「がん体験者の悩み」調査と群馬県立がんセンターの地域特性

群馬県立がんセンター

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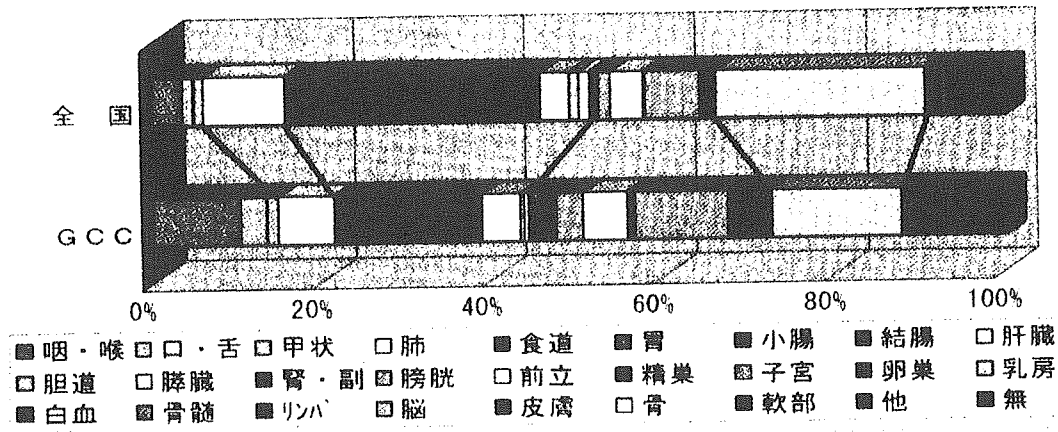
[目　　的]

がんは死亡原因の一位であり，診療技術の進歩により治癒する患者数は増加しているものの，なお約6割の患者は再発し，医療を受けつつ不幸な転帰をたどる．これら「がん体験者」は治癒する人も再発する人も様々な問題を抱えて生活しているが，必要とする支援を得られているとはいえない．個々の患者・家族の苦悩の要因を把握して初めて，支援の方向性を明らかにすることが可能となる．この支援ツール作成を目的とした全国規模のアンケート調査に関し，分担研究したので報告する．

[方　　法]

厚生労働省のがん研究に関する3つの研究班は，「がんの社会学」に関する合同研究班（主任研究者：山口　建・静岡がんセンター総長）として，平成15年4～12月，連結不可能匿名化アンケートを行った．対象は，全国がん協議会加盟全施設を含む53医療機関に通院する患者，および15の患者会・患者支援団体に所属する人々である．

調査項目は，診断（年齢，再発，治療）苦悩（種類，相談・相手，支援要望）生活状況（家族，職業，収入，医療費）などからなる．また悩みとそれに対する要望には「自由記載」の欄が付け加えられている．具体的には，がん体験者の身体的・精神的な悩みをはじめ，社会的・経済的，さらには人間としての生き方におよぶ悩みとその対処方法に関する調査となっている．



	頭頸部	消化器	生殖器	乳腺
全　国	5.9%	35.9%	14.9%	24.3%
GCC	14.7%	22.9%	28.4%	15.1%

図1　疾患部位別の対象者の構成
（GCC；群馬県立がんセンター，以下同）

このうち群馬県立がんセンターで説明，同意確認しアンケート用紙を配布，本部研究機構に回収された調査結果を，全国集計の結果と比較検討した．また，当院で平成14年に行った通院がん治療患者に対する調査結果[3]との比較考察も試みた．なおアンケート調査の内容と方法は当院の倫理委員会で承認されたものである．

[結　　果]

1）調査票の配布と回収の状況は，全国で配布数12,345通，回収率63.9%（医療機関10,200通，71.3%，患者会等2,145通，28.6%）であり，当院では配布数279通，回収率78.1%であった．

2）当院の対象者では，調査時年齢は60歳代が最も多く34.9%，診断時の年齢でもがん年齢といわれる60歳以上が過半数を占めていた．性別比は男性が52.3%と多く，全国統計（女性が53.8%）と異なっていた．

対象者の構成を疾患部位別に大別すると，頭頸部（14.7%）生殖器（28.4%）は全国より比率が高く，消化器(22.9%)乳腺(15.1%)は低率であった（図1）．

現在の状況は，治療中が34.4%であったが，日常生活行動に制約を受けない人（PS＝0）が過半数（55.5%）で全国と同等であった．

3）苦悩の種類は全国データと類似の分布を示し，痛み・後遺症などの肉体的苦痛は53.2%，再発不安や恐怖などの精神的な苦悩は56.9%とやや高率であり，生きる意味などに関すること33.0%はやや低率であった（表1）．相談の希望および相手，支援要望分類では全国とほぼ同様であった（表2，表3）．

4）仕事の内容を勤め人と回答した人のうち41.3%（全国34.7%：表4）が依願退職・

表1　苦悩の種類

	全　国	GCC
痛み・副作用，後遺症などの身体的な苦痛	48.1%	53.2%
落ち込みや不安，恐怖などの精神的なこと	52.9%	56.9%
夫婦間，子供との関係など家庭・家族のこと	29.1%	33.5%
仕事，地位，人間関係などの社会との関わり	20.5%	23.9%
医師や看護師などとの関わり	8.0%	7.8%
収入，治療費，蓄えなどの経済的なこと	35.1%	39.9%
今後の生き方，生きる意味などに関すること	37.6%	33.0%

表2　悩みが軽減した相談相手
（三時点における評価，全国データ[1]より）

	診断された頃	診断後，現在に至るまで	現在
家　　族	59.1%	53.4%	52.2%
担　当　医	25.6%	34.9%	34.4%
相談室＋役所	1.5%	2.0%	1.7%

表3　支援要望分類
（悩みの緩和に何が必要か，全国データ[1]より）

・医療者との関係	18.7%
・自身の努力による解決	18.3%
・相談・心のケア	11.3%
・家族の協力・理解・支え	8.3%
・同病者との交流・患者会	8.1%
・行政・医療機関への要望	6.6%
・情報提供・情報公開	6.0%
・経済面での制度・支援	5.3%
・医学の進歩	3.2%
・友人の協力・理解・支え	2.6%
・宗教	0.9%
・就職・職場環境	0.6%

表4　職業の継続について（全国データ[1]より）

勤労者		自営業者（家族）	
現在も勤務	47.6%	現在も営業	68.0%
休　職　中	8.7%	休　業　中	7.7%
依願退職	30.5%	従事せず	5.7%
解雇された	4.2%	廃業した	13.2%
そ　の　他	9.0%	そ　の　他	5.4%
	(2,625人)		(1,021人)

解雇で失職していた．患者の（家族の収入も合わせた）年間収入は，約半数（47.7%）が400万円未満であった（図2）．

［考　　察］

わが国では毎年新たに44万人ががんと診断されており，1999年の時点で治療後のがん生存者数は276万人と推計されている．この数は高齢化社会を迎えて増加し，2015年には533万人になると予測され，多くの人々ががんの治療を受け，様々な不安を抱えながら生活することになる．これらがん体験者や家族が不安を克服し，社会復帰するために必要な支援方法を確立することは急務であろう．

これまで外来患者を対象とした調査やがん体験者の心理研究は，個別の施設ではなされていたが[2)3)]，エビデンスとなり得る全国規模の調査は国内では皆無であった．今回，がん体験者の悩みを1万人規模で調査し，プライバシーを確保しながら患者の言葉で語ってもらい，科学的な分析を加えた上でデータベース化する作業が開始した[1)]．「がんの社会学」に関する合同研究班の一次報告のうち，分担研究者として群馬県立がんセンターの地域特性について検討した．

1）集計結果として，がん体験者の背景因子（年齢，治療状況，日常生活状況）および苦悩の種類，支援要望項目などでは，全国データとほぼ同等であった．対象患者に男性が多かった理由は，疾患部位別の構成比に差があったためである．当院ではありのままの患者構成を反映させるべく，通院患者実数の比率に応じて担当医に調査票を割り当てた．しかし配布数と回収状況を全国データと比べると，内科・外科とも消化器患者の回答が少なくなってしまった．男性患者の多い頭頸部が全国の約3倍と多く，乳腺の患者が全国の約6割と少なかったことが当院の特徴であった．

2）悩みの種類は全国データとほぼ同様の結果で，精神的な苦痛，身体的な苦痛を過半数の人が訴え，次いで経済的な不安が挙げられた．「今後の生き方，生きる意味」という項目は回答者にとって必ずしも「霊的な苦悩」と理解されるとは限らないが，全国では「家庭」や「社会との関わり」より頻度が高く37.6%であり，群馬県では33.0%であった．いわゆる「霊的な苦悩」という設問の設定は非常に難しく，2002年に当院で行った調査では「自分の存在意義」とか「生きてきたあか

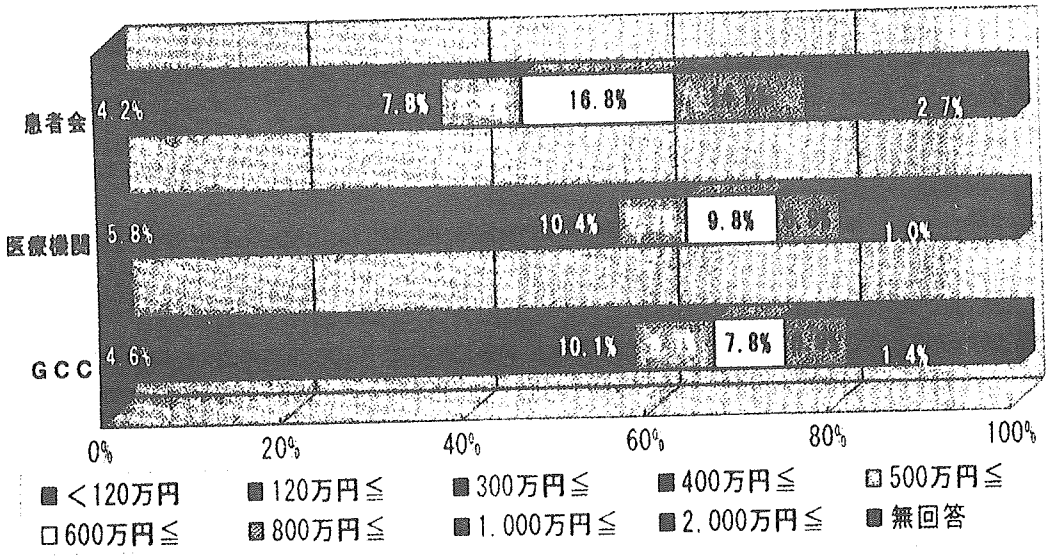


図2　家庭の収入

し」という問い方に対し，不安があると回答したのは20%に過ぎなかった[3]．しかし少なからず，「魂の救済」を訴えている人がいることは事実で，自由記載の内容が詳細に検討されることにより，その実態は明らかになると考えられる．

3）「誰かに相談したか」という項目では，診断時点では約3分の2の人が相談を求めていた．自分の病状が分かるに連れ，あるいは治療・退院という時間の推移によって減少するものの，現時点でさえも半数近くが相談相手を必要としていた．悩みを少しでも和らげるために必要なこととして，「医療者との良好な関係」と「自助努力」がほぼ同数で最も多く，「同病者との交流」や「行政機関」を求める声も比較的多くあった．医療者との関係とは「医師から温かい一言をかけてほしい」など，むしろ現状への不満があり関係性の改善を要望する内容が中心であったようだ．自由記載では「自助努力」が最も頻度が高く自分の体験を綴るなど，がん体験者は術後障害や合併症を自ら克服しようとしている．自助努力をサポートする様々なツールを収集し，開発することが重要と思われる．悩みの緩和には，医学の進歩や宗教は頼りにされていない．

「悩みの相談相手」で，群馬県と全国との間に大きな差はなく，三時点（診断時，経過中，現在）とも家族，担当医の順であった．悩みの相談相手に医療者を求めていることは以前当院で行われた調査結果とも一致している．「病院の相談室」や「県や市町村など公的機関」は現時点では2%以下と全く頼りにされていない．担当医が頼りにされていることは明らかだが，時間的に大きな制約がある．ここにソーシャル・ワーカー（S・W）の存在意義が認められよう．患者の救済のためには，担当医がS・Wに仕事を渡すこと，公的機関との橋渡しになってもらうことが重要と考えられる．診療機関依存の傾向は当院にも当てはまり，終末期まで患者に密着した医療が求められている．当院の診療圏は栃木県南部および埼玉県北部を含むものの2004年現在，人口120万人以上の都市を診療圏に持たない．このため地域行政からの支援にも制約が感じられ，患者は当院・医療者との関係を重視することになる．同時に，在宅療養の支援には地域一次医療機関・行政組織との連携の充実は必要不可欠である．また当院では患者会も組織されている頭頸部領域や，泌尿器，婦人科のがん体験者も多い．この領域を含め（下部

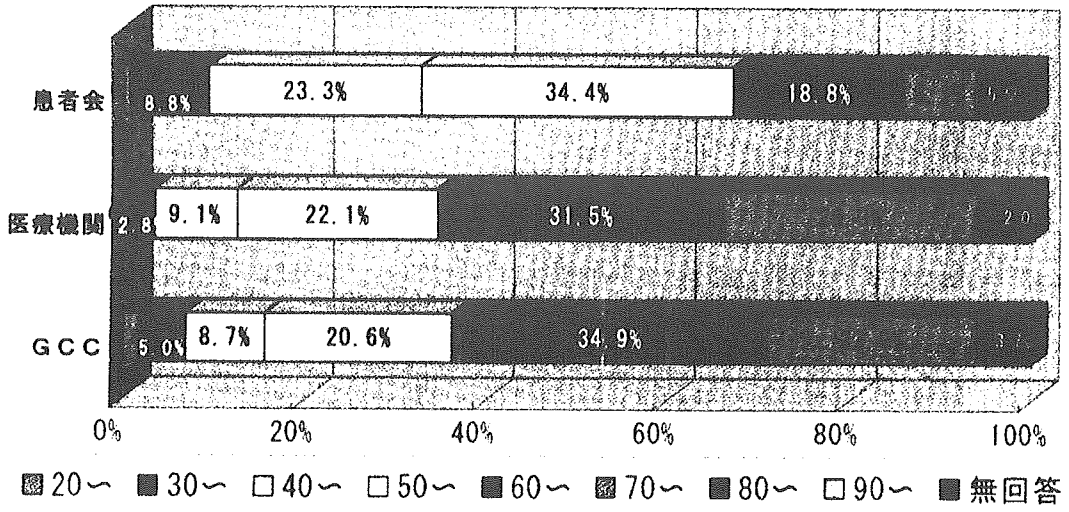


図3　対象者の年齢構成

消化器，乳腺など）例えばストーマやリンパ浮腫のような機能障碍が発症しやすい疾患に対する支援策の作成も重要であると思われた．

4）依願退職または解雇により失職した人は全国値より高く41.3%であった．がんが職業継続に影響を与えていることは容易に推測できる．患者家庭の年間収入400万円未満の比率は，全体では44.5%（医療機関45.8%，患者会29.5%）であった[1]．当院では47.7%と，さらに所得の低い人が多い傾向にあった．群馬県のがん体験者は職業継続が困難になることが多く（全国データに比べ）やや所得が低く，霊的な苦悩より現実的な苦悩に直面している傾向にあった．公的機関による積極的な支援が強く望まれる．患者会の所得水準が高かった理由は，患者会の年齢構成は60歳未満の若い人が多い，すなわち働いて収入を得られる人が多いためと考えられた（図3）．「医療機関への支払金額」は50万円未満が47.1%と約半数であったが，がん年齢といわれる60歳代以降の人は，一方で年金生活者が多いことから経済的負担は必ずしも小さくはない．

本研究の成果は，「第一に，がん体験者の悩みと解決法を伝えることにより，新たな体験者の孤独感を癒しがんとの闘いの道しるべとなる．第二に，医療技術者や行政担当者が，患者の悩みをより深く把握するのに役立つ．第三に，社会が身近な問題としてがんを理解するのに役立ち，社会的基盤（医療資源）の整備が進む．」ことによりがん患者のQOLの向上に役立てられるであろう[1]．近く「肝臓編」が発刊され，臓器別に継続されていく予定となっている．その中に本研究から導き出された「支援ツール」が具現されることが期待される．

[文　献]

1）山口　建（がんの社会学に関する合同研究班），がんと向き合った7885人の声，がん体験者の悩みや負担等に関する実態調査報告書，2004.

2）小林国彦，在宅癌治療―通院化学療法を中心として，Biotherapy，15(4)：455，2001.

3）塚越俊夫，福田敬宏，角田愉香理，上出美嘉，荻原弘子，伊藤典子，がん生存者の精神的ケアに関する調査研究（通院治療患者の療養環境について），群馬県立がんセンター年報，30：117，2002.

Thymidine phosphorylase expression and efficacy of adjuvant doxifluridine in advanced colorectal cancer patients

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Abstract. To clarify the correlation between the expression level of thymidine phosphorylase (TP) and efficacy of doxifluridine (5'-DFUR) and 5-fluorouracil (5-FU), samples from 177 colorectal cancer patients who underwent curative resection were evaluated by immunohistochemical staining using a newly developed monoclonal antibody 1C6-203. Patients were randomly given either oral 5'-DFUR or 5-FU as postoperative adjuvant chemotherapy. In Dukes' C staged colon cancer patients treated with 5'-DFUR, better survival was observed in the high TP patients than the low TP patients (P=0.025 by the log-rank test). The observed 5-year survival rates were 91.2 and 74.8%, respectively. No correlation between TP expression and patient prognosis was detected in the 5-FU group. In Dukes' C staged colon patients with high TP expression, the 5'-DFUR group had slightly better survival than the 5-FU group. These findings suggest that TP may be a chemosensitive marker for 5'-DFUR as postoperative adjuvant chemotherapy for advanced colon cancer patients.

Introduction

Although intravenous administration of 5-fluorouracil (5-FU) + leucovorin (LV) was introduced worldwide in the mid-1990s as the standard postoperative adjuvant therapy for colorectal cancer (1), oral cancer drugs such as capecitabine have recently been re-evaluated due to a favorable benefit:risk ratio compared with 5-FU+LV (2,3), while in Japan, oral fluoropyrimidines have been widely used as postoperative adjuvant chemotherapy since the early 1990s, due to better compliance and minimal toxicity. However, a major retardation of cancer chemotherapy is the lack of predictive markers for responsiveness, and selecting effective chemotherapy might not only offer a survival benefit but also avoid unnecessary adverse effects caused by unsuitable drugs.

Thymidine phosphorylase (TP), which is predominantly observed in tumor tissue (4-7), is a key enzyme in the metabolic activation of fluoropyrimidine by conversion of doxifluridine (5'-DFUR), which is an intermediate metabolite of capecitabine, to 5-FU (8). Thus, administration of 5'-DFUR against tumors with high TP expression is expected to yield high concentrations of 5-FU in tumor tissue, and thereby a good chemotherapeutic response. In fact, the clinical efficacy of 5'-DFUR has been demonstrated in colorectal cancer patients with high-TP-expression tumors compared to patients with low-TP tumors (9,10). However, the efficacy of 5'-DFUR has not previously been compared to that of other therapies.

Immunohistochemical staining of TP has been widely adopted to evaluate its relationships with clinicopathologic features and prognosis. However, these studies have produced conflicting results, and may not be definitive, as monoclonal antibody (MAb) 654-1, which stains stromal cells stronger than cancer cells, was used (11-14). A newly developed MAb, 1C6-203, is reportedly more sensitive to colorectal cancer cells than MAb 654-1 in 10% formalin-fixed specimens (15). Thus, MAb 1C6-203 appears to be more suitable than MAb 654-1 to assess TP expression.

We previously conducted a randomized controlled trial (RCT) to compare the usefulness of 5'-DFUR to oral 5-FU as postoperative adjuvant chemotherapy for 558 patients with colorectal cancer, and reported a survival benefit in 5'-DFUR therapy in Dukes' B or C staged patients, particularly in colon cancer patients (16). In the present study, we assessed colorectal cancer patients from the previous RCT who were strictly followed for more than 5 years, and examined the predictive value of TP expression for patients' prognoses by immunohistochemistry using the new sensitive antibody.

Materials and methods

Patients. A total of 177 colorectal cancer patients (103 men and 74 women, median age at surgery; 62.0 years (range; 42-78 years), who had enrolled in the previously mentioned RCT and whose paraffin-embedded specimens were available for immunohistochemical staining, were assessed. All patients were diagnosed as having TNM stage II or III primary colorectal cancer, and underwent curative resection at institutes

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Key words: colorectal cancer, thymidine phosphorylase, adjuvant chemotherapy, doxifluridine, 5-fluorouracil

affiliated with the Department of General Surgery, Graduate School of Medicine, Chiba University, Japan, from April 1993 to September 1996. No patient had any history of treatment for other colorectal cancers, other active cancers, or serious concurrent disease. Patients were randomly assigned to two groups; either the 5'-DFUR group (84 patients) or 5-FU group (93 patients) at each institute. No differences in patient characteristics were observed between the two groups (Table I). The present study was approved by the institutional review boards at participating centers. All patients provided fully informed consent.

Treatment schedule. Both groups were given 6.0 mg/m^2 of mitomycin C intravenously on the day of surgery and on the following day. Oral chemotherapy was started from 2 weeks after surgery; the 5'-DFUR group was given 460 mg/m^2/day and the 5-FU group was given 115 mg/m^2/day, daily for 1 year. Both groups were concomitantly given 3 g/day of polysaccharide kureha for 1 year. All patients were followed for more than 5 years after surgery, and the median follow-up period after surgery was 5.5 years. No patient had additional treatment unless the cancer recurred or another cancer developed.

Immunohistochemical staining. After reviewing hematoxylin-eosin-stained slides of surgical sections, we selected a paraffin block including the deepest invasion site. Immunohistochemical staining was performed using avidin-biotin-peroxidase complex (Peroxidase Vectastain ABC Kit®, Vector Laboratories Inc., Burlingame, CA, USA). The sections were deparaffinized with xylene and rehydrated with ethanol. The specimens were washed with phosphate buffered saline (PBS) for 5 min, and endogenous peroxidase was then blocked by incubating the preparations with 0.3% hydrogen peroxide in methanol for 30 min. After washing the preparations 3 times with PBS for 5 min, they were incubated for 15 min with biotin blocking solution, rinsed again with PBS for 5 min, and re-incubated with PBS containing 3% skim milk for 30 min. These preparations were then incubated with anti-TP mouse antibody, 1C6-203 (Nippon Roche Research Center, Kamakura, Japan), which were diluted 1:1000 with 0.5% normal horse serum/PBS at 4°C overnight in a moist chamber. The sections were then washed 3 times with PBS and incubated with peroxidase-labeled horse anti-mouse IgG monoclonal antibody for 30 min at room temperature. These were rinsed again and then incubated with avidin-biotin-peroxidase complex for 30 min at room temperature. After washing 3 more times, the preparations were incubated with diaminobenzidine substrate for 1-7 min. The specimens were rinsed again with distilled water, counterstained with Mayer's hematoxylin and mounted.

Evaluation of stained sections. TP expression was simultaneously evaluated on x100 and x50 fields using a two-headed light microscope by 2 investigators (Drs S. Hasegawa and N. Takiguchi) with no prior knowledge of the patients' clinico-pathologic characteristics and prognosis. Specimens with ≥5% cancer cells stained in the cytoplasm or nucleus were regarded as having high TP expression, while the rest were regarded as having low TP expression.

Table I. Patient characteristics.

	5'-DFUR (n=84)	5-FU (n=93)	P-value
Age (y)	61.2	62.7	0.252
Gender (M/F)	53/31	50/43	0.269
Location of tumor			
Colon	56	61	0.427
Rectum	28	32	
Histologic differentiation			
Well	47	50	0.727
Moderate	33	40	
Poor	3	3	
Mucinous	1	0	
Depth of tumor			
pT1	1	1	0.954
pT2	9	9	
pT3	47	56	
pT4	27	27	
Dukes' stage			
A	8	8	0.860
B	39	47	
C	37	38	

Statistical analysis. Statistically significant differences in continuous variables between groups were assessed by the t-test, and with categorical variables either by the χ^2 test or Mann-Whitney U-test. P-values <0.05 were considered significant. The survival curve was calculated by the Kaplan-Meier method, and differences between two groups were evaluated using the log-rank and Wilcoxon tests.

Results

Relationship between TP expression and clinicopathological characteristics. TP staining was observed in the nucleus and/or cytoplasm of cancer cells (Fig. 1). Most normal colorectal mucosal cells were not stained with anti-TP antibody. TP was highly expressed in 92 (52.0%) colorectal cancer patients.

Table II summarizes the relationships between TP expression and some clinicopathological characteristics. In the 5-FU group, patients with low TP had more advanced tumor depth than those with high TP, but no difference in Dukes' stage was observed between them. No other correlation between TP expression and clinicopathological characteristics was observed.

Correlation between TP expression and outcome of adjuvant chemotherapy. A comparison of overall survival time of the colorectal cancer patients and tumor TP expression revealed no correlation in either the 5'-DFUR or 5-FU group (Fig. 2).

Figure 1. Immunohistochemical staining with 1C6-203 for TP in colon cancer tissue. TP staining was seen in the cytoplasm and/or nucleus of cancer cells (original magnification x50).

Table II. Relationships between TP expression and clinicopathologic features.

	High TP (n=92)		Low TP (n=85)	
	5'-DFUR (n=42)	5-FU (n=50)	5'-DFUR (n=42)	5-FU (n=43)
Age (y)	60.7	63.3	61.8	61.9
Gender (M/F)	25/17	22/28	28/14	28/15
Location of tumor				
Colon	26	34	30	27
Rectum	16	16	12	16
Histologic differentiation				
Well	20	27	27	23
Moderate	21	21	12	19
Poor	1	2	2	1
Mucinous	0	0	1	0
Depth of tumor				
pT1	0	1	1	0
pT2	6	6	3	3
pT3	25	36	22	20
pT4	11	7	16	20
		[a]		
Dukes' stage				
A	4	6	4	2
B	18	27	21	20
C	20	17	17	21

[a]P=0.0062.

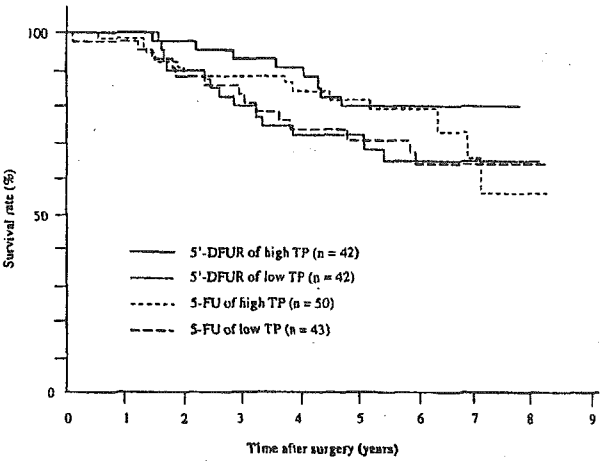

Figure 2. Overall survival curve between patients with high and low TP expression of the colorectal cancer patients in the two chemotherapy groups. Estimated overall 5-year survival rates in patients with high and low TP expression were 79.5 and 71.4% in the 5'-DFUR group, respectively, and 81.6 and 70.5% in the 5-FU group, respectively. No significant difference was detected among each group.

However, in the colorectal cancer patients with Dukes' C stage, slightly better survival was observed in the 5'-DFUR group with high TP expression than in the 5-DFUR group with low TP expression, while no significant difference was detected in the 5-FU group (Fig. 3). Evaluation of Dukes' C patients with only colon cancer revealed significantly better survival in the 5'-DFUR group with high TP expression than low TP expression. In addition, Dukes' C staged colon cancer patients with high TP expression had slightly better survival in the 5'-DFUR group than the 5-FU group, while in the low

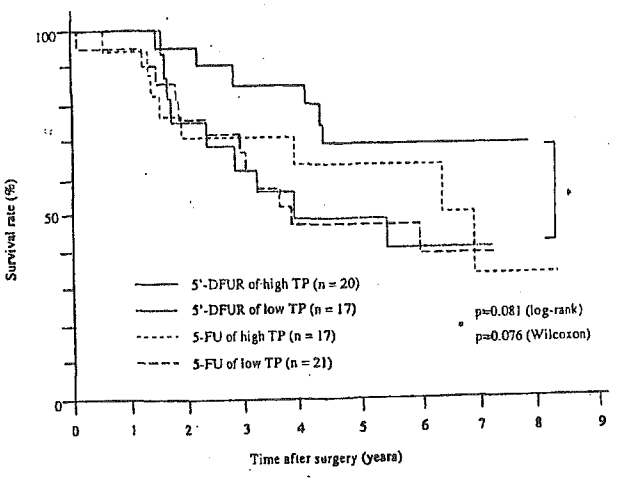

Figure 3. Overall survival curve between patients with high and low TP expression in Dukes' C staged colorectal cancer patients in the two chemotherapy groups. Estimated overall 5-year survival rates in patients with high and low TP expression were 69.1 and 49.2% in the 5'-DFUR group, respectively, and 63.5 and 47.6% in the 5-FU group, respectively. In the 5'-DFUR group, patients with high TP expression had slightly better survival than those with low TP expression.

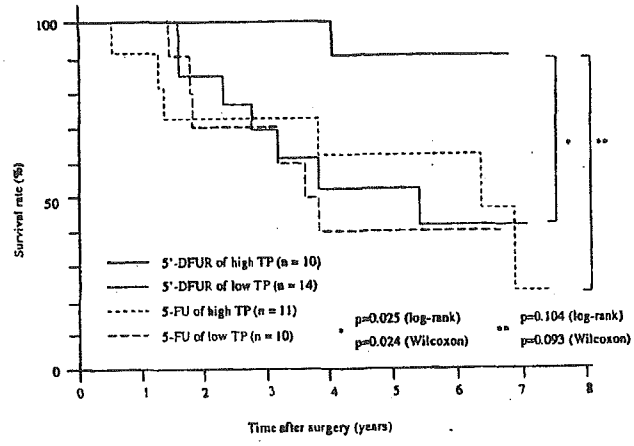

Figure 4. Overall survival curve between patients with high and low TP expression in Dukes' C staged colon cancer patients in the two chemotherapy groups. Estimated overall 5-year survival rates in patients with high and low TP expression were 91.2 and 74.8% in the 5'-DFUR group, respectively, and 82.2 and 71.1% in the 5-FU group, respectively. In the 5'-DFUR group, patients with high TP expression had significantly better survival than those with low TP expression. Of the patients with high TP expression, the 5'-DFUR group had slightly better survival than the 5-FU group.

TP patients, no difference in survival was observed between the 5'-DFUR and 5-FU groups (Fig. 4).

Discussion

TP activates 5'-DFUR to the active drug 5-FU by cleaving the 5-deoxyribose moiety, while by addition of 2-deoxyribose-1-phosphate TP can activate 5-FU to 5-fluoro-2'-deoxyuridine, a precursor of FdUMP, which inhibits thymidylate synthase, responsible for *de novo* thymidylate synthesis. Therefore, high levels of TP overexpression affect 5-FU sensitivity. However, several reports have demonstrated that high TP expression correlates with low sensitivity to 5-FU (17,18). Similarly, the present study was unable to demonstrate 5-FU efficacy in patients with high TP expression. We previously demonstrated that TP expression has no significant relationship with prognosis in gastric cancer patients treated with 5-FU, but that 5'-DFUR treated staged III gastric cancer patients with high tumor TP expression receive a significant survival benefit (19,20). The present study produced similar results, in that administration of 5'-DFUR contributed a higher survival benefit in Dukes' C staged colorectal cancer patients with high TP expression than those with low TP expression. In addition, slightly better survival was observed in the 5'-DFUR treated Dukes' C colon cancer patients than the 5-FU treated ones.

In the present study, a newly developed MAb, 1C6-203, was used for immunohistochemical analysis. In previous studies, MAb 654-1, which stained stromal cells stronger than cancer cells, was used, and thus TP expression was often evaluated by staining of cancer stromal cells not cancer cells (11-14,21,22). MAb 1C6-203 was raised against recombinant human TP, while MAb 654-1 was directed against human TP refined from a human colon cancer HCT 116 xenograft. The sensitivity of the new developed MAb 1C6-203 to colorectal cancer cells is 60%, whereas that of MAb 654-1 is 20% in 10% formalin-fixed specimens. In addition, this new antibody produced a 67% expression rate in stromal immune cells, while MAb 654-1 had a lower frequency of 47% in 10% formalin-fixed specimens (15). We also assessed TP expression in stromal cells (data not shown), and found that the relationships between TP expression and patient survival were similar to the results in cancer cells. Thus, MAb 1C6-203 appears more suitable than MAb 654-1 to assess TP expression in cancer cells, and contributes to the reliability of the results of the present study.

Our study demonstrated the clinical efficacy of 5'-DFUR in Dukes' C staged patients with high TP expression, particularly in colon cancer. In contrast to colon cancer, rectal cancer reportedly has different behavior in relapse, such as intrapelvic recurrence associated with or without anastomosis, even with the same histology and staging (23,24), and thus the operative outcome might significantly influence patient prognosis even if a curative resection was macroscopically performed. Thus, interpreting the results of postoperative adjuvant chemotherapy in rectal cancer patients requires caution, and it might be preferable to analyze only colon cancer patients if the chemotherapeutic responses are being strictly evaluated.

In general, the benefit from adjuvant chemotherapy has been clearly established in Dukes' C staged colon cancer patients. Mamounas *et al* (25) demonstrated the clinical efficacy of adjuvant chemotherapy in patients with Dukes' B staged colon cancer, as lymph node micro-metastasis, present in more than half the patients with Dukes' B staged colon cancer, significantly correlated with patient prognosis. Our study showed no survival difference in patients with Dukes' B staged colon cancer, but indicated the efficacy of 5'-DFUR in Dukes' C staged colon cancer patients with high TP expression, which supports the chemotherapeutic potential of 5'-DFUR as postoperative adjuvant treatment for advanced colon cancer.

TP is also an enzyme known as platelet-derived endothelial cell growth factor (26) or tumor-associated angiogenic factor (27), and correlates with tumor growth and metastasis not only in colorectal cancer but also in stomach and ovarian cancer (5,6,28,29). However, the relationship between TP expression and tumor malignant potential remains controversial. Studies have shown that TP expression in liver metastasis is higher than in primary colorectal cancer (30,31) and high TP expression is a risk factor for hematogenous metastasis in patients with Astler Coller B1/B2 colorectal cancer (17). In contrast to these studies, better survival in patients with high TP expression has been demonstrated in several studies (11,21). In the present study, no correlation was detected between TP expression and clinicopathologic features. This discrepancy was probably due to the various chemotherapy regimens or different analysis methods.

Based on these findings, immunohistochemical evaluation of TP expression might help predict patient response to oral 5'-DFUR. There is an increasing need for defining new factors that might be used to predict prognosis in colorectal cancer and its response to therapy. Several enzymes, such as thymidylate synthase, dihydropyrimidine dehydrogenase and orotate phosphoribosyl dehydrogenase, have been reported to be useful in predicting the sensitivity of colorectal cancer to 5-FU based chemotherapy (32,33). Hotta *et al* reported that TP level could be evaluated from preoperative endoscopic biopsy specimens (34). Establishing a predicting marker for chemo-sensitivity should contribute to effective neo-adjuvant chemotherapy in patients with high-risk of recurrence.

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References

1. International Multicentre Pooled Analysis of Colon Cancer Trials (IMPACT) Investigators: Efficacy of adjuvant fluorouracil and folic acid in colon cancer. Lancet 345: 939-944, 1995.
2. Hoff PM, Ansari R, Batist G, Cox J, Kocha W, Kupermic M, Maroun J, Walde D, Weaver C, Harrison E, Burger HU, Osterwalder B, Wong AO and Wong R: Comparison of oral capecitabine versus intravenous fluorouracil plus leucovorin as first-line treatment in 605 patients with metastatic colorectal cancer: result of a randomized phase III study. J Clin Oncol 19: 2282-2292, 2001.
3. Van Custem E, Twelves C, Cassidy J, Allman D, Bajetta E, Boyer M, Bugat R, Findlay M, Frings S, Jahn M, McKendrick J, Osterwalder B, Perez-Manga G, Rosso R, Rougier P, Schmiegel WH, Seitz JF, Thompson P, Vieitez JM, Weitzel C and Harper P: Oral capecitabine compared with intravenous fluorouracil plus leucovorin in patients with metastatic colorectal cancer: results of a large phase III study. J Clin Oncol 19: 4097-4106, 2001.
4. Takebayashi Y, Yamada K, Miyadera K, Sumizawa T, Furukawa T, Kinoshita F, Aoki D, Okumura Y, Yamada Y, Akiyama S and Aikou T: The activity and expression of thymidine phosphorylase in human solid tumors. Eur J Cancer 32A: 1227-1232, 1996.
5. Takebayashi Y, Akiyama S, Akiba S, Yamada Y, Murata F and Aikou T: Clinicopathologic and prognostic significance of an angiogenic factor, thymidine phosphorylase, in human colorectal carcinoma. J Natl Cancer Inst 88: 1110-1117, 1996.
6. Takebayashi Y, Miyadera K, Akiyama S, Hokita S, Yamada K, Akiba S, Yamada Y, Sumizawa T and Aikou T: Expression of thymidine phosphorylase in human gastric carcinoma. Jpn J Cancer Res 87: 288-295, 1996.
7. Toi M, Hoshina S and Taniguchi T: Expression of platelet-derived endothelial cell growth factor/thymidine phosphorylase in human breast cancer. Int J Cancer 64: 79-82, 1996.
8. Kono A, Hara Y, Sugata S, Karube Y, Matsushima Y and Ishitsuka H: Activation of 5'-deoxy-5-fluorouridine by thymidine phsphorylase in human tumors. Chem Pharm Bull 31: 175-178, 1983.
9. Yamaue H, Tanimura H, Kono N, Aoki Y, Tabuse K, Uchiyama K, Takifuji K, Iwahashi M and Tani M: Clinical efficacy of doxifluridine and correlation to *in vitro* sensitivity of anticancer drugs in patients with colorectal cancer. Anticancer Res 23: 2559-2564, 2003.
10. Nishimura G, Terada I, Kobayashi T, Ninomiya I, Kitagawa H, Fishida S, Fujimura T, Kayahara M, Shimizu K, Ohta T and Miwa K: Thymidine phosphorylase and dihydropyrimidine dehydrogenase levels in primary colorectal cancer show a relationship to clinical effects of 5'-deoxy-5-fluorouridine as adjuvant chemotherapy. Oncol Rep 9: 479-482, 2002.
11. Saito S, Tsuno N, Nagawa H, Sunami E, Zhengxi J, Osada T, Kitayama J, Shibata Y, Tsuruo T and Muto T: Expression of platelet-delived endothelial cell growth factor correlates with good prognosis in patients with colorectal carcinoma. Cancer 88: 42-49, 2000.
12. Takahashi Y, Bucana CD, Liu W, Yoneda J, Kitadai Y, Cleary KR and Ellis LM: Platelet-derived endothelial growth factor in human colon cancer angiogenesis: role of infiltrating cells. J Natl Cancer Inst 88: 1146-1151, 1996.
13. Haba A, Monden T, Sekimoto M, Ikeda K, Izawa H, Kanou T, Amano M, Kanayama H and Monden M: PyNPase expression in human colon cancer. Cancer Lett 122: 85-92, 1998.
14. *Shomori K, Sakatani T, Goto A, Matsuura T, Kiyonari T and Ito H: Thymidine phosphorylase expression in human colorectal mucosa, adenoma and carcinoma: role of p53 expression. Pathol Int 49: 491-499, 1999.*
15. Kono T, Nishida M, Hino A, Inagaki N, Tanaka Y, Yoneda M and Kasai S: *Development and characterization of 1C6-203, a new monoclonal antibody specific to human thymidine.* J Histochem Cytochem 49: 131-138, 2001.
16. Koda K, Miyazaki M, Sarashina H, Suwa T, Saito N, Suzuki M, Ogawa K, Watanabe S, Kodaira S and Nakazato H: A randomized controlled trial of postoperative adjuvant immunochemotherapy for colorectal cancer with oral medicines. Int J Oncol 23: 165-172, 2003.
17. Salonga D, Danenberg KD, Johnson M, Metzger R, Groshen S, Tsaowei DD, Lenz HJ, Leichman CG, Leichman L, Diasio RB and Danenberg PV: Colorectal tumors responding to 5-fluorouracil have low gene expression levels of dihydropyrimidine dehydrogenase, thymidylate synthase and thymidine phosphorylase. Clin Cancer Res 6: 1322-1327, 2000.
18. Yoshinare K, Kubota T, Watanabe M, Wada N, Nishibori H, Hasegawa H, Kitajima M, Takechi T and Fukushima M: Gene expression in colorectal cancer and *in vitro* chemosensitivity to 5-fluorouracil: a study of 88 surgical specimens. Cancer Sci 94: 633-638, 2003.
19. Ishii R, Takiguchi N, Oda K, Koda K and Miyazaki M: Thymidine phosphorylase expression is useful in selecting adjuvant chemotherapy for stage III gastric cancer. Int J Oncol 19: 717-722, 2001.
20. Takiguchi N, Ishii R, Koda K, Oda K and Miyazaki M: Thymidine phosphorylase expression correlate with malignant potential and anti-tumor effect of doxifluridine on gastric cancer: multivariate analysis for adjuvant chemotherapy doxifluridine vs. 5-fluorouracil. Oncol Rep 10: 1105-1111, 2003.
21. Tsuji T, Sawai T, Yamashita H, Takeshita H, Nakagoe T, Shindou H, Fukuoka H, Yoshinaga M, Hidaka S, Yasutake T, Nagayasu T and Tagawa Y: Platelet-derived endothelial cell growth factor expression is an independent prognostic factor in colorectal cancer patients after curative surgery. Eur J Surg Oncol 30: 296-302, 2004.
22. Matsumura M, Chiba Y, Lu C, Amaya H, Shimomatsuya T, Horiuchi T, Muraoka R and Tanigawa N: Platelet-derived endothelial cell growth factor/thymidine phosphorylase expression correlated with tumor angiogenesis and macrophage infiltration in colorectal cancer. Cancer Lett 128: 55-63, 1998.

23. Havenga K, Enker WE, Norstein J, Moriya Y, Heald RJ, van Houwelingen HC and van de Velde CJ: Improved survival and local control after total mesorectal excision or D3 lymphadenectomy in the treatment of primary rectal cancer: an international analysis of 1411 patients. Eur J Surg Oncol 25: 368-374, 1999.
24. Hermanek P and Hermanek PJ: Role of the surgeon as a variable in the treatment of rectal cancer. Semin Surg Oncol 19: 329-335, 2000.
25. Mamounas E, Wieand S, Wolmark N, Bear HD, Atkins JN, Song K, Jones J and Rockette H: Comparative efficacy of adjuvant chemotherapy in patients with Dukes' B versus Dukes' C colon cancer: results from four national surgical adjuvant breast and bowel project adjuvant studies (C-01, C-02, C-03 and C-04). J Clin Oncol 17: 1349-1355, 1999.
26. Furukawa T, Yoshimura A, Sumizawa T, Haraguchi M and Akiyama S: Angiogenic factor. Nature 356: 668, 1992.
27. Ishikawa F, Miyazono K and Hellman U: Identification of angiogenic activity and cloning and expression of platelet-derived endothelial cell growth factor. Nature 338: 557-562, 1989.
28. Raynolds K, Farazaneh F, Collins WP, Campbell S, Bourne TH, Lawton F, Monghaddam A, Harris AL and Bicknell R: Correlation of ovarian malignancy with expression of platelet-derived endothelial cell growth factor. J Natl Cancer Inst 86: 1234-1238, 1994.
29. Maeda K, Chung Y, Ogawa Y, Takatsuka S, Kang SM, Ogawa M, Sawada T, Onoda N, Kato Y and Sawa M: Thymidine phosphorylase/platelet-derived endothelial cell growth factor expression associated with hepatic metastasis in gastric carcinoma. Br J Cancer 73: 884-888, 1996.
30. Collie-Duguid ES, Johnston SJ, Boyce L, Smith N, Cowieson A, Cassidy J, Murray GI and McLeod HL: Thymidine phosphorylase and dihydropyrimidine dehydrogenase protein expression in colorectal cancer. Int J Cancer 94: 297-301, 2001.
31. Inokuchi M, Uetake H, Shirota Y, Yamada H, Tajima M and Sugihara K: Gene expression of 5-fluorouracil metabolic enzymes in primary colorectal cancer and corresponding liver metastasis. Cancer Chemother Pharmacol 53: 391-396, 2004.
32. Farrugia DC, Ford HE, Cunningham D, Danenberg KD, Danenberg PV, Brabender J, McVicar AD, Aherne GW, Hardcastle A, McCarthy K and Jackman AL: Thymidylate synthase expression in advanced colorectal cancer predicts for response to raltitrexed. Clin Cancer Res 9: 792-801, 2003.
33. Van Halteren HK, Peters HM, van Krieken JH, Coebergh JW, Roumen RM, van der Worp E, Wagener JT and Vreugdenhil G: Tumor growth pattern and thymidine phosphorylase expression are related with the risk of hematogenous metastasis in patients with Astler Coller B1/B2 colorectal carcinoma. Cancer 91: 1752-1757, 2001.
34. Hotta T, Taniguchi K, Kobayashi Y, Johata K, Sahara M, Naka T, Watanabe T, Ochiai M, Tanimura H and Tsubota YT: Preoperative endoscopic analysis of thymidine phosphorylase and dihydropyrimidine dehydrogenase in gastrointestinal cancer. Oncol Rep 11: 1233-1239, 2004.

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新しい肝区域概念に基づいた肝前背側区域切除

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永井書店

手術手技

新しい肝区域概念に基づいた肝前背側区域切除

Anterodorsal segmentectomy based on reclassification of the anterior segment of the liver

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趙らの肝前区域を腹側区域と背側区域に分ける概念に基づいて，肝門部で流入血管を先行処理した後，背側区域の単独切除を2例に実施したので，報告する．肝尾側面を縦切開，開放し，前区域グリソン鞘の背面の視野を良好に保ちながら，背側のグリソン枝を尾側から順次，結紮，切離した後，阻血域に沿った肝離断を行い，確実な背側区域切除が実施可能であった．

はじめに

Couinaudの肝区域の概念では前区域は上下のS8，S5に2分されており[1]，この概念が肝臓外科医に広く，受け入れられている．しかし，造影CTによる肝内門脈の分岐様式の分析から，肝前区域門脈の分岐は腹側枝と背側枝に分けられ，その分布する領域をそれぞれ，前腹側区域と前背側区域として捉える方が臨床的に妥当であることを趙らが報告してきた[2)-6)]．今回われわれはその概念に沿って，肝門部で流入血管処理を先行した後，背側区域の単独切除を実施したので，その手術手技を報告する．

I. 症　例

症例 1

子宮癌の転移巣を肝前背側区域に認めた(図1)．

手術手技

①胆嚢摘出後，右，前区域，後区域グリソン鞘(GR，GA，GP)をテーピング．②肝右葉の脱転．③肝尾側面で胆嚢床右端より約1cm右側を切開(図2)，開放し，GA背側に沿って肝切離を進めた．④前背側枝グリソン鞘(GAd)の尾側の1本を確認し(図3)，これを一時的に阻血し，肝表面の変色域が腫瘍付近にあることを確かめた後，結紮切離．⑤さらに背側の剝離を進め，頭背側に向かう枝を確認，これも腫瘍付近を支配する枝であることを確かめた後，結紮(図4)．⑥肝表面の変色域(図5)を電気メスでマーキングし，肝切離線を決定．⑦変色域の腹側(左側)から肝門に向かって肝切離を開始．⑧肝門部に達したら，結紮のみを行っていたGAdの枝の結紮糸を肝切離面より腹側に引き出し(図6)，切離．⑨肝切離を尾背側に進め，右肝静脈本幹を露出．切除領域から右肝静脈に入る数本の枝を処理し，右肝静脈に沿って

千葉県がんセンター消化器外科 *聖隷佐倉市民病院外科 **千葉県立佐原病院外科
Key words：肝切除／肝区域／前区域／前背側区域

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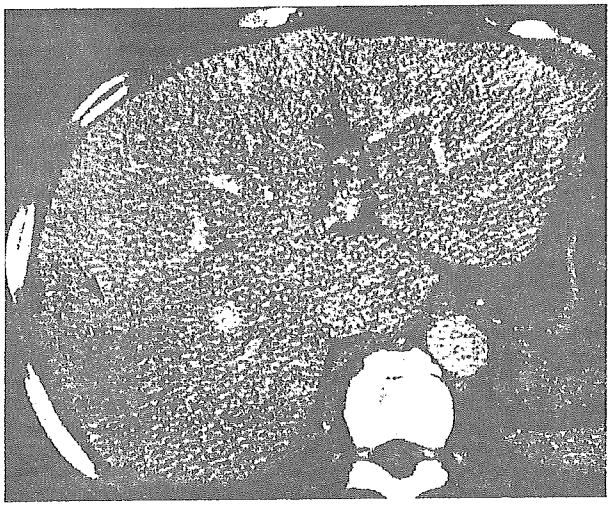

図1 症例1のCT像
矢印：腫瘍

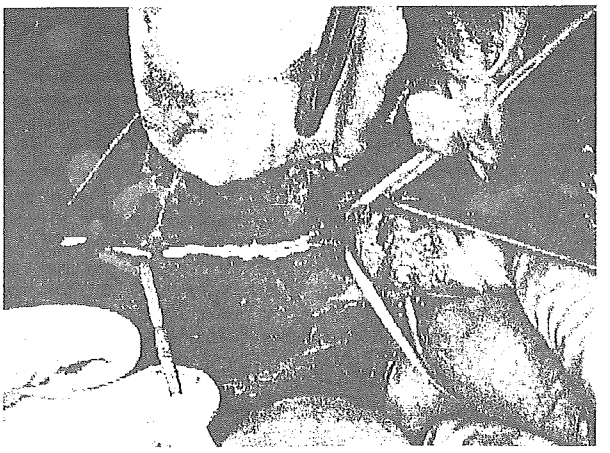

図2 電気メスで肝尾側面に切開を加える

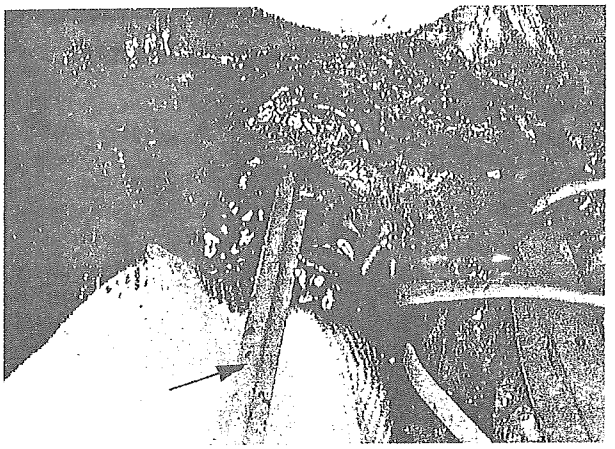

図3 GAdの尾側の枝
矢印のテープで保持されている.

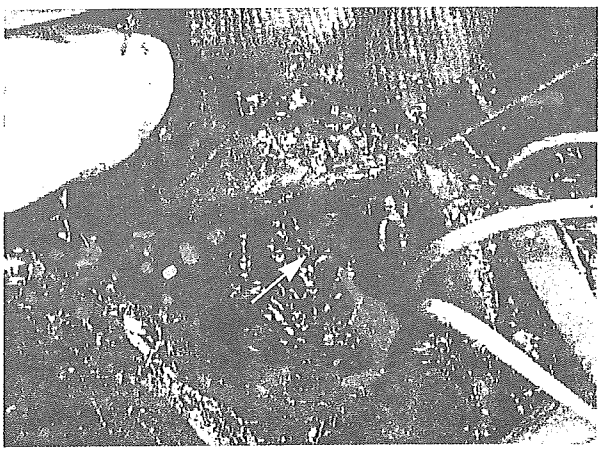

図4 GAdの頭側の枝(矢印)
結紮糸で牽引されている.

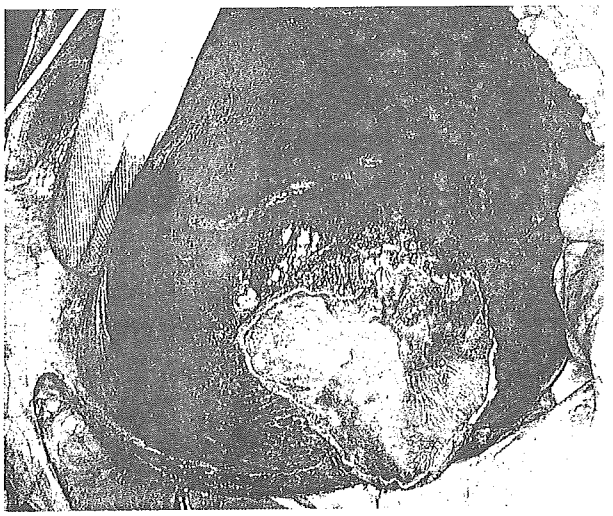

図5 GAdを結紮後の肝表面の変色域
本症例では横隔膜浸潤があり横隔膜部分切除を付加した.

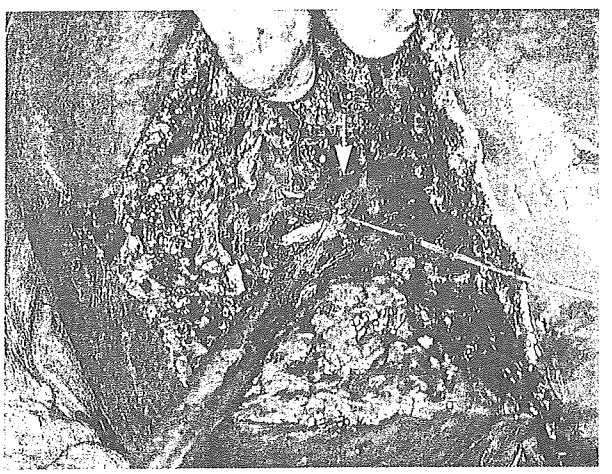

図6 GAdの頭側の枝(矢印)
結紮糸が腹側に引き出されている.

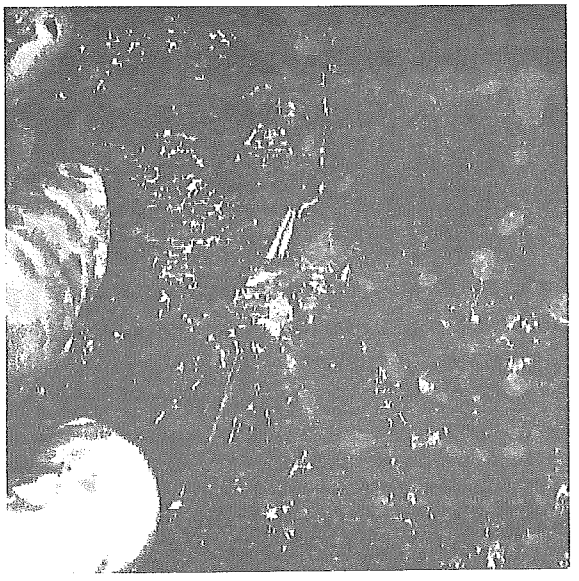

図7　前背側区域の静脈枝に右肝静脈流入部で剝離鉗子が通されている.

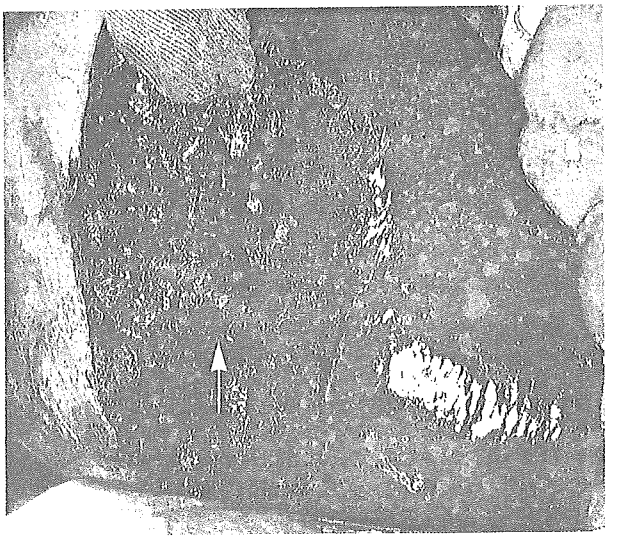

図8　標本摘出後の断端部
矢印：右肝静脈

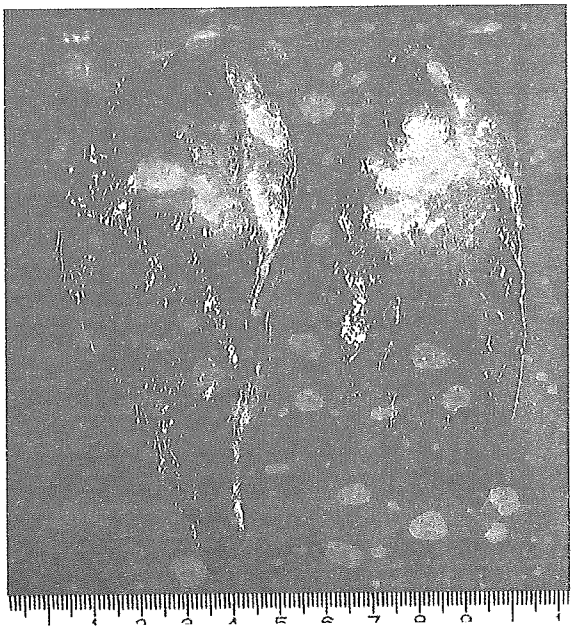

図9　症例1の摘出標本割面像

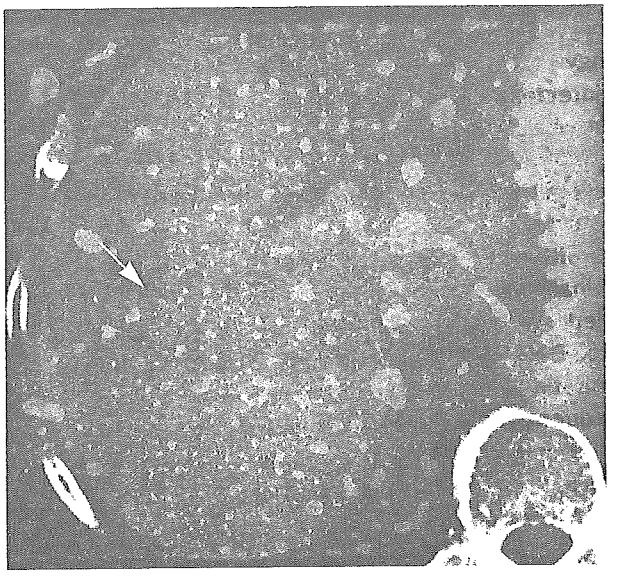

図10　症例2のCT像
矢印：腫瘍

肝切離を頭側に進めた．⑩右肝静脈に流入する前背側区域をドレナージする太い静脈枝を右肝静脈流入部で結紮切離(図7)．⑪切除する前背側区域の右側の境界に向かって右肝静脈の走行面で肝切離を行い，標本を摘出(図8，9)．

症　例　2

肝細胞癌を肝前背側区域に認め，前区域，後区域門脈の間に存在した(図10)．

手術手技

①本症例においても症例1で示した手順に沿って手術を進めた．②肝尾側面での切開を広げ，GA の背側に沿って肝切離を進め，GAd の枝を

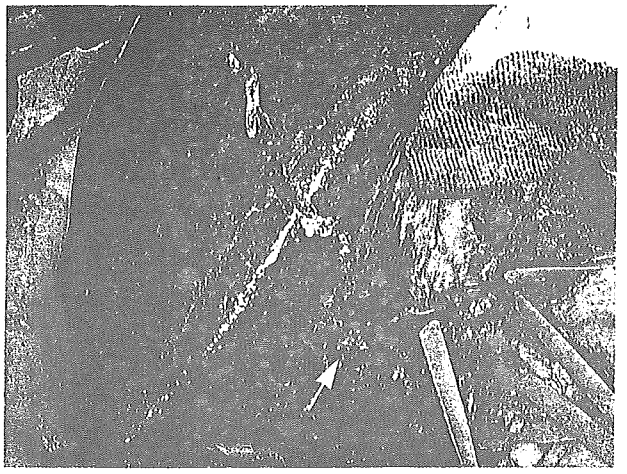

図11 GAd の枝の結紮によって変色域が anterior fissure の右尾側から認められ，変色域の左側で切離を始めた．
矢印：肝尾側面の切開創

図12 変色域が帯状に頭側に広がっている．

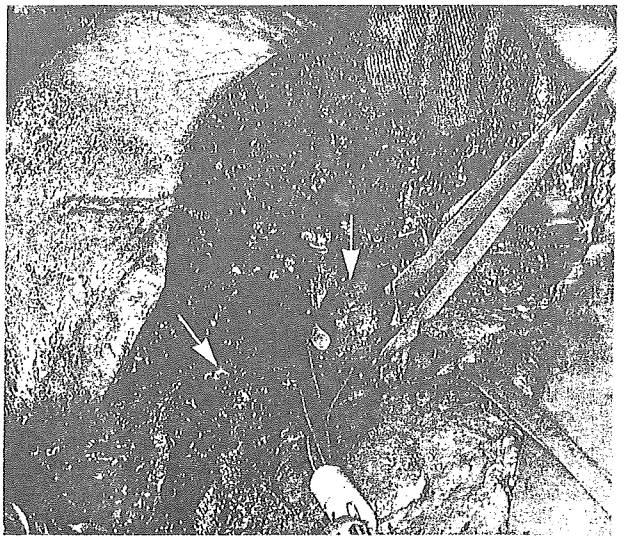

図13 標本摘出後の断端部を左右に開いた時の写真
矢印左：右肝静脈
矢印右：前枝グリソン

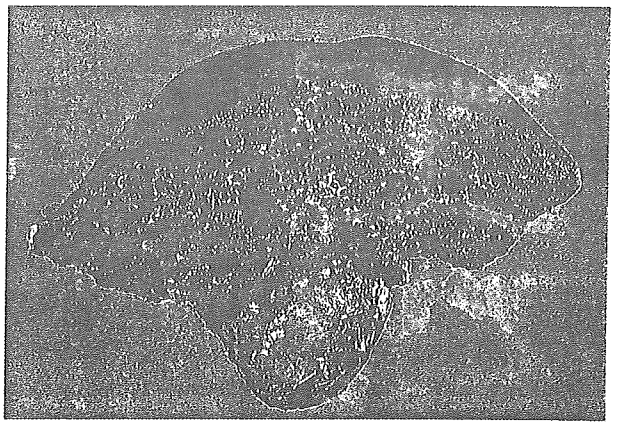

図14 症例2の摘出標本

確認し，尾側の枝から結紮していく．(本症例においては数本の枝を確認)．③本症例においては症例1と異なって，GAd の処理によって変色域が肝尾側面で最初の切開線の右側に認められ，変色域の左側で切離を腹側に進めた(図11)．④頭側の枝を順次処理することによって肝表面の変色域が頭側に広がり，その左側の切離を頭側に進めた(図12)．⑤変色域の右側の肝切離を開始．⑥右肝静脈の枝の処理は症例1と同様に進め，標本を摘出(図13，14)．

II. 考　　察

趙らは肝前区域を上下のS8とS5の亜区域に分類するよりも，腹側，背側区域の2つに分類する方が合理的であることを主張してきた[2)-6)]．門脈の分岐形態の分析を基にする考え方であり，前区域門脈枝はP8とP5ではなく，腹側枝と背側枝に分けられるとするものである．P8がほぼ腹側枝と背側枝に2分岐し，P5は前区域門脈本幹ある

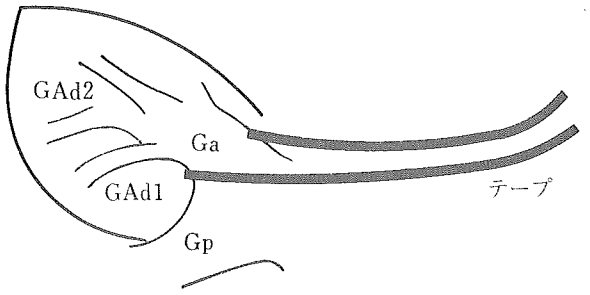

図15 前背側グリソン枝の処理
症例1においてはGad1 Gad2の2本の主たる前背側グリソン枝が結紮切離された.

いは，P8腹側枝から分岐していることが多いことから，前腹側区域がS8腹側域＋S5，前背側区域がS8背側域にほぼ相当するとした．

Kogure[7]らは解剖で得られた肝標本で門脈枝，肝静脈枝を分析し，Hjortsjoの提唱した，肝左葉のumbilical fissureに相当する右葉のlongitudinal portal fissure(前腹側区域と前背側区域を境する)が存在することを報告している．趙らはそれをanterior fissureと呼称し，その離断によって，肝実質内に存在するGAへのアプローチが容易になると報告している[8]．今回の症例で，肝尾側面に入れた切開部(図2）がanterior fissure尾側面に相当することになる．

また，趙らは区域概念にわずかな修正を加え，前腹側区域はS8の腹側域＋S5の大部分，前背側区域はS8の背側部＋S5の一部とした[8]．前背側区域尾側，つまり，anterior fissureの右尾側領域はS5の一部ということになる．症例1および2では，同様にGAの背側に確認できるグリソン鞘の枝をGAdの枝として尾側から処理していくという方法で手術を進めたが，症例1においては前背側区域の尾側領域が阻血にならず，その部分は標本に含まれなかった(図8)．一方，症例2においては尾側領域が阻血され，その部分も標本に含まれた(図13)．この結果から前背側区域の尾側端領域がほとんどないか，あってもわずかである場合があることを示していると推測される．

前背側区域切除のポイントは，いかにGAの背側面の視野を良く保つかである．anterior fissureの肝尾側面を切開，開放し，テーピングしたGAを右腹側に牽引することにより，GAの背面の視野が良好になり，背側に確認できるGAdの枝を尾側から確実に結紮切離していくことが可能となる(図3，4，15)．

肝前区域は非常に広い領域であり，前区域を主座とする腫瘍の肝切除を想定した時，肝機能の制約から切除領域を絞り込まなければならない場合がある．背側区域にある腫瘍は通常，従来の考えからするとS8に存在する腫瘍と判断され，門脈に色素を注入するなどして切除領域を決め，肝表面の離断から切除が始まる場合が多い．しかし，背側区域に分布するGAdの枝が症例2のように数本に及ぶ症例もあり，今回の報告のように肝門部からGAdを確認しながら処理する方が確実な系統的肝切除になりうると考えられる．

文　献
1) Couinaud C: Surgical anatomy of the liver revisited: Couinaud C, Paris, 1989.
2) Cho A, Okazumi S, Takayama W, et al: Anatomy of the right anterosuperior area (segment 8) of the liver: evaluation with helical CT during arterial portography. Radiology 214: 491-495, 2000.
3) 趙　明浩，竜　崇正，岡田　正ほか：新しい肝区域概念に基づいた肝S5切除．手術　56：627-630，2002.
4) 竜　崇正，趙　明浩，高山　亘ほか：新しい肝区域概念に基づいた肝前腹側上亜区域切除S5切除．手術　57：727-730，2003.
5) 趙　明浩，岡住慎一，牧野治文ほか：系統的拡大肝左葉切除術．手術　57：599-601，2003.
6) Cho A, Okazumi S, Makino H, et al: Relation between hepatic and portal veins in the right paramwdian sector: Proposal for anatomical reclassification of the liver. World J Surg 28: 8-12, 2004.
7) Kogure K, Kuwano H, Fujimaki N, et al: Reproposal for Hjortsjo's segmental anatomy on the anterior segment in human liver. Arch Surg 137: 1118-1124, 2002.
8) 趙　明浩，岡住慎一，三浦文彦ほか：新しい肝区域概念に基づいた拡大肝後区域切除術．手術　58：521-523，2004.
9) Cho A, Okazumi S, Miyazawa Y, et al: Limited resection of the right hemiliver based on reclassification of the right anterior segment of the liver. Hepatogastroenterology, 51: 820-821, 2004.

A New Method for Isolating Colonocytes From Naturally Evacuated Feces and Its Clinical Application to Colorectal Cancer Diagnosis

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Background & Aims: The early detection of colorectal cancer is desired because this cancer can be cured surgically if diagnosed early. The purpose of the present study was to determine the feasibility of a new methodology for isolating colonocytes from naturally evacuated feces, followed by cytology or molecular biology of the colonocytes to detect colorectal cancer originating from any part of the colorectum. *Methods:* Several simulation studies were conducted to establish the optimal methods for retrieving colonocytes from any portion of feces. Colonocytes exfoliated into feces, which had been retrieved from 116 patients with colorectal cancer and 83 healthy volunteers, were analyzed. Part of the exfoliated colonocytes was examined cytologically, whereas the remainder was subjected to DNA analysis. The extracted DNA was examined for mutations of the APC, K-*ras*, and p53 genes using direct sequence analysis and was also subjected to microsatellite instability (MSI) analysis. *Results:* In the DNA analysis, the overall sensitivity and specificity were 71% (82 of 116) of patients with colorectal cancer and 88% (73 of 83) of healthy volunteers. The sensitivity for Dukes A and B was 72% (44 of 61). Furthermore, the sensitivity for cancers on the right side of the colon was 57% (20 of 35). The detection rate for genetic alterations using our methodology was 86% (80 of 93) when the analysis was limited to cases in which genetic alterations were present in the cancer tissue. *Conclusions:* We have developed a new methodology for isolating colonocytes from feces. The present study describes a promising procedure for future clinical evaluations and the early detection of colorectal cancers, including right-side colon cancer.

Colorectal cancer is one of the most common malignancies worldwide. In Japan, colorectal cancer is the third and second leading cause of death from cancer in men and women, respectively.[1] However, colorectal cancer is curable by surgical resection if diagnosed at a sufficiently early stage. This incentive has prompted investigators to develop new methods enabling the early diagnosis of colorectal cancer and has led to the introduction of cancer screening programs in many countries. For mass cancer screenings, a simple, economic, and noninvasive method of cancer detection is desired. The Hemoccult test is currently used in many countries for this purpose.[2–6] However, this test is nonspecific and is not sufficiently sensitive to detect early stage colorectal cancer, although a higher sensitivity has been reported for advanced-stage colorectal cancer.[7] Radioimmunoassays using tumor markers, such as carcinoembryonic antigen, also are not suitable for the detection of early cancer, although such tests can be used to monitor patients for an increasing tumor burden or tumor recurrence. Diagnosis by barium enema study and fiberoptic colonoscopy is accurate but time-consuming, expensive, and invasive. Therefore, an urgent need exists to establish a sensitive, reliable, and noninvasive method for the detection of colorectal cancer at an early stage.

To date, several screening methods for colorectal cancer based on the detection of mutated DNA in feces have been reported.[8–20] These methods, however, are time-consuming and are not sufficiently sensitive. The major reason for this inaccuracy is the fact that

Abbreviations used in this paper: APC, adenomatous polyposis coli; MSI, microsatellite instability; OMIM, Online Mendelian Inheritance in Man.

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nucleic acids in feces are derived from an enormous number and variety of bacteria and normal cells. Accordingly, the proportion of genes derived from cancer cells in feces is as low as 1%, at most.[9] This makes the application of gene-detecting methods difficult in clinical practice.

We previously reported that the expression of CD44 variants in exfoliated colonocytes isolated from feces according to the Percoll centrifugation method could serve as a noninvasive diagnostic marker for early colorectal cancer.[21] However, the repetition of the Percoll centrifugation method was found to distort the morphology of the exfoliated colonocytes. Accordingly, the sensitivity of this method also appeared to be unsatisfactory because of the low retrieval rate of the exfoliated colonocytes. Another study described a processing method that involved scraping or washing the stool's surface with a buffer to collect exfoliated colonocytes.[22] In the ascending colon, however, the feces remains unformed. Therefore, most cancer cells exfoliated from the walls of the ascending colon would be incorporated into the inner core of the feces during the course of its formation. Thus, recovering cancer cells that originated from the ascending colon might be difficult using methods that involve scraping or washing solid feces.

Under these circumstances, we succeeded in developing a new, very effective methodology that allows the simple isolation of exfoliated colonocytes from not only the surface but also the central portion of feces while maintaining the colonocytes' initial morphology. Currently, we are attempting to apply a molecular biologic tool to purified colonocytes exfoliated into feces to detect cells from early colorectal cancers, including right-side colon cancer.

Table 1. Characteristics of Patients and Healthy Volunteers

Characteristic	Patient (N = 116)	Healthy volunteer (N = 83)
Age, *y*		
Mean	62.0	58.4
Range	32–82	40–70
Sex, no (%)		
Male	69 (59.5)	37 (44.6)
Female	47 (40.5)	46 (55.4)
DNA, ng/gram of stool		
Mean	570.8	175.3
Range	2.0–7462.8	0.2–1907.5
Tumor location, no (%)		
Cecum	6 (5.2)	
Ascending colon	23 (19.8)	
Transverse colon	6 (5.2)	
Descending colon	7 (6.0)	
Sigmoid colon	21 (18.1)	
Rectum	53 (45.7)	
Size, mm		
Mean	40.0	
Range	4.0–120.0	
Histology, no (%)		
W/D	55 (47.4)	
M/D	56 (48.3)	
P/D	2 (1.7)	
Mucinous carcinoma	2 (1.7)	
Carcinoid tumor	1 (0.9)	
Depth, no (%)		
T1	10 (8.6)	
T2	32 (27.6)	
T3	71 (61.2)	
T4	3 (2.6)	
Dukes' stage, no (%)		
A	30 (25.9)	
B	31 (26.7)	
C	53 (45.7)	
D	2 (1.7)	

W/D, Well-differentiated adenocarcinoma; M/D, moderately differentiated adenocarcinoma; P/D, poorly differentiated adenocarcinoma.

Materials and Methods

Study Design

This was a prospective study conducted between December 2002 and August 2004. The study protocol was reviewed and approved by the Institutional Review Board of the National Cancer Center, Japan. Written informed consent was obtained from all patients and healthy volunteers. No modifications to the protocol procedures were made during the course of the study.

Study Population

A total of 116 patients with histologically confirmed colorectal cancer and 83 healthy volunteers were enrolled. The healthy volunteers consisted of 37 men and 46 women with no apparent abnormalities, such as adenoma or carcinoma (including hyperplastic polyps), found during a total colonoscopy performed at the National Cancer Center Research Center for Cancer Prevention and Screening. The median age of these volunteers was 58.4 years (range, 40–70 years). The characteristics of the patients and healthy volunteers are summarized in Table 1. All the patients with colorectal cancer had undergone surgical resection of their primary tumor at the National Cancer Center Hospital, Tsukiji, or at Hospital East, Kashiwa, Japan. The median age of the patients was 62.0 years (range, 32–82 years). There were 69 men and 47 women patients. The primary tumors were located in the following sites: rectum in 53 patients, sigmoid colon in 21 patients, descending colon in 7 patients, transverse colon in 6 patients, ascending colon in 23 patients, and cecum in 6 patients. The clinical stage of the patients according to Dukes' classification was as follows: Dukes' stage A in 30 patients, stage B in 31 patients, stage C in 53 patients, and stage D in 2 patients.

Stool Samples

Before surgical resection, stool samples were obtained from 116 patients with colorectal cancer. Stool sam-