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The fibrotic focus in advanced colorectal carcinoma: a hitherto unrecognized histological predictor for liver metastasis

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Abstract A fibrotic focus (FF) is a clearly defined area consisting of fibroblasts and/or collagen fibres arranged in irregular or storiform patterns within tumours. We looked to see whether FF in advanced colorectal carcinoma was associated with distant organ metastasis especially to the liver. The correlation between FF and the presence of synchronous or total (synchronous and/or metachronous) liver metastasis and tumour recurrence was assessed in 77 patients with Dukes B and C advanced colorectal carcinoma treated by resection. The median follow-up period was 21 months. In multivariate analysis, FF significantly increased the relative risk (RR) of synchronous liver metastasis (RR=4.9, $P<0.05$) and total liver metastasis (RR=4.6, $P<0.05$). FF also increased the RR of tumour recurrence (RR=2.4), but the increase was not statistically significant. FF is a newly recognized histological indicator of liver metastasis in advanced colorectal carcinoma.

Key words Colon carcinoma · Fibrotic focus · Liver metastasis · Tumour recurrence

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Introduction

It is very important to assess the metastatic potential of advanced colorectal carcinoma and to predict early tumour recurrence after the initial operation, because patients with highly metastatic tumours require immediate adjuvant chemotherapy. Nevertheless, no clinicopathological factors associated with early tumour recurrence have yet been identified, although clinicopathological factors associated with long-term survival (5–10 years) have been reported [3, 4, 6–9, 16, 21, 24].

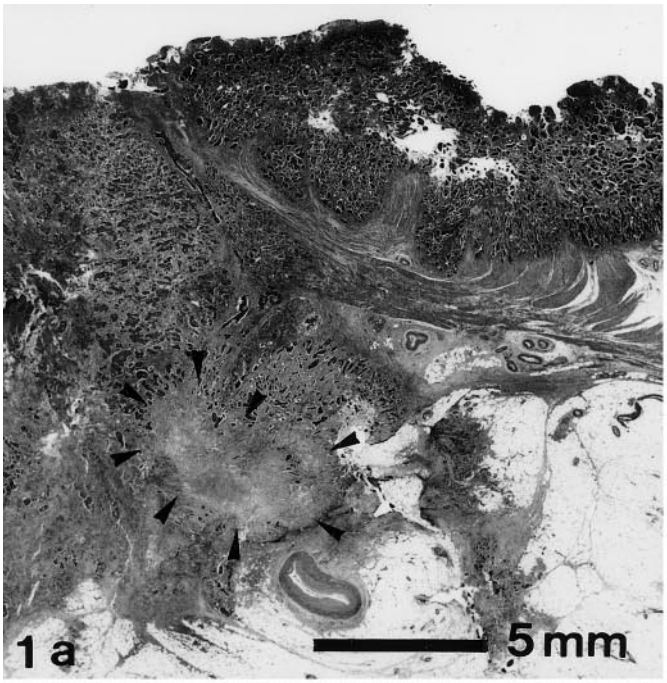
A fibrotic focus (FF) is a clearly defined area consisting of fibroblasts and/or collagen fibres arranged in irregular or storiform patterns within tumours. FF is known to be an unfavourable prognostic factor in lung adenocarcinoma [23], invasive ductal carcinoma of the breast [11, 13] and adenocarcinoma of the pancreatic head [20]. FF is also a predictor of high aggressiveness of invasive ductal carcinoma of the breast [10]. These findings suggested that FF might be an excellent histological predictor of the outcome of adenocarcinoma in other organs.

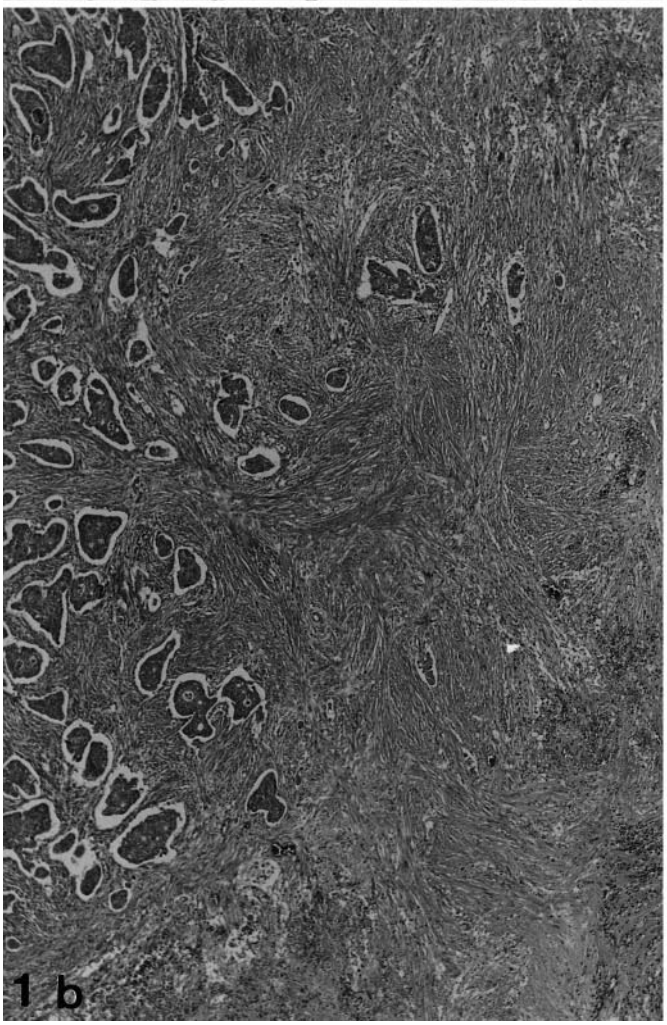
We analysed whether FF in advanced colorectal carcinoma is associated with a high rate of metastasis, especially to the liver, or with early tumour recurrence within 3 years after the initial operation.

Materials and methods

Seventy-seven patients with advanced colorectal carcinoma (Dukes B, 45 cases; Dukes C, 32 cases; Stages II, 41 cases; III; 26 cases; IV, 10 cases, according to the UICC stage classification [26]) underwent curative resection between February 1993 and October, 1994 at the National Cancer Centre Hospital East. Cases diagnosed as adenocarcinoma of the ordinary histological type were used in this study; cases with special histological types (for example mucinous or signet-ring cell carcinoma) were excluded. Clinical information at the time of the operation (age, sex, tumour location, operation date, presence or absence of liver metastasis) and during follow-up (date and site of tumour recurrence, date of death) were obtained from the patients' medical records. Metastatic sites were detected by ultrasound and/or computed tomography in most cases. Biopsy was performed in some patients to confirm local recurrence of the tumours in the pelvic region.

There were 48 male and 29 female patients, with ages ranging from 40 to 87 years (average: 63 years). The follow-up period was 8–34 months (median: 21 months). Sixty patients (77.9%) were alive and well without tumour recurrence and 9 were alive with disease. Eight patients (10.4%) died of their disease from 8 to 24 months after the initial operation (median: 10 months). Seventeen patients (22.1%) developed tumour recurrence in the liver (10 cases), locally (3 cases), and in brain, bone, peritoneum, or lymph nodes (1 case each). Eight (10.4%) of the 17 patients, whose sites of recurrence were local (3), liver (2), and brain, bone, peritoneum (1 each), died of their disease. Ten patients (13.0%) had had liver metastases, which were completely resected at the time of the initial operation (synchronous liver metastasis, SLM). Liver metastasis after the initial operation (metachronous liver metastasis, MLM) was observed in 5 (6.4%) of the 10 cases with SLM, and 5 of the 67 cases without SLM. Thus, total liver metastasis (TLM; synchronous and/or metachronous liver metastasis) was present in 15 of the 77 cases (19.5%).





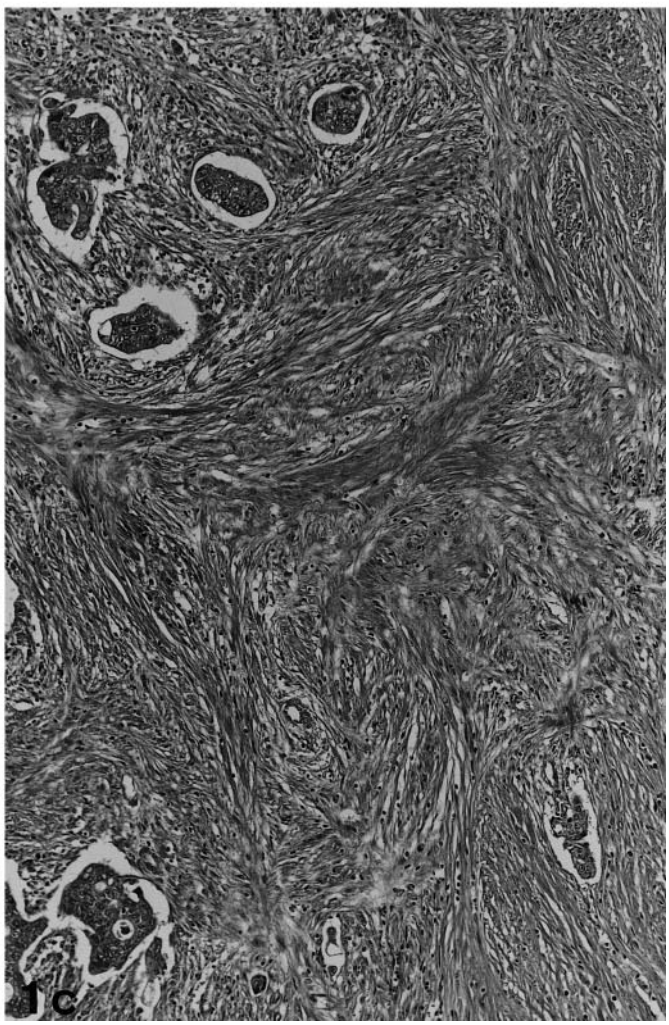
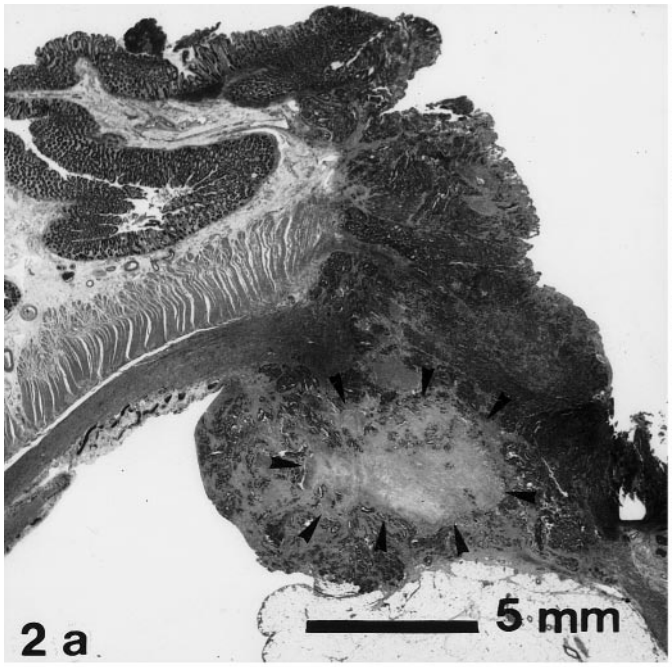


Fig. 1a–c A large (fibrotic focus) (FF) in adenocarcinoma of the colon. **a** The FF, 6 mm in largest diameter (*arrowheads*), is seen in a tumour invading the subserosa. **b** Fibroblasts are arranged in an irregular or storiform pattern admixed with small foci of carcinoma cells. HE, original magnification ×34. **c** Storiform-type pattern of fibroblasts is clearly seen in the FF. HE, original magnification ×85



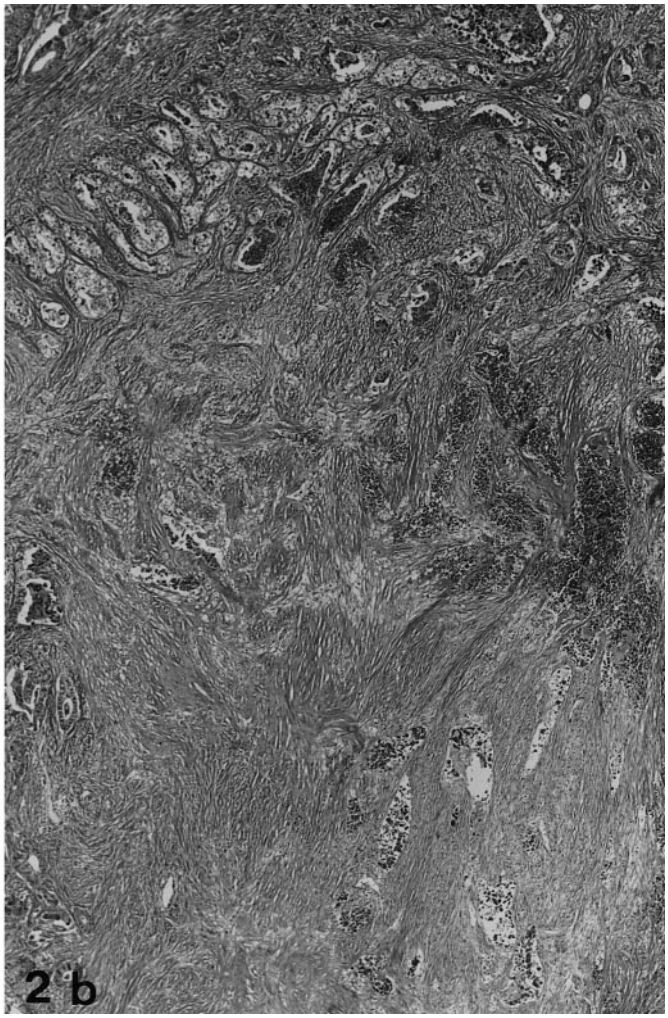


Fig. 2a, b A large FF in adenocarcinoma of the colon. **a** The FF, 7 mm in largest diameter (*arrowheads*), has formed in a tumour invading the subserosa. **b** The FF consists of dense collagen fibres and fibroblasts arranged in storiform pattern. Tumour necrosis is seen in the FF. HE, original magnification ×34

Fresh surgical specimens were fixed overnight in 10% formalin at room temperature, and the gross appearance and the size of the tumour were recorded. The entire tumour was then serially cut into 1-cm-wide strips. Representative tissue sections were taken from the centre of each tumour for microscopic examination. The presence of FF within the tumor was not macroscopically evident in many cases. Therefore, almost all slices of the tumour were routinely processed and embedded in paraffin. Histological sections were stained with haematoxylin and eosin (HE), and also stained with alcian blue and periodic acid–Schiff (AB-PAS) to detect mucin, and with an elastic stain to evaluate the presence or absence of vascular invasion.

The following factors were assessed: depth of invasion [15], invasive growth pattern (whether expanding, minimally infiltrating or highly infiltrating), vascular invasion (none, slight, occasional, or frequent), neural invasion (present or absent), histological differentiation of the tumour at the invading edge [28], the degree of inflammation in the stroma, the presence or absence of a Crohn's-like lymphoid reaction [8] and the presence or absence of FF. The first four factors were evaluated according to the General Rules for Clinical and Pathological Studies on Cancer of the Colon, Rectum and Anus of the Japanese Research Society for Cancer of the Colon and Rectum [15]. All pathological factors were assessed by two of the authors (R.N. and T.H.) without knowledge of the patients' clinical outcome.

FF has the following features [10–13, 20]. It is clearly defined areas within a tumour that consists of an increased number of fibroblasts and/or collagen fibres. FF often consists of fibrous bands expanding radially into the surrounding area, and appears as radiating fibrosclerotic cores or scars. FF is surrounded by a more cellular zone of infiltrating carcinoma cells and occupies various percentages of the tumour (Figs. 1a, 2a, 3a). When FF is 3 mm or smaller tumour cells are infrequently seen within them (Fig. 3b); however, tumour cells growing in a scirrhous pattern or in solid nests are seen as the size increases (Figs. 1b, 2b). Fibroblasts or collagen fibers in FF are arranged in irregular or storiform patterns with increased fibroblast cellularity and/or collagenization. The arrangement of the fibroblasts or collagen fibres forming FF is less orderly than that of the surrounding tumour stroma (Fig. 1c). "FF with necrosis" is defined as a FF containing cells that have undergone coagulation necrosis and are surrounded by fibroblasts or collagen fibres (Figs. 2b, 3b). The area of coagulation necrosis of the tumour within the FF is smaller than the area occupied by fibroblasts or collagen fibres. Coagulation necrosis within tumours unaccompanied by proliferation of fibroblasts or collagen fibres is not FF.

Data from 77 patients were subjected to statistical analysis. The Chi-square test was used to evaluate the correlation between individual clinicopathological factors and liver or other distant organ metastasis. The logistic regression model was used to estimate the multivariate relative risks (RRs) of SLM, MLM TLM, and total tumour recurrence (TTR, distant metastasis and/or local recurrence) within 3 years after the initial operation [with 95% confidence intervals (CI)]. All calculations were performed using Statistica/DOS software (Stat Soft, Tulsa, Okla).

Results

The size of FF (diameter) ranged from 1 to 10 mm. In tumours with FF, 13 of 21 had one FF and 8 had two or more. There was no significant correlation between the size or number of FF within a tumour and SLM, TLM or TTR (data not shown). FF with tumour necrosis were observed in only two cases.

SLM was significantly associated with Dukes C (P=0.019), FF (P=0.013), and occasional or frequent vas-

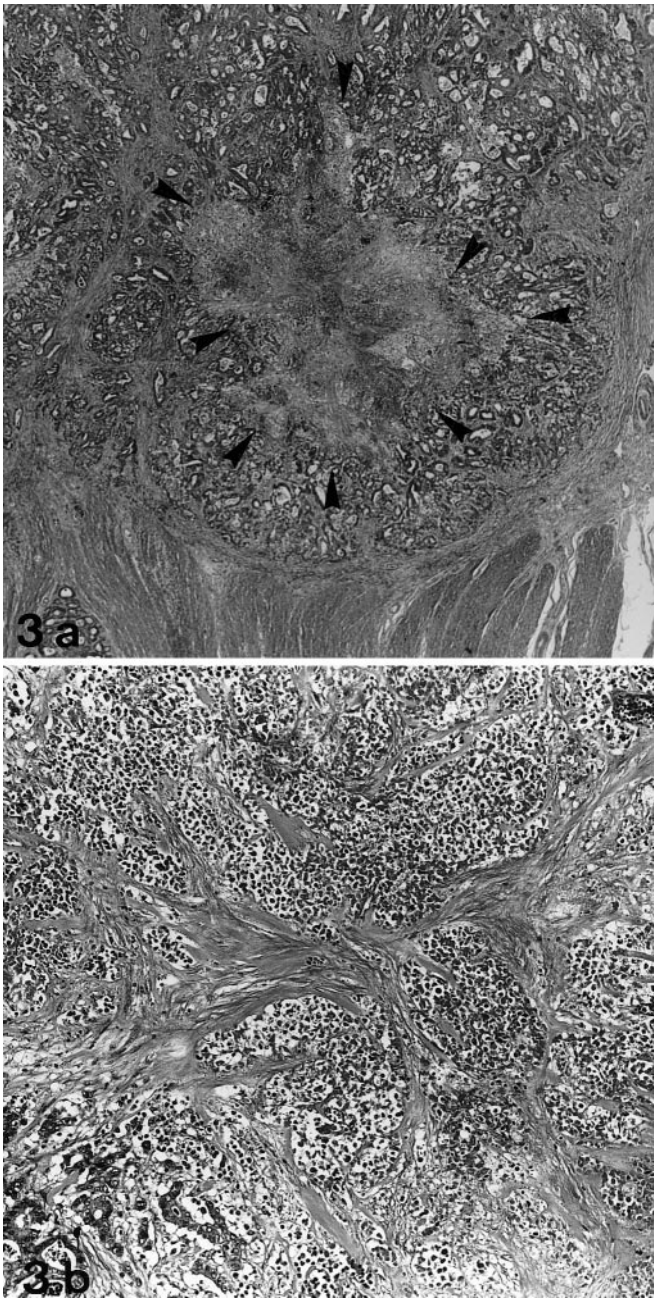


Fig. 3a, b A small FF in adenocarcinoma of the colon. **a** The FF, 2 mm in largest diameter (*arrowheads*), is seen within the tumour. HE, original magnification ×17. **b** The FF consists of increased fibroblasts arranged in a storiform pattern. Necrotic tumour cells are admixed with fibroblasts. HE, original magnification ×85

cular invasion (P=0.036; Table 1). FF (P=0.012) and occasional or frequent vascular invasion (P=0.008) were the significant factors for TLM (Table 1). Only occasional or frequent vascular invasion (P=0.037) was significantly associated with MLM. There was no correlation between the various clinicopathological factors mentioned above (Dukes classification, FF and vascular invasion).

TTR was significantly associated with Dukes C (P=0.005), FF (P=0.014), poorly differentiated histology (P=0.005), occasional or frequent vascular invasion (P=0.002), and a primary tumour in the rectum (P=0.031) (Table 1).

Other pathological factors (size of the tumour, an invasive growth pattern, neural invasion, degree of inflammation in the tumour stroma, Crohn's-like lymphoid reaction) and demographic factors (age and sex) did not show statistically significant correlations with SLM, MLM, or TTR (data not shown).

Multivariate analysis was conducted using the histological factors (Dukes classification, FF, histology, vascular invasion) found to be significantly associated with a risk of SLM, TLM or TTR in univariate analysis.

Multivariate analysis demonstrated that the presence of FF was the only factor significantly associated with increased risk of liver metastasis (SLM and TLM, Table 2). Among the other factors, vascular invasion was significantly associated with TLM and TTR (Table 2). Dukes' classification increased RRs to higher than 2.0 for SLM and TLM, but the increases was not statistically significant (Table 2). Although cases of rectal cancer had RRs higher than 2.0 for SLM, TLM and TTR, and those with poorly differentiated histology at the invading edge had a RR higher than 2.0 for TTR, the increases in the RRs were not statistically significant (Table 2).

Discussion

This study demonstrates that the presence of FF in advanced colorectal carcinoma is an important histological factor associated with an increased risk of SLM and TLM. Occasional or frequent vascular invasion was associated with TLM, but not with SLM. FF is probably the only histological factor that is strongly associated with the liver metastasis. The presence of FF has been shown to be associated a poor prognosis in small peripheral lung adenocarcinoma [23], and FF have also been reported as an important histological factor in determining the short- and long-term survival with invasive ductal carcinoma of the breast [11, 13]. In addition, FFs is a significant prognostic factor for patients with advanced adenocarcinoma of the pancreatic head [20]. FF seems to be a marker of high aggressivity of adenocarcinoma in various organs.

We found no significant correlation between the presence of FF and MLM. Since MLM occurred in only 10 patients in this series, the presence of FF failed to show a significant association with MLM. In addition, we performed statistical analysis of TLM using a logistic regression model, since the SLM patients included some with no clinical period for liver metastasis in TLM. We performed statistical analyses using the log-rank test and Cox proportional hazard regression model in another large series of patients with advanced colorectal cancers to determine whether the presence of FF significantly correlated with future MLM.

Invasive growth pattern, neural invasion, degree of inflammation and Crohn's-like lymphoid reaction did not increase RRs in this study. These factors are probably useful as predictors of long-term survival [3, 7–9, 16], but they seemed to be less effective in predicting short-term tumour recurrence in advanced colon carcinoma.

Table 1 Univariate analysis of correlations between clinicopathological factors and liver metastatsis or tumour recurrence (*Dukes* Duke's classification, *FF* fibrotic focus, *W/D* well differentiated, *M/D* moderately differentiated, *P/D* poorly differentiated, *VI* vascular invasion, *0* none, *1* slight, *2* occasional, *3* frequent, *SLM* synchronous liver metastasis, *TLM* total liver metastasis, *TTR* total tumour recurrence, – absent, + present)

Factors	No. of patients (%)								
	SLM		*P*-value	TLM		*P*-value	TTR		*P*-value
	–	+		–	+		–	+	
Dukes	67	10	62	15	56	21			
B	40 (95)	2 (5)		37 (88)	5 (12)		36 (86)	6 (14)	
C	27 (77)	8 (23)	0.019	25 (71)	10 (29)	0.066	20 (57)	15 (43)	0.005
FF									
Absent	52 (93)	4 (7)		49 (88)	7 (12)		45 (80)	11 (20)	
Present	15 (71)	6 (29)	0.013	13 (62)	8 (38)	0.012	11 (52)	10 (48)	0.014
Histology									
W/D and M/D	60 (88)	8 (12)		55 (81)	13 (19)		53 (78)	15 (22)	
P/D	7 (78)	2 (22)	0.380	7 (78)	2 (22)	0.825	3 (33)	6 (67)	0.005
VI									
0/1	58 (91)	6 (9)		55 (86)	9 (14)		51 (80)	13 (20)	
2/3	9 (69)	4 (31)	0.036	7 (54)	6 (46)	0.008	5 (38)	8 (62)	0.002
Tumour site									
Colon	44 (92)	4 (8)		41 (85)	7 (15)		39 (81)	9 (19)	
Rectum	23 (79)	6 (21)	0.118	21 (72)	8 (28)	0.163	17 (59)	12 (41)	0.031

Table 2 Multivariate analysis of correlations between clinicopathological factors and liver metastasis or tumour recurrence. [*LMR* liver metastatic ratio, *RR* relative risk, (simultaneously adjusted for the variables listed), 95% CI 95% confidence interval]

Factors	Total	SLM			TLM			TTR		
	76	LMR (%)	RR	95% CI	LMR (%)	RR	95% CI	LMR (%)	RR	95% CI
Dukes										
B	41	5	1.0		12	1.0		15	1.0	
C	35	23	4.4	0.7–26.2	29	2.2	0.6–8.7	43	1.9	0.5–7.1
FF										
Absent	55	7	1.0		13	1.0		20	1.0	
Present	21	29	4.9*	1.0–23.6	38	4.6*	1.2–17.5	48	2.4	0.6–9.4
Histology										
W/D and M/D	67	12	1.0		19	1.0		22	1.0	
P/D	9	22	0.4	0.02–4.0	22	0.1	0.0–1.8	67	6.0	0.9–38.7
VI										
0/1	63	10	1.0		14	1.0		21	1.0	
2/3	13	31	4.6	0.7–29.5	46	8.1*	1.5–42.7	62	5.0*	1.0–23.8
Tumour site										
Colon	47	9	1.0		15	1.0		19	1.0	
Rectum	29	21	2.4	0.4–13.3	28	2.5	0.6–10.8	41	2.1	0.5–8.2

The mechanism of formation of FF within tumours is not understood, but there are two possibilities. The first is that FF is induced by tumour necrosis, as a reparative reaction. The cohesive growth of tumour cells in masses growing in solid nests results in decreased blood flow or hypoxia within the tumour, resulting in coagulation necrosis. The necrotic area is probably replaced by FF in the same way as in the wound-healing response [5, 19]. The second possibility is that FF arises from the interaction between tumour cells and stromal fibroblasts by an autocrine or paracrine mechanism. It is possible that certain growth factors (EGF, TGF alpha or beta), which are potent stimulators of fibroblasts, are produced by the tumour cells [2, 12, 14, 25] or fibroblasts themselves [14, 29]. In invasive ductal carcinoma of the breast, we have reported that tumour cells growing in a scirrhous pattern with FF showed a significantly higher frequency of bFGF expression than those within FF [12]. This pathway may be responsible for the formation of FF in scirrhous tumours but is probably less important in solid tumours [12, 25, 29].

Why is FF associated with the aggressivity of colorectal carcinoma? The presence of necrosis indicates that a solid tumour has high proliferative activity, and is thus highly malignant. However, tumour–stroma interaction, mainly mediated by growth factors, seems to be the mechanism of FF formation, especially in scirrhous tumours [12]. The

growth factors produced by stromal fibroblasts have been shown to stimulate the growth of breast cancer cells [17] and the invasiveness of scirrhous gastric cancer cells [14]. Many growth factors also have angiogenetic effects in cancer tissue [27]. Highly angiogenetic colorectal cancers are prone to develop distant organ metastasis, especially to the liver [22]. Colorectal cancer with FF probably produces several growth factors whose source is either the neoplastic cells or stromal fibroblasts, and these factors increase the metastatic potential by stimulating growth, increasing motility and inducing neovascularization.

It is important to investigate the histological factors associated with early liver metastasis after initial operation in colorectal cancer, as the liver is the initial metastatic site in more than 60% of patients who die of the disease [18]. In this study, FF showed a significant correlation with SLM and TLM. This suggests that FF is a specific histological factor associated with liver metastasis. Patients who have colorectal carcinoma with FF must be closely followed up for the detection of early liver metastasis.

FF is a hitherto unrecognized histological predictor of liver metastasis in advanced colorectal carcinoma. Although the presence of TLM was not associated with early tumour death (data not shown), the metastatic potential of the tumour cells may be found to be correlated with the outcome in long-term follow-up. Further clinicopathological study with long-term follow-up and a large number of samples is necessary to determine the biological significance of FF in colorectal carcinoma.

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