Tumor Necrosis in Patients with TNM Stage IV Colorectal Cancer without Residual Disease (R0 Status) Is Associated with a Poor Prognosis

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Abstract. *Aim: To examine the usefulness of the histopathological finding of tumor necrosis for stratifying TNM stage IV colorectal cancer in R0 status. Patients and Methods: We enrolled 98 patients with stage IV colorectal cancer, without residual disease after resection. The extent of necrosis was assessed using published thresholds, the extent was graded as "absent", "moderate" (<30% of tumor area), or "severe" (≥30%) in each section. Results: In multivariate analysis, the only significant difference in the disease-free survival rate was related to tumor necrosis (p=0.01) and the significant differences in the overall survival rates were related to the maximum tumor size and the degree of tumor necrosis (p=0.02 and p=0.001, respectively). Conclusion: Tumor necrosis is associated with a poor prognosis in colorectal cancer and may allow the stratification of TNM stage IV patients without residual disease after surgery.*

The use of specific histopathological findings, for example "tumor budding", in resected specimens to predict poor prognosis in colorectal cancer has been assessed in a number of studies (1, 2). In recent years, tumor necrosis has become recognized as a potential prognostic marker for a variety of solid tumor types, including those of the breast (3), lung (4), pancreas (5), kidney (6), and upper urinary tract (7, 8), as well as for soft tissue sarcomas (9). Non-clinical studies have shown that tumor necrosis is correlated with local and systemic inflammation especially the one caused by IL-6, apoptosis, and microsatellite instability (10, 11). More recently, there have been a number of clinical studies on tumor necrosis in colorectal cancer (12, 13). However, previous reports have included only patients with TNM stage II or III colorectal cancer (12, 13). Here, we report the findings of the first study, to our knowledge, on tumor necrosis in TNM stage IV colorectal cancer.

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Patients and Methods

We enrolled 98 patients who underwent resection for stage IV colorectal cancer without any residual cancer being detected at the end of surgery. This study took place at the Department of Gastroenterological Surgery, Aichi Cancer Center Hospital, Nagoya, Japan, between January 1980 and December 2006. None of the patients had received chemotherapy or radiation therapy prior to surgery. Complete dissection of all the regional lymph nodes was performed in all cases.

In cases where liver and/or lung metastases were present, the metastatic lesion was removed non-concurrently, usually between two and three months after the primary tumor had been removed. In cases of metachronous liver metastasis, it is generally accepted at our Institution, that delaying resection allows for a more accurate assessment of the number and location of hepatic metastases, which in turn is of benefit in determining which patients should undergo surgery, and in selecting the most appropriate surgical procedure (14, 15). In cases of resectable peritoneal and distant lymph node metastases, the primary tumor was removed synchronously with the metastatic lesion.

The resected specimens were fixed with 10% formalin for several days, and the tumor-containing tissue samples were sliced into 4-μm sections in the region with the deepest tumor invasion. Histopathological diagnoses were established on the basis of hematoxylin and eosin staining at low magnification (×40) using standard procedures without specific immunostaining.

The extent of necrosis was assessed semi-quantitatively and, by using published thresholds, this extent was graded as "absent" (none), "moderate" (<30% of tumor area), or "severe" (>30% of tumor area) in each section before an assessment was made of the overall extent of necrosis (Figures 1 and 2).

We reviewed the hospital records to obtain clinicopathological information regarding the patients, including their gender and age (median, 61 years), lesion location, maximum tumor size (median, 5

cm), greatest depth of invasion of the tumor (pT1+pT2+pT3 vs. pT4), histological type of tumor, presence/absence of lymphatic and venous invasion, the number of metastasis-positive lymph nodes, and use of adjuvant chemotherapy, with regimens including oral 5-fluorouracil, 5'-doxifluridine, carmofur or uracil-tegafur with leucovorin, as the most commonly used drugs, for about 6 to 12 months (16-18). Adenocarcinoma of the rectum was graded predominantly on the basis of glandular appearance, and classified as well/moderately differentiated or "other", according to the WHO histopathological classification of tumors of the colon and rectum (19) and the Japanese Classification of Colorectal Carcinoma (20). Lesions were classified according to whether they were located in the colon or rectum, with the latter defined as a tumor whose lowest border was located between the anal verge and the sacral promontory.

All data are expressed as the mean±SD. Statistical analysis was performed using the Chi-square independence test. Multivariate stepwise logistic regression analysis was subsequently performed to identify factors that might have influenced the outcome. The log-rank test was used to evaluate differences in the overall survival rates and disease-free survival rates. Statistical significance was set at $p<0.05$ and confidence intervals (CIs) were determined at the 95% level.

Results

The data presented in Table I show that no significant difference in the extent of necrosis was observed with respect to gender, average patient age, histological type, maximum tumor size, and lymphatic or venous invasion. There was a significant difference with respect to tumor location, with tumors in the moderate group mainly being located in the rectum, and absent group tumors mainly being located in the colon ($p=0.009$). In an analysis of the greatest invasion depth, moderate group tumors were mainly scored pT1+pT2+pT3, whereas severe group tumors were mainly scored pT4 ($p=0.041$).

There were no significant differences with respect to the presence of synchronous hepatic metastasis, synchronous peritoneal metastasis, metastasis in distant lymph nodes, or adjuvant chemotherapy between the groups (Table II). There were, however, significant differences between absent and moderate groups with respect to metastasis to regional lymph nodes ($p=0.012$), being more frequent in the latter, and between moderate and severe groups for synchronous pulmonary metastasis ($p=0.025$), again being more frequent in the latter.

Table III shows the results of univariate and multivariate analysis performed to identify factors that might be correlated with the disease-free survival rate. In univariate analysis, no significant differences in the disease-free survival rate were observed in relation to gender, age, cancer location, greatest depth of tumor invasion, presence/absence of lymphatic or venous invasion, synchronous hepatic metastasis, synchronous pulmonary metastasis, synchronous peritoneal metastasis, metastasis to distant lymph nodes, or adjuvant chemotherapy. However, the rates differed significantly in relation to the histological type, maximum tumor size, metastasis to regional lymph nodes, and tumor necrosis status ($p=0.039$, $p=0.047$, $p=0.031$, and $p=0.016$, respectively). In multivariate analysis, the only significant difference in the disease-free survival rate were observed with respect to tumor necrosis status ($p=0.011$).

Table IV shows the results of univariate and multivariate analyses performed to identify factors that might be correlated with the overall survival rates. In univariate analysis, no significant differences in the overall survival rates were observed in relation to gender, age, cancer location, greatest depth of tumor invasion, presence/absence of lymphatic or venous invasion, metastasis in distant lymph nodes, or adjuvant chemotherapy. However, the rates differed significantly in relation to the histological type, maximum tumor size, metastasis in regional lymph nodes, synchronous hepatic metastasis, synchronous pulmonary metastasis, synchronous peritoneal metastasis, adjuvant chemotherapy, and tumor necrosis status ($p=0.017$, $=0.033$, <0.0001, $=0.009$, $=0.028$, $=0.040$, and <0.0001, respectively). In multivariate analysis, significant differences in the overall survival rates were observed in the maximum tumor size and tumor necrosis status ($p=0.019$ and $=0.001$).

Figure 3 shows the disease-free and overall survival rates after surgery, of patients who were found not to have residual cancer. Significant differences in the disease-free survival rates were observed between the moderate group and the severe group ($p=0.023$), and in the overall survival rates between the absent group and the moderate group ($p=0.005$), as well as the moderate group and the severe group ($p=0.023$) with survival being poorer in the latter.

Discussion

The present study confirmed that the presence of tumor necrosis, as part of the pathological findings of resected tumor specimens, is a potential stage-independent prognostic factor in TNM stage IV colorectal cancer without residual cancer after resection and might help in determining whether a follow-up with high-potency adjuvant therapy is warranted.

However, this study had two potential problems that need to be considered. One is that tumor necrosis was measured using a semi-quantitative technique; however, it is very difficult to estimate necrosis quantitatively, and all previous reports have used the same semi-quantitative estimation with the same classifications (absent, focal, moderate, or extensive) (12, 13). The second potential difficulty is the lack of subjective cases, and this, we hope, will be addressed by future prospective trials where the significance of tumor necrosis status is assessed in more patients with prognostic information.

In recent years, histopathologically-identified tumor necrosis has been recognized as a potential prognostic marker for a variety of solid tumors including those of the

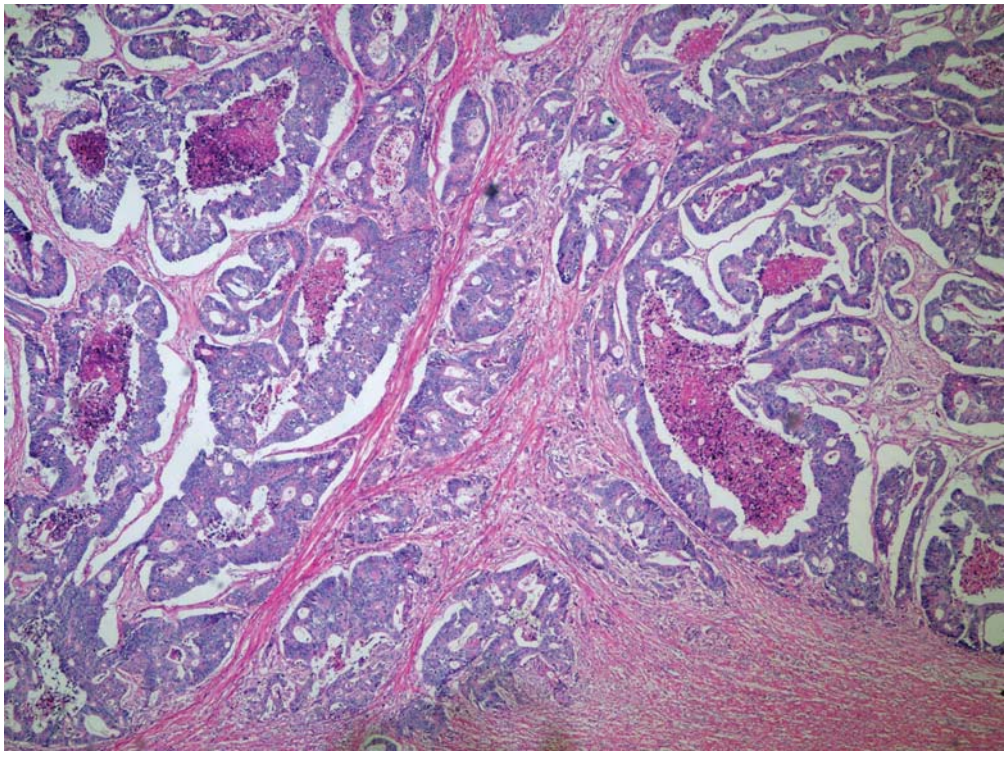

Figure 1. *Moderate necrosis: Nuclear fragmentation is present without any structures consistent with ductal carcinoma. The area of necrotic cells is <30% of the total tumor area. Original magnification, ×200.*

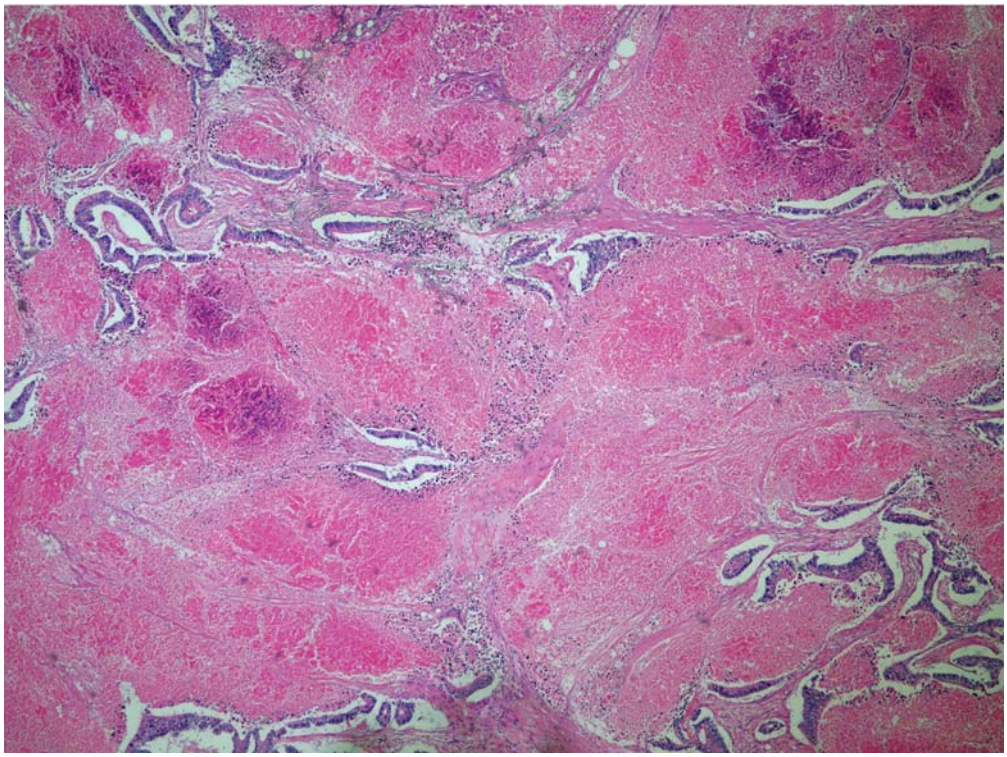

Figure 2. *Nuclear fragmentation is present without any structures consistent with ductal carcinoma. The area of necrotic cells is >30% of the total tumor area. Original magnification, ×200.*

Table I. *Clinicopathological fingings*.

	None (n=15)	Moderate (n=69)	Severe (n=14)	*p*-Value	
				None *vs*. Moderate	Moderate *vs*. Severe
Gender					
Male	5 (33.3%)	39 (56.5%)	10 (71.4%)	0.089	0.223
Female	10 (66.7%)	30 (43.5%)	4 (28.6%)		
Age, years					
≥61	6 (40.0%)	37 (53.6%)	9 (64.3%)	0.215	0.334
<60	9 (60.0%)	32 (46.4%)	5 (35.7%)		
Cancer location					
Colon	14 (93.3%)	41 (59.4%)	10 (71.4%)	0.009	0.229
Rectum	1 (6.7%)	28 (40.6%)	4 (28.6%)		
Histological type					
W/M	13 (86.7%)	60 (87.0%)	10 (71.4%)	0.627	0.146
others	2 (13.3%)	9 (13.0%)	4 (28.6%)		
Greatest invasion depth					
pT1+pT2+pT3	7 (46.7%)	45 (65.2%)	5 (35.7%)	0.148	0.041
pT4	8 (53.3%)	24 (42.0%)	9 (64.3%)		
Maximum tumor size, cm					
<5	5 (33.3%)	29 (58.0%)	3 (21.4%)	0.375	0.125
≥5	10 (66.7%)	40 (42.9%)	11 (78.6%)		
Lymphatic invasion					
Present	11 (73.3%)	55 (79.7%)	11 (78.6%)	0.405	0.586
Absent	4 (26.7%)	14 (20.3%)	3 (21.4%)		
Vascular invasion					
Present	10 (66.7%)	47 (68.1%)	11 (78.6%)	0.568	0.333
Absent	5 (33.3%)	22 (31.9%)	3 (21.4%)		

W/M: Well- and moderately-differentiated adenocarcinoma.

Table II. *Clinicopathological fingings*.

	None (n=15)	Moderate (n=69)	Severe (n=14)	*p*-Value	
				None *vs*. Moderate	Moderate *vs*. Severe
Metastasis in regional lymph nodes					
None	1 (6.7%)	13 (18.8%)	2 (14.3%)		
≤3	11 (73.3%)	22 (31.9%)	6 (42.9%)	0.012	0.723
≥4	3 (20.0%)	24 (49.3%)	6 (42.9%)		
Synchronous hepatic metastasis					
Present	9 (53.3%)	32 (57.1%)	4 (41.7%)	0.251	0.177
Absent	6 (46.7%)	37 (42.9%)	10 (58.3%)		
Synchronous pulmonary metastasis					
Present	2 (13.3%)	4 (5.8%)	4 (41.7%)	0.290	0.025
Absent	13 (86.7%)	65 (94.2%)	10 (58.3%)		
Synchronous peritoneal metastasis					
Present	4 (26.7%)	17 (24.6%)	3 (21.4%)	0.551	0.550
Absent	11 (73.3%)	52 (75.4%)	11 (78.6%)		
Metastasis in distant lymph nodes					
Present	3 (20.0%)	22 (31.9%)	5 (35.7%)	0.281	0.503
Absent	12 (80.0%)	47 (68.1%)	9 (64.3%)		
Adjuvant chemotherapy					
Present	7 (46.7%)	24 (34.8%)	5 (35.7%)	0.281	0.587
Absent	8 (53.3%)	45 (65.2%)	9 (64.3%)		

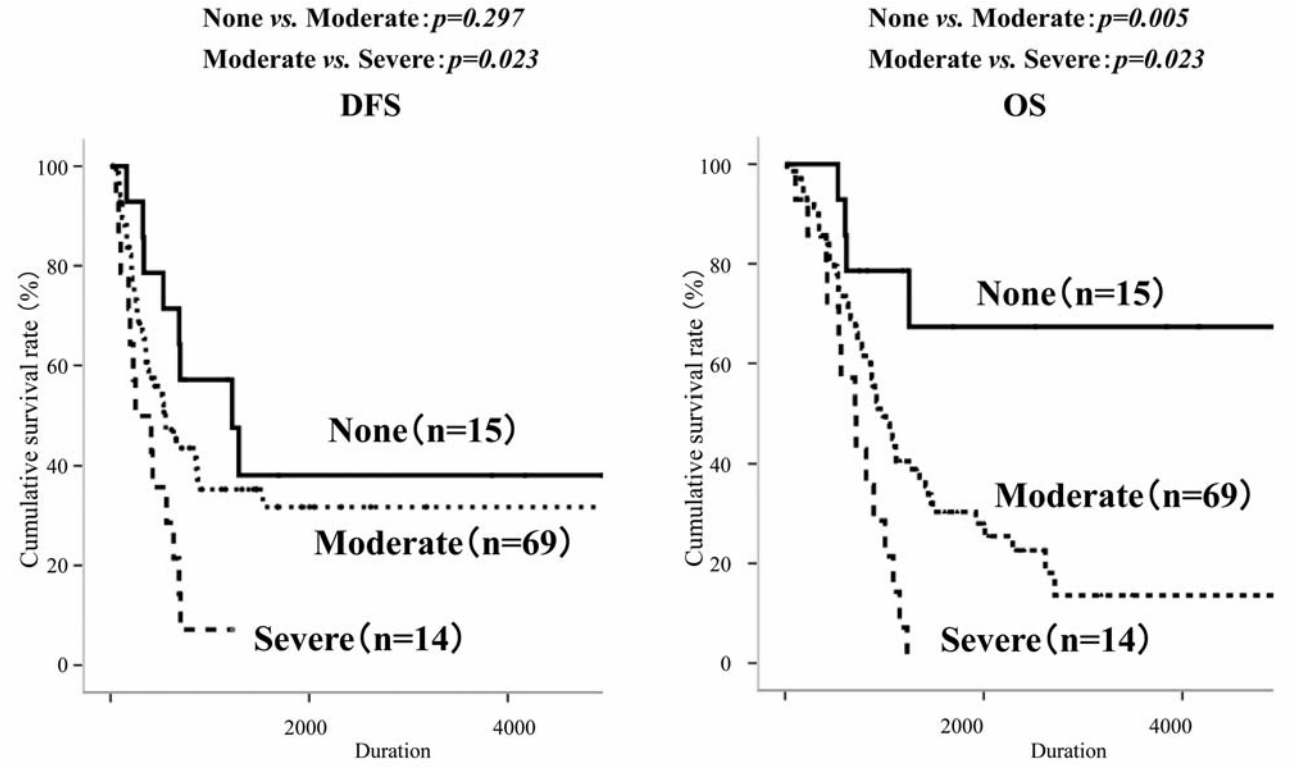

Figure 3. *Disease-free survival (left) and the overall survival (right) rates for the "absent" , "moderate", and "severe" categories of tumor necrosis.*

Table III. *Factors associated with disease-free survival rates.*

	p-Value	
	Univariate	Multivariate
Gender	0.743	–
Age	0.675	–
Cancer location	0.063	–
Histological type	0.039	0.102
Greatest invasion depth	0.725	–
Maximum tumor size	0.047	0.055
Lymphatic invasion	0.269	–
Vascular invasion	0.566	–
Metastasis to regional lymph nodes	0.031	0.256
Synchronous hepatic metastasis	0.754	–
Synchronous pulmonary metastasis	0.118	–
Synchronous peritoneal metastasis	0.647	–
Metastasis to distant lymph nodes	0.131	–
Tumor necrosis	0.016	0.011
Adjuvant chemotherapy	0.801	–

Table IV. *Factors associated with overall survival rates.*

	p-Value	
	Univariate	Multivariate
Gender	0.213	–
Age	0.782	–
Cancer location	0.244	–
Histological type	0.017	0.250
Greatest invasion depth	0.645	–
Maximum tumor size	0.033	0.019
Lymphatic invasion	0.715	–
Vascular invasion	0.273	–
Metastasis to regional lymph nodes	<0.0001	0.127
Synchronous hepatic metastasis	0.009	0.357
Synchronous pulmonary metastasis	0.088	–
Synchronous peritoneal metastasis	0.028	0.552
Metastasis to distant lymph nodes	0.058	–
Tumor necrosis	<0.0001	0.001
Adjuvant chemotherapy	0.040	0.128

breast (3), lung (4), pancreas (5), kidney (6), and upper urinary tract (7, 8), as well as for soft tissue sarcomas (9). Studies on tumor necrosis in colorectal cancer are few in number, but this area has been the focus of much attention recently. It has been established that tumor necrosis is the result of two distinct pathways, one of which is the conventional route involving apoptosis, whereas the other results from the stimulation of the inflammatory pathway

due to rapid tumor growth, resulting in vascular insufficiency and tissue hypoxia (12). The results of our study show that the maximum tumor size and tumor necrosis status were statistically significant factors for predicting a poor prognosis with regard to overall survival, indicating the possible importance of the latter pathway. We also showed that tumor necrosis is a stage-independent prognostic factor in colorectal cancer, the inference being that if tumors have outgrown their blood supply, histological tumor necrosis is consequently a marker of tumor aggressiveness and poor prognosis.

Another study has made reference to inflammation with tumor necrosis, in which the presence of tumor necrosis, itself associated with a weak local inflammatory cell infiltrate, may represent a trigger for the host to initiate a systemic inflammatory response and an attenuation of the local inflammatory cell infiltrate (12). It has also been reported that tumor necrosis status is closely associated with expression of the urokinase-type plasminogen activator (21). There is also a reported association between inflammatory infiltration and microsatellite instability (10).

High concentrations of IL-6 in the tumor have also been shown to be directly associated with increased necrosis, proliferation, differentiation, and vascular invasion, whereas circulating concentrations of IL-6 are directly associated with T-stage, C-reactive protein concentrations, and poor survival. Thus, IL-6 has emerged as a key mediator in the relationship between tumor necrosis, local and systemic inflammatory responses, and outcome in patients with colorectal cancer (11, 22).

There have been a number of previous reports on the significance of tumor necrosis in colorectal cancer, but these have only involved patients with TNM stage II or III disease (12, 13, 23). To our knowledge, our study gives the first detailed description of TNM stage IV colorectal cancer in patients with no apparent residual cancer after surgery, and who, as a result, generally have a good prognosis (24). We excluded patients who did have residual disease after surgery as in these cases, the prognosis is far less certain (25). It is also noteworthy that the use of hematoxylin and eosin staining for the assessment of tumor necrosis status is straightforward and highly reproducible in terms of histopathological diagnosis. A poor prognosis predicted on the basis of the presence of tumor necrosis after surgery indicates the need for intensive follow-up with high-potency adjuvant therapy.

The results of our study imply that the extent of tumor necrosis should be considered during clinical review as a potential indicator of disease prognosis and, hence, the future treatment of the patient. Further, large-scale prospective studies are warranted to confirm these findings and also to further evaluate whether they can be extended to other disease grades and possibly other types of cancer.

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