

Cytochrome P450 Profile of Colorectal Cancer: Identification of Markers of Prognosis

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Abstract **Purpose:** The cytochromes P450 (P450) are a multigene family of enzymes with a central role in the oxidative metabolism of a wide range of xenobiotics, including anticancer drugs, carcinogens, and endogenous compounds. The purpose of this study was to define the P450 profile of colorectal cancer and establish the prognostic significance of expression of individual P450s in colorectal cancer.

Experimental Design: Immunohistochemistry for a panel of 23 P450s was done on a colorectal cancer tissue microarray consisting of 264 primary colorectal cancers, 91 lymph node metastasis, and 10 normal colorectal samples. The intensity of immunoreactivity in each sample was established by light microscopy.

Results: The most frequently expressed form of P450 in normal colon was CYP3A4. In primary colorectal cancer, several P450s (CYP1B1, CYP2S1, CYP2U1, CYP3A5, and CYP51) were present at a significantly higher level of intensity compared with normal colon. P450 expression was also detected in lymph node metastasis and the presence of several P450s (CYP1B1, CYP2A/2B, CYP2F1, CYP4V2, and CYP39) in the lymph node metastasis strongly correlated with their presence in corresponding primary tumors. The presence of strong CYP51 (log-rank = 12.11, $P = 0.0005$) or strong CYP2S1 (log-rank = 6.72, $P = 0.0095$) immunoreactivity were associated with poor prognosis. CYP51 was also an independent marker of prognosis ($P = 0.009$).

Conclusions: The expression of individual P450s has been established in colorectal cancer. Several P450s show increased expression in colorectal cancer. High expression of CYP51 or CYP2S1 were associated with poor prognosis and CYP51 is an independent marker of prognosis.

Colorectal cancer is one of the most common cancers in the Western world. The 5-year survival rate, although slowly improving, is still relatively poor at 40% (1). A significant proportion of patients present with locally advanced disease and current therapy for advanced colorectal cancer, which is based on a 5-fluorouracil regimen, results only in a modest improvement in survival (1, 2). Most large bowel cancers arise from adenomas and ~5% of these adenomatous polyps progress to malignant tumors within 5 to 10 years (3). Environmental factors and genetic susceptibility both make important contributions to the development of colorectal cancer (3, 4).

The cytochromes P450 (P450) are a multigene family of constitutive and inducible enzymes that have a central role in the oxidative metabolism of a wide range of xenobiotics and biologically active endogenous compounds (5, 6). The P450s

are classified into families and subfamilies based on nucleic acid homology; there are currently 57 known human P450s. Some have been very well characterized, whereas little is known about the biology of the more recently identified P450s. Individual P450s show cell type- and tissue-specific patterns of expression (7, 8).

The P450s have a major role in tumor development via their metabolism of many carcinogens (9). Compounds implicated in the etiology of colon cancer include polycyclic aromatic hydrocarbons and more especially heterocyclic amines, many of which require metabolic activation by P450s before exerting their genotoxic effect (10).

Specific P450s have also been shown to be expressed in tumors; in particular, CYP1B1 is overexpressed in a range of tumors (7, 11, 12). Because the P450s are involved in the oxidative metabolism (activation and deactivation) of many anticancer drugs, they are capable of influencing the response of tumors to anticancer therapy (12, 13). The outcome in terms of activation (i.e., cytotoxicity) or deactivation (i.e., resistance) is determinant upon the relative amount and activity of specific P450s in individual tumor cells (7).

Several therapeutic strategies are now being developed to exploit the presence, overexpression, and activity of P450s in tumors (14, 15). These approaches include P450 vaccines (16), P450-mediated prodrug activation (12, 17–19), and P450 inhibitors (20). The presence of CYP1B1 in tumors is currently being exploited as a tumor antigen. A phase 1 trial of a CYP1B1

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DNA vaccine has been successfully completed (16) and a phase 2 trial of this vaccine will begin patient recruitment shortly. A number of prodrugs designed to be selectively activated by P450 enzymes are also currently being evaluated (12, 14, 17, 19). The bioreductive prodrug AQ4N, a topoisomerase

inhibitor, is activated to the cytotoxic amine AQ4 by cytochrome P450-mediated bioreduction selectively under the hypoxic conditions found in tumor tissue. CYP3A4, CYP1A1, and CYP1B1 all contribute to bioreduction of AQ4N (17, 18). Several inhibitors of individual P450s are also

Table 1. Details of the cytochrome P450 antibodies

P450 antibody	Source	Type	Immunogen	Antigen retrieval* and antibody dilution for immunohistochemistry
CYP1A1	Chemicon	Polyclonal	Peptide, amino acid sequence not stated in datasheet	20 min, 1/1,000
CYP1B1	Own laboratory (23)	Monoclonal	Peptide, PENFDPARFLDKDGL (amino acids 437-451)	20 min, undiluted tissue culture supernatant
CYP2A6/2B6	Own laboratory	Monoclonal	C terminal peptide, RNYTMSFLPR (CYP2A6 sequence)	20 min, undiluted tissue culture supernatant
CYP2C8/9/19	Chemicon	Polyclonal	Peptide, amino acid sequence not stated in datasheet	No antigen retrieval, 1/500
CYP2D6	BD Bioscience	Monoclonal	Expressed human CYP2D6	No antigen retrieval, 1/20
CYP2E1	Oxford Biomedical Research	Polyclonal	Expressed human CYP2E1	20 min, 1/2,000
CYP2F1	Own laboratory	Polyclonal	COOH-terminal peptide, RPFQLCLRPR	20 min, 1/1,000
CYP2J2	Own laboratory	Polyclonal	COOH-terminal peptide, SHRLCAVPOV	20 min, 1/200
CYP2R1	Own laboratory	Polyclonal	COOH-terminal peptide, QPYLICAERR	20 min, 1/1,000
CYP2S1	Own laboratory	Polyclonal	COOH-terminal peptide, TDLHSTTQTR	20 min, 1/1,000
CYP2U1	Own laboratory	Polyclonal	COOH-terminal peptide, HPFNITISRR	20 min, 1/1,000
CYP3A4	Own laboratory (24)	Monoclonal	Purified human CYP3A4	20 min, undiluted tissue culture supernatant
CYP3A5	Own laboratory	Monoclonal	COOH-terminal peptide, DSRDGTLSGE	20 min, undiluted tissue culture supernatant
CYP3A7	Own laboratory	Monoclonal	COOH-terminal peptide, ESRDETVSGA	20 min, undiluted tissue culture supernatant
CYP3A43	Own laboratory	Polyclonal	COOH-terminal peptide, HLRDGITSGP	20 min, 1/1,000
CYP4F11	Own laboratory	Monoclonal	COOH-terminal peptide, RVEPLGANSQ	20 min, 1/10
CYP4V2	Own laboratory	Polyclonal	COOH-terminal peptide, KLKRRNAADER	20 min, 1/1,000
CYP4X1	Own laboratory	Polyclonal	COOH-terminal peptide, NGMYLHLKKL	20 min, 1/1,000
CYP4Z1	Own laboratory	Polyclonal	COOH-terminal peptide, NGIHVFHKVV	20 min, 1/1,000
CYP24	Own laboratory	Polyclonal	COOH-terminal peptide, RELPIAFCQR	20 min, 1/1,000
CYP26A1	Own laboratory	Monoclonal	COOH-terminal peptide, PARFTHFHGE	20 min, 1/10
CYP39	Own laboratory	Polyclonal	COOH-terminal peptide QCRIEYKQRI	20 min, 1/1,000
CYP51	Own laboratory	Polyclonal	COOH-terminal peptide CPVIRYKRRSK	20 min, 1/1,000

*The antigen retrieval step consisted of microwaving the sections in 0.01 mol/L citrate buffer (pH 6.0) for 20 minutes in an 800 W microwave oven operated at full power.

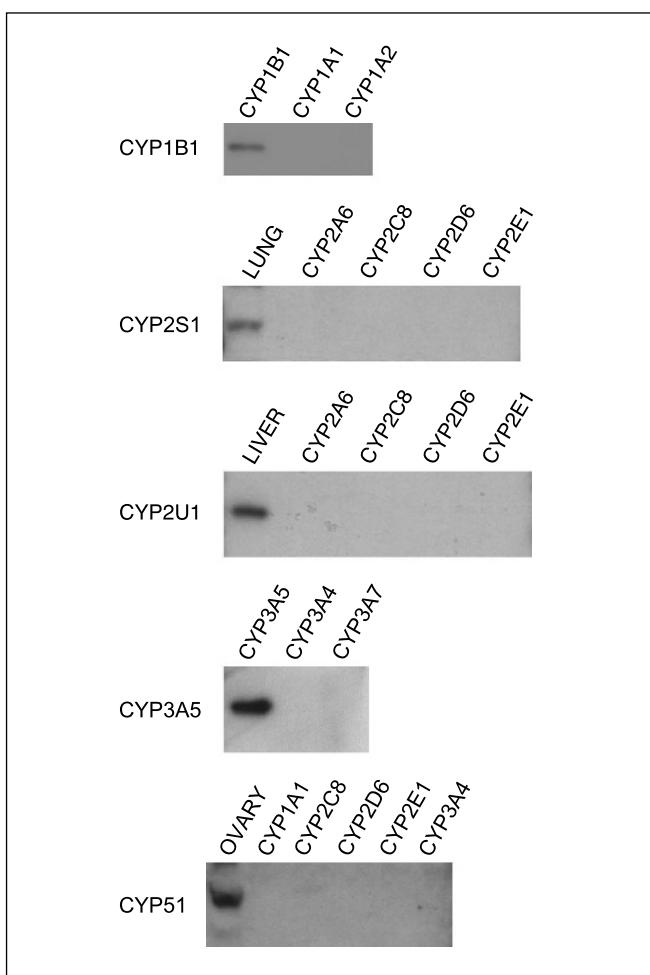


Fig. 1. Representative immunoblots for the P450 antibodies (*CYP1B1*, *CYP2S1*, *CYP2U1*, *CYP3A5*, and *CYP51*), which showed a significantly greater intensity of immunostaining in colorectal cancer. The specificity of *CYP1B1*, *CYP2S1*, *CYP2U1*, and *CYP3A5* was shown by immunoblotting against related P450 forms. Lung and liver were positive controls for *CYP2S1* and *CYP2U1*, respectively. Ovary was used as a positive control for *CYP51*, which was also immunoblotted against a range of expressed P450s.

currently in development (20) and AVI-4557, an antisense construct specifically targeted against *CYP3A4*, has recently completed a phase 1 study (21). Gene-directed prodrug therapy is also being used to deliver exogenous P450s (21, 22). The presence of other P450s that may interact with prodrug activation would, therefore, have important clinical implications.

In this study, we have conducted a comprehensive analysis of the expression of P450s in colorectal cancer and defined the expression profile of P450s in primary colorectal cancer, metastatic colorectal cancer, and normal colon. We have identified P450s that are overexpressed in colorectal cancer and those associated with poor prognosis.

Materials and Methods

Antibodies. A panel ($n = 23$) of P450 antibodies was used in this study. The development of monoclonal antibodies to *CYP1B1* (23) and *CYP3A4* (24) has been described previously. Polyclonal antibodies to the following P450s—*CYP2J2*, *CYP2R1*, *CYP2S1*, *CYP2U1*, *CYP3A43*,

CYP4V2, *CYP4X1*, *CYP24*, *CYP39*, and *CYP51*—were produced by immunizing rabbits with the relevant COOH-terminal peptide (Table 1) conjugated to ovalbumin. The use of COOH-terminal peptides as immunogens has been previously used to successfully develop antibodies to individual P450 forms (25). Animals received two booster immunizations at 4- to 6-week intervals after the initial immunization. Animals were bled 7 to 10 days after the last injection and serum obtained by centrifugation of the clotted blood. Monoclonal antibodies to *CYP2A6*, *CYP3A5*, *CYP3A7*, *CYP4F11*, and *CYP26A1* were produced as previously described (23, 24). In each case, the appropriate COOH-terminal peptide conjugated to ovalbumin was the immunogen. Briefly, mice were immunized with the relevant peptide conjugate and received booster immunizations. Spleens from mice that showed the highest antibody titers as assessed by ELISA using the peptide immunogen were fused with myeloma cells. After cloning of the hybridomas, antibody titers were again assessed by ELISA.

The specificity of the antibodies was confirmed by immunoblotting (23) against microsomes prepared from appropriate human tissues (Fig. 1). Some antibodies were also immunoblotted against the relevant expressed P450. Expressed human P450s (*CYP1A1*, *CYP1A2*, *CYP1B1*, *CYP2A6*, *CYP2B6*, *CYP2C8*, *CYP2D6*, *CYP2E1*, *CYP3A4*, *CYP3A5*, *CYP3A7*, and *CYP4A11*) were purchased from BD Biosciences (Bedford, MA). The *CYP2A* antibody recognized both *CYP2A6* and *CYP2B6*, reflecting the very close sequence similarity of these two P450s and in particular the almost identical COOH-terminal amino acid sequences of these P450s. Therefore, this antibody has been designated *CYP2A/CYP2B*. All the antibodies described above are available from Aviuation, Ltd. (Aberdeen, United Kingdom).

Polyclonal antibodies to *CYP1A1* and *CYP2C* were purchased from Chemicon Europe (Chandlers Ford, United Kingdom), whereas a monoclonal antibody to *CYP2D6* was bought from BD Biosciences (Oxford, United Kingdom) and a polyclonal antibody to *CYP2E1* was obtained from Oxford Biomedical Research (Oxford, MI).

Tumor samples. This project had the permission of the Grampian Research Ethics Committee. There were 264 patients in the study (26) and the cases were selected from the Aberdeen colorectal tumor bank. All the patients had a diagnosis of primary colorectal cancer

Table 2. Clinicopathologic characteristics of the patients in this study

Gender	
Male	51.9% (137)
Female	48.1% (127)
Mean age (range)	69 (33–92)
≤70	51.1% (135)
>70	48.9% (129)
Tumor site	
Proximal	34.5% (91)
Distal	32.2% (85)
Rectum	33.3% (88)
Tumor differentiation	
Well	4.2% (11)
Moderate	85.6% (226)
Poor	10.2% (27)
MSI status	
Low (intact)	82.6% (218)
High (defective)	17.4% (46)
Stage	
Dukes A (stage I)	25.4% (67)
Dukes B (stage II)	239.4% (104)
Dukes C (stage III)	235.2% (93)

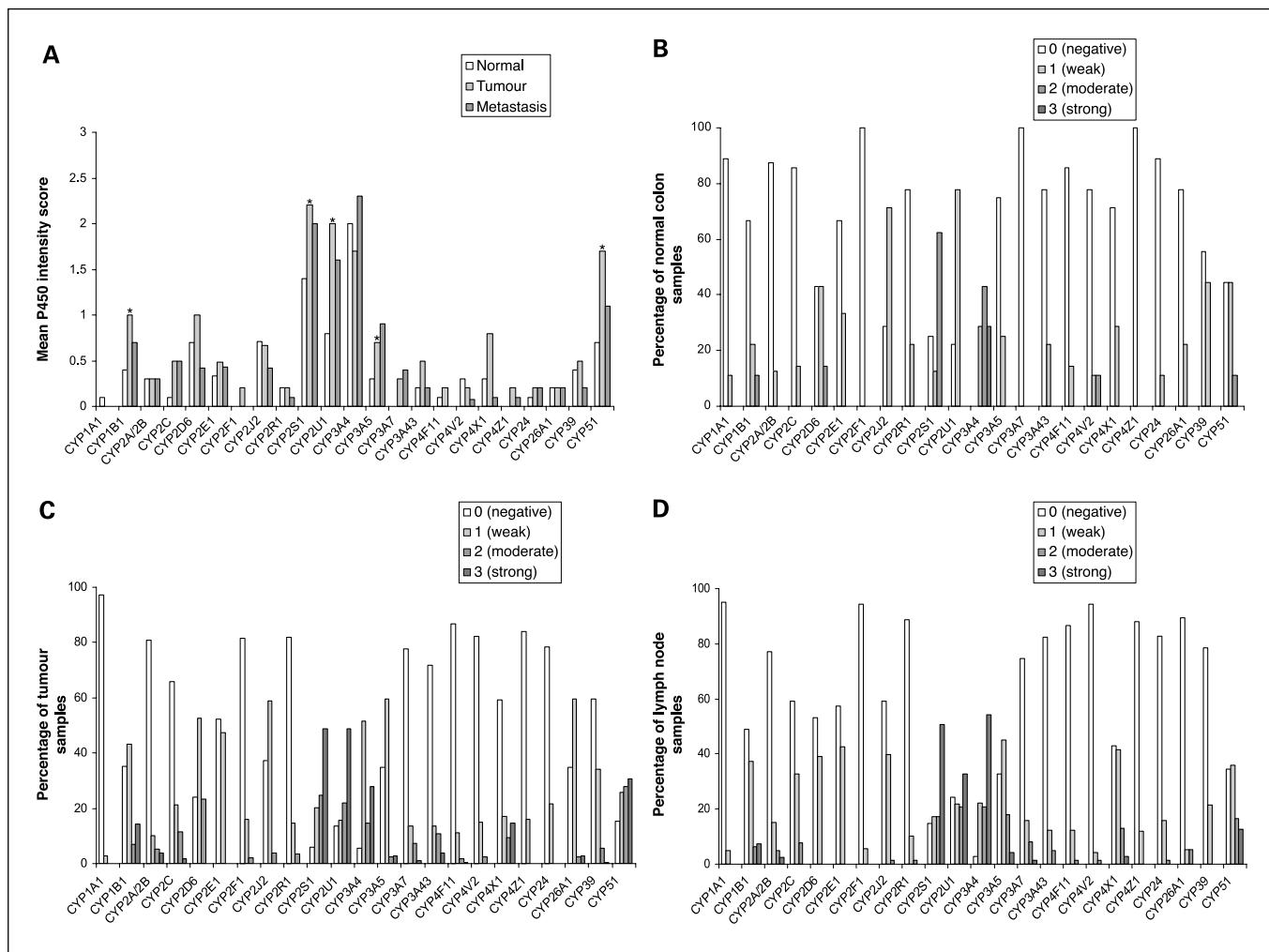


Fig. 2. A, mean cytochrome P450 intensity scores in normal colon, colon cancer, and lymph node metastasis. P450s that showed a significantly greater intensity of immunohistochemical staining in colorectal cancer compared with normal colon are marked with an asterisk (*CYP1B1*, *CYP2S1*, *CYP2U1*, *CYP3A5*, and *CYP51*). Frequency distribution (percentage) of the intensity of individual P450s in normal colon (B), colorectal cancer (C), and lymph node metastasis (D).

and had undergone elective surgery for colorectal cancer in Aberdeen between 1994 and 2003. The tumor samples had been submitted to the Department of Pathology, University of Aberdeen, for diagnosis. The tumor excision specimens were fixed in formalin, representative blocks were embedded in wax, and sections were stained with H&E. The clinicopathologic characteristics [age, gender, site of primary tumor, degree of primary tumor differentiation, microsatellite instability (MSI) status, and Dukes stage] of the patients included in this study are detailed in Table 2. Complete follow-up was available for all patients and ranged from 1 to 105 months. There were 71 deaths (26.9%) in the patient group with a median survival of >105 months. Fifty-four (58%) of Dukes C patients had received adjuvant chemotherapy, all with a 5-fluorouracil-based regimen.

Tissue microarray. An eight-block tissue microarray was constructed as described (26). The tissue microarray contained 264 primary colorectal cancer (Dukes A = 67, Dukes B = 104, and Dukes C = 93), 91 lymph node metastasis, and 10 normal morphologically colonic mucosal samples. The lymph node metastasis were from the corresponding Dukes C cases (adequate nodal metastatic tissue to sample was not available in two cases). The arrayed tumors reflected the distribution of the anatomic locations and Dukes stage of colorectal cancer in this population. A single normal colon sample was obtained from each of 10 of the colorectal cancer resection

specimens (proximal = 2, distal = 3, and rectum = 5) and each sample was acquired from at least 10 cm distant from the tumor as previously described (27). One representative 1.6 mm core of tissue was taken from each donor block using a steel Menghini needle and arrayed into the recipient wax block. One section from each microarray was stained with H&E to confirm the histopathologic diagnosis and the adequacy of sampling.

Immunohistochemistry. Immunohistochemistry for each antibody was carried out using a Dako autostainer (DakoCytomation, Ely, United Kingdom). Sections (5 μ m) of the tissue microarray were dewaxed, rehydrated, and an antigen retrieval step was done when required. The antigen retrieval step consisted of microwaving the sections in 0.01 mol/L citrate buffer at pH 6.0 for 20 minutes in an 800 W microwave oven operated at full power. The sections were then allowed to cool to room temperature. Primary antibody appropriately diluted (Table 1) in antibody diluent (DakoCytomation) was applied for 60 minutes at room temperature, washed with buffer (DakoCytomation), followed by peroxidase blocking for 5 minutes (DakoCytomation), and followed by a single 2-minute buffer wash. Prediluted peroxidase-polymer-labeled goat anti-mouse/rabbit secondary antibody (Envision, DakoCytomation) was applied for 30 minutes at room temperature, followed by further washing with buffer to remove unbound antibody. Sites of peroxidase activity were then shown with diaminobenzidine as the chromogen applied

for three successive 5-minute periods. Finally, sections were washed in water, lightly counterstained with hematoxylin, dehydrated, and mounted. Tissues known to express the relevant P450 were used as positive controls. Omitting the primary antibody from the immunohistochemical procedure and replacing it with antibody diluent or nonimmune rabbit serum acted as negative controls. For the newly developed P450 antibodies, preincubating each antibody with their corresponding unconjugated peptide immunogen before performing immunohistochemistry with positive control tissues acted as a further control and resulted in a loss of immunohistochemical staining.

The sections were evaluated by light microscopic examination and the intensity of immunostaining in each section was assessed independently by two observers (M.K and G.M) using the scoring system described. There were very few discrepancies (<5% of cases) and these were resolved by simultaneous reevaluation. The intensity of immunostaining in each core was scored as negative = 0, weak = 1, moderate = 2, or strong = 3 (26).

The MSI status of the tumors was determined by immunohistochemistry as previously described (28). Sections were immunostained with monoclonal antibodies to hMLH1 (clone G168-728, BD Biosciences, Oxford, United Kingdom) used at a dilution of 1 in 50 and hMSH2 (clone Fe11, Oncogene, Merck Biosciences, Nottingham, United Kingdom) also used at a dilution of 1 in 50. Immunohistochemistry, including an antigen retrieval step for each antibody, was done as described above. Loss of expression when none of the tumor nuclei stained with either hMLH1 or hMSH2 was regarded as MSI-high (MSI defective), whereas staining of tumor nuclei for either hMLH1 or hMSH2 was considered as microsatellite stable (MSI low; ref. 28).

Statistical analysis. Statistical analyses including χ^2 test, *t* test, Kaplan-Meier survival analysis, and Cox multivariate regression analysis were done using SPSS version 11.5 for Windows XP (SPSS UK, Ltd., Woking, United Kingdom). The log-rank test was used to determine survival differences between individual groups. We regarded $P < 0.05$ as significant.

Results

P450s in normal colon. All the P450s with the exception of CYP2F1, CYP3A7, and CYP4Z1 showed immunoreactivity in normal colon (Figs. 2 and 3). Most of the P450s displayed low frequency and weak immunoreactivity in normal colon. Several P450s, CYP2S1, CYP2U1, CYP3A4, and CYP51, showed immunoreactivity in >50% of cores. Only CYP3A4 immunoreactivity was detected in all cores and this P450 also showed the highest intensity of immunoreactivity in normal colon. All P450s that showed positive immunohistochemical staining displayed cytoplasmic immunoreactivity in colonic epithelium with stronger staining in surface epithelial cells compared with crypt epithelial cells. CYP2S1 also displayed cytoplasmic staining of chronic inflammatory cells (lymphocytes, plasma cells, and macrophages) present in the lamina propria. There was no relationship between the expression of individual P450s and the anatomic site within the colon.

P450s in colorectal cancer. All P450s showed some immunoreactivity in colorectal cancer (Figs. 2 and 3). There was significantly greater intensity of immunohistochemical staining for CYP1B1 ($P = 0.05$), CYP2S1 ($P = 0.02$), CYP2U1 ($P = 0.003$), CYP3A5 ($P = 0.02$), and CYP51 ($P = 0.0001$) in colorectal cancer compared with normal colon. The highest percentage of strong immunoreactivity was observed for CYP2S1 with 48.9% of the tumors showing strong immunohistochemical staining. Whereas for CYP1A1, CYP2F1, CYP2R1, CYP4F11, CYP4V2, and CYP4Z1, >80% of the cores were negative for the respective P450.

All P450s that showed immunohistochemical staining displayed diffuse cytoplasmic immunoreactivity in tumor cells (Fig. 3). CYP2S1 also displayed staining of chronic inflammatory cells present in the surrounding tumor stroma. Comparison of the presence of individual P450s and Dukes

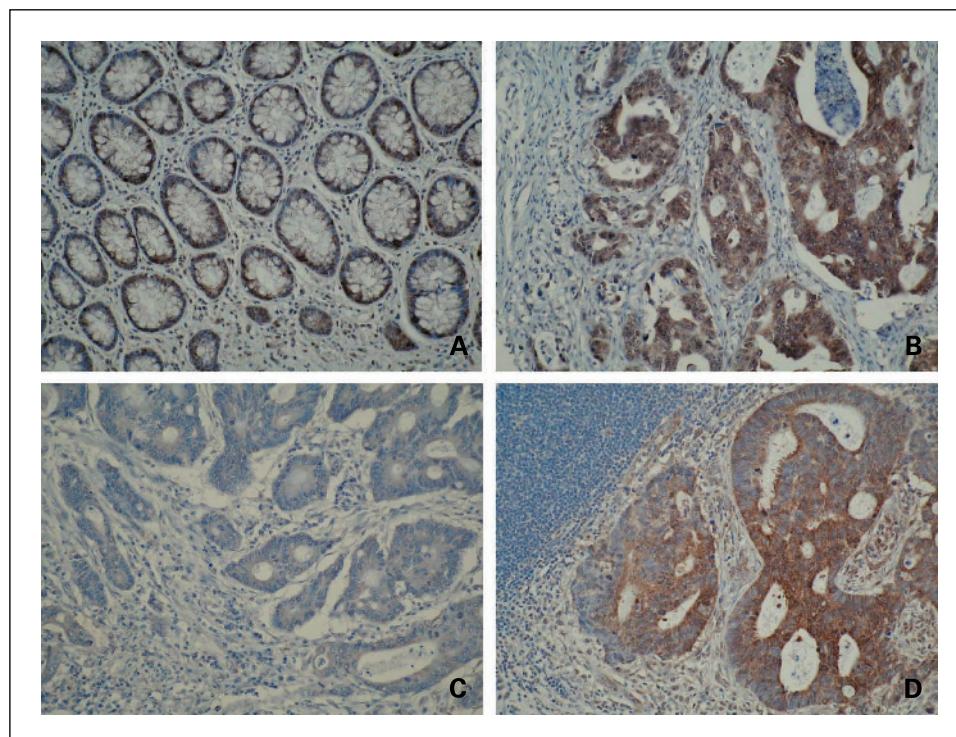


Fig. 3. Immunolocalization of cytochrome P450 CYP51 in normal colon (A), primary colorectal cancer showing strong CYP51 immunoreactivity (B), no CYP51 immunostaining (C), and strong immunoreactivity in a lymph node metastasis (D).

stage showed that there were significant correlations for CYP2S1 ($\chi^2 = 19.2, P = 0.004$), CYP2U1 ($\chi^2 = 14.8, P = 0.02$), CYP3A4 ($\chi^2 = 24.7, P = 0.001$), CYP3A5 ($\chi^2 = 20.7, P = 0.002$), CYP3A43 ($\chi^2 = 17.8, P = 0.007$), CYP4X1 ($\chi^2 = 37.1, P = 0.001$), and CYP51 ($\chi^2 = 40.1, P = 0.001$). Other P450s did not show any correlation between their expression and tumor stage.

P450s in lymph node metastasis. All the P450s showed some degree of immunoreactivity in lymph node metastasis (Figs. 2 and 3). The highest frequency of strong immunoreactivity was observed for CYP2S1 and CYP3A4. All P450s that showed immunohistochemical staining in lymph nodes displayed diffuse cytoplasmic immunoreactivity in tumor cells (Fig. 1).

Comparison of the Dukes C carcinomas and their corresponding metastases showed that there were significant correlations for CYP1B1 ($\chi^2 = 27.3, P = 0.001$), CYP2A/2B ($\chi^2 = 29.0, P = 0.001$), CYP2C ($\chi^2 = 18.1, P = 0.006$), CYP2F1 ($\chi^2 = 11.1, P = 0.001$), CYP4V2 ($\chi^2 = 10.7, P = 0.005$), and CYP39 ($\chi^2 = 8.2, P = 0.042$) between the presence of each of these P450s in the primary tumor compared with their expression in the secondary tumors. Other P450s did not show any correlation between their expression in the primary tumor and the lymph node metastases.

P450 expression and survival in colorectal cancer. There was a significant survival difference for patients whose tumors showed strong CYP51 immunoreactivity compared with those patients whose tumors showed negative, weak, or moderate CYP51 immunoreactivity (log-rank = 12.11, $P = 0.0005$; Fig. 4A). The median survival in the poor survival group was 61 months, whereas the median survival in the good survival group was >105 months. Similarly, there was a significant survival difference for patients whose tumors showed strong CYP2S1 immunoreactivity compared with those patients whose tumors showed negative, weak, or moderate CYP2S1 immunoreactivity (log-rank = 6.72, $P = 0.0095$; Fig. 4B). The median survival in the poor survival group was 81 months, whereas in the good survival group it was >105 months. The other significant survival factors by univariate analysis were Dukes stage (log-rank = 15.9, $P = 0.003$), tumor site (log-rank = 7.9, $P = 0.02$), and age (log-rank = 5.4, $P = 0.02$).

CYP51 (hazard ratio, 0.46; 95% confidence interval, 0.26–0.82; $P = 0.009$) remained independently prognostically significant after multivariate analysis with the prognostic model including Dukes stage (hazard ratio, 0.52; 95% confidence interval, 0.27–0.99; $P = 0.05$), age (hazard ratio, 1.60; 95% confidence interval, 0.92–2.76; $P = 0.1$), and tumor site (hazard ratio, 1.63; 95% confidence interval, 0.85–3.12; $P = 0.14$). CYP2S1 was not independently significant (hazard ratio, 1.5; 95% confidence interval, 0.88–2.57; $P = 0.14$).

Discussion

In this study, we have analyzed, by immunohistochemistry, P450 expression in colorectal cancer, corresponding lymph node metastasis, and normal colon. We have also defined the expression profile for individual P450s and established the prognostic significance of individual P450 expression in colorectal cancer.

CYP3A4 was the main form (most frequently expressed and at the highest intensity) of P450 present in normal colon and this finding is consistent with previous studies (29, 30).

CYP2S1 was one of the other P450s that also showed frequent high-intensity expression in normal colon. This P450 has only recently been cloned and although assigned to the CYP2 family on the basis of nucleic acid and amino acid sequence homology, it is dioxin inducible (31, 32). All other dioxin-inducible P450s are members of the CYP1 family and this suggests that CYP2S1 may have similar functional characteristics and metabolic substrates as CYP1 P450s (31, 32). The immunohistochemical localization of CYP2S1 protein in normal colon is a novel finding although a high level of CYP2S1 mRNA has previously been identified by real-time quantitative PCR in this tissue (33). CYP2S1 was localized to both epithelial cells and chronic inflammatory cells present in the lamina propria. This finding contrasts with other P450s that were only localized to epithelial cells of normal colon.

It is becoming increasingly recognized that individual forms of P450, most notably CYP1B1 (11), are overexpressed in specific types of cancer and that the P450s are emerging as important cancer therapeutic targets (12, 14), both as a

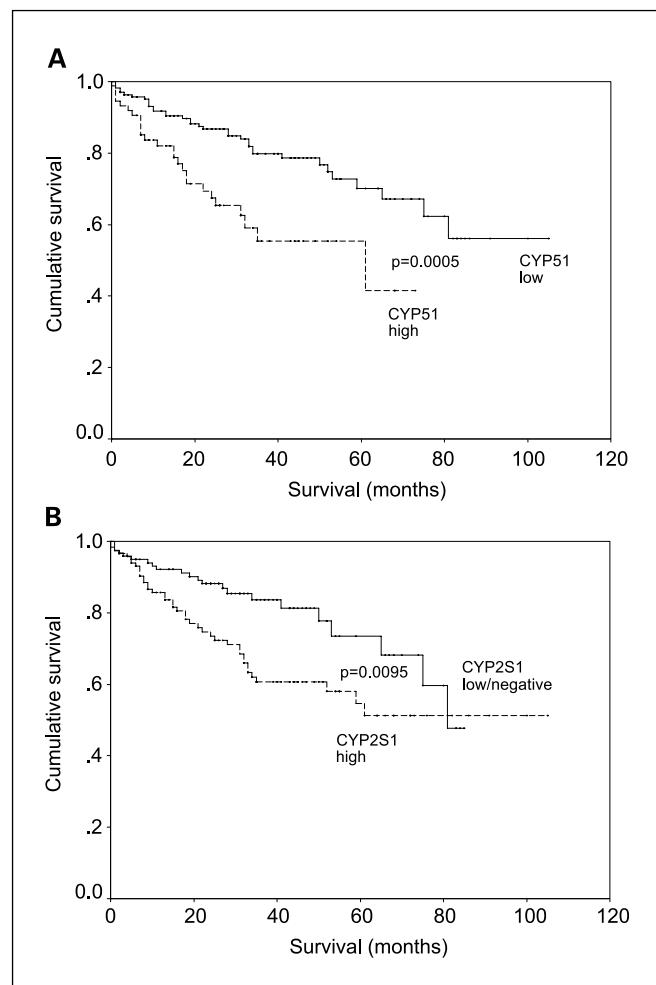


Fig. 4. *A*, comparison of survival in patients whose tumors showed strong CYP51 immunoreactivity and those tumors that have moderate, low, or negative CYP51 immunoreactivity. There is poorer survival in those patients whose tumors showed strong CYP51 immunoreactivity (log-rank test 12.11, $P = 0.0005$). *B*, comparison of survival in patients whose tumors showed strong CYP2S1 and those tumors that had moderate, low, or negative CYP2S1 immunoreactivity. There is poorer survival in those patients whose tumors showed strong CYP2S1 immunoreactivity (log-rank test, 6.72; $P = 0.0095$).

consequence of their overexpression and because of the distinct microenvironment in which tumors exists. Hypoxia is one of the main features of the tumor microenvironment that is currently being exploited by P450-targeted therapy. AQ4N is a CYP3A-activated prodrug that, in hypoxic conditions, is activated to a highly potent topoisomerase inhibitor and is currently being evaluated in clinical trials (17).

It is, therefore, important to define the expression profile of P450 in colorectal cancer and identify which P450s are overexpressed and also establish their prognostic significance. Previous studies of P450 expression and activity in colon cancer have studied only a limited number of P450s (30, 34–36). CYP3A immunoreactivity (30) and CYP3A-associated activity (34) have both been detected in colon cancer. In this study, we found that a range of P450s were present in colorectal cancer and several P450s showed more frequent and greater intensity of expression compared with normal colon. Importantly, we confirmed that CYP1B1 was overexpressed in colorectal cancer, consistent with both our previous study that used a polyclonal antibody to CYP1B1 (11) and the findings of others (37). We also found that CYP2S1, CYP2U1, CYP3A5, and CYP51 were significantly overexpressed. In particular, we identified several P450s that have not previously been identified in colon cancer, most notably CYP51 and CYP2S1. Not only are these distinct P450s overexpressed but the patients with strongly expressing tumors showed poorer prognosis compared with patients whose tumors showed less or no immunoreactivity and CYP51 expression is independently prognostically significant.

Biologically, CYP51 is involved in the synthesis of sterols (38, 39) that are incorporated into membranes. Greater activity of this P450 may allow tumor cells to retain a greater degree of membrane integrity and/or membrane fluidity, thus promoting tumor cell survival and motility and hence invasive capacity (40, 41). Clinically, this would be ultimately reflected in poorer patient survival.

We also found that CYP2S1 was overexpressed in tumors and that high expression of CYP2S1 was associated with poor prognosis. There have been no previous studies of this P450 in tumors.

CYP2U1 was also overexpressed in primary colorectal cancer. This novel P450, which has only recently been identified, catalyses the ω and $\omega - 1$ hydroxylation of fatty acids,

suggesting a role for this P450 in intracellular signaling pathways (42, 43). There have been no previous studies of this P450 in tumors.

The expression pattern of P450s in lymph node metastasis have not previously been studied in colorectal cancer. This is important because it cannot necessarily be assumed that the pattern of expression in the primary tumors will be reflected in the lymph node metastasis. Because most chemotherapy is targeted at metastatic tumors, it is important to have knowledge of the expression profile of P450s in the lymph node metastasis and how this relates to the expression pattern in the corresponding primary tumors. In this study, we found a correlation between the expression of a subset of P450s including CYP1B1, CYP2A/2B, CYP2C, CYP2F1, CYP4V2, and CYP39 in the primary tumors and the corresponding lymph node metastases. We have previously shown that CYP1B1 expression in primary and secondary ovarian carcinoma correlate (44). However, for most P450s, there was no relationship between their presence in primary tumor compared with their immunoreactivity in the secondary tumors, suggesting that the tumor microenvironment is an important factor in influencing the expression of many individual P450s. Several P450s have recently been identified in hepatic metastases of colorectal cancer by one-dimensional gel electrophoresis and mass spectrometry (45). However, in that study, only six metastases were investigated and comparative data for the corresponding primary colorectal cancers was not available. We believe that to maximize therapeutic efficiency, it will be necessary to directly phenotype lymph node metastasis for many individual P450s because expression cannot be inferred from the corresponding primary tumor.

In conclusion, we have defined the P450 profile of colorectal cancer, lymph node metastasis, and normal colon. We have identified overexpression of several P450s in colorectal cancer, most notably CYP51 and CYP2S1. Both of these enzymes show prognostic significance and CYP51 is an independent marker of prognosis in colorectal cancer.

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