Jpn. J. Cancer Res. 85, 1280–1287, December 1994

Risk Factors Related to Liver Metastasis in Colorectal Carcinoma: A Multivariate Analysis of Clinicopathologic and Immunohistochemical Variables

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Specimens from 48 consecutive patients undergoing surgery for colorectal carcinoma and having synchronous or metachronous liver metastases (Group 1) and those from 52 consecutive patients who had no evidence of hepatic metastases within at least 5 year after colorectal resection (Group 2) were selected and compared using a multiple logistic regression analysis. A multivariate analysis using a stepwise logistic regression revealed six independent risk factors significantly related to hepatic metastases. In addition, the following logistic regression model was obtained from this analysis. $P = \exp a/(1+\exp a)$: $a = 3.524(\text{SM-V}) + 2.731(\text{Ex-V}) + 2.718(\text{E/M}) + 2.562(\text{Lo}) + 1.858(\text{p53}) + 1.941(\text{HIR}) - 4.397$, where P is the probability of hepatic metastasis given six independent risk factors (E/M, Ex/M ratio; Lo, location; HIR, host inflammatory cell reaction). When the estimated probability "P" in the above logistic regression model is more than 0.55 after an examination of surgical specimens, we must consider adjuvant chemotherapy and closely monitor the patient to ensure early detection of hepatic metastases.

Key words: Multivariate analysis — Colorectum — Liver metastasis — p53 — Venous invasion

Hepatic metastases are found in as many as one-fifth[1] to a quarter[2] of patients operated on for colorectal carcinoma and are an important contributory cause of death. The technical aspects of hepatic resection have become relatively standardized, while previous studies[3–5] have shown the benefits of the surgical removal of liver metastases from patients with colorectal carcinoma. Adson and van Heerden[6] concluded that the excision of small, apparently solitary metastatic lesions could be justified on the basis of the low operative risk and the prolonged survival. To improve survival in patients with hepatic metastases from colorectal carcinomas, an early diagnosis of a metastatic liver tumor is therefore important. Venous invasion is considered to be closely related to liver metastases,[7, 8] though occasionally venous invasions cannot be identified even in surgical specimens of patients with hepatic metastasis. In addition, some patients with colorectal carcinomas with venous invasions survive for more than 5 years without liver metastasis. It is thus difficult to regard "venous invasion" alone as useful in selecting cases with metachronous liver metastases.

The aim of this study was to evaluate multivariately the effects of several clinicopathologic variables on the hepatic metastases and to aid prediction of whether or not hepatic metastases will occur in patients operated on for colorectal carcinoma.

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MATERIALS AND METHODS

Specimens We examined specimens from 48 consecutive patients undergoing surgery for colorectal carcinoma and having synchronous or metachronous liver metastases in the Second Department of Pathology, Kyushu University during the period from 1974 to 1992 (Group 1). Surgical specimens from 52 consecutive patients who had no evidence of hepatic metastases within at least 5 years after colorectal resection were also selected (Group 2) from 2266 cases of primary colorectal carcinoma that had been registered at our department during the same period. The two groups were compared (Table I). All the 100 tumors involved the entire thickness of the colorectal wall; however, an adequate radical resection margin was obtained. All liver metastases were investigated using surgical or autopsy specimens.

Histologic study The resected tissue specimens were fixed with 10% formalin. A strip running the entire length of the tumor was cut to include the point of greatest panetration through the colorectal walls, and it was divided into several pieces, which were embedded in paraffin. Histologic sections cut from the paraffin blocks were stained with hematoxylin and eosin (HE) and according to the elastica van Gieson (EVG) method.

Immunohistochemical study For immunohistochemical examination using anti-CA19-9 (monoclonal; 1:1; TFB, Tokyo) and anti-p53 (PAb1801) (monoclonal; 1:100; Oncogene Science, Uniondale, NY) antibodies, 5-μm-

Table I. Classification of 100 Primary Colorectal Carcinomas

	No. of cases	
		Time to recurrence (Avg.) (years)
Colorectal carcinoma with liver metastasis	48	
Synchronous	32	
Metachronous	16	0.5–4.5 (2.0)
		Follow-up period (Avg.) (years)
Colorectal carcinoma without evidence of liver metastasis	52	5.3–11.5 (9.3)
Death from other cause	5	5.3–10.4 (7.4)
Survivors	47	7.0–11.5 (9.5)

thick sections of 10% formalin-fixed, paraffin-embedded materials were mounted on poly-L-lysine-coated slides and deparaffinized. The endogenous peroxidase activity was blocked by incubation in 0.3% methanolic hydrogen peroxide. The sections to be stained by anti-p53 antibody were placed in jars containing distilled water for 10 min at 97°C and the specimens to be stained by anti-CA19-9 antibody were predigested with 0.1% trypsin in tris buffer for 30 min at 37°C to help unmask the antigen. Non-specific protein binding was inhibited by treatment with 10% goat serum before exposure to the primary antibodies. The detection of binding in the primary reagents was achieved by use of the avidin-biotin-peroxidase complex (ABC) method of Hsu *et al.*[9] Diaminobenzidine was used to visualize peroxidase deposition at the antigenic sites, and these sections were counterstained with methyl green.

According to the distribution of tumor cells showing a positive reaction, the results were expressed as negative, scatteredly positive (less than 10% of the cells had a positive reaction), focally positive (more than 10% of the cells had a positive reaction), and diffusely positive (more than 50% of the cells had a positive reaction).

Statistical analysis When testing the effect of the risk factors for liver metastases in the comparison between Groups I and II, a chi-square test with Yates correction for continuity was used. Furthermore, the influence of various clinical and morphologic variables on liver metastasis was considered in a multivariate analysis using a multiple logistic regression model. This model has the general form

$$P_r(Y=1|X_1,X_2,\cdots,X_k)=\frac{\exp(\beta_0+\beta_1X_1+\beta_2X_2+\cdots+\beta_kX_k)}{1+\exp(\beta_0+\beta_1X_1+\beta_2X_2+\cdots+\beta_kX_k)}$$

where $Y=1$ if liver metastasis occurred within 5 years after surgery and $Y=0$ if not, X_i ($i=1, 2, 3, ..., k$) represents a distinctive variable and β_i ($i=1, 2, 3, ..., k$) is the regression coefficient; the 15 variables used in this analysis are shown in Tables II and III. P_r ($Y=1|X_1, X_2, ... X_k$) is the probability of liver metastasis ($Y=1$), given $X_1, X_2, ... X_k$. The model selection for identifying the variables with an important and statistically significant effect of liver metastasis was based on a forward stepwise procedure (enter limit 0.05). The final estimates of the weights were based on maximum likelihood solutions.[10] The statistical analysis was made using the BMDP statistical package (BMDP Statistical Software, University of California Press, Los Angeles) and an IBM 3090 computer.

RESULTS

Liver metastasis Sixteen (33.3%) patients out of 48 had metachronous liver metastasis of the colorectum. All the metachronous liver metastases had occurred within 5 years after resection of colorectal carcinomas. The average period to recurrence in the 16 patients with metachronous metastasis was 2.0 years (range, 0.5 to 4.5 years). The remaining 32 cases had synchronous liver metastasis. In Group 2, all the patients either lived or have been living for five years or more without hepatic metastasis after operation, while five of them died of other causes after that.

Clinical findings The patients studied included 62 male and 38 female patients; the male-to-female ratio was 1.4:1 in Group 1 and 1.9:1 in Group 2. They ranged in age from 28 to 89 yr, the median being 60 yr. When this median value was used as the determinative line, age did not affect liver metastasis.

Histopathological findings

1) Histological grade: The grade of differentiation of the tumor is classified according to WHO's classification into well, moderately and poorly differentiated adenocarcinoma and other types. Thirty-eight and 46 tumors were categorized as "well or moderately differentiated adenocarcinoma" in Groups 1 and 2, respectively.

2) Tumor size: There was no statistically significant difference in mean tumor size between the two groups (5.8 cm in Group 1 vs. 6.3 cm in Group 2). When the median tumor size of 6.0 cm was used as the determinative line, tumor size was also found not to affect liver metastasis.

Table II. Clinical and Immunohistochemical Variables and Their Significance (chi-square test)

Variable	Category	Code (X_i)	Group 1 (48 cases)	Group 2 (52 cases)	P value
Age	<60	0	23	23	0.866
	≥ 60	1	25	29	
Sex	Male	0	28	34	0.603
	Female	1	20	18	
Size (cm)	≤ 6.0	0	30	29	0.631
	>6.0	1	18	23	
Location	Colon	0	26	39	0.049
	Rectum	1	22	13	
p53	Negative, scatteredly or focally	0	28	44	0.0069
	Diffusely	1	20	8	
CA19-9	Negative	0	28	43	0.0138
	Positive	1	20	9	

Group 1: colorectal carcinoma with liver metastasis; Group 2: colorectal carcinoma without evidence of hepatic metastasis.

Table III. Histopathological Variables and Their Significance (chi-square test)

Variable	Category	Code (X_i)	Group 1 (48 cases)	Group 2 (52 cases)	P value
Histologic grade	Well & moderate	0	38	46	0.320
	Poorly & others	1	10	6	
Ex/M ratio	Low ($0<$, $<2/3$)	0	26	40	0.023
	High ($2/3\leq$)	1	22	12	
Degree of tumor ulceration	Grade 1	0	22	17	0.219
	Grade 2	1	26	35	
Venous invasion					<0.0001
SM-V	$-$	0	24	50	
	$+$	1	24	2	
PM-V	$-$	0	36	51	0.001
	$+$	1	12	1	
Ex-V	$-$	0	17	45	<0.0001
	$+$	1	31	7	
A host inflammatory cell reaction	Grade 1	0	42	30	0.002
	Grade 2	1	6	22	
Fibrosis	Grade 1	0	38	32	0.089
	Grade 2	1	10	20	
Degenerative change	Grade 1	0	42	39	0.198
	Grade 2	1	6	13	

Group 1: colorectal carcinoma with liver metastasis; Group 2: colorectal carcinoma without evidence of hepatic metastasis.

3) Primary site: The tumors were categorized as colonic or rectal with the rectosigmoid junction being included with the rectum. In Group 1, 22 tumors (46%) were located in the rectum, while in Group 2 13 tumors (25%) were in the rectum.

4) Extent of tumor (Ex/M ratio): We defined 'Ex' as the maximal width of the tumor at the extramural layer in the cross section. Likewise, 'M' was evaluated as the maximal width of the tumor surface in the same section. The ratio of 'Ex' to 'M' was calculated in each case (Fig. 3) to express the spread of tumor cells in the extramural layer. Twenty-two (45.8%) out of the 48 cases in Group 1 had a high Ex/M ratio (more than 2/3), compared with only 12 (23.1%) of the 52 cases in Group 2.

5) Incidence of blood vessel invasion: Vascular invasion was examined in both the EVG-stained sections and HE sections (Fig. 1). Elastic tissue stains such as EVG stain have been emphasized to be useful for both the detection

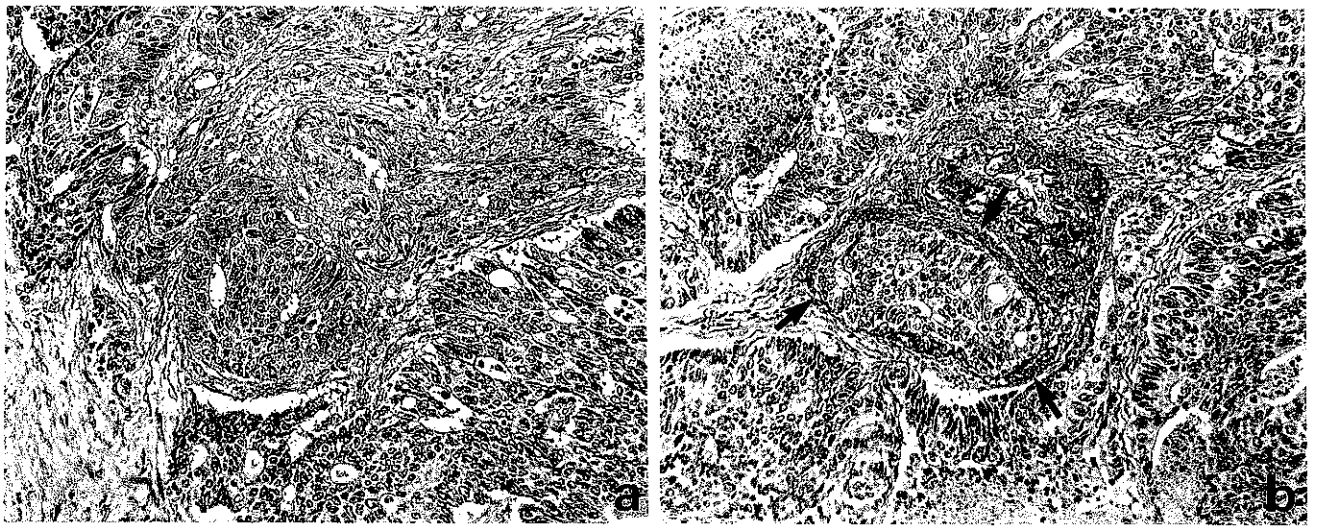

Fig. 1. Group 1 (a 63-year-old man with sigmoid colon carcinoma). Both sections are taken from the same block of tissue. (a) It is difficult to detect any intravenous spread of carcinoma tissue (HE, ×150). (b) Section stained with EVG stain. Compare with the HE-stained section in A for the selective merits of the two stains. Note that the lumen is almost completely occluded (EVG stain, ×150). This vein invaded by tumor cells is classified as SM-V.

of blood vessels and their differentiation from lymphatic vessels.[11, 12] The veins invaded by the tumor were classified according to their location; the veins outside the proper muscle of the colorectal wall were designated 'Ex-V', while those in the submucosal layer and in the muscular layer were classified as 'SM-V' and 'PM-V', respectively. In Group 1, 24 out of 48 cases (50%) had SM-V, whereas only 2 out of 52 cases (3.8%) had SM-V in Group 2. PM-V was recognized in 12 of 48 cases (25.0%) of Group 1 but in only 1 of 52 (1.9%) of Group 2. Moreover, with respect to Ex-V, there were 31 positive cases (64.6%) in Group 1, but only 7 positive cases (13.5%) in Group 2. Venous invasion was a significant factor (SM-V, $P<0.0001$; PM-V, $P=0.001$; Ex-V, $P<0.0001$).

6) Degree of tumor ulceration: The degree of ulceration in the tumor was classified into two groups: grade 1 (either a shallow ulceration or no ulceration at all was seen, while the tumor cells sporadically permeated the muscularis propriae) (Fig. 2) and grade 2 (deeply excavated central crater and a widely separated proper muscle layer were recognized) (Fig. 3). Grade 2 ulcerations were frequently seen in both groups; 26 and 35 cases in Groups 1 and 2, respectively.

7) Host inflammatory cell response at the tumor edge: Host inflammatory cell response at the advancing margin of the tumors was analyzed. Typical reactions were represented by a marked inflammatory infiltrate over the entire advancing margin of the tumor and appeared as a band-like area (Fig. 4). The degree of reaction was classified into two groups: grade 1 (no stromal reaction or mild reaction without a band-like appearance), and grade 2 (typical reaction with a band-like appearance). A typical host inflammatory cell reaction was recognized in 22 cases (42.4%) of Group 2, compared with 6 cases (12.5%) of Group 1.

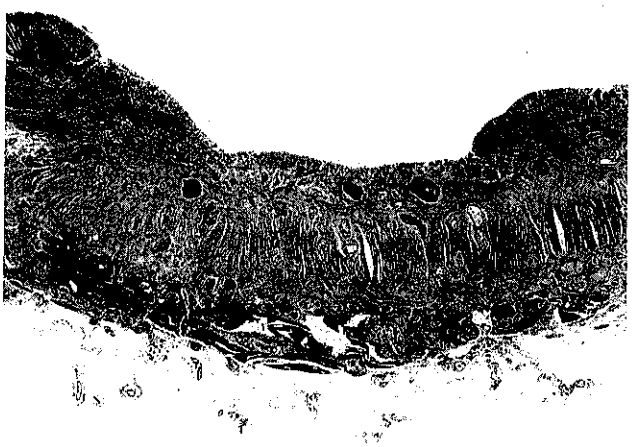

Fig. 2. Grade 1 ulcer (Group 1, a 42-year-old woman with rectal carcinoma). Malignant cells forming small and irregular tubules involve the entire thickness of the colonic wall; associated with a shallow ulceration (HE, ×3).

8) Fibrosis: The appearance of fibrosis at the advancing margin of the tumors was analyzed. Typical fibrosis exhibited a band-like appearance at the whole advancing margin. The degree of fibrosis was also classified as a host inflammatory cell response; grade 1 (no fibrosis or mild fibrosis without a band-like appearance), and grade 2 (fibrosis with a band-like appearance). Grade 2 fibrosis was more frequently recognized in carcinomas of Group 2 than in those of Group 1.

9) Degenerative and destructive changes: These features were occasionally recognized in the front carcinoma tubules in specimens with a stromal reaction. The distribution of carcinoma tubules in degenerative and destructive processes at the whole advancing margin of the tumor was distinguished into the following two groups: grade 1 (negatively or focally recognized) and grade 2 (diffusely recognized). Tumor cells undergoing degenerative change were more frequently present in Group 2 than Group 1.

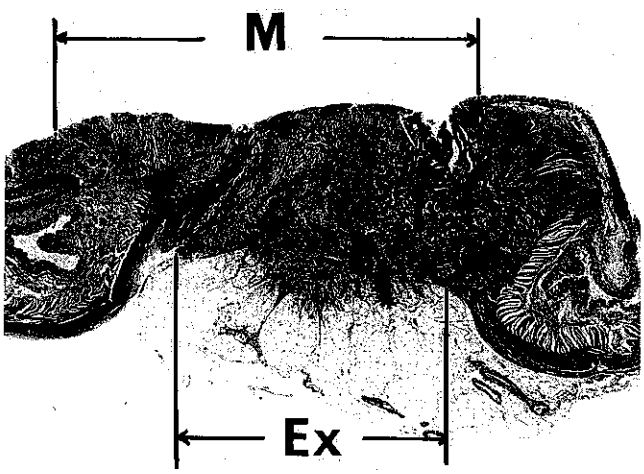

Fig. 3. Grade 2 ulcer (Group 1, an 89-year-old woman with cecal carcinoma). The proper muscle layer is widely separated by carcinoma, and malignant cells have invaded down to the serosa (HE, ×3).

Immunohistochemical findings p53 expression was detected in 49 (49%) of 100 cases of colorectal carcinomas: 18 (34.6%) in Group 1 and 27 (56.3%) in Group 2. The p53 was localized in the nuclei of the carcinoma cells, while the normal epithelial cells adjacent to carcinomas were completely negative for staining with p53 (Fig. 5). The percentage and distribution of stained nuclei were analyzed. p53 was diffusely positive within the tumor in 20 (41.7%) out of 48 cases in Group 1, and in 8 (15.4%) out of 52 cases in Group 2 ($P=0.0069$). CA19-9 caused a positive reaction in 20 (41.7%) tumors of Group 1, and in 9 (17.3%) tumors of Group 2 ($P=0.0138$).

Univariate analysis The chi-square test results on the clinico-pathological and immunohistochemical factors of the patients are shown in Tables II and III. Among 15 factors, location ($P=0.049$), Ex/M ratio ($P=0.023$), SM-V ($P<0.0001$), PM-V ($P=0.001$), Ex-V ($P<0.0001$), p53 ($P=0.0069$), CA19-9 ($P=0.0138$), and

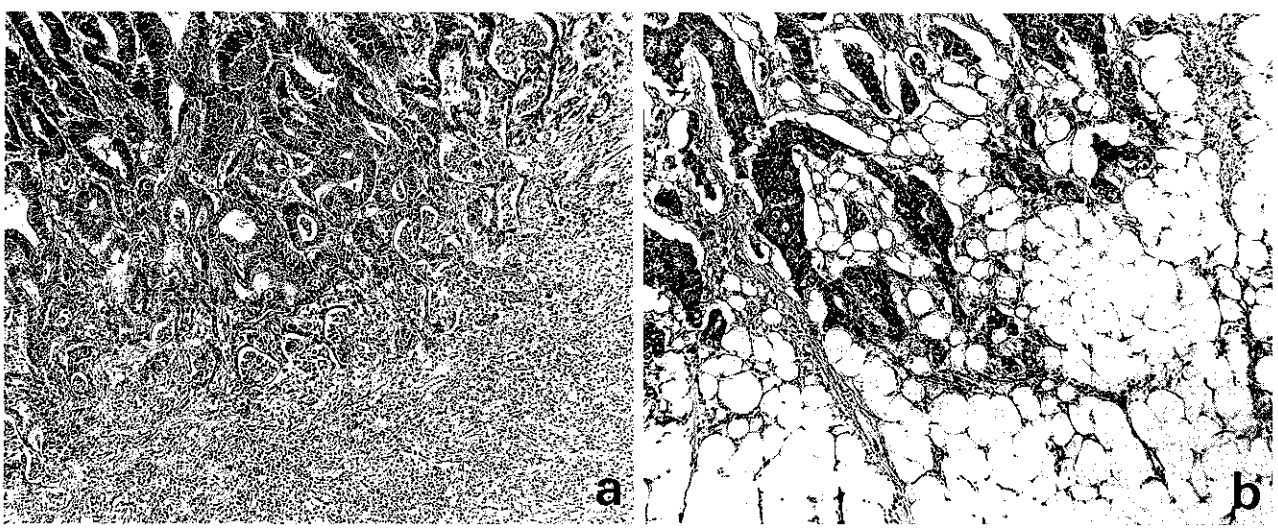

Fig. 4. A host inflammatory cell reaction at the advancing margin of the tumor is prominent, and degenerative and destructive changes are recognized in the front malignant tubules (a, HE, ×74, Group 2, a 53-year-old man with rectal carcinoma). Neither exudative stromal reactions nor degenerative changes are recognized in another case (b, HE, ×74, Group 1, an 89-year-old woman with cecal carcinoma).

host inflammatory cell reaction ($P=0.002$) were strongly related to hepatic metastasis of colorectal carcinomas.

Multivariate analysis A multivariate analysis using a stepwise logistic regression was employed to evaluate the influence of the foregoing 15 clinicopathologic variables on liver metastasis. Six factors were significant, i.e., location, extent of tumor, host inflammatory cell reaction, SM-V, Ex-V, and p53 (Table IV). We obtained the following equation from this analysis:

$$P(Y=1)=\exp a/(1+\exp a)$$
$$a=3.524\,(\text{SM-V})+2.731\,(\text{Ex-V})+2.718\,(\text{E/M})+2.562(\text{Lo})+1.858\,(\text{p53})+1.941\,(\text{HIR})-4.397$$

(E/M, Ex/M ratio; Lo, location; HIR, host inflammatory cell reaction).

When the cut-off point was set at 0.55 units, the sensitivity and specificity were determined to be 81.3% and 92.3%, respectively.

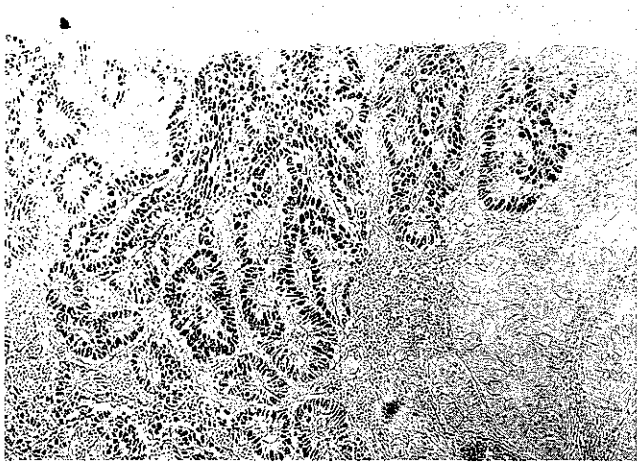

Fig. 5. The immunohistochemical method demonstrates diffuse immunoreactivity for p53 in the tumor cells and a negative reaction for p53 in the non-neoplastic epithelium (×100) (Group 1, a 51-year-old woman with rectal carcinoma).

DISCUSSION

The liver is the most common site of metastasis.[13] About 50% of the patients with colorectal carcinoma demonstrate recurrence, while 40 to 70% of them experience metastases to the liver.[13,14] The overall five-year survival rate of the patients undergoing hepatic resection for metastasis from colorectal carcinoma was 25%, which is significantly higher than that of a group of historical controls who had resectable metastases that were not removed.[15] Surgical resection is at present the most effective (potentially curative) treatment for metastatic liver tumors; the risk factors that influence the criteria for resection and which also have an impact on survival have been evaluated and consist of the number, size and distribution of metastases.[16] The previous data[17] revealed a favorable outcome for the patients with a smaller number and smaller size of metastatic foci or with unilobular lesions when compared with the patients who had a larger number and larger size of metastatic foci or bilobular lesions, respectively. Early detection of secondary liver tumor from colorectal carcinoma, or better means for predicting its development, should contribute to improving the outcome. The present series has indicated the independent influence of six risk factors on hepatic metastases after the resection of colorectal carcinomas, and we have obtained a multivariate equation which may be helpful in earlier detection of hepatic recurrence or a more judicious selection of patients for trials of adjuvant chemotherapy.

A high incidence of hepatic metastases in venous invasion-positive cases has also been reported[18,19] and the independent effect of venous invasion was confirmed in our multivariate analysis. Lui[11] considered that, at first, tumor cells invaded veins in the submucosa and passed through the extramural veins, resulting in hepatic metastases. Talbot *et al.*[7] and others indicated that the spread into the extramural veins had the greatest adverse influence on hepatic metastases. In our series, tumor invasion into the veins in the submucosal layer (SM-V) was shown to have a significant effect on hepatic metastases

Table IV. Risk Factors for Liver Metastasis of Colorectal Carcinoma (Stepwise Logistic Regression)

Variable	Coefficient	Standard error (SE)	Coefficient/SE	*P* value
Location	2.562	0.851	3.011	0.0006
Ex/M ratio	2.718	0.97	2.802	0.0012
Host inflammatory cell reaction	−1.941	0.827	−2.347	0.0103
SM-V	3.524	1.09	3.233	<0.0001
Ex-V	2.731	0.77	3.547	0.0001
p53	1.858	0.823	2.258	0.0177
Constant	−4.397	1.11	−3.950	

independently of Ex-V. SM-V is an important variable, as is Ex-V. The multivariate effect of PM-V was lost, because such cases tended to have SM-V and/or Ex-V.

The Ex/M ratio, which reflects the extent of the direct spread of a tumor through the intestinal wall, significantly increased the incidence of hepatic metastases. It was thus considered to be a useful and practical factor for routine diagnosis.

Inflammatory cell infiltration in the tumor stroma, particularly lymphocytic stromal infiltration, has been recognized and is considered to be a favorable prognostic factor in various neoplasms such as breast carcinoma[20] and gastric carcinoma.[21] Sakurai *et al.*[22] examined the exudative stromal reactions at the advancing margin of 168 colorectal carcinomas and found band-like stromal reactions in 28 tumors. The inflammatory infiltrate in typical stromal reactions was composed mostly of lymphocytes, plasma cells, and neutrophils. The patients with tumors having typical stromal reactions showed a higher survival rate than those having non-typical reactions. In the present study, it was confirmed that the typical stromal reaction also reduced the incidence of hepatic metastases of colorectal carcinomas. These results suggested that stromal reactions represented a host response against the spread or invasion of carcinoma cells.

Mutation of p53, a tumor-suppressor gene product,[23] leads to immunohistochemically detectable overexpression of the product.[24] The mutated form of p53 does not have a tumor-suppressor function, and in some neoplasms (e.g., in the breast),[25] p53 expression is considered to be a significant prognostic factor. The influence of p53 expression on the prognosis of colorectal carcinomas, however, is still unclear. No significant correlation has been reported between the expression of p53 and the histologic grade, venous invasion, liver metastasis[26] or survival.[27] However, others have found that p53 expression is related to early relapse and death.[28, 29]

The reported positive rate of p53 protein in colorectal carcinoma has ranged from 42 to 61%.[26, 27, 30] In our study, a similar value was obtained; 49% of 100 colorectal carcinomas. Our results also revealed no relation between p53 expression and hepatic metastases, when we only considered whether p53 was positive or not. However, the p53 staining showed a heterogeneity in all the positive cases. In view of the percentage and distribution of the p53-positive nuclei, the patients whose tumors demonstrated diffusely positive immunoreactivities of p53 were more frequently found in Group 1 than in Group 2 ($P=0.0069$). Our findings thus suggested that diffuse positivity of p53 influenced the incidence of hepatic metastases, which is in accordance with previous reports.[28, 29]

The results of multivariate analysis thus highlight the need for a careful assessment of surgical specimens. The above equation might be useful in predicting the behavior of colorectal carcinoma and in designing an optimum treatment regimen for patients with colorectal carcinoma. We believe that adjuvant chemotherapy should be considered when $Y \geq 0.055$ in the above equation, and close monitoring should be conducted in such cases to ensure the early detection of hepatic metastases.

ACKNOWLEDGMENTS

The authors thank Mr. Brian T. Quinn for advice concerning the manuscript.

(Received June 27, 1994/Accepted September 1, 1994)

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