similar results were obtained by Western blotting with mouse monoclonal antibodies to decorin core protein (Fig. 3, rows *1-6*). A clear-cut reduction of the expression of the protein part of decorin was observed in mammary tumor tissue in comparison with control tissue. Moreover, preliminary treatment of tumor tissue homogenate with chondroitin-AS-liase led to the appearance of a 40-kDa band corresponding to nonglycosylated core protein of decorin on electrophoregram (Fig. 3, rows *7*, *8*). These data confirm the results obtained in electrophoresis of summary PG and show that decorin carbohydrate chains in mammary tumor tissue are presented mainly by chondroitin sulfate-AS. These data attest to disorders at the level of the core protein expression and at the level of DSPG (decorin) carbohydrate chain biosynthesis. Decorin participates in many mechanisms regulating cell proliferation and is virtually not detected in tumor cells [1-3]. The causes of reduced DSPG level in tumor cells remain unknown. Presumably, disorders in the function of D-glucuronyl-C5-epimerase enzyme realizing epimerization of D-glucuronic acid into L-iduronic acid essential for DSPG maturation are responsible for inadequate synthesis of mature DSPG carbohydrate chains and accumulation of their precursors (CSPG) in tumor cells. One more hypothesis is that disorders involve one of dermatan sulfate-sulfotransferases, acting after epimerization stage and preventing reverse transformation of DSPG into CSPG.

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