



INVESTIGATION OF GLUTATHIONE LEVEL IN HOMOGENATES OF TUMOR AND NORMAL COLORECTAL TISSUES

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ABSTRACT

The aim of the current study is to determine the levels of reduced glutathione in homogenates from tumor and normal tissues obtained from 12 patients with colorectal cancers (CRC) and to compare them with histological and clinical tumor characteristics. The results showed a tendency for higher content of GSH in homogenates from normal mucosa compared to that from corresponding tumor tissues, however a considerable interindividual variations were found. Patients with advanced tumor stage and those who died before the end of the follow-up period had lower intracellular level of GSH in tumor tissue. Based on the results of our preliminary study we can propose that the intracellular GSH content in tumor tissue of primary CRC can have some prognostic significance for survival of the patients.

Key words: colorectal cancer, glutathione, homogenates, survival

INTRODUCTION

The development of resistance to chemotherapeutic agents is considered to be a major obstacle in clinical management of malignant tumor diseases. One of the mechanisms mediating such resistance is the altered activity of metabolizing and detoxifying enzymes, which protect the cells from the cytotoxic effect of antitumor drugs and from the reactive oxygen species (ROS) formed during their metabolism. This mechanism in a great deal is carried out by the glutathione/glutathione-S-transferase (GSH/GST) detoxification system [9].

The glutathione (GSH, γ -glutamyl cysteinylglycine) is the most abundant water-soluble small thiol molecule in the cells. The thiol group in the structure of GSH is a potent reducing agent, rendering GSH the

most important intracellular reductant [16].

In the cell GSH plays different roles due to his function as a co-factor of variety of enzymes. The GSH is the most important intracellular antioxidant. With the catalytic activity of glutathione peroxidases (GPXs) and glutathione-S-transferases (GSTs), the GSH play a role in the conversion of H_2O_2 to water and in the reduction of lipid hydroperoxides [5,15]. The GSH is involved in the metabolism of several xenobiotics, including carcinogens and anticancer drugs [14,15]. It can be conjugated to such electrophilic compound via GSTs, a reaction which increases their hydrophilic properties favoring the elimination of the xenobiotics [4]. In addition to detoxification, GSH plays a role in many other cellular reactions [8,13,16]

In the cells, glutathione can exist in either an oxidized (GSSG) or in reduced (GSH) state. The maintaining optimal GSH:GSSG ratios is critical to survival of the cells, since the deficiency of reduced glutathione put the cells in increased risk of damage because of oxidative stress. On the other hand, the high level of GSH in tumor cells may determine the development of resistance to chemotherapeutics metabolized via GST catalyzed conjugation with GSH. [9,14,15].

The aim of the current preliminary study is to determine and compare the levels of reduced glutathione in homogenates from tumor and normal tissues obtained from patients with colorectal cancers (CRC). This work is a part from larger prospective study focused on the prognostic role of GST-pi and GSH for the therapeutic response and survival of patients with CRC.

MATERIALS AND METHODS

Patients: The 5% homogenates in 0,1 M phosphate buffer, pH 6,5 were prepared from fresh tumor and normal colorectal tissue biopsies obtained from 12 patients with CRC. These patients had undergone tumor resections for adenocarcinoma of the colon and rectum at the II Surgical Clinic of University Hospital, Trakia University, Stara Zagora. Informed consent was obtained from all patients. The operations were carried out according to the accepted protocols in Bulgaria for surgical interventions and obtaining of human biopsy materials.

The patient population consisted of 6 males and 6 females, aged between 45 and 81 years (mean of $65,7 \pm 10$). Three of the patients suffered from rectal and 9 from colon cancer.

Determination of GSH content: The level of GSH was measured in 5% tissue homogenates containing 1 mM DTT according to the modified method of Ellman [3,11]. The method is based on the interaction of reduced glutathione with the reagent of Ellman [5,5'-dithiobis-(2-nitrobenzoic acid)] (DTNB), which creates, an intensive yellow color and can be measured spectrophotometrically at 412 nm. The concentration of GSH was determined with a standard curve and the content of GSH in the

homogenates was expressed nmol/mg protein. The concentration of the total protein in homogenates was measured with the adapted method of Lowry [7].

Statistical analyses: Statistical analyses were performed using StatView™ v.4.53. for Windows (Abacus Concepts, Inc). The descriptive statistical tests, the Student *t*-test, Mann-Whitney U test, pair *r*-test and correlation Fisher *r* to *z* test were applied. Factors with $p < 0.05$ were considered statistically significant,

RESULTS

The levels of GSH in the homogenates from tumor and normal colorectal tissues appeared to vary from 45,3 to 99,2 nmol/mg protein. There was a tendency for higher content of GSH in homogenates from normal mucosa compared to that from corresponding tumor tissues ($p=0.092$, pair *t*-test) (Figure 1), however a considerable interindividual variations were found (Figure 2)

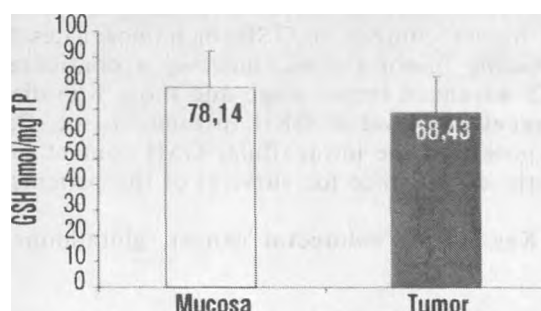


Figure 1. Comparison of GSH content in the homogenates from tumor and normal colorectal tissues from patients with CRC.

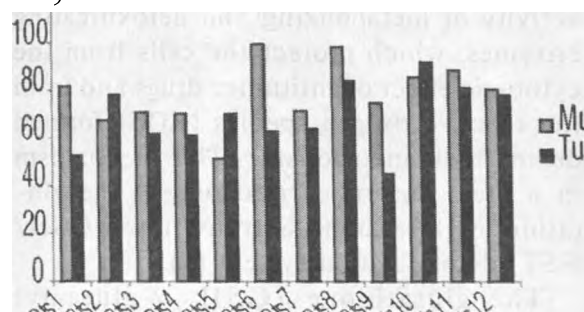


Figure 2. Interindividual differences of the GSH content in the homogenates from tumor and normal colorectal tissues from patients with CRC

The comparison of different histological tumor characteristics and of the clinical data of the patients with the level of GSH in the homogenates either from normal unaffected mucosa or from tumor tissues with did not showed significant associations. There was only observed a tendency for lower GSH content in tumors of patients with more advanced stage (IV) compared to those with stage II ($p=0.098$, II vs. IV) (Figure 3).

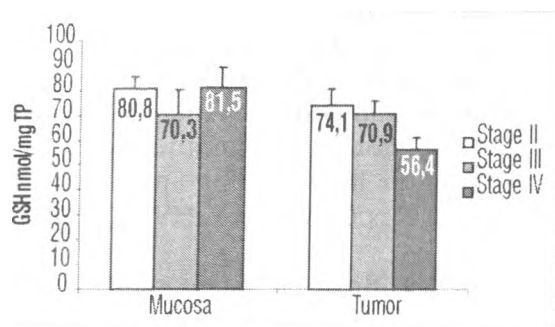


Figure 4. Association of the GST level in the homogenates from tumor and normal colorectal tissues with the tumor stage of the patients with CRC

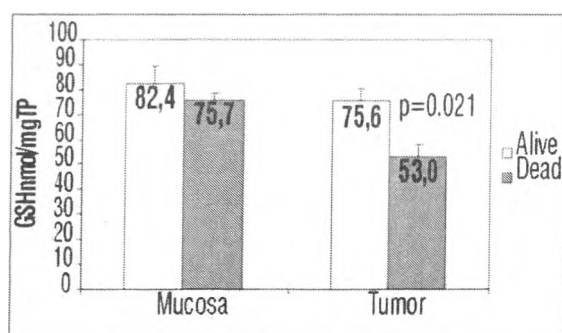


Figure 5. Association of the GST level in the homogenates from tumor and normal colorectal tissues with the survival of the patients with CRC

Moreover, we found significantly lower GST level in tumor homogenates of patients who had died before the end of the follow-up period compared to those who survived ($p = 0.021$, Student *t*-test, as well as $p=0.020$, Mann-Whitney U test) (Figure 4)

DISCUSSION

Colorectal cancer is an important example of the most spread carcinomas observed in humans. In spite of the abundance of research studies, the cellular mechanisms

of carcinogenesis, progression and therapeutic resistance of colorectal cancers has not been elucidated yet. However, there is considerable evidence that the GSH/GST detoxification system has an important role in these processes [9,14,15]. There are several studies focused on the level and role of GSH in colorectal tissue, but the results are very inconsistent. Investigators have reported increased [2,10], decreased [12] and unchanged [12] GSH levels in colorectal cancer tissue compared to the normal mucosa. In the current study we observed decreased level of GSH in the tumor tissue that suggest an impaired balance of intracellular thiol/disulfide homeostasis, probably because of increased oxidative product formation during the tumor cell metabolism and/or increased consumption of GSH in the processes of detoxification of electrophilic compounds.

According to our knowledge there are only few studies on the role of intracellular GSH as a biological marker for patient outcome. Most of these reports have linked raised intracellular levels of GSH with chemoresistance and worse prognosis [1,6]. The results of our preliminary study showed an opposite association: patients with lower intracellular GSH content in the tumor tissue had higher tumor progression and worse outcome at the end of the follow-up period. We suggest that the depletion of intracellular GSH could create an increase of oxidative stress in tumor cells and the enhanced concentration of ROS could induce further DNA damages and mutations in tumor cell leading to increased of tumor aggressiveness. Thus, we can propose that the decreased GSH content in tumor tissue of primary CRC can have some prognostic significance for survival of the patients. However, we do not also exclude the possibility that the discrepancy of our results with those of other studies could be attributed to the limited sample number in our current work.

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