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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1 cm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

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Key words：肝切除／肝区域／前区域／前背側区域

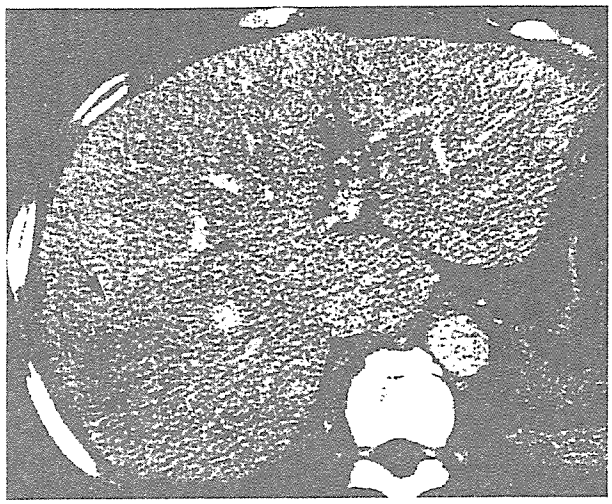

図1 症例1のCT像
矢印：腫瘍

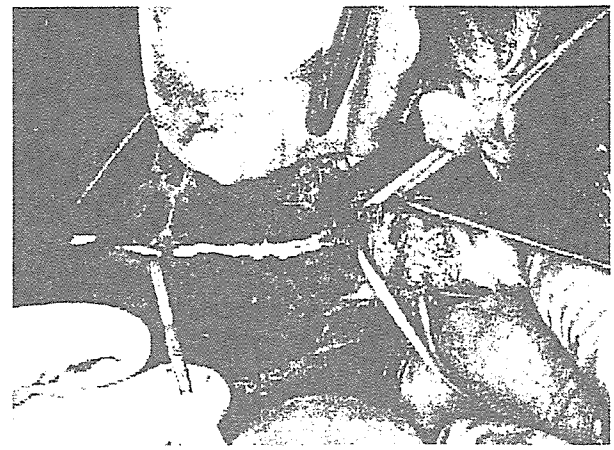

図2 電気メスで肝尾側面に切開を加える

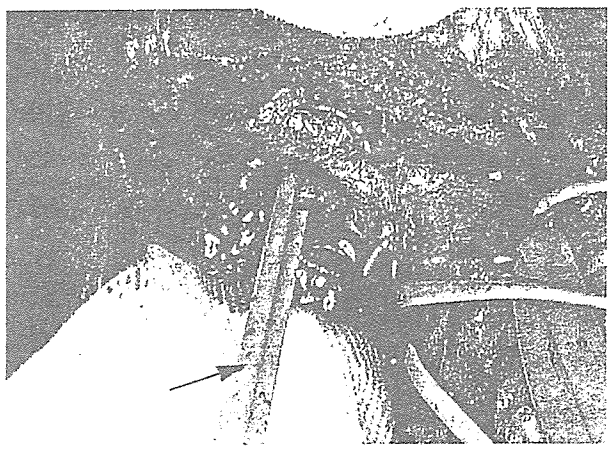

図3 GAdの尾側の枝
矢印のテープで保持されている.

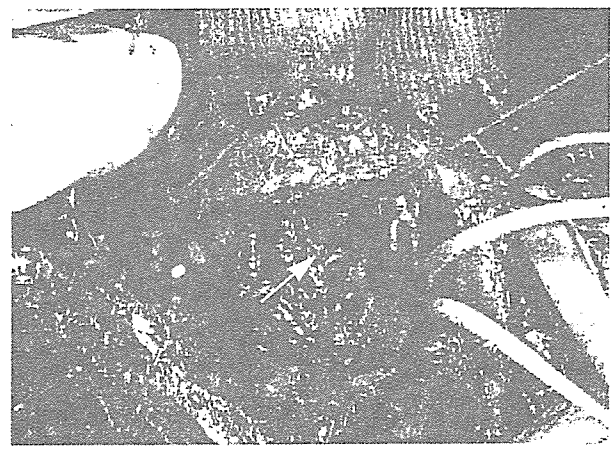

図4 GAdの頭側の枝(矢印)
結紮糸で牽引されている.

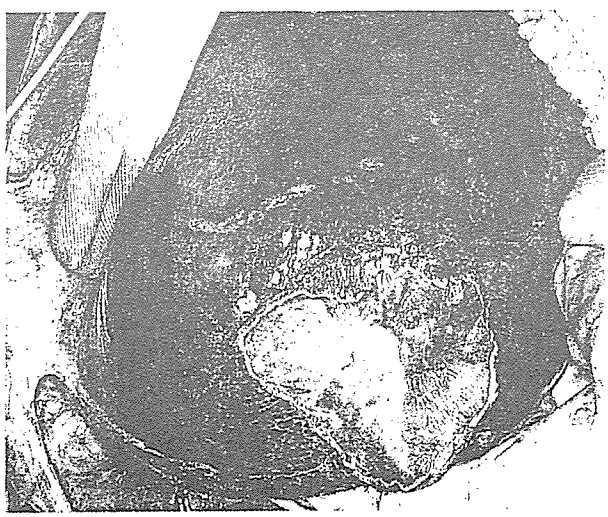

図5 GAdを結紮後の肝表面の変色域
本症例では横隔膜浸潤があり横隔膜部分切除を付加した.

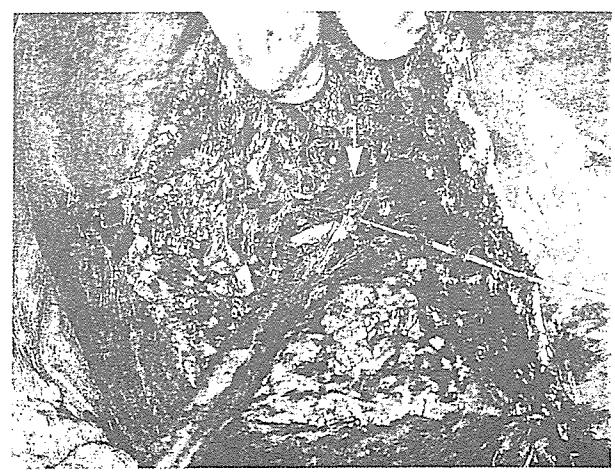

図6 GAdの頭側の枝(矢印)
結紮糸が腹側に引き出されている.

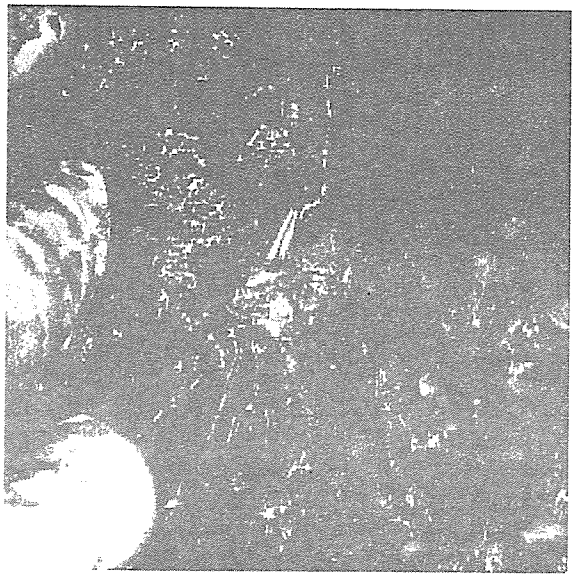

図7　前背側区域の静脈枝に右肝静脈流入部で剝離鉗子が通されている.

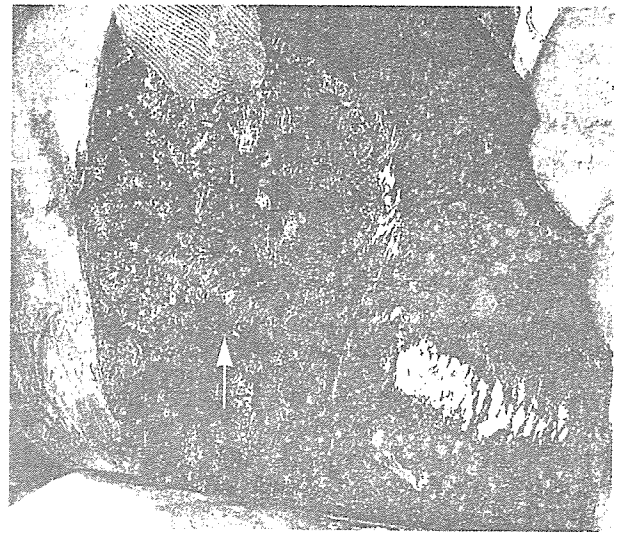

図8　標本摘出後の断端部
矢印：右肝静脈

図9　症例1の摘出標本割面像

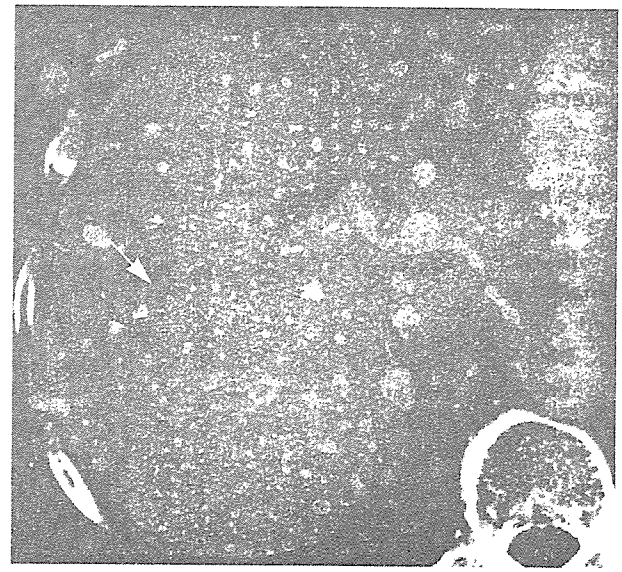

図10　症例2のCT像
矢印：腫瘍

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

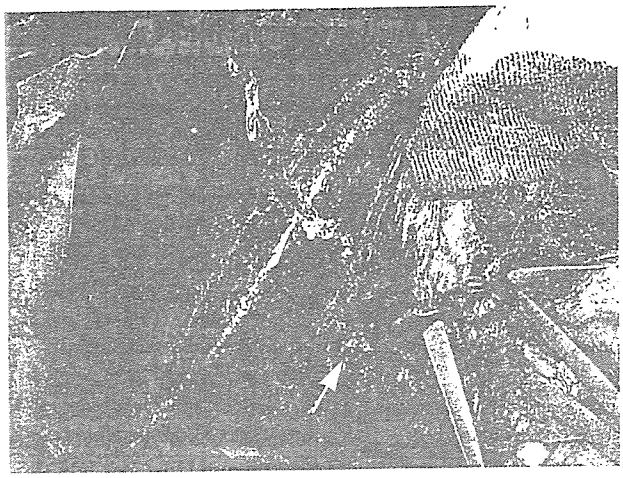

図11 GAd の枝の結紮によって変色域が anterior fissure の右尾側から認められ，変色域の左側て切離を始めた．
矢印：肝尾側面の切開創

図12 変色域が帯状に頭側に広がっている．

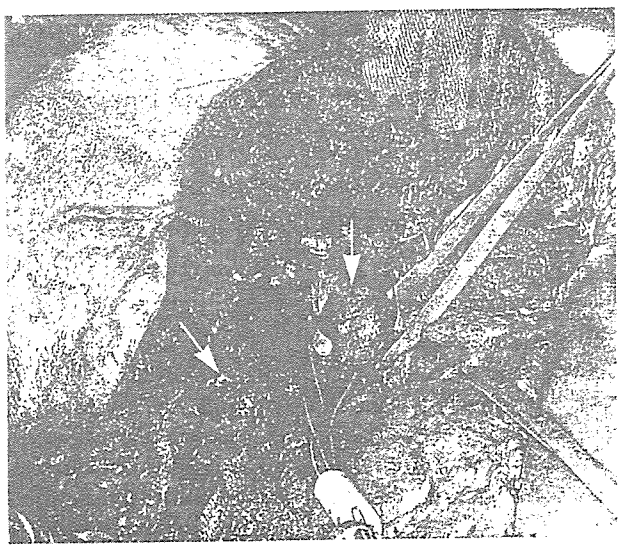

図13 標本摘出後の断端部を左右に開いた時の写真
矢印左：右肝静脈
矢印右：前枝グリソン

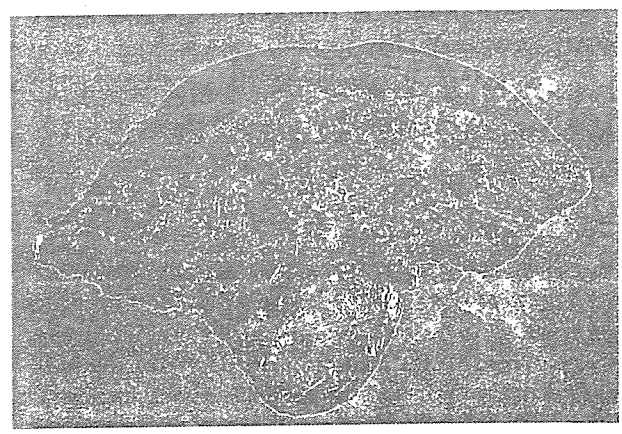

図14 症例 2 の摘出標本

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

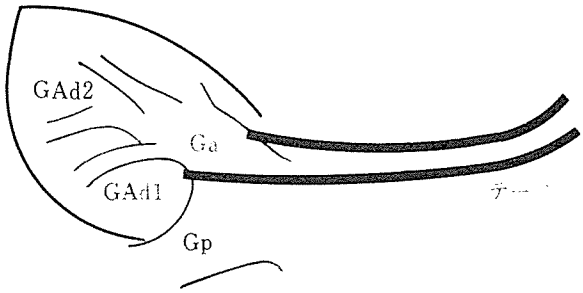

図15 前背側クリソン枝の処理
症例1においてはGad1 Gad2の2本の主たる前背側クリソン枝が結紮切離された.

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.

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Original Paper

Postsurgical Surveillance for Recurrence of UICC Stage I Colorectal Carcinoma: Is Follow-up by CEA Justified?

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KEY WORDS: UICC stage I colorectal carcinoma; Follow-up; Surveillance; CEA

ABBREVIATIONS: Carcinoembryonic Antigen (CEA)

ABSTRACT

Background/Aims: This study was undertaken to investigate whether it will be possible to reduce the times and types of postoperative examinations for surveillance in patients with UICC stage I colorectal carcinoma. In addition, the value of CEA in postoperative surveillance is discussed.

Methodology: A review was performed of 541 patients who underwent curative resection for UICC stage I colorectal carcinoma between January, 1985 and December, 1998. Periodic check-up was routinely conducted to identify recurrence.

Results: The median follow-up was 82 months. The recurrence rate was 2.9% in the UICC stage Ia (pT1N0M0) group, and 5.6% in the Ib (pT2N0M0) group. Cancer-specific survival rates at 5 years were 99.3% and 97.6%, respectively ($p=0.0354$). Recurrences occurred more frequently in patients with lower rectal carcinoma ($p=0.0415$). Curative-intent salvage surgery was performed in 61.9% (13/21) for recurrent lesions. Between the patients who were CEA positive (13/21; 61.9%) and those who were CEA negative at the time of recurrence, there was no significant difference in the prognosis.

Conclusions: The incidence of recurrence was low after curative surgery in patients with UICC stage I colorectal carcinoma, and it is therefore possible to reduce times and types of postoperative examinations. CEA measurement alone appears to be sufficient.

INTRODUCTION

Currently, a main topic for discussion with regard to the surveillance after colorectal carcinoma surgery is whether intensive follow-up for detecting recurrence earlier and initiating the treatment of it practically contributes to the improvement in prognosis for colorectal carcinoma patients. In nonrandomized cohort studies and randomized studies, significant differences in the time of confirming recurrence, the surgical resectability of recurrent lesion, and the 5-year survival rate between intensive follow-up group and control group (traditional follow-up or no follow-up group) were reported (1-5). At the same time, there are other studies that have reported no significant difference in these points (6-12). However, in those previous studies, the numbers of cases that were reviewed ranged from 98 to 1247, and there were a variety of disease stages from UICC stages I through IV. One study reported that although the resectability after recurrence was higher by more than 10% in an intensive follow-up group than in the control group, no significant difference was obtained, probably due to the small number of cases (13). In two studies using meta-analysis that were reported lately, the 5-year survival rates were 9% to 14% greater in the intensive follow-up group than in the control group (14,15).

Recently, advances in diagnostic techniques have enabled the detection of colorectal carcinoma at earlier stages in Japan (16). At our institution, the proportion of UICC stage I cases in all colorectal carcinoma patients receiving the first-line treatment was 14% (12/86) in 1980, but it increased to 25% (71/284) in 2000. It is important to conduct a cost-effective follow-up in view of the risk for recurrence (17,18). In fact, for UICC stage I colorectal carcinoma patients, the rate of recurrence is lower, and hence fewer times and screening examinations may be reasonable and warranted for the postoperative surveillance, compared with UICC stages II-IV colorectal carcinoma patients (19).

In the present study, we utilized the prospective follow-up database at a single institution to analyze the long-term outcomes of UICC stage I colorectal carcinoma patients, and to investigate whether it will be possible to reduce the times and types of screening examinations for postoperative surveillance. In addition, the present study discusses the value of CEA (carcinoembryonic antigen) in performing surveillance after curative surgery for UICC stage I colorectal carcinoma.

METHODOLOGY

Between January, 1985 and December, 1998,

Hepato-Gastroenterology 2005; 52:444-449

2,550 primary colorectal carcinoma patients were treated at our institution. Patient information and follow-up data were prospectively collected and added to the department database. Of those patients, the present study selected 541 (21.2%) cases of UICC stage I colorectal carcinoma undergoing curative resection combined with surgical lymph node clearance, in order to review the time and form of recurrence, the changes in CEA levels at recurrence, and the rate of re-resectability. For analysis, the 541 cases of UICC stage I colorectal carcinoma were divided into two groups: 313 patients with stage Ia colorectal carcinoma (pT1N0M0) and 228 patients with stage Ib colorectal carcinoma (pT2N0M0).

In terms of the follow-up of a patient with stage I colorectal carcinoma, we routinely conducted a periodic check-up every six months until two years after the operation, and subsequently once per year from the 3rd to 5th postoperative year. Clinical examination, abdominal ultrasound, and CEA measurement were performed at each visit, and chest X-ray was performed once per year. CEA was defined as positive when the level was increased above the cut-off value. Colonoscopy or barium enema was conducted once within one year of the first surgery, and was repeated at intervals of one to two years depending on the findings of the prior examination. When a patient complained of a symptom that suggested recurrence or had an increased level of CEA without symptoms, we employed other types of examinations in addition to the periodic check-up.

The clinicopathologic parameters were compared using Student's t test and the Fisher's exact test as appropriate. Cancer-specific survival curves and disease-free survival curves were estimated using the Kaplan-Meier technique and were compared by means of the log-rank test. For cancer-specific survival, only cancer-related deaths were considered; data on the patients who died from other causes or who were still alive at the end of the study were censored. A P value of less than 0.05 was considered significant.

TABLE 1 Patient's Characteristics

		UICC stage Ia patients	UICC stage Ib patients	P value
Number of patients		313	228	
Sex ratio (Male:Female)		201:112	129:99	0.0750
Age (yr; mean and range)		60.7 (33-88)	62.0 (23-91)	0.1641
Location	Cecum	16	14	0.0003*
	Ascending colon	23	15	
	Transverse colon	18	7	
	Descending colon	7	5	
	Sigmoid colon	122	53	
	Upper rectum	28	23	
	Middle rectum	34	31	
	Lower rectum	65	80	
Operative procedures	Partial resection	45	4	
	Ileocecal resection	11	4	
	Right hemicolectomy	15	25	
	Transverse colectomy	3	5	
	Descending colectomy	7	2	
	Left hemicolectomy	0	4	
	Sigmoid colectomy	105	49	
	Anterior resection	91	93	
	Abdominoperineal resection	14	35	
	Abdominosacral resection with coloanal anastomosis	4	2	
	Transsacral partial resection	17	0	
	Hartmann's operation	1	4	
	Total pelvic exenteration	0	1	
Follow-up time (mo; range and median)		3-189 (80)	1-201 (85)	
Recurrence	Positive	9	12	0.1793
	Negative	304	216	
Sites of First Tumor Recurrence	Liver	7	5	
	Lung	1	6	
	Local			
	Pelvis	1	2	
	Anastomosis	1	1	
	Para-aortic lymph node	0	1	
Oncologic outcome	5-Year disease-free survival (%)	96.9	94.9	0.1575
	5-Year cancer-specific survival (%)	99.3	97.6	0.0354

*colon and upper/middle rectum *vs.* lower rectum.

RESULTS

The patient demographics are summarized in **Table 1**. Compared with the UICC stage Ia group, the UICC stage Ib group included significantly more patients with lower rectal carcinoma ($p=0.0003$). Recurrence occurred in 9 of 313 (2.9%) UICC stage Ia group, and in 12 of 216 (5.6%) UICC stage Ib group. However, the difference between the two groups was not significant ($p=0.1793$). Disease-free survival rates at 5 years were 96.9% for the UICC stage Ia group and 94.9% for the UICC stage Ib group (**Figure 1a**), with no significant difference between the two groups ($p=0.1575$). Cancer-specific survival rates at 5 years were 99.3% for the UICC stage Ia group and 97.6% for the UICC stage Ib group (**Figure 1b**); there was a significant difference between the two groups ($p=0.0354$).

The performance rate of curative-intent salvage surgery for recurrent lesions in these recurrent carcinoma patients was 61.9% (13/21) (**Table 2**). Recurrence was found at a median time of 19 months (range 6-66) after primary carcinoma resection. Only one patient with pelvic and hepatic recurrence was found after five-year routine follow-up.

Since the proportion of lower rectal carcinoma patients was significantly elevated in the UICC stage Ib group, we divided the sites of carcinoma into the lower rectum and other parts to evaluate recurrence rates and prognoses (**Table 3**). Recurrences occurred in 10 of 145 (6.9%) patients with lower rectal carcinoma, and in 11 of 396 (2.8%) patients with colon or upper/middle rectal carcinoma. Between these two groups, the difference in the recurrence rate was significant ($p=0.0415$). Disease-free survival rates at 5

years in patients with lower rectal carcinoma were 92.6%, and 97.3% in patients with colon or upper/middle rectal carcinoma (**Figure 2a**), with the difference between the two groups significant ($p=0.0304$). However, the cancer-specific survival rates at 5 years were not significantly different between the groups ($P=0.2402$) (**Figure 2b**).

Among the 21 recurrent cases, 13 (61.9%) individuals were CEA positive at the time of recurrence (**Table 4**). With regard to the recurrent site and CEA positive rate, patients with hepatic recurrence showed a significantly higher rate of CEA positivity, compared with the patients with recurrence at other sites ($p=0.0272$). Between the patients who were CEA positive and those who were CEA negative at the time of recurrence, no significant difference in the prognosis after the detection of recurrence was found (**Figure 3a**), in addition to in the prognosis after the first surgery (**Figure 3b**).

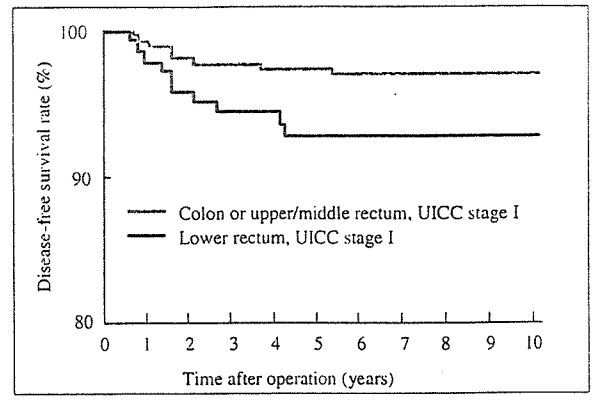

FIGURE 2a Cumulative disease-free survival curves for patients with lower rectal carcinoma and colon or upper/middle rectal carcinoma. The difference between the two groups was significant ($p=0.0304$).

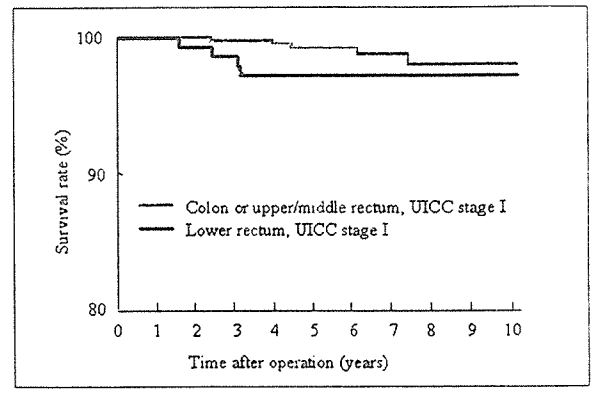

FIGURE 2b Cancer-specific survival curves for patients with lower rectal carcinoma and colon or upper/middle rectal carcinoma. The difference between the two groups was not significant ($p=0.2402$).

FIGURE 1a
Cumulative disease-free survival curves for UICC stage Ia group and UICC stage Ib group. The difference between the two groups was not significant ($p=0.1575$).

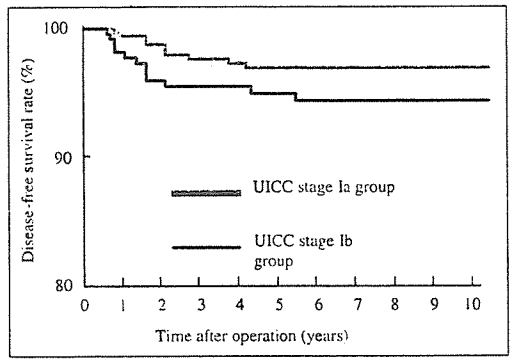

FIGURE 1b
Cancer-specific survival curves for UICC stage Ia group and UICC stage Ib group. The difference between the two groups was significant ($p=0.0354$).

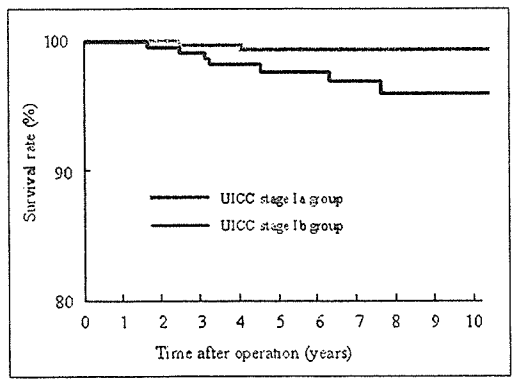

TABLE 2 Treatment of Recurrent Cancers

Treatment	No. of patients
Resection	
APR+radiation	3 (2*)
TPE+combined resection of sacrum	1 (1)
hepatic resection	9 (7*)
lung resection	5 (5)
Systemic chemotherapy	2
Hepatic artery infusion	2
Pelvic radiotherapy	1

( ), number of patients having curative-intent salvage surgery. *two patients underwent curative-intent salvage surgery for pelvic and hepatic recurrences.

DISCUSSION

For surveillance after curative surgery for colorectal carcinoma, a cost-effective method of follow-up should be established for consideration of the risk for recurrence. The probable subjects that the numbers of times and follow-up examinations can be reduced are UICC stage I patients. In the present study, we carried out follow-up examinations of a large number of UICC stage I patients over a long period at a single institution, and analyzed the data to clarify an appropriate method of surveillance. The present findings demonstrated that compared with the UICC stage Ia group, the UICC stage Ib group had a significantly lower rate of 5-year cancer-specific survival. In addition, lower rectal carcinoma involved a significantly higher incidence of recurrence. A recent study by Wichmann *et al.* (19) reported that between UICC stages Ia and Ib, there was an approximately 10% difference in the 5-year survival rate, although the difference did not achieve significance due to the small number of study patients. In the present study, however, the number of UICC stage I patients who were investigated was

much larger compared with the numbers reported in former studies, suggesting that the present study findings may help establish a method of follow-up for UICC stage I patients in the future.

In most carcinomas other than colorectal carcinoma, when recurrence is discovered after resection of the primary lesion, they are treated as a systemic disease and salvage surgery is infrequently indicated for the recurrent lesion. However, in colorectal carcinoma, resection of the recurrent lesion may improve patient prognosis. In this respect, research is required to determine whether intensive follow-up for detecting recurrence earlier and initiating the treatment of it will lead to improvement in prognosis for colorectal carcinoma patients. In earlier studies, the numbers of examinations and times of the check-up conducted were different (1-13). As a matter of course, it should be recognized that with advances in technologies, the precisions diagnostic examinations are being enhanced, and new effective methods of examination are being developed. Moreover, the treatment regimens have been changing rapidly; in recent years the indications for aggressive surgical resection for recurrent lesions have been expanded, and new chemotherapies that are useful for improving patient prognosis have been identified (20-23). For the reasons mentioned above, a study that retrospectively confirms the usefulness of follow-up will not able to avoid a bias caused by the times when the study was performed.

TABLE 3 Site of the Primary Tumor and Recurrence

	Colon and upper/ middle rectum	Lower rectum	*P* value
Number of patients	396	145	
Recurrence			
Positive	11	10	0.0415
Negative	385	135	
Oncologic outcome			
5-Year disease-free survival (%)	97.3	92.6	0.0304
5-Year cancer-specific survival (%)	99.1	97.1	0.2402

TABLE 4 Recurrent Disease and Results of Tumor Marker Monitoring at the Time of Recurrence

Tumor marker monitoring	Elevation	No elevation	*P* value
Number of patients	13	8	
Sites of recurrence			
Liver	11	1	0.0272
Lung	2	5	
Local (Pelvis and anastomosis)	3	2	
Para-aortic lymph node	1	0	
Interval to recurrence (mo; range and median)	6-66 (19)	9-32 (18)	0.3348
Oncologic outcome 5-Year survival following first recurrence (%)	52.7	87.5	0.2734
5-Year survival after primary surgery (%)	61.5	87.5	0.3558

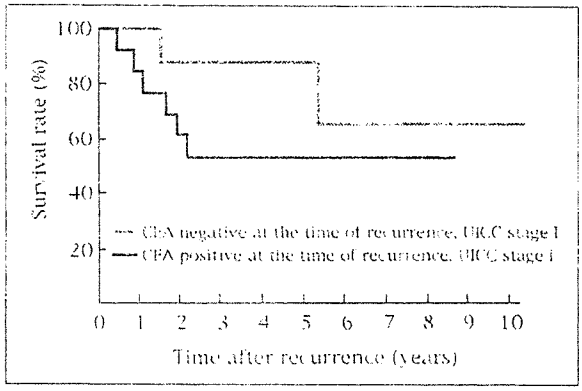

FIGURE 3a Cancer-specific survival curves after the detection of recurrence for patients who were CEA positive and CEA negative at the time of recurrence. The difference between the two groups was not significant ($p=0.2734$).

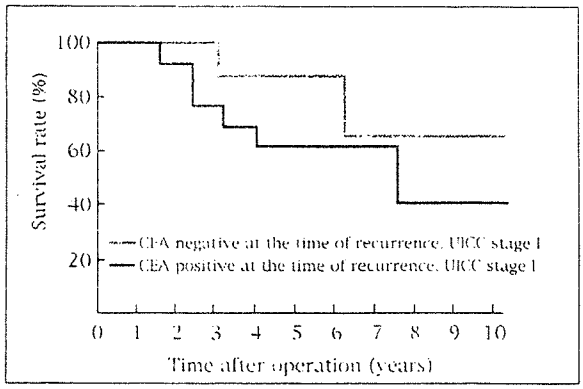

FIGURE 3b Cancer-specific survival curves after the first surgery for patients who were CEA positive and CEA negative at the time of recurrence. The difference between the two groups was not significant ($p=0.3558$).

With regard to the value of CEA in the postoperative surveillance, some benefits have been reported from the viewpoint of earlier detection of recurrence and cost-effectiveness in detecting potentially curable recurrent disease (24-26). However, no conclusion has been reached whether the earlier detection of recurrence using CEA may influence the prognosis. In the present study, 62% (13/21) of patients with recurrence showed an increased CEA level at the time of recurrence. In these patients, the follow-up that used CEA alone might have enabled the confirmation of recurrence if diagnostic imaging was performed at the point when an increased level of CEA was recorded. However, the question here is about those cases in which recurrence was confirmed first by diagnostic imaging without showing an increased level of CEA. Of these patients, 75% (6/8) remain disease-free to date, and there is a possibility that with the follow-up using CEA alone, asymptomatic recurrences without CEA elevation may not be detected. However, these 6 patients comprised only 1.1% (6/541) of all study patients, and it may therefore be inefficient to conduct the usual postoperative surveillance while burdening the remaining 99% patients with huge costs and effort. In all UICC stage I carcinoma patients, there was a low recurrence rate of 3.9% (21/541), and in addition,

because two-thirds of recurrences could be identified using CEA, the CEA test alone may be adequate at each visit, at least for UICC stage I patients.

Another problem in the CEA examination is that encountering a patient who shows false-positivity is inevitable. Moertel *et al.* (27) reported that when the preoperative CEA level was 5ng/mL or higher, false-positivity may appear approximately in 30% of such cases. If a UICC stage I patient shows an increased CEA level during the follow-up that uses CEA alone, it may be necessary to perform examinations for other carcinoma occurrences in addition to the metastasis and recurrence of the primary colorectal carcinoma.

A noteworthy aspect of the present study was that the patients with lower rectal carcinoma showed a significantly higher incidence of recurrence. Wichmann *et al.* (19) also reported that although there was no significant difference across UICC stage I patients, rectal carcinoma involved a higher rate of recurrence, with particularly more local recurrence, compared with colon carcinoma. The CEA positive rate in patients with local recurrence of rectal carcinoma was not as high as that in patients with hepatic metastasis (2,27,28). Hence, especially in conducting follow-up examinations of patients with lower rectal carcinoma, special attention should be paid to local recurrence, and when any symptom such as pain, hemorrhage, or change in bowel habit appears, necessary examinations should be performed early.

In the present study, the UICC stage Ia group included a significantly smaller number of patients with lower rectal carcinoma. This may be because some patients who had pT1 carcinoma at the lower rectum were followed up after undergoing trans-anal resection alone. The treatment of T1 and T2 carcinoma of the lower rectum is controversial, and several studies have suggested satisfactory tumor control after local excision for lower rectal T1 and T2 carcinoma (29,30). However, recent studies suggested that local excision of T1 and T2 rectal carcinoma is followed by a much higher recurrence rate than previously reported (31,32). In our institution, a radical surgery of low anterior resection or abdominoperineal resection is often indicated for T2 lesions and most T1 lesions with adverse risk factors, especially poorly differentiated carcinoma, lymphovascular invasions, incomplete excision, or massive invasion of carcinoma to the submucosal layer. Although most patients with T1 and T2 carcinoma lesions in the lower rectum in whom local recurrence develops after local excision can be salvaged by radical resection, the long-term outcome remains unknown (33).

In the field of the postoperative follow-up examination, the value of colonoscopy has been discussed. Periodic colonoscopy may be useful for detecting anastomotic and locoregional recurrences after colorectal carcinoma operation in addition to finding metachronous colorectal carcinoma (34,35). However, in UICC stage I patients, the anastomotic and locoregional recurrences have involved a very low proportion of 1% to 3%, according to previous and the present study (19). Particularly in patients with colonic carcinoma, there have been no anastomotic or locoregional recurrences observed at our institution. Performing colonoscopy is not warranted for the purpose of detecting anastomotic and locoregional recurrences in UICC stage I patients.

In conclusion, for UICC stage I patients, the incidence of recurrence was lower, and it is therefore possible to reduce the times and screening examinations for the postoperative surveillance. Regarding screening examinations, the CEA measurement every six months until two years after the operation, and subsequently once per year until the 5th postoperative year appears to be sufficient. Nevertheless, for patients with UICC stage Ib disease and those with lower rectal carcinoma, oncologists need to pay special attention because the rates of recurrence are significantly higher.

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転移・再発時の治療戦略

藤田　伸*

はじめに

転移・再発が生じるとその治療成績は大幅に低下する．しかし，大腸がんはほかのがんに比べると，転移・再発に対して外科治療が有効であり，外科的切除できるかどうかは大きな治療上の分岐となり，当然，治療成績も大きく異なる．ここでは，大腸がんの転移・再発の部位と頻度，そして肝，肺，局所，その他の比較的まれな転移・再発の治療戦略について述べてみたい．

転移・再発部位と頻度

大腸がんの転移頻度を，大腸癌研究会の大腸癌全国登録(1998年登録症例)の結果を表1に示す．転移例の半数以上が肝転移，次いで腹膜播種，肺転移であることがわかる．次いで，国立がんセンター中央病院の1985～1995年の10年間の治癒切除症例(遠隔転移がなく，がんをすべて切除できた症例)の再発部位を表2に示す．再発部位も再発例の約半数が肝臓で，次いで肺，局所再発の順である．大腸がんに限らず消化器のがんで転移・再発頻度の高い臓器は肝臓であり，治療戦略を考えるときに最大の問題となる．

▼表1　大腸がんの転移部位と頻度

転移部位	症例数(%) 5,573(100%)
転移なし	4,451(80%)
肝	603(11%)
腹　膜	277(5%)
肺	104(2%)
その他	58(1%)

その他の転移は，骨，脳，副腎など．(大腸癌全国登録1998年症例)

▼表2　大腸がん治癒切除後の再発部位と頻度

再発部位	症例数(%) 1,955(100%)
再発なし	1,448(74%)
肝	229(12%)
肺	155(8%)
局所再発	124(6%)
腹　膜	37(2%)
その他	47(2%)

その他の転移は，骨，脳，リンパ節など
(国立がんセンター中央病院1985年から1995年症例)

肝転移・再発の治療戦略(図1)

以上述べたように，大腸がんの肝臓への転移・再発は，転移・再発症例の半数を占めるため，この治療戦略はきわめて重要である．肝転移が発見されたときの治療戦略は，まず，肝転移が切除可能かどうかを判断する．切除可能と判断されれば，手術が原則である．肝転移に対する手術の有効性に関しての臨床試験は行われていないものの，過去

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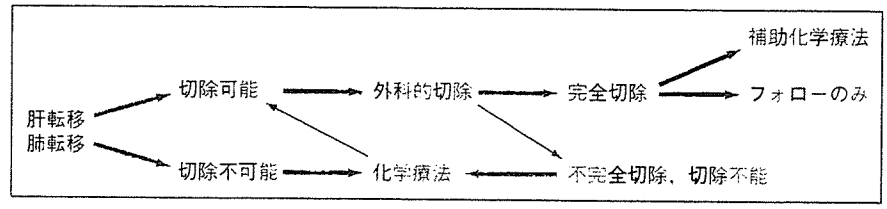

▶図1　肝転移，肺転移の治療戦略
太い矢印は現在の主流を示し，細い矢印は主流ではないか，臨床試験が必要なものを示している．

の多数の肝転移切除の成績が5年生存率で30～40％を示していることと，他の治療法ではそのような高い生存率は示されていないため，現段階では他の治療法との比較試験は，倫理的に実施できない．その点は，ほかの転移・再発の治療も同様である．

肝切除が可能な条件は

(1)　適度の残肝量(術後の侵襲に耐え生体を維持できる量のことで，術前ICG値と切除予定量で判断する)を保って，肝転移を切除可能なこと

(2)　原発大腸がんが完全切除可能なこと，あるいはされていること

(3)　肝臓以外に転移・再発がないこと

の以上3点で，単純に肝転移個数，大きさで決めているわけではない．もちろん，転移個数が増えるにつれて切除の可能性は低下するので，一般的には，転移個数4～6個程度が切除の適応であると理解しておくのがわかりやすい．肝切除法は，その部位，個数により異なるが，できるだけ正常肝を温存して，病巣を切除するので，原則的に部分切除を行い，必要に応じて区域切除，葉切除，拡大葉切除を行う．個数が多ければ，これらを組み合わせた，たとえば，部分切除と葉切除という切除も行うこともある．

肝転移切除後の補助化学療法の有効性は証明されていないため，補助化学療を行うのであれば，臨床試験として行うか，患者に十分説明し，インフォームド・コンセントを得る必要がある．

肝転移切除後の再発パターンは，表2と同様の傾向で，再発例の約半数が残肝に再発している．残肝再発の場合でも切除可能と判断されれば切除を行うことで，肝臓の1回のみの切除例と同等の治療成績が得られている．

切除できない場合には抗がん剤治療となり，全身化学療法が行われる．転移巣が肝転移のみの場合には，肝動注療法が積極的に行われていたが，これまでの臨床試験の結果では，残肝再発率は全身化学療法に比べ明らかに低下するものの，生存率の改善効果は示されていない．このため，現在この治療を行うのであれば，臨床試験か，インフォームド・コンセントを得た上で行う必要がある．一般に大腸がんにおける化学療法の位置づけは，がんの治癒を目指すというより，QOLを保ったまま延命を目指すものであるが，ごく少数例ではあるが，化学療法により肝転移の大きさ，数が縮小し，肝転移が切除可能となる症例があるので奏効例では肝臓外科医との連携が重要となる．

●ICG：インドシアニン・グリーン indocyanine green．

肺転移・再発の治療戦略(図1)

肺転移・再発の治療戦略は，肝転移・再発の治療戦略とほぼ同様である．肝転移と同様，切除可能と判断されたならば外科的手術を行う．切除可能の条件は，肝転移とよく似ており，

(1)　必要な肺機能を保って，肺転移が切除可能なこと

(2)　原発大腸がんが完全切除可能なこと，あるいはされていること

(3)　肺以外に転移・再発がないこと

の以上3点である．やはり肝転移と同様，転移個数，大きさで，切除可能かどうかを決められない．一般的には，転移個数は1～3個程度が切除適応であると理解しておくとわかりやすい．肺切除法も，肝切除と同様，転移個数，部位により異なるが，正常肺をできるだけ温存するため，部分切除が原則で，それで困難であれば，区域切除，葉切除を行う．肺全摘術は，手術侵襲，予後からあまり行われない．肺切除後の治療成績も肝転移とほぼ同等で，5年生存率は30～40％である．

補助化学療法も，肝転移と同様，その効果は証明されていないため，術後は，無治療でのフォローアップを行う．

また，肝転移切除後の肺転移・再発例，あるいは数は少ないが肺転移切除後の肝転移例は，先に示した切除可能条件が満たせば，切除を行うことで，症例数は少ないものの長期生存した症例もある．

腹膜播種・再発の治療戦略

表1，2に示したように，腹膜播種・再発の頻度は，転移部位としては，肝転移についで2番目，全大腸がん症例の5%，再発部位としては肝・肺再発に次いで3番目，治癒切除例の2%に生じる．腹膜再発の診断は画像上困難であるため，実際にはもっと頻度は高いと考えられる．

原発大腸がん切除時の術前から腹膜播種と診断される場合は少なく，ほとんど開腹時に診断される．腹膜播種が，わずかに存在する程度であれば，切除をすることが多いが，肝転移，肺転移と異なり，切除で治癒することはなく，あくまでも腹膜再発までの期間を延長して，QOLの向上をはかる姑息的な切除である．また多数の腹膜播種がある場合には，原発巣の切除をしないでバイパス術や人工肛門のみを作製する場合もある．したがって，腹膜播種・再発時には，化学療法が原則となる．

腹膜再発の診断は困難で，腹水や腸閉塞の症状で発見されることが多く，一般に多発再発のため，治癒的な切除が可能なことはほとんどないが，きわめてまれに1個所だけの再発であれば，切除により長期生存が得られる場合がある．腸閉塞症状がある場合は，人工肛門の作製を考慮する．

がんが漿膜まで浸潤している場合には，手術時に，肉眼的に腹膜播種が認められなくても，手術開始時の腹腔内洗浄液の細胞診でがん細胞が見つかることがある．当院での頻度は約5%である．この場合，約半数の症例で腹膜播種・再発が生じるため，大きな予後不良因子であるが，術中の腹腔内への抗がん剤の投与や補助化学療法の有効性は明らかでない．

局所再発の治療戦略

大腸がんで局所再発をきたすのは，ほとんど直腸がんであり，結腸がんでは吻合部再発がまれに見られる程度である．吻合部再発は，ほかに転移がなければ，吻合部を含めた腸管を切除して再度吻合する，直腸の吻合部再発で再吻合が困難である場合には，永久人工肛門とする．

直腸がんの局所再発率は5～10%で，がんが肛門に近いほど頻度が高くなり，下部直腸がんの局所再発率は上部直腸がんの約4倍の頻度である．直腸がんの局所再発は，いま述べた吻合部再発もあるが，ほとんどが仙骨前面や骨盤側壁，会陰創の再発である．この局所再発に対して，大腸骨盤外科，腫瘍内科，放射線治療科の3科が連携した治療，すなわち集学的治療が必要ある．

骨盤内の局所再発で切除可能と判断されれば，切除を考える．切除の適応は，

(1)　がんを遺残なく切除できること
(2)　遠隔転移がないこと
(3)　手術に耐えられること

などは，肝・肺転移の切除適応と同様であるが，さらに

(4)　再発巣は1個所のみであること
(5)　骨盤内に再発巣が広範に存在することを示唆する所見(仙骨上部あるいは骨盤側方壁への進展，下肢の浮腫，坐骨神経痛など)がないことである．

ただし，切除可能と判断されても，骨盤内臓全摘術，さらにほとんどの症例で中下部仙骨の合併切除を加えた手術が必要となるため，手術可能と判断された場合には，患者に，

(1)　手術時間が平均12時間，出血量も平均3,500 ml(国立がんセンターの平均)と大きな侵襲を伴う手術であること
(2)　人工肛門，人口膀胱(回腸導管)となること
(3)　完全に切除できたとしても5年生存率は30～40%であること

を十分に説明し，インフォームド・コンセントを得ることが必要となる．

手術前後に，放射線療法，あるいは放射線化学療法を加えることがある．術前の放射線治療は，腫瘍辺縁のがん細胞を死滅させ，切除断端にがんが遺残しないように，また場合により縮小手術を可能とする，たとえば，仙骨合併切除をしなくて済む，あるいは，骨盤内臓全摘術を行わなくて済むようにする目的で行うが，その効果は明らかでない．術後の放射線療法は，手術で再発巣の完全切除ができなか

った場合に行われる.

手術不能例，手術拒否例に対しては，放射線療法と化学療法を行う．これらの治療の目的は，一般の大腸がんの化学療法と同様，症状の緩和と延命であるが，最近，放射線医学総合研究所(千葉市)での重粒子線による局所再発の治療が比較的良い成績をあげており，まだ長期フォロー症例が少ないため予後の改善効果は不明であるが，期待できる治療である．とくに手術拒否例には勧めたい治療の1つであるが，保険適用外で高度先進医療として行われているため，患者の自己負担額が314万円と高額なのが難点である.

その他再発の治療戦略

リンパ節再発の頻度は，表2にも示したように，さほど高いものではない．リンパ節再発の場所としては，腹部動脈周囲リンパ節，骨盤内リンパ節である閉鎖リンパ節，鼠径リンパ節である．画像上1個だけの再発でほかに再発を認めなければ切除を考えてもよい．ほかの転移を認める場合には，例外もあるが化学療法を行う．いずれの再発も初回手術から時間を経過しての孤発性の再発で切除可能であれば，比較的予後はよい.

脳転移・再発の頻度は低いものの，神経症状が前面に出るため患者のQOLを大きく損ねる．大腸がんからの脳転移は，すべての脳転移例の約5%といわれている．術後，外来検査では脳転移の検索は行わないため，頭痛やさまざまな神経麻痺，痙攣などで発症して，脳CT検査ではじめて発見される場合がほとんどである．単発性の転移でほかに転移がなければ，切除も考えるが，一般に多発であることが多く，また他臓器にも転移があることが多いため，治療は放射線療法が主体となる．脳転移に対しては，化学療法は一般には有効でない.

骨転移・再発も，脳転移同様，転移頻度は高くはないが，患者のQOLを大きく損ねる再発である．椎骨への転移頻度が高く，病的骨折により不可逆性の神経麻痺が生じるので，診断がついた時点で早急な対応をしなければならない．治療は，脳転移同様，放射線治療である．また，病的骨折に対しては，コルセット，必要であれば骨の固定を行う.

おわりに

転移・再発は，切除できれば，一般に30～40%の5年生存率が得られるが，切除できなかった場合には，化学療法，放射線療法，あるいはその併用療法となる．しかしながら，長期生存例は少なく，これらの治療の目的は，延命，症状の緩和である．このような患者には医師よりも看護師の役割が大きく，病態，治療を理解して，積極的にかかわってもらいたい.

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Involvement of cyclin D3 in liver metastasis of colorectal cancer, revealed by genome-wide copy-number analysis

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The question of whether any genetic differences exist between primary and colorectal cancers (CRCs) and their metastatic foci is controversial. To look for genetic aberrations involved in metastasis of CRCs to the liver, we performed subtractive comparative genomic hybridization (CGH) experiments using paired samples from 20 CRC patients with primary tumors and synchronous or metachronous liver metastases. Relatively frequent gains in DNA copy number were detected at 6p, suggesting the presence of one or more metastasis-related genes in the region. Analysis of 11 CRC cell lines using array-based CGH (CGH-array) revealed one 6p candidate gene, *CCND3*. Quantitative reverse transcriptase-polymerase chain reaction experiments showed that *CCND3* was significantly upregulated in liver-metastatic lesions compared with primary lesions ($P<0.0152$). In addition, immunohistochemical analysis of 120 primary CRC tumors demonstrated that cyclin D3 expression in the region of rolled edge was significantly associated with total recurrence, especially hematogenous recurrence ($P=0.0307$). The results implied involvement of cyclin D3 in liver metastasis of CRC, and the data may contribute to the development of a novel therapy or diagnostic agent for this currently intractable disease. Our experiments also confirmed the power of subtractive CGH and CGH-array analysis for identifying cancer-related genes.

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Keywords: colorectal cancer; metastasis; comparative genomic hybridization (CGH); CGH-array; gene amplification

Colorectal cancer (CRC) is one of the most common forms of human malignancy, especially in industrialized countries, and metastasis of those tumors to the liver is a major cause of death among patients with CRC. The progression of cancer cells to a metastatic phenotype is thought to occur in multiple steps that include cytoskeletal changes, loss of adhesion, enhanced mortality, expression of proteolytic enzymes that degrade the basement membrane, adhesion to endothelial cells, anchorage-independent growth, and angiogenesis. Given the highly complicated nature of the molecular pathogenesis of metastasis, much remains to be learned about this lethal process.

Multiple genetic alterations occurring sequentially in a cell lineage underlie the carcinogenetic process in solid tumors.[1] Among those genetic alterations, amplification of chromosomal DNA is one of the mechanisms capable of activating genes whose aberrant expression is critical in the development and progression of cancer. Comparative genomic hybridization (CGH) analysis[2] has proven to be useful for identifying novel regions of gene amplification in various types of cancers.[3–6] Moreover, a recent development, the CGH-array technique, allows high-throughput and quantitative

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analysis of copy-number changes at high resolution throughout cancer genomes, providing many advantages over conventional methods and often allow precise and rapid identification of tumor suppressor genes as well as oncogenes.[7–10]

Whether any genetic differences exist between primary colorectal carcinomas and liver metastases remains controversial even in the postsequence era. CGH analysis has been performed in primary tumors and metastatic tumors of CRC to compare CGH profiles between those two groups. However, only a few published studies have analyzed paired samples, and only in limited numbers.[11–14] To determine genetic aberrations responsible for the metastatic phenotype in CRC, it is necessary to compare paired tumor tissues obtained from original and metastatic sites in the same patient.

In the work reported here, we examined 20 primary CRC tumors and their corresponding metastatic foci in the liver by conventional CGH and subtractive CGH analyses, to explore genomic alterations that might be associated with metastasis of CRC. With this approach, we identified 6p as a candidate region for harboring one or more metastasis-related genes. A CGH-array analysis of CRC cell lines using our custom-made array ('MCG Cancer Array-800'; Inazawa *et al*,[8] Sonoda *et al*[9] and Takada *et al*[10]), and analysis of gene expression by means of real-time quantitative reverse transcriptase-polymerase chain reaction (RT-PCR) experiments revealed *CCND3* as the most probable target of additional amplification during metastasis. To clarify the significance of cyclin D3 overexpression in colorectal carcinogenesis and metastasis to the liver, we examined associations between the expression of cyclin D3 protein and clinicopathological characteristics of 120 primary CRCs.

Materials and methods

Cell Lines and Primary Tumors

Among the 11 human CRC cell lines selected for our experiments, COLO205, HCT-15, and SW480 were generously provided by Cell Resource Center for Biomedical Research, Institute of Development, Aging and Cancer, Tohoku University (Sendai, Japan). CCK-81, CoCM-1, COLO201, COLO320DM, DLD-1, CaR-1, and WiDr were purchased from the Japanese Collection of Research Bioresources (Osaka, Japan), and HT-29 from the American Type Culture Collection (Manassas, VA, USA). CCK-81, COLO320DM, and WiDr were maintained in Dulbecco's modified Eagle's MEM supplemented with 10% fetal bovine serum (FBS) and 100 U/ml penicillin/100 μg/ml streptomycin (P/S). HT-29 and CoCM-1 were maintained in McCoy's 5a and RPMI-1640 (50%)/F-12 (50%), respectively, each supplemented with 10% FBS and P/S. All others were maintained in RPMI-1640 supplemented with 10% FBS and P/S. HSC-4 and HO-1-u-1 cell lines derived from oral squamous cell carcinoma were maintained in Dulbecco's modified Eagle's MEM supplemented with 10% FBS and P/S.

Paired samples of primary CRCs and corresponding normal colonic mucosa and metastatic foci (14 with synchronous liver metastases and six with metachronous liver metastases) for CGH experiments were obtained from 20 unrelated patients being treated at the Tokyo Medical and Dental University Hospital, after written consent from each patient in the formal style and approval by the local ethics committee. Tissues from these patients were frozen immediately in liquid nitrogen and stored at −80 C until required. Clinicopathological data were collected on the basis of General Rules for Clinical and Pathological Studies on Cancer of the Colon, Rectum, and Anus by the Japanese Society for Cancer of the Colon and Rectum, and clinical stages of the disease were classified according to the tumor-node metastasis (TNM) classification of the International Union Against Cancer (Table 1).

Paraffin-embedded specimens of primary pT3 CRCs to be used for immunohistochemistry (IHC) were obtained from 120 unrelated patients treated at the National Defense Medical College Hospital, with written consent from each patient in the formal style and after approval by the local ethics committee. Clinicopathological data were collected on the basis of General Rules for Clinical and Pathological Studies on Cancer of the Colon, Rectum, and Anus by the Japanese Society for Cancer of the Colon and Rectum. Clinical stages of the disease were classified according to the TNM classification of the International Union Against Cancer; 58 patients were at stage II, 39 at stage III, and 23 at stage IV. All patients were periodically followed up at outpatient clinics and monitored for postoperative recurrence every 3 months by chest X-rays and measurements of serum carcinoembryonic antigen and carbonhydrate antigen 19-9 levels; every 6 months by abdominal ultrasonography; and every year by colonoscopy. Contrast-enhanced computed tomography was performed when recurrence of cancer was suspected. Whenever no findings suggestive of cancer relapse appeared during 5 years of follow-up, the procedure was changed to an annual physical check without other detailed examinations. Once a year, we confirmed by telephone the conditions of patients who did not visit the clinic. The duration of overall survival was calculated for each patient from the date of primary surgery to the date of the last follow-up or death. Of the 120 patients, 48 died of cancer during the study period, with a median interval of 26.3 months (range 1.8–72.7 months) from surgery to death. The median follow-up period of the 72 survivors was 79.0 months (range 40.0–135.9 months). The others were dead, but not by cancer.

Table 1 Clinicopathological features of primary CRC tumors analyzed in CGH and CGH-array

	Age	*Gender*	*Location*	*TNM stage*	*Liver metastasis*[a]	*Histologic subtype*[b]
1	74	Male	Ascending colon	IV	Synchronous	mod
2	61	Male	Sigmoid colon	IV	Synchronous	muc
3	68	Male	Rectum	III	Metachronous	por
4	36	Male	Cecum	IV	Synchronous	por
5	66	Female	Rectum	III	Metachronous	well
6	77	Male	Transverse colon	IV	Synchronous	well
7	62	Male	Rectum	IV	Synchronous	well
8	59	Female	Sigmoid colon	IV	Synchronous	mod
9	69	Male	Rectum	IV	Synchronous	well
10	63	Female	Sigmoid colon	IV	Metachronous	mod
11	80	Male	Rectum	IV	Synchronous	well
12	66	Male	Rectum	IV	Synchronous	well
13	49	Male	Sigmoid colon	II	Metachronous	well
14	61	Male	Cecum	IV	Synchronous	mod
15	54	Male	Rectum	IV	Synchronous	mod
16	66	Male	Rectum	III	Metachronous	mod
17	61	Female	Transverse colon	IV	Metachronous	mod
18	76	Male	Cecum	IV	Synchronous	well
19	74	Male	Sigmoid colon	IV	Synchronous	well
20	79	Female	Rectum	IV	Synchronous	well

[a]Synchronous or metachronous liver metastases.
[b]Well = well-differentiated adenocarcinoma; mod = moderately differentiated adenocarcinoma; por = poorly differentiated adenocarcinoma; muc = mucinous adenocarcinoma.

CGH and Subtractive CGH

We used directly fluorochrome-conjugated DNA for CGH experiments as described by Kallioniemi *et al*,[2] with minor modifications.[3] DNAs extracted from normal colonic mucosa corresponding to tumor samples were used as reference. Briefly, test and reference DNAs were labeled, respectively, with Spectrum Green- and Orange-dUTP (Vysis, Chicago, IL, USA) by nick translation, denatured, and hybridized to normal male metaphase chromosome spreads together with Cot-1 DNA. Shifts in CGH profiles were rated as gains and losses if they reached at least the 1.2 and 0.8 thresholds, respectively, and over-representations were considered to be high-level gains (HLGs) when the fluorescence ratio exceeded 1.5, as described elsewhere.[3] In subtractive CGH, DNAs from metastatic tumors and primary tumors were labeled, respectively, with Spectrum Green- and Orange-dUTP by nick translation. Heterochromatic regions near centromeres, and Y chromosomes, were excluded from these analyses.

CGH-Array Analysis

Our MCG Cancer Array-800[8–10] was used for CGH-array experiments, which were carried out as described elsewhere.[9,10] *Dpn*II-restricted test and reference DNAs were labeled by random priming with Cy3- and Cy5-dCTP (Amersham Biosciences, Tokyo, Japan), respectively, precipitated together with ethanol in the presence of Cot-1 DNA, redissolved in a hybridization mix (50% formamide, 10% dextran sulfate, 2 × standard saline citrate (SSC), 4% sodium dodecyl sulfate (SDS), pH 7), and denatured at 75°C for 10 min. After incubation at 37°C for 30 min, the mixture was applied to array slides set up in custom-made hybridization chambers, and incubated at 42°C on a rocking table for 48–72 h. The hybridized slides were washed once in a solution of 50% formamide, 2 × SSC (pH 7.0) for 15 min at 50°C, once in 2 × SSC, 0.1% SDS for 15 min at 50°C, and once in a 0.1 mol/l sodium phosphate buffer containing 0.1% Nonidet P-40 (pH 8) for 15 min at room temperature.

The arrays were scanned with a GenePix 4000B (Axon Instruments, Foster City, CA, USA), and the acquired images were analyzed with GenePix Pro 4.1 imaging software (Axon Instruments). Fluorescence ratios were normalized so that the mean of the middle third of log 2 ratios across the array was zero. Average ratios that deviated significantly (> 2 s.d.) from zero were considered abnormal.

Fluorescence *In Situ* Hybridization (FISH)

Metaphase chromosome slides were prepared in the manner described previously.[3] BACs containing the *CCND3* gene (RP11-720D9) were labeled with biotin-16-dUTP by nick translation (Roche Diagnostics, Tokyo, Japan), and hybridized to metaphase chromosome spreads together with Cot-1. The chromosomes were counterstained with 4′,6-diamidino-2-phenylindole.

Real-Time Quantitative RT-PCR

Expression levels of *CCND1*, *CCND2*, *CCND3*, and *CDK6* mRNA were measured by means of a real-time

fluorescence detection method.[5] Single-stranded cDNAs were generated from total RNAs using the SuperScript™ First-Strand Synthesis System (Invitrogen, Carlsbad, CA, USA). Real-time quantitative PCR was performed with an ABI PRISM 7900HT Sequence Detection System (Applied Biosystems, Foster City, CA, USA) using SYBR Green, according to the manufacturer's protocol; primer sequences are available on request. The glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*) gene served as an endogenous control (Applied Biosystems); the level of mRNA expression for each gene in each sample was normalized on the basis of the respective *GAPDH* content and recorded as a relative expression level. PCR amplification was performed in duplicate for each sample.

Immunohistochemistry

Indirect IHC was performed on formalin-fixed, paraffin-embedded tissue sections, as described elsewhere.[15] Using tissue blocks prepared from pT3 primary CRCs resected from 120 patients, we constructed tissue-microarray (TMA) blocks of tissue-core specimens taken from the submucosal invasive front, subserosal invasive front, central area, and rolled edge of each tumor.[16] To construct TMA blocks, four tissue cores were taken from each representative tissue block, and these cores were transferred to a recipient block using a Tissue Microarrayer (Beecher Instruments, Silver Spring, MD, USA). We used cores 2.0 mm in diameter and arranged them 0.7–0.8 mm apart in a recipient block, to construct a total of 15 TMA sets comprising 480 core specimens. The sections were dewaxed and rehydrated in graded concentrations of ethanol. Antigens were retrieved by microwave pretreatment in 10 mM citrate buffer (pH 6.0) for 10 min. After cooling, the sections were treated with 3% hydrogen peroxide to block endogenous peroxidase, then reacted overnight at 4°C with antihuman cyclin D3 monoclonal antibody (1:50, DSC-22; DAKO, Santa Barbara, CA, USA) or normal rabbit serum. The sections were rinsed, incubated with rabbit EnVision + peroxidase (Dako, Carpinteria, CA, USA), stained with 0.05% hydrogen peroxide and 3,3′-diaminobenzidine, and counterstained with hematoxylin. HSC-4 and HO-1-u-1 cell lines served as positive controls, and also as negative controls where the primary antibody was omitted. In the HSC-4 and HO-1-u-1 cells, cyclin D3 was strongly stained in about 70% and about 5% of carcinoma cell nuclei, respectively.

Expression levels of cyclin D3 were divided into four categories according to the percentage of cyclin D3-positive cells (nuclei) in a sample as follows: irrespective of the intensity of immunoreaction, no positive cells = 0; positive in <5% of constituent carcinoma cells = +1; positive in 5–50% of constituent carcinoma cells = +2; and positive in >50% of constituent carcinoma cells = +3. CRC samples that registered levels 0 or +1 were defined as negative expression, and samples containing levels +2 or +3 were defined as positive.

Statistical Analysis

Differences in levels of *CCND3* mRNA expression between subgroups were tested by the nonparametric Wilcoxon's rank test with the determination of associated probability (*P*). Correlations between cyclin D3 expression in primary CRCs and clinicopathological variables were analyzed for statistical significance by χ^2 or Fisher's exact tests. Survival data were analyzed according to the method of Kaplan and Meier. The log-rank test was used to compare survival data with cyclin D3 expression patterns. *P*-values of less than 0.05 were considered significant.

Results

Chromosomal Regions Frequently Involved in DNA Copy-Number Aberrations in CRCs

To explore chromosomal regions that might harbor genes critical to metastatic progression of CRC, we first carried out a conventional CGH analysis of 20 primary CRC tumors and their corresponding liver metastases. DNA extracted from normal colonic mucosa corresponding to the tumor sample served as a reference for each case. Overviews of genetic changes in 20 primary (P) and 20 metastatic (M) tumors are shown in Figure 1a and b, respectively. Most of the samples showed copy-number aberrations (P, 15/20, 75%; M, 18/20, 90%). On average, we observed 9.3 (range, 0–27) aberrations in primary tumors per patient: 4.5 (range, 0–10) gains and 4.8 (range, 0–19) losses. Metastatic tumors showed an average of 9.5 (range, 0–19) aberrations: 5 (range, 0–8) gains and 4.5 (range, 0–12) losses.

Regions with the most frequent copy-number gains in primary tumors were at 13q (70%), 20q (65%), 8q (55%), 20p (40%), 7p and 7q (25% each), and 1q (20%). Common losses in primary tumors were seen at 18q (55%), 18p and 8p (50% each), 1p (40%), 17p (35%), 4q (30%), 4p (25%), and 3p (35%). The smallest regions of HLGs in primary tumors involved 8q23-qter (five cases), 13q32-qter and 20q12-qter (four cases each), and 20p (three cases). Common regions for the most frequent copy-number gains in metastatic tumors were at 8q (70%), 20q and 13q (60% each), 20p (45%), 7p (40%), 7q (35%), Xq (25%), 1q (20%), and 6p (15%). Common losses in metastatic tumors were seen at 8p (60%), 18p and 18q (55% each), 14q (30%), 17p (25%), 4p and 22q (20% each), 4q (15%), and 1p (15%). The smallest regions of HLGs in metastatic tumors involved 8q13-q21.3, 13q14-qter, 20q12-qter and