

RESEARCH ARTICLE

GSTT1 is Deregulated in Left Colon Tumors

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Abstract

Our aim was to determine GSTT1 expression levels in left colon tumors and paired normal tissue in order to identify specific alterations in GSTT1 mRNA levels. Alterations in GSTT1 expression in twenty-four left-sided colon tumors and paired cancer free tissue were determined by qRT-PCR. Significant fold changes were determined with t-test. When compared with cancer free tissue, left colon cancers showed a significant decrease in GSTT1 expression. However, GSTT1 mRNA levels among different grades increased gradually in correlation with tumor grade. Our results suggest that downregulation of GSTT1 in left-sided colon cancers is an early event and is reversed with cancer progression, probably due to cellular defense mechanisms as a response to changes in the microenvironment.

Keywords: Deregulation - GSTT1 expression - left colon cancer

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Introduction

Colorectal cancer (CRC) is the third most common cancer for both genders worldwide (Ferlay et al., 2010). CRC are categorized as left, right sided and rectum tumors according to tumor location with distinct symptoms and genetic profiles (Glebov et al., 2003; Sugai et al., 2006). Even though environmental and demographic factors are the most prominent effects on CRC risk, there is also strong evidence for genetic susceptibility to CRC. While some of the inter individual differences in CRC susceptibility can be attributed to sequence variations in xenobiotic metabolizing genes including glutathione S-transferase theta-1 (GSTT1), altered expression levels as a result of epigenetic or transcriptional regulation also effect colorectal cancer susceptibility.

Glutathione S-transferases (GSTs) catalyze the conjugation of GSH with various electrophilic and hydrophobic xenobiotics including exogenous polycyclic aromatic hydrocarbons and endogenous hydroperoxides in order to facilitate their detoxification and excretion (Lee et al., 2004; Tunali et al., 2011; van Eijl et al., 2012). GSTs show differential expression dependent on the tissue type and even on different parts of the same tissue. Different expression levels of GST expression result in different exposure to carcinogens (Grootjans et al., 2010; Mannervik, 2012; Rotunno et al., 2012). In addition to that, studies conducted so far indicate that the rate of activation and deactivation of xenobiotics also effect carcinogenesis. The shift in the balance of activation/deactivation of

xenobiotics is a result of deregulated expression of various enzymes in addition to genomic variations resulting in non-functional proteins (Cotton et al., 2000). This process is particularly important in digestive and excretory system tissues which are directly exposed to carcinogens.

GSTT1 gene belongs to the theta family of GSTs. It is located on 22q11.23 and codes for a 239 amino acid long enzyme which is crucial for metabolism of various carcinogens (Singh et al., 2008). Its main function is to catalyze the conjugation of glutathione to hydrophobic electrophiles (Mainwaring et al., 1996). This gene is polymorphic for a very common deletion which has been investigated in relation to various cancers. Although studies conducted so far have conflicting findings, results in general indicate a significant increased risk for colorectal cancer, Caucasian population while East Asian populations appear to be minimally effected (Economopoulos et al., 2010; Xu et al., 2011).

A null GSTT1 phenotype clearly can lead to increased risk of cancer, for example in the esophagus (Yi and Li, 2012) and colon (Xu et al., 2011). In addition, deregulated GSTT1 expression has been observed in some cancers including bladder cancer, breast cancer and glioma (Dialyna et al., 2001; Diedrich et al., 2006; Ha et al., 2011; Chen et al., 2013). A previous study comparing GSTT1 protein levels in paired normal tissue and colorectal and gastric tumors has found a mild decrease in GSTT1 protein levels (de Bruin et al., 1999). Despite its significant effect on carcinogenesis there are few studies with conflicting results investigating the GSTT1 expression

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in CRC especially in case of left colon cancers. The aim of our study was to determine the mRNA expression levels of GSTT1 in left colon tumors and paired normal tissue in order to identify specific alterations in GSTT1 mRNA levels associated with different tumor grades and carcinogenesis. Hereby we report the deregulation of GSTT1 expression in left colon cancers.

Materials and Methods

Patients and tissues

Twenty-four patients with left-sided colon cancer were enrolled in our study. Patients consisted of 10 women and 14 men with a median age of 61 (Table 1). An informed consent form was obtained from each patient. Cancerous and normal tissue samples obtained during surgery were frozen immediately in liquid nitrogen and stored at -80°C until RNA isolation. Matched normal tissues were obtained from at least 2 cm away from cancerous tissue samples.

RNA extraction and RT-PCR

Total RNA was extracted using Ambion Purelink RNA extraction kit (Ambion) according to manufacturers instructions. Isolated RNA samples were quantified by NanoDrop[®] ND-1000 spectrophotometer (Thermo Fisher Scientific) and checked for integrity by running on a 1.5% gel. cDNA synthesis was performed using RevertAid First Strand cDNA Synthesis Kit (Fermentas). GSTT1 mRNA levels were quantified by qRT-PCR using Light Cycler 1.5 (Roche) and performing each reaction in triplicate. The β -Actin (ACTB) gene was used as housekeeping gene and Livak's delta delta Ct' ($\Delta\Delta\text{Ct}$) method was used to evaluate qRT-PCR results by using an Excel sheet.

Statistical analysis

Expression ratios were transformed into fold changes and reported as relative expression. Obtained fold changes were compared between different tumor grades in addition to normal tissue samples. Significant fold changes were determined with t-test using SPSS 17. An alpha of <0.05 was considered as statistically significant.

Results

Left colon cancers have shown a significant decrease in GSTT1 mRNA levels (Figure 1). Comparison of tumors according to their grade indicated to an increase in GSTT1 mRNA levels with correlation to tumor grade as shown in Figure 2. When compared with normal tissue, left colon tumors have exhibited a 0.3 fold decrease in GSTT1 expression ($p<0.005$). On the other hand GSTT1 mRNA

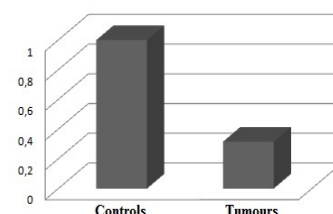


Figure 1. Relative Expression of GSTT1 in Controls and Tumours

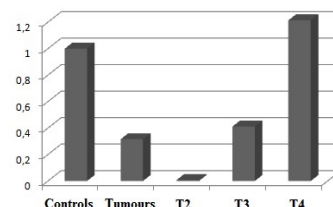


Figure 2. Relative Expression of GSTT1 in Grade T2, T3 and T4 Tumors in Addition to Normal Tissues

levels among different grades increased gradually from T2 to T4 (FC_{T_2} : 0,007, FC_{T_3} : 0,041, FC_{T_4} : 1, 214; p value for all comparisons <0.05).

Discussion

Our study demonstrates the downregulation of GSTT1 in left colon tumors. GSTT1 functions as a crucial part of xenobiotic metabolism and provides protection against carcinogenic compounds and oxidative stress (Hayes et al., 1995; Talalay et al., 2000). Because of this, downregulation of GSTT1 at the beginning of carcinogenesis and in early-stage tumors is expected since nearly all of the protective cellular mechanisms tend to diminish during the development of cancer. This loss of function can be a result of gross chromosomal alterations including deletions or epigenetic and transcriptional silencing. Although GSTT1 deletion is a common event (Zhao et al., 2009), our results indicate that loss of GSTT1 function substantiates at a transcriptional level in a portion of left colon cancers.

When we compared GSTT1 mRNA levels in samples significant overexpression was detected in correlation with increasing tumor grade. This marked change in the direction of deregulation can be attributed to specific conditions under which more invasive tumors have to survive. Invasive tumors are exposed to a more inimical microenvironment, especially in terms of increased exposure to oxidative stress due to enhanced proliferation and migration rate (Whiteside et al., 2008). These conditions require the activation of previously suppressed defense mechanisms including various phase II detoxification enzymes in addition to GSTT1 which known to be upregulated by oxidative stress (Ito et al., 2011). Therefore GSTT1 mRNA levels can be considered as a probable indicator of invasiveness in left colon tumors. In addition to that, overexpression of GSTT1 in high grade left colon tumors can generate a selective treatment option for high grade tumors by inducing cell death due to toxicity with the use of certain GSTT1 substrates which become more toxic following GSH conjugation (de Bruin et al., 1999).

Table 1. Patient Data

Age	Mean	60.2
	Median	61
Smoking status	None-smoker (%)	23
	Smoker (%)	77
Alcohol use	User (%)	10
	None-user (%)	90
Neoadjuvant therapy	Treated (N)	21
	None-treated (N)	3

The associations of both transcriptional and sequential alterations resulting in the loss of GSTT1 function with colorectal cancers have been the focus of numerous studies across various populations. A large part of these studies have reported conflicting findings. But none of the studies conducted so far have analyzed all means of GSTT1 silencing in conjunction with each other, including ours. More coherent results can be achieved when different mechanisms for the loss of GSTT1 function involving hypermethylation, deletion, transcriptional silencing, enhanced protein degradation and compartmentalisation, are investigated together.

Acknowledgements

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