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ORIGINAL RESEARCH

Mutational and Clinical Predictors of Pathologic Complete Response in the Treatment of Locally Advanced Rectal Cancer

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Abstract
Purpose Preoperative chemoradiation (CRT) for locally advanced rectal adenocarcinoma achieves pathologic complete response (pCR) in 8–20 % of patients. Mutations in critical cancer genes may contribute to lack of pCR. We retrospectively evaluated our institutional experience to determine potential mutational and clinical predictors of pCR in patients treated with CRT.
Methods Patients with locally advanced rectal adenocarcinoma treated with preoperative CRT ($n=79$) were identified. A clinical cancer genotyping assay evaluated 140 hotspot mutation sites across 15 cancer genes in 47 patients with sufficient tissue. Mutational profiles were compared in pre- and post-CRT specimens and with pCR rate. Clinical variables were evaluated using logistic regression.
Results Genotyping identified mutations in *KRAS* (43 %), *APC* (17 %), *BRAF* (4 %), *NRAS* (4 %), *PIK3CA* (4 %), and *TP53* (11 %). In the entire cohort, 21.5 % had a pCR. No patients with *BRAF*, *NRAS*, *APC*, or *TP53* achieved a pCR. pCR rate was 23.5 % (4/17) in wild-type tumors versus 3.3 % (1/30) in those with a mutation. There was no difference in the mutation rates in pre- versus post-CRT specimens. On univariate analysis, clinical predictors of pCR included post-RT carcinoembriogenic antigen level of ≤2.5 and smaller tumor size. No patients with a pCR developed recurrence.

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Conclusion Patients without mutations in commonly mutated cancer genes may be associated with a higher likelihood of having a pCR after preoperative CRT. This should be confirmed in a prospective study.

Keywords Rectal cancer · Mutation · Pathologic complete response

Introduction

Colorectal cancer is the third most common cancer and the fourth most common cause of cancer death worldwide [1]. Advanced stage rectal cancer is typically treated with preoperative chemotherapy and radiation followed by surgery and adjuvant chemotherapy. Preoperative, compared to postoperative, radiation therapy improves local control and sphincter preservation and has been shown to increase disease-free survival (DFS) [2, 3]. Importantly, preoperative chemoradiation (CRT) allows for tumor downstaging and the ability to assess pathologic complete response (pCR), which has been correlated with 5-year DFS rates [4]. A meta-analysis of 1,913 patients showed that pCR is associated with improved local and distant control, DFS, and overall survival [5]. Given this correlation, pCR is often used as a surrogate for outcomes in clinical trials, particularly in trials incorporating novel agents. Of note, there is also some data showing a lack of correlation between pCR and outcome [6].

Identifying patients who may or may not achieve a pCR would allow for treatment with alternative approaches in the preoperative setting. Several studies have identified clinical and biologic predictors of pCR, including a longer interval from completion of CRT to surgery, pre- and post-CRT carcinoembriogenic antigen (CEA) levels, *KRAS* status, and expression of *EGFR* or *VEGF* [7–10]. We sought to assess both clinical and biologic predictors of pCR, focusing specifically on targetable genetic mutations in order to identify patients who may benefit from more intensive or alternative treatment.

Patients and Methods

Patient Population and Treatment

We retrospectively reviewed medical records of 79 consecutive patients with stage II or III rectal adenocarcinoma treated at our institution between July 2005 and June 2010. Study variables including pretreatment tumor (T) and nodal (N) stage, grade, circumferentiality, distance to the anal verge, pretreatment tumor size (determined by colonoscopy, MRI, and/or EUS), pre and post-CRT CEA levels, treatment duration, type of concurrent chemotherapy, and recurrence data were reported. This study was approved by the Institutional Review Board.

All patients received preoperative CRT prior to total mesorectal excision. Three-dimensional conformal radiation therapy was delivered to a dose of 45 Gy to the pelvis followed by a 5.4-Gy cone down to the tumor. Radiation was given in 1.8-Gy daily fractions, 5 days a week. Concurrent 5-fluorouracil (5-FU)-based chemotherapy was given in the form of continuous infusion of 5-FU (97 %) or capecitabine (3 %). Four patients received 5-FU with bevacizumab and four received 5-FU with oxaliplatin.

Pathology and Treatment Response

Tumor specimens were evaluated at the time of initial resection for pCR, reported as ypT0N0, obtained from the patient's medical record. Subsequently, for purposes of this study, a single pathologist, blinded to patients' clinical outcome, assessed pCR by the Dworak tumor regression grade (TRG). TRG was scored as follows: grade 0, no regression; grade 1, minor regression; grade 2, moderate regression; grade 3, good regression; and grade 4, total regression, where grade 4 was equivalent to pCR [4, 11].

Mutational Analysis

Mutations were analyzed on pre- ($n=26$) and post-CRT ($n=21$) primary tumors available for 47 patients; 32 patients did not have sufficient tissue for analysis. Genotyping was performed on formalin-fixed and paraffin-embedded tissue with a multiplexed PCR and single-base extension, followed by capillary electrophoresis (SNaPshot version 3), previously described [12]. This tests for 60 frequently mutated loci across 15 cancer genes, which detects 140 previously described mutations including *APC*, *BRAF*, *CTNNB1*, *EGFR*, *FLT3*, *IDH1*, *IDH2*, *JAK2*, *KIT*, *KRAS*, *NOTCH1*, *NRAS*, *PIK3CA*, *PTEN*, and *TP53* [13]. Specific mutations assessed using SNaPshot are shown in Supplemental Table 1. Of note, *TP53* testing was limited to only several common mutation sites. These genes were selected because they either have targeted drugs available or have potential clinical implications.

Statistics

Fisher's exact test was used to compare rates of mutations in pre- and post-CRT specimens and to compare the pCR rate in patients with wild-type tumors to those with any mutations in both the pre- and post-CRT tumors. Univariate logistic regression was used to assess clinical predictors of pCR. Distance from the anal verge (in centimeter), pretreatment tumor size (per centimeter), age at treatment, and duration of treatment (in days) were treated as continuous variables. Multivariate analysis was not performed due to the limited number of

Table 1 Overall patient and tumor characteristics

Variable	
Patient characteristics ($n=79$)	
Age at treatment, mean (range)	58 (18–86)
Male, *n* (%)	52 (65.8)
Stage II, *n* (%)	12 (15)
Stage III, *n* (%)	67 (85)
T-stage 3–4, *n* (%)	75 (94.9)
Tumor size, mean (cm, range)	4.9 (1.4–12)
Treatment characteristics ($n=79$)	
Days of RT≥40, *n* (%)	23 (29.1)
Treated with 5-FU+other[a], *n* (%)	8 (10.1)
CEA values	
Mean pre-RT CEA (range), $n=73$	7.3 (0.4–133.4)
Mean post-RT CEA (range), $n=57$	2.6 (0.6–18.1)

T-stage tumor stage, *RT* radiation therapy, *5-FU* fluorouracil, *CEA* carcinoembryonic antigen

[a] Other included bevacizumab in four patients and oxaliplatin in four patients

events. The Kaplan–Meier method was used to estimate distant metastasis (DM), DFS, and event-free survival (EFS). Overall survival, disease-specific survival, and local control could not be analyzed due to low event rates. All p values were two-sided.

Results

Patient Characteristics

Patient characteristics are listed in Table 1. All patients completed neoadjuvant treatment. Overall, 17 (21.5 %) patients had a pCR. With reanalysis by Dworak TRG, 57 (83.8 %) had at least a good response (TRG 3 or 4). Nine patients had more than one mutation, including five patients with both *KRAS* and *APC* mutations, one with *BRAF* and *APC*, one with *TP53* and *APC*, one with *KRAS* and *PIK3CA*, and one patient with *TP53* and *PIK3CA*. The distribution of pre- and post-CRT mutations is shown in Table 2. There was no significant difference in the rates of mutations when pre-CRT specimens were compared to post-CRT specimens. Of the four patients treated with 5-FU plus oxaliplatin, one patient had a pCR, and of the four treated with 5-FU plus bevacizumab, one patient had a pCR.

pCR by Mutational Status

Among the 47 patients with mutations assessed, five (11 %) had a pCR. Table 2 shows frequencies of mutations by pCR and by whether tissue was obtained pre- or post-CRT. Of note, no patients with an *APC*, *BRAF*, *NRAS*, or *TP53* mutation achieved a pCR and only one patient with combined *KRAS* and *PIK3CA* mutations achieved a pCR. In the pre-CRT group, 3 of 10 patients (30 %) with wild-type tumors had a pCR compared to 1 of 16 in those with any mutation (6 %). In the post-CRT group, one of seven (14 %) patients with wild-type tumors had a pCR compared to none of 14 patients with any mutation (0 %). Given the small number of patients with mutational analysis and the lack of differences in the mutation rates between pre- and post-CRT specimens, the rate of pCR between patients with any mutation and wild-type tumors was compared for the combined pre- and post-CRT groups. There were significantly more patients who achieved a pCR in the wild-type group (4/17, 23.5 %) compared to those with any mutation (1/30, 3.3 %, $p=0.05$).

Predictors of pCR

Table 3 demonstrates the univariate analysis of factors associated with pCR. Patients with a post-RT CEA level of ≤2.5 (odds ratio (OR) 8.61, 95 % confidence interval (95 % CI) 1.03–71.9, $p=0.047$) were significantly more likely to achieve a pCR. Increasing tumor size was associated with a decreased rate of pCR (OR 0.69, 95 % CI 0.48–1.0, $p=0.050$). The association of wild-type tumors and pCR was borderline significant (OR 8.92, 95 % CI 0.91–87.8, $p=0.06$) in both the pre-CRT and post-CRT specimens.

Patient Outcomes

With a median follow-up of 30.9 months (IQR 18.0–44.5 months), seven (8.9 %) patients had any recurrence. One (1.3 %) patient had an isolated local recurrence, four (5.1 %) had an isolated distant recurrence, and two patients (2.5 %) had both local and distant recurrences. No patients with a pCR had a local or distant failure. There were four deaths, two of which were due to disease. On univariate Cox regression modeling, only duration of radiation treatment ≥40 days was of borderline significance in predicting DFS (HR 4.45, 95 % CI 0.99–20.1, $p=0.052$). No clinical variables or mutations were associated with DM, DFS, or EFS.

Discussion

This study investigated clinical and biologic factors in patients with rectal adenocarcinoma as a means to predict response to preoperative chemotherapy and radiation. Overall 21.5 % of patients achieved a pCR. We identified both post-RT CEA level of ≤2.5 and having decreasing tumor size as clinical predictors of pCR. In assessing mutations of pre- and post-CRT tumors, only one patient with a mutation had a pCR, and the patients without mutations in commonly mutated cancer

Table 2 Mutations and pathologic complete response by pre- and postchemoradiation

	Pre-CRT (n=26)			Post-CRT (n=21)			p value (overall)
Mutation	Total	pCR	No pCR	Total	pCR	No pCR	
APC	7 (27 %)	0	7	1 (5 %)	0	1	0.06
BRAF	0 (0 %)	0	0	2 (10 %)	0	2	0.19
KRAS	12 (46 %)	1	11	8 (38 %)	0	8	0.77
NRAS	1 (4 %)	0	1	1 (5 %)	0	1	1.00
PIK3CA	2 (8 %)	1	1	0 (0 %)	0	0	0.50
TP53	2 (8 %)	0	2	3 (14 %)	0	3	0.64
Any mutation	16 (62 %)	1 (6 %)	15 (94 %)	14 (67 %)	0	14 (100 %)	0.77
Wild type	10 (38 %)	3 (30 %)	7 (70 %)	7 (33 %)	1 (14 %)	6 (86 %)	0.77

Five patients of the 47 analyzed had a pCR. One patient with a pCR had both *KRAS* and *PIK3CA* mutations. Overall, five patients had both APC/KRAS (pre-), one patient had KRAS/PIK3CA (pre-), one had APC/TP53 (pre-), one had PIK3CA/TP53 (pre-), and one patient had APC/BRAF (post-)

CRT chemoradiation, *pCR* pathologic complete response

genes were significantly more likely to have a pCR in both the pre- and post-CRT groups combined. This supports prior findings that patients with mutations in *KRAS*, *BRAF*, *PIK3CA*, *APC*, and *TP53* have poorer outcomes [14–16].

There have been several publications assessing clinical and biologic predictors of pCR, summarized in Supplemental Tables 2 and 3. Others have found either a pre-CRT CEA level of ≤2.5–5 ng/mL or a post-CRT CEA level of <5 ng/mL to be associated with pCR [17]. We did not find pre-CRT CEA values to be of significance. However, we did find a post-CRT CEA level of ≤2.5 ng/mL to be significantly associated with pCR, consistent with previous studies [8]. We also found that increasing pretreatment tumor size was significantly associated with decreased likelihood of achieving pCR. Size was previously assessed in a meta-analysis and was found not to be predictive [5]. This discrepancy could be from the large average tumor size in our population (4.9 cm) compared to the smaller average tumor size in the meta-analysis (3.9 cm).

Table 3 Univariate logistic regression predicting pCR

Variable	OR (95 % CI)	p value
Patient and tumor characteristics		
Age≥50	0.91 (0.28, 2.96)	0.87
Age at treatment	1.01 (0.97, 1.06)	0.55
Male	0.50 (0.17, 1.49)	0.21
Stage II	0.29 (0.03, 2.42)	0.25
Distance from anal verge (cm)	1.07 (0.94, 1.22)	0.33
Pretreatment tumor size (cm)	0.69 (0.48, 1.00)	0.05
Days of RT≥40	1.02 (0.31, 3.31)	0.98
Days of treatment	0.99 (0.81, 1.21)	0.94
Treated with 5FU+other	1.24 (0.23, 6.80)	0.80
Post-RT CEA≤2.5	8.61 (1.03, 71.93)	0.047
Pre-RT CEA>5	0.95 (0.27, 3.44)	0.94
Pre-RT CEA≤2.5	1.33 (0.42, 4.17)	0.62
Mutations		
APC mutation	–	–
BRAF mutation	–	–
KRAS mutation	0.30 (0.03, 2.94)	0.30
PIK3CA mutation	10.25 (0.53, 197.03)	0.12
TP53 mutation	–	–
Wild type	8.92 (0.91, 87.84)	0.06

pCR pathologic complete response, *OR* odds ratio, *CI* confidence interval, *T-stage* tumor stage, *RT* radiation therapy, *CEA* carcinoembryonic antigen

Biologic predictors of pCR offer the potential to identify possible targets for enhancing treatment approaches and have been previously investigated. Unfortunately, because of the heterogeneity of the markers and methods of assessment, it is difficult to compare studies. These are shown in Supplemental Table 3. Our study found a subset of patients without mutations in commonly mutated cancer genes, were more likely to have a pCR. In particular, no patients with a *BRAF*, *NRAS*, *APC*, or *TP53* mutation achieved a pCR, and only one patient with a combined *KRAS/PIK3CA* mutation had a pCR. Similarly, others have shown that mutations in *KRAS* predict for lack of pCR and *p53* wild type to be predictive of pCR [9, 10].

This was a small, retrospective study and, as such, information is not available for all variables and all patients. There was inadequate pre- and post-CRT tissue to perform mutational analysis in 32 patients; therefore, this was only conducted on 47 specimens. Our subgroups of pre- and post-CRT on which mutational analysis was performed were small (n=26 and n=21, respectively); however, we did not see any significant difference in the mutation rates between these groups. Other investigators have shown that fewer mutations are detected in post-CRT specimens, due to the paucity of cellular material after CRT [18]. However, it is likely that mutational profiles are similar between pre- and post-CRT

specimens as has been demonstrated by others where it has been shown that there is a high concordance of mutations in *KRAS*, *NRAS*, *BRAF*, and *PIK3CA* between pre- and post-FOLFOX treated colorectal cancers, and as was shown in this study [19]. A larger study comparing pre- and post-CRT tissue could further address this question. This study also does not include a validation set of tumors as it was strictly hypothesis-generating; however, we are currently planning a multi-institutional trial that incorporates testing of these mutations on which we can validate our results. Lastly, the method of surveying for mutations is not comprehensive and could potentially miss mutations that would be identified if whole genome sequencing was performed. However, the benefit of the method of analysis used in this study is that these genes and mutations were preselected to include those which have potential clinical implications, many of which can be targeted by drugs. The platform allows for high-throughput analysis and is in contrast to most other studies of molecular markers in rectal cancer, which have primarily relied on immunohistochemistry.

The ability to predict which patients will respond to treatment is important to appropriately counsel patients and offer treatments to maximize response. Furthermore, there may be a subset of patients who may not require surgery if a pCR is attained. Habr-Gama et al. published on 99 patients with a clinical complete response (cCR) who were observed for a mean of 59.9 months after CRT and had a recurrence rate of 13.1 % with five local recurrences salvaged and a 5-year DFS of 85 % [20]. Recently, Maas et al. demonstrated excellent control with a 2-year DFS of 89 % in a group of 21 patients with a cCR selected for a wait-and-see policy after CRT [21]. The use of clinical and biologic predictors of treatment response may further help to define this selected group who may not require definitive surgery.

In summary, we found that the rates of mutations in important cancer genes including *KRAS*, *NRAS*, *BRAF*, *APC*, *TP53*, and *PIK3CA* were not different between pre- and postchemoradiation specimens. Furthermore, patients with mutations in these genes were less likely to achieve a pCR compared to patients whose tumors did not have mutations. In addition, our findings that both post-CRT CEA levels and decreasing tumor size are associated with pCR are concordant with the current literature. Future studies utilizing prospective assessment of mutations on pre- and posttreatment specimens may help to better identify patients who are less responsive to treatment or to potentially de-escalate treatment in patients who have achieved a pCR in the appropriate setting. Furthermore, given that this was strictly a hypothesis-generating study, we are currently planning a prospective multi-institutional study that will mandate genotyping be performed on all pre-CRT tumor specimens as a study entry criterion to further assess this hypothesis.

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Conflict of Interest Coauthor Darrell Borger has a stated conflict of interest, as a paid consultant for Bio-Reference Laboratories, the licensee of SNaPshot which is the technology used in this study. No other conflicts of interest exist.

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