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Expression of CYP and GST in human normal and colon tumor tissues

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ABSTRACT

We investigated the immunohistochemical staining characteristics of cytochrome P450 1A1 (CYP1A1), CYPB1, CYP2E1, and glutathione S-transferase P1 (GSTP1), GSTT1, GSTO1, GSTK1 in colon tumor and surrounding normal colon tissues. Tissues were obtained from 47 patients with colon adenocarcinoma and the staining intensity of tumor and control tissues was compared. CYP1A1, CYP1B1, CYP2E1, GSTP1, GSTT1, GSTO1 and GSTK1 expressions in colon cancer cells were significantly greater than those in normal colon epithelial cells. No significant relation was found between the isoenzyme expressions and age, gender, smoking status, tumor grade and tumor stage. The higher expressions of CYP1A1, CYP1B1, CYP2E1, GSTP1, GSTO1, GSTT1 and GSTK1 in tumor than in normal colon tissues may be important for colon cancer progression and development.

KEYWORDS

Adenocarcinoma; colon; cytochrome P450; glutathione-S-transferase; human; smoking status

Colon cancer is the most common malignant tumor of the gastrointestinal tract; it is a common cause of death. The colonic epithelium is in continuous contact with numerous ingested xenobiotics, especially alcohol and potential procarcinogens including heterocyclic amines, N-nitrosamines and polycyclic aromatic hydrocarbons (PAHs). These xenobiotics may be activated or detoxified by phase I and phase II biotransformation enzymes (Powis and Prough 1987; Le Blanc and Waxman 1989).

Cytochrome P450s (CYPs) are a superfamily of enzymes that play an important role in oxidation reactions. Although CYPs are found primarily in liver, some specific isoforms also are expressed in colon tissue. CYP1A1, CYP1B1, CYP2C, CYP2E1, CYP3A4 and CYP3A5 expression has been detected in human colon (Ding and Kaminsky 2003). The CYP1 family consists of three members, CYP1A1, CYP1A2 and CYP1B1. CYP1A1 and CYP1B1 are extrahepatic enzymes that are involved primarily in metabolic oxidation of procarcinogens to their electrophilic reactive intermediates. CYP1A1 and CYP1B1 are inducible enzymes, especially by PAHs, that are found in barbequed or grilled meat. Another member of the CYP family, CYP2E1, is an alcohol inducible enzyme that plays an important role in activation of several procarcinogens, such as nicotine-derived nitrosamine ketone (NNK) in tobacco smoke. CYP2E1 also produces reactive oxygen species by oxidizing ethanol to acetaldehyde via hydroxyl radicals (Lieber 1997). CYP2E1 is induced by high lipid diets and chronic alcohol intake (Murray 2006). On the other hand, CYP2E1 is inhibited by cruciferous vegetables that contain isothiocyanates (IARC 2004). CYPs are important not only for detoxification of xenobiotics, but also for activation of potential procarcinogens into toxic metabolites. Therefore, overexpression of CYPs is linked to the development of various types of tumors (Kapucuoglu et al. 2003; Androutsopoulos et al. 2013; Beyerle et al. 2015). Heterocyclic amines, N-nitrosamines and PAHs are activated by CYPs to form carcinogenic intermediates that are implicated in the etiology of colorectal cancer (Gonzalez and Gelboin 1994).

Glutathione S-transferases (GSTs) are a family of phase II enzymes that detoxify xenobiotics by catalyzing the conjugation of glutathione (GSH) to a variety of electrophilic carcinogenic compounds including active metabolites of PAHs in tobacco smoke and charcoaled meat. GSTs also participate in detoxification of chemotherapeutic agents used for cancer therapy (Hayes et al. 2005; Oakley 2011). GSTs are classified into eight iso-enzyme groups: alpha (A), mu (M), pi (P), theta (T), omega (O), kappa (K),

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zeta (Z) and sigma (S) (Hayes et al. 2005). GSTP1 and GSTT1 are required for deactivation of carcinogenic intermediates of PAHs. GSTP1 and GSTK1 are important for protecting cellular macromolecules against oxidative stress. GSTO1 is a potential reservoir of intracellular GSH, which protects cells against oxidative stress (Ada et al. 2013). Altered levels of GSTs in tumor tissues have been reported in colorectal cancer (Vlaykova et al. 2012). Also, specific GST isoenzymes are responsible for development of drug resistance of neoplastic cells (Naidu et al. 2003).

Differences in expression of CYPs and GSTs in colorectal tissues are important for cancer formation, differentiation and therapy. Chemicals derived from food also have been shown to alter the expressions of CYPs and GSTs (Murray 2006). In addition, CYPs and GSTs that are overexpressed in tumors may participate in activation or inactivation of chemotherapeutics and alter the efficacy or toxicity of many anticancer drugs used for treating colonic neoplasia (Kivisto et al. 1995; Patterson and Murray 2002; Bergheim et al. 2005). Therefore, differences in CYP and GST enzyme expression may affect prognosis and could be useful for developing individualized therapeutic strategies. CYPs have been investigated as possible prognostic markers for colon cancer (Kumarakulasingham et al. 2005).

The separate expression of CYPs and GSTs in human colorectal carcinoma and normal mucosa tissues has been reported (De Waziers et al. 1991; Peters et al. 1992; Chang et al. 2005); we have found only one report in which phase I and phase II isozymes were assessed in the same samples (McKay et al. 1993). Also, we have found few reports concerning differences in tissue expression of CYPs or GSTs between colon tumor and normal colon tissue in the same patient (Howie et al. 1990; Hengstlera et al. 1998; Gibson et al. 2003; Bergheim et al. 2005).

We investigated the expression levels of CYPs and GSTs in the same patients to obviate inter-individual differences of CYPs and GSTs protein expressions. To do this, we determined using immunohistochemistry the expression of CYP1A1, CYP1B1, CYP2E1, GSTP1, GSTT1, GSTK1 and GSTO1 isoenzymes in cancerous and normal samples from the same patients; we also investigated their relations to clinicopathological parameters including age, gender, smoking status, tumor grade and tumor stage.

Material and methods

Patients

We obtained institutional approval for our study. Archived colon adenocarcinoma and surrounding normal colon mucosa tissue blocks were obtained during surgery from 26 male and 21 female patients, 68.34 ± 12.72 years old, who were diagnosed at Kecioren Training and Research Hospital, Ankara, Turkey between 2009 and 2012. Questionnaires concerning tumor stage, tumor grade, age, gender and smoking status were completed for all patients (Table 1). We confirmed that none of the patients had received anticancer chemotherapy before surgery. Tumors were staged according to the American Joint Committee on Cancer (AJCC) Clinicopathological Staging System for colorectal cancer. Tumor stages were classified as stage 1 (pT1 and pT2, N0, M0; three samples), stage 2 (pT3 and pT4, N0, M0; 26 samples), stage 3 (pT3 and pT4, N1, N2, M1; 18 samples), lymph node negative, no metastasis (29 samples) or lymph node metastatis positive (18 samples). Tumor grades were classified as well differentiated (G1) (24 samples), moderately differentiated (G2) (16 samples) or poorly differentiated (G3) (seven samples).

Immunohistochemical staining

Immediately after removal of the biopsies, colon mucosa specimens were fixed overnight in 10% buffered formalin. The tissues were dehydrated through a series of graded ethanol baths, then embedded in paraffin. Sections were cut at 4 μm from the archived tissue blocks. One section was stained with hematoxylin and eosin (H & E) to observe tissue morphology and to score the tumor (Bancroft and Stevens 1995). For immunohistochemistry (Basak et al. 2016), endogenous peroxidase activity was blocked by incubating the sections in 1% hydrogen peroxide (v/v) in methanol for 10 min at room temperature. The sections subsequently were washed in distilled water for 5 min and antigen retrieval was performed for 3 min using 0.01 M citrate buffer, pH 6.0, in a domestic pressure cooker. The sections were transferred to 0.05 M Tris-HCl, pH 7.6, containing 0.15 M sodium chloride Tris buffered saline (TBS).

Table 1. Clinicopathologic characteristics of patients.

	n	%
Colon adenocarcinoma	47	100
Gender		
Male	26	55.3
Female	21	44.7
Age mean = 68.3. range = 45–90		
< 68	23	49
≥ 68	24	51
Tumor differentiation		
G1	24	51
G2	16	34
G3	7	15
Tumor stage		
Stage 1	3	6.4
Stage 2	26	55.3
Stage 3	18	38.3

n, number of patients; %, percent of patients; G1, well differentiated adenocarcinoma; G2, moderately differentiated adenocarcinoma; G3, poorly differentiated adenocarcinoma.

After washing in water, the sections were incubated at room temperature for 10 min with super block (SHP125; ScyTek Laboratories Inc., Logan, UT) to block nonspecific background staining. The sections then were covered with the primary antibodies diluted 1:250 for anti-GSTP1, 1:400 for anti-GSTK1, 1:500 for anti-GSTT1, 1:250 for anti-GSTO1, 1:50 for anti-CYP1A1, 1: 400 for anti-CYP1B1, 1:250 for anti-CYP2E1 in TBS at 4 °C overnight. Anti-GSTK1 (EPR1939) was obtained from Origene Technologies Inc., Rockville, MD; GSTO1 (ab88604) was obtained from Abcam Inc., Cambridge, MA; anti-CYP1A1 (sc-20,772) and anti-GSTP1 (sc-28,494) were obtained from Santa Cruz Biotechnology Inc., Dallas, TX; anti-CYP1B1 (sc-32,882) was obtained from Santa Cruz Biotechnology Inc.; anti-CYP2E1 (PA1116) was obtained from BOSTER Biological Technology, Ltd., Pleasanton, CA). After washing in TBS for 15 min, the sections were incubated at room temperature with biotinylated link antibody (SHP125; ScyTek Laboratories) followed by streptavidin/HRP complex (SHP125; ScyTek Laboratories). Diaminobenzidine was used to visualize peroxidase activity in the tissues. Nuclei were lightly counterstained with hematoxylin, then the sections were dehydrated through 95 and 100% ethanol, and cleared with xylene for 10 sec each. The sections then were mounted with coverslips. Both positive and negative controls were included with each run. Positive controls consisted of sections of gall bladder for CYP1A1 and skeletal muscle for CYP1B1, liver tissue for GSTK1 and GSTP1, lung for GSTT1 and CYP2E1, and colon for GSTO1. TBS was used instead of the primary antibody for negative controls.

Scoring of immunostained sections was performed at x 200 magnification by two qualified pathologists, who had no knowledge of the patients' clinical information. Distribution, localization and characteristics of immunostaining were recorded. For immunohistochemical evaluation, for a group of cells, the highest intensity of epithelial cells was recorded as the score for the group. The reaction product (brown color) of all proteins examined located in the cytoplasm and/or nuclei of epithelial cells in the mucosa of the colon was evaluated as positive staining. For each antibody, the intensity of the reaction was determined as: (-) negative, (1+) weak, (2+) moderate or (3+) strong (Gibson et al. 2003; Kumarakulasingham et al. 2005; Jankova et al. 2012).

Statistical analysis

MINITAB 14 statistical software (MINITAB® release 14.12.0; MINITAB Inc., State College, PA) was used for statistical evaluation. Expression differences of CYP and GST isoenzymes between tumor and normal tissues were examined using the Mann-Whitney U test and relations between CYP and GST isoenzyme expressions and the clinicopathological data for the patients (age, gender, smoking status, tumor grade and stage) were examined using the Spearman correlation rank test. Values for $p \leq 0.05$ were considered significant.

Results

Paired samples of colon adenocarcinoma and adjacent normal colon tissue were examined from 47 patients. Normal colon mucosa was characterized by low frequency of expression of CYP1A1, CYP1B1, CYP2E1, GSTP1, GSTT1, GSTO1 and GSTK1. By contrast, colon cancer cells exhibited a high frequency of expression of all CYP and GST proteins studied (Table 2). Among the proteins studied, GSTP1 expression was found in 46 (97.87%) and GSTK1 expression was found in 45 (95.74%) of tumor tissues from 47 patients (Table 2).

CYP1A1 expression was stronger in tumor epithelium than in normal epithelium in 32 samples (68.08%). Similarly, CYP1B1 and CYP2E1 expression was higher in tumor epithelium than in normal epithelium (63.83 and 55.32%, respectively) (Table 3). Stronger GSTP1 staining was observed in tumor epithelium than in normal epithelium; 78.72% of the tumors exhibited greater GSTP1 expression than normal colon tissue. GSTT1 (76.59%), GSTO1 (57.44%) and GSTK1 (76.59%) expression was stronger in tumor epithelium than in normal epithelium (Table 3).

Tumors and normal colon tissues were immunostained for CYP1A1, CYP1B1, CYP2E1, GSTP1, GSTT1, GSTO1 and GSTK1. Strong nuclear and cytoplasmic CYP1A1staining was observed in colon adenocarcinoma cells (Figure 1A) and weak expression was observed in normal epithelium (Figure 1B); 63.83% of tumors exhibited higher CYP1B1 expression compared to normal tissue (Table 3, Figure 1C, D). Similarly,

Table 2. The number and percentages of CYP and GST expressions in tumor and normal tissues.

	Tumor n/%	Normal n/%
CYP1A1	41/87.23	25/53.19
CYP1B1	40/85.10	40/85.10
CYP2E1	33/70.21	9/19.14
GSTP1	46/97.87	43/91.48
GSTT1	43/91.48	13/27.65
GSTO1	42/89.36	25/53.19
GSTK1	45/95.74	33/70.21

Total n = 47. n = number stained/% of total; staining scores were calculated based on the sum of the staining intensity of positively stained tumor and normal tissues.

Table 3. The number and percentages of patients with high CYP and GST expressions when matching tumorous tissues with normal tissues and matching normal tissue with tumor tissues.

	Tumor n/%	Normal n/%
CYP1A1	32/68.08	0/0
CYP1B1	30/63.83	0/0
CYP2E1	26/55.32	0/0
GSTP1	37/78.72	0/0
GSTT1	36/76.60	0/0
GSTO1	27/57.44	0/0
GSTK1	36/76.60	0/0

Total n = 47. n = number stained/% of total; staining scores were calculated based on the sum of the staining intensity of positively stained tumor and normal tissues.

55.32% of tumors exhibited higher CYP2E1 expression than normal tissue (Table 2, Figure 1E, F).

We found strong nuclear and cytoplasmic staining of GSP1 and GSTO1 in colon adenocarcinoma cells (Figure 2A–D). Similarly, GSTT1 and GSTK1 expression was higher in tumor epithelium than in normal epithelium (Figure 2E–H).

Expression of CYP1A1, CYP1B1 and CYP2E1 was significantly higher in tumors than in the adjacent normal tissue ($p < 0.05$) (Table 3). Tumor tissues exhibited significantly higher GSTP1, GSTT1, GSTO1 and GSTK1 expression compared to normal tissue ($p < 0.05$) (Table 3).

Expression of CYP1A1, CYP1B1 and CYP2E1 was high in tissues of well differentiated adenocarcinoma (WDCA) (G1) and moderately differentiated in adenocarcinoma (MDCA) (G2) compared to normal tissues ($p < 0.05$). Expression of CYP1A1, CYP1B1 and CYP2E1, however, was higher in tumor tissues of WDCA, MDCA and poorly differentiated adenocarcinoma (PDCA) than in normal tissues ($p < 0.05$) (Table 4). GSTP1 and GSTK1 expression was higher in tumor tissues of WDCA, MDAC and PDCA than in normal tissues ($p < 0.05$). GSTT1 and GSTO1

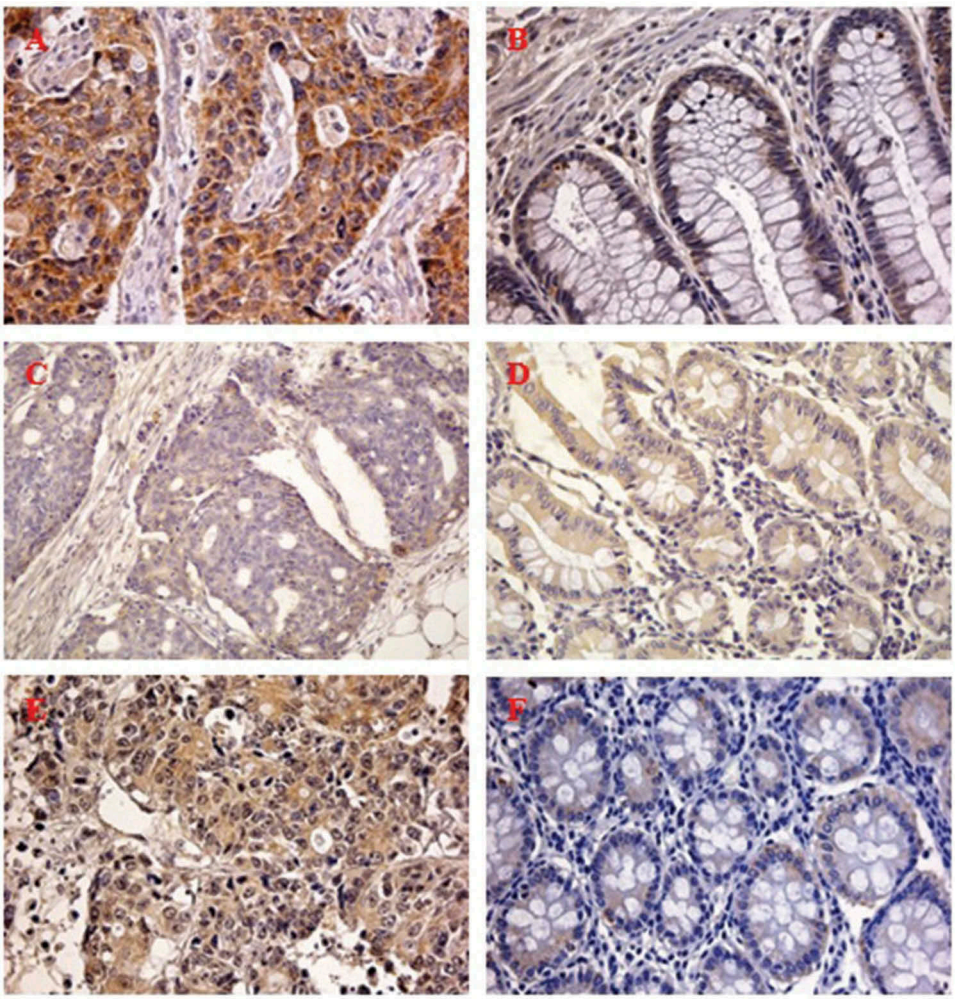

Figure 1. Immunohistochemical expression of CYP isoenzymes in patients with colon adenocarcinoma. A) CYP1A1 expression. x 400. B) CYP1A1 negative control for adenocarcinoma. x 400. C) CYP1B1 expression. x 200. D) CYP1B1 negative control for colon adenocarcinoma. x 400. E) CYP2E1 expression. x 400. F) CYP2E1 negative control for colon adenocarcinoma. x 400.

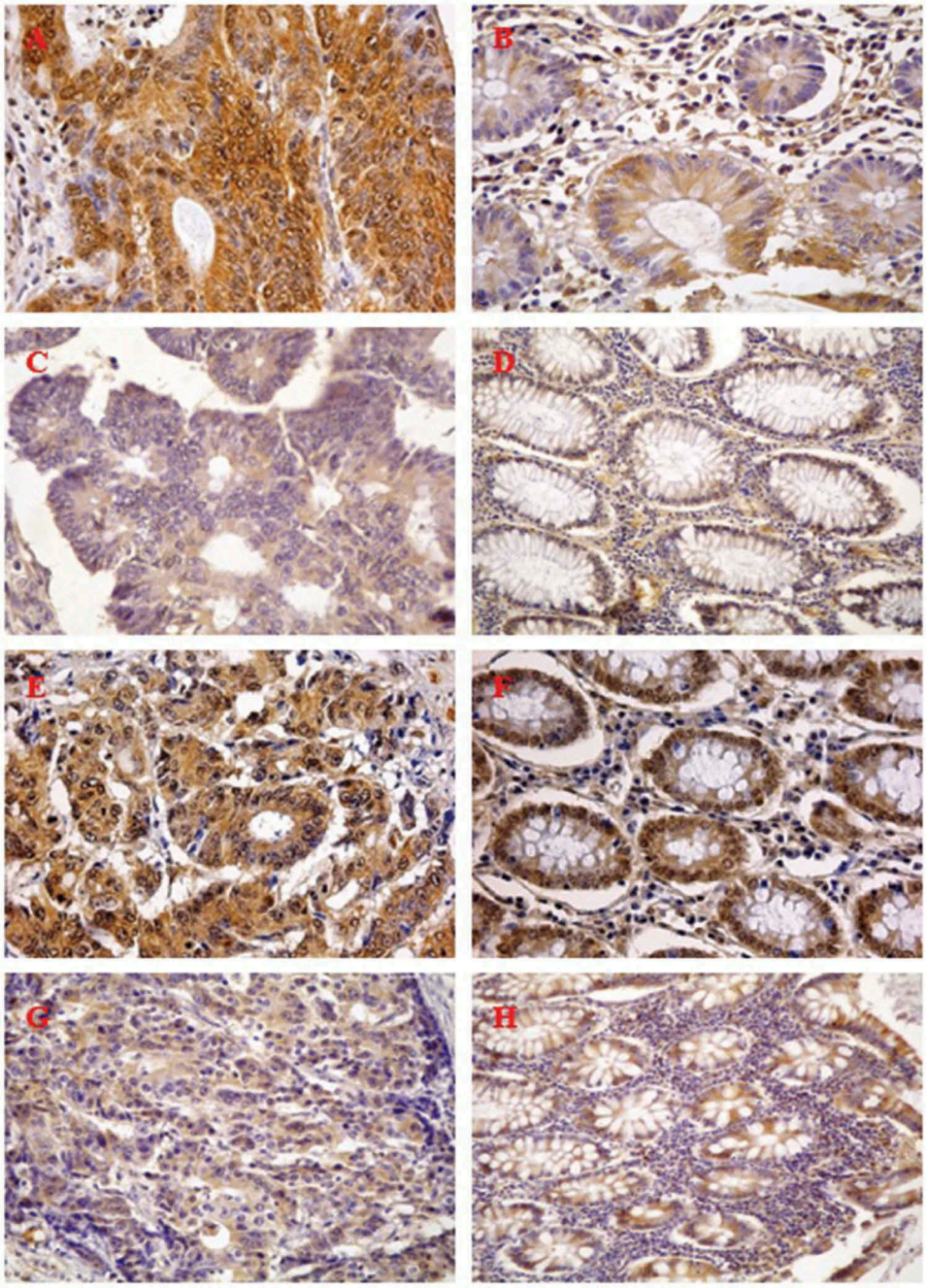

Figure 2. Immunohistochemical expression of GST isoenzymes in patients with colon adenocarcinoma. A) GSTP1 expression. x 400. B) GSTP1 negative control for colon adenocarcinoma. x 400. C) GSTO1 expression. x 400. D) GSTO1 negative control for colon adenocarcinoma. x 400. E) GSTT1 expression. x 400. F) GSTT1 negative control for colon adenocarcinoma. x 400. G) GSTK1 expression. x 400. H) GSTK1 negative control for colon adenocarcinoma. x 400.

expression, however, was higher in tumor tissues of MDCA and WDCA than in normal tissue ($p < 0.05$) (Table 5).

No statistically significant correlation was observed between the expressions of CYP1A1, CYP1B1, CYP2E1, GSTT1, GSTO1 and GSTK1, and the age, gender and smoking status of the patients. We found no significant correlation between CYP1B1, CYP1A1, CYP2E1, GSTT1, GSTO1, GSTP1 and GSTK1 expressions and tumor stage and tumor grade (Table 6).

Discussion

Ours is the first comprehensive description of tissue specific expression of both CYPs and GSTs in colon tumor tissue and normal colon tissue. We observed

Table 4. Protein expression differences of CYP proteins between tumors and normal tissues of patients with colon adenocarcinoma.

		CYP1A1			CYP1B1			CYP2E1		
	Total (n)	Tumor	Normal	T/N* **(*p* value)	Tumor	Normal	T/N (*p* value)	Tumor	Normal	T/N (*p* value)
Colon adenocarcinoma	47	1.47 ± 0.13[a] (0–3)[b]	0.53 ± 0.07 (0–1)	2.77 (0.0000)	1.92 ± 0.16 (0–3)	1.00 ± 0.08 (0–2)	1.92 (0.0000)	0.87 ± 0.09 (0–2)	0.19 ± 0.05 (0–1)	4.58 (0.0000)
G1	24	1.42 ± 0.20 (0–3)	0.42 ± 0.10 (0–1)	3.38 (0.0006)	1.83 ± 0.25 (0–3)	0.96 ± 0.13 (0–2)	1.91 (0.0109)	0.83 ± 0.14 (0–2)	0.25 ± 0.09 (0–1)	3.32 (0.0067)
G2	16	1.50 ± 0.20 (0–3)	0.75 ± 0.11 (0–1)	2.00 (0.0136)	2.00 ± 0.26 (0–3)	1.00 ± 0.13 (0–2)	2.00 (0.0088)	0.94 ± 0.17 (0–2)	0.13 ± 0.09 (0–1)	7.23 (0.0019)
G3	7	1.57 ± 0.20 (1–2)	0.43 ± 0.20 (0–1)	3.65 (0.0127)	2.00 ± 0.31 (1–3)	1.14 ± 0.14 (1–2)	1.75 (0.0639)	0.88 ± 026 (0–2)	0.14 ± 0.14 (0–1)	6.29 (0.0736)

[a]Mean ± SEM,[b]min.–max staining intensity.*Rate of tumor and normal.**$p < 0.05$. G1, well differentiated adenocarcinoma; G2, moderately differentiated adenocarcinoma; G3, poorly differentiated adenocarcinoma.

stronger staining intensity for all CYPs (CYP1A1, CYP1B1 and CYP2E1) and GSTs (GSTP1, GSTO1, GSTK1 and GSTT1) in tumor epithelium compared to normal epithelium. Because tumor cells overexpress certain proteins during malignant transformation (Korc et al. 1992), it is likely that the greater than normal expression levels of CYPs and GSTs in the tumor cells that we observed were a result of this transformation.

Earlier reports concerning CYP1A1 expression in human colon indicated no significant differences in CYP1A1 level between tumor and normal colon mucosa samples (De Waziers et al. 1991; Kumarakulasingham et al. 2005). We found that CYP1A1 expression was stronger in tumor epithelium than normal epithelium in the majority of the samples (68.08%).

Gibson et al. (2003) reported higher CYP1B1 expression in colon adenocarcinoma tissue than in adjacent normal mucosa by immunohistochemistry. McKay et al. (1993) reported high CYP1B1 expression in colon cancer, but none in normal colon. Kumarakulasingham et al. (2005) and Chang et al. (2005) reported that immunohistochemical staining of CYP1B1 was more intense in tumor tissue of colorectal carcinoma patients compared to normal colorectal samples from healthy individuals. Elevated expression of CYP1B1 in colon carcinoma is a consistent finding.

Bergheim et al. (2005) detected CYP2E1 protein in colonic adenoma tissues using western blot. These investigators reported no difference between the mean levels of CYP2E1 in normal and neoplastic tissues of patients with colon adenocarcinoma. We found a significant difference using immunohistochemistry, however, in CYP2E1 expression between normal and colon adenocarcinoma tissue.

GSTs are an important class of enzymes involved in the protection of cells from the toxic effects of reactive electrophiles. Both increased and reduced expression levels of specific GST isoenzymes in tumors, particularly in those that have become resistant to anti-cancer drugs, suggest a role for these proteins in development of resistance to chemotherapy. Determination of the GST isoenzyme profile of a cancer tissue could have value for prognosis and selection of treatment (Oguztuzun et al. 2009).

Kodate et al. (1986) investigated GSTP1 expression in normal and colon carcinoma tissue using immunohistochemistry. These investigators reported higher GSTP1 expression in colon carcinoma than in normal tissue. Similarly, Ranganathan and Tew (1991) reported that increased expression of GSTP in 21 of 30 carcinoma specimens. Howie et al. (1990) reported elevated levels of GSTP1 in colon tumor tissue compared to adjacent normal colon mucosa using immunohistochemistry. Similarly, Hengstlera et al. (1998) reported a nearly two-fold increase in expression of GSTP1 protein in colon cancer tissue compared to adjacent normal colon tissue by immunoblotting. Our findings concerning GSTP1 expression in colon tissue are consistent with previous studies.

Earlier reports indicated that the level of GSTP1 expression could be a prognostic factor for colon cancer (Sutoh et al. 2000, Tan et al. 2011). We have found no previous reports concerning the expression of GSTT1, GSTO1 and GSTK1 in colon cancer. An interesting finding of our study is the high expression of GSTP1, GSTK1, GSTT1 and GSTO1 in tumor tissues, which may, therefore, have potential prognostic value for colon cancer.

We found no significant differences in CYP and GST expressions related to patient age, gender, tumor stage status, which indicates that the expression differences of these isoenzymes between tumor and normal tissues do not depend on variables such as age, gender, tumor stage status of patients. Our findings suggest that higher expression of GST, CYP and especially CYP1A1, CYP1B1, CYP2E1, GSTP1, GSTO1, GSTK1 and GSTT1 can play a role in tumor growth and carcinogenesis of colon adenoarcinoma. We found a wide variability in CYP and GST protein expression in colon

Table 5. Protein expression differences of GST proteins between tumors and normal tissues of patients with colon adenocarcinoma.

		GSTP1			GSTT1			GSTO1			GSTK1		
	Total (n)	Tumor	Normal	T/N* *p* value**	Tumor	Normal	T/N *p* value	Tumor	Normal	T/N *p* value	Tumor	Normal	T/N *P* value
Colon adenocarcinoma	47	2.28 ± 0.10[a] (0–3)[b]	1.11 ± 0.08 (0–2)	2.05 0.0000	1.34 ± 0.12 (0–3)	0.28 ± 0.07 (0–1)	4.79 0.0000	1.34 ± 0.11 (0–3)	0.62 ± 0.094 (0–2)	2.16 0.0000	2.11 ± 0.12 (0–3)	1.00 ± 0.12 (0–3)	2.11 0.0000
G1	24	2.29 ± 0.17 (0–3)	1.00 ± 0.10 (0–2)	2.29 0.0000	1.21 ± 0.15 (0–3)	0.29 ± 0.09 (0–1)	4.17 0.0001	1.25 ± 0.16 (0–3)	0.63 ± 0.15 (0–2)	1.98 0.0126	2.25 ± 0.14 (1–3)	1.21 ± 0.16 (0–3)	1.86 0.0001
G2	16	2.19 ± 0.16 (1–3)	1.06 ± 0.11 (0–2)	2.07 0.0001	1.44 ± 0.18 (0–2)	0.19 ± 0.10 (0–1)	7.58 0.0001	1.56 ± 0.20 (0–3)	0.56 ± 0.16 (0–2)	2.79 0.0023	2.06 ± 0.25 (0–3)	0.88 ± 0.24 (0–3)	2.34 0.0044
G3	7	2.43 ± 0.20 (2–3)	1.58 ± 0.20 (1–2)	1.55 0.0409	1.57 ± 0.43 (0–3)	0.43 ± 0.20 (0–1)	3.65 0.0639	1.14 ± 0.14 (1–2)	0.71 ± 0.18 (0–1)	1.61 0.2502	1.71 ± 0.36 (0–2)	0.57 ± 0.20 (0–1)	3.00 0.0350

[a]Mean ± SEM, [b]min.–max staining intensity.*Rate of tumor and normal.**$p < 0.05$. G1, well differentiated adenocarcinoma; G2, moderately differentiated adenocarcinoma; G3, poorly differentiated adenocarcinoma.

Table 6. Relations between CYP and GST expressions and clinical data.

	Age	Gender	Smoking status	Tumor stage	Tumor grade
CYP1A1	0.090*	−0.212	0.122	−0.018	0.068
	0.548**	0.512	0.415	0.906	0.651
CYP1B1	0.211	−0.110	0.193	0.227	0.069
	0.154	0.463	0.194	0.060	0.547
CYP2E1	0.025	0.084	−0.144	0.068	0.036
	0.868	0.575	0.334	0.649	0.810
GSTP1	−0.001	−0.175	−0.170	−0.099	0.029
	0.995	0.395	0.255	0.508	0.846
GSTT1	0.025	−0.047	0.043	0.039	0.180
	0.869	0.755	0.776	0.792	0.227
GSTO1	−0.048	0.193	−0.030	0.088	0.031
	0.750	0.076	0.839	0.557	0.838
GSTK1	−0.009	−0.039	−0.234	−0.081	−0.218
	0.950	0.792	0.114	0.587	0.141

*Spearman's rank correlation (r ratio),**$p < 0.05$, (-), negative correlation

adenocarcinoma. Using our approach for larger trials may elucidate the roles of these proteins in carcinogenesis and identify potential targets for chemoprevention.

Disclosure statement

No potential conflict of interest was reported by the authors.

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