ONCOLOGY

Activity of Glutathione-Metabolizing and Antioxidant Enzymes in Malignant and Benign Tumors of Human Lungs

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We measured the content of glutathione and activity of glutathione-metabolizing and antioxidant enzymes superoxide dismutase and catalase in samples obtained from 52 patients with malignant lung tumors and 20 patients with benign lung tumors. The content of glutathione and activity of glutathione-metabolizing enzymes underwent similar changes, but these changes were most pronounced in malignant tumors. Antioxidant enzyme activity changed insignificantly in benign tumors, but significantly decreased in malignant tumors (squamous cell carcinoma and adenocarcinoma). The severity of changes in malignant tumors depended on the degree of malignancy. Most pronounced changes were observed in adenocarcinoma, which often metastasizes and is resistant to chemotherapy. These changes were least pronounced in bronchoalveolar carcinoma sensitive to chemotherapy.

Key Words: *lung cancer; glutathione; glutathione-metabolizing enzymes; superoxide dismutase; catalase*

Studies of glutathione-metabolizing enzymes in tumors attracted much attention over several years. Glutathione plays an important role in DNA synthesis and antioxidant and radiational protection and acts as a sulfhydryl buffer in cells.

Our previous studies [2] and published data [5, 7,13] show that activity of glutathione-metabolizing enzymes glutathione reductase (GR), glutathione S-transferase (GST), glutathione peroxidase (GSH-Px), and γ-glutamyltranspeptidase (γ-GT) and glutathione content increase in malignant lung tumors. A considerable increase in activity of membrane-bound glutathione-degrading γ-GT in lung tumors is of particular interest. These changes are probably associated with consumption of glutathione or constituent γ-amino acids during tumor growth and intensive release of glutathione into the tumor tissue [5]. We found that the increase in γ-GT activity is most pronounced in malignant thymoma (70-fold higher than in normal tissues) [2].

Attempts were made to evaluate the relationship between changes in activity of glutathione-metabolizing enzymes in lung tumors and the resistance of cancer cells to chemotherapy. These experiments were performed with cultured cells from human lung cancer [8,15].

Published data show that activity of antioxidant enzymes decreases in malignant lung tumors [9,12]. Here we compared glutathione content and activity of glutathione-metabolizing and antioxidant enzymes superoxide dismutase (SOD) and catalase in lung tumors.

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MATERIALS AND METHODS

We examined specimens of malignant and benign lung tumors obtained from 72 patients during surgery. Fifty-two patients (47 men and 5 women, 42-78 years) had malignant tumors, including squamous cell carcinoma (n=32), adenocarcinoma (n=12), and bronchoalveolar carcinoma (n=8). We examined 20 patients (12 men and 8 women, 34-59 years) with benign tumors, including tuberculoma (n=10) and hamartoma (n=10).

The specimens taken from individuals died due to accidents served as the control. These samples were obtained from the N. F. Sklifasovsky Institute of Emergency 12-24 h after death.

Tissue samples were immediately frozen and stored at -20°C. Before the experiment the tissues were minced and homogenized in cold physiological saline in a Potter-Eveljem homogenizer. Glutathione content in the homogenate was measured as described elsewhere [4]. Activities of glutathione-metabolizing enzymes GR (EC 1.6.4.2) [6], GSH-Px (EC 1.11.1.9) [11], GST (EC 2.5.1.18) [10], and γ-GT (EC 2.3.2.4) [14] were estimated. We measured activities of Cu/Zn-SOD (EC 1.15.1.1) [16] and catalase (EC 1.11.1.6) [1].

The measurements were performed in 2 replicants. The results were analyzed by Student's t test.

RESULTS

The content of glutathione and activity of glutathione-metabolizing and antioxidant enzymes in benign and malignant tumors considerably differ from the control (Table 1). It should be emphasized that the severity of changes in malignant tumors was higher than in benign tumors.

Activities of GR, GST, and GSH-Px in benign tumors increased by 90-120, 85-100, and 100-150%, respectively, compared to the control. γ-GT activity in malignant and benign tumors increased to a different degree. Enzyme activity in tuberculoma 2.6-fold surpassed the control, while in hamartoma this parameter increased by 50%. Glutathione content in tuberculoma and hamartoma increased by 65 and 40%, respectively. Activity of antioxidant enzymes in tumor tissues only insignificantly changed. SOD activity insignificantly decreased in hamartoma and remained practically unchanged in tuberculoma. Catalase activity tended to increase in these tissues (particularly, in tuberculoma). Various changes in activity of antioxidant enzymes are probably related to different histological characteristics of tumors. Hamartoma is atypical hyaline cartilage surrounded by fatty and connective tissue layers. Tuberculoma consists of dense caseous structures separated by connective tissue, which attests to alternation of exacerbation and remissions [3].

TABLE 1. Glutathione Content and Activities of Glutathione-Metabolizing and Antioxidant Enzymes in Lung Tumors ($M\pm m$)

Parameter	Control	Tuberculoma	Hamartoma	Squamous cell carcinoma	Adeno-carcinoma	Bronchoalveolar carcinoma
GR, nmol NADPH/mg protein/min	0.869±0.194	1.924±0.271*	1.656±0.180*	2.618±0.252*	3.113±0.304*	2.255±0.367*
GST, nmol conjugated glutathione and 1-chloro-2,4-dinitrobenzene/mg protein/min	7.207±0.632	12.749±1.538*	13.374±1.314*	14.444±1.316*	16.133±1.692*	16.486±3.876*
GSH-Px, nmol NADPH/mg protein/min	15.152±1.498	38.702±4.642*	31.843±6.808*	42.312±4.360*	40.206±5.100*	32.310±4.745*
γ-GT, nmol γ-glutamyl-p-nitroanilide/mg protein/h	3.473±0.591	9.142±1.346*	5.123±1.323*	21.397±3.199*	45.941±4.317*	14.555±2.091*
Glutathione, nmol/mg protein	1.809±0.135	2.979±0.380*	2.540±0.310*	4.650±0.323*	5.166±0.630*	2.836±0.521*
SOD, U/mg	86.887±3.372	89.447±11.589	71.613±7.386	51.116±3.798*	51.989±4.350*	74.984±5.418
Catalase, mU/mg	3.673±0.322	5.149±1.575	4.141±1.907	2.373±0.448	1.449±0.320	2.468±0.602

In malignant tumors changes in activity of glutathione-metabolizing enzymes and glutathione content were more pronounced and also depended on individual characteristics of tumors.

Most pronounced changes in test parameters were observed in adenocarcinoma. This malignant tumor often metastasizes and is resistant to chemotherapy. Pathological changes were least pronounced in bronchoalveolar carcinoma highly sensitive to chemotherapy.

In squamous cell carcinoma, adenocarcinoma, and bronchoalveolar carcinoma we observed an increase in activities of GR (by 201.3, 258.2, and 159.5%, respectively), GST (by 100.4, 123.8, and 128.4%, respectively), and GSH-Px (by 179.2, 165.4, and 113.2%, respectively). γ-GT activity underwent most pronounced changes. In squamous cell carcinoma, adenocarcinoma, and bronchoalveolar carcinoma enzyme activity increased by 516.1, 1222.8, and 319.1%, respectively. The total content of glutathione in squamous cell carcinoma, adenocarcinoma, and bronchoalveolar carcinoma increased by 157.0, 182.8, and 56.8%, respectively, compared to the control.

SOD activity in squamous cell carcinoma and adenocarcinoma decreased by 41.2 and 40.2%, respectively. In these tissues catalase activity decreased by 35.4 and 60.6%, respectively. Catalase activity in bronchoalveolar carcinoma decreased, while SOD activity only tended to decrease.

When comparing changes in activity of glutathione-metabolizing enzymes in benign and malignant lung tumors we found that enzyme activities and glutathione content in tuberculoma and hamartoma only insignificantly increased compared to malignant tumors. This is probably associated with consumption of glutathione or constituent γ-glutamyl amino acids during benign tumor growth (similarly to cancer).

In rapidly growing malignant tumors we observed a marked increase in activity of glutathione-metabolizing enzymes. γ-GT activity increased most significantly in squamous cell carcinoma, adenocarcinoma, and bronchoalveolar carcinoma (by 6, 13, and 4 times, respectively). The increase in enzyme activities depended on tumor malignancy and its sensitivity to antitumor drugs.

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