Epidermal Growth Factor Receptor Gene Polymorphisms Predict Pelvic Recurrence in Patients with Rectal Cancer Treated with Chemoradiation

Wu Zhang,1 David J. Park,1 Bo Lu,3 Dong Yun Yang,2 Michael Gordon,1 Susan Groshen,2 Jim Yun,1 Oliver A. Press,1 Daniel Vallböhmer,1 Katrin Rhodes,1 and Heinz-Josef Lenz1,2
1Division of Medical Oncology and 2Department of Preventive Medicine, University of Southern California/Norris Comprehensive Cancer Center, Keck School of Medicine, Los Angeles, California; and 3Department of Radiation Oncology, Vanderbilt University Medical Center, Nashville, Tennessee

ABSTRACT

An association between epidermal growth factor receptor (EGFR) signaling pathway and response of cancer cells to ionizing radiation has been reported. Recently, a polymorphic variant in the EGFR gene that leads to an arginine-to-lysine substitution in the extracellular domain at codon 497 within subdomain IV of EGFR has been identified. The variant EGFR (HER-1 497K) may lead to attenuation in ligand binding, growth stimulation, tyrosine kinase activation, and induction of proto-oncogenes myc, fos, and jun. A (CA)n repeat polymorphism in intron 1 of the EGFR gene that alters EGFR expression in vitro and in vivo has also been described. In the current pilot study, we assessed both polymorphisms in 59 patients with locally advanced rectal cancer treated with adjuvant or neoadjuvant chemoradiation therapy using PCR-RFLP and a 5′-end [γ-32P]ATP-labeled PCR protocol. We tested whether either polymorphism alone or in combination can be associated with local recurrence in the setting of chemoradiation treatment. We found that patients with HER-1 497 Arg/Arg genotype or lower number of CA repeats (both alleles <20) tended to have a higher risk of local recurrence (P = 0.24 and 0.31, respectively). Combined analysis showed the highest risk for local recurrence was seen in patients who possessed both a HER-1 497 Arg allele and <20 CA repeats (P = 0.05, log-rank test). Our data suggest that the HER-1 R497K and EGFR intron 1 (CA)n repeat polymorphisms may be potential indicators of radiosensitivity in patients with rectal cancer treated with chemoradiation.

INTRODUCTION

Colorectal cancer is the third most prevalent cancer in the United States. In the year 2004, an estimate of 147,000 new cases will be diagnosed and 56,730 people will die from this disease. Over 40,000 new cases of rectal cancer are predicted in the year 2004 (1). Local recurrence after curative resection continues to be a significant problem in the management of rectal cancer even with neoadjuvant or adjuvant chemoradiation therapy. In addition to the traditional anatomic stage for prognosis and selection of treatment, the identification of molecular markers for prediction of local recurrence would be helpful in defining those who may benefit from pre- or postoperative chemoradiation therapy.

The epidermal growth factor receptor (EGFR), also known as HER-1 or erbB-1, is a member of the human epithelial receptor tyrosine kinase family. It is a transmembrane protein with intrinsic tyrosine kinase activity of which activation leads to downstream effects in gene expression, cellular proliferation, inhibition of apoptosis, and angiogenesis (2). Its signaling system is often dysregulated in cancer, and its overexpression has been noted in a variety of epithelial tumors, including colorectal cancer (3). High EGFR expression has been associated with tumor aggressiveness and poor clinical outcome and has been linked with radioresistance in a number of neoplasms (4–7). Moreover, several studies have shown that blocking EGFR ligand binding via a monoclonal antibody (e.g., C-225) can enhance tumor radiosensitivity (8, 9).

A polymorphic variant EGFR arising from a single nucleotide change (G→A) leading to an arginine (Arg)→ lysine (Lys) substitution in codon 497 (HER-1 R497K) in the extracellular domain within subdomain IV of the EGFR gene has been identified. An in vitro study has shown that the variant HER-1 497K has attenuated functions in ligand binding, growth stimulation, tyrosine kinase activation, and induction of proto-oncogenes myc, fos, and jun compared with the “wild-type” HER-1 497R (10).

Also, there is evidence that a highly polymorphic region in intron 1 of the EGFR gene is associated with transcription levels of EGFR in vitro and in vivo (11, 12). The length of this (CA)n dinucleotide polymorphism correlates inversely with the transcriptional activity of the gene. In vitro studies have shown that the transcriptional activity in cell lines containing a prolonged polymorphic region (>20 CA repeats) was markedly reduced compared with cells containing a shorter allele (16 repeats; ref. 11). These findings were confirmed in human breast cancer samples. A constant decline of intratumoral EGFR protein expression was also observed to be associated with increase in allele length. Furthermore, hemizygote tumors showed higher EGFR expression if the longer allele was lacking compared with tumors with the longer allele remaining (12).

Based on these findings, we tested the hypothesis whether the above-mentioned polymorphisms of the EGFR...
gene, alone or in combination, could be associated with increased likelihood of recurrence in patients with rectal cancer treated with adjuvant or neoadjuvant chemoradiation.

PATIENTS AND METHODS

Eligible Subjects

Fifty-nine patients with locally advanced rectal cancer who were treated with neoadjuvant or adjuvant chemoradiation therapy at the University of Southern California/Norris Comprehensive Cancer Center (USC/NCCC) or the Los Angeles County/University of Southern California Medical Center (LAC/USCMC), between 1991 and 2000, were eligible for the present study. This study was done at the USC/NCCC and approved by the Institutional Review Board of the University of Southern California for Medical Sciences. Out of 59 patients, 43 patients were treated with adjuvant and 16 patients with neoadjuvant 5-fluorouracil chemotherapy in combination with pelvic radiation (50.4–54 Gy). During radiation, patients received 5-fluorouracil as either a 4-day infusion (1000 mg/m²) at the beginning and end of radiation treatment or as a daily continuous infusion (200 mg/m²). A tumor was considered to be a rectal cancer if a portion of the tumor was located below the peritoneal reflection or if the lower margin of the tumor was within 12 cm of the anal verge on endoscopy. Patient data were collected retrospectively through chart review and informed consent was signed by all patients involved in the study.

In addition, a secondary analysis was done for 31 patients with rectal cancer who had received either adjuvant (24/31) or neoadjuvant (7/31) chemoradiation at outside facilities. These patients were referred to USC/NCCC or LAC/USCMC either after their recurrence (28/31) or for routine follow-up (3/31). This cohort had a very high rate (85%) of 5-year local recurrence compared with historical controls.

HER-1 R497K Polymorphism

A tissue sample was collected from each patient and genomic DNA was extracted from paraffin-embedded tissue using the QiaAmp kit (Qiagen, Valencia, CA). HER-1 R497K polymorphism was done by PCR-RFLP method as previously described (13). Briefly, forward primer 5′-TGCTGTGACCACAATCTGTCT-3′ and reverse primer 5′-CCAGAAGGTTCACCTTGCCC-3′ were used for PCR amplification. After initial denaturation at 95°C for 3 minutes, the reaction was carried out at 94°C denaturation for 1 minute, 59°C annealing for 1 minute, and 72°C extension for 1 minute for 35 cycles. PCR product was digested by BstN1 restriction enzyme (New England Biolabs, Beverly, MA) at 60°C overnight and alleles were separated on 4% Nusieve ethidium bromide-stained agarose gel.

EGFR Intron 1 (CA)n Repeat Polymorphism

The (CA)n repeat polymorphism in intron 1 of the EGFR gene was determined as previously described with the following modifications (14). Briefly, 100 ng genomic DNA were used in a final PCR volume of 15 μl together with 200 μmol/L deoxynucleotide triphosphate, 1.0 μmol/L 5′-end [γ-32P]ATP-labeled reverse primer, 1.0 μmol/L unlabeled forward primer, 0.75 U Taq polymerase (Perkin-Elmer, Boston, MA), and PCR buffer (10 mmol/L Tris-HCl pH 8.3, 50 mmol/L KCl, 1.5 mmol/L MgCl2). The reaction was carried out for 28 cycles with 94°C (1 minute) denaturation, 55°C (1 minute) annealing, and 72°C (2 minutes) extension. The reaction products were separated on a 6% denaturing polyacrylamide DNA sequencing gel, vacuum blotted for 1 hour at 80°C, and exposed to an XAR film (Eastman-Kodak Co., Rochester, NY) overnight. The exact number of the CA repeats was confirmed by direct sequencing.

Statistical Analysis

The primary outcome of interest in this study was time to local recurrence. Time to local recurrence was defined as the time from the date of completion of chemoradiation to the date of first pelvic recurrence, or until last contact if the patient was free of any pelvic recurrence. If a patient developed distant metastasis, but did not have any local recurrence, the time to local recurrence was censored at the date of first distant metastasis.

The associations of time to local recurrence with patient’s clinico-pathologic characteristics (age, sex, race, tumor grade, T-stage, N-stage, and type of surgery) were assessed using univariate survival analyses (log-rank test).

Initially, associations of HER-1 R497K and EGFR intron 1 (CA)n repeat polymorphisms with demographic, clinico-pathologic characteristics, and outcome were analyzed separately. The number (range: 16-23) of EGFR intron 1 (CA)n repeat in each allele was categorized at the sample median. To be consistent, the higher sample median in two alleles was used for both alleles. There were few patients with both alleles that had 20 repeats or more, so they were grouped with those who had one allele that had 20 or more repeats together. The Fisher’s exact test was used to evaluate the relationship between patient demographic and clinico-pathologic characteristics and EGFR genotypes. Univariate survival analyses (Kaplan-Meier plots and log-rank tests) were used to evaluate the relationship between EGFR polymorphisms and time to local recurrence. The probability of recurrence at 5 years with its Greenwood standard error and the Pike estimate of relative risk with 95% confidence intervals (CI) based on the log-rank test were used to provide quantitative summaries of the relationship.

For the combination analysis of HER-1 R497K and intron 1 (CA)n repeat polymorphisms in relationship of time to local recurrence, the log-rank test was used. Association between HER-1 R497K and EGFR intron 1 (CA)n repeat polymorphisms was assessed with Fisher’s exact test.

Within our primary study cohort, tissue DNA samples were not adequate for HER-1 R497K polymorphism analysis in 13 of 59 patients. To compare the data between patients with HER-1 R497K polymorphism (n = 46) and those without it (n = 13), the log-rank test was used to examine whether completeness of HER-1 R497K polymorphism was associated with time to local recurrence. There was no statistically significant difference in the time to local recurrence between those two groups of patients (P = 0.90). The EGFR intron-1 CA repeat polymorphism assay was successful in all 59 patients.

Differences in 5-year local recurrence rates, as well as preponderance of “favorable polymorphisms” (i.e., the HER-1 497 Lys/Lys genotype between patients in the main cohort and Lys/Thr and Thr/Thr genotypes).
those subsequently referred to our center), were assessed using the Fisher’s exact test.

All reported P values were two sided. All analyses were done using the SAS statistical package version 8.2 and Epilog Plus Version 1.0 (15).

RESULTS

Our cohort consisted of 24 women (41%) and 35 men (59%) with a median age of 54 years (range: 25-79 years). There were 36 Caucasian (61%), 13 Hispanic (22%), 7 Asian (12%), and 3 African-American (5%) study participants. The median follow-up was 57.9 months (range: 2.8-143.8). Of the 59 patients treated at the USC/NCC and LAC/USC, 12 patients experienced local recurrence, 8 developed distant metastases, and 16 died. No significant associations between demographic and clinicopathologic parameters and time to local recurrence were observed, although a trend could be seen for ethnicity (Caucasian versus others) and treatment modality (adjuvant versus neoadjuvant; Table 1).

EGFR polymorphisms were not associated with demographic (gender and ethnicity), clinical (surgery and chemotherapy), or pathologic characteristics (tumor stage, grade, or lymph node status; data not shown).

HER-1 R497K Polymorphism

Fifty-nine patients were evaluated for HER-1 R497K polymorphism and the assay was successful in 46 patients. Forty-three percent (20/46) of the patients were homozygous for the Arg/Arg variant. Thirty-nine percent (18/46) were heterozygous Arg/Lys, and 17% (8/46) were homozygous for the Lys/Lys genotype. The distribution of the HER-1 R497K genotypes was not statistically significantly different across gender and ethnic categories.

Patients with the Arg/Arg genotype showed the highest probability of 5-year local recurrence. Comparing the patients with the Arg/Arg genotype, those with the Arg/Lys genotype had a decreased risk of local recurrence (RR = 0.66, 95% CI: 0.18-2.35, Table 2). None of the patients with the Lys/Lys genotype experienced local recurrence throughout the follow-up period of the study (P = 0.24, log-rank test).

EGFR Intron 1 (CA)n Repeat Polymorphism

Alleles corresponding to each of the previously reported (CA)n repeat lengths were observed (n = 16-21). In addition, a novel (CA)n repeat length of n = 22 was determined in three patients and another rare (CA)n repeat length n = 23 was found in one patient. In both scenarios, (CA)n allele or (CA)n allele was found to be in combination with a (CA)16 allele. Heterozygosity in this series was 81% (48/59). Seventeen different (CA)n repeat EGFR genotypes could be determined with frequencies ranging between 1% and 24%. The most common genotypes in this study were 16/20 (24%, 14/59), 16/18 (10%, 6/59), and 16/16 (10%, 6/59). The most frequently observed dinucleotide repeat alleles were (CA)16 36% (42/59 \times 2), (CA)18 14% (16/59 \times 2), and (CA)20 30% (35/59 \times 2). In 62% (30/48) of the heterozygous genotypes the shorter allele consisted of 16 CA repeats.

To evaluate the effect of the number of (CA)n repeats on time to local recurrence among all study participants, we separated patients into two subgroups: 23 patients (39%) possessed both (CA)n repeats <20 and 36 patients (61%) had any (CA)n repeats ≥20. Patients with both (CA)n repeats <20 showed a higher risk of local recurrence compared with those with any CA repeats ≥20 (P = 0.31, log-rank test; Table 2). If we use patients with both (CA)n repeats <20 group as reference, patients with any (CA)n repeats ≥20 group showed a trend for decreased relative risk of local recurrence (RR = 0.56, 95% CI: 0.17-1.79).

The distribution of patients with any (CA)n repeats ≥20 and both (CA)n <20 was not statistically significantly different

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Time to local recurrence in rectal cancer based on demographic and clinical parameters</th>
</tr>
</thead>
<tbody>
<tr>
<td>Parameter</td>
<td>n</td>
</tr>
<tr>
<td>Total patients</td>
<td>59</td>
</tr>
<tr>
<td>Age</td>
<td></td>
</tr>
<tr>
<td>&lt;50 y</td>
<td>19</td>
</tr>
<tr>
<td>≥50 y</td>
<td>40</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>35</td>
</tr>
<tr>
<td>Female</td>
<td>24</td>
</tr>
<tr>
<td>Race</td>
<td></td>
</tr>
<tr>
<td>Caucasian</td>
<td>36</td>
</tr>
<tr>
<td>Other</td>
<td>23</td>
</tr>
<tr>
<td>Therapy</td>
<td></td>
</tr>
<tr>
<td>Adjuvant</td>
<td>43</td>
</tr>
<tr>
<td>Neoadjuvant</td>
<td>16</td>
</tr>
<tr>
<td>Surgery type</td>
<td></td>
</tr>
<tr>
<td>APR</td>
<td>15</td>
</tr>
<tr>
<td>LAR</td>
<td>36</td>
</tr>
<tr>
<td>TR</td>
<td>8</td>
</tr>
</tbody>
</table>

*Greenwood SE.

†If the relative risk is <1, then the relative risk can be thought of as the average decreased risk of recurrence at any point in time compared with the reference group.

‡Based on log-rank test statistics.

§APR, abdominal perineal resection; LAR, lower anterior resection; TR, transanal resection.
across demographic (gender and ethnicity), clinical (surgery, chemotherapy), or pathologic characteristics (tumor stage, grade, or lymph node status; data not shown).

Combination Analysis

When we considered these two polymorphisms together, there was a statistically significant relationship between the two polymorphisms and time to local recurrence (P = 0.05, log-rank test; Fig. 1). Patients with the HER-1 497 Lys/Lys variant had the lowest risk of local recurrence, regardless of CA repeats (Table 2). Compared to patients with a HER-1 497 Arg allele and both (CA)<20, those with a HER-1 497 Arg allele and any repeat ≥20 were at lower risk of local recurrence (RR = 0.36, 95% CI: 0.10-1.33).

Linkage Disequilibrium

No statistically significant evidence for linkage disequilibrium between the HER-1 R497K polymorphism and EGFR (CA)<n repeat polymorphisms was found in our cohort of patients (data not shown).

Secondary Analysis for Patients Treated at Outside Facilities

There were 31 patients treated at outside facilities of which demographic characteristics (age, sex, and race), treatment modality (neoadjuvant and adjuvant therapy), type of surgery, and clinico-pathologic variables (tumor stage, lymph node status, and histologic grade) did not differ significantly from patients treated at our center (P > 0.05, Fisher’s exact test, data not shown). However, a much higher proportion of these patients (85%, 22/26) had local recurrence compared with patients treated at our center (40%, 12/30) within 5 years of completion of chemoradiotherapy (P < 0.001, Fisher’s exact test). The assays for the HER-1 R497K and for the EGFR intron 1 (CA)<n repeat polymorphism were successful in 24 and 29 patients, respectively. In combination analysis, only 1 of 24 (4%) patients treated at outside facilities carried the most “favorable” genotype

Table 2  Analysis of recurrence of patients with rectal cancer: association with EGFR polymorphisms (univariate and combined analysis)

<table>
<thead>
<tr>
<th>Factor</th>
<th>n</th>
<th>Probability ± SE* of 5-y local recurrence</th>
<th>Relative risk†</th>
<th>P ‡</th>
</tr>
</thead>
<tbody>
<tr>
<td>HER-1 R497K</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lys/Lys</td>
<td>8</td>
<td>0.05</td>
<td>0.05</td>
<td></td>
</tr>
<tr>
<td>Arg/Lys</td>
<td>18</td>
<td>0.29 ± 0.13</td>
<td>0.66 (0.18, 2.35)</td>
<td>0.24</td>
</tr>
<tr>
<td>Arg/Arg</td>
<td>20</td>
<td>0.38 ± 0.14</td>
<td>1 (Reference)</td>
<td>0.31</td>
</tr>
<tr>
<td>EGFR (CA)&lt;n repeat</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Both (CA)&lt;n repeats &lt;20</td>
<td>23</td>
<td>0.26 ± 0.10</td>
<td>1 (Reference)</td>
<td>0.05</td>
</tr>
<tr>
<td>Any (CA)&lt;n repeats ≥20</td>
<td>36</td>
<td>0.25 ± 0.08</td>
<td>0.56 (0.17, 1.79)</td>
<td></td>
</tr>
<tr>
<td>Combined analysis</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lys/Lys and any (CA)&lt;n repeats</td>
<td>8</td>
<td>0.05</td>
<td>0.05</td>
<td></td>
</tr>
<tr>
<td>Arg/Arg or Arg/Lys and any (CA)&lt;n repeats ≥20</td>
<td>23</td>
<td>0.27 ± 0.11</td>
<td>0.36 (0.10, 1.33)</td>
<td></td>
</tr>
<tr>
<td>Arg/Arg or Arg/Lys and both (CA)&lt;n repeats &lt;20</td>
<td>15</td>
<td>0.37 ± 0.13</td>
<td>1 (Reference)</td>
<td></td>
</tr>
</tbody>
</table>

*Greenwood SE.
†If the relative risk is <1, then the relative risk can be thought of as the average decreased risk of recurrence at any point in time compared with the reference group.
‡Based on log-rank test statistics.
§None of patients with Lys/Lys developed local recurrence.

Fig. 1  Recurrence-free survival of patients with rectal cancer by EGFR polymorphisms. Vertical hash marks, time of last follow-up for those patients who were still recurrence-free at the time of the analysis of data. All censored patients and those who were locally recurrent are accounted for.
(HER-1 R497K Lys/Lys) compared with 8 of 46 (17%) patients treated at our center ($P = 0.15$, Fisher’s exact test).

**DISCUSSION**

In this study we show that two novel polymorphisms, HER-1 R497K and (CA)$_{n}$ repeat in intron 1 of the EGFR gene, are associated with local recurrence in patients with rectal cancer treated with adjuvant or neoadjuvant chemoradiation.

Both EGFR gene polymorphisms showed trend associations with local recurrence in the univariate analysis. For the HER-1 R497K polymorphism, patients with the Arg/Lys or Arg/Arg genotypes had an increased risk of local recurrence compared with those with Lys/Lys genotype. In fact, no patients in our cohort with the Lys/Lys genotype experienced local recurrence during the follow-up period. In the intron 1 of the EGFR gene polymorphism analysis, patients with shorter (CA)$_{n}$ repeat (CA repeat $<20$) also showed a trend for a shorter time to local recurrence compared with those with longer (CA)$_{n}$ repeat (CA repeat $\geq 20$). In the combined analysis, patients with HER-1 497 Arg/Arg or Arg/Lys genotypes combined with CA repeats $<20$ had the highest risk of local recurrence whereas those with Arg/Arg or Arg/Lys genotypes but CA repeats $\geq 20$ had an intermediate risk of local recurrence. No patients with the HER-1 497 Lys/Lys genotype, regardless of CA repeats, developed a local recurrence.

Patients treated at outside facilities showed a much higher rate for local recurrence probably due to referral bias. In comparison with our main cohort of which 5-year local recurrence rates were within the range of historical controls, the “favorable” HER-1 497 Lys/Lys genotype was less prevalent. No significant differences in demographic and clinico-pathologic characteristics were observed. This finding is in agreement with our hypothesis.

Several studies have reported an association between high EGFR expression and resistance to ionizing radiation. For instance, in a study including mammary, ovarian, squamous cell, hepatocellular, and adenocarcinoma carcinoma xenografts, a significant relationship between EGFR expression and radioresistance was observed (16, 17). This observation has been confirmed in clinical studies in various neoplasms, including rectal cancer (18–20).

Although the exact mechanism by which EGFR activation leads to radioresistance is unclear, it is believed to be due to downstream activation of its signaling pathway. Ionizing radiation has shown to activate EGFR and lead to increased levels of phosphorylation of downstream substrates involved in cell survival such as Raf-1, mitogen-activated protein kinase, phosphatidylinositol 3’ kinase, and Akt pathways (17).

The mechanism through which the variant human EGFR (HER-1 497K) may account for lower local failures after chemoradiation is unknown. A study with Chinese hamster ovary cells, the variant HER-1 497K cell line, showed an attenuated growth response to EGF and transforming growth factor-α, and a reduced induction of the protooncogenes fos, jun, and myc. The authors suggested that the amino acid substitution in the extracellular domain may modulate ligand binding and transmembrane signaling to the intracellular domain. Thus, variant EGFR receptor may be less efficient in the recruitment of intracellular substrates and/or cause downstream activation of alternative signaling pathways with decreased proto-oncogene induction or growth stimulation, affecting radioresensitivity (10).

Recent reports have shown a proportional inverse association between the number of CA repeats at the 5’- regulatory sequence of intron 1 and EGFR expression. Higher EGFR expression in the breast cancer cell lines, later confirmed in breast tumor tissues, was associated with a shorter length of the CA repeat polymorphism. In addition, tumors with loss of heterozygosity in intron 1 of EGFR were shown to have a higher EGFR expression when the longer CA repeat allele was lost compared with the loss of the shorter allele. Regulatory sequences of the EGFR gene have been shown to be located within the 5’-flanking region and intron 1, and the CA repeat sequence of intron 1 is located close to one of the enhancer sequences of this gene. The exact mechanism through which the polymorphic CA repeats of intron 1 modulate EGFR transcription is not fully known. However, it has been posited that the bendability of the CA repeat sequence may lead to secondary DNA structures that may affect binding of neighboring enhancer sequences by regulatory factors (11, 12). To date, there are no reports linking the intron 1 CA repeat polymorphism of EGFR and the clinical outcome to chemoradiation.

To conclude, we have assessed the association between local recurrence and two novel polymorphisms of the EGFR gene, namely HER-1 R497K and the dinucleotide CA repeat in intron 1. Our results show that these polymorphisms may be associated with pelvic recurrence in rectal cancer patients treated with chemoradiation, especially when used in combination. This is probably through its effect in EGFR ligand binding and/or EGFR gene expression, although the mechanism is not clear at the present time. To our knowledge, this is the first study that shows a relationship between EGFR gene polymorphisms and local recurrence in rectal cancer patients treated with chemoradiation therapy.

Recent studies have shed light to the importance of intratumoral genetic mutations within functional domains of the EGFR gene in relation to response to gefitinib in lung cancer (21, 22). It would be of great interest to assemble a more comprehensive picture which includes functional polymorphic variations as well as mutations, and assess their individual and/or collective predictive value to a given therapy.

Our retrospective, pilot study presents several limitations. First, our findings are based on retrospective analyses on a small number of patients treated at a single institution who underwent two different treatment modalities, adjuvant and neoadjuvant chemoradiation. This could lead to spurious associations between EGFR polymorphisms and clinical outcome. In addition, negative associations between EGFR polymorphisms and established markers for tumor aggressiveness such as T and N-status may be missed due to the limited number of patients. Therefore, the results of this pilot study should be interpreted with caution. Notwithstanding the aforementioned limitations, we believe that assessment of EGFR polymorphisms and future haplotype analysis may aid in the prediction of chemoradiation failure in patients treated with adjuvant or neoadjuvant therapy. Currently, we are in the
process of confirming our findings in a much larger cohort of patients with rectal cancer treated with adjuvant chemoradiation (SWOG 9304).

REFERENCES
Epidermal Growth Factor Receptor Gene Polymorphisms Predict Pelvic Recurrence in Patients with Rectal Cancer Treated with Chemoradiation

Wu Zhang, David J. Park, Bo Lu, et al.


Updated version
Access the most recent version of this article at:
http://clincancerres.aacrjournals.org/content/11/2/600

Cited articles
This article cites 21 articles, 9 of which you can access for free at:
http://clincancerres.aacrjournals.org/content/11/2/600.full.html#ref-list-1

Citing articles
This article has been cited by 15 HighWire-hosted articles. Access the articles at:
/content/11/2/600.full.html#related-urls

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.