DOI:10.1158/1078-0432.CCR-07-0343

Clinical Cancer Research

Caspase-3 Activity Predicts Local Recurrence in Rectal Cancer

Pieter de Heer, Elza C. de Bruin, Elma Klein-Kranenbarg, et al.

Clin Cancer Res 2007;13:5810-5815. Published online October 1, 2007.

DOI:10.1158/1078-0432.CCR-07-0343

Caspase-3 Activity Predicts Local Recurrence in Rectal Cancer

Pieter de Heer,[1] Elza C. de Bruin,[2] Elma Klein-Kranenbarg,[1] Remco I.J.M. Aalbers,[1] Corrie A.M. Marijnen,[6] Hein Putter,[3] Hans J. de Bont,[5] J. Fred Nagelkerke,[5] J. Han J.M. van Krieken,[7] Hein W. Verspaget,[4] Cornelis J.H. van de Velde,[1] and Peter J.K. Kuppen,[1] for the Dutch Colorectal Cancer Group

Abstract

Purpose: Radiotherapy followed by total mesorectal excision surgery has been shown to significantly reduce local recurrence rates in rectal cancer patients. Radiotherapy, however, is associated with considerable morbidity. The present study evaluated the use of biochemical detection of enzymatic caspase-3 activity as preoperative marker for apoptosis to preselect patients that are unlikely to develop a local recurrence to spare these patients from overtreatment and the negative side effects of radiotherapy.

Experimental Design: Nonirradiated freshly frozen tissue samples from 117 stage III rectal cancer patients were collected from a randomized clinical trial that evaluated preoperative radiotherapy in total mesorectal excision surgery. Additional frozen archival tissues from 47 preoperative biopsies and corresponding resected colorectal tumors were collected. Level of apoptosis was determined by measuring the enzymatic activity of caspase-3 in a biochemical assay.

Results: In tumor tissue, caspase-3 activity lower than the median was predictive of 5-year local recurrence (hazard ratio, 7.4; 95% confidence interval, 1.7-32.8; $P = 0.008$), which was unaffected by adjustment for type of resection, tumor location, and T status (adjusted hazard ratio, 7.5; 95% confidence interval, 1.7-34.1; $P = 0.009$). Caspase-3 activity in preoperative biopsies was significantly correlated with caspase-3 activity in corresponding resected tumors ($r = 0.56$; $P < 0.0001$).

Conclusion: Detection of tumor apoptosis levels by measuring caspase-3 activity, for which a preoperative biopsy can be used, accurately predicted local recurrence in rectal cancer patients. These findings indicate that caspase-3 activity is an important denominator of local recurrence and should be evaluated prospectively to be added to the criteria to select rectal cancer patients in which radiotherapy is redundant.

Local recurrence is a major problem after rectal cancer surgery, as it is the cause of severely disabling symptoms and is difficult to treat (1, 2). Local recurrence rates historically vary between 15% and 45% (3–5). The introduction of total mesorectal excision (TME) as treatment for patients with rectal cancer has led to an improved local control and survival when compared with historical controls (2, 6, 7). In addition to improved surgery, the administration of preoperative radiation therapy has further decreased local recurrence rates in several randomized clinical trials (8–12). Radiation therapy, however, is associated with considerable morbidity. Several studies have evaluated the short-term and long-term morbidity of radiation therapy. Preoperative radiation therapy is associated with fecal incontinence, urgency, and anal blood loss (13). In addition to the general bowel dysfunction, an increase in sexual dysfunction has been reported (14, 15). These negative side effects of radiation therapy emphasize the need for finding predictive factors for local recurrence to exclude patients with a very high probability for cure with surgery alone.

Among the predictive factors for survival and local tumor recurrence are lymph node metastasis, stage of the disease, and the presence of a tumor-positive circumferential margin (12). Most criteria, however, can only be determined postoperatively. Several recent studies in rectal cancer have shown that tumors with high levels of apoptosis show low local recurrence rates and favorable prognosis (16–19). These results indicate that only patients with low levels of apoptosis may benefit from radiation therapy with respect to the development of local recurrences. Therefore, the level of apoptosis in tumors may provide a criterion to select patients for radiation therapy. The present study evaluated the use of biochemical detection of caspase-3 activity as a simple and quantitative technique to measure apoptosis in tissue samples and preoperative biopsies

Authors' Affiliations: Departments of [1]Surgery, [2]Clinical Oncology, [3]Medical Statistics, and [4]Gastroenterology, Leiden University Medical Centre; [5]Department of Toxicology, Leiden Amsterdam Centre for Drug Research, Leiden, the Netherlands; [6]Department of Radiotherapy, the Netherlands Cancer Institute, Amsterdam, the Netherlands; and [7]Department of Pathology, Radboud University Medical Centre, Nijmegen, the Netherlands

Received 2/9/07; revised 6/9/07; accepted 7/18/07.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Note: C.J.H. van de Velde and P.J.K. Kuppen were responsible for this study.

Role of the funding source: We declare no funding sources that may influence the writing, interpretation of the data, or the decision to submit the current manuscript for publication. The authors have not been paid by a pharmaceutical company or other agency to write this article. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Requests for reprints: Peter J.K. Kuppen and Cornelis J.H. van de Velde, Department of Surgery, Leiden University Medical Center, P. O. Box 9600, 2300 RC Leiden, the Netherlands. Phone: 31-71-5262309; Fax: 31-71-5266750; E-mail: P.J.K.Kuppen@lumc.nl and C.J.H.van_de_Velde@lumc.nl.

doi:10.1158/1078-0432.CCR-07-0343

DOI:10.1158/1078-0432.CCR-07-0343

Table 1. Patient characteristics of the 117 included rectal cancer patients

Patient characteristic	No. patients (%)	Caspase-3 activity (pmol AMC/min/mg protein)	*P*
	N = 117 (100%)	Median (IQR)	
Gender			0.32
Male	81 (69)	14.5 (9.8-26.6)	
Female	36 (31)	16.1 (13.1-27.2)	
Age (median, 65; range, 26-85)			0.94
< Median	61 (52)	15.5 (5.8-26.5)	
> Median	56 (48)	15.1 (5.2-27.2)	
Preoperative CEA levels (median, 3.0; range, 2-41)			0.20
< Median	47 (40)	17.8 (12.2-30.4)	
> Median	70 (60)	14.5 (9.5-23.6)	
Maximum tumor diameter (median, 4.5; range, 0-11)			0.09
< Median	51 (44)	13.8 (9.3-22.7)	
> Median	64 (56)*	16.2 (12.7-31.5)	
Distance of tumor from anal verge (cm)			0.73
10.1-15	38 (33)	15.3 (11.6-26.4)	
5.1-10	49 (42)	15.0 (9.7-24.8)	
≤5	30 (25)	16.3 (9.9-31.2)	
Grade of differentiation			0.12
Well	4 (3)	13.7 (12.4-31.1)	
Moderate	79 (68)	16.0 (12.0-29.8)	
Poor	34 (29)	13.3 (5.8-20.2)	
WHO classification			**0.04**
Adenocarcinoma	105 (90)	16.2 (10.4-28.6)	
Mucinous carcinoma	12 (10)	12.8 (11.2-14.0)	
No. positive lymph nodes			0.64
1-3	71 (61)	16.0 (10.1-29.8)	
≥4	46 (39)	14.2 (11.0-22.8)	
T status			0.62
T_1-T_2	22 (19)	14.7 (8.5-27.9)	
T_3	89 (76)	16.2 (11.0-27.1)	
T_4	6 (5)	12.7 (9.9-22.7)	
Circumferential margin			**0.04**
Negative	81 (69)	16.4 (11.8-30.1)	
Positive	36 (31)	13.4 (9.5-17.6)	
Type of resection			0.18
Abdominoperineal	40 (34)	17.4 (12.2-26.8)	
Low anterior	77 (66)	14.5 (9.4-27.1)	
Adjuvant therapy			0.37
None	83	16.2 (12.0-29.8)	
Chemotherapy	6	16.2 (10.4-22.1)	
Radiation therapy	24	13.1 (19.4-17.6)	
Chemoradiation therapy	4	11.2 (6.1-55.6)	

NOTE: Association of clinical and pathologic variables with caspase-3 activity as determined in Materials and Methods. Significant associations are stated in bold.
Abbreviation: CEA, carcinoembryonic antigen.
*Tumor diameter of two tumors was not determined.

of rectal cancer to predict local recurrences in rectal cancer. The proapoptotic enzyme caspase-3 is activated at a point of convergence for the intrinsic and extrinsic apoptosis induction pathways (20), so its activity should give a reliable measure of ongoing levels of apoptosis in tumor samples. The study was done in stage III rectal cancer patients, as these are the patients that are most likely to benefit from preoperative radiation therapy (11, 12).

Materials and Methods

Patients. The study population consisted of two sources of pathologic material.

The first consisted of stage III rectal cancer patients who participated in the Dutch TME trial. These were collected and analyzed for caspase-3 activity to evaluate the prognostic value of caspase-3 activity. In the TME study, patients were randomized to receive radiation therapy before undergoing surgery according to a standardized TME protocol (12). Patients were selected from the trial arm that did not receive preoperative radiation therapy. All stage III patients who complied with the eligibility criteria of the TME trial (12) and of whom frozen tumor material was available were selected for this study, resulting in a study cohort of 117 patients. Frozen tissue samples of adjacent normal rectum tissue were available from 29 of the patients. A pathology review committee reviewed all tumors (12). Any specimen that had tumor (i.e., primary tumor or lymph node metastasis) ≤1 mm from the circumferential margin was recorded as having a positive resection margin (21).

The second source consisted of 47 frozen biopsies and corresponding frozen rectal and rectosigmoidal, nonirradiated tumor tissues. These were collected and analyzed for caspase-3 activity to evaluate the feasibility of caspase-3 detection in biopsies and to assess

DOI:10.1158/1078-0432.CCR-07-0343

whether caspase-3 activity in preoperative biopsies is representative for that of the primary tumor. As preoperative biopsies were not collected in the TME trial, biopsies and corresponding nonirradiated tumor specimens were collected from the tissue archives of the Leiden University Medical Center. As these patients did not receive TME surgery and therefore have poor local control, they were not included in survival analyses.

The study was conducted following the regulations according to Dutch law for human material for research. Ethics board approval was obtained for gathering all study material and patient data in the current study.

Measurement of caspase-3 activity. The enzymatic activity of caspase-3 in tissue samples was measured as described previously (22). Briefly, five 10-μm cryostat sections of tumor or normal tissue were suspended in a lysis buffer consisting of 10 mmol/L HEPES (pH 7.0), 40 mmol/L β-glycerophosphate, 50 mmol/L NaCl, 2 mmol/L $MgCl_2$, and 5 mmol/L EGTA. After 10 min on ice, the cells were disrupted by 10 s of sonification followed by four cycles of freezing and thawing and stored at -80°C. Protein concentration was determined using the method described by Bradford (23). For measurement of caspase-3 enzymatic activity, samples containing 15 μg protein were incubated with 2.5 nmol of the enzyme substrates DEVD-AMC (Bachem) in a 100-mmol/L HEPES buffer (pH 7.25) containing 10% (w/v) sucrose, 0.1% (v/v) NP40, and 10 mmol/L DTT. During incubation at 37°C, fluorescent AMC was cleaved off by active caspases, corresponding with the level of caspase activity in the sample. The fluorescent AMC was monitored at an excitation of 360 nm and emission of 460 nm using a FLUOstar Optima plate reader (BMG Labtech GmbH). Calibration curves were constructed using free AMC. Caspase-3 activity was indicated in pmol AMC/min/mg protein.

Tumor sections and preoperative biopsies may contain various proportions of tumor epithelium and tumor stroma. To assess whether caspase-3 activity depended on the ratio of tumor epithelium and tumor stroma in sections of the tumor tissues used for analysis, the percentage of tumor epithelium was assessed by two independent observers (R.I.J.M.A. and N.G.E.) in adjacent H&E-stained slides.

Statistical analysis. Analysis was done with Statistical Package for the Social Sciences statistical software (version 11.0 for Windows; SPSS, Inc.). Mann-Whitney *U*, Kruskal-Wallis, Wilcoxon signed rank, and Spearman's Rho tests were used to compare continuous variables. The entry date for the recurrence analyses was the time of surgery of the primary tumor. To guarantee sufficient number of patients in both groups, the patients were dichotomized at the median level of apoptosis by caspase-3 activity. Kaplan-Meier analyses and log-rank tests were done to compare recurrence rates in patients from the high and low apoptotic groups. Cox regression analyses were used to calculate hazard ratios with 95% confidence intervals for categorical variables. Variables with a P value of ≤0.10 in the univariate analyses were subjected to a multivariate analysis. To enable comparisons of the outcome of caspase-3 data with recurrence rate of stage III rectal cancer patients treated with radiation therapy, Kaplan-Meier analysis was done for these patients in the follow-up data of the Dutch TME trial (12).

Results

Level of apoptosis in rectal cancer. We determined caspase-3 activity in rectal cancer specimens and adjacent normal tissue in a biochemical cleavage assay. Caspase-3 activity in the rectal tumor specimens was significantly higher than in the 29 normal tissue samples: median, 15.2 pmol AMC/min/mg protein; interquartile range (IQR), 10.6-26.9 compared with median, 4.9 pmol AMC/min/mg protein; IQR, 3.4-10.5 ($P < 0.0001$, Wilcoxon signed rank). There was no significant correlation between caspase-3 activity in tumor tissue and adjacent normal tissue (correlation coefficient = 0.04; $P = 0.85$, Spearman's Rho test).

Correlation between clinical variables and apoptosis. The characteristics of the stage III rectal cancer patients included in this study are summarized in Table 1. Patient characteristics and several markers that have an effect on disease recurrence in rectal cancer, or that can be used as diagnostic tool (24–26), were evaluated for their association with level of apoptosis caspase-3 activity.

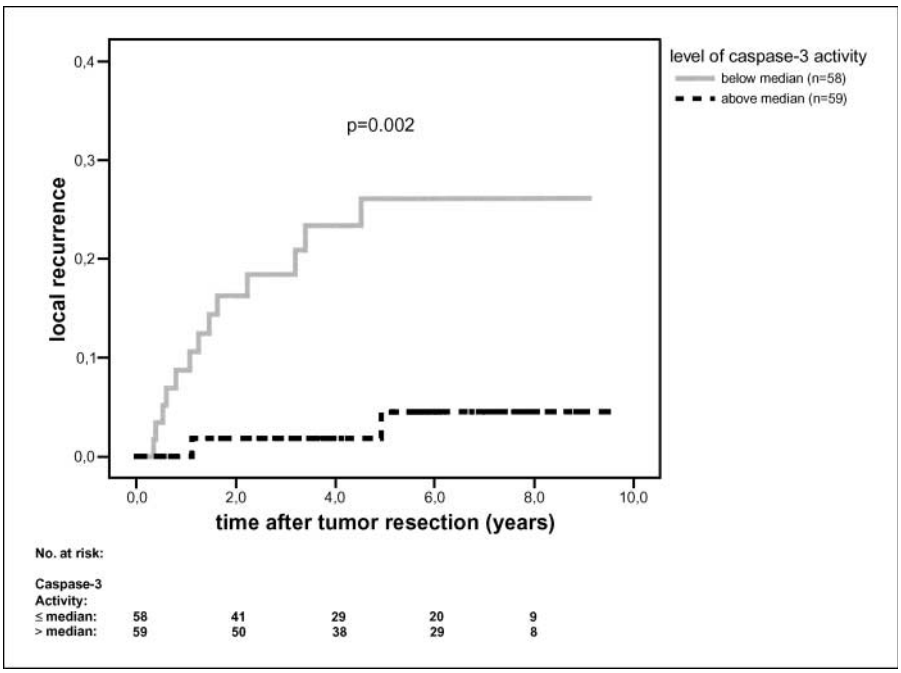

Fig. 1. Recurrence rates since TME surgery for low (*gray*) and high (*black, dotted*) levels of apoptosis in tumors of 117 rectal cancer patients. Levels of caspase-3 activity above the median were associated with significantly lower local recurrence rates (5-y risks: 5.9% versus 28.1%; $P = 0.002$, log-rank analysis).

DOI:10.1158/1078-0432.CCR-07-0343

Table 2. Univariate Cox regression analysis of the effect of clinical and pathologic variables on local recurrence rates in stage III rectal cancer

Variable	Association with local recurrence	
	HR (95% CI)	*P*
Age (y)	1.0 (0.98-1.03)	0.50
Preoperative CEA levels (ng/mL)	1.0 (0.98-1.03)	0.58
Maximum tumor diameter (cm)	0.91 (0.77-1.10)	0.26
Gender		0.24
Male	1	
Female	0.73 (0.42-1.25)	
Distance of tumor from anal verge (cm)		**0.007**
10.1-15	1	
5.1-10	3.7 (1.6-8.7)	
≤5	2.3 (1.0-5.3)	
Grade of differentiation		0.30
Well	1	
Moderate	0.49 (0.2-1.2)	
Poor	0.63 (0.2-1.7)	
WHO classification		0.82
Adenocarcinoma	1	
Mucinous carcinoma	1.1 (0.7-1.7)	
No. positive lymph nodes		0.68
1-3	1	
≥4	1.1 (0.6-1.9)	
T status		0.10
T_1-T_2	1	
T_3	2.6 (1.0-6.6)	
T_4	3.4 (0.9-12.5)	
Circumferential margin		0.13
Negative	1	
Positive	1.6 (0.9-2.7)	
Caspase-3 activity		**0.008**
> Median	1	
≤ Median	7.4 (1.7-32.8)	
Type of resection		**0.06**
Abdominoperineal	1	
Low anterior	1.7 (1.0-3.0)	
Adjuvant therapy		0.24
No	1	
Yes	1.9 (0.7-5.2)	

Abbreviations: HR, hazard ratio; 95% CI, 95% confidence interval.

Mucinous type of carcinoma and tumors with tumor-positive circumferential margins were associated with lower caspase-3 activity (P = 0.04 and 0.04, respectively); all other variables were not significant (Table 1).

Predictive value of caspase-3 activity for local recurrence. In the current study, caspase-3 activity with the median as cutoff point was accurately predictive for local tumor recurrence (P = 0.002, log-rank test; Fig. 1). After 5 years of follow-up, the local recurrence rates were 28.1% in the tumors with caspase-3 activity below the median and 5.9% in tumors with caspase-3 activity above the median. Caspase-3 activity below the median was also associated with high distant recurrence rates (69.0% versus 42.2% in the group with high caspase-3 activity after 5 years; P = 0.05, log-rank test) but did not have an effect on patient survival (P = 0.10, log-rank test).

To evaluate the benefit of short-term radiotherapy for the above-described recurrence rates of low and high levels of apoptosis, we calculated the 5-year local recurrence rate of all stage III rectal cancer patients treated with preoperative radiation therapy included in the Dutch TME trial (N = 243). Stage III patients receiving preoperative radiation therapy had a 5-year local recurrence rate of 11.4%. This percentage shows that patients with high levels of apoptosis have sufficiently low recurrence rates (5.9%) to make preoperative radiation therapy redundant.

Univariate and multivariate analysis. To assess the independent predictive value of caspase-3 activity on local recurrences, variables with a significant effect on local recurrence, shown in Table 2, were analyzed in a multivariate analysis. Proximal location of the tumor from the anal verge (P = 0.007), abdominoperineal resection (P = 0.06), T status (P = 0.10), and high caspase-3 activity (P = 0.008) proved to be associated with low local recurrence rates in stage III rectal cancer and were subjected to a multivariate analysis.

In the multivariate Cox regression analysis (Table 3), caspase-3 levels below the median proved to be an independent predictor of a high risk of local recurrence in stage III rectal cancer (P = 0.009; hazard ratio, 7.5; 95% confidence interval, 1.7-34.1), whereas type of operation, T status, and distance of tumor from anal verge had no independent prognostic value with regard to this end point (Table 3).

If the circumferential margin (P = 0.13 in the univariate analysis) was included in the multivariate analysis, caspase-3 levels below the median remained an independent predictor of a low risk of local recurrence in stage III rectal cancer (P = 0.007; hazard ratio, 8.0; 95% confidence interval, 1.8-36.5).

Correction for percentage of tumor epithelium. Because the cleavage assay used in the current study did not discriminate between caspase-3 activity in tumor epithelium and tumor stroma, we corrected the caspase-3 activity for the percentage tumor epithelium to evaluate the influence variety in the percentages of epithelium and stroma. Caspase-3 levels were compared with levels corrected for percentage tumor epithelium. Median caspase-3 levels before and after adjustment were 15.2 (IQR, 10.6-26.9) and 17.3 (IQR, 10.4-38.3) pmol AMC/min/mg protein, respectively.

All statistical analyses in the current study were repeated using caspase-3 levels adjusted for percentage tumor epithelium in the frozen tissue sections. Results were unaffected whether the original or adjusted levels were used, indicating that caspase-3 activity can be assessed in tumor tissue specimens without previous knowledge of the tumor epithelium to stroma ratio in the tissue specimen.

Evaluation of possible selection bias. A total of 271 stage III patients was included in the TME trial. To investigate whether the patients in the current study were subject to a selection bias, patient and tumor characteristics of the 117 included patients were compared with all remaining eligible nonirradiated stage III patients included in the TME trial (N = 154). No significant differences in patient age (P = 0.80) and gender (P = 0.10) were found. No differences in tumor characteristics as T stage (P = 0.49), N stage (P = 0.30), grade of differentiation (P = 0.22), localization (P = 0.72), WHO classification (P = 0.65), carcinoembryonic antigen levels (P = 0.07), or circumferential margins (P = 0.43) were found. The maximum tumor diameters in the current study were significantly larger (5.0 cm versus 4.5 cm; P = 0.02) than in nonincluded tumors. This can be explained by the fact that tumors with sufficient material for study tended to be large specimens. As tumor size was not of prognostic value in the current study, we concluded that patients were not subject to a selection bias.

DOI:10.1158/1078-0432.CCR-07-0343

Caspase-3 activity in preoperative biopsies and resected rectal tumors. Finally, we determined the feasibility of measuring caspase-3 activity in preoperative biopsies in archive samples of 47 fresh-frozen preoperative biopsies and corresponding resected rectal tumors. Caspase-3 activity levels in preoperative biopsies and in tumor samples were highly correlated (correlation coefficient = 0.56; $P < 0.0001$, Spearman's Rho test). Levels were significantly higher in the tumor specimens [median, 31.4 pmol AMC/min/mg protein (IQR, 15.1-109.9)] than in the preoperative biopsies [median, 23.3 pmol AMC/min/mg protein (IQR, 8.7-48.5); $P = 0.002$, Wilcoxon signed rank].

These results indicate that determination of levels of apoptosis by caspase-3 activity in preoperative biopsies can be used in predicting level of apoptosis in rectal tumors.

Discussion

Preoperative radiation therapy has shown to be of benefit for the prevention of local recurrence rates in rectal cancer patients (11, 12). Long-course preoperative chemoradiotherapy is of benefit in stage T_3/T_4 rectal cancer patients, and long-course preoperative chemoradiation is the standard of care in the United States (27). However, considering the extensive morbidity of preoperative radiation therapy (13–15), it is of great importance to identify patients with a low risk of local recurrence in which radiation therapy is redundant. With this intention, the current study was done in patients with stage III rectal cancer, as these patients are at the highest risk for local recurrence (12). Our results show that biochemical detection of caspase-3 levels can be used as a marker to identify patients with a very high probability for local cure with surgery alone.

To select patients who can be refrained from preoperative radiation therapy, a marker should provide accurate prediction of clinical behavior and it must be applicable in a pretreatment biopsy of the tumor. Several markers for prediction of radiation therapy efficacy have been suggested in previous studies, including analysis of Ki-67, BAX, cyclooxygenase-2, survivin, and M30 staining (17–19, 28–30). As the benefit of radiation therapy is, at least in part, mediated by the induction of tumor cell apoptosis and other forms of cell death (20, 31, 32), it is not surprising that the majority of these markers involve proapoptotic or antiapoptotic proteins. A recent study evaluated the predictive value of tumor cell apoptosis as quantified by immunohistochemical staining of paraffin-embedded tumor tissue arrays with the M30 antibody in rectal cancer specimens from the Dutch TME trial (19). In this study, the number of M30-positive cells showed to be a significant predictor of local recurrence but with at much lower levels of statistical significance than we found measuring caspase-3 activity. This may be due to limited accuracy of quantifying cells by immunohistochemical staining or the influence of apoptotic infiltrative cells that were included in measured caspase activity. Preoperative biopsies may yield a limited number of tumor cells (16), thus further limiting the use of immunohistochemical markers such as M30 to detect the number of apoptotic tumor cells to select patients for radiation therapy. By measuring enzymatic caspase-3 activity, apoptosis can be determined even before the phenotypic changes of these cells are clearly detectable. In addition, our study showed that only 10 μg of tumor-derived protein were necessary for a single assay, making biochemical detection of caspase-3 activity a feasible assay to determine apoptotic levels in preoperative biopsies of rectal tumors.

Caspase-3 activity in the current study was evaluated in nonirradiated tumors, as several studies have convincingly showed that radiation therapy–induced apoptosis is not of prognostic value (17, 19). Evaluation of enzymatic caspase-3 activity as an indicator of apoptotic cell death seemed in this study an accurate variable for prediction of local recurrences. Preoperative biopsies are routinely taken for diagnosis in diseases of the large bowel. For biochemical quantification of caspase-3 activity, an additional biopsy can be taken and either processed immediately or freshly frozen for analysis later.

A highly significant association between caspase-3 activity in the biopsies and corresponding tumor suggests that determining levels of caspase-3 activity in pretreatment biopsies can be used in predicting level of apoptosis in tumors. It must be taken into account that caspase-3 levels were significantly lower in adjacent normal tissue and a risk of misinterpretation of caspase-3 levels can be apparent if a biopsy contains normal tissue. Caspase-3 activity in the archival biopsies that we studied was significantly lower than in the corresponding resected tumor specimens, suggesting an effect of tumor resection on apoptosis.

One of the factors predictive for local recurrences in rectal cancer is a positive circumferential resection margin (33), and short-term preoperative radiation therapy is of limited effect in patients with a circumferential margin of ≤1 mm (21). In the nonirradiated stage III rectal cancer patients evaluated in this study, the presence of positive resection margins was not a prognostic factor. Apparently, other factors are of more importance in this subset of stage III patients. A possible explanation could be lymphatic spread beyond the surgical resection. In this study, a positive resection margin was associated with low caspase-3 activity in the residual tumor specimens, suggesting that tumors with low levels of apoptosis do not have a clear invasive front and, therefore, are difficult to resect completely. Several clinical and pathologic factors as

Table 3. Results of multivariate Cox regression analysis of local recurrence among 117 nonirradiated stage III rectal cancer patients

Variable	Association with local recurrence (Cox regression analysis)	
	HR (95% CI)	*P*
Caspase-3 activity		**0.009**
> Median	1	
≤ Median	7.5 (1.7-34.1)	
Type of resection		0.87
Abdominoperineal	1	
Low anterior	1.12 (0.3-4.6)	
Distance of tumor from anal verge (cm)		0.36
10.1-15	1	
5.1-10	2.3 (0.3-16.3)	
≤5	3.2 (0.7-15.3)	
T status		0.11
T_1-T_2	1	
T_3	4.4 (0.2-72.1)	
T_4	5.7 (0.7-39.7)	

DOI:10.1158/1078-0432.CCR-07-0343

lymph node metastases and tumors located within 10 cm from anal verge are predictors of local recurrence (12, 24, 25, 33). It has already been shown that magnetic resonance imaging can improve the selection of patients who may have a positive circumferential margin (34). Preoperative detection of positive lymph nodes by magnetic resonance imaging in combination with ultrasmall particles of iron oxide is currently being reviewed and tested in clinical studies (34, 35). This is likely to result in a better preoperative staging of patients and this will enable accurate identification of stage III patients, which are candidates for preoperative radiation therapy. To establish a caspase-3 activity level that can be generalized to a broader population of rectal cancer patients, we are currently prospectively collecting preoperative rectal cancer biopsies for analyses of caspase-3 activity. Selection of patients who will not benefit from preoperative radiation therapy by determination of caspase-3 activity will drastically further decrease the number of patients who receive unnecessary preoperative radiation therapy.

In conclusion, the present study shows that caspase-3 activity is an important denominator of local recurrence in rectal cancer and suggests that identification of patients with a low risk of recurrence can be achieved by caspase-3 measurement in preoperative biopsies. If an independent study can confirm our results, determination of caspase-3 levels should be added to other selection criteria to select rectal cancer patients in whom radiation therapy is redundant.

Acknowledgments

We thank N. Geeske Dekker-Ensink and Connie Janssen-van Rhijn for their excellent technical assistance.

References

1. Kapiteijn E, Marijnen CA, Colenbrander AC, et al. Local recurrence in patients with rectal cancer diagnosed between 1988 and 1992: a population-based study in the west Netherlands. Eur J Surg Oncol 1998;24:528–35.
2. Kapiteijn E, Putter H, van de Velde CJ. Impact of the introduction and training of total mesorectal excision on recurrence and survival in rectal cancer in The Netherlands. Br J Surg 2002;89:1142–9.
3. Phillips RK, Hittinger R, Blesovsky L, Fry JS, Fielding LP. Local recurrence following 'curative' surgery for large bowel cancer. II. The rectum and rectosigmoid. Br J Surg 1984;71:17–20.
4. Porter GA, Soskolne CL, Yakimets WW, Newman SC. Surgeon-related factors and outcome in rectal cancer. Ann Surg 1998;227:157–67.
5. MacFarlane JK, Ryall RD, Heald RJ. Mesorectal excision for rectal cancer. Lancet 1993;341:457–60.
6. Enker WE. Total mesorectal excision—the new golden standard of surgery for rectal cancer. Ann Med 1997;29:127–33.
7. Aitken RJ. Mesorectal excision for rectal cancer. Br J Surg 1996;83:214–6.
8. Gerard A, Buyse M, Nordlinger B, et al. Preoperative radiotherapy as adjuvant treatment in rectal cancer. Final results of a randomized study of the European Organization for Research and Treatment of Cancer (EORTC). Ann Surg 1988;208:606–14.
9. Goldberg PA, Nicholls RJ, Porter NH, Love S, Grimsey JE. Long-term results of a randomised trial of short-course low-dose adjuvant pre-operative radiotherapy for rectal cancer: reduction in local treatment failure. Eur J Cancer 1994;30A:1602–6.
10. Cedermark B, Johansson H, Rutqvist LE, Wilking N. The Stockholm I trial of preoperative short term radiotherapy in operable rectal carcinoma. A prospective randomized trial. Stockholm Colorectal Cancer Study Group. Cancer 1995;75:2269–75.
11. Improved survival with preoperative radiotherapy in resectable rectal cancer. Swedish Rectal Cancer Trial. N Engl J Med 1997;336:980–7.
12. Kapiteijn E, Marijnen CA, Nagtegaal ID, et al. Preoperative radiotherapy combined with total mesorectal excision for resectable rectal cancer. N Engl J Med 2001;345:638–46.
13. Peeters KC, van de Velde CJ, Leer JW, et al. Late side effects of short-course preoperative radiotherapy combined with total mesorectal excision for rectal cancer: increased bowel dysfunction in irradiated patients—a Dutch colorectal cancer group study. J Clin Oncol 2005;23:6199–206.
14. Holm T, Singnomklao T, Rutqvist LE, Cedermark B. Adjuvant preoperative radiotherapy in patients with rectal carcinoma. Adverse effects during long term follow-up of two randomized trials. Cancer 1996;78:968–76.
15. Marijnen CA, Kapiteijn E, van de Velde CJ, et al. Acute side effects and complications after short-term preoperative radiotherapy combined with total mesorectal excision in primary rectal cancer: report of a multicenter randomized trial. J Clin Oncol 2002;20:817–25.
16. Adell GC, Zhang H, Evertsson S, et al. Apoptosis in rectal carcinoma: prognosis and recurrence after preoperative radiotherapy. Cancer 2001;91:1870–5.
17. Marijnen CA, Nagtegaal ID, Mulder-Stapel AA, et al. High intrinsic apoptosis, but not radiation-induced apoptosis, predicts better survival in rectal carcinoma patients. Int J Radiat Oncol Biol Phys 2003;57:434–43.
18. Rodel F, Hoffmann J, Distel L, et al. Survivin as a radioresistance factor, and prognostic and therapeutic target for radiotherapy in rectal cancer. Cancer Res 2005;65:4881–7.
19. de Bruin EC, van de Velde CJH, van de Pas S, et al. Intrinsic and radiotherapy-induced apoptosis in rectal cancer patients of the Dutch TME trial: no need for radiotherapy in intrinsically high apoptotic tumours. Clin Cancer Res 2006;12:6432–6.
20. Brown JM, Attardi LD. The role of apoptosis in cancer development and treatment response. Nat Rev Cancer 2005;5:231–7.
21. Marijnen CA, Nagtegaal ID, Kapiteijn E, et al. Radiotherapy does not compensate for positive resection margins in rectal cancer patients: report of a multicenter randomized trial. Int J Radiat Oncol Biol Phys 2003;55:1311–20.
22. Jonges LE, Nagelkerke JF, Ensink NG, et al. Caspase-3 activity as a prognostic factor in colorectal carcinoma. Lab Invest 2001;81:681–8.
23. Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem 1976;72:248–54.
24. Wibe A, Syse A, Andersen E, et al. Oncological outcomes after total mesorectal excision for cure for cancer of the lower rectum: anterior vs. abdominoperineal resection. Dis Colon Rectum 2004;47:48–58.
25. Gunderson LL, Sargent DJ, Tepper JE, et al. Impact of T and N stage and treatment on survival and relapse in adjuvant rectal cancer: a pooled analysis. J Clin Oncol 2004;22:1785–96.
26. Nagtegaal ID, Marijnen CA, Kranenbarg EK, et al. Local and distant recurrences in rectal cancer patients are predicted by the nonspecific immune response; specific immune response has only a systemic effect—a histopathological and immunohistochemical study. BMC Cancer 2001;1:7.
27. Sauer R, Becker H, Hohenberger W, et al. Preoperative versus postoperative chemoradiotherapy for rectal cancer. N Engl J Med 2004;351:1731–40.
28. Pachkoria K, Zhang H, Adell G, Jarlsfelt I, Sun XF. Significance of Cox-2 expression in rectal cancers with or without preoperative radiotherapy. Int J Radiat Oncol Biol Phys 2005;63:739–44.
29. Nehls O, Okech T, Hsieh CJ, et al. Low BAX protein expression correlates with disease recurrence in preoperatively irradiated rectal carcinoma. Int J Radiat Oncol Biol Phys 2005;61:85–91.
30. Rodel C, Grabenbauer GG, Papadopoulos T, et al. Apoptosis as a cellular predictor for histopathologic response to neoadjuvant radiochemotherapy in patients with rectal cancer. Int J Radiat Oncol Biol Phys 2002;52:294–303.
31. Garcia-Barros M, Paris F, Cordon-Cardo C, et al. Tumor response to radiotherapy regulated by endothelial cell apoptosis. Science 2003;300:1155–9.
32. Paris F, Fuks Z, Kang A, et al. Endothelial apoptosis as the primary lesion initiating intestinal radiation damage in mice. Science 2001;293:293–7.
33. Nagtegaal ID, Marijnen CA, Kranenbarg EK, van de Velde CJ, van Krieken JH. Circumferential margin involvement is still an important predictor of local recurrence in rectal carcinoma: not one millimeter but two millimeters is the limit. Am J Surg Pathol 2002;26:350–7.
34. Beets-Tan RG, Lettinga T, Beets GL. Pre-operative imaging of rectal cancer and its impact on surgical performance and treatment outcome. Eur J Surg Oncol 2005;31:681–8.
35. Koh DM, Brown G, Temple L, et al. Rectal cancer: mesorectal lymph nodes at MR imaging with USPIO versus histopathologic findings—initial observations. Radiology 2004;231:91–9.