ISSN 0006-2979, Biochemistry (Moscow), 2007, Vol. 72, No. 9, pp. 1016-1020. © Pleiades Publishing, Ltd., 2007.
Original Russian Text © T. Yu. Eshchenko, V. I. Rykova, A. E. Chernakov, S. V. Sidorov, E. V. Grigorieva, 2007, published in Biokhimiya, 2007, Vol. 72, No. 9, pp. 1248-1254.

# Expression of Different Proteoglycans in Human Breast Tumors

**T. Yu. Eshchenko[1]*, V. I. Rykova[2], A. E. Chernakov[3], S. V. Sidorov[3], and E. V. Grigorieva[1]**

*[1]Institute of Molecular Biology and Biophysics, Siberian Branch of the Russian Academy of Medical Sciences, ul. Akademika Timakova 2, 630117 Novosibirsk, Russia; fax: (383) 332-3147; E-mail: Tatiana_E@ngs.ru*
*[2]Institute of Cytology and Genetics, Siberian Branch of the Russian Academy of Sciences, ul. Akademika Lavrentieva 10, 630090 Novosibirsk, Russia; fax: (383) 333-1277; E-mail: Dymshits@niboch.nsc.ru*
*[3]1st Municipal Clinical Hospital, ul. Zalesskogo 6, 630047 Novosibirsk, Russia*

Received April 18, 2007
Revision received May 15, 2007

**Abstract**—The composition of proteoglycans and their changes during malignant transformation are important factors influencing adhesive properties and mitotic activity of tumor cells. In this study, expression level of different proteoglycans (decorin, syndecan-1, lumican, glypican-1, and aggrecan) in tumors and normal human breast tissue was investigated. Multiplex RT-PCR data revealed different expression changes for different proteoglycans in human breast tumors—syndecan expression was activated compared to almost no expression in normal breast tissue, expression of decorin and lumican decreased 2-5- and 2-3-fold, respectively, and aggrecan transcription seems to be unaffected. A change of expression level of decorin correlated with expression of D-glucuronyl-C5-epimerase, a key enzyme responsible for the biosynthesis of idurone-containing glycosaminoglycans, possessing antimitotic activity. The results suggest that changes in decorin, lumican, and syndecan-1 expession in tumor tissue could induce a distortion of proteoglycan composition and mitotic activity of cells in human breast tumor.

**DOI**: 10.1134/S0006297907090143

*Key words*: breast cancer, proteoglycans, D-glucuronyl-C5-epimerase, proteoglycan biosynthesis

Various molecules influence cell proliferation, and among them there are proteoglycans (PG)—complex macromolecules consisting of protein core and covalently attached glycosaminoglycan chains (GAG). As shown previously, some proteoglycan species stimulate cell proliferation, whereas others inhibit it. For example, decorin retards cell division via a mechanism mediated by EGF-receptor/MAP-kinase/p21 [1, 2], lumican also suppresses cell proliferation [3, 4], syndecan-1 stimulates cell division [5, 6], and glypican-3 is able to inhibit or stimulate cell division depending on the tissue [7].

In the course of malignant transformation the proteoglycan composition of tumor changes, and for different tumors either decrease or increase in the content of proteoglycan species have been shown. Thus, lumican is expressed in cells of pancreatic adenocarcinoma [8] and colon tumor [9], though it is not expressed in epithelial cells of corresponding normal tissue, while in human breast tumor lumican expression decreases and its level correlates with rapid tumor growth and poor prognosis [10]. The level of decorin expression changes in different types of tumors; moreover, the structure and composition of its carbohydrate chain, sulfation sites, and the extent of epimerization change as well. For example, decorin content in pancreas cancer cells increases six times, and its GAG component is mainly chondroitin sulfates (CS) (the CS content is increased 11 times compared with normal level, when dermatan sulfate (DS) is prevalent [11]). Eight-fold increase in the decorin protein content was registered in intestinal adenocarcinoma cells, when GAG was present mainly as CS (86%) compared with normal level, where DS represents 90-93% [12]. The decorin content in stomach carcinoma cells increases eight times compared with the normal level, and in this case the CS content is doubled [13]. It was noted that in rectum carcinoma cells the content of iduronic acid residues decreases compared with the normal level and the respective decrease from 73 to 30% in the content of dermatan sulfates, characteristic of quiescent tissues, was noted [14].

*Abbreviations*: CS) chondroitin sulfate; DS) dermatan sulfate; ECM) extracellular matrix; GAG) glycosaminoglycans; HS) heparan sulfate; PG) proteoglycans; RT-PCR) reversed-transcription polymerase chain reaction.

* To whom correspondence should be addressed.

Thus, according to data published in the literature, various changes appear in tumor tissue concerning expression of individual PG and the structure of their carbohydrate chains (GAG). We have supposed that the disturbance in the ratio of PG species, capable of cell proliferation stimulation/inhibition, takes place in tumor. This may be due to the alteration of expression level of their protein core and/or to structural distortion of their carbohydrate chains, such as glucuronic acid epimerization to iduronic acid. To test this supposition, we studied the level of expression of the main proteoglycan species in extracellular matrix (ECM) and cell surface, as well as expression of epimerase—the enzyme responsible for biosynthesis of idurone-containing GAG/PG [15] exhibiting antimitotic activity.

## MATERIALS AND METHODS

**Clinical material.** The breast tumor tissue, removed during radical surgery and stored in liquid nitrogen, was used in this work. Samples of tumor and those of corresponding normal tissue, maximally remote from the tumor center, were obtained from each patient.

Samples taken during surgery were obtained in the 1st Municipal Clinical Hospital, where the diagnosis was verified by histological investigation. The prevalent histological type of tumor is duct infiltrating cancer (22 patients of 24) of different degree of malignancy: four patients had tumors of degree I, 12 patients were at degree II, and six patients at degree III of malignancy. Stages of malignancy progression were determined using the formula $T_xN_xM_x$, where T corresponds to the tumor size, N indicates the existence of metastases in regional lymph nodes, and M shows the existence of metastases in remote lymph nodes. Most patients were at the second stage of malignancy progression: 16 patients at the stage $T_2N_{1\text{-}2}M_0$, six patients at $T_1N_{1\text{-}2}M_0$, and two patients at $T_3N_{1\text{-}2}M_0$. All patients presented their written informed consents concerning their participation in the investigation, and experiments were performed in accordance with ethical principles of the Helsinki Declaration and standards of the Committee of Bioethics of the State Research Institution of Molecular Biology and Biophysics, Siberian Branch of the Russian Academy of Medical Sciences.

**Isolation of RNA.** RNA was isolated from 50-100 mg tissue using TRIZOL reagent (Invitrogen, Great Britain) according to the manufacturer's instructions.

Reverse transcription was carried out with 2 μg isolated RNA using oligo(dT) primers and M-MLV reverse transcriptase (Promega, USA) as recommended by the manufacturer. The decorin, syndecan-1, lumican, glypican-1, aggrecan, and GAPDH gene fragments were amplified on the Tercik (DNA Technologies, Russia) amplifier in 20 μl of solution containing ~200 ng cDNA, 2 μl 10× PCR buffer (10 mM Tris-HCl, 1.5 mM $MgCl_2$, 50 mM KCl, pH 8.3), 20 pmol of each primer, 0.2 mM of each dNTP, and 1 activity unit of Taq polymerase. The PCR conditions were as follows: 5 min at 95°C, 30 sec at 95°C, 30 sec at 59°C, 60 sec at 72°C, and 10 min at 72°C. To amplify the *GAPDH* gene fragments, 16-18 cycles of PCR were carried out, for decorin and lumican genes—22 cycles, and for epimerase, syndecan-1, glypican-1, and aggrecan genes—32 cycles were necessary. The following primers were used:

GAPDH: 5′-GGGCGCCTGGTCACCAG-3′ (F)
and
5′-AACATGGGGGCATCAGCAGAG-3′ (R);

decorin: 5′-GGCCACTATCATCCTCCTTCTGC-3′ (F)
and
5′-ATGGCAGAGCGCACGTAGACAC-3′ (R);

lumican: 5′-CTCTCTTCCTGGCATTGATTGGTGG-3′; (F)
and
5′-GACAGATCCAGCTCAACCAGGG-3′ (R);

glypican-1: 5′-GAGCTGCGGCGAGGTCCG-3′ (F)
and
5′-CTGGTCTACTGTGCTCACTGCC-3′ (R);

syndecan-1: 5′-GCCCCCTGAAGATCAAGATGGC-3′ (F)
and
5′-CCTCCTGTTTGGTGGGCTTCTG-3′ (R);

aggrecan: 5′-CTTCTACCGCCCCACTGGCC-3′ (F)
and
5′-GCCAGCCGGCGTCACACTG-3′ (R);

5′-epimerase: 5′-AAGGGAGACGAGAGGGGAAC-GAA-3′ (F)
and
5′-GCCACCTTTCTCATCCTGGTTCC-3′ (R).

**Horizontal electrophoresis of nucleic acids in agarose gel.** PCR products were analyzed by horizontal gel electrophoresis in 0.9% agarose gel in TAE buffer (0.04 M Tris-HCl, 0.05 M EDTA, pH 8.0), 1 μg/ml ethidium bromide, 20 min at electric field intensity 6-8 V/cm. Marker DNA of 100 and 1000 bp (Sibenzyme, Russia) were used as standards.

**Data processing.** Semiquantitative analysis of fragments obtained by multiplex PCR-amplification was carried out using the TotalLab program (Nonlinear Dynamics, Great Britain). The household gene *GAPDH* was used as control, and the intensity of amplified DNA bands of D-glucuronyl-C5-epimerase and GAPDH were designated as $A_{epim}$ and $A_{GAPDH}$, respectively. Their ratio in tumor ($A_{epim.tum.}/A_{GAPDH.tum.}$) to that in control ($A_{epim.contr.}/A_{GAPDH.contr.}$) was designated as $A_{alt.}$:

$$\frac{A_{\text{epim.tum.}}/A_{\text{GAPDH.tum.}}}{A_{\text{epim.contr.}}/A_{\text{GAPDH.contr.}}} = A_{\text{alt.}}. \quad (1)$$

## RESULTS

**Expression of different proteoglycans.** The goal at the first stage was to characterize human breast tumor by the level of expression of proteoglycan genes involved in maintenance of intercellular contact and cell interaction with extracellular matrix (ECM). The main types of ECM and cell surface proteoglycans were chosen for investigation, such as decorin, syndecan-1, lumican, glypican, and aggrecan. We also determined the expression level of the epimerase gene. This enzyme of proteoglycan biosynthesis is responsible for production of idurone-containing proteoglycans exhibiting antimitotic activity. Expression level was semiquantitatively evaluated (Fig. 1).

The data show that in breast tumor the syndecan-1 gene expression is activated, expression of decorin and lumican genes decreases 2-5 and 2-3 times, respectively, whereas the glypican-1 gene is practically not expressed either in tumor or in corresponding normal tissue. Such diversely directed changes in mRNA amounts as a decrease in the amount of mRNA of some genes and an increase in the amount of mRNA of others may be the likely cause of the distortion of proteoglycan composition in tumor tissue.

In this experiment a correlation was noted between the level of decorin expression (and to a lesser extent of lumican) with expression of D-glucuronyl-C5-epimerase (Fig. 1); therefore, we decided to study their expression in more detail and to determine whether there is any correlation between them.

**Decorin expression in human breast tumor.** The level of decorin gene expression in samples of human breast was determined by multiplex RT-PCR. Clinical samples of control and tumor tissue from 24 patients were studied (Fig. 2). The prevalent histological type among them was duct infiltrating cancer of different extent of malignancy (I-III) with metastases into local lymph nodes and without remote metastases (22 patients), cancer of the dispersed cell type, and scirrhous cancer (two patients). All

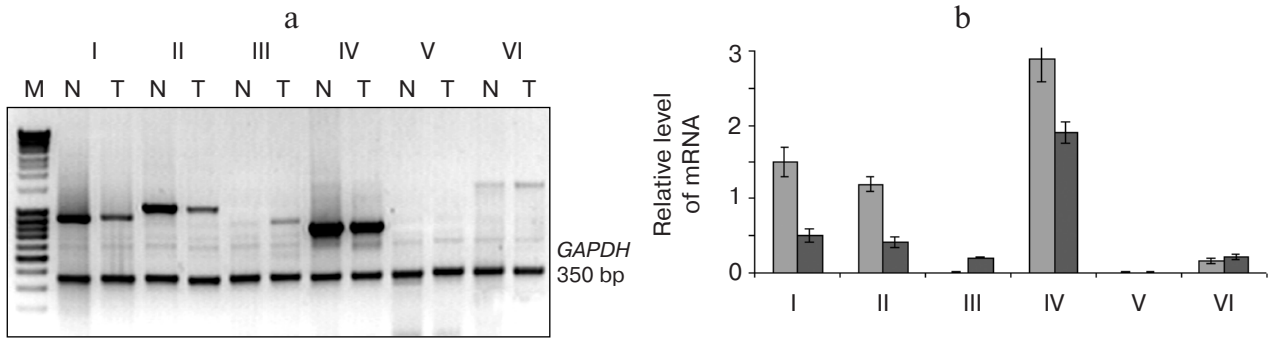


**Fig. 1.** Expression of different proteoglycans in human breast tumor and normal tissue. a) Electrophoregram of multiplex PCR products for epimerase, decorin, syndecan, lumican, glypican, and aggrecan genes. Here and in Figs. 2 and 3: N, normal tissue; T, tumor; M, marker 1000-bp DNA. b) Semiquantitative evaluation of the epimerase gene expression level relative to expression of the *GAPDH* gene (TotalLab program). Here and in Figs. 2 and 3, gray columns correspond to the norm, black to the tumor. I-VI, epimerase, decorin, syndecan, lumican, glypican, and aggrecan, respectively.

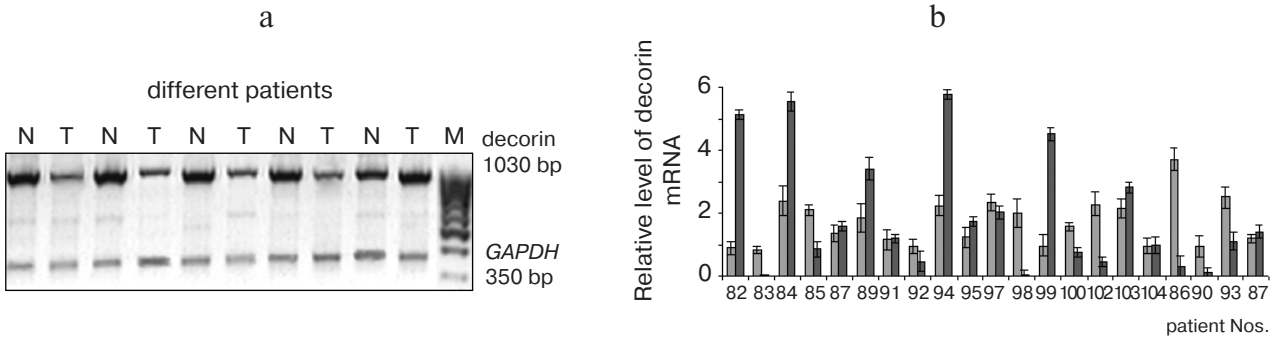


**Fig. 2.** Relative change of decorin gene expression in human breast tumor compared with normal breast tissue. a) Electrophoregram of products of multiplex PCR-amplification with primers for decorin and *GAPDH* genes. b) Level of decorin gene expression in normal and tumor tissue relative to the *GAPDH* expression (TotalLab program).

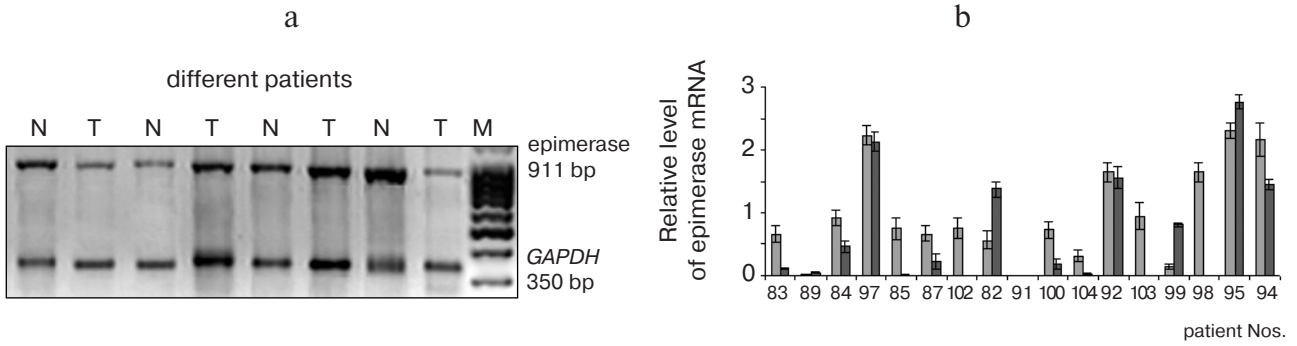


**Fig. 3.** Epimerase gene expression in tumor compared with control in different patients. a) Electrophoregram of products of multiplex PCR-amplification with primers for *GAPDH* and epimerase genes. b) Level of epimerase gene expression in normal and tumor tissue relative to *GAPDH* expression (TotalLab program).

examined tumors were similar histologically and were at approximately the same clinical stage of tumor progression.

According to our data, decorin gene expression in breast tumor of most patients decreases 2-5 times or does not change significantly compared with normal breast tissue (in this case the criterion was more than 50% increase or decrease in decorin gene expression). However, there were individual distinctions between patients by the decorin expression in tumor: in 10 patients out of 24, a sharp increase in mRNA amount (3-4 times compared with normal level) was noted.

**Expression of D-glucuronyl-C5-epimerase in human breast tumors.** In parallel, the amount of D-glucuronyl-5-epimerase mRNA was determined in the same clinical samples (Fig. 3). The level of decorin and epimerase mRNA was simultaneously determined for 24 patients.

In most of the patients a decrease in the level of epimerase gene expression in tumor was observed comparing to the normal breast tissue. However, like in the case of decorin, there were certain individual peculiarities in different patients: in some cases increased amount of epimerase mRNA was observed in tumor compared with normal tissue (Nos. 82, 99), whereas in other patients the level of epimerase expression was noticeably increased both in tumor and in control sample (Nos. 95, 97).

To determine whether the change in the decorin gene expression in human breast tumor correlates with expression of D-glucuronyl-C5-epimerase gene, we compared quantities of mRNA of decorin and epimerase genes in tumors of the same patients. To evaluate alterations that happen in tumor, we determined the ratio of the decorin and epimerase expression levels in tumor to their expression in normal human breast tissue (Fig. 4).

The $A_{alt}$ value allowed us to estimate approximately how many times the D-glucuronyl-C5-epimerase gene expression in tumor exceeds that in control. The value of 1 shows that there are no changes in gene expression, value > 1 is indicative of increased level of gene expression in tumor, and value < 1 shows that gene expression in tumor is less than that in control.

To reveal the coordination in expression of D-glucuronyl-C5-epimerase and decorin genes in the human breast tumor tissue, the correlation coefficient of these parameters was determined using formula:

$$\frac{\Sigma(A_{\text{epim.}\,i} - \overline{A}_{\text{epim.}})\,(A_{\text{dec.}\,i} - \overline{A}_{\text{dec.}})}{N\,\sigma_{\text{dec.}}\,\sigma_{\text{epim.}}}, \qquad (2)$$

where σ is square (standard) deviation determined by the formula:

$$\sigma = \sqrt{\frac{\Sigma\,(A_i - \overline{A})^2}{N}}, \qquad (3)$$

where $N$ is the number of patients and $\overline{A}$ is mean value of the feature.

The calculations show that compared to normal tissue, the change in decorin expression in human breast tumors correlates with that of D-glucuronyl-C5-epimerase with the level of reliability of 95%.

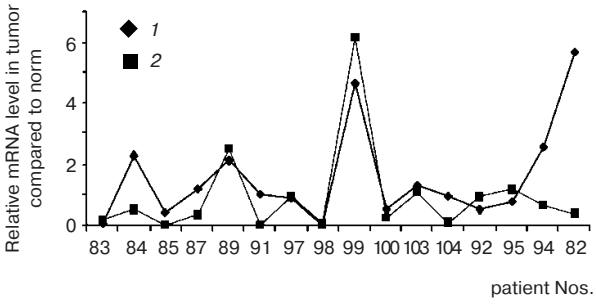


**Fig. 4.** Changes of decorin (*1*) and epimerase (*2*) gene expression levels in tumor compared to their expression in normal human breast tissue in different patients.

Thus, the data indicate the following changes in human breast tumors concerning expression of different proteoglycans: the level of syndecan-1 expression increases, that of decorin and lumican decreases 2-5 and 2-3 times, respectively, whereas aggrecan expression remains practically unchanged. Changes in decorin expression in human breast tumor correlate with changes of D-glucuronyl-C5-epimerase expression with the level of reliability of 95%.

## DISCUSSION

The levels of expression of different proteoglycans in human breast tumors really change—both decrease (decorin, lumican) and increase (syndecan-1) are observed. Data on decrease in the lumican expression level well agree with results obtained previously for this type of tumor [10], but changes in decorin expression are not so unambiguous. It was shown previously that decorin expression in breast tumors decreased [10], and addition of decorin protein to breast cells grown in culture and to breast tumor cells caused 70% inhibition of their growth and reduced the level of metastasizing; injection of an adenovirus construct into the tumor retarded growth of the latter by 70% [16].

According to our data, the 2-5-fold decrease in the decorin expression level was also registered in most clinical samples, although in some samples the expression level remained unchanged or even increased.

The fact that the decorin expression level in human breast tumor correlated with expression of D-glucuronyl-C5-epimerase was to some extent unexpected. However, it should be noted that this correlation strongly depended on the gene region for which primers were chosen. During preliminary work, we checked six different pairs of primers for different regions of the epimerase gene, and some of them (mainly located in the fourth exon) exhibited a stable level of epimerase expression both in tumor and control breast tissue. In this case, no correlation between decorin and epimerase gene expression levels was observed (data not shown). At the same time, primers concerning the third exon pointed to a significant decrease in the epimerase mRNA content, more precisely, in the content of the sequence corresponding to the third exon of the gene under investigation. Based on these data, we supposed an alteration in the epimerase mRNA structure in tumor tissue, due to the deletion/alternative splicing of a sequence corresponding to the third exon. Owing to this, primers located just in this region of the epimerase gene DNA were used for further work.

Although it is known that the extent of decorin epimerization is strongly changed in tumor tissue [14], nevertheless the presence/absence of epimerase should not directly influence the level of expression of the decorin core protein. It may affect mainly functional activity of decorin, which is supported by the fact of replacement of DS chains by CS chains in tumor tissue [11-13]. The existence of correlation between the decorin and epimerase expression levels at this stage only suggests that these genes may be expressed in a single transcription group, but this supposition requires further investigations.

The authors are grateful to Prof. G. M. Dymshits for support and discussion of this work, to V. S. Titova, A. V. Proskura, and O. Z. Goldinstein for collection of clinical material for investigation, to A. D. Grigoriev for help in preparation of this article.

This work was supported by ISCC Intermedbiofizkhim (grants 19/05-Gene-M and 19/06-Gene-M).

## REFERENCES

1. Moscatello, D. K., Santra, M., Mann, D. M., McQuillan, D. J., Wong, A. J., and Iozzo, R. V. (1998) *J. Clin. Invest.*, **101**, 406-412.
2. Santra, M., EiCStetter, I., and Iozzo, R. V. (2000) *J. Biol. Chem.*, **275**, 35153-35161.
3. Vij, N., Roberts, L., Joyce, S., and Chakravarti, S. (2004) *Exp. Eye. Res.*, **78**, 957-971.
4. Vuillermoz, B., Khoruzhenko, A., D'Onofrio, M. F., Ramont, L., Venteo, L., Perreau, C., Antonicelli, F., Maquart, F. X., and Wegrowski, Y. (2004) *Exp. Cell Res.*, **296**, 294-306.
5. Maeda, T., Alexander, C. M., and Friedl, A. (2004) *Cancer Res.*, **64**, 612-621.
6. Barbareschi, M., Maisonneuve, P., Aldovini, D., Cangi, M. G., Pecciarini, L., Angelo Mauri, F., Veronese, S., Caffo, O., Lucenti, A., Palma, P. D., Galligioni, E., and Doglioni, C. (2003) *Cancer*, **98**, 474-483.
7. Filmus, J. (2001) *Glycobiology*, **11**, 19-23.
8. Ping Lu, Y., Ishiwata, T., and Asano, G. (2002) *J. Pathol.*, **196**, 324-330.
9. Lu, Y. P., Ishiwata, T., Kawahara, K., Watanabe, M., Naito, Z., Moriyama, Y., Sugisaki, Y., and Asano, G. (2002) *Pathol. Int.*, **52**, 519-526.
10. Troup, S., Njue, C., Kliewer, E. V., Parisien, M., Roskelley, C., Chakravarti, S., Roughley, P. J., Murphy, L. C., and Watson, P. H. (2003) *Clin. Cancer Res.*, **9**, 207-214.
11. Skandalis, S. S., Kletsas, D., Kyriakopoulou, D., Stavropoulos, M., and Theocharis, D. A. (2006) *Biochim. Biophys. Acta*, **1760**, 1217-1225.
12. Theocharis, A. D. (2002) *Biochim. Biophys. Acta*, **1588**, 165-172.
13. Theocharis, A. D., Vynios, D. H., Papageorgakopoulou, N., Skandalis, S. S., and Theocharis, D. A. (2003) *Cell Biol.*, **35**, 376-390.
14. Tsara, M. E., Theocharis, A. D., and Theocharis, D. A. (2002) *Anticancer Res.*, **22**, 2893-2898.
15. Tiedemann, K., Larsson, T., Heinegard, D., and Malmstrom, A. (2001) *Arch. Biochem. Biophys.*, **391**, 65-71.
16. Reed, C. C., Waterhouse, A., Kirby, S., Kay, P., Owens, R. T., McQuillan, D. J., and Iozzo, R. V. (2005) *Oncogene*, **24**, 1104-1110.