Published OnlineFirst November 30, 2010; DOI: 10.1158/1078-0432.CCR-10-2592

Clinical Cancer Research

Predictive Biomarkers and Personalized Medicine

Failure of Downregulation of Survivin Following Neoadjuvant Radiochemotherapy in Rectal Cancer Is Associated with Distant Metastases and Shortened Survival

Thilo Sprenger[1], Franz Rödel[5], Tim Beissbarth[2], Lena-Christin Conradi[1], Hilka Rothe[3], Kia Homayounfar[1], Hendrik A. Wolff[4], B. Michael Ghadimi[1], Müjdat Yildirim[5], Heinz Becker[1], Claus Rödel[5], and Torsten Liersch[1]

Abstract

Purpose: Valid molecular markers need to be implemented in clinical trials to fulfill the demand of a risk-adapted and more individualized multimodal therapy of locally advanced primary rectal cancer. In this study, the expression of the inhibitor-of-apoptosis (IAP) protein survivin was evaluated in pretreatment biopsies and corresponding posttreatment resection specimens, and was correlated to histo-pathological tumor characteristics and clinical follow-up.

Patients and Methods: One hundred sixteen patients with stage II/III rectal cancer treated with 5-FU–based neoadjuvant radiochemotherapy (RCT) at a single university medical centre within the German Rectal Cancer Trials were investigated. Survivin expression in pretreatment biopsies and surgical resection specimens were determined by immunohistochemistry by two independent institutions and correlated with histopathologic parameters, tumor recurrences, disease-free (DFS), and overall cancer-specific survival (CSS).

Results: In pretreatment biopsies, a higher survivin expression correlated with advanced ypT ($P = 0.026$) and ypUICC ($P = 0.05$) stage as well as DFS ($P = 0.038$) after preoperative RCT. High posttreatment survivin levels were associated with advanced ypT stage ($P = 0.03$) and residual lymph node metastases ($P = 0.04$). Moreover, neoadjuvant RCT resulted in a significant downregulation of survivin expression ($P < 0.0001$). A failure of RCT-induced downregulation was associated with development of distant metastases ($P = 0.0056$) and cancer-related death ($P = 0.026$), and correlated significantly with DFS ($P = 0.011$*/0.02**) and CSS ($P = 0.0017$*/0.01**) in uni-* and multivariate** analyses.

Conclusions: Survivin expression displays a marker with prognostic utility in rectal cancers. These results underline the potential of survivin to monitor individual response to RCT and encourage anti-survivin strategies in multimodal rectal cancer therapy within future randomized clinical trials. *Clin Cancer Res; 17(6); 1623–31. ©2010 AACR.*

Introduction

Neoadjuvant radiochemotherapy (RCT) followed by total mesorectal excision (TME) has become standard treatment in locally advanced UICC (Union International contre le Cancer) II/III rectal cancer within the last decade (1). Individual treatment response, however, varies considerably. As shown in our previous studies, histopathological tumor regression ranges from a complete response with no viable tumor cells detectable to virtually no regression at all (2). Despite uniform treatment protocols, this observed variance in tumor response is most probably caused by differences in the genetic profile expressed in the individual lesions (3, 4), and might influence long-term survival (5). Reliable molecular markers may help to individually tailor multimodal treatment regimens by risk-adapted or novel therapeutic approaches.

Survivin, the smallest member of the inhibitor-of-apoptosis (IAP) family is a multifunctional protein that is reported to interact at the crossroads of disparate molecular networks of cellular division, intracellular signaling, and apoptosis (6, 7). In this context, one of the signature features of survivin is not only its ability to associate with multiple protein partners, but also to localize to disparate subcellular compartments (8, 9). Abundantly, overexpressed in nearly every tumor tested so far, survivin is associated with a more aggressive tumor behavior and

Authors' Affiliations: Departments of [1]General and Visceral Surgery, [2]Medical Statistics, [3]Pathology, and [4]Radiotherapy and Radiooncology, University Medical Center, Gottingen; and [5]Department of Radiotherapy and Oncology, Johann Wolfgang Goethe-University, Frankfurt am Main, Germany

Note: Supplementary data for this article are available at Clinical Cancer Research Online (http://clincancerres.aacrjournals.org/).

Note: Thilo Sprenger and Franz Rödel contributed equally to this work.

Corresponding Author: Thilo Sprenger, Department of General and Visceral Surgery, University Medical Center, Georg-August-University, Göttingen 37099, Göttingen 0049-551-3910480, Germany. Phone: +49-551-3910480; Fax: +49-551-3914155;
E-mail: tsprenger@chirurgie-goettingen.de

doi: 10.1158/1078-0432.CCR-10-2592

Published OnlineFirst November 30, 2010; DOI: 10.1158/1078-0432.CCR-10-2592

Translational Relevance

Overexpression of survivin has been shown to be associated with poor clinical outcome and aggressive behavior in a variety of human tumors, indicating its relevance as a prognostic marker and its potential as a target for molecular cancer therapy. Our actual data indicate that rectal cancer patients with low pretreatment survivin levels show a significantly prolonged disease-free survival after neoadjuvant radiochemotherapy (RCT). Moreover, by comparison of pre- and posttreatment survivin levels, an elevated expression and failure of RCT-induced downregulation of the protein was related to a higher incidence of distant metastatic disease and cancer-related death. Thus, survivin seems to be relevant not only for response of the primary lesion, but also for tumor dissemination and systemic tumor control within multimodal treatment of rectal cancer. As a growing number of survivin antagonists recently entered clinical phase I/II studies, these results provide a further rationale for the implementation of anti-survivin strategies within future randomized clinical trials in rectal cancer patients.

worse clinical outcome (10). In addition, survivin is involved in resistance to both chemotherapy and ionizing irradiation (11). Several preclinical *in vitro* studies have demonstrated that targeting survivin's expression by the use of small interfering RNAs, antisense-oligonucleotides and small molecule repressors radiosensitize tumor cells and reduces tumor growth *in vivo*. Because of these properties, survivin has been proposed as a molecular target for anticancer therapies (12, 13).

Although the role of survivin as a prognostic marker is consistent within the literature, its value as a marker for the prediction of treatment response toward radiation therapy or RCT remains controversial (14). In rectal cancer, a high survivin expression in pretreatment biopsies of patients treated with 5-FU–based preoperative RCT or short-course radiotherapy was significantly associated with a higher risk of local tumor recurrences (15) and decreased survival (16), respectively. However, other studies with smaller and more heterogeneous patient cohorts reported that there were no correlations between survivin mRNA or protein expression and histopathological tumor regression grades as well as clinical outcome (17, 18). This discrepancy may in part arise from the use of different methods of survivin evaluation, the use of different clinical endpoints or different quantitative scoring systems of the expression characteristics, as well as variable stratification of the patient groups.

Recent data indicate that in oesophageal and ovarian cancer intratumoral survivin expression decreased significantly during neoadjuvant chemotherapy or RCT (19, 20). In contrast, elevated postoperative survivin levels after neoadjuvant treatment were significantly associated with a higher tumor stage, poor histopathological response, and reduced cancer-specific survival.

In this study, survivin expression was—for the first time—evaluated in both pretreatment biopsies and corresponding surgical specimens from patients with locally advanced rectal cancer, treated with standardized preoperative RCT within randomized clinical phase III trials. Methodical and interobserver accordance were evaluated between 2 independent institutions, and survivin expression levels were correlated with clinicopathologic characteristics and clinical follow-up.

Patients and Methods

Patient collective

One hundred and sixteen patients with locally advanced UICC II/III rectal adenocarcinoma were included in this study. All patients underwent standardized preoperative RCT and quality-assessed curative surgery within the randomized phase III German Rectal Cancer Trials CAO/ARO/AIO-94 and CAO/ARO/AIO-04 (1). Approval from the ethics committee of the University of Göttingen and informed consent for additional translational research projects were obtained from all patients before enrollment into this study. Staging procedures including rigid rectoscopy with endorectal ultrasonography; contrast enhanced CT scans of thorax, abdomen, and pelvis; and MRI imaging of the pelvis were performed in all cases to confirm locally advanced tumor stage and to exclude patients with evidence of distant metastatic disease at the time of initial rectal cancer diagnosis.

Treatment and pathological staging procedures

Treatment procedures consisted of neoadjuvant RCT according to the protocol of the German Rectal Cancer Trials (CAO/ARO/AIO-94 and -04). All patients received a total irradiation dose of 50.4 Gy in 28 fractions of 1.8 Gy with concomitant application of 5-fluorouracil (73 patients;63%) or combined 5-flourouracil/oxaliplatin (43 patients; 37%) chemotherapy. Surgery was scheduled within 6 weeks after completion of RCT. Surgical procedures comprised 83 low anterior resections (72%), 32 abdomino-perineal resections (28%), and 1 discontinuous resection (Hartmann procedure), all including quality-assessed TME.

Pretreatment biopsy material was taken from different representative luminal tumor sites and stored within the prospective translational biobank of the Department of General and Visceral Surgery of the University Medical Center Göttingen. Pathological staging in posttreatment tumor resections was performed according to the respective TNM classification (21). Histopathological tumor regression was denoted on the basis of a semiquantitative 5-point grading system described by Dworak and colleagues (22): Grade 0: no regression; Grade 1: dominant tumor mass with obvious fibrosis or mucin; Grade 2: dominantly fibrotic or mucinous changes with few tumor cells or cell groups; Grade 3: very few tumor cells in fibrotic or mucinous tissue; and Grade 4: no tumor cells, complete fibrotic or mucinous regression.

Published OnlineFirst November 30, 2010; DOI: 10.1158/1078-0432.CCR-10-2592

Immunohistochemical staining of survivin

Immunhistochemical staining of survivin was performed on rectal cancer biopsies and on the corresponding surgical specimens. In 11 patients (9.5%) with pathologically confirmed complete response (pCR) after neoadjuvant RCT staining for survivin was only accessible on the pretreatment biopsies. Immunhistochemical staining was performed by alternative methods in 2 institutions (Göttingen and Frankfurt) on consecutive tissue slides using the polyclonal rabbit antihuman survivin antibody AF886 (R&D Systems).

Staining procedure Göttingen

Tissue sections were mounted on microscope slides (Star Frost) and subjected to an automatic staining procedure with standardized tissue preparation, epitope retrieval (98°C for 60 minutes) and antibody incubation (42°C for 90 minutes, 1:750) using a Benchmark XT Autostainer (Ventana Medical Systems SA). Alkaline phosphatase (Red Detection Kit, Ventana Medical Systems) was used for color development and visualization of the epitope–antibody reaction product.

Staining procedure Frankfurt

Staining of survivin was performed as described previously (23). Briefly, before labeling, tissue sections heated for 20 minutes in a pressure cooker, rinsed in Tris-puffered saline (TBS) and nonspecific binding sites were blocked with 5% nonfat dry milk in TBS. Primary anti-survivin antibodies were applied at a 1:50 dilution and incubated at 4°C overnight. The slices were next incubated with biotinylated goat anti-rabbit secondary antibody (Dianova, 1:50 dilution) followed by a strepavidin/biotinylated horseradish perioxidase complex (Dako). Finally, 3-amino-9-ethyl-carbazole (AEC) solution was used as chromogen and hematoxylin (37%) for counterstaining.

Scoring of survivin expression

Survivin expression was analyzed by an individual labeling score considering percent of positive cells and staining intensity. Intensity was scored as: 1+ (weak), 2+ (moderate), and 3+ (intense). The fraction of tumor cells with survivin positivity was assigned to: 1 (0–25%), 2 (25–50%), 3 (50–75%), and 4 (>75%). The individual labeling score for survivin results from the multiplication of staining intensity and number of positive cells, and ranges from 0 (no positive tumor cells) to 12 (>75% of tumor cells with intense staining). Initial scoring was independently performed in each institution. Next, results of both institutions were correlated to evaluate intratumor and interobserver variances as well as availability and comparability of the survivin labeling score (Supplementary Fig. S1). Subsequently, a consensual score was performed based on collaborative re-evaluation of all cases by 2 investigators of each institution (T.S., F.R.). This score served for correlation of survivin expression with clinicopathological parameters and survival.

Clinical follow-up and criteria for relapse

All patients were re-evaluated at 3-month intervals for 2 years and every 6 months thereafter. Evaluations consisted of pertinent medical history, physical examination, blood cell counts, and blood chemistry including carcinoembryonic antigen (CEA) levels at every follow-up visit. Rigid recto-sigmoidoscopy (in patients treated with anterior resection) was performed at 3-month intervals in the first year, at 6-month intervals in the second year, and once per year thereafter. A follow-up schedule for abdominal ultrasound, computerized tomography studies of the abdomen and pelvis, and chest X-rays was defined at regular intervals within the protocol of the German Rectal Cancer Trials. Histological confirmation of local recurrence and distant relapse (defined as tumor manifestation outside the pelvis) was encouraged. Alternate acceptable criteria included sequential enlargement of a mass in radiologic studies with simultaneous increase of serum CEA levels. DFS was defined as the time from surgery to detection of any tumor relapse. Accordingly, CSS was defined as the time from surgery to death due to malignant disease. Median follow-up time was 66.5 months.

Statistical methods

Statistical analysis was performed using the open-source statistical computing software R and the packages *exactRankTests* and *survival*. Correlation of the survivin labeling score with clinical outcome variables was computed by the Spearman Rank correlation using the function *cor.test*. Two group comparisons were performed using a Wilcoxon Rank Test. Time to event data were visualized using Kaplan-Meyer analysis and significance was computed based on Cox proportional hazards regression models using the function *coxph* when using the ordinal survivin score (0–12) or based on a log-rank test using the function *survdiff* when using the dichotomized survivin score. In addition, the influence on the following variables: gender, age, TRG, ypT, ypN, and ypUICC stage on survival was assessed in univariate analysis, because of these variables only gender and ypN reached significance, multivariate analysis was performed using these variables and posttreatment survivin score in a Cox model. The level of significance was set to $\alpha = 5\%$ for all tests.

Results

Clinical characteristics

Clinical staging results of all 116 patients are presented in Table 1. Therapy- and surgery-related 30-day morbidity and mortality were 22% and 0%, respectively. In initial staging, 85 patients (73%) had evidence of mesorectal lymph node involvement (cN+). After preoperative RCT, 44 patients (38%) presented with residual nodal metastases (ypN+). Within the follow-up period, cancer relapse occurred in 26 patients (22%). Isolated distant metastatic disease occurred in 22 patients (19%), whereas simultaneous local and distant recurrence was observed in an additional 4 patients (3%). There was no case of isolated

Published OnlineFirst November 30, 2010; DOI: 10.1158/1078-0432.CCR-10-2592

Table 1. Clinicopathological findings

Feature	Number of patients, $n = 116$	%*
Gender		
Male	82	71
Female	34	29
Age (y)		
Mean ± SD	62.4 ± 9.9	
Median	63	
Tumor distance from anal verge (cm)		
0–6	55	47
>6–12	60	52
>12–16	1	1
cT stage		
2	3	2
3	105	91
4	8	7
cN stage		
Positive	85	73
Negative	31	27
Neoadjuvant treatment regime		
50.4 Gy + Standard 5-FU	73	63
50.4 Gy + Intensified 5-FU/Oxaliplatin	43	37
Surgical procedure (including TME)		
Low anterior resection	83	72
Abdominoperineal resection	32	28
Hartmann's procedure	1	1
Resection status		
R0	116	100
R1	0	0
R2	0	0
Circumferential resection margin (CRM) <1 mm		
Negative	116	100
Positive	0	0
Tumor regression grading		
0	0	0
1	13	11
2	29	25
3	63	54
4	11	9
ypT stage		
0	12	10
1	8	7
2	25	22
3	63	54
4	8	7
ypN stage		
0	72	62
1	30	26
2	14	12
ypM stage		
0	108	93
1	8	7
Cancer recurrence		
No cancer recurrence	90	76
Isolated local recurrence	0	0
Isolated distant recurrence	22	19
Combined local + distant recurrence	4	3

local cancer recurrence. Cancer-related death within the follow-up period occurred in 13 patients (11%). Pathologic staging results and tumor recurrences are summarized in Table 1.

Immunostaining of survivin on pretreatment biopsies and surgical resections

Survivin immune-reactivity was observed in all investigated tumor biopsies and the corresponding surgical specimens. Survivin positivity was predominately homogeneous within biopsy as well as posttreatment tumor samples. The fraction of tumor cells with positive survivin immunoreactivity ranged between 25% and 100% in all but 1 case. Staining intensity varied between different cases as well as between the matched biopsy–resection specimen pairs of individual patients. Staining of noncancerous tissue became apparent as slight positivity of mucosal epithelium in the apex of the rectal crypts and as lymphocytic nuclear positivity in a few cases. Table 2 displays percentages of immunopositive cells, staining intensity, and individual labeling score in all 116 rectal cancer biopsies and the corresponding 105 surgical resection specimens.

There was a high concordance of the intratumor staining characteristics (not shown) and interobserver variability results obtained by 2 different institutions. For the survivin score in pretreatment biopsies, the interobserver correlation coefficient was 0.742 ($P < 0.0001$) and 0.831 ($P < 0.0001$) for the corresponding posttreatment tumors (Supplementary Fig. S1).

Association of pretherapeutic survivin expression with clinicopathologic parameters and survival

The survivin score in pretherapeutic biopsies was significantly correlated with posttherapeutical tumor stage (ypT) ($P = 0.026$) as well as ypUICC stage ($P = 0.05$), indicating that high pretreatment survivin expression was associated with unfavorable histopathological characteristics following preoperative RCT. Moreover, patients with low pretreatment survivin levels (individual labeling score: ≤ 6) showed increased DFS-rates compared with patients with high initial (>6) survivin scores ($P = 0.038$, Fig. 1).

Published OnlineFirst November 30, 2010; DOI: 10.1158/1078-0432.CCR-10-2592

Table 2. Results of *survivin* immunoreactivity

Feature	Number of patients			
	Biopsy, *n* = 116	**%***	**Tumor, *n* = 105**	**%***
Percentage of immunopositive cells				
0–25%	1	1	0	0
>25–50%	4	3	5	5
>50–75%	14	12	23	22
>75–100%	97	84	77	73
Staining intensity				
1+	21	18	48	46
2+	57	49	50	48
3+	38	33	7	7
Individual labeling score				
1–3	11	9	19	18
4–6	18	16	38	36
7–9	49	42	41	39
10–12	38	33	7	7

*Percentages might not result in 100 because of rounding.

Downregulation of survivin expression following neoadjuvant radiochemotherapy

As compared with pretherapeutic biopsies, a highly significant downregulation of survivin expression ($P < 0.0001$) was observed after neoadjuvant RCT (Fig. 2A). Figure 2B shows an example of an intensively stained pretreatment biopsy with more than 75% of positive tumor cells (individual labeling score: 12) and its corresponding surgical resection specimens, showing decreased survivin staining intensity (individual labeling score: 4). Stratified by neoadjuvant treatment arm 50.4 Gy + 5-FU ($n = 73$) versus 50.4 Gy + 5-FU + oxaliplatin ($n = 43$) no significant difference in downregulation of survivin expression was observed ($P = 0.3828$).

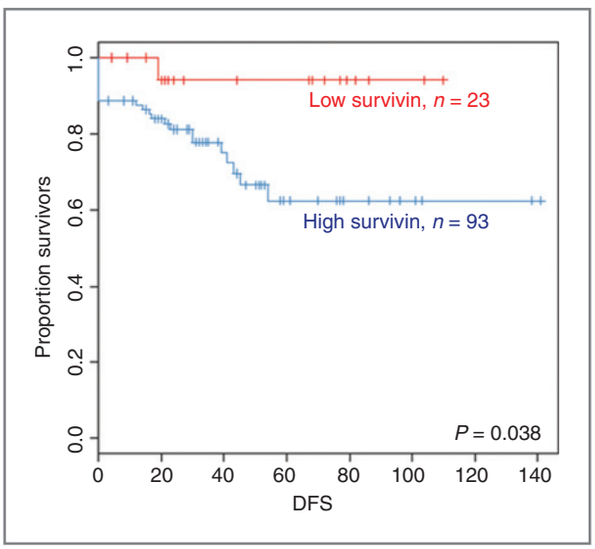

Figure 1. DFS according to low (labeling score ≤ 6) vs. high survivin expression (labeling score >6) in pretreatment biopsies of patients with rectal cancer treated with neoadjuvant RCT. DFS is defined as the time from surgery to detection of any tumor relapse.

Association of posttherapeutic survivin levels with clinicopathologic parameters and survival

In rectal cancer specimens after neoadjuvant RCT, high expression levels of survivin were significantly associated with advanced ypT stage ($P = 0.03$) and the presence of residual nodal metastases (ypN+) ($P = 0.04$). In addition, a high survivin level correlated to the development of distant metastases ($P = 0.0056$) and cancer-related death ($P = 0.026$) (Supplementary Table). In the subgroup of patients with a high posttreatment labeling score of 12, distant metastases were evident in 5 of 7 patients (71%) within the follow-up period, whereas there were only 15% (7 of 47 patients) developing metastases in the subgroup with low survivin levels ≤6, indicating that survivin expression may display a useful marker to distinguish patients at high risk for distant cancer relapse.

In patients with a downregulation of survivin during preoperative RCT, a significant decrease of distant cancer relapse ($P = 0.022$) was observed. Moreover, a significant correlation between high survivin expression levels in residual tumor after RCT and shortened DFS ($P = 0.011$, Fig. 3A) as well as CSS ($P = 0.0017$, Fig. 3B) was evident. In multivariate analyses intratumoral survivin expression was confirmed as an independent prognostic factor with significant influence on DFS ($P = 0.02$) and CSS ($P = 0.01$, Table 3).

Published OnlineFirst November 30, 2010; DOI: 10.1158/1078-0432.CCR-10-2592

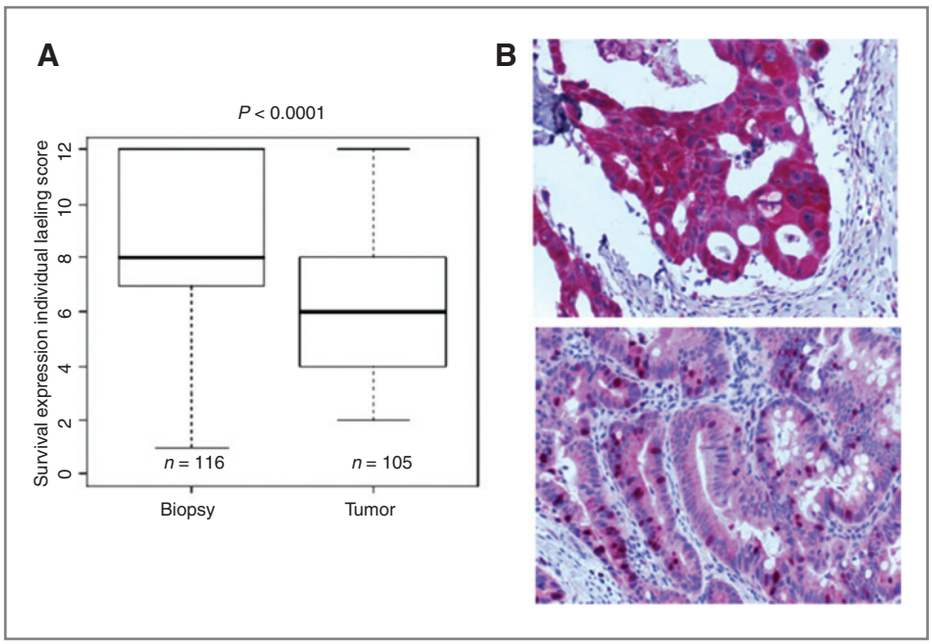

Figure 2. A, downregulation of survivin expression (labeling score) following neoadjuvant radiochemotherapy in 116 patients with advanced rectal cancer (11 patients had complete pathologic response). B, example of a pretreatment tumor biopsy with intensive survivin staining (individual labeling score: 12) and the corresponding posttreatment surgical specimen with decreased staining intensity (individual labeling score: 4)

Discussion

In this study, we investigated the predictive and prognostic relevance of survivin expression both in pretreatment biopsies and corresponding surgical specimens of 116 patients with locally advanced rectal cancer treated with standardized 5-FU based neoadjuvant RCT. Comparing pretreatment biopsies and surgical resection specimens we observed a significant downregulation of survivin in cancer cells following RCT (Fig. 2). This finding is in line with a significant downregulation of survivin expression, as recently described in ovarian and oesophageal cancer treated with neoadjuvant chemotherapy or RCT (19, 20). In the latter study, patients with elevated survivin score after RCT

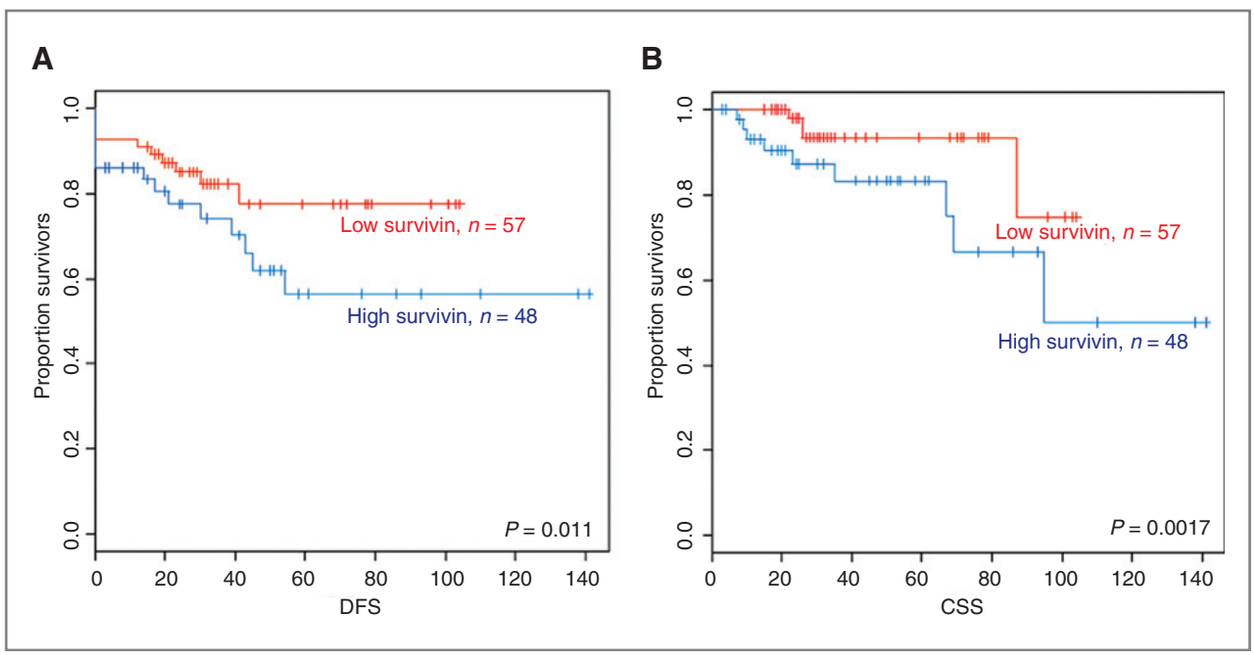

Figure 3. Cox model for DFS (A) and CSS (B) based on survivin labeling score in posttreatment tumor specimens. The cut-off in the Kaplan-Meier curves represents the median survivin score and serves the purpose of visualization. DFS and CSS are defined as the time from surgery to detection of any tumor relapse or tumor-related death.

Published OnlineFirst November 30, 2010; DOI: 10.1158/1078-0432.CCR-10-2592

Table 3. Survivin expression and survival: uni- and multivariate analyses

Variable	Survivin score tumor		Relatio	DFS			CSS		
	High >6	Low ≤6	*P*	Univariate	Multivariate		Univariate	Multivariate	
	N = 48	*N* = 57		*P*	HR (95% CI)	*P*	*P*	HR (95% CI)	*P*
Gender									
Female	32	44	0.54	0.04	0.21 (0.05–0.91)	0.04	0.7	0.9 (0.2–3.4)	0.88
Male	16	13							
ypN									
0	24	37	0.05	0.01	2.34 (1.03–5.29)	0.04	0.001	7.9 (1.7–36.3)	0.01
1/2	24	20							
Survivin score Tumor									
High >6				0.01	1.19 (1.02–1.38)	0.02	0.002	1.3 (1.1–1.7)	0.01
Low ≤6									

and deficient downregulation were reported to have minor histomorphological tumor regression and decreased survival rates.

In our present investigation, a low survivin expression in pretreatment biopsies was significantly related to lower tumor (ypT) and ypUICC stages after preoperative RCT. As these parameters display strong prognostic factors in recent investigations (2, 24), survivin expression is assumed to be not only a predictive marker for local tumor control but also for disease-free survival after preoperative RCT and surgical resection. The predictive relevance of survivin expression in rectal cancer, however, is controversial as conflicting data exist concerning the relationship between survivin expression and response to RCT. Most investigations, including our own previous studies, reported a significant association between a low survivin expression in pretreatment biopsies and decreased distant and local recurrence rates as well as increased survival (15, 16). Although our present results further strengthen a predictive value of pretreatment survivin levels for patients treated with neoadjuvant RCT, other studies with smaller study cohorts did not report on any relevance of intratumoral survivin expression (17, 18).

The discrepancies between results of different studies may be associated with variable methods of evaluation of survivin expression (mRNA vs. protein expression), the use of antibodies with divergent staining characteristics, or may be due to variable study collectives and treatment regimens, scoring criteria as well as follow-up periods. However, these are well-known methodical problems in quantitative immunhistochemical evaluations. Thus, we tried to minimize these effects by implementation of 2 different staining techniques in different institutions with independent interpretation of the results and a consensual re-evaluation of all cases. Statistical analysis of interobserver variability further strengthened a high concordance of the results exposing a methodically solid basis for the performed analyses.

High survivin levels after neoadjuvant RCT were correlated to advanced ypT and lymph node positive disease (ypN+). Given the generally tight associations between (y)pT, (y)pN, and (y)pUICC stage it is surprising that the posttreatment survivin level correlated with ypT and ypN, but not ypUICC stage ($P = 0.11$). This discrepancy might be based on a statistical problem as 11 patients (9.5%) with pCR (representing ypUICC 0) were not included into statistical analyses due to a lack of cancer tissue for immunhistochemistry and Spearman rank correlation was performed only with ypUICC I to IV stages.

Individual tumor response to multimodal therapy in rectal cancer is highly heterogeneous and to date unpredictable. Although 8% to 20% of patients have pCR, others patients show intrinsic or acquired resistance for which, at least in part, elevated intratumoral survivin levels might play a substantial role. This hypothesis is strengthened by *in vitro* results from pancreatic and colorectal tumor cell lines with divergent intrinsic radio-responsiveness: a high constitutive survivin as well as radiation-induced upregulation of survivin was associated with a more radioresistant phenotype (25, 26). Notably, in previous studies, we have shown an inverse relationship between survivin expression and spontaneous apoptosis in rectal cancer biopsies (15) as well as radiation-induced apoptosis in a panel of colorectal cancer cell lines (26). Moreover, an elevated level of apoptosis displays an significant prognosticator for increased histopathologic response and lower rates of local tumor recurrences in rectal cancer patients both treated with or without radiation therapy (23, 27–29) further indicating an impact of apoptotic pathways on clinical outcome in rectal cancer.

As shown in this study, tumor cells may downregulate survivin expression during RCT in rectal cancer. The mechanisms that are responsible for impaired survivin expression or a failure in downregulation of survivin during (radio-) chemotherapy remain elusive. However, efficient downregulation of survivin in tumors likely to respond to

Published OnlineFirst November 30, 2010; DOI: 10.1158/1078-0432.CCR-10-2592

DNA-damaging radiation and chemotherapy may result in increased tumor regression and improved survival. Accordingly, this study demonstrates that tumors with a sufficient RCT-induced downregulation of survivin and lower posttreatment expression levels developed significantly fewer cancer relapse and distant metastases, which constitutes an important clinical endpoint in curatively intended treatment of primary rectal cancer. On the contrary, a failure of survivin downregulation was associated with an increased risk to develop distant metastases, that was in line with recent reports on a correlation between a high survivin expression and distant recurrence in T1/T2 prostate (30) and colon cancer (31). This underlines the potential role of survivin in metastatic pathways—beside its antiapoptotic abilities. More recently, a intermolecular cooperation between X-linked inhibitor of apoptosis protein (XIAP) and survivin was reported to stimulate cell invasion and to promote metastases (32). Thus, both IAPs are considered to be metastasis genes, possibly orchestrating a cellular network of transcription factor nuclear factor kappa B (NF-KB) dependent expression of fibronectin, β1-integrin signaling, and activation of the cell motility kinases focal adhesion kinase (FAK) and Src (32). To the best of our knowledge, this is the largest evaluation of survivin expression in rectal cancer undergoing neoadjuvant RCT based on a well documented patient cohort and tumor material available. Thus, we consider the results as representative for a larger group of patients. Nevertheless, a prospective validation of the impact of survivin expression in rectal cancers has to be performed on the basis of a clinical trial including a suitable and cost-efficient immunhistochemical evaluation of the protein in both pretreatment biopsies and resection specimens.

In summary, survivin is relevant not only for individual response of the primary lesion and local control, but also for tumor dissemination and systemic tumor control after multimodal treatment of rectal cancers. By comparison of pre and posttherapeutic intratumoral survivin, our results implicate that therapeutic strategies to downregulate survivin expression during preoperative therapy or inhibit survivin mediated pathways may further increase individual local and distant tumor response and, as a consequence, patient's prognosis (12, 13). Concretely, patients with low pretreatment survivin levels (individual labeling score ≤ 6) show a significantly prolonged DFS compared with patients with high initial survivin expression (<6). Based on these results, patients with high baseline survivin levels might benefit from additional anti-survivin strategies to decrease the number of patients with impaired downregulation of intratumoral survivin expression, being correlated with higher incidences of distant metastatic disease and cancer-related death as well as decreased survival. Because of a variety of survivin antagonists including antisense-oligonucleotides (Eli-Lilly compound LY2181308) and transcriptional repressors (Ym155) recently entered phase I/II clinical trials (33, 34), our data display a further rationale to combine survivin inhibitors with radio(chemo)therapy in future clinical trials for rectal cancer.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Acknowledgments

The authors thank Birgit Jünemann for excellent technical support and high-quality performance of laboratory procedures as well as Johanna Kreutzer, MA, and Elke Stauffer (study nurse) for organizational support. In addition, the authors thank David M. Goldenberg, ScD, MD (Belleville, NJ) for his critical and helpful comments on the manuscript.

Grant Support

This study was supported by the Deutsche Forschungsgemeinschaft (KFO 179 and DFG RO 3482/1-1) and the German Federal Ministry of Education and Research (01ES0808 and 01EX1021).

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.

Received September 27, 2010; revised November 10, 2010; accepted November 18, 2010; published OnlineFirst November 30, 2010.

References

1. Sauer R, Becker H, Hohenberger W, Rödel C, Wittekind C, Fietkau R, et al. Preoperative versus postoperative chemoradiotherapy for rectal cancer. N Engl J Med 2004;351:1731–40.
2. Rödel C, Martus P, Papadoupolos T, Füzesi L, Klimpfinger M, Fietkau R, et al. Prognostic significance of tumor regression after preoperative chemoradiotherapy for rectal cancer. J Clin Oncol 2005;23:8688–96.
3. Smith FM, Reynolds JV, Miller N, Stephens RB, Kennedy MJ. Pathological and molecular predictors of the response of rectal cancer to neoadjuvant radiochemotherapy. Eur J Surg Oncol 2006;32:55–64.
4. Ghadimi BM, Grade M, Difilippantonio MJ, Varma S, Simon R, Montagna C, et al. Effectiveness of gene expression profiling for response prediction of rectal adenocarcinomas to preoperative chemoradiotherapy. J Clin Oncol 2005;23:1826–38.
5. Liersch T, Grade M, Gaedcke J, Varma S, Difilippantonio MJ, Langer C, et al. Preoperative chemoradiotherapy in locally advanced rectal cancer: correlation of a gene expression-based response signature with recurrence. Cancer Genet Cytogenet 2009;190:57–65.
6. Altieri DC. Survivin, cancer networks and pathway-directed drug discovery. Nat Rev Cancer 2008;8:61–70.
7. Altieri DC. New wirings in the survivin networks. Oncogene 2008;27:6276–84.
8. Li F, Yang J, Ramnath N, Javle MM, Tan D. Nuclear or cytoplasmic expression of survivin: what is the significance? Int J Cancer 2005;114:509–12.
9. Stauber RH, Mann W, Knauer SK. Nuclear and cytoplasmic survivin: molecular mechanism, prognostic, and therapeutic potential. Cancer Res 2007;67:5999–6002.
10. Altieri DC. Validating survivin as a cancer therapeutic target. Nat Rev Cancer 2003;3:46–54.
11. Li F, Ling X. Survivin study: an update of "what is the next wave"? J Cell Physiol 2006;208:476–86.
12. Mita AC, Mita MM, Nawrocki ST, Giles FJ. Survivin: key regulator of mitosis and apoptosis and novel target for cancer therapeutics. Clin Cancer Res 2008;14:5000–5.

Published OnlineFirst November 30, 2010; DOI: 10.1158/1078-0432.CCR-10-2592

13. Pennati M, Folini M, Zaffaroni N. Targeting survivin in cancer therapy. Expert Opin Ther Targets 2008;12:463–76.
14. Capalbo G, Rödel C, Stauber RH, Knauer SK, Bache M, Kappler M, et al. The role of survivin for radiation therapy. Prognostic and predictive factor and therapeutic target. Strahlenther Onkol 2007;183: 593–9.
15. Rödel F, Hoffmann J, Grabenbauer GG, Papadopoulos T, Weiss C, Günther K, et al. High survivin expression is associated with reduced apoptosis in rectal cancer and may predict disease-free survival after preoperative radiochemotherapy and surgical resection. Strahlenther Onkol 2002;178:426–35.
16. Knutsen A, Adell G, Sun XF. Survivin expression is an independent prognostic factor in rectal cancer patients with and without preoperative radiotherapy. Int J Radiat Oncol Biol Phys 2004;60:149–55.
17. McDowell DT, Smith FM, Reynolds JV, Maher SG, Adida C, Crotty P, et al. Increased spontaneous apoptosis, but not survivin expression, is associated with histomorphologic response to neoadjuvant chemoradiation in rectal cancer. Int J Colorectal Dis 2009;24:1261–9.
18. Terzi C, Canda AE, Sagol O, Atila K, Sonmez D, Fuzun M, et al. Survivin, p53, and Ki-67 as predictors of histopathologic response in locally advanced rectal cancer treated with preoperative chemoradiotherapy. Int J Colorectal Dis 2008;23:37–45.
19. Vallbohmer D, Kuhn E, Warnecke-Eberz U, Brabender J, Hoffmann AC, Metzger R, et al. Failure in downregulation of intratumoral survivin expression following neoadjuvant chemoradiation in esophageal cancer. Pharmacogenomics 2008;9:681–90.
20. Dutta T, Sharma H, Kumar L, Dinda AK, Kumar S, Bhatla N, et al. Neoadjuvant chemotherapy for epithelial ovarian cancer—role of apoptosis. Cancer Chemother Pharmacol 2005;56:427–35.
21. Sobin LH. TNM, sixth edition: new developments in general concepts and rules. Semin Surg Oncol 2003;21:19–22.
22. Dworak O, Keilholz L, Hoffmann A. Pathological features of rectal cancer after preoperative radiochemotherapy. Int J Colorectal Dis 1997;12:19–23.
23. Rödel F, Hoffmann J, Distel L, Herrmann M, Noisternig T, Papadopoulos T, et al. Survivin as a radioresistance factor, and prognostic and therapeutic target for radiotherapy in rectal cancer. Cancer Res 2005;65:4881–7.
24. Kaminsky-Forrett MC, Conroy T, Luporsi E, Peiffert D, Lapeyre M, Boissel P, et al. Prognostic implications of downstaging following preoperative radiation therapy for operable T3-T4 rectal cancer. Int J Radiat Oncol Biol Phys 1998;42:935–41.
25. Asanuma K, Moriai R, Yajima T, Yagihashi A, Yamada M, Kobayashi D, et al. Survivin as a radioresistance factor in pancreatic cancer. Jpn J Cancer Res 2000;91:1204–9.
26. Rödel C, Haas J, Groth A, Grabenbauer GG, Sauer R, Rödel F. Spontaneous and radiation-induced apoptosis in colorectal carcinoma cells with different intrinsic radiosensitivities: survivin as a radioresistance factor. Int J Radiat Oncol Biol Phys 2003;55:1341–7.
27. Rödel C, Grabenbauer GG, Papadopoulos T, Bigalke M, Günther K, Schick C, 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.
28. de Heer P, de Bruin EC, Klein-Kranenbarg E, Aalbers RI, Marijnen CA, Putter H, et al. Caspase-3 activity predicts local recurrence in rectal cancer. Clin Cancer Res 2007;13:5810–5.
29. de Bruin EC, van de Velde CJ, van de Pas S, Nagtegaal ID, van Krieken JH, Gosens MJ, et al. Prognostic value of apoptosis in rectal cancer patients of the dutch total mesorectal excision trial: radiotherapy is redundant in intrinsically high-apoptotic tumors. Clin Cancer Res 2006;12:6432–6.
30. Zhang M, Coen JJ, Suzuki Y, Siedow MR, Niemierko A, Khor LY, et al. Survivin is a potential mediator of prostate cancer metastasis. Int J Radiat Oncol Biol Phys 2010;15:1095–103.
31. Fang YJ, Lu ZH, Wang GQ, Pan ZZ, Zhou ZW, Yun JP, et al. Elevated expressions of MMP7, TROP2, and survivin are associated with survival, disease recurrence, and liver metastasis of colon cancer. Int J Colorectal Dis 2009;24:875–84.
32. Mehrotra S, Languino LR, Raskett CM, Mercurio AM, Dohi T, Altieri DC. IAP regulation of metastasis. Cancer Cell 2010;17:53–64.
33. Tolcher AW, Mita A, Lewis LD, Garrett CR, Till E, Daud AI, et al. Phase I and pharmacokinetic study of YM155, a small-molecule inhibitor of survivin. J Clin Oncol 2008;26:5198–203.
34. Giaccone G, Zatloukal P, Roubec J, Floor K, Musil J, Kuta M, et al. Multicenter phase II trial of YM155, a small-molecule suppressor of survivin, in patients with advanced, refractory, non-small-cell lung cancer. J Clin Oncol 2009;27:4481–6.

Published OnlineFirst November 30, 2010; DOI: 10.1158/1078-0432.CCR-10-2592

Clinical Cancer Research

Failure of Downregulation of Survivin Following Neoadjuvant Radiochemotherapy in Rectal Cancer Is Associated with Distant Metastases and Shortened Survival

Thilo Sprenger, Franz Rödel, Tim Beissbarth, et al.

Clin Cancer Res 2011;17:1623-1631. Published OnlineFirst November 30, 2010.