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Gene Polymorphisms Predict Toxicity to Neoadjuvant Therapy in Patients with Rectal Cancer

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Abstract

Background—Toxicity from neoadjuvant chemoradiation therapy (NT) increases morbidity and limits therapeutic efficacy in patients with rectal cancer. Our objective was to determine whether specific polymorphisms in genes associated with rectal cancer response to NT correlate with NT-related toxicity.

Methods—One hundred thirty-two patients with locally advanced rectal cancer were treated with NT followed by surgery. All patients received 5-fluorouracil (5-FU) and radiation (RT), and 80 patients also received modified FOLFOX-6 (mFOLFOX-6) chemotherapy. Grade 3 adverse events (AEs) that occurred during 5-FU/RT and 5-FU/RT+mFOLFOX-6 were recorded. Pretreatment biopsies and normal rectal tissue were collected from all patients. DNA was extracted and screened for 22 polymorphisms in 17 genes associated with response to NT. Polymorphisms were correlated with treatment-related Grade 3 AEs.

Results—Overall 27 of 132 (20%) patients had Grade 3 AEs; 24 of 132 (18%) patients had Grade 3 AEs during 5-FU/RT, 9 of 80 (11%) patients had Grade 3 AEs during mFOLFOX-6, and 6 (5%) patients had Grade 3 AEs during both 5-FU/RT and mFOLFOX-6. Polymorphisms in *XRCC1*, *XPB*, and *TP53* were associated with Grade 3 AEs during NT ($p < 0.05$). Specifically, the *XRCC1* Q399R and *XPB* K751Q polymorphisms were associated with increased toxicity to 5-FU/RT ($p < 0.05$), and the *TP53* R72P polymorphism was associated with increased toxicity to mFOLFOX-6 ($p = 0.008$).

Conclusion—Specific polymorphisms in *XRCC1*, *XPB*, and *TP53* are associated with increased toxicity to NT in rectal cancer. Polymorphism screening may help tailor treatment for patients by selecting therapies with the lowest risk of toxicity thus increasing patient compliance.

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Disclosures:

The authors declare no conflicts of interest associated with this manuscript.

Keywords

Gene polymorphisms; toxicity; rectal cancer; neoadjuvant chemoradiation

Introduction

Neoadjuvant chemoradiation therapy (NT), including 5-fluorouracil and radiation (5-FU/RT) followed by total mesorectal excision (TME) is the standard of care for patients with locally advanced rectal cancer.¹ The oncologic benefits of this treatment approach have been well-established, and patients who respond to treatment achieve excellent local tumor control and low rates of recurrence.^{1–4} However, not all patients respond equally to NT. Approximately one third of patients will have no viable cancer cells in the resected surgical specimen after treatment and have a pathologic complete response (pCR). However, other patients will have only a partial response or no response at all.³

An important limiting factor that can impede the efficacy of NT is the development of treatment-related toxicity. Some patients tolerate therapy well with few complications, but other patients experience severe adverse events (Grade 3 AEs) that cause significant morbidity. This can lead to a truncated course of therapy limiting the full efficacy of treatment.^{5, 6} The ability to identify those patients most likely to develop toxicity to NT is therefore clinically important.

Gene polymorphisms are genetic variations that contribute to the genetic diversity observed between individuals. They can also alter the expression and function of the encoded protein by changing a single amino acid.^{7, 8} Recent studies suggest that specific polymorphisms in genes related to DNA repair, drug metabolism, cell cycle progression, cell growth, and inflammation are associated with response to NT, and that patients with different polymorphisms in these genes have different tumor response.^{9–14} We have recently shown that rectal cancer patients harboring select polymorphisms in methylenetetrahydrofolate reductase (*MTHFR*), which functions in the metabolism of 5-FU, and cyclin D1 (*CCND1*), which regulates cell cycle progression are unlikely to achieve a pCR to CRT.¹⁵ Studies in breast, ovarian, prostate, lung, esophageal, gastric, and colorectal cancers have also demonstrated that specific polymorphisms associated with response to NT also predict toxicity to treatment.^{9, 11, 14, 16–21} However, few studies have examined this association in patients with rectal cancer.^{22, 23}

We studied a large cohort of rectal cancer patients treated with NT - 5-FU/RT, with or without modified FOLFOX-6 (mFOLFOX-6) chemotherapy - to determine whether specific polymorphisms correlate with NT-related toxicity.

Patients and Methods

Patients and treatment

This study included 132 patients with Stage II/III rectal cancer enrolled in a multicenter clinical trial investigating the effect of increasing the chemoradiation-to-surgery time interval and adding chemotherapy (mFOLFOX-6) during the waiting period on tumor

response ([ClinicalTrials.org](https://clinicaltrials.org) Identifier: NCT00335816). This study was designed as a series of sequential Phase II trials or study groups (SGs) each with a progressively longer chemoradiation-to-surgery time interval and increasing cycles of pre-operative mFOLFOX-6. This study was approved by an Institutional Review Board (IRB) at each participating institution, and by a central IRB. Informed written consent was obtained from each patient prior to enrollment in the trial. Patients in the present study were from SG1 (n=52), SG2 (n=58) and SG3 (n=22). Further details of patient eligibility for this trial are presented elsewhere.²⁴

All patients were treated with 5-FU/RT as described previously.²⁴ Patients in SG1 (n=52) then underwent TME an average of 6 weeks after completing 5-FU/RT. The remaining patients (SG2 and SG3, n=80) received 2 (SG2) or 4 (SG3) cycles of additional chemotherapy (mFOLFOX-6) as described previously.²⁴ These patients underwent TME an average of 11 (SG2) and 16 (SG3) weeks after completing 5-FU/RT.

Assessment of NT-related adverse events

An AE was defined as a complication that occurred during 5-FU/RT or mFOLFOX-6 treatment. Adverse events were graded according to the National Cancer Institute (NCI) Common Terminology Criteria for Adverse Events, Version 3.0 (CTCAE 3.0). A severe AE was defined as any Grade 3 toxicity (Grade 3 AEs) that occurred during 5-FU/RT or mFOLFOX-6 treatment. Severe AEs were recorded prospectively for all patients and compiled in a central database.

Sample preparation and polymorphism screening

Unstained formalin fixed paraffin-embedded tissue blocks were retrieved from all participating institutions. Benign colonic epithelial cells were microdissected from the proximal resection margins of the surgical specimen >10cm in distance from the primary tumor. DNA was extracted using the QIAamp DNA FFPE Tissue kit (Qiagen Inc, Valencia, CA) according to the manufacturer's instructions as described.¹⁵ Standard polymerase chain reaction (PCR) and Direct Sanger sequencing were performed to detect gene polymorphisms. We examined 22 polymorphisms in 17 genes associated with DNA repair (*ERCC1*, *XRCC1*, *RAD23B*, *XPA*, *XPD*, *OGG1*, and *PARP*), drug metabolism (*MTHFR*, *TS*, *DPD*, and *OPRT*), cell cycle progression (*CCND1* and *TP53*), cell growth (*EGFR*), and inflammation (*VEGF*, *IL6*, and *TLR2*). Primers were used to amplify genomic sequences using established conditions (Table 1).¹⁵ All polymorphisms were confirmed by two independently-derived PCR products.

Statistical analysis

Univariate analysis using the Chi-square test was performed to determine the association between gene polymorphisms and Grade 3 AEs resulting from 5-FU/RT, mFOLFOX-6, or both therapies, and to determine the association between Grade 3 AEs and clinical/pathologic factors. No adjustment for multiple comparisons was made. A two-sided p-value of 0.05 indicated a significant association.

Results

Patient characteristics and tumor response

Patient characteristics and tumor response to NT for all 132 patients are shown in Table 2. Over half of the patients were male (n=78; 59%) and the mean age was 57 years old. Most patients (n=102, 77%) had clinical stage III disease. Of 132 patients who received NT, 52 patients received 5-FU/RT alone and 80 patients received 5-FU/RT+mFOLFOX-6. Final pathology revealed that 33 of 132 (25%) patients achieved a pCR to NT; 10 (19%) patients who received 5-FU/RT alone, and 23 (29%) patients who received 5-FU/RT+mFOLFOX-6. There were no differences in clinical and pathological characteristics between treatment groups (p=0.22).

Rate of adverse events

A total of 27 of 132 (20%) patients had Grade 3 AEs related to NT. Of the 27 patients who experienced a Grade 3 AE, 18 patients had a complication associated only with 5-FU/RT, 3 patients experienced toxicity only during mFOLFOX-6, and 6 patients had Grade 3 AEs associated with both treatments (Table 3). The most common AEs observed were gastrointestinal symptoms including diarrhea, rectal pain, severe nausea, and vomiting.

To determine whether AE rates differed according to treatment response, we compared the rate of Grade 3 AEs for patients with a pCR to patients with non-pCR (residual disease). In patients with a pCR (n=33), 6 (18%) had a Grade 3 AE after NT, while 21 of 99 (21%) patients with non-pCR experienced toxicity from NT. There was no statistical difference in the rate of Grade 3 AEs in patients with or without a pCR after NT (p=0.81). There was also no difference in the rate of Grade 3 AEs between patients who received 5-FU/RT alone (n=11 of 52, 21%) and patients who received 5-FU/RT+mFOLFOX-6 (n=17 of 80, 21%) (p=1.0).

Association of gene polymorphisms with treatment toxicity

We examined the association of Grade 3 AEs with gene polymorphisms during NT (Table 4). Gene polymorphisms in *XRCC1* (Q399R), *XPB* (Q751K), and *TP53* (R72P) were associated with Grade 3 AEs during NT. Specifically, we found that patients with the *XRCC1* Q399 allele had an increased rate of toxicity to NT compared to patients who were homozygous for the *XRCC1* R399 allele (27% versus 5%; p=0.003). Additionally, we found that patients with the *XPB* Q751 allele had a lower rate of Grade 3 AEs to NT compared to patients only with *XPB* K751 allele (14% versus 31%; p=0.02). Finally, patients with the *TP53* P72 allele had an increased rate of Grade 3 AEs compared to patients only with the *TP53* R72 allele (32% versus 16%; p=0.05).

We next examined the association of gene polymorphisms with toxicity in each treatment arm (5-FU/RT; mFOLFOX-6) (Table 4). In the patients who received 5-FU/RT (n=132), those with the *XRCC1* Q399 allele had a significantly higher rate of Grade 3 AEs compared to patients only with *XRCC1* R399 allele (24% versus 5%; p=0.01). Additionally, patients only with the *XPB* K751 allele had an increased risk of developing Grade 3 AEs to 5-FU/RT compared to patients with the *XPB* Q751 allele (27% versus 12%; p=0.02).

Finally, we determined whether gene polymorphisms correlated with toxicity specifically to mFOLFOX-6 (Table 4). Univariate analysis showed that patients with the *TP53* P72 allele had an increased risk of toxicity to mFOLFOX-6 compared to patients only with the *TP53* R72 allele (26% versus 5%; $p=0.008$).

Discussion

Severe toxicity from NT contributes to patient morbidity and may limit the delivery and efficacy of treatment. Predictive factors may help identify patients who are at risk for increased toxicity and thus guide multimodal therapy. We found that specific polymorphisms in the *XRCC1* (Q399R), *XPB* (Q751K), and *TP53* (R72P) genes are associated with increased toxicity to NT in patients with locally advanced rectal cancer. The identification of these genetic polymorphisms as predictive factors is clinically relevant because they may help identify patients who are more likely to develop severe AEs to NT. Treatment regimens with the lowest risk of toxicity depending on these genetic factors could then be selected, which could increase treatment compliance and efficacy.

Gene polymorphisms have been linked to differential response to neoadjuvant therapies in lung, colon, esophageal, breast, ovarian, and prostate cancers.^{9, 11, 13, 14, 16, 19, 20, 25} Gene polymorphisms can affect protein function and alter biologic pathways integral in response to NT in tumor cells.^{7–8} Polymorphisms can also attenuate pathways, such as DNA damage repair, drug metabolism, and cell cycle progression, impairing the survival of normal cells under stress from treatment from either RT or chemotherapy.^{7–8} As such, polymorphisms may contribute to significant treatment-related toxicity in patients.

Although little is known about the functional consequences of the *XRCC1* R399Q polymorphism, previous reports have examined its association with toxicity to RT and/or chemotherapy in other malignancies.^{11, 17, 18, 20, 26} The *XRCC1* protein acts as a scaffold that coordinates the base excision repair mechanism, which repairs DNA after damage from RT. Monaco et al. described the effect of the *XRCC1* R399Q gene polymorphism on *XRCC1* protein function and observed that the R to Q amino acid substitution produces significant conformational changes at the BRCT1 binding domain, which interacts with the *BRCA1* gene during DNA damage, and decreases protein interaction. The overall effect may result in attenuated DNA repair, especially after RT.²⁶ Chang-Claude et al. found that breast cancer patients harboring the *XRCC1* R399 allele had increased acute toxicity from RT.^{17, 18} Other studies have shown that the *XRCC1* R399 allele correlates with severe AEs after chemoradiation therapy and an increased rate of Grade 3–4 neutropenia in ovarian cancer patients treated with cisplatin-based chemotherapy.¹¹ In contrast, Giachino et al. showed that the *XRCC1* Q399 allele was associated with a significantly increased risk of Grade 3–4 complications in lung cancer patients treated with platinum-based chemotherapy.²⁰ Our results support the later association of the *XRCC1* Q399 allele with the development of severe toxicity to NT in patients with rectal cancer. In our study, the *XRCC1* Q399 allele was strongly associated with toxicity during 5-FU/RT treatment.

The *XPB* protein is a DNA helicase involved in the nucleotide excision repair pathway. Monaco et al. showed that the *XPB* K751Q polymorphism results in a conformational

change in the C-terminal of the XPD protein.²⁷ The C-terminal is responsible for the activation of XPD helicase during DNA repair, and it also affects the interaction with other proteins involved in DNA repair. Previous studies have reported contrasting roles for the XPD K751 and Q751 alleles.^{28, 29} Our results, which demonstrated an increased risk of AEs in patients with the XPD K751 allele, are in agreement with the results of Boige et al., who previously reported increased toxicity in metastatic colorectal cancer patients with the XPD K751 allele treated with 5-FU-based regimens.¹⁶ Homozygosity of the XPD K751 allele may result in attenuated XPD function and diminished response to DNA damage. This could further lead to decreased DNA damage repair in normal cells after chemoradiation and result in treatment toxicity. Other studies also support the association of the XPD K751 allele with increased risk of toxicity from RT in patients with lung cancer.^{9, 30}

Mutations in the TP53 gene has been well studied, but genetic polymorphisms in the TP53 gene have only recently been evaluated and have been shown to associate with increased risk of cancers of the stomach, breast, lung, and esophagus.^{31–33} Studies have demonstrated that the TP53 R72 allele may encode a TP53 variant that increases the induction of programmed cell death. However, in cancer cells, the effectiveness of the TP53 R72 allele in promoting cell death may depend on the mutation status of the TP53 gene.^{35–37} Specifically, the pro-apoptotic effects of the TP53 R72 allele may be lost when an inactivating TP53 gene mutation is present. This has been described in 70% of patients with non-melanoma skin cancers.³⁸ However, few studies have examined the role of the TP53 R72P polymorphism in determining toxicity to chemoradiation therapy. Khrunin et al. observed an increased risk of severe neutropenia in ovarian cancer patients who were homozygous for TP53 P72 allele and received platinum-based chemotherapy.¹¹ These findings are consistent with our results, which demonstrate an association of the TP53 P72 allele with toxicity to NT, especially in patients treated with mFOLFOX-6. Further studies may be necessary to investigate the association of the TP53 R72P polymorphism with toxicity to platinum-based regimens.

Screening patients for specific gene polymorphisms that may predict toxicity to different components of NT could help tailor the management of rectal cancer patients. Specifically, patients carrying polymorphisms associated to the RT-CT may benefit from a chemotherapy based (mFOLFOX-6) and avoiding radiation. Additionally, patients that harbor polymorphisms associated with platinum-based toxicity may be spared unnecessary morbidity by selecting non-platinum containing regimens. This potential impact to the clinical decision making process must be weighed against the limitations of our study. First, our cohort size is relatively small with heterogeneity in the mFOLFOX-6 regimen. Consequently, we support validation of our results in a larger series of patients treated within the confines of a prospective trial. Second, our clinical trial consisted of 3 different treatment groups and it is feasible that our current investigation was underpowered to detect small differences between groups. Finally, our list of polymorphisms was extensive, but not exhaustive. Others polymorphisms that were not assessed in this study could be associated with treatment toxicity. Future sequencing of the whole genome may identify other polymorphisms associated with specific outcomes.

In conclusion, polymorphisms in the XRCC1, XPD, and TP53 genes may predict the development of severe AEs in rectal cancer patients receiving NT. Screening for these

polymorphisms before treatment may help identify patients who are at increased risk of developing toxicity to treatment. This may help guide clinicians in choosing the optimal treatment regimen, by individualizing therapeutic regimens for maximum efficacy with minimal morbidity. By screening patients for the presence of these genetic markers, we may increase the likelihood that patients recommended for NT tolerate and complete therapy, and receive the maximal benefit from treatment.

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Table 1

Primers and annealing temperatures for gene polymorphisms included in the analysis

Polymorphism	Forward Primer (5'-3')	Reverse Primer (5'-3')	Size (bp)	Tm (°C)
<i>CCND1</i> G870A	TGAAGTTCATTTCCTCAATC	TCAGTAAGTTCTAGGAGCAG	337	48
<i>DPD</i> F632F	ATCAGTGAGAAAACGGCTGC	TGCATCAGCAAAGCAACTGG	205	60
<i>EGFR</i> R497K	TCTGTCACTGACTGCTGTGAC	CAACGCAAGGGGATTAAAG	204	64
<i>ERCC1</i> N118N	GTGGTTATCAAGGGTCATCC	TGCCCTTCCTGAAGTCTG	197	60
<i>TP53</i> R72P	CGTTCTGGTAAGGACAAGGGT	AAGAAATGCAGGGGATACGG	446	64
<i>IL6</i> 572	TGGAGACGCCTTGAAGTAAC	TGACCAGATTAAACAGGCTAG	237	60
<i>IL6</i> 174	ACTTCGTGCATGACTTCAGC	GGGCTGATTGGAAACCTTAT	211	60
<i>MTHFR</i> C677T	CCAAAGGCCACCCGAAG	GAAAGATCCCGGGGACGATG	180	60
<i>MTHFR</i> A1298C	CTTTGGGGAGCTGAAGGACTACTAC	CACTTTGTGACCATTCCGGTTTG	163	76
<i>OGG1</i> S326C	CAACACTGTCTAGTCTCAC	CCAAGGACTCTTCCACCTC	166	62
<i>OPRT</i> G213A	TGAGACAGTTGGGAGAGTGA	TGAGTTCTTTGGGTGCTTCCTT	104	60
<i>PARP1</i> V762A	TTGGACCTTCTCTGCATG	TCCAGGAGATCCTAACACAC	313	54
<i>RAD23B</i> A249V	ATTTTGCATGATGGGATATCT	ATGAACGTCATTTCTGAAGTAT	272	56
<i>TLR2</i> R753Q	AGTGAGTGGTGCAAGTATG	AAATATGGGAACCTAGGAC	240	56
<i>TS</i> Del6bp	CAAATCTGAGGGAGCTGAGT	GCAGATAAGTGGCAGTACAGA	149	60
<i>VEGF</i> 634	TGGAAACCAGCAGAAAGAGG	TCAGCGCGACTGGTCAG	203	60
<i>VEGF</i> 936	TCACCATCGACAGAACAGTC	TGTGTCTACAGGAATCCCAG	228	60
<i>VEGF</i> 2578	TGACTAGGTAAGCTCCCTG	ATTCCTAGCTGGTTTCTGAC	227	58
<i>XPA</i> R23G	TTAACTGCGCAGGCGCT	TTCCGCTCGATACTCGC	228	54
<i>XRCC1</i> R194Y	GGACCTTAGAAGGTGAC	AGGAGTCCAGGACTCCAC	252	52
<i>XRCC1</i> R399Q	TCAGATCACACCTAACTGG	CAGGTCCTCCTTCCCTC	341	56
<i>XPB</i> K751Q	CCTCTCCCTTTCCTCTGT	AATGTCACCTGACTTCATAAGAC	227	56

bp: Base pairs; Tm: Annealing temperature.

Table 2

Patient Characteristics and tumor response

Characteristics	Total patients n=132
Gender	
Female	54 (41%)
Male	78 (59%)
Age, years (mean, range)	57 (25–87)
Pre-operative clinical stage	
Stage II	30 (23%)
Stage III	102 (77%)
Neoadjuvant treatment	
5-FU/RT	52 (39%)
5-FU/RT and mFOLFOX-6	80 (61%)
Tumor response	
pCR	33 (25%)
Non-pCR	99 (75%)

5-FU: 5-fluorouracil; RT: radiation; mFOLFOX-6: Modified FOLFOX-6; pCR: Pathologic complete response.

Table 3

Grade 3 AEs stratified by treatment

Grade 3 AEs	5-FU/RT n=132	mFOLFOX-6 n=80
Patients with Grade 3 AEs	24 (18%)	9 (11%)
Most common Grade 3 AEs		
Gastrointestinal	10 (8%)	0 (0%)
Abdominal/Rectal pain	6 (5%)	1 (1%)
Vascular	0 (0%)	2 (3%)
Infection	2 (2%)	0 (0%)
Hematologic	3 (2%)	4 (5%)
Constitutional symptoms	3 (2%)	0 (0%)
Cardiac event	0 (0%)	1 (1%)
Other (Allergy/skin/rash)	8 (6%)	3 (4%)

AE: Adverse event; 5-FU: 5-fluorouracil; RT: radiation therapy; mFOLFOX-6: modified FOLFOX-6 chemotherapy.

Table 4

Association of gene polymorphisms with treatment-related toxicity

	Treatment Arm							
	NT				5-FU/RT			
Gene polymorphism	Patients n=132	Grade 3 AE n=27	p-value	Patients n=132	Grade 3 AE n=24	p-value	Patients n=80	mFOLFOX-6 Grade 3 AE n=9
<i>XRCC1</i> R399Q								
R399 alone	40	2 (5%)	0.003	40	2 (5%)	0.01	17	0 (0%)
Q399	92	25 (27%)		92	22 (24%)		63	9 (14%)
<i>XPB</i> K751Q								
K751 alone	55	17 (31%)	0.02	55	15 (27%)	0.02	32	6 (19%)
Q751	77	11 (14%)		77	9 (12%)		48	3 (6%)
<i>TP53</i> R72P								
R72 alone	95	15 (16%)	0.05	95	14 (15%)	0.10	57	3 (5%)
P72	37	12 (32%)		37	10 (27%)		23	6 (26%)

AE: Adverse events; 5-FU: 5-fluorouracil; RT: Radiotherapy; NT: neoadjuvant chemoradiation therapy; mFOLFOX-6: Modified FOLFOX-6 chemotherapy