# Expression Patterns of Xenobiotic-Metabolizing Enzymes in Tumor and Adjacent Normal Mucosa Tissues among Patients with Colorectal Cancer: The ColoCare Study

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## ABSTRACT

**Background:** Xenobiotic-metabolizing enzymes (XME) play a critical role in the activation and detoxification of several carcinogens. However, the role of XMEs in colorectal carcinogenesis is unclear.

**Methods:** We investigated the expression of XMEs in human colorectal tissues among patients with stage I–IV colorectal cancer ($n = 71$) from the ColoCare Study. Transcriptomic profiling using paired colorectal tumor and adjacent normal mucosa tissues of XMEs (*GSTM1, GSTA1, UGT1A8, UGT1A10, CYP3A4, CYP2C9*, *GSTP1*, and *CYP2W1*) by RNA microarray was compared using Wilcoxon rank-sum tests. We assessed associations between clinicopathologic, dietary, and lifestyle factors and XME expression with linear regression models.

**Results:** *GSTM1, GSTA1, UGT1A8, UGT1A10*, and *CYP3A4* were all statistically significantly downregulated in colorectal tumor relative to normal mucosa tissues (all $P \leq 0.03$). Women had significantly higher expression of *GSTM1* in normal tissues compared with men ($\beta = 0.37$, $P = 0.02$). By tumor site, *CYP2C9* expression was lower in normal mucosa among patients with rectal cancer versus colon cancer cases ($\beta = -0.21$, $P = 0.0005$). Smokers demonstrated higher *CYP2C9* expression levels in normal mucosa ($\beta = 0.17$, $P = 0.02$) when compared with nonsmokers. Individuals who used NSAIDs had higher *GSTP1* tumor expression compared with non-NSAID users ($\beta = 0.17$, $P = 0.03$). Higher consumption of cooked vegetables (>1×/week) was associated with higher *CYP3A4* expression in colorectal tumor tissues ($\beta = 0.14$, $P = 0.007$).

**Conclusions:** XMEs have lower expression in colorectal tumor relative to normal mucosa tissues and may modify colorectal carcinogenesis via associations with clinicopathologic, lifestyle, and dietary factors.

**Impact:** Better understanding into the role of drug-metabolizing enzymes in colorectal cancer may reveal biological differences that contribute to cancer development, as well as treatment response, leading to clinical implications in colorectal cancer prevention and management.

## Introduction

Colorectal cancer is the third most commonly diagnosed cancer among men and second most commonly diagnosed cancer among women globally, with an estimated 1.1 million cases diagnosed in 2018 (1). Colorectal cancer is a multifactorial disease with complex etiology and is traditionally divided into sporadic and familial (hereditary) cases, with some overlapping of clinical features (2). Most commonly, colorectal cancer arises sporadically (>80%), and is influenced by environmental and/or lifestyle factors such as alcohol intake, obesity, physical inactivity, smoking, and consumption of processed and red meat (3, 4). The majority of exogenous compounds, such as polycyclic aromatic hydrocarbons (PAH) present in tobacco smoke or heterocyclic amines (HA) formed during high-temperature cooking of meat, require metabolic activation to become carcinogenic (5).

The gastrointestinal tract is the major path of entry for a wide variety of these compounds including food, orally administered

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drugs, and also compounds with neither nutritional nor other functional value, such as carcinogens. Variations exist between individuals as to how the metabolism of carcinogens may occur via the activation of xenobiotic-metabolizing enzymes (XME), including those of the cytochrome P450s (CYP; refs. 6–8), glutathione *S*-transferases (GST; refs. 9–11), and uridine 5′-diphospho-glucuronosyltransferases (UGT; refs. 12–14). Phase I XMEs (e.g., CYPs) activate procarcinogens, predominantly through oxidation, and convert them into reactive metabolites that react irreversibly with macromolecules to cause mutations and potentially carcinogenesis (8, 15). Phase II metabolic enzymes (e.g., GSTs and UGTs) tend to inactivate or detoxify these reactive metabolites—GSTs bioactivate vicinal dihalides and glucuronidation by UGTs is the prevailing conjugative pathway for the metabolism of xenobiotics (16–19). Alterations in this conjugative pathway, including genetic polymorphisms, have been shown to significantly influence responsiveness to drugs and drug-induced toxicities as well as cancer susceptibility (16, 19).

Aligned with the carcinogenic potential of these enzymes, previous studies have demonstrated that XMEs, including CYPs, GSTs, and UGTs, are commonly expressed in both normal and tumor tissues (14, 20–23). Extrahepatic tissues also demonstrate metabolic activity (e.g., colon, rectum, and kidney), prompting a need to better understand the role of XMEs in these tissues. Previous work by McKay and colleagues showed that XME expression was almost entirely confined, independent of stage of carcinogenic progression, to epithelial cells in normal colonic and colon carcinoma biopsy specimens using IHC staining (24). However, paired samples, clinicopathologic, dietary and lifestyle characteristics of patients, as well as mRNA expression of XMEs, have not been evaluated. Thus, we sought to investigate the role of XMEs in the colon and rectum by evaluating mRNA expression in paired tissues among patients with colorectal cancer enrolled in the prospective ColoCare Study.

Based upon the enzymatic activity of these genes toward carcinogens (25, 26), we profiled the expression of eight XMEs [cytochrome P450 family 2 subfamily C member 9 (*CYP2C9*), cytochrome P450 family 2 subfamily W member 1 (*CYP2W1*), cytochrome P450 family 3 subfamily A member 4 (*CYP3A4*), glutathione S-transferase alpha 1 (*GSTA1*), glutathione S-transferase mu 1 (*GSTM1*), glutathione S-transferase pi 1 (*GSTP1*), UDP glucuronosyltransferase family 1 member A8 (*UGT1A8*), and UDP glucuronosyltransferase family 1 member A10 (*UGT1A10*)], that are regulated by: antioxidants present in fruits and vegetables, PAHs present in tobacco smoke, and by HAs formed during high-temperature cooking of meat (ref. 25, 27–29; Supplementary Table S1).

## Materials and Methods

### Study design and study population

This study population includes patients from the international prospective ColoCare Study (Clinicaltrials.gov Identifier: NCT02328677). The primary purpose of the ColoCare Study, a cohort of men and women ages 18–89 years who were diagnosed with a primary invasive colorectal cancer (stage I–IV) undergoing surgery at clinics and sites internationally, is to investigate predictors of colorectal cancer survival, recurrence, treatment toxicities, and health-related quality of life (30–34). This study was approved by the Institutional Review Board of the Medical Faculty at the University of Heidelberg (Heidelberg, Germany), and written informed consent was obtained by all participants. This study included $n = 71$ patients with colorectal cancer, recruited between November 2010 and May 2014 at the Division of Preventive Oncology, National Center for Tumor Diseases in Heidelberg, (Heidelberg, Germany), with available paired colorectal tumor and normal mucosa tissue samples collected peri-operatively.

Electronic medical charts, including pathologic reports, were reviewed to document other clinical parameters. Height and weight were obtained from surgical anesthesia records; body mass index (BMI) was calculated as kg/m$^2$. Medical history, treatment information on neoadjuvant and adjuvant therapy, and tumor characteristics (e.g., tumor site and stage), were abstracted from medical records. Self-administered ColoCare and interview-based DACHS (Darmkrebs: Chancen der Verhütung durch Screening) questionnaires were used to collect data on a set of multiple exposures among study participants, as well as on a variety of potential colorectal cancer risk factors, including lifestyle characteristics (35). In this study, we evaluated: current alcohol consumption, current smoking status, recent use of NSAIDs, consumption of red and processed meat, and raw and cooked vegetable consumption (36). For each lifestyle and dietary factor, we analyzed contrasting gene expression levels of patients with colorectal cancer between high and low levels of exposure or dietary intake. The consumption of red meat, processed meat, and raw and cooked vegetables were categorized into two groups based on the empirical distributions in the population: low intake (patients who consumed one or fewer servings of meat or vegetables per week) or high intake (patients who consumed more than one serving per week) in the 12 months prior to colorectal cancer surgery. Cigarette smoking was categorized as: nonsmoker (never and former smoker who stopped smoking more than 2 years ago) or smoker (current smoker). Use of NSAIDs was categorized as either being a recent user (defined as use of anti-inflammatory drugs within the month prior to colorectal cancer surgery) or a nonuser. Mean daily alcohol intake (in the 12 months prior to colorectal cancer surgery) was calculated assuming an ethanol content of 4 g in 100 mL of beer, 8.6 g of ethanol in 100 mL of wine, and 33 g ethanol in 100 mL of spirit. Alcohol consumption was categorized as: nondrinker/light drinker (0–4.7 g/day) or moderate/heavy drinker (>4.7 g/day).

### Tissue collection

Colorectal tumor and adjacent healthy mucosal tissues ("normal," >10 cm from the primary tumor) from each patient were collected during primary tumor resection at the University Hospital Heidelberg following standardized protocols. Tissue samples were processed by the Tissue Bank of the National Center for Tumor Diseases (NCT, Heidelberg, Germany) in accordance with the regulations of the tissue bank and the approval of the ethics committee at the University of Heidelberg. Samples were placed into vials, snap-frozen in liquid nitrogen, and stored at −80°C until use. One sample aliquot was placed in 4% formalin fixative and sent to the Department of Pathology for histopathologic evaluation in each case.

### RNA extraction, gene expression measurements, and data preprocessing

RNA was isolated from 50 mg of tumor and adjacent normal tissue for $n = 71$ patients with colorectal cancer using QIAGEN AllPrep DNA/RNA Mini Kit according to the manufacturer's instructions. Total RNA integrity was assessed by Agilent Bioanalyzer and the RNA Integrity Number (RIN) was calculated; all RNA samples had a RIN > 6.0. Gene expression patterns were measured using Illumina HumanHT-12 Expression BeadChips, which includes *GSTP1, GSTA1,*

*GSTM1, UGT1A10, UGT1A8, CYP2W1, CYP2C9*, and *CYP3A4* transcripts (Supplementary Table S1).

While the *CYP1A* family is a major isoform that bioactivates PAHs, expression levels of enzymes of the CYP1A subfamily (*CYP1A1* and *CYP1A2*) were nearly undetectable in colorectal tissues in our previous work (37) such that expression of these genes was not considered for further evaluation in this study. Raw gene expression data were transformed using variance stabilization transformation and normalized using robust spline normalization (lumi package; ref. 38). Data were adjusted for possible batch effects using ComBat (sva package; ref. 39). All preprocessing steps were conducted using the statistical software R 3.1.0 (www.r-project.org).

### Statistical analysis

Standard descriptive methods were used to assess sociodemographic, lifestyle, and dietary factors of the study population. Continuous variables were reported as mean (SD) and categorical variables were reported as frequencies and percentages. Expression of genes linked to drug metabolism from colorectal tumor and normal mucosa tissues of patients with colorectal cancer was visualized with box plots. Expression differences between colorectal tumor and normal mucosa were tested by the paired Wilcoxon-Rank sum test.

For each gene (*GSTP1, GSTA1, GSTM1, UGT1A10, UGT1A8, CYP2W1, CYP2C9*, and *CYP3A4*), linear regression models were used to evaluate potential associations between sociodemographic, lifestyle, and dietary factors and the relative gene expression in colorectal tumor and normal mucosa tissue as continuous outcome variables, respectively. After evaluating univariate associations in unadjusted linear regression models, multivariable models were used to adjust for potential confounding variables, including age (years, continuous), sex (male and female), current smoker (yes and no), tumor site (colon and rectum), and neoadjuvant therapy (yes and no). Further potential confounding variables were selected separately for each gene by backward elimination using an alpha value of 0.1. The following covariates were included in the backward elimination: BMI (kg/m$^2$, continuous), alcohol intake (0–4.7 and >4.7 g/day), clinical stage (I–II and III–IV), regular NSAID use (yes and no), and consumption of red meat, processed meat, cooked vegetables, and raw vegetables ($\leq$1×/week and >1× week).

Because of our investigation of candidate genes and our specific hypothesis (Supplementary Table S1) partly based upon previously reported associations (25, 26), we presented nominal *P* values. However, also to address the potential impact of multiple comparisons, we applied the Benjamini–Hochberg method to FDR-adjusted *P* values (40) and also reported these adjusted *P* values. FDR-adjusted $P < 0.05$ (*q* value) was considered to be statistically significant. All statistical tests were two-sided, and $P < 0.05$ was considered to be statistically significant. Analyses were performed using SAS, version 9.4 (SAS Institute, Inc.).

Hierarchical clustered heatmaps of XME expression in the colorectum by metabolic enzyme phase (I vs. II) and tissue type (colorectal tumor vs. normal mucosa) with dietary patterns (consumption of red meat, processed meat, cooked vegetables, and raw vegetables) by patient with colorectal cancer were generated using Clustergrammer software (41). Hierarchical clustering was performed using the Scipy library in Python with cosine distance and average linkage.

**Table 1.** Baseline clinicodemographic, lifestyle, and pathologic characteristics of patients with colorectal cancer from the ColoCare study.

| Characteristic | *N* (%) |
|---|---|
| Total | 71 |
| Age at surgery | |
| <50 years | 8 (11.3) |
| 50+ years | 63 (88.7) |
| Mean, years (SD) | 63.9 (12.7) |
| Sex | |
| Female | 23 (32.4) |
| Male | 48 (67.6) |
| Race/ethnicity | |
| Caucasian | 71 (100.0) |
| BMI | |
| Underweight/normal weight (<25 kg/m$^2$) | 22 (31.0) |
| Overweight (25–29.99 kg/m$^2$) | 31 (43.7) |
| Obese (30+ kg/m$^2$) | 18 (25.4) |
| Mean, kg/m$^2$ (SD) | 27.4 (4.2) |
| Tumor site | |
| Colon | 38 (53.5) |
| Rectosigmoid junction and rectum | 33 (46.5) |
| Tumor stage | |
| Early stage (I/II) | 31 (43.7) |
| Late stage (III/IV) | 40 (56.3) |
| Smoking history | |
| Never/former smoker | 54 (76.1) |
| Current smoker | 11 (15.5) |
| Unknown | 6 (8.5) |
| Alcohol consumption[a] | |
| Nondrinker/light drinker (0–4.7 g/day) | 22 (31.0) |
| Moderate/heavy drinker (>4.7 g/day) | 41 (57.7) |
| Unknown | 8 (11.3) |
| NSAID use[b] | |
| None | 48 (67.6) |
| Yes | 17 (23.9) |
| Unknown | 6 (8.5) |
| Neoadjuvant therapy | |
| None | 59 (83.1) |
| Radiotherapy | 6 (8.5) |
| Chemotherapy | 2 (2.8) |
| Radiochemotherapy | 4 (5.6) |
| Adjuvant therapy | |
| None | 41 (57.7) |
| Chemotherapy | 27 (38.0) |
| Radiochemotherapy | 2 (2.8) |
| Unknown | 1 (1.4) |
| Red meat consumption[c] | |
| >1×/week | 47 (74.6) |
| $\leq$1×/week | 16 (25.4) |
| Processed meat consumption[c] | |
| >1×/week | 52 (82.5) |
| $\leq$1×/week | 11 (17.5) |
| Raw vegetables consumption[c] | |
| >1×/week | 51 (81.0) |
| $\leq$1×/week | 12 (19.0) |
| Cooked vegetables consumption[c] | |
| >1×/week | 56 (88.9) |
| $\leq$1×/week | 7 (11.1) |

[a]Alcohol consumption in the last 12 months.
[b]NSAID use in the last month.
[c]Eight patients had unknown information on dietary patterns.

# Results

## Patient characteristics

Seventy-one patients diagnosed with primary invasive colorectal cancer from the ColoCare Study comprise our cohort. Mean age at surgery among these individuals was 63.9 years, ranging from 27 to 85 years (**Table 1**). Over two-thirds of individuals were men (67.6%, 48/71 cases). The majority of our population presented with primary colorectal cancer in the colon (53.5%, 38/71 cases), and with late-stage (stage III–IV) disease (56.4%, 40/71 cases). A total of 85.9% of individuals did not undergo neoadjuvant therapy prior to surgery (**Table 1**). Evaluation of patient BMI revealed that 69.1% of individuals (49/71 cases) were classified as overweight or obese, with a mean BMI in this cohort of 27.4 kg/m$^2$ (SD 4.2). Investigation of other established colorectal cancer risk factors demonstrated that 16.9% (11/65 cases) were current smokers at diagnosis, 65.1% (41/63 cases) of individuals consumed greater than 4.7 g of alcohol daily, and 26.2% (17/65 cases) used NSAIDs within the month prior to colorectal cancer surgery (**Table 1**). Nearly three-quarters of patients consumed red meat more frequently than once per week (74.6%, 46/63 cases). In addition, over 80% of individuals consumed multiple servings of processed meat, raw, and cooked vegetables weekly.

## Expression of xenobiotic metabolism–related genes

Gene expression of eight xenobiotic metabolism–related enzymes was measured in paired colorectal normal mucosa and tumor tissues from patients with colorectal cancer. For each of the 71 individuals diagnosed with colorectal cancer, the relative expression of each XME gene was compared in paired normal and tumor tissues. As shown in **Fig. 1**; Supplementary Table S2, all XMEs evaluated, except *CYP2C9* ($P = 0.06$), showed statistically significant differences in expression between normal mucosa and colorectal tumor tissues ($P \leq 0.03$). *GSTM1, GSTA1, UGT1A8, UGT1A10*, and *CYP3A4* were significantly downregulated in colorectal tumor tissue as compared with tissues of the normal mucosa. In contrast, *GSTP1* and *CYP2W1* were observed to be upregulated in colorectal tumor relative to normal mucosal tissues. The largest difference in the expression between colorectal tumor and normal mucosal tissues was detected for *GSTP1* (mean effect 0.60, $P < 0.001$), whereas the smallest difference was present in *CYP3A4* gene expression (mean effect 1.09, $P = 0.03$).

## Associations of demographic, lifestyle, and dietary factors with the expression of drug metabolism–related genes

To evaluate what clinical- and lifestyle-related factors are associated with XME expression among patients with colorectal cancer, we performed multivariable adjusted regression analysis. Statistically significant estimates for each clinical- and lifestyle-related characteristic are individually reported in **Fig. 2**, and overall findings are summarized in **Fig. 3**.

## Sex-specific differences in colorectal expression of drug metabolism–related genes

Associations between demographic characteristics and XME expression revealed that women had significantly higher expression of *GSTM1* in normal mucosa tissues compared with men, although

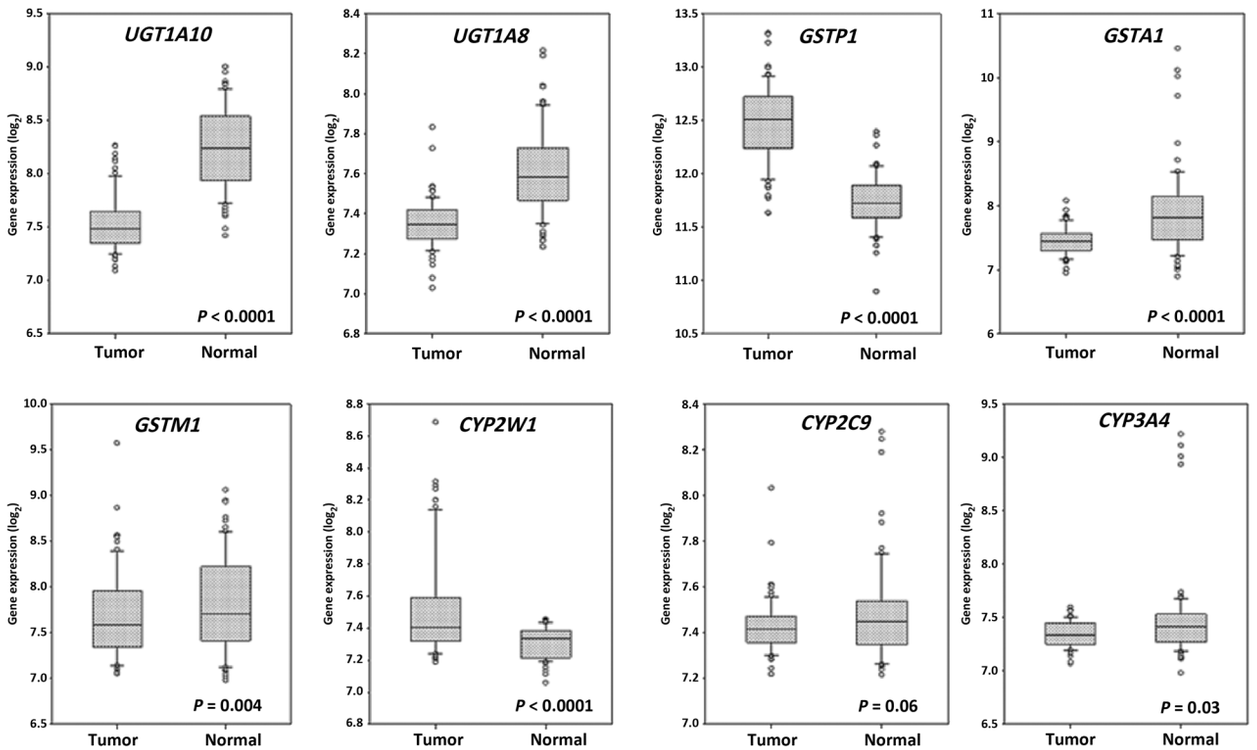


**Figure 1.**

Expression of genes linked to drug metabolism in tumor versus normal mucosa tissues from patients with colorectal cancer in the ColoCare Study. Boxes represent the interquartile range (IQR), and the line within the box represents the median gene expression for each tissue. Whiskers extend from the [75th percentile + (1.5 × IQR)], and from the [(25th percentile – (1.5 × IQR)]. Outliers are presented as dots. *P* values correspond to the Wilcoxon Rank sum test.

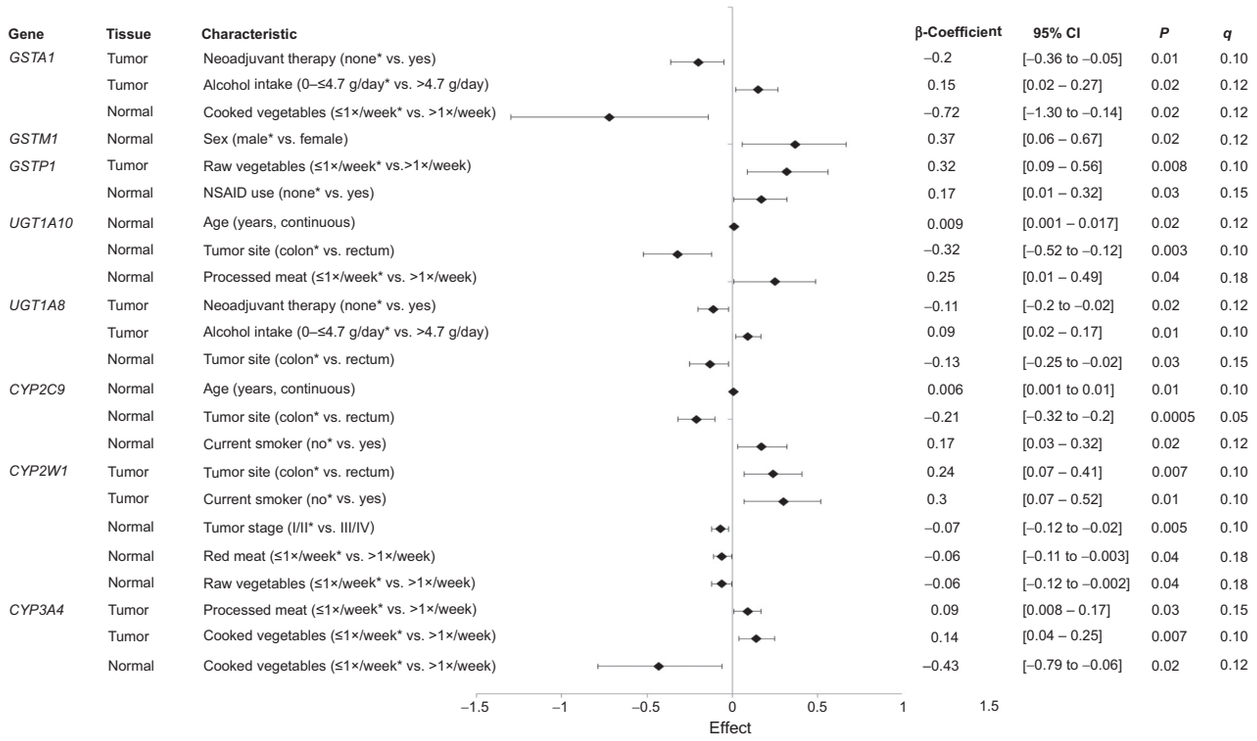


**Figure 2.**

Significant associations of clinicopathologic, dietary, and lifestyle factors with the expression of XMEs in colorectal tumor and normal mucosa tissues. Multivariable linear regression models (adjusted for age, sex, smoking status, neoadjuvant therapy, and tumor site) were used to calculate β-coefficients of estimates represented as black diamonds. Bars, 95% confidence intervals (CI). Further potential confounding variables were selected separately for each gene by backward elimination using an alpha value of 0.1. *, referent group characteristic. All data shown are nominally statistically significant with $P < 0.05$. *P*, *P* value; *q*, FDR-adjusted *q* value.

these findings did not persist after adjustment for multiple comparisons ($\beta = 0.37$; $P = 0.02$; $q = 0.12$; **Fig. 2**; Supplementary Tables S3–S10).

## Clinical patterns of XME expression in the colorectum

By clinical features, we observed that *CYP2C9* expression was significantly lower in normal mucosa among patients diagnosed with rectal cancer versus colon cancer ($\beta = -0.21$; $P = 0.0005$; $q = 0.05$). Similarly, patients diagnosed with rectal cancer also had lower normal mucosal tissue expression levels of *UGT1A10* ($\beta = -0.32$; $P = 0.003$; $q = 0.10$) and *UGT1A8* ($\beta = -0.13$; $P = 0.03$; $q = 0.15$) compared with patients with tumors in the colon (**Figs. 2** and **3**; Supplementary Tables S3–S10). In comparison, patients with rectal cancer also had higher *CYP2W1* expression detected in colorectal tumor tissues ($\beta = 0.24$; $P = 0.007$; $q = 0.10$). Patients diagnosed with late-stage disease (stage III–IV) were noted to have lower normal mucosal tissue expression of *CYP2W1* ($\beta = -0.07$; $P = 0.005$; $q = 0.10$) compared with patients diagnosed with early-stage (stage I–II) colorectal cancer (**Figs. 2** and **3**; Supplementary Tables S3–S10). We also observed that patients who underwent treatment (chemo- and/or radiotherapy) prior to surgery had lower colorectal tumor tissue expression of *GSTA1* ($\beta = -0.20$; $P = 0.01$; $q = 0.10$) and *UGT1A8* ($\beta = -0.11$; $P = 0.02$; $q = 0.12$) when compared with patients with no treatment prior to surgery (**Figs. 2** and **3**; Supplementary Tables S3–S10).

## Smoking history, NSAID use, and alcohol consumption on XME expression in colorectal tissues

Assessment of lifestyle-related colorectal cancer risk factors revealed associations with various XMEs in colorectal tumor and normal mucosa tissues. Smokers were found to have higher *CYP2W1* expression in tumor tissues ($\beta = 0.30$; $P = 0.01$; $q = 0.10$) and higher expression levels of *CYP2C9* in normal mucosa ($\beta = 0.17$; $P = 0.02$; $q = 0.12$), when compared with nonsmokers (**Figs. 2** and **3**). Alcohol consumption was associated with expression of *GSTA1* and *UGT1A8* in colorectal tumor tissues. Individuals who drank more than 4.7 g of alcohol daily had higher expression levels of these genes in colorectal tumor tissues ($\beta = 0.15$, $P = 0.02$, $q = 0.12$; and $\beta = 0.09$, $P = 0.01$, $q = 0.10$; respectively) relative to individuals who drank less than 4.7 g of alcohol every day. Individuals who used NSAIDs had higher *GSTP1* tumor expression compared with non-NSAID users ($\beta = 0.17$; $P = 0.03$; $q = 0.15$; **Figs. 2** and **3**; Supplementary Tables S3–S10).

## Clustering of drug metabolism–related genes with dietary patterns

To examine how the expression of XMEs in colorectal tumor and normal mucosa tissues clusters by dietary factors, hierarchical clustering is presented in Supplementary Fig. S1. Dietary consumption was associated with five XMEs (*CYP3A4, GSTA1, GSTP1, CYP2W1*, and *UGT1A10*) in normal mucosa and/or tumor tissues of the colon and

| Tissue | Gene | Age (years) | BMI (kg/m$^2$) | Sex (male* vs. female) | Tumor site (colon* vs. rectum) | Tumor stage (I/II* vs. III/IV) | Current smoker (no* vs. yes) | Alcohol intake (0–4.7 g/day* vs. >4.7 g/day) | NSAID use (none* vs. yes) | Neoadjuvant therapy (none* vs. yes) | Red meat (≤1×/week* vs. >1×/week) | Processed meat (≤1×/week* vs. >1×/week) | Raw vegetables (≤1×/week* vs. >1×/week) | Cooked vegetables (≤1×/week* vs. >1×/week) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Normal mucosa | *GSTA1* | – | | – | – | | – | | | – | | | | ↓ |
| | *GSTM1* | – | | ↑ | – | | – | | | – | | | | |
| | *GSTP1* | – | | – | – | | – | | ↑ | – | | | | |
| | *UGT1A10* | ↑ | | – | ↓ | – | – | | | – | – | ↑ | – | |
| | *UGT1A8* | – | | – | ↓ | | – | | | – | | – | | |
| | *CYP2C9* | ↑ | | – | ↓ | | ↑ | | | – | | | | – |
| | *CYP2W1* | – | – | – | – | ↓ | – | | | – | ↓ | | ↓ | |
| | *CYP3A4* | – | | – | – | | – | | | – | | | | ↓ |
| Colorectal tumor | *GSTA1* | – | | – | – | | – | ↑ | | ↓ | – | | | |
| | *GSTM1* | – | | – | – | | – | | | – | | | | |
| | *GSTP1* | – | | – | – | | – | | | – | | | ↑ | |
| | *UGT1A10* | – | | – | | | – | | | – | | | | |
| | *UGT1A8* | – | | – | – | | – | ↑ | – | ↓ | | | | |
| | *CYP2C9* | – | | – | – | | – | | | – | | | | |
| | *CYP2W1* | – | | – | ↑ | | ↑ | – | | – | – | | | |
| | *CYP3A4* | – | – | – | – | | – | | | – | | ↑ | | ↑ |

**Figure 3.**

Summary study findings for expression patterns of XMEs with clinicopathologic, dietary, and lifestyle factors in colorectal tumor and normal mucosa tissues among patients with colorectal cancer. *, referent group. Up arrows represent a β-coefficient greater than zero in the multivariable linear regression model with $P < 0.05$. Down arrows represent a β-coefficient less than zero in the multivariable linear regression model with $P < 0.05$. Dashes indicate an association that did not reach nominal statistical significance. Gray boxes indicate that the variable was not a significant covariate in the backward elimination for each individual XME.

rectum (**Figs. 2** and **3**). Higher consumption of red meat or raw vegetables (>1×/week) was significantly associated with lower *CYP2W1* expression in normal tissues ($\beta = -0.06$, $P = 0.04$, $q = 0.18$; and $\beta = -0.06$, $P = 0.04$, $q = 0.18$; respectively). Higher intake of raw vegetables weekly was also associated with higher tumor expression of *GSTP1* ($\beta = 0.32$; $P = 0.008$; $q = 0.10$; **Figs. 2** and **3**; Supplementary Tables S3–S10). Increased intake of processed meats was associated with higher *UGT1A10* expression in normal mucosal tissue ($\beta = 0.25$; $P = 0.04$; $q = 0.18$), as well as increased expression of *CYP3A4* in colorectal tumor tissue ($\beta = 0.09$; $P = 0.03$; $q = 0.15$).

The strongest dietary associations with XME gene expression were observed with the consumption of cooked vegetables (**Figs. 2** and **3**; Supplementary Tables S3–S10). Higher consumption of cooked vegetables (>1×/week) was found to be associated with lower *CYP3A4* and *GSTA1* expression in normal mucosa tissue ($\beta = -0.43$, $P = 0.02$, $q = 0.12$; and $\beta = -0.72$, $P = 0.02$, $q = 0.12$; respectively). In contrast, more frequent consumption of cooked vegetables was associated with higher *CYP3A4* expression in colorectal tumor tissues ($\beta = 0.14$; $P = 0.007$; $q = 0.10$). However, these findings did not remain significant after adjustment for multiple testing.

## Discussion

Our study of 71 patients diagnosed with primary invasive colorectal cancer from the ColoCare Study who underwent surgical resection of their tumors demonstrated that in colorectal tumor tissues, the expression of *GSTM1, GSTA1, UGT1A8, UGT1A10*, and *CYP3A4* were statistically significantly downregulated, while *GSTP1* and *CYP2W1* were statistically significantly upregulated, relative to adjacent normal mucosa tissues. Women had significantly higher expression of *GSTM1* in normal mucosa tissues compared with men. *CYP2C9* expression was lower in normal mucosa among patients with rectal cancer versus individuals diagnosed with colon cancer, and the expression of XMEs was associated with various colorectal cancer

lifestyle-related risk factors (alcohol intake, smoking status, NSAID use, and dietary consumption). These findings are novel given that our study is the first to define expression patterns of XMEs, and to identify associations between XMEs and clinicopathologic, demographic, lifestyle-, and dietary-related factors, using paired colorectal tumor and adjacent normal mucosa tissues from patients with colorectal cancer.

The colorectum is exposed to diverse compounds based on diet composition, where xenobiotics and endogenous compounds are absorbed from intestinal circulation, transported to the liver, and then returned to the intestine via bile ducts for reabsorption or metabolism by the gut microbiome (42). Glucuronidation by UGTs is not only the most prevalent conjugative pathway for the metabolism of xenobiotics, but also for endogenous compounds (17). CYPs tend to play both protective and activating roles in the metabolism of xenobiotics, and a defined physiologic role in the metabolism of endogenous substrates (43). Emerging evidence suggests that the human gut microbiome can impact activation of eukaryotic phase I and phase II XMEs via entero–endocrine cross-talk and interference with eukaryotic regulatory networks (44). Here, we observed the strongest dietary pattern—XME expression associations with the consumption of cooked vegetables, where more frequent consumption of cooked vegetables among patients with colorectal cancer was associated with lower *CYP3A4* expression in normal mucosa, and with higher *CYP3A4* expression in colorectal tumor tissues. We also noted that more frequent consumption (greater than once per week) of cooked vegetables was associated with lower expression levels of *GSTA1* in normal mucosa tissues. Our finding of lower *GSTA1* expression in normal mucosa tissues of patients diagnosed with colorectal cancer contrasts with previous work by Navarro and colleagues that reported cruciferous vegetable supplementation induced circulating expression of *GSTA1/2* among healthy individuals (45). Moreover, Tijhuis and colleagues previously reported that *GSTA1* and *GSTP1* genotypes may modulate the association between colorectal adenomas and cruciferous vegetable consumption (46), with polymorphisms in *GSTA1* also previously linked to risk of colorectal adenoma in a nested case–control study of the EPIC–Heidelberg cohort (47). Together, these findings emphasize the importance of identifying dysregulated XMEs in the colorectum, as well as other organs (e.g., liver) that impact colorectal carcinogenesis, to elucidate key regulators and mechanisms, including the xenobiotic–microbiome interactions, which could play a fundamental role in colorectal carcinogenesis.

Phase I XMEs (e.g., CYPs) can activate procarcinogens, and convert them into reactive metabolites that react irreversibly with macromolecules, such as proteins and DNA to cause mutations and potentially cancer. Consistent with the function of these enzymes, we observed that expression of CYPs, in particular *CYP2W1*, was higher in colorectal tumor relative to adjacent normal mucosa tissues among patients with colorectal cancer. The *CYP2W1* enzyme is one of the orphan cytochrome P450 genes and may play an important role in localized retinoid metabolism that may be intimately linked to its involvement in tumorigenesis (48). *CYP2W1* is known to activate procarcinogens, including aflatoxin B1, aromatic amines, and polycyclic aromatic hydrocarbon dihydrodiols. Indeed, previous studies have demonstrated high mRNA and protein expression levels of *CYP2W1* in colon and rectal tumors, or low *CYP2W1* expression in normal mucosa tissues (49–51). High CYP2W1 protein expression is associated with poorer survival outcomes among patients with colon cancer (49, 52), suggesting that *CYP2W1* could be a promising independent prognostic marker for colorectal cancer. For phase II enzymes, we report here that the expression of most GSTs and UGTs (*GSTM1, GSTA1, UGT1A8*, and *UGT1A10*) was significantly lower in colorectal tumor relative to normal mucosa tissues among patients with colorectal cancer. In contrast, we noted that *GSTP1* expression was significantly higher in colorectal tumor versus normal mucosa tissues among patients with colorectal cancer—aligned with previous studies that have reported significant increases in *GSTP1* mRNA and/or protein expression in colorectal cancer, as well as in aberrant crypt foci, precursor lesions of colorectal cancer (20–23, 53–55). By patient sex, we also found higher expression of *GSTM1* in normal tissues among women compared with men. Together, our findings are the first to define the expression patterns of these XMEs in paired colorectal tumor and normal mucosa tissues from patients with colorectal cancer, further implicating XMEs as key modulators of colorectal carcinogenesis.

The most abundant cytochrome P450 isoform of subfamily C that is expressed in the intestine, and is minorly involved in the metabolism of procarcinogens, including HA 2-amino-3,4-dimethylimidazo(4,5-f) quinolone and PAH dibenzo(a, h)anthracene, is *CYP2C9*. Tobacco smoke contains a variety of carcinogenic compounds such as PAHs, HAs, and nitrosamines that require metabolic activation via enzymatic pathways initiated by CYP enzymes, including *CYP2C9* (56–58). Variants of *CYP2C9* have been found to increase the risk of colorectal adenomas and modify associations with smoking among wild-type individuals (25, 37), and *CYP2C9* has been found to be significantly induced in tissues of the lung and larynx among smokers versus nonsmokers (58, 59). As such, a higher CYP expression may contribute to an increase in the activation of carcinogens that could lead to increased colorectal cancer risk among smokers. Aligned with these expression patterns, our evaluation of *CYP2C9* revealed that smokers had higher expression levels of *CYP2C9* in normal mucosa tissues compared with nonsmokers. We also observed that *CYP2C9* expression was lower in normal mucosa among patients with rectal cancer versus individuals diagnosed with colon cancer. These findings are aligned with previous studies that have also detected lower *CYP2C9* expression in healthy rectal versus colon biopsy samples (60, 61). Further studies that investigate the association between *CYP2C9* and smoking, as well as mechanisms underlying these procarcinogenic processes, are warranted.

XMEs provide a major route of detoxification of drugs, including cytotoxic therapies (62). The presence of CYPs in tumor cells is hypothesized to be part of a pleiotropic response to tumor development, as CYPs may either inactivate antitumor compounds or activate tumor-promoting compounds (62, 63). Indeed, studies have reported that taxanes can induce their own degradation through the induction of *CYP3A* (64), and that *UGT1A* can compromise cytotoxicity of anticancer agents by reducing its intracellular exposure and triggering the redox cycle to metabolic elimination in colon cancer cell lines (65). Here, we noted that patients with cytostatic chemotherapy and/or radiotherapy prior to colorectal cancer surgery had significantly lower colorectal tumor expression of *UGT1A8* compared with patients who did not undergo neoadjuvant therapy. Depending on the cytostatic therapy used, differences in the expression of drug-metabolizing enzymes in colorectal tumor versus normal mucosa tissue could compromise cytotoxicity of anticancer agents and lead to higher detoxification of cytostatic agents. These findings highlight the promising potential of the cellular pharmacokinetics by which XMEs could influence the side effects and therapeutic potential of cytotoxic drugs that are XME substrates.

This study has several strengths: our review of clinical records and baseline questionnaires to collect clinicodemographic data (e.g., BMI

and treatment regimens) and environmental information on lifestyle-related (e.g., smoking, alcohol, and NSAID use) and dietary-related (e.g., red meat consumption) colorectal cancer risk factors is a strength of this study, as it allowed for the comprehensive evaluation of associations with XME expression. Our selection of candidate genes was based on known biological roles of these XMEs in drug metabolism and review of the colorectal cancer literature (25, 27–29, 37). We acknowledge limitations of our study, including: the limited sample size that yielded insufficient power in our investigations to adjust for multiple testing, no available genotype data, and insufficient human tissues to examine protein levels or functional enzyme activity of XMEs. Furthermore, detailed information on dietary patterns prior to colorectal cancer surgery was not collected and could potentially influence the expression profile of XMEs in the colorectum, as well as other organs (e.g., liver) that impact colorectal carcinogenesis. The concordance of our findings with previous literature, and the evaluation of XME expression patterns from paired tumor and adjacent normal tissues together with clinicopathologic, dietary, and lifestyle characteristics of patients diagnosed with colorectal cancer, provided us with a unique study population to examine the role of XMEs in the colon and rectum.

Taken together, our findings demonstrate that XMEs have lower expression in colorectal tumor versus normal mucosa tissues. Furthermore, we demonstrate that associations of XMEs with environmental and clinicodemographic factors are critical to untangle the complex mechanisms underlying colorectal carcinogenesis. Identification of these patterns in our study provides novel insight into the role of XMEs in colorectal carcinogenesis and implicates key modulators in colorectal cancer development and progression. A better understanding into the role of drug-metabolizing enzymes in colorectal cancer may reveal some of the biological differences that contribute to cancer development, as well as treatment response, leading to clinical implications in colorectal cancer prevention and management.

## Disclosure of Potential Conflicts of Interest

The authors declare no conflict of interest with this work. C.M. Ulrich has, as cancer center director, oversight over research funded by several pharmaceutical companies but has not received funding directly herself.

## Disclaimer

The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.

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