Accumulation of collagen in ovarian benign tumours

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Extracellular matrix components of benign ovarian tumours (cystadenoma, adenofibroma, cystadenofibroma) were analysed. The investigated tumours contained twice as much collagen than control ovarian tissues. Significant alterations in mutual quantitative relationships between collagens of various types were observed. The proportion of type I collagen decreased and that of type III collagen increased. The accumulation of collagen was accompanied by a reduction in sulphated glycosaminoglycan content whereas the amount of hyaluronic acid was not changed. Dermatan sulphate was the most abundant glycosaminoglycan component. It is suggested that the accumulation of collagen (natural barrier to the migration of tumour cells) and underexpression of glycosaminoglycans/proteoglycans (binding some growth factors and interleukins) may exert an inhibitory effect on tumour growth.

Malignant neoplasms are composed of two discrete components: tumour cells and stromal connective tissue. The latter constitutes a significant proportion of the neoplastic mass and provides the infrastructure required by tumour cells for growth, gas exchange, and waste disposal. Proteoglycans (PG) are intrinsic constituents of both the cellular and extracellular environment associated with proliferating neoplasms. They are present at the cell surface of most, if not all, of the tumour cells and in the cell membranes. PG influence the surface charge of plasma membranes, the movement of molecules across tissues, structure of biological filters, and migration of cells along defined routes. Proteoglycans and glycosaminoglycans (GAGs) modulate biological activities of tumour cells. They may serve as a storage site to concentrate and stabilise growth factors in

the vicinity of tumour cells. Some of them modify binding of the tumour cells to extracellular matrix-components and affect secretion of hydrolytic enzymes, i.e., proteinases, type IV collagenases, and heparinases, that degrade the matrix components. Other PGs and GAGs modulate tumour metastasis and angiogenesis. In general, underexpression of PGs or decreased GAG biosynthesis inhibit tumour growth. In some cases an inhibitory effect of PGs on tumour growth has been observed [1, 2].

Neoplastic transformation of fibroblasts invariably results in changes of their phenotypic expression. For example, in some fibroblastic cells grown *in vitro* and transformed with oncogenic viruses, the ability to synthesise collagen was significantly reduced [3–5]. Similarly, SV-40-transformed fibroblasts [6] and Rous sarcoma virus-transformed chondroblasts [7] were reported to synthesise lower amounts of proteoglycans. On the other hand, some experimental tumours, like fibrosarcoma, induced by subcutaneous injection of methylcholanthrene, were reported to contain high amounts of types I, III and V collagens [8–10]. Furthermore, a variety of epithelial and mesenchymal neoplasms express abnormal amounts of PGs and GAGs [2].

This report describes the collagen and GAG composition of the ovarian benign tumours in comparison with that of normal ovaries. Accumulation of collagen in tumour tissues has been observed.

MATERIALS AND METHODS

Tissue material. Studies were performed on benign epithelial serous tumours removed during surgery. The material was taken from 15 patients aged 42–57. In each case the diagnosis was confirmed by histopathological examination. The following tumours were examined: 12 serous cystadenomas, 2 adenofibromas and 1 cystadenofibroma. Samples of control tissues were obtained from 12 pa-

tients subjected to ovariectomy accompanied the excision of uterus because of cervical or myometrial carcinoma.

Collagen determination. Collagen contents in individual tumours were measured by the assay of hydroxyproline (Hyp) as described by Prockop & Udenfriend [11]. Since hydroxyproline constitutes about 1/8 of collagen weight, the approximate amount of this protein in tumour tissue may be calculated by multiplying of the hydroxyproline content by 8. Collagen:GAGs index was evaluated by the assay of hydroxyproline:uronic acid ratio.

Fractionation of collagen. The control tissue and the tumours of the two groups were cut into small pieces and homogenized in 0.2 M acetic acid with the use of a glass homogenizer at 4°C. The homogenate was supplemented with pepsin (1 mg/ml) and incubated at 4°C for 24 h with continuous stirring. The mixture was centrifuged at 25000 × *g* for 30 min. The supernatant was collected and the sediment was further digested with pepsin in the same conditions. This procedure was repeated five times. During the pepsin treatment the noncollagenous proteins were digested to low molecular products and collagen become soluble in 0.5 M acetic acid [10]. The solubilised collagen was fractionated as described by Murata *et al.* [12]. Electrophoresis of collagen subunits and cyanogen bromide peptide mapping described in our previous paper demonstrated that such a procedure makes possible to achieve a full separation of types I, III and V collagens [13]. The quantities of isolated collagens were measured by the assay of hydroxyproline [11] and presented as percentage of total collagen content.

Isolation of GAGs. The investigated tissues were cut into small pieces and washed with physiological saline, suspended in anhydrous acetone, homogenized with a knife homogenizer, and dehydrated in the same solvent (4°C, 48 h). The homogenate was defatted by extraction with mixtures of acetone/ether (1:1, v/v) and methanol/chloroform (1:1, v/v) at room temperature for 48 h. The

defatted material was dried at room temperature to constant weight and GAGs were isolated as described in a previuos paper [14].

Electrophoresis of GAGs. The samples of GAG solutions containing 1.5 μg of uronic acid were applied to cellulose acetate strips and submitted to electrophoresis in 0.1 M barium acetate buffer, pH 5.0, at a constant current of 1 mA/cm, at room temperature, for 2 h [15]. The electrophoregrams were stained with Alcian blue and submitted to densitometric analysis with the use of a Beckman (Paragon - type) densitometer. Commercial preparations of highly purified GAGs (Sigma) were used as standards. Proportion between particular GAG fractions were calculated.

Fractionation of GAGs. The purified GAGs were separated on a CF11 cellulose microcolumn (0.3 cm × 6 cm), equilibrated with 1% cetylpyridinium chloride with a slight modification described in a previous paper [14]. Commercial preparations of highly purified GAGs (Sigma) were used as standards. The concentration of keratan sulphate in the eluate from the column was estimated by the assay of hexosamines [16] and that of other GAGs by the assay of uronic acid by the method of Bitter & Muir [17].

Isolation of elastin. The control tissue and the tumours of the two groups were extracted and defatted in the same way as described for isolation of GAGs. From the defatted and lyophilized material elastin was extracted with 0.25 M oxalic acid at 100°C for 1 h. The insoluble sediment was separated by centrifugation. The supernatant was collected and the sediment was extracted again in the same conditions. The extraction was repeated 4 times, until the test for elastin in the supernatants gave a negative result. Such a procedure makes possible to convert the insoluble elastin into a soluble product, called α-elastin. All supernatants were combined, dialysed against distilled water and submitted to the assay of elastin [18].

Elastin assay. Elastin was determined according to Fastin Elastin Assay Instructions

[18] with the use of a commercial kit containing the dye label: 5,10,15,20-tetraphenyl-21-23 porphyrine sulphonate (Biocolor Ltd., Belfast).

Statistical analysis. Mean values from 12 assays (control) or 15 assays (tumours) and standard deviations (S.D.) were calculated. The results were submitted to statistical analysis with the use of Student's t-test, accepting $P < 0.05$ as significant.

RESULTS

The mean amount of collagen present in normal ovary (Fig. 1) was 311.2 mg per gram of dry, defatted tissue. About 80% more collagen was found in tumour tissues.

The relationships between collagens of various type are presented in Fig. 2. Both in control ovarian tissues and in the investigated tumours, type I and type III collagens were found to be the most abundant. Type V collagen was found in small amounts. However, the proportion between them were significantly different. In normal ovaries collagen I and III constituted about 70% and 20% of total collagen, respectively. In the investigated tumours the proportion of type I collagen was distinctly lower and of type III collagen significantly higher.

In contrast to collagen, the amount of elastin in the tumours was only slightly lower than in control ovaries (Fig. 3).

Both normal ovaries and the investigated tumours contained small amounts of glycosaminoglycans. The mean amount of GAGs-bound uronic acid in normal ovaries was 3.02 mg per gram of dry deffated tissue (Fig. 4). Distinctly lower amounts (1.84 mg/g) of these polysaccharides were found in the ovarian tumours.

Fractionation of the isolated GAGs by chromatography on a CF11 cellulose microcolumn made possible to demonstrate the heterogeneity of ovarian GAGs and to compare the composition of ovarian and tumour GAGs. The
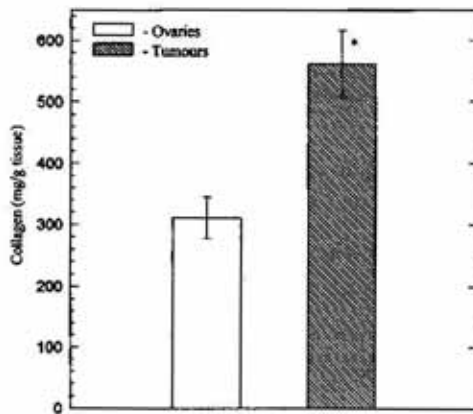
Figure 1. The amounts of total collagen in human ovaries and in ovarian tumours (in mg per gram of dry defatted tissue).
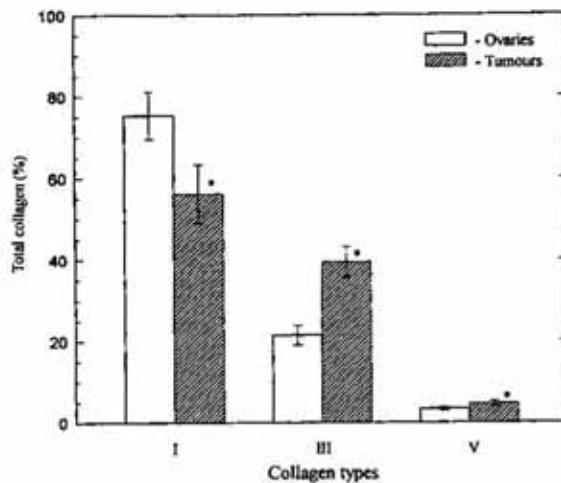
*$P < 0.001$

Figure 3. The amounts of elastin in human ovaries and in ovarian tumours (in mg per gram of dry defatted tissue).

*$P < 0.05$

Figure 2. The relationships between collagens of various types in ovaries and ovarian tumours.

*$P < 0.001$

Figure 4. The amounts of total GAGs in ovaries and ovarian tumours (in mg of uronic acid per gram of dry defatted tissue).

*$P < 0.001$

ovaries contained all the GAGs present in most human tissues but significant quantitative differences were apparent (Fig. 5). The amount of hyaluronic acid was very low (about 0.2 mg/g) and constituted less than 7% of total GAGs. Sulphated glycosaminoglycans were found to be much more abundant, as they constituted above 90% of total GAGs. Dermatan sulphate was the most abundant GAG component in the ovarian tissue, constituting about 50% of total GAGs. The ovaries contained, moreover, sulphated GAGs: keratan sulphate, heparan sulphate, chondroitin-
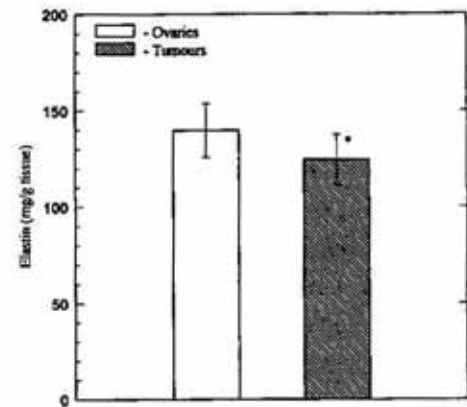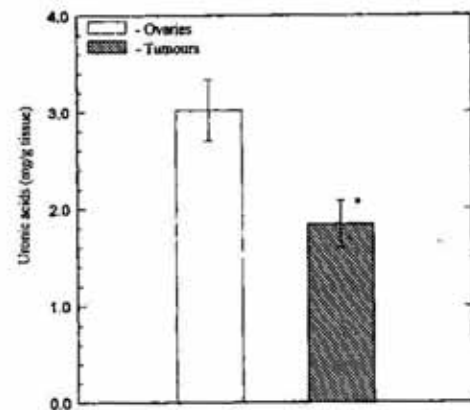
4-sulphate, chondroitin-6-sulphate and heparin (Fig. 5).

The investigated tumours contained the same amounts of hyaluronic acid as normal ovarian tissues but the quantities of sulphated GAGs were distinctly lower. The reduction in dermatan sulphate content was rather slight but the amounts of other sulphated GAGs were lower by 50% to 70% than in normal tissue.

Similar conclusions can be drawn from electrophoretic analysis of GAGs isolated from normal ovaries and ovarian tumours. As can be seen from Fig. 6, sulphated GAGs predominate over hyaluronic acid both in normal ovaries (Fig. 6 A) and in the tumours (Fig. 6 B).
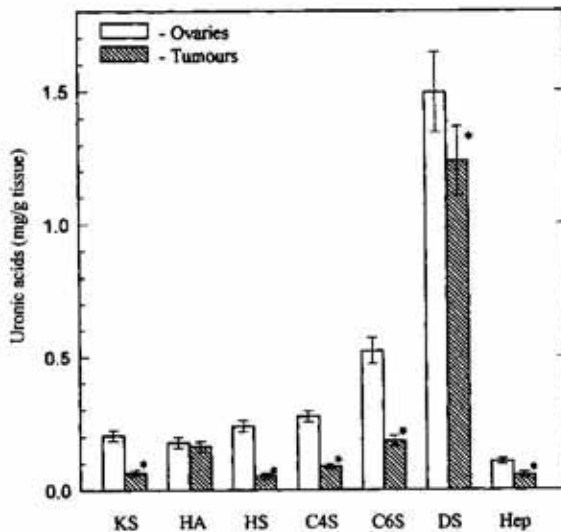
Figure 5. The amounts of various glycosamino-glycans in ovaries and ovarian tumours (in mg of uronic acids [17] per gram of dry defatted tissue).

*$P < 0.001$

They constituted more than 90% of total glyco-saminoglycans. Hyaluronic acid constituted 6.7% and 8.1% of total GAGs in control ovaries and in the tumours, respectively.

As shown in Fig. 7, the ratio of collagen to glycosaminoglycans in ovarian tumours was about 3 times as high as in control ovarian tis-sues.

DISCUSSION

Human ovary contains a significant amount of collagen. In ovarian tumours the amount of collagen was found distinctly increased reach-ing a value which is twice as high as in normal ovarian tissue. The increase of collagen con-tent was accompanied by a change in relation-ships between collagens of various types: the tumours contained relatively less type I colla-gen and more type III collagen.

Connective tissue stroma probably presents a natural barrier to the migration of tumour cells. Collagen represents the major struc-tural protein of most tissues and provides the chief obstacle to such a migration [19].

It is apparent from our studies that normal human ovary contains a rather low amount of
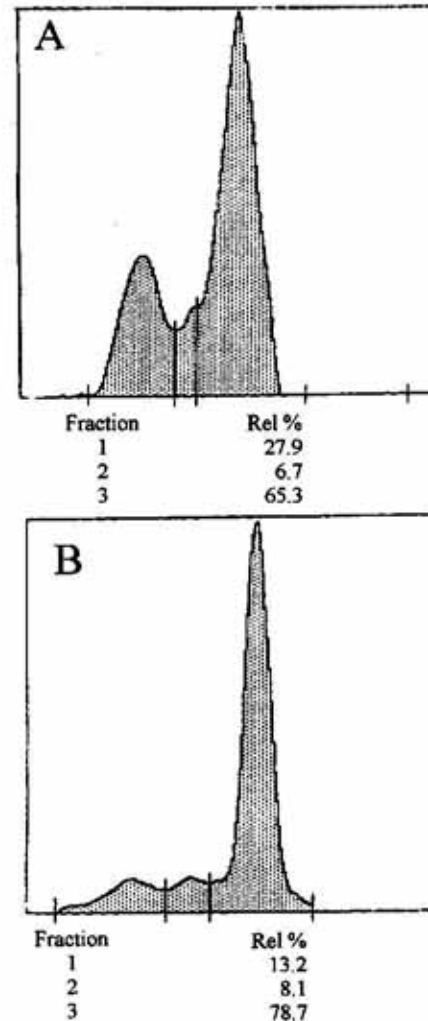

Figure 6. Typical densitometric scans of electrophoregram of total GAGs isolated from ova-ries (A) and ovarian tumours (B).

Composition of the fractions: 1, KS, C4S, C6S; 2, HA; 3, HS, DS, Hep.

glycosaminoglycans. In contrast to many other tissues, the amount of hyaluronic acid is very low in comparison to that of sulphated glycosaminoglycans. Dermatan sulphate is the major glycosaminoglycan component.

It is of interest that the accumulation of col-lagen in tumour tissues is accompanied by a significant reduction in sulphated glycosa-minoglycans content. In general, under-expression of proteoglycans or decreased GAG biosynthesis inhibit tumour growth [1, 2].

No doubt such a decrease in sulphated GAGs content, especially of HS, Hep and DS is im-
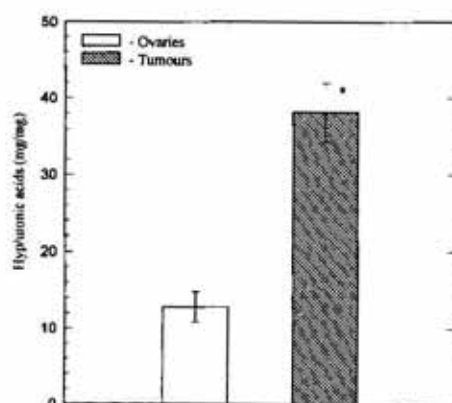
Figure 7. Collagen:glycosaminoglycans ratio in human ovaries and in ovarian tumours (mg hydroxyproline:mg uronic acids).

*$P < 0.001$

portant for the tumour biology as they can bind various growth factors and interleukins [20]. HS and Hep bind interleukins 3, 4 and 8, fibroblasts growth factors, platelet-derived growth factors and some others. Rapid and localized changes in the activity of these factors can be induced by their release from matrix storage and by activation of latent forms. These growth factors, in turn, control cell proliferation, differentiation and synthesis, as well as remodeling of the extracellular matrix [21].

Dermatan sulphate is a glycosaminoglycan component of small proteoglycans called biglycan and decorin which bind to the transforming growth factor and inhibit the mitogenic activity of this factor [22]. Both decorin and biglycan bind to interstitial collagens and inhibit collagen fibrillogenesis [23]. Also chondroitin sulphate proteoglycans strongly interact with type I collagen *via* glycosaminoglycan chains and by protein–protein interactions, and they inhibit fibrillogenesis [24].

It may be concluded that the accumulation of collagen (natural barrier to the migration of tumour cells) and underexpression of glycosaminoglycans/proteoglycans (binding sites for growth factors and interleukins) may exert an inhibitory effect on tumour growth. Low amounts of sulphated GAGs in the tu-

mour may enhance the fibrillogenesis process and promote the accumulation of collagen. The accumulated collagen may constitute a barrier which protects normal ovarian tissue against infiltration by tumour cells.

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