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p27 Expression in Posttreatment Rectal Cancer: A Potential Novel Approach for Predicting Residual Nodal Disease

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Abstract

Background: Expression profiles of p21, p27, p53, Ki-67, and thymidylate synthase (TS) may be associated with response to neoadjuvant chemoradiation. The relationship between posttreatment protein expression and regional lymph node involvement has not been fully explored.

Method: Surgical specimen tumor cores from 126 rectal cancer patients underwent immunohistochemical (IHC) analysis for p21, p27, p53, Ki-67, and TS. A staining index (SI = percentage of stained cells x staining intensity [1=weak, 2=moderate, 3=strong], maximum score=300) was calculated for 10 tumor cores per patient. Mean and median SI for each marker was compared between node negative (ypN₀) and node positive (ypN₊) patients.

Results: Twenty-six (20.6%) cancers had a pathologic complete response and 37 cancers had tissue loss or lack of cancer cells, leaving 63 cancers for analysis. Thirty-seven (58.7%) cancers were staged ypN₀ and 26 (41.3%) cancers were staged ypN₊. There was an association between increased p27 SI and lymph node positivity ($P=0.04$).

Conclusion: Increased p27 expression in posttreatment rectal cancer is associated with nodal positivity and may help determine which patients are suitable for local excision.

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Introduction

Local excision of rectal cancer has evolved from an option for patients with early stage cancer or for patients not suitable for major resection, to one offered to carefully-selected patients with locally advanced rectal cancer demonstrating a favorable response to neoadjuvant therapy.^{1–4} However, a favorable response of the primary tumor to neoadjuvant therapy does not ensure lymph node negativity, as even ypT₀ specimens can have residual lymph node involvement. Since the incidence of lymph node metastases after neoadjuvant therapy parallels ypT stage (ypT₀: 2%–7%, ypT₁: 6%–17%, ypT₂: 9%–38%, ypT₃: 30%–38%, ypT₄: 33%–67%),^{5–9} ypT staging through analysis of locally-excised residual cancer/scar provides an estimate of residual lymph node involvement. However, reliance on this approach is limited because a wide range of residual lymph node involvement is reported per ypT stage.^{5–9} Furthermore, since imaging modalities commonly used for initial rectal cancer staging, such as endorectal ultrasound (ERUS) and magnetic resonance imaging (MRI), are unable to accurately determine lymph node involvement after neoadjuvant therapy, there is currently no known method to reliably determine posttreatment lymph node involvement.^{10–14}

Gene expression signatures, polymorphisms, and expression profiles of p21, p27, p53, Ki-67, and thymidylate synthase (TS) have been shown to be associated with rectal cancer response to neoadjuvant therapy and prognosis.^{15–38} Some of these studies have evaluated protein expression in both pretreatment or control (no neoadjuvant treatment) rectal cancer specimens and posttreatment specimens as a means to predict prognosis.^{16, 18, 19, 21, 22, 25–28, 31} However, the relationship between posttreatment protein expression and lymph node status has not been fully explored. We hypothesized that the expression of p21, p27, p53, Ki-67, and TS in residual rectal cancer following neoadjuvant chemoradiation (CRT) might help predict lymph node status.

Materials and Methods

Patient Population

As part of a National Cancer Institute-sponsored, prospective trial designed to compare PET and CT assessment of rectal cancer response to neoadjuvant CRT, we enrolled 150 patients with locally advanced (cT_{3/4} and/or cN₊, or clinically bulky) primary rectal cancer between September 1999 and December 2005. To be eligible for the trial, patients had to be diagnosed with biopsy-proven rectal adenocarcinoma, receive neoadjuvant CRT, and undergo rectal resection. Patients with metastatic disease were included if they received neoadjuvant CRT according to the study protocol requirements and underwent primary rectal cancer resection (N=18). Of 150 enrolled patients, 24 were excluded for the following reasons: 8 patients did not receive neoadjuvant radiation and/or chemotherapy, 5 patients did not undergo a resection because of widely-spread metastatic disease, 4 patients withdrew from the study, 3 patients underwent surgery at other institutions, 1 patient refused surgery, 1 patient underwent transanal local excision, and 1 patient died before treatment initiation. This study was approved by the Institutional Review Board at Memorial Sloan-Kettering Cancer Center. Informed consent was obtained from all participants.

Neoadjuvant Chemoradiotherapy

All patients received neoadjuvant external beam radiation therapy (EBRT) according to previously published techniques.³⁹ The most common regimen was 26 fractions of 180 cGy followed by a 360 cGy boost to the primary tumor bed, for a total dose of 5040 cGy. The median dose of EBRT was 5040 cGy (range: 4500 to 5400 cGy).

All patients received neoadjuvant 5-fluorouracil (5-FU)-based chemotherapy: bolus infusion, n=37 (29.4%); continuous infusion, n=68 (54.0%), or unknown, n=21 (16.6%). The most common regimen for bolus infusion chemotherapy was 5-FU (325 mg/m²/day) with leucovorin (20 mg/m²/day) given for 2 cycles on 5 consecutive days during week 1 (days 1 to 5) and week 5 (days 29 to 33) of radiation therapy. Leucovorin was administered by intravenous (IV) infusion for 30 minutes and was followed 20 minutes later with IV bolus 5-FU. The most common regimen for continuous infusion chemotherapy was 5-FU (225 mg/m²/day) for a 6-week continuous cycle. Five patients in the continuous infusion chemotherapy group received cetuximab (400mg/m² IV over 120 minutes on day 1, followed by weekly doses of 250 mg/m² IV over 60 minutes; following completion of CRT, cetuximab was administered alone weekly for 4 weeks before surgery) in addition to 5-FU.

Rectal Resection

Patients underwent rectal resection according to the principles of TME at a median of 46 days (range: 19 to 100 days) after completion of neoadjuvant CRT. For middle and low rectal cancer (less than 10 centimeters from the anal verge), a TME was performed. For high rectal cancer (greater than 10 centimeters from the anal verge), the mesorectum was transected at a right angle 5 centimeters distal to the distal-most mucosal edge of the rectal cancer. All resections were performed in an open fashion, with the exception of two laparoscopically performed cases.

Pathological Assessment

Detailed pathological analysis was performed by a single pathologist (J.S.) using standardized whole mount sections, in the majority of patients (N=106 [84%]), as previously described.⁴⁰ Pathological analysis was performed on all resection specimens. The rectal cancer was staged according to the AJCC Cancer Staging Manual, 6th edition. Pathological features such as lymphovascular invasion (LVI), perineural invasion (PNI), distal and circumferential resection margin involvement, and extent of cancer response were documented. Areas of cancer response were characterized by the replacement of neoplastic glands with loosely collagenized fibrous tissue and scattered chronic inflammatory cells, as previously reported from our institution.⁴¹ Due to matters of subjectivity related to the use of tumor regression grade systems, pathological response of the rectal cancer was recorded as the percentage of histological response, ranging from no evidence of treatment effect (0%) to a complete response with no viable cancer identified (100%). The pathologist was blinded to the results of the immunohistochemical analysis and nodal status.

Tissue Array Construction

Rectal cancer specimens were formalin-fixed and embedded in paraffin. Five micron-thick sections stained with hematoxylin and eosin stain were obtained to confirm the diagnosis

and to identify viable, representative areas of each specimen. From these defined areas, tissue cores were taken with a precision instrument (Beecher Instruments, Silver Spring, MD, USA), as previously described.^{42, 43} For each specimen, 10 tissue cores with a diameter of 0.6 mm were punched and arrayed on 2 recipient paraffin blocks. Five- μ m sections of these tissue array blocks were cut and used for immunohistochemical analysis.

Immunohistochemical Analysis

Sections from tissue arrays were placed on Fisherband Superfrost Plus Microscope Slides (Thermo Fisher Scientific, Waltham, MA, USA). Slides were deparaffinized, hydrated and processed using the immunoperoxidase method. For antigen retrieval, citric acid (p27, p53, Ki-67, and TS) at pH 6.0 or EDTA (p21) at pH 8.0 at 97 degrees for 30 minutes (steamer) was used. Endogenous peroxidase was quenched with 5 minute incubation in 3% hydrogen peroxide diluted in distilled water. Slides were washed in distilled water for 1 minute and transferred to a bath of phosphate buffered saline (PBS) at pH 7.2 for 2 changes of 5 minutes each. Slides were then placed in 0.05% bovine serum albumin (BSA) diluted in PBS for a minimum of 1 minute. Normal serum was applied at a 1:20 dilution in 2% BSA/PBS. The slides were then incubated overnight in a humidity chamber at 4°C in appropriately-diluted primary antibody.

Mouse anti-human monoclonal antibodies to p21 (p21^{Waf1/cip1}, clone SX118, 1:1000, Dako Cytomation, Glostrup, Denmark), p27 (p27^{Kip1}, clone SX53G8, 1:1000, Dako Cytomation, Glostrup, Denmark), p53 (clone D0-7; 1:500; Dako Cytomation, Glostrup, Denmark), Ki-67 (Mib-1; 1:50; Immunotech, Marseille, France), and TS (clone TS106/4H4B1, 1:400, Zymed, San Francisco, CA, USA) were used. The anti-p53 antibody detected both wild-type and mutated p53. Samples were then incubated with biotinylated anti-mouse secondary antibody at 1:500 dilution (Vector Laboratories, Inc., Burlingame, CA, USA) followed by peroxidase-conjugated streptavidin (1:500, Dako Cytomation, Glostrup, Denmark) in 1% BSA/PBS for 45 to 60 minutes. Diaminobenzidine was used as the chromogen, and Fisherbrand Harris Modified Hematoxylin (Thermo Fisher Scientific, Waltham, MA, USA) was used as the nuclear counterstain.

An estimated percentage of stained cells (0 to 100%), staining intensity (weak, moderate, or strong) and a staining index (SI = percentage of stained cells x staining intensity [1=weak, 2=moderate, 3=strong], maximum score=300) was recorded for each core. Tissues and cell lines known to express the antigens under study were used as positive controls. Arrayed normal tissue served as a control.

Statistical Analysis

Only patients with at least 2 tissue array cores with representative cancer cells were included in the immunohistochemical (IHC) analysis. Patients were stratified into lymph node negative (ypN₀) and lymph node positive (ypN₊) groups. The mean and median SI for each marker among the cores were calculated and grouped based on each specimen's lymph node status. The association between SI and nodal status was assessed using logistic regression models with generalized estimating equations to correct for correlations among multiple cores within patients. The associations between clinical factors such as pretreatment T and N

stages as well as histologic features and nodal status were assessed using Fisher's exact test for categorical variables and Mann-Whitney U test for ordinal variables.

Results

Of the 126 surgical specimens included in this study, 26 (20.6%) demonstrated a pathologic complete response of the primary tumor (ypT₀). Among these 26 specimens, 2 (8%) had residual lymph node involvement (ypN₊). Thus, 100 specimens with residual primary tumor were evaluated by tissue array. Thirty-seven of these 100 specimens could not be included in the IHC analysis because of tissue damage incurred during cutting and staining or because of lack of representative cancer cells in the tissue cores. This included the five patients who were treated with neoadjuvant cetuximab and did not have enough viable tissue for IHC analysis secondary to pathological responses of 80% and greater. Therefore, 63 residual primary tumor specimens were available for IHC analysis. The clinicopathologic characteristics of the whole study group as well as the subgroup of patients eligible for IHC analysis were similar, with the exception of posttreatment stage, due to the exclusion of patients lacking representative cancer cells in the tissue cores (Table 1). Pretreatment T and N stages as assessed by endorectal ultrasound and/or rectal MRI were not associated with nodal positivity in the resected specimen, though histologic features such as lymphovascular and perineural invasion were associated with ypN₊ cancers (Table 2).

The median number of tissue cores with representative cancer cells per patient was 6 (range 2 to 10). Out of the 63 cancers analyzed by IHC, 37 (58.7%) were staged ypN₀ and 26 (41.3%) were staged ypN₊. The mean and median SI for all examined markers was grouped by ypN₀ and ypN₊ cancers. The SI for p27 was higher in ypN₊ specimens (mean 41.8, median 31.0) compared to ypN₀ specimens (mean 25.5, median 17.1, $P=0.04$, Table 3). There were no statistical differences in SI for p21, p53, Ki-67, or TS. An example of varied IHC staining intensity for p27 is demonstrated in Figure 1.

Discussion

Local excision is being explored as a treatment alternative to rectal resection in rectal cancers that demonstrate a pCR or a near-complete pathological response after neoadjuvant CRT.^{1–4, 44} However, the recurrence rate after local excision appears to be higher than the recurrence rate after rectal resection, even among early rectal cancers.^{45–47} The etiology of this higher recurrence rate is likely multifactorial, but may include the presence of unresected lymph node metastases. Among patients with a pCR of the primary tumor after neoadjuvant CRT (ypT₀), regional lymph nodes harbor metastatic disease in 2%–7% of cases.^{5–9} Therefore, while patients who have a posttreatment cancer or scar smaller than 2 centimeters in diameter (33% in the present study) could technically be considered eligible for local excision, there is a small but meaningful risk of residual regional lymph node metastasis. Although the results of pilot studies using novel MRI contrast agents such as USPIO and gadofosveset show promise for improving nodal staging in rectal cancer,^{48, 49} standard imaging cannot accurately predict regional lymph node status.^{10–14} This study was therefore conducted in order to determine if expression of cell cycle-regulatory proteins and

TS in residual rectal cancer following neoadjuvant CRT is associated with regional lymph node metastases.

Our results show that increased expression of p27 in rectal cancer treated by neoadjuvant CRT is associated with higher regional lymph node involvement. As such, positive p27 expression as measured by IHC in residual rectal cancer following neoadjuvant CRT, in conjunction with other clinical prognosticators such as lymphovascular or perineural invasion, could potentially aid in identifying patients who may be suboptimal candidates for local excision because of the presence of residual lymph node disease. If validated, this approach may be widely applicable because IHC is affordable and universally available. The association between p27 expression and lymph node status supports our previous finding that positive p27 expression in posttreatment rectal cancer is associated with decreased 5-year recurrence-free survival.²² Since p27 is a cyclin-dependent kinase (CDK) inhibitor that inhibits cell-cycle progression from G₁ to S phase by binding to cyclin-CDK complexes, we and others have proposed that increased p27 expression may decrease sensitivity to chemoradiation by inducing cells to arrest at the G₁ phase, which enables DNA repair and thus might allow cancerous cells to avoid apoptosis.^{22, 50}

Studies looking at pretreatment (as opposed to posttreatment) rectal cancer biopsies have demonstrated conflicting results on whether p27 expression is associated with response to neoadjuvant therapy.^{18, 27, 37, 51, 52} The discrepancy in results may be due to differences in neoadjuvant therapy regimens, grading of pathological response (Dworak versus Mandard's versus TNM), and quantification of p27 expression (percentage of stained cancer cells as a continuous variable or as a discrete category [ie. 1/2/3/4 versus +/-] and inclusion or exclusion of staining intensity). Since we examined p27 expression in posttreatment tissue where the amount of intervening stroma can vary greatly, we quantitated p27 expression by estimating the percent of tumor cell staining within the tissue core rather than counting positive cells per high power field.

Studies that have investigated whether cell cycle proteins and proliferation markers are associated with treatment response in rectal cancers treated by neoadjuvant therapy conflict in their conclusions on whether pretreatment p21,^{17, 21, 27, 28, 32, 35, 37, 52} p53,^{7, 16–18, 20, 21, 24, 27, 28, 31, 32, 37, 52} Ki-67^{21, 28, 31, 35} and TS,^{25, 28, 31, 32, 35, 52–54} protein and RNA expression are associated with treatment response. In the present study, we found no association between p21, p53, Ki-67, and TS posttreatment expression and ypN positivity. Similar to that noted for p27, the conflicting results between studies may be due to differences in neoadjuvant therapy regimens, grading of pathological response, and quantification of protein expression.

One limitation of evaluating rectal cancer after completion of neoadjuvant CRT was the inability to perform IHC analysis in cancers that achieved a pCR (20.6%) or in cancers that, after the cutting and staining procedures, had an inadequate amount of residual cancer cells (29.4%). Thus, in half of the patients in our study, there was not enough residual cancer tissue to complete IHC analysis. Our study is limited by a small sample size, which did not allow for a subanalysis based on pathological stage (ie. ypT₂ versus ypT₃). Additionally, there were not enough ypT₁ cancers in our study (n=3, none adequate for IHC analysis) for

us to comment on the utility of IHC analysis among patients with ypT₁ cancers. As a consequence of the resultant sample size (63 patients remained eligible for IHC analysis), we were not able to estimate the added value of IHC analysis to ypT staging, a calculation which could have potentially increased the accuracy of predicting regional lymph node involvement. Further, the small sample size precludes the use of dichotomized analysis to assess specific levels of p27 expression in relation to nodal status. As such, the overlap of reported staining index scores amongst the ypN₀ and ypN₊ groups may be attributable to this size limitation. If our results are validated by larger studies, then it is possible that the level of p27 expression in a locally excised rectal cancer following neoadjuvant CRT may help identify patients who have a high likelihood of residual mesorectal lymph node involvement and, therefore, in need of a TME-based rectal resection.

In conclusion, our preliminary study is novel in that it utilizes the residual rectal cancer following neoadjuvant CRT in order to select the extent of subsequent surgical interventions by predicting regional lymph node involvement. Future studies will need to confirm our observation that suggests that IHC analysis for p27 expression could potentially add incremental information to assist in predicting the risk of lymph node metastasis. In the era of personalized medicine, our results are timely and important because, if validated by others, may change patient management by influencing the clinical decision of whether to perform a local excision or a rectal resection.

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References

1. Kim CJ, Yeatman TJ, Coppola D, et al. Local excision of T2 and T3 rectal cancers after downstaging chemoradiation. *Ann Surg*. 2001; 234:352–8. [PubMed: 11524588]
2. Ruo L, Guillem JG, Minsky BD, et al. Preoperative radiation with or without chemotherapy and full-thickness transanal excision for selected T2 and T3 distal rectal cancers. *Int J Colorectal Dis*. 2002; 17:54–8. [PubMed: 12018456]
3. Lezoche E, Guerrieri M, Paganini AM, et al. Long-term results in patients with T2–3 N0 distal rectal cancer undergoing radiotherapy before transanal endoscopic microsurgery. *Br J Surg*. 2005; 92:1546–52. [PubMed: 16252312]
4. Belluco C, De Paoli A, Canzonieri V, et al. Long-Term Outcome of Patients with Complete Pathologic Response after Neoadjuvant Chemoradiation for cT3 Rectal Cancer: Implications for Local Excision Surgical Strategies. *Ann Surg Oncol*. 2011; 18:3686–93. [PubMed: 21691880]
5. Stipa F, Zerneck A, Moore HG, et al. Residual mesorectal lymph node involvement following neoadjuvant combined-modality therapy: rationale for radical resection? *Ann Surg Oncol*. 2004; 11:187–91. [PubMed: 14761922]
6. Pucciarelli S, Capirci C, Emanuele U, et al. Relationship between pathologic T-stage and nodal metastasis after preoperative chemoradiotherapy for locally advanced rectal cancer. *Ann Surg Oncol*. 2005; 12:111–6. [PubMed: 15827790]
7. Guillem JG, Diaz-Gonzalez JA, Minsky BD, et al. cT3N0 rectal cancer: potential overtreatment with preoperative chemoradiotherapy is warranted. *J Clin Oncol*. 2008; 26:368–73. [PubMed: 18202411]
8. Mignanelli ED, de Campos-Lobato LF, Stocchi L, et al. Downstaging after chemoradiotherapy for locally advanced rectal cancer: is there more (tumor) than meets the eye? *Dis Colon Rectum*. 2010; 53:251–256. [PubMed: 20173469]

9. Kundel Y, Brenner R, Purim O, et al. Is local excision after complete pathological response to neoadjuvant chemoradiation for rectal cancer an acceptable treatment option? *Dis Colon Rectum*. 2010; 53:1624–31. [PubMed: 21178856]
10. Chen C-C, Lee R-C, Lin J-K, et al. How accurate is magnetic resonance imaging in restaging rectal cancer in patients receiving preoperative combined chemoradiotherapy? *Dis Colon Rectum*. 2005; 48:722–8. [PubMed: 15747073]
11. Denecke T, Rau B, Hoffmann KT, et al. Comparison of CT, MRI and FDG-PET in response prediction of patients with locally advanced rectal cancer after multimodal preoperative therapy: is there a benefit in using functional imaging? *European radiology*. 2005; 15:1658–66. [PubMed: 15806369]
12. Maretto I, Pomerri F, Pucciarelli S, et al. The potential of restaging in the prediction of pathologic response after preoperative chemoradiotherapy for rectal cancer. *Ann Surg Oncol*. 2007; 14:455–61. [PubMed: 17139456]
13. Huh JW, Park YA, Jung EJ, et al. Accuracy of endorectal ultrasonography and computed tomography for restaging rectal cancer after preoperative chemoradiation. *J Am Coll Surg*. 2008; 207:7–12. [PubMed: 18589355]
14. Al-Sukhni E, Milot L, Fruitman M, et al. Diagnostic accuracy of MRI for assessment of T category, lymph node metastases, and circumferential resection margin involvement in patients with rectal cancer: a systematic review and meta-analysis. *Ann Surg Oncol*. 2012; 19:2212–23. [PubMed: 22271205]
15. Johnston PG, Fisher ER, Rockette HE, et al. The role of thymidylate synthase expression in prognosis and outcome of adjuvant chemotherapy in patients with rectal cancer. *J Clin Oncol*. 1994; 12:2640–7. [PubMed: 7989939]
16. Luna-Perez P, Arriola EL, Cuadra Y, et al. p53 protein overexpression and response to induction chemoradiation therapy in patients with locally advanced rectal adenocarcinoma. *Ann Surg Oncol*. 1998; 5:203–8. [PubMed: 9607618]
17. Qiu H, Sirivongs P, Rothenberger M, et al. Molecular prognostic factors in rectal cancer treated by radiation and surgery. *Dis Colon Rectum*. 2000; 43:451–9. [PubMed: 10789738]
18. Esposito G, Pucciarelli S, Alaggio R, et al. P27kip1 expression is associated with tumor response to preoperative chemoradiotherapy in rectal cancer. *Ann Surg Oncol*. 2001; 8:311–8. [PubMed: 11352304]
19. Sogawa N, Takiguchi N, Koda K, et al. Value of expression of p21WAF1/CIP1 as a prognostic factor in advanced middle and lower rectal cancer patients treated with preoperative radiochemotherapy. *Int J Oncol*. 2002; 21:787–93. [PubMed: 12239617]
20. Kandioler D, Zwrtok R, Ludwig C, et al. TP53 genotype but not p53 immunohistochemical result predicts response to preoperative short-term radiotherapy in rectal cancer. *Ann Surg*. 2002; 235:493–8. [PubMed: 11923604]
21. Rau B, Sturm I, Lage H, et al. Dynamic expression profile of p21WAF1/CIP1 and Ki-67 predicts survival in rectal carcinoma treated with preoperative radiochemotherapy. *J Clin Oncol*. 2003; 21:3391–401. [PubMed: 12885834]
22. Moore HG, Shia J, Klimstra DS, et al. Expression of p27 in residual rectal cancer after preoperative chemoradiation predicts long-term outcome. *Ann Surg Oncol*. 2004; 11:955–61. [PubMed: 15525823]
23. Ghadimi BM, Grade M, Difilippantonio MJ, et al. Effectiveness of gene expression profiling for response prediction of rectal adenocarcinomas to preoperative chemoradiotherapy. *J Clin Oncol*. 2005; 23:1826–38. [PubMed: 15774776]
24. Lopez-Crapez E, Bibeau F, Thezenas S, et al. p53 status and response to radiotherapy in rectal cancer: a prospective multilevel analysis. *Br J Cancer*. 2005; 92:2114–21. [PubMed: 15956964]
25. Jakob C, Liersch T, Meyer W, et al. Immunohistochemical analysis of thymidylate synthase, thymidine phosphorylase, and dihydropyrimidine dehydrogenase in rectal cancer (cUICC II/III): correlation with histopathologic tumor regression after 5-fluorouracil-based long-term neoadjuvant chemoradiotherapy. *Am J Surg Pathol*. 2005; 29:1304–9. [PubMed: 16160472]

26. Liersch T, Langer C, Ghadimi BM, et al. Lymph node status and TS gene expression are prognostic markers in stage II/III rectal cancer after neoadjuvant fluorouracil-based chemoradiotherapy. *J Clin Oncol*. 2006; 24:4062–8. [PubMed: 16943523]
27. Lin LC, Lee HH, Hwang WS, et al. p53 and p27 as predictors of clinical outcome for rectal-cancer patients receiving neoadjuvant therapy. *Surg Oncol*. 2006; 15:211–6. [PubMed: 17360176]
28. Bertolini F, Bengala C, Losi L, et al. Prognostic and predictive value of baseline and posttreatment molecular marker expression in locally advanced rectal cancer treated with neoadjuvant chemoradiotherapy. *Int J Radiat Oncol Biol Phys*. 2007; 68:1455–61. [PubMed: 17445998]
29. Kim IJ, Lim SB, Kang HC, et al. Microarray gene expression profiling for predicting complete response to preoperative chemoradiotherapy in patients with advanced rectal cancer. *Dis Colon Rectum*. 2007; 50:1342–53. [PubMed: 17665260]
30. Spindler KL, Nielsen JN, Lindebjerg J, et al. Germline polymorphisms may act as predictors of response to preoperative chemoradiation in locally advanced T3 rectal tumors. *Dis Colon Rectum*. 2007; 50:1363–9. [PubMed: 17661145]
31. Jakob C, Liersch T, Meyer W, et al. Predictive value of Ki67 and p53 in locally advanced rectal cancer: correlation with thymidylate synthase and histopathological tumor regression after neoadjuvant 5-FU-based chemoradiotherapy. *World J Gastroenterol*. 2008; 14:1060–6. [PubMed: 18286688]
32. Negri FV, Campanini N, Camisa R, et al. Biological predictive factors in rectal cancer treated with preoperative radiotherapy or radiochemotherapy. *Br J Cancer*. 2008; 98:143–7. [PubMed: 18087284]
33. Kuremsky JG, Tepper JE, McLeod HL. Biomarkers for response to neoadjuvant chemoradiation for rectal cancer. *Int J Radiat Oncol Biol Phys*. 2009; 74:673–88. [PubMed: 19480968]
34. Garcia-Aguilar J, Chen Z, Smith DD, et al. Identification of a biomarker profile associated with resistance to neoadjuvant chemoradiation therapy in rectal cancer. *Ann Surg*. 2011; 254:486–92. [PubMed: 21865946]
35. Kim K, Chie EK, Wu HG, et al. High survivin expression as a predictor of poor response to preoperative chemoradiotherapy in locally advanced rectal cancer. *Int J Colorectal Dis*. 2011; 26:1019–23. [PubMed: 21424389]
36. Tan BR, Thomas F, Myerson RJ, et al. Thymidylate Synthase Genotype-Directed Neoadjuvant Chemoradiation for Patients With Rectal Adenocarcinoma. *J Clin Oncol*. 2011; 29:875–83. [PubMed: 21205745]
37. Edden Y, Wexner SD, Berho M. The use of molecular markers as a method to predict the response to neoadjuvant therapy for advanced stage rectal adenocarcinoma. *Colorectal Dis*. 2012; 14:555–61. [PubMed: 21689364]
38. Grade M, Wolff HA, Gaedcke J, et al. The molecular basis of chemoradiosensitivity in rectal cancer: implications for personalized therapies. *Langenbecks Arch Surg*. 2012; 397:543–55. [PubMed: 22382702]
39. Grann A, Feng C, Wong D, et al. Preoperative combined modality therapy for clinically resectable uT3 rectal adenocarcinoma. *Int J Radiat Oncol Biol Phys*. 2001; 49:987–95. [PubMed: 11240239]
40. Guillem JG, Chessin DB, Shia J, et al. A prospective pathologic analysis using whole-mount sections of rectal cancer following preoperative combined modality therapy: implications for sphincter preservation. *Ann Surg*. 2007; 245:88–93. [PubMed: 17197970]
41. Ruo L, Tickoo S, Klimstra DS, et al. Long-term prognostic significance of extent of rectal cancer response to preoperative radiation and chemotherapy. *Ann Surg*. 2002; 236:75–81. [PubMed: 12131088]
42. Kononen J, Bubendorf L, Kallioniemi A, et al. Tissue microarrays for high-throughput molecular profiling of tumor specimens. *Nat Med*. 1998; 4:844–7. [PubMed: 9662379]
43. Hoos A, Urist MJ, Stojadinovic A, et al. Validation of tissue microarrays for immunohistochemical profiling of cancer specimens using the example of human fibroblastic tumors. *Am J Pathol*. 2001; 158:1245–51. [PubMed: 11290542]
44. Garcia-Aguilar J, Shi Q, Thomas CR Jr., et al. A phase II trial of neoadjuvant chemoradiation and local excision for T2N0 rectal cancer: preliminary results of the ACOSOG Z6041 trial. *Ann Surg Oncol*. 2012; 19:384–91. [PubMed: 21755378]

45. Paty PB, Nash GM, Baron P, et al. Long-term results of local excision for rectal cancer. *Ann Surg.* 2002; 236:522–29. [PubMed: 12368681]
46. Bentrem DJ, Okabe S, Wong WD, et al. T1 adenocarcinoma of the rectum: transanal excision or radical surgery? *Ann Surg.* 2005; 242:472–7. [PubMed: 16192807]
47. Nash GM, Weiser MR, Guillem JG, et al. Long-term survival after transanal excision of T1 rectal cancer. *Dis Colon Rectum.* 2009; 52:577–82. [PubMed: 19404055]
48. Lahaye MJ, Engelen SM, Kessels AG, et al. USPIO-enhanced MR imaging for nodal staging in patients with primary rectal cancer: predictive criteria. *Radiology.* 2008; 246:804–11. [PubMed: 18195379]
49. Lambregts DM, Beets GL, Maas M, et al. Accuracy of gadofosveset-enhanced MRI for nodal staging and restaging in rectal cancer. *Ann Surg.* 2011; 253:539–45. [PubMed: 21239980]
50. St Croix B, Florenes VA, Rak JW, et al. Impact of the cyclin-dependent kinase inhibitor p27Kip1 on resistance of tumor cells to anticancer agents. *Nat Med.* 1996; 2:1204–10. [PubMed: 8898746]
51. Gunther K, Dimmler A, Rodel F, et al. P27 does not predict histopathological response to radiochemotherapy in rectal cancer. *J Surg Res.* 2003; 113:179–88. [PubMed: 12957127]
52. Shinto E, Hashiguchi Y, Ueno H, et al. Pretreatment CD133 and cyclooxygenase-2 expression as the predictive markers of the pathological effect of chemoradiotherapy in rectal cancer patients. *Dis Colon Rectum.* 2011; 54:1098–106. [PubMed: 21825889]
53. Conradi LC, Bleckmann A, Schirmer M, et al. Thymidylate synthase as a prognostic biomarker for locally advanced rectal cancer after multimodal treatment. *Ann Surg Oncol.* 2011; 18:2442–52. [PubMed: 21347782]
54. Unger KR, Romney DA, Koc M, et al. Preoperative chemoradiation for rectal cancer using capecitabine and celecoxib correlated with posttreatment assessment of thymidylate synthase and thymidine phosphorylase expression. *Int J Radiat Oncol Biol Phys.* 2011; 80:1377–82. [PubMed: 20656421]

Figure 1: Examples of colorectal adenocarcinoma with varied immunohistochemical staining intensity for p27

(A: 3+ intensity; B: 2+ intensity; C: 1+ intensity; D: negative)

Table 1:

Clinicopathologic Characteristics

Characteristic	Whole Study Group	Patients Eligible for IHC Analysis
Patients, n	126	63
Gender, n (%)		
Male	81 (64)	39 (62)
Female	45 (36)	24 (38)
Age, year		
Median (range)	60 (27–80)	58 (27–80)
Tumor Location, n (%)		
Anterior	45 (36)	17 (27)
Posterior	39 (31)	22 (35)
Lateral	18 (14)	8 (13)
Circumferential	24 (19)	16 (25)
Distance from Anal Verge, n (%)		
0–6cm	59 (47)	34 (54)
7–12cm	67 (53)	29 (46)
Circumferential Involvement, Pretreatment, n (%)		
0–33%	30 (24)	13 (21)
34–66%	66 (52)	31 (49)
67–100%	30 (24)	19 (30)
M Stage, n (%)		
0	108 (86)	57 (91)
1	18 (14)	6 (9)
Type of Surgery, n (%)		
Low Anterior Resection	100 (79)	48 (76)
Abdominoperineal Resection	25 (20)	14 (22)
Hartmann Procedure	1 (1)	1 (2)
Residual Tumor/Scar, Posttreatment, n (%)		
2 cm	41 (33)	16 (25)
> 2 cm	85 (67)	47 (75)
ypT Stage, n (%)		
0	26 (21)	0 (0)
in situ	1 (1)	0 (0)
1	3 (2)	0 (0)
2	38 (30)	21 (33)
3	54 (43)	39 (62)
4	4 (3)	3 (5)
ypN Stage, n (%)		
0	89 (71)	37 (59)

Characteristic	Whole Study Group	Patients Eligible for IHC Analysis
1	29 (23)	20 (32)
2	8 (6)	6 (9)
Pathologic Response, n (%)		
0–33%	9 (7)	9 (14)
34–66%	25 (20)	21 (33)
67–99%	66 (52)	33 (52)
100%	26 (21)	N/A

IHC = immunohistochemical.

Table 2:

Clinical factors, Stratified by Lymph Node Status

Clinical factors	ypN0 n = 37	ypN+ n = 26	<i>P</i> value ^a
Pretreatment cT stage ^b , n (%)			0.28
0	0	0	
1	0	0	
2	2 (5)	2 (8)	
3	34 (92)	20 (77)	
4	1 (3)	4 (15)	
Pretreatment cN stage ^b , n (%)			0.32
0	12 (32)	6 (23)	
1	25 (68)	19 (73)	
2	0	1 (4)	
+LVI, n (%)	2 (5)	10 (38)	0.002
+PNI, n (%)	5 (14)	13 (50)	0.004

AV = anal verge. LVI = lymphovascular invasion. PNI = perineural invasion.

^aThe association between clinical factors and nodal status was assessed using Fisher's exact test for categorical variables and Mann-Whitney U test for ordinal variables.

^bClinical T and N stages were assessed by endorectal ultrasound or magnetic resonance imaging of the rectum.

Table 3:

Immunohistochemical Analysis Using Mean Staining Index per Patient, Stratified by Lymph Node Status

Marker	ypN0 n = 37		ypN+ n = 26		P value ^a
	Mean (SD)	Median (range)	Mean (SD)	Median (range)	
p21	2.3 (4.5)	0 (0–22.5)	1.3 (2.8)	0 (0–13.3)	0.45
p27	25.5 (22.8)	17.1 (0–91.3)	41.8 (35.1)	31.0 (0–113.3)	0.04
p53	79.7 (85.4)	53.3 (0–287.1)	121.5 (89.9)	144.5 (0–258.0)	0.16
Ki-67	51.6 (71.8)	10.0 (0–237.5)	29.1 (47.0)	3.9 (0–148.6)	0.22
TS	8.5 (23.3)	0 (0–136.3)	2.1 (5.1)	0 (0–22.0)	0.12

SD = standard deviation; TS = thymidylate synthase.

^aThe association of staining index (SI = percentage of stained cells x staining intensity [1=weak, 2=moderate, 3=strong]) with nodal status was assessed using a logistic regression model with generalized estimating equations to correct for correlations within patients.