Trakia Journal of Sciences, Vol. 3, No. 3, pp 39-44, 2005

Available online at:
http://www.uni-sz.bg

ISSN 1312-1723

Original Contribution

EXPRESSION OF GST-PI AND ITS IMPACT ON THE SURVIVAL OF COLORECTAL CANCER PATIENTS UNDERGOING CHEMOTHERAPY

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ABSTRACT

Resistance to chemotherapeutic agents is a problem facing the oncologist. The glutathione-S-transferase (GST) enzyme system has been implicated in the development of resistance to several anticancer drugs. Our present study is aimed at evaluating the expression of GST-pi in colorectal carcinoma and to determine its impact on the survival of patients treated with particular chemotherapy for colorectal cancer. Immunohistochemistry for GST-pi was performed on 67 biopsies obtained from patients with colon and rectal cancers. Seventeen of the biopsies (25%) appeared to have strong immunohistochemical signal for GST-pi, whereas the remaining 50 were negative (6, 9%) or with weak staining properties (44, 66%). Patients with strong GST-pi immune disposition appeared to have shorter survival after surgery compared to those negative for or with weak GST-pi staining (p=0.005, Log-rang test). Thirty (45%) of the patients received adjuvant mono- or polychemotherapy. Consequently, strong GST-pi expression produced unfavourable prognostic factor for both patients on adjuvant chemotherapy (p=0.031, Log-rank test) and without p=0.049, Log-rank test).
We propose here that strong GST-pi expression may lead to reduced efficacy of anticancer drugs or to inhibition of apoptosis, thus jeopardising survival of the patients.

Key word: colorectal carcinoma, GST-pi, chemotherapy, prognosis, immunohistochemistry.

INTRODUCTION

Primary colon and rectal cancers (CRC) are tumours that occur at high frequency in the United States and in all European countries, including Bulgaria (1-3) Each year more than 3000 new cases of colorectal cancers are diagnosed in Bulgaria. Despite the progress in early diagnosis and the improvement of treatment modalities, more than 2200 cancer-related deaths continue to occur each year (3).

Resistance to chemotherapeutic agents has been a formidable problem to the oncologist (4). Lately a growing amount of reports has been accumulated on drug metabolism and disposition and on the effect of adaptive xenobiotic-metabolising enzymes upon cytotoxicity of anticancer drugs (5, 6).

Some of the enzymes, involved in phase II of the biotransformation of xenobiotics are the isoenzymes of Glutathione-S-transferase (GST, EC. 2.5.1.18) (5, 6). GSTs are a large group of dimeric enzymes, playing an important role in cell defence system by catalysing the conjugation of reduced glutathione with a variety of endogenic and exogenic toxic electrophilic compounds, including several carcinogens and antineoplastics, (5, 6). So far in the human, 24 isoenzymes have been described, which are classified into 11 cytosolic, mitochondrial or microsomal subclasses GSTs: alpha (α), pi (π), mu (μ), theta (θ), kappa (κ), sigma (σ), zeta (ζ), omega (ω), and three groups microsomal MAPEG (5). The isoenzyme of class pi, GST-pi, is an acidic cytosolic protein, which possesses unique enzymatic properties: broad substrate specificity (e.g. alkylating antitumour agents, cisplatin derivatives), glutathione peroxidase activity towards lipid hydroperoxides, and high sensitivity to reactive oxygen species (ROS) (7-9). GST-pi acts also non-catalytically as intracellular binding protein for a large number of nonsubstrate molecules

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of either endogeneous or exogeneous origin, thus contributing to their intracellular transport, sequestration and disposition (5, 8,10). Besides that, GST-pi plays a regulatory role in the MAP kinase pathway that participates in cellular survival and death signals via physically protein:protein interaction with c-Jun-N-terminal Kinase 1 (JNK1) and Apoptosis Signal-regulating Kinase (Ask1) (5, 6, 11-13).

Therefore, the increased protein levels and activity of GST-pi found in a variety of neoplastic cancers with different histological origins, including colorectal carcinoma (4, 8, 14-16), are debated as factors responsible, at least partly, for the chemotherapy resistance observed in many cancers (6, 12, 13, 17, 18).

The aim of the current preliminary retrospective study was to assess by immunohistochemistry the expression of GST-pi in colorectal carcinoma and to elucidate its potential role as a prognostic factor in patients with adjuvant chemotherapy.

MATERIALS AND METHODS

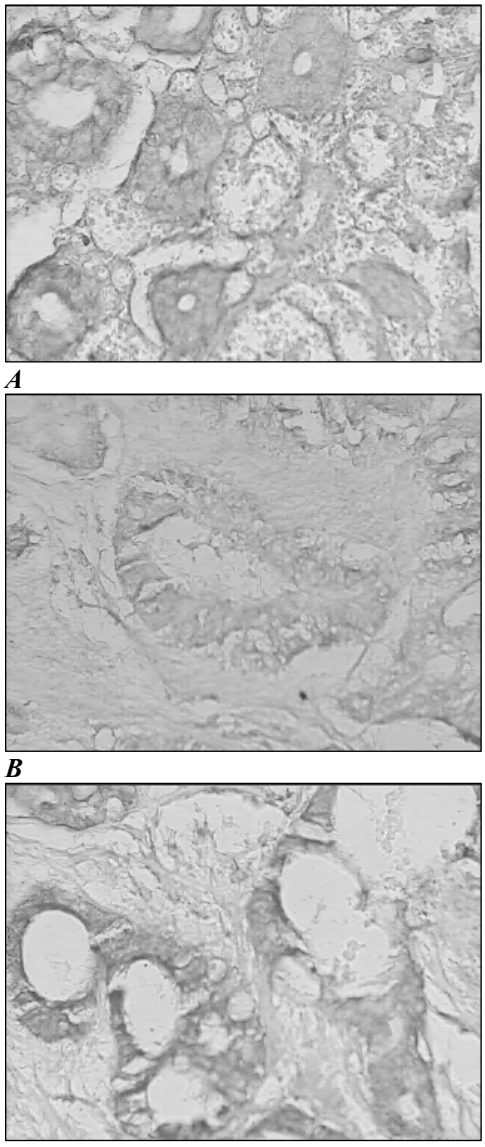
Sixty-seven patients with primary colorectal carcinoma were enrolled in the current study. These patients underwent tumour resection in the period of 2001-2003. All patients were followed up till 06.08.2004. The patients' clinical data and histological characteristics of the tumour biopsies are given on **Table 1**.

The level of expression of GST-pi was assessed by applying the standard streptavidin-biotin immunohistochemical method on formalin-fixed paraffin-embedded routinely processed tumour biopsy 10 μm slides as described earlier (19). The primary antibody used was mouse monoclonal anti-human GST-pi (*DAKO A/S, Denmark*). The detection system was StreptABComplex/HRP Duet, Mouse/Rabbit kit (*DAKO A/S, Denmark*) and the chromogen was 3'3'-diaminobenzidine (DAB).

For analysing the results we used a package of statistical programs StatView for Windows v.4.53 (*Abacus Consept Inc, USA*). There were applied the standard descriptive analyses, ANOVA test, $\chi 2$ test, Fisher exact test and Log-rank test

RESULTS

The immunohistochemical reaction for GST-pi was detected as brown staining in the cytoplasm of cells expressing this protein. In all tissue specimens containing both tumour and adjacent non-involved mucosa, the cytoplasmic reaction for GST-pi was observed in the cells of normal glands (**Figure 1A**).



A

B

C

Figure 1*. Intensive cytoplasmic reaction in the cells of normal colon glands. (**A**). Low (**B**) and strong expression of GST-pi (**C**) in tumour glands (x 400)*

Intensive immune staining was found in tumour infiltrating inflammatory cells. This reaction served for us as an internal positive control.

Table 1. *Clinical and histological data for the patients and their primary tumour biopsies.*

Parameters	Colon n=36 (%)	Rectum n=31 (%)	Total n=67 (%)
Clinical data			
Gender			
Male	*18 (50)*	*20 (65)*	*38 (57)*
Female	*18 (50)*	*11 (35)*	*29 (43)*
Age			
median (years)	*63.9*	*6.7*	*64.5*
(range)	*(40 –82)*	*(42 –76)*	*(40 – 82)*
Presence of metastases			
no metastases	*27 (75)*	*22 (71)*	*49 (73)*
in regional lymph nodes	*7 (19)*	*7 (23)*	*14 (21)*
in distant organs	*4 (11)*	*3 (10)*	*7 (10)*
pTNM staging			
I	*2 (6)*	*4 (13)*	*6 (9)*
IIA	*16 (44)*	*12 (39)*	*28 (42)*
IIB	*9 (25)*	*6 (19)*	*15 (22)*
IIIA	*1 (3)*	*2 (6)*	*3 (4)*
IIIB	*4 (11)*	*3 (10)*	*7 (11)*
IIIC	*0 (0)*	*1 (3)*	*1 (1)*
IV	*4 (11)*	*3 (10)*	*7 (11)*
Survivors at the end of follow-up			
alive	*25 (69)*	*22 (71)*	*47 (70)*
dead	*11 (31)*	*9 (29)*	*20 (30)*
Survival after the operation			
median (months)	*15.7*	*16.8*	*15.7*
(range)	*(1.2-43.0)*	*(1.4-43.5)*	*(1.2-43.5)*
Postoperative chemotherapy			
no chemotherapy	*20 (56)*	*17 (55)*	*37 (55)*
monochemotherapy (5FU/FA)	*11 (30)*	*10 (32)*	*21 (31)*
polychemotherapy (5FU/FA + camtothecin or mitomicin C or oxaliplatin)	*5 (14)*	*4 (13)*	*9 (14)*
Histological data			
Differentiation of the primary tumor			
low	*10 (28)*	*9 (29)*	*19 (28)*
moderate	*22 (61)*	*16 (52)*	*38 (57)*
high	*4 (11)*	*6 (19)*	*10 (15)*
Invasion in blood vessels			
no	*34 (94)*	*31 (100)*	*65 (97)*
yes	*2 (6)*	*0 (0)*	*2 (3)*
Invasion in lymph vessels			
no	*26 (72)*	*25 (81)*	*51 (76)*
yes	*10 (28)*	*6 (19)*	*16 (24)*
Perineural invasion			
no	*28 (78)*	*28 (90)*	*56 (84)*
yes	*8 (22)*	*3 (10)*	*11 (16)*
Infammatory infiltrate			
no (-)	*2 (6)*	*9 (29)*	*11 (17)*
weak (+)	*16 (44)*	*9 (29)*	*25 (37)*
strong (++)	*18 (50)*	*13 (42)*	*31 (46)*

The levels of immune reaction were very heterogeneous in the cytoplasm of tumour cells: 6 (9%) of the tumours were negative, 44 (66%) had weak staining (**Figure 1B**), 16 (24%) exhibited strong (**Figure 1C**), and 1 (1%) very strong immune reaction for GST-pi (**Figure 2).**

For further statistical analyses the biopsies and patients were dichotomised into a group without or with weak expression (-/+, n=50, 75%), and in a group with strong expression (++/+++, n=17, 25%).

The level of expression did not associate with any of the histological characteristics of the tumours: degree of differentiation (p=0.312, $\chi 2$ test), presence of invasion in blood vessels (p=0.062, Fisher exact test), in lymph vessels (p>0.999, Fisher exact test), perineural invasion (p=0.266, Fisher exact test), or with the level of inflammatory infiltrate (p=0.887, $\chi 2$ test).

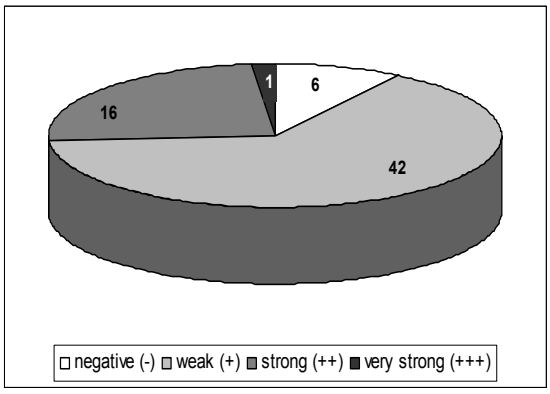


Figure 2. *Distribution of studied tumour biopsies according to the level of immune staining for GST-pi in the cytoplasm of tumour cells*

When the survival of the patients after the surgery was analysed according to the level of expression of GST-pi, a highly significant association was obtained (p=0.005, Log-rank test). The patients with strong expression of this xenobiotic-metabolising enzyme in the tumour tissues had significantly shorter survival after operation (mean of 14 months) in comparison with the patients without or with weak level of GST-pi (mean of 23.5 months) (**Fig. 3A**). This significant association persisted also after stratification for pTNM staging (stage I/II vs. Stage III/IV, p=0.008, Log-rank test).

Interesting observation was that the strong expression of GST-pi retained its impact as unfavourable prognostic factor either for the patients who received an adjuvant chemotherapy (p=0.031, Log-rank test) (**Fig. 3B**), or for those without such treatment p=0.049, Log-rank test) (**Figure 3C**).

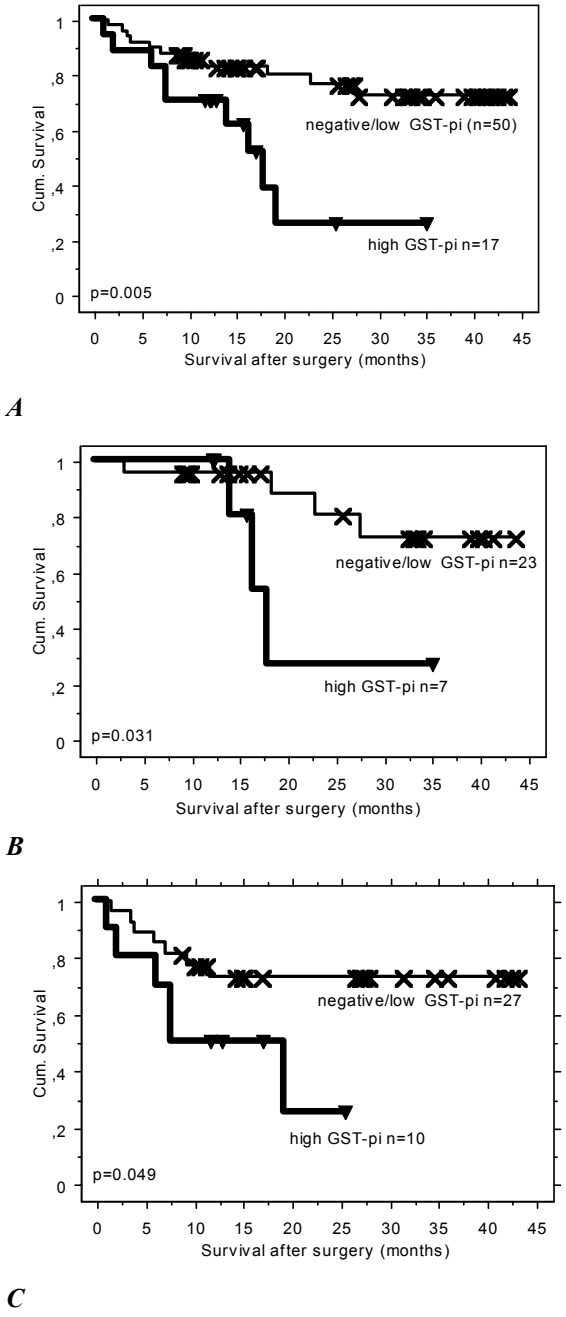


Figure 3. *Survival of the patient population with colorectal carcinoma after the surgical treatment for the primary tumours according to the level of expression of GST-pi in tumour cells (**A**). Survival of the patients subjected to adjuvant chemotherapy according to the GST-pi expression (**B**). Association between the level of expression of GST-pi and survival of the patients, who did not receive adjuvant chemotherapy (**C**)*

DISCUSSION

In the current study we found quite heterogeneous levels of expression of GST-pi in the cytoplasm of tumour cells of colorectal carcinoma, which could be due to different genetic or epigenetic factors. We suppose that such factors resulting in overproduction of the enzyme protein could be the reactive oxygen species, which are generated in high amount

during the metabolism of tumour cells. These ROS are found to induce the expression of the genes of GST-pi and other phase II xenobiotic-biotransforming enzymes (7, 13, 18). There is growing evidence that these genes have regulatory sequences recognised by Nrf2 transcription factor, which in turn is regulated by the antioxidant response element (ARE) (7, 13, 18). For the induction by ROS of genes coding GST-pi and other antioxidant enzymes another mechanism has been proposed, which implies participation of Zn (20).

Another factor, resulting in overproduction of GST-pi, could be the gene amplification. Such genetic change has been proved for squamous cell carcinoma of head and neck. *GST-pi* amplification has been shown to be a common event and proposed to be associated with cisplatin resistance and poor clinical outcome in head and neck cancer patients treated with cisplatin-based therapy (21, 22).

On the other hand, the lack of or the low expression of GST-pi could be due to the somatic inactivation by hypermethylation of promoter sequences of GST-pi gene. Such hypermethylation is the most common event (about 90%) described in prostate adenocarcinoma (23, 24).

An important result of our current study is the association found between the high expression level of GST-pi and the unfavourable prognosis of the patients with coloreactal carcinoma. This association was valid both for patients who had received adjuvant chemotherapy and for those without such treatment. We suppose that the shorter survival of the patients with higher GST-pi could be due to lowering of the effectiveness of administered antineoplastic agents. The high protein level of GST-pi could contribute to this process either via its direct detoxifying effect towards some of the drugs (oxaliplatin) (6, 18), or via the inhibitory effect of GST-pi on MAP kinase signal pathways of apoptosis, triggered by 5-FU, mitomycin C, camtothecin or other antitumour drugs included in mono- or polychemotherapeutic regiments (5,6,11,12).

The observed association of the high GST-pi level with the worse prognosis of the patients who did not received chemotherapy could also be explained by the ability of this enzyme protein physically to interact with and inhibit proteins involved in regulation of apoptosis (JNK1 and Ask1) (5,6,11,12). In tumours the high levels of free radicals, which in general are triggering factors and mediators of apoptosis, probably stimulate the expression of GST-pi that can lead to suppression of apoptosis. In turn, the decreased apoptosis can result in increase of tumour burden, which can negatively affect tumour survival.

In conclusion, we suggest that the expression level of GST-pi in primary tumours could be a valuable prognostic factor for patients with colorectal carcinoma both treated with adjuvant chemotherapy and those not subjected to such therapy. We consider that, for more accurate definition and proof of the role of GST-pi expression as prognostic and predictive marker, further expansion of the current study is needed and more thorough analyses of the therapeutic schemes and response should be performed.

ACKNOWLEDGEMENTS

This work was performed with the financial support of National Science Foundation of Bulgaria for the project VU-L-05/10.06.05.

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