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Cancer Detection and Prevention 29 (2005) 562–569

Cancer Detection and Prevention

www.elsevier.com/locate/cdp

Using a combination of cytochrome P450 1B1 and β-catenin for early diagnosis and prevention of colorectal cancer

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Accepted 19 September 2005

Abstract

Background: Although fecal occult blood test and invasive endoscopic examination are common used to detect colorectal adenomas and cancers, non-invasive and specific biomarkers are still under investigation. The objective is to evaluate the biomarker CYP1B1 alone or in combination with aryl hydrocarbon receptor (AhR), nuclear β-catenin, p53 or bcl-2 for early diagnosis and prevention of colorectal cancer. *Methods:* These biomarkers were analyzed semi-quantified across 231 colonic tissues including 97 adenocarcinomas, 85 adenomas and 49 non-neoplastic colons using immunohistochemistry. In order to differentiate non-neoplastic colons from colorectal neoplasms (adenoma and carcinoma), the values for CYP1B1, AhR, nuclear β-catenin, p53 and bcl-2 expressions were subjected to discrimination analysis, then the cross-validation, sensitivity and specificity of these models were calculated. *Results:* Expressions of CYP1B1, p53, nuclear β-catenin and bcl-2 were significantly associated with colorectal carcinogenesis ($p < 0.01$ for the trend test). The overexpression rates for CYP1B1, p53, nuclear β-catenin and bcl-2 were significantly higher in the adenoma and carcinoma groups than in the non-neoplastic colon group ($p < 0.05$). The discrimination models showed that a combination of two biomarkers was better than a single biomarker, and provided specificity ranging from 39% to 100% and sensitivity ranging from 43% to 82% for colorectal carcinoma. *Conclusions:* The increase in expression of CYP1B1 occurred not only in colorectal carcinoma and but also in adenoma. Moreover, a screening panel of CYP1B1 in combination with nuclear β-catenin was the most suitable marker pair to screen for colorectal carcinoma based on this study.

Keywords: Cytochrome P450; CYP1B1; Colorectum; Diagnosis; Prevention; Combined diagnostic test; Early diagnosis; Aryl hydrocarbon receptor; AhR; beta-catenin; bcl-2 expression; p53; Colorectal cancer; Adenomas; Dysplasia; Differentiation; Lymph node metastasis; Immunohistochemistry

1. Introduction

Colorectal cancer is one of the most common cancers in developed countries [1]. The development of colorectal cancer is regarded as an ''adenoma-carcinoma'' sequence [2,3]. Therefore, colorectal cancer is preventable when this sequence is interrupted at the preneoplastic or adenomatous stage. Indeed, Winawer et al. [4] reported that endoscopic removal of colorectal adenomas reduced the incidence of colorectal cancer by 76–90%. Although colorectal adenomas may be asymptomatic, many of them were found during anemia evaluation or occult bleeding. Thus, some screening programs have been designed with the aim of detecting asymptomatic adenomas before they progress to carcinomas.

Current screening methods for detecting colorectal adenoma or cancer include fecal occult blood tests and a flexible sigmoidoscopy or colonoscopy [4,5]. Fecal occult blood (FOB) tests are the only effective, non-invasive

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0361-090X/$30.00
doi:10.1016/j.cdp.2005.09.007

screening method. However, these tests are used to detect bleeding arising from a range of gastrointestinal diseases and are not specific to the diagnosis of colorectal neoplasms. Therefore, patients with intestinal occult bleeding are subsequently examined by a sigmoidoscopy or colonoscopy, which is used to excise adenomas or to diagnose early-stage cancers. However, this endoscopic procedure is invasive and uncomfortable for patients. Furthermore, the cost of an endoscopic examination is too expensive for large-scale screening, and patient compliance with this procedure is low [6]. Thus, non-invasive and specific biomarkers for the early detection and prevention of colorectal neoplasms are still under investigation.

Several molecular biomarkers have been identified for the different stages of colorectal carcinogenesis, including the *β-catenin*, *bcl-2* and *p53* gene mutations [7–9]. Mutational inactivation of *β-catenin*, involving the initiation stage of colorectal carcinogenesis, leads to abnormal accumulation of cytoplasmic β-catenin, which translocates into the nucleus, where β-catenin triggers T cell factor (Tcf)-mediated gene expression [8]. The *Bcl-2* oncogene is related to the inhibition of apoptosis. It has been suggested that during colorectal carcinogenesis the bcl-2 protein plays a role at an early stage because of bcl-2 overexpression in colorectal adenomas [7,10]. It is well known that *p53* gene is commonly inactivated during tumor progression. Mutated *p53* gene product loses its normal functions and inactivates the regulatory machinery for *p21WAF1/CIP1* expression, leading to cell proliferation [9]. In contrast to *β-catenin* and *bcl-2*, *p53* gene mutations are frequently found in colorectal carcinomas [11]. Therefore, p53 is considered to be involved in the late stages of colorectal carcinogenesis.

Several studies have shown an association between cytochrome P450 (CYP) polymorphisms and the risk of colorectal cancers [12]. CYP families are important enzymes for drug metabolism [13]. Recently, some studies have demonstrated that expression or activity of certain CYP enzymes is higher in tumor tissues than in adjacent normal tissues [14,15]. The activation of certain prodrugs by CYP enzymes preferentially occurs in specific tumors but not in normal tissues [16–18]. CYP-directed prodrugs have become a new concept not only to improve the effectiveness of anticancer treatment but also to reduce anticancer drug-induced side effects. Cytochrome P450 1B1 (CYP1B1) is a major member of extrahepatic xenobiotic-metabolizing CYP enzymes. CYP1B1 has been shown to activate several human promutagens and procarcinogens via an aryl hydrocarbon receptor-aryl hydrocarbon receptor nuclear translocator (AhR-ARNT) pathway [19,20]. Several studies have demonstrated that CYP1B1 is overexpressed in malignant cells including kidney, breast, brain and lung, but is only expressed to a limited extent in corresponding normal cells [14,21,22]. The differential expression of CYP1B1 between cancer and normal cells provides a potential chemopreventive property for the development of CYP1B1-based prodrugs. Furthermore, several clinical trials have been done on CYP1B1-based anticancer therapy. Recently, Gibson et al. [23] showed that CYP1B1 is also overexpressed in colorectal adenocarcinomas relative to normal colon. This implies that CYP1B1-activated or targeted anticancer drugs may be useful for treatment of colorectal cancer.

Although colorectal cancer-related gene alterations have been extensively investigated to study the molecular mechanisms of colorectal carcinogenesis, few researchers have described the applications of these biomarkers to early diagnosis and prevention of colorectal cancer. Since colorectal cancer can be prevented if adenoma is diagnosed and removed, adenoma biomarkers are most applicable for early diagnosis or prevention in colorectal cancer. Our present study examined the expression of CYP1B1 as well as other cancer-related genes in normal colon, adenoma and adenocarcinoma specimens using immunohistochemistry. Using a diagnostic discrimination model, we evaluated the possibility of combining two biomarkers for early diagnosis and prevention of the development of colorectal cancer.

2. Materials and methods

2.1. Subjects

Two hundred and thirty-one colonic tissues were collected from the Department of Pathology, Chung Shan Medical University Hospital, Taiwan, between 1999 and 2001. Chung Shan Institutional Review Board approved this study protocol with informed consents from all patients. These specimens were obtained by hemicolectomy, colonoscopic polypectomy and hemorrhoidectomy. After formalin fixation, paraffin embedding and routine staining with hematoxylin and eosin, their morphology and grading were diagnosed according to the World Health Organization Classification [24], including 97 colorectal adenocarcinomas (ADC), 53 adenomas with low-grade dysplasia (ADLG), 32 adenomas with high-grade dysplasia (ADHG) and 49 non-neoplastic colons (NC). Clinical variables were also collected from the subjects' medical records and these consisted of age, gender, smoking status and cancer staging. Smoking status was divided into smoker and non-smoker. A non-smoker was defined as a subject who had never smoked. The staging was defined according to Dukes' classification [25]. The 49 NC specimens from subjects with internal hemorrhoids and formed a non-neoplastic control group.

2.2. Immunohistochemistry

Tissue sections (4–5 μm thick) were cut and mounted on Silane-coated microscope slides (DakoCytomation, Denmark). Sections were deparaffinized in xylene and rehydrated through graded ethanol to distilled water. For antigen detection, sections were microwaved in citrate buffer (pH 6) for 20–30 min. These sections were cooled for

20 min at room temperature and washed with distilled water. To block endogenous peroxidase activity, sections were then incubated with 3% hydrogen peroxidase in distilled water for 20 min. In this study, the biomarker antibodies included anti-AhR (1:90 dilution, Biomol, Plymouth Meeting), anti-CYP 1B1 (1:1500 dilution, clone WB-1B1, Gentest), anti-β-catenin (1:600 dilution, clone 14, BD transduction laboratory), anti-p53 (1:100 dilution, clone DO-7, Novocastra) and anti-bcl-2 (1:50 dilution, clone bcl-2/100/D5, Novocastra). After incubation with the primary antibodies overnight at 4 °C in a moist chamber, an immunoenzymatic reaction was carried out using an avidin-biotinylated horseradish peroxidase complex (Universal LSAB2 kit, DakoCytomation). Finally, the brown color was developed with 3′-3′-diaminobenzidine as the chromogen substrate (DakoCytomation), and Gill's hematoxylin was used for nuclear counterstaining.

2.3. Quantification of aryl hydrocarbon receptor (AhR) expression

AhR immunoreactivity revealed a cytoplasmic staining in normal and tumor cells. The basal cells of hyperplastic prostatic glands have been known as AhR-rich cells [26], so hyperplastic prostatic glands were used as positive controls. The immunointensities of control prostate and the studied colonic tissues were all quantified using the Image Pro Plus 3.0 image analysis program (Media Cybernetic, Inc., Silver Spring, MD). The mean digital density of the control prostates was 56.5 ± 26.6 (mean ± standard deviation). Mean plus one standard deviation was 83.1 and this was defined as the gate value for the AhR digital density. The colonic tissues under study showing a digital density of more than 83.1 were scored as overexpressers and positives. Colonic tissues showing a digital intensity of less than or equal to 83.1 were scored as low-expressers and negatives. The rate of AhR overexpression was then calculated.

2.4. Scoring expressions of CYP 1B1, β-catenin, p53 and bcl-2

Immunohistochemically, CYP1B1 (Fig. 1A and B) and bcl-2 showed granular cytoplasmic staining. In addition, β-catenin (Fig. 1B and C) and p53 were investigated for nuclear expression. Overexpressers were defined as those showing greater than 20% stained cells with positive immunoreactivities and this measure was used for CYP1B1, β-catenin, p53 and bcl-2, because the intensity of expression of these genes was stronger than adjacent stroma. The rates of overexpression were evaluated. All slides were reviewed by three observers (H. Chang, C.H. Tsai and L.C. Liu) individually. If a great discrepancy was found, they read these slides using a multi-headed microscope (Olympus) in order to obtain a consistent result. Negative controls were

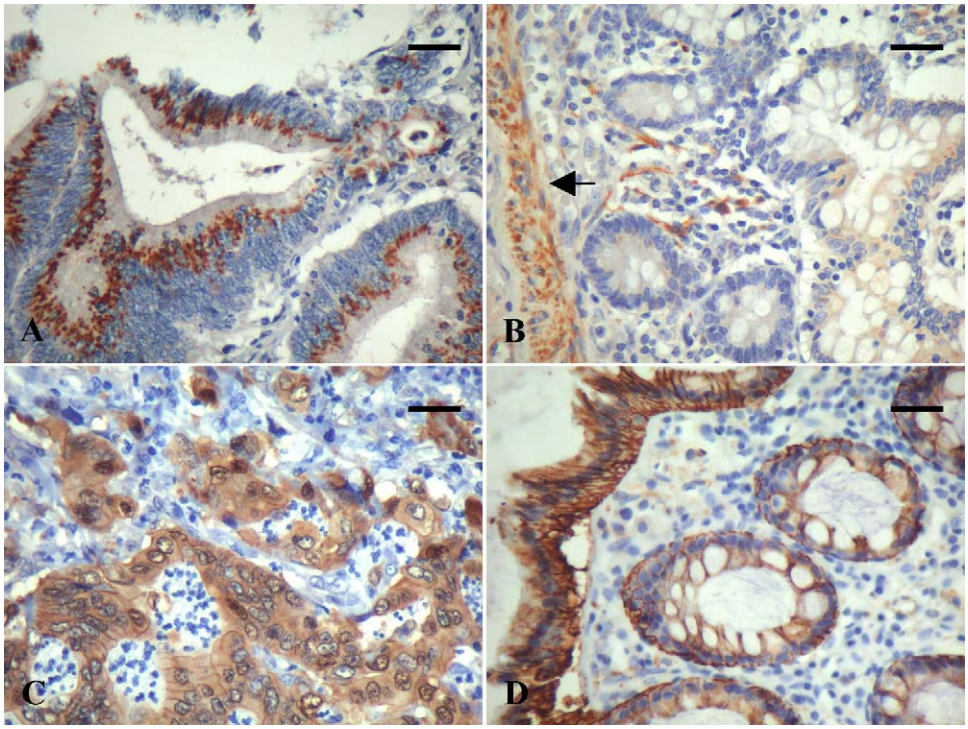

Fig. 1. Microphotographs of CYP1B1 and β-catenin immunohistochemistry in colorectal adenocarcinoma and non-neoplastic colon. (A) Adenocarcinoma overexpressed CYP1B1 protein in a granular cytoplasmic pattern. (B) Non-neoplastic colon epithelial cells were CYP1B1-negative in comparison with adenocarcinoma (see (A)). Arrow indicates a constitutive expression of CYP1B1 in muscularis mucosa. (C) Increased cytoplasmic and nuclear expressions of β-catenin were both noted in the colon adenocarcinoma. (D) Distinct membrane staining of β-catenin was shown in non-neoplastic epithelial cells. Bar represents 25 μm.

performed using normal rabbit or mouse serum instead of the primary antibodies (data not shown).

2.5. Statistical analysis

Student's *t*-test was used for the analysis of variance between the studied groups to compare AhR digital intensity. Trends for these overexpression rates of biomarkers in multistep carcinogenesis of the colorectum were determined by Chi-square for linear trend tests (Epi Inf 2000 software statistical package). Fisher's exact test was used for the difference in biomarker overexpression between groups. In order to differentiate non-neoplastic colons from colorectal neoplasms, including adenomas and carcinomas, the values for AhR, CYP1B1, β-catenin, p53 and bcl-2 expression were subjected to discrimination analysis to assess their significance by multiple statistical indices in order to create various discrimination models. The models were then used to recheck the studied subjects. The cross-validation, sensitivity and specificity of these models were calculated (SPSS 8.0 software statistical package). Statistical significance was considered to occur with a value of $p < 0.05$.

3. Results

A total of 112 males and 119 females were recruited for this study (Table 1). According to the histopathological changes, the patients were divided into three groups: NC, AD and ADC. The AD group included 53 tubular adenomas, 23 tubulovillous adenomas and 9 villous adenomas. Most adenomas (68%) were smaller than 1 cm. The ADC group was the oldest, followed by the AD group and the NC group was the youngest. More than 50% of the patients were females in the ADC and AD groups, while the NC group, which had more males (Table 1). The majority of patients in all three groups were non-smokers, although smoking information was not available for some patients. In the ADC group, more than 50% of the patients were at the early stage without lymph node metastasis (Table 1).

Table 1
Characteristics of studied subjects

Variables	Adenocarcinomas	Adenomas	Non-neoplastic colons
Total numbers	97	85	49
Age, mean	64.5	57.3	50.3
(range, years)	(28–98)	(27–87)	(24–80)
Gender			
Male	45	40	27
Female	52	45	22
Smoking status[a]			
Smoker	32	11	1
Non-smoker	61	41	10
Dysplasia			
Low-grade dysplasia		53	
High-grade dysplasia		32	
Duke's stage			
A	16		
B	42		
C/D	39		
Differentiation			
Well	20		
Moderate	51		
Poor	26		
Depth of invasion			
Mucosa	6		
Muscularis propria	10		
Subserosa	81		
Lymph node metastasis			
Absent	58		
Present	39		

[a] Four missing cases in adenocarcinoma group, 33 missing cases in adenoma group and 38 missing cases in non-neoplastic colon group.

3.1. Expression of biomarkers in non-neoplastic colons

The overexpression frequencies for the five biomarkers, AhR, CYP1B1, p53, nuclear β-catenin and bcl-2, in the studied subjects are summarized in Table 2. In the NC group, 31% and 32% of them overexpressed AhR and CYP1B1. Almost all NC specimens were p53 negative. β-Catenin immunostaining showed diffuse staining on the membrane of normal epithelial cells without nuclear staining, which

Table 2
Overexpression of AhR, CYP1B1, p53, nuclear β-catenin and bcl-2 in multistage carcinogenesis of colorectal cancer

	Total	Numbers of biomarkers (%)				
		AhR	CYP1B1[a]	P53[a]	β-Catenin[a]	Bcl-2
NC	49	15 (31)	16 (32)	1 (2)	0 (0)	31 (63)
ADLG	53	16 (30)	21 (65)	4 (8)	11 (21)[b]	31 (58)
ADHG	32	6 (19)	24 (75)[b,c]	3 (9)	11 (34)[b]	27 (84)[c]
ADC	97	19 (20)	55 (57)[b,c]	42 (43)[b,c,d]	56 (58)[b,c,d]	25 (26)[b,c,d]

NM, non-neoplastic colon; ADLG, adenoma with low-grade dysplasia; ADHG, adenoma with high-grade dysplasia; ADC, adenocarcinoma.
[a] Statistically significant for linear trend test, $p < 0.05$.
[b] Compared with NM, $p < 0.05$.
[c] Compared with ADLG, $p < 0.05$.
[d] Compared with ADHG, $p < 0.05$.

was defined as negative. In contrast, more than 60% of non-neoplastic epithelia expressed bcl-2 in the basal cells of the colorectal crypts.

3.2. Overexpression of biomarkers in adenomas

According to the severity of dysplasia, the AD group was further divided into low-grade (ADLG) and high-grade (ADHG) groups. Compared to the NC group (Table 2), the frequency of AhR overexpression was similar in the ADLG group, and slightly decreased in the ADHG group, although the difference between the NC and ADLG or ADHG groups was not statistically significant. The frequencies of CYP1B1 overexpression increased and peaked significantly in the ADHG group ($p < 0.05$). P53 expression was uncommon (8–9%) in both adenoma groups. Nuclear β-catenin expression clearly started in the ADLG group ($p < 0.05$). Bcl-2 overexpression was more common in the ADHG group than in ADLG group ($p < 0.05$). Similar to CYP1B1 overexpression, the peak (84%) occurred in the ADHG group. In summary, CYP1B1, nuclear β-catenin and bcl-2 overexpression was more common in the adenoma groups than in the NC group ($p < 0.05$). These results suggest that CYP1B1, nuclear β-catenin and bcl-2 may be involved in the early stages of colorectal carcinogenesis. As adenoma groups were stratified by histopathology or size of adenomas, overexpression of nuclear β-catenin was more prevalent in tubular adenomas and adenomas smaller than 1 cm ($p < 0.05$, data not shown). These associations were not demonstrated in other markers (data not shown).

3.3. Overexpression of biomarkers in adenocarcinomas

The frequency of AhR overexpression in the ADC group was similar to that of the ADHG group (Table 2). CYP1B1 overexpression in the ADC group was slightly less common than in both adenoma groups, but significantly more common than in the NC group ($p < 0.05$). The frequencies of p53 and nuclear β-catenin expression both sequentially increased in the order NC, ADLG, ADHG and ADC. The overexpression of p53 increased markedly in the ADC group (43%). Most of the ADC specimens showed a loss of bcl-2 protein. CYP1B1, p53, nuclear β-catenin and bcl-2 expression frequencies were significantly associated with the various stages of colorectal carcinogenesis ($p < 0.01$ for trend test), but AhR expression was not associated with the development of colorectal cancer.

Table 3
Sensitivity and specificity of single biomarker for colorectal neoplasms

Biomarkers	Sensitivity (%)	Specificity (%)
CYP1B1	86	28
P53	98	26
β-Catenin	100	32
Bcl-2	73	24

Sensitivity = the probability that overexpression was present given that the examined case showed the neoplasm. Specificity = the probability that overexpression was not present given the examined case did not show the neoplasm.

3.4. Diagnostic discrimination models

The specificity and sensitivity of these biomarkers, except AhR, were calculated to evaluate their applicability to the early diagnosis of colorectal cancer. Each biomarker showed high sensitivity (73–100%), but very low specificity (24–32%) (Table 3). Therefore, biomarkers were randomly combined to improve their sensitivity and specificity, which was evaluated using a discrimination model. CYP1B1, nuclear β-catenin, p53 and Bcl-2 were accepted into this analysis. The discrimination models, which combined two biomarkers showed better cross-validated rates (Table 4) than those that combined three or four biomarkers, where the cross-validated rates were around 50% (data not shown). The combination of CYP1B1 and nuclear β-catenin biomarkers showed a 72% cross-validation rate, a 67%

Table 4
Diagnostic discrimination models of two biomarkers for colorectal neoplasms

Combined biomarkers	Discrimination models[a]	Cross-validation (%)	Sensitivity (%)	Specificity (%)
CYP1B1 + p53	$Y(1) = -7.431 + 5.357X_{\text{CYP1B1}} + 6.243X_{\text{p53}}$ $Y(2) = -10.469 + 6.251X_{\text{CYP1B1}} + 7.773X_{\text{p53}}$	66	66	65
CYP1B1 + β-catenin	$Y(1) = -6.735 + 5.339X_{\text{CYP1B1}} + 5.001X_{\beta\text{-catenin}}$ $Y(2) = -10.632 + 6.208X_{\text{CYP1B1}} + 7.181X_{\beta\text{-catenin}}$	72	74	67
CYP1B1 + Bcl-2	$Y(1) = -8.692 + 4.658X_{\text{CYP1B1}} + 6.014X_{\text{bcl-2}}$ $Y(2) = -8.857 + 5.167X_{\text{CYP1B1}} + 5.682X_{\text{bcl-2}}$	73	82	39
p53 + β-catenin	$Y(1) = -5.456 + 5.360X_{\text{p53}} + 4.056X_{\beta\text{-catenin}}$ $Y(2) = -9.085 + 6.423X_{\text{p53}} + 6.043X_{\beta\text{-catenin}}$	63	53	98
p53 + Bcl-2	$Y(1) = -11.689 + 8.555X_{\text{p53}} + 8.123X_{\text{bcl-2}}$ $Y(2) = -12.602 + 9.978X_{\text{p53}} + 7.659X_{\text{bcl-2}}$	62	62	63
β-Catenin + Bcl-2	$Y(1) = -9.791 + 7.336X_{\text{bcl-2}} + 6.219X_{\beta\text{-catenin}}$ $Y(2) = -10.632 + 6.869X_{\text{bcl-2}} + 8.352X_{\beta\text{-catenin}}$	55	43	100

[a] By Fisher's discriminant analysis: $Y(1)$ for non-neoplastic group, $Y(2)$ for neoplastic group, X_i for individual overexpresser.

concordance rate for the non-neoplastic group (specificity) and 74% for the neoplastic groups (sensitivity) (Table 4). Thus, the combination of CYP1B1 and nuclear β-catenin biomarkers might be suitable for the early diagnosis of colorectal cancers.

4. Discussion

This study evaluated if CYP1B1 alone or in combination with other biomarkers was suitable for early detection of colorectal adenoma and prevention of colorectal carcinoma. Our results demonstrated that CYP1B1, p53, nuclear β-catenin and bcl-2 expressions were significantly associated with colorectal carcinogenesis ($p < 0.01$ for trend test). The overexpression rates for CYP1B1, p53, nuclear β-catenin and bcl-2 were significantly higher in the adenoma and carcinoma groups than in the non-neoplastic colon group ($p < 0.05$). Our data supported earlier research that overexpression of p53 and bcl-2, as well as the accumulation of nuclear β-catenin occurs in colorectal adenomas and carcinomas [7,27,28]. The differential immunohistochemical expressions of CYP1B1, p53, nuclear β-catenin and bcl-2 might be potential biomarkers for screening colorectal adenoma and carcinoma. Using single biomarkers for screening colorectal neoplasm, it was found that CYP1B1, p53, nuclear β-catenin and bcl-2 showed high sensitivity (73–100%) but low specificity (24–32%). The combination of two biomarkers was able to improve the specificity (from 30% to 70%) but decreased the sensitivity (from 90% to 70%) using discrimination analysis. The discrimination functions that were established were used to calculate cross-validation rates ranging from 55% to 73%. Among these combinations, the combination of CYP1B1 and nuclear β-catenin was the best and most appropriate.

Discrimination analysis is used to explore the choice of variables among many and identify those that are most useful for discriminating between groups, and to build functions for identifying new cases among groups [29–31]. Cross-validation provides the frequency of correct classification using the discrimination functions. Recently, Wen et al. [32] used discrimination analysis to select biomarkers for the diagnosis of colorectal cancer. Compared to sensitivity and specificity, which are used conventionally to evaluate the effectiveness of a screening test, the discrimination analysis is more informative. In our present study, the value calculated was cross-validation rate, which acts as a predicator for differentiating colorectal neoplasms from normal colon.

CYP1B1 has been reported to overexpress in colorectal carcinoma [14,23,33]. In our present study, we also demonstrated that CYP1B1 overexpression occurs not only in carcinoma but also in adenoma. This new finding supports a recent idea that CYP1B1-directed anticancer prodrugs might be used for cancer chemoprevention [16–18]. Resveratrol is a well-known example. Resveratrol has been reported to possess an antiproliferative effect against colorectal cancer [34]. Later, it was found that CYP1B1 metabolizes resveratrol into two major metabolites, that is, piceatannol and 3,4,5,4′-tetrahydroxystilbene. 3,4,5,4′-Tetrahydroxystilbene could up-regulate Bax in transformed cells resulting in apoptosis, but not in their normal counterparts [35,36]. Thus, CYP1B1 may bioactivate resveratrol, which demonstrates anticancer properties. Our present results suggest that CYP1B1-directed anticancer agents may be used to prevent the transition of CYP1B1-overexpressed colorectal adenoma into carcinoma.

Until now, FOB tests are the most common screening test for colorectal cancer, although FOB tests show only moderate sensitivity (37–79%), but high specificity (90%) for the detection of adenoma and carcinomas [5]. DNA mutations associated with colorectal carcinogenesis are potentially valuable as indicators for both colorectal adenoma and carcinoma [37,38]. However, in comparison with FOB and the stool DNA mutation tests, measuring the CYP1B1 expression is more informative. CYP1B1 overexpression could not only be used as a biomarker for early diagnosis, but could also be used to suggest a choice of CYP1B1-based chemoprevention. Quantitative real-time RT-PCR assay of CYP1B1 mRNA have been performed in human airway epithelial cells and blood cells [39,40]. Therefore, further investigation is needed to evaluate the possibility of quantifying stool CYP1B1 mRNA as a screen for colorectal adenoma.

Although nuclear β-catenin in combination with CYP1B1 expression may be an appropriate biomarker panel, the localization of β-catenin cannot be determined in the stool or the blood. It has been shown that β-catenin is constitutively expresses in the membrane of normal and neoplastic colonic epithelia. During colorectal carcinogenesis β-catenin is activated via nuclear translocation. Therefore, nuclear β-catenin is not reflected by total β-catenin expression in the stool exofoliated enterocytes. Studies have been demonstrated that nuclear β-catenin triggers the expression of several gene, including *c-myc*, cyclin D1, gastrin, cyclooxygenase (COX)-2, matrix metalloproteinase-7 and P-glycoprotein [8]. Therefore, the detection of *COX-2* gene expression in the stool may be a better approach to the representation of nuclear β-catenin. Recently, a small clinical trial by Kanaoka et al. [41] showed that *COX-2* mRNA levels in stool was detectable in 90% of subjects with colorectal cancers, but never in non-neoplastic subjects. Similar to CYP1B1, a COX-2 inhibitor, rofecoxib, has been developed as a cancer chemopreventive agent [42]. Rofecoxib significantly reduced the numbers and sizes of colorectal adenomas compared to the placebo controls during a 9-month treatment period [43]. Thus, the combination of stool CYP1B1 and *COX-2* (instead of nuclear β-catenin) mRNA may be used potentially to screen for colorectal adenoma and carcinoma.

In our investigation, the overexpression rate for AhR showed no significant difference between non-neoplastic

colon and colorectal neoplasms. This result suggests that AhR overexpression may be not involved in development of colorectal carcinoma. Although some studies showed that AhR activation up-regulates CYP1B1 expression [20,44,45], we observed no association between AhR and CYP1B1 expression in non-neoplastic colon or colorectal neoplasms. A possible explanation for this result is that CYP1B1 expression is increased via an AhR-independent mechanism [44,46,47]. Another possibility is that endogenous AhR ligands are increased in colorectal neoplasms, leading to subsequent up-regulation of CYP1B1. Therefore, more studies are needed to elucidate the up-regulation mechanism of CYP1B1 in colorectal cancers.

In summary, our data showed that CYP1B1, p53, nuclear β-catenin and bcl-2 expression was significantly associated with colorectal carcinogenesis. Among these biomarkers, the combination of CYP1B1 and nuclear β-catenin was the most appropriate for early diagnosis and prevention of colorectal cancer.

Acknowledgements

This work was supported in part by the National Science Council and Chung Shan Medical University (ROC) under Grant numbers NSC93-2320-B-040-020 and CSMU 91-OM-B-035.

References

[1] Boring CC, Squires TS, Tong T, Montgomery S. Cancer statistics. CA Cancer J Clin 1994;44:7–26.
[2] Fearon ER, Vogelstein B. A genetic model for colorectal tumorigenesis. Cell 1990;61:759–67.
[3] Vogelstein B, Fearon ER, Hamilton SR, et al. Genetic alterations during colorectal-tumor development. N Engl J Med 1988;319:525–32.
[4] Winawer SJ, Zauber AG, Ho MN, et al. Prevention of colorectal cancer by colonoscopic polypectomy. The National Polyp Study Workgroup. N Engl J Med 1993;329:1977–81.
[5] Ransohoff DF, Lang CA. Screening for colorectal cancer with the fecal occult blood test: a background paper. American College of Physicians. Ann Intern Med 1997;126:811–22.
[6] Subramanian S, Klosterman M, Amonkar MM, Hunt TL. Adherence with colorectal cancer screening guidelines: a review. Prev Med 2004;38:536–50.
[7] Bosari S, Moneghini L, Graziani D, et al. Bcl-2 oncoprotein in colorectal hyperplastic polyps, adenomas, and adenocarcinomas. Hum Pathol 1995;26:534–40.
[8] Kikuchi A. Tumor formation by genetic mutations in the components of the Wnt signaling pathway. Cancer Sci 2003;94:225–9.
[9] Levine AJ. p53, the cellular gatekeeper for growth and division. Cell 1997;88:323–31.
[10] Saleh HA, Jackson H, Khatib G, Banerjee M. Correlation of bcl-2 oncoprotein immunohistochemical expression with proliferation index and histopathologic parameters in colorectal neoplasia. Pathol Oncol Res 1999;5:273–9.
[11] Ito T, Kaneko K, Makino R, et al. Clinical significance in molecular detection of p53 mutation in serum of patients with colorectal carcinoma. Oncol Rep 2003;10:1937–42.
[12] Kiss I, Sandor J, Pajkos G, Bogner B, Hegedus G, Ember I. Colorectal cancer risk in relation to genetic polymorphism of cytochrome P450 1A1, 2E1, and glutathione-S-transferase M1 enzymes. Anticancer Res 2000;20:519–22.
[13] Guengerich F, Shimada T. Activation of procarcinogens by human cytochrome P450 enzymes. Mutat Res 1998;400:201–13.
[14] Murray GI, Taylor MC, McFadyen MC, et al. Tumor-specific expression of cytochrome P450 CYP1B1. Cancer Res 1997;57:3026–31.
[15] Lin P, Chang H, Ho W, Wu M, Su J. Association of aryl hydrocarbon receptor and cytochrome P4501B1 expressions in human non-small cell lung cancers. Lung Cancer 2003;42:255–61.
[16] Aziz MH, Kumar R, Ahmad N. Cancer chemoprevention by resveratrol: in vitro and in vivo studies and the underlying mechanisms (review). Int J Oncol 2003;23:17–28.
[17] Leong CO, Gaskell M, Martin EA, et al. Antitumour 2-(4-aminophenyl)benzothiazoles generate DNA adducts in sensitive tumour cells in vitro and in vivo. Br J Cancer 2003;88:470–7.
[18] McFadyen MC, Melvin WT, Murray GI. Cytochrome P450 enzymes: novel options for cancer therapeutics. Mol Cancer Ther 2004;3:363–71.
[19] Shimada T, Hayes CL, Yamazaki H, et al. Activation of chemically diverse procarcinogens by human cytochrome P-450 1B1. Cancer Res 1996;56:2979–84.
[20] Shehin SE, Stephenson RO, Greenlee WF. Transcriptional regulation of the human CYP1B1 gene. Evidence for involvement of an aryl hydrocarbon receptor response element in constitutive expression. J Biol Chem 2000;275:6770–6.
[21] Hakkola J, Pasanen M, Pelkonen O, et al. Expression of CYP1B1 in human adult and fetal tissues and differential inducibility of CYP1B1 and CYP1A1 by Ah receptor ligands in human placenta and cultured cells. Carcinogenesis 1997;18:391–7.
[22] Muskhelishvili L, Thompson PA, Kusewitt DF, Wang C, Kadlubar FF. In situ hybridization and immunohistochemical analysis of cytochrome P450 1B1 expression in human normal tissues. J Histochem Cytochem 2001;49:229–36.
[23] Gibson P, Gill JH, Khan PA, et al. Cytochrome P450 1B1 (CYP1B1) is overexpressed in human colon adenocarcinomas relative to normal colon: implications for drug development. Mol Cancer Ther 2003;2:527–34.
[24] Hamilton S, Aaltonen L. Carcinoma of the colon and rectum. In: Hamilton S, Aaltonen L, editors. World health organization classification of tumors. Pathology and genetics of tumors of the digestive system. Lyon: IARC Press; 2000. p. 103–47.
[25] Greene FL, Page DL, Fleming ID, et al. Colon and rectum, in AJCC cancer staging manual. New York: Springer, 2002. p. 113–30.
[26] Kashani M, Steiner G, Haitel A, et al. Expression of the aryl hydrocarbon receptor (AhR) and the aryl hydrocarbon receptor nuclear translocator (ARNT) in fetal, benign hyperplastic, and malignant prostate. Prostate 1998;37:98–108.
[27] Polyak K, Hamilton SR, Vogelstein B, Kinzler KW. Early alteration of cell-cycle-regulated gene expression in colorectal neoplasia. Am J Pathol 1996;149:381–7.
[28] Jen J, Powell SM, Papadopoulos N, et al. Molecular determinants of dysplasia in colorectal lesions. Cancer Res 1994;54:5523–6.
[29] Beard M. Predictors of performance on national board examinations. Part II. J Dent Educ 1984;48:458–62.
[30] Kramer GA, DeMarais DR. Reliability and validity of the pilot National Board Dental Examination. J Dent Educ 1992;56:236–41.
[31] Stip E, Mancini-Marie A. Cognitive function and depression in symptom resolution in schizophrenia patients treated with an atypical antipsychotic. Brain Cogn 2004;55:463–5.
[32] Wen J, Gao M-Q, Lin Z-W, Yang G-X. Quantitative study of multiple biomarkers of colorectal tumor with diagnostic discrimination model. World J Gastroenterol 2004;10:439–42.
[33] Kumarakulasingham M, Rooney PH, Dundas SR, et al. Cytochrome p450 profile of colorectal cancer: identification of markers of prognosis. Clin Cancer Res 2005;11:3758–65.

[34] Schneider Y, Vincent F, Duranton B, et al. Anti-proliferative effect of resveratrol, a natural component of grapes and wine, on human colonic cancer cells. Cancer Lett 2000;158:85–91.
[35] Lu J, Ho CH, Ghai G, Chen KY. Resveratrol analog 3,4,5,4′-tetrahydroxystilbene, differentially induces pro-apoptotic p53/Bax gene expression and inhibits the growth of transformed cells but not their normal counterparts. Carcinogenesis 2001;22:321–8.
[36] Potter GA, Patterson LH, Wanogho E, et al. The cancer preventive agent resveratrol is converted to the anticancer agent piceatannol by cytochrome P450 enzyme CYP1B1. Br J Cancer 2002;86:774–8.
[37] Ahlquist DA, Skoletsky JE, Boynton KA, et al. Colorectal cancer screening by detection of altered human DNA in stool: feasibility of a multitarget assay panel. Gastroenterology 2000;119:1219–27.
[38] Levin B, Bond JH. Colorectal cancer screening: recommendations of the U.S. Preventive Services Task Force. American Gastroenterological Association. Gastroenterology 1996;111:1381–4.
[39] Martinez J, Afshari C, Bushel P, Masuda A, Takahashi T, Walker N. Differential toxicogenomic responses to 2,3,7,8,-tetrachlorodibenzo-*p*-dioxin in malignant and nonmalignant human airway epithelial cells. Toxicol Sci 2002;69:409–23.
[40] Wu MF, Wu WJ, Chang GC, et al. Increased expression of cytochrome P4501B1 in peripheral leukocytes from lung cancer patients. Toxicol Lett 2004;150:211–9.
[41] Kanaoka S, Yoshida K, Miura N, Sugimura H, Kajimura M. Potential usefulness of detecting cyclooxygenase 2 messenger RNA in feces for colorectal cancer screening. Gastroenterology 2004;127:422–7.
[42] Chan CC, Boyce S, Brideau C, et al. Rofecoxib [Vioxx MK-0966; 4-(4′-methylsulfonylphenyl)-3-phenyl-2-(5H)-furanone]: a potent and orally active cyclooxygenase-2 inhibitor. Pharmacological and biochemical profiles. J Pharmacol Exp Ther 1999;290: 551–60.
[43] Higuchi T, Iwama T, Yoshinaga K, Toyooka M, Taketo MM, Sugihara K. A randomized, double-blind, placebo-controlled trial of the effects of rofecoxib, a selective cyclooxygenase-2 inhibitor, on rectal polyps in familial adenomatous polyposis patients. Clin Cancer Res 2003;9:4756–60.
[44] Kerzee JK, Ramos KS. Constitutive and inducible expression of Cyp1a1 and Cyp1b1 in vascular smooth muscle cells: role of the Ahr bHLH/PAS transcription factor. Circ Res 2001;89:573–82.
[45] Spink BC, Hussain MM, Katz BH, Eisele L, Spink DC. Transient induction of cytochromes P450 1A1 and 1B1 in MCF-7 human breast cancer cells by indirubin. Biochem Pharmacol 2003;66: 2313–21.
[46] Zheng W, Brake PB, Bhattacharyya KK, Zhang L, Zhao D, Jefcoate CR. Cell selective cAMP induction of rat CYP1B1 in adrenal and testis cells. Identification of a novel cAMP-responsive far upstream enhancer and a second Ah receptor-dependent mechanism. Arch Biochem Biophys 2003;416:53–67.
[47] Guo J, Sartor M, Karyala S, et al. Expression of genes in the TGF-beta signaling pathway is significantly deregulated in smooth muscle cells from aorta of aryl hydrocarbon receptor knockout mice. Toxicol Appl Pharmacol 2004;194:79–89.