Background: Expression of epidermal growth factor receptor is observed in 50 – 70% of colorectal carcinomas and is associated with poor prognosis. The objective of this study was to analyze whether epidermal growth factor receptor expression predicts tumor response and sphincter preserving in patients treated with preoperative chemoradiation therapy.

Methods: This study was conducted on 34 patients with locally-advanced rectal adenocarcinoma who were treated with preoperative chemoradiation therapy. The patients had histologically-proven adenocarcinoma of the rectum with the inferior margin of the tumor located no farther than 6 cm from the anal verge. Preoperative radiotherapy was delivered to the pelvis with 60Co to 50.4 Gy. All patients received simultaneous chemotherapy with 5-fluorouracil, 300 mg/m² IV infused over 24 hr during radiotherapy on days 1 – 5 every week; 28 patients received oxaliplatin 50 – 60 mg/m² weekly during radiotherapy. The patients were restaged by physical examination and pelvic CT, between four and six weeks later. Then, they were referred to a surgeon who was expert in gastrointestinal cancer surgery. Subsequently, postsurgical specimen was histopathologically examined and graded according to the Mandard criteria for assessment of pathologic response after neo-adjuvant chemoradiation. Immunohistochemistry for epidermal growth factor receptor was determined at the preradiation biopsy and was evaluated according to the extension and staining intensity.

Results: Fourteen (41%) out of 34 tumors were epidermal growth factor receptor positive. Twenty (59%) patients responded to pelvic preoperative chemoradiation. Sixteen (47%) patients achieved complete response with no residual tumor in the resected specimen; four (12%) were downstaged (partial response). Response to pelvic radiotherapy was observed in 80% of those negative for epidermal growth factor receptor and in 28% of those with positive epidermal growth factor receptor (P = 0.005). Only two of 14 positive epidermal growth factor receptor patients achieved a complete response, while 14 of 20 of the negative epidermal growth factor receptor patients developed complete response. In our study, the sphincter preservation rate was 43% in positive epidermal growth factor receptor patients and 80% in those who did not express epidermal growth factor receptor (P = 0.036).

Conclusion: Epidermal growth factor receptor expression in the diagnostic biopsy of locally-advanced rectal cancer treated with chemoradiation therapy may serve as an important predictor of complete response to preoperative treatment.

Keywords: Colorectal cancer • epidermal growth factor receptor (EGFR) • sphincter preserving

Introduction

Rectal cancer, accounting for 35% of all colorectal cancers (CRCs), is the second cause of cancer death. In those with locally-advanced rectal cancer treated with surgery alone, local recurrence occurs in 20 – 60% of patients. In this group of patients, adjuvant chemoradiotherapy improves local control and
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survival. Several research groups have studied the benefits of preoperative radiotherapy with or without chemotherapy. When comparing the benefits of neo-adjuvant therapy vs. adjuvant, the former approach decreases tumor bulk, enhances sphincter preservation, and reduces acute toxicity. Preoperative pelvic radiotherapy produces 4 – 30% pathologic complete response and downstaging in 30 – 60% of cases.

Tumor downstaging after neo-adjuvant chemoradiation therapy occurs in 46.7% including 17.9% complete response. Response to preoperative radiotherapy varies depending on clinical factors such as tumor stage, total dose, fractionation schedules, concomitant chemotherapy treatment, or time between radiation and surgery.

Although TNM classification is useful for staging patients and selecting them for specific treatment, it is not sufficient, as many patients at the same stage may have various outcomes. Therefore, additional prognostic biomarkers are really needed for the management of patients with CRC.

Increasing effort has been directed towards developing molecular targeted therapies or searching for molecular markers that are useful either in predicting treatment outcome or in selecting patients for specific molecular targeted therapies, based on particular tumor characteristics.

There are several reports suggesting that expression of epidermal growth factor receptor (EGFR) may be associated with a reduction in survival in primary CRC. EGFR is a member of the tyrosine kinase family. It is a membrane glycoprotein composed of an extracellular ligand-binding domain, a transmembrane lipophilic segment, and an intracellular protein kinase domain with a regulatory segment. After ligand binding, EGFR dimerization occurs, which produces activation of the intrinsic protein tyrosine kinase activity. This leads to the activation of a biochemical cascade and physiologic responses involved in the mitogenic signal transduction of the cells that regulate cell division, proliferation, and differentiation.

EGFR is often expressed at high levels in human cancer and has been associated with more aggressive tumors. In CRC, EGFR is expressed in 50 – 70% of cases and is associated with a poor prognosis.

There are few data on the effect of EGFR expression on response to pelvic radiotherapy in the preoperative setting.

The objectives of this study were:
• To determine the rate of expression of EGFR in locally-advanced rectal cancer and to analyze whether EGFR expression predicts tumor response in patients treated with preoperative chemoradiation therapy.
• To determine the magnitude of their relationship.
• To determine whether the addition of chemotherapy can improve the outcome, if there is a poor pathologic response in EGFR+ patients after preoperative radiotherapy.
• To determine whether expression of EGFR predicts sphincter preserving in patients treated with preoperative chemoradiation therapy.

**Patients and Methods**

**Patient selection and pretreatment evaluation**

The inclusion criteria were as follows:
(1) Histologically-confirmed diagnosis of adenocarcinoma of the rectum, with the inferior margin of the tumor being no farther than 6 cm from the anal verge, (2) clinically staged as locally-advanced rectal cancer, (3) administration of preoperative chemoradiation therapy followed by surgical resection, and (4) availability of tissue samples of the diagnostic biopsy and tumor specimen for reviewing and immunostaining.

From September 2003 through October 2005, 34 patients with locally-advanced rectal adenocarcinoma were given preoperative chemoradiation therapy in our institution. There were 22 males and 12 females with a median age of 52 (range: 28 – 80) years. Locally-advanced rectal cancer was defined as tumor extension through the bowel wall (T3-T4) or with lymph node involvement (LN+). All patients were free of distant metastases at the diagnosis. Assessment of the local extension was based on clinical and/or radiographic evaluations. Diagnostic studies consisted of colorectal endoscopy, abdominopelvic computed tomography (CT), chest X-ray, endoscopic ultrasonography, and routine laboratory studies. The patients were staged according to the American Joint Committee on Cancer Staging.

**Immunohistochemical (IHC) assay of EGFR**

Paraffin-embedded blocks of diagnostic biopsies and surgical specimens were cut into 5-µm thick sections. Sections of the tissue blocks were deparaffined with xylene and rehydrated.
Endogenous peroxidase activity was blocked with 0.2% hydrogen peroxidase solution, and nonspecific labeling was blocked in serum blocking solution. Antigen retrieval was achieved by boiling sections in 0.01 µM citrate buffer (pH = 6.0) in microwave oven — four cycles, 10 min each, 300 W. Sections were incubated in complete medium for one hour at room temperature with EGFR rabbit monoclonal antibody (MU 207-UC; Biogenex, San Ramon, CA) at a dilution of 1:20 (v/v). The reaction was confirmed by avidin-biotin complex peroxidase method (ABC Elite kit, Vector Burlingame, CA) followed by staining with the peroxidase substrate 3,3 diaminobenzidine tetrachloride (DAB; Sigma GmbH, Deisenhofen, Germany). The slides were then counterstained with 50% hematoxylin.

Specimens were examined by light microscopy. All slides were assessed for EGFR expression by a well-trained histopathologist blinded to the tumor response to pelvic radiotherapy. IHC staining results were evaluated according to extension and intensity. Extension was defined as the percentage of positive tumor cells. A score of 1 to 5 was assigned according to the percentage of positivity of the stained tumor cells: 1 = positive staining <5%; 2 = positive staining between 5% and 25%; 3 = 25 – 50% positive; 4 = 50 – 75% positive, and 5 = >75% positively stained tumor cells. Staining intensity was graded qualitatively as 0 = not detectable; 1 = weak; 2 = moderate; and 3 = intense. EGFR staining was considered positive (EGFR+) when extension was 5% or more (score 2 – 4). When the extension was less than 5% (score 1), staining was considered negative (EGFR−).

Preoperative chemoradiation therapy and surgical modalities

Preoperative radiotherapy was delivered to the pelvis with 60Co. Clinical target volume included the tumor and the entire rectum, the anterior wall of the sacrum, the posterior wall of the prostate or vagina, and perirectal, presacral, hypogastric, actuator, and iliac lymph nodes. The standard AP-PA fields to 45 Gy, and then two opposed lateral boost fields up to 50.4, were used. All patients received conventional fractionation of 1.80 Gy/day, five fractions per week. All patients received simultaneous chemotherapy with 300 mg/m² IV 5-fluorouracil (5-FU) infused over 24 hr during radiotherapy on days 1 – 5 every week. Twenty-eight patients received 50 – 60 mg/m² oxaliplatin weekly during radiotherapy. The

patients were restaged by physical examination and pelvic CT, between four and six weeks later. They then were referred to a surgeon who was expert in gastrointestinal cancer surgery. Subsequently, the pathologic response was evaluated in postsurgical specimen and graded according to the method described by Mandard et al25 in 1994. This method was first developed to assess the pathologic response after neo-adjuvant chemoradiation therapy in esophageal cancer on a scale of 1 – 5, based on the presence of residual tumor cells and the extent of fibrosis. We considered grade 1 which is defined as the absence of residual tumor cells and fibrosis extending through the different layers of rectum, as “complete pathologic response.” We considered grade 2 – 4 which is characterized by the presence of various amounts of residual tumor cells, as “partial response.” And, grade 5 which is defined as tumor regression, was considered as “no response.”

Statistical analysis

Possible associations between EGFR immunexpression and clinical/histopathologic characteristics were determined using the χ² test. Data analyses were performed with SPSS (SPSS, Inc., Chicago, IL). The significance level (α level) was set to 0.05 in all statistical analyses used in this study.

Results

Twenty patients (59%) responded to pelvic preoperative chemoradiation therapy. Sixteen (47%) patients achieved pathologic complete response with no residual tumor in the resected specimen; four (12%) were downstaged (partial response). In our series, EGFR proved to be positive in 14 biopsies, so the EGFR expression rate was 41%.

No significant differences were found in gender, tumor location, tumor stage, lymph node stage, tumor differentiation, or response to treatment with chemotherapy. However, there were significant differences with regard to the response rate. Response to pelvic radiotherapy was observed in 80% of EGFR− and in 28% of EGFR+ patients (P = 0.005). Only two (14%) of the 14 EGFR+ patients achieved pathologic complete response, in comparison with 70% (14/20) of EGFR− patients. Multivariate analysis showed that the only significant predictor of complete remission was EGFR status (Table 1).
In our study, the sphincter preserving rate was 43% in EGFR+ patients compared to 80% in those who did not express EGFR ($P = 0.036$).

**Discussion**

EGFR is now recognized as an important target for investigation because of the prognostic significance of its expression in a wide variety of tumors and its possible implication for selective therapies.11 A positive correlation between EGFR status, as determined by IHC staining, and many human carcinomas has been shown. In breast cancer, EGFR protein expression has been associated with poor prognosis20 and non-responsiveness to endocrine therapy.21 Grandis et al22 quantified EGFR levels in squamous cell carcinomas of the head and neck and found that the level can predict the disease-free and cause-specific survival. Ang et al23 studied EGFR expression in 155 patients enrolled in a phase III randomized trial, who were treated with conventional radiotherapy. EGFR expression was a robust predictor of survival and local control. EGFR amplification was also found to be an independent predictor of prolonged survival in patients older than 60 years with glioblastoma multiforme.24 In prostate cancer, EGFR expression correlates with disease relapse and progression to androgen-independence.25

So far, the available data support a role for EGFR expression in the development of CRC. Although some negative results were reported, most investigators have shown that EGFR levels correlate with a more aggressive disease and a poor prognosis.11 Steele et al14 assessed EGFR expression in 50 patients with invasive CRC using immunohistochemistry. Dukes’ C tumors were found to exhibit significantly higher EGFR levels than Dukes’ A or B. It was then concluded that high EGFR expression is associated with poor prognosis. Mayer et al15 found 72 (88%) of 82 cases with resected colorectal adenocarcinomas of EGFR+. The extent of EGFR expression revealed significant differences in survival times; the patients with >50% stained tumor cells had poorer prognosis than those with <50% staining ($P < 0.01$). Radinsky et al26 found that increased tumor EGFR mRNA levels were associated with a higher incidence of liver metastases. Goldstein et al29 analyzed EGFR status in 102 patients with metastatic colon adenocarcinoma and found EGFR reactivity in 75% of patients. EGFR reactivity appeared to have the strongest correlation in the deep parts of colon adenocarcinoma and closer correlation with survival length. Not all investigators reported a significant link between EGFR status and outcome. McKay et al30 found that EGFR expression did not influence the prognosis in a cohort of 249 patients with colorectal adenocarcinomas. Khorana et al31 analyzed EGFR expression in 131 consecutive patients with stage II–III colon carcinoma treated with surgical resection. High grades of EGFR expression were found to be associated with a non-statistically significant trend towards a worsen survival.

Exposure of human cancer cells to radiation

| Table 1. Relationship between EGFR expression and other clinical and pathologic factors. |
|-----------------------------------------------|---|---|---|---|---|
|                                | Total | EGFR | EGFR | $P$ value |
|                                | No. | %  | No. | %  | No. | %  |
| Total                          | 34  | 100| 20  | 59 | 14  | 41 |
| Gender                         |     |    |     |    |     |    |
| Male                           | 22  | 65 | 13  | 65 | 9   | 64 |
| Female                         | 12  | 35 | 7   | 35 | 5   | 36 |
| Treatment                      |     |    |     |    |     |    |
| Rt-Cht(–Ox)                    | 6   | 18 | 4   | 20 | 2   | 14 |
| Rt-Cht(+Ox)                    | 28  | 82 | 16  | 80 | 12  | 86 |
| Pathologic stage               |     |    |     |    |     |    |
| T3                             | 20  | 59 | 12  | 60 | 8   | 57 |
| T4                             | 14  | 41 | 8   | 40 | 6   | 43 |
| LN−                            | 10  | 26 | 6   | 30 | 4   | 29 |
| LN+                            | 24  | 74 | 14  | 70 | 10  | 71 |
| Pathologic response            |     |    |     |    |     |    |
| Yes                            | 20  | 59 | 16  | 80 | 4   | 29 |
| No                             | 14  | 41 | 4   | 20 | 10  | 71 |
| Sphincter preserving           |     |    |     |    |     |    |
| Yes                            | 22  | 65 | 16  | 80 | 6   | 43 |
| No                             | 12  | 35 | 4   | 20 | 8   | 57 |

NS = not significant; Rt = radiotherapy; Cht = chemotherapy; Ox = Oxaliplatin.
activates EGFR, which mediates a cytoprotective response reducing the cells’ sensitivity to radiation. EGFR activation mediates clonogenic repopulation. A temporary coincidence of increasing EGFR expression and clonogenic repopulation has been shown in experimental tumors. Lammering et al concluded that radiation-induced EGFR activation contributes, at least in part, to the mechanism of accelerated proliferation. This cellular proliferation response to repeated radiation leads to increased renewal of tumor clonogens. Therefore, preoperative radiotherapy may be able to eradicate EGFR tumor cells, whereas EGFR tumor cells may remain active because of their radio-resistance.

Hickey et al analyzed EGFR expression in 14 chemoradiotherapy-treated esophageal squamous cell carcinomas and found a statistically significant relationship between EGFR expression and lack of response to chemoradiotherapy. Bensadoun et al evaluated 92 patients with unresectable pharyngeal carcinoma treated with bid radiotherapy and concomitant chemotherapy (three courses of 5-FU). The EGFR expression at pretreatment biopsy was a significant prognostic factor for response, disease-free survival, and overall survival, suggesting that EGFR expression may be an important marker for tumor radiosensitivity. Our results agree with these findings.

EGFR has been found to be elevated in CRCs, with expression rates ranging from 8% to 100% (50 – 70% in average). In our study, the expression rates was 41%.

Preoperative pelvic radiotherapy produces 4 – 30% pathologic complete response and downstaging in 30 – 60% of patients. Tumor downstaging after neo-adjuvant chemoradiation therapy occurs in 46.7% of patients including 17.9% complete response.

In our study, the overall pathologic response was 59%. This high pathologic response that has not been reported in any other study, can be attributed to concurrent chemoradiation therapy, especially with oxaliplatin that is currently under evaluation in a phase II trial. On the other hand, the lower rate of EGFR compared with other trials may be the cause.

Probably, only one trial similar to ours has been performed by Giralt et al in which they analyzed EGFR expression in 45 patients with locally-advanced rectal cancer treated with preoperative radiotherapy and total mesorectal resection. IHC for EGFR was determined at the preradiation diagnostic biopsy and in the resected specimens. Preoperative treatment resulted in pathologic complete remission in seven (15%) patients, downstaging in 13 (29%) patients, and no response in 25 (56%) patients. EGFR positivity was observed in 29 (64%) of 45 tumors and was associated with neither clinical tumor stage nor with clinical nodal stage. The overall response rate was 34% in EGFR-positive patients vs. 62% in those who did not express EGFR (P = 0.07). Only one of the seven patients with pathologic complete remission was EGFR positive (P = 0.003).

In our study, the overall response rate was 28% in EGFR vs. 80% in EGFR patients (P = 0.005). Like other trials, EGFR positivity correlated with poor outcome. Pathologic response rate in EGFR patients in our study (28%) did not significantly differ from the rate reported by Giralt et al (34%). In EGFR patients, however, the pathologic response rate in our trial was higher than that of Giralt et al’s trial (80% vs. 62%). This difference shows that addition of chemotherapy to radiotherapy can improve the outcome in EGFR but not in EGFR patients.

In view of our second objective, the magnitude of EGFR effect, in a meta-analysis published in 2001, more than 200 studies were evaluated that analyzed relapse-free interval or survival data directly in relation to EGFR levels in over 20,000 patients. Analysis of the data showed that ten cancer types both express elevated levels of EGFR relative to normal tissues and have been studied sufficiently to allow sound judgments to be made concerning the association between EGFR and patient outcome. The EGFR was found to act as a strong prognostic indicator in head and neck, ovarian, cervical, bladder, and esophageal cancers. In these cancers, increased EGFR expression was associated with reduced recurrence-free or overall survival rates in 70% (52 of 74) of studies. In gastric, breast, endometrial, and colorectal cancers, the EGFR had modest prognostic value, correlating to poor survival rates in 52% (13 of 25) of studies, while in nonsmall-cell lung cancer, EGFR expression only rarely (three of ten studies) correlated to the outcome of patients.

The situation in CRC is not entirely clear, as the data pertaining to EGFR and survival are currently very limited. Furthermore, the patient populations in the published studies were heterogeneous, including both early and advanced cases. However, EGFR expression has been associated with tumor grade and stage, relapse-free, and
overall survival.\textsuperscript{16, 42} In three studies, human epidermal growth factor receptor-2 expression showed a better correlation with survival than did EGFR expression.\textsuperscript{45 – 47} while in another study, transforming growth factor-α expression was found to be a better prognostic indicator.\textsuperscript{42}

In our study, the overall response rate was 80% in EGFR$^+$ patients vs. 28% in those with EGFR$^-$ (\(P = 0.05\)). On the other hand, several recent studies showed that tumor regression grade (TRG) is a predictor for local failure, metastases-free, disease-free, and overall survival.\textsuperscript{10, 48, 49} Therefore, we hope by continuing this study, we can show that EGFR expression is a strong prognostic factor in rectal cancer.

Regarding the last objective, to the best of our knowledge, this is the first study to address EGFR expression in relation to the chance of sphincter preservation. In our study, the sphincter preserving rate was 43% in EGFR$^+$ vs. 80% in EGFR$^-$ patients (\(P = 0.036\)). The sphincter preservation rate in EGFR$^+$ patients (80%) was equal to the pathologic response rate in this group. In EGFR$^-$ patients, the sphincter preservation rate (43%) was higher than their pathologic response rate (28%), which may be due to some errors in determining the distance between the lower edge of the tumor and the anal verge as measured by endoscopic ultrasonography so that in some patients this distance was estimated shorter than that it really was. The rate of sphincter preservation in EGFR$^+$ patients encouraged us to find a way such as addition of cetuximab in the neo-adjuvant therapy at least for EGFR$^+$ patients, for improving the response.

Ultimately, there is some limitations in our study. Although we determined the sample size according to results of previous studies, it is better to conduct similar studies with larger sample sizes. On the other hand, we defined the pathologic response according to the Mandard criteria that had been used generally in previous studies; however, it is not unlikely that our results would be changed if another criteria were used. All slides of surgical specimen were evaluated by two expert pathologists. However, for limited number of sections of specimen, it is not unlikely that some cases were falsely categorized as complete response rather than partial response. Currently, the most acceptable method for preoperative staging of rectal cancer is endoscopic ultrasonography that has some inaccuracy in determining the nodal (N) stage and that may be overestimate the distance between the lower edge of the tumor to the anal verge. Therefore, it is likely that some cases with tumor distance further than 6 cm from the anal verge included in our study, which disturbed our results.

In summary, our study indicates that EGFR expression in locally-advanced rectal cancer treated with radiotherapy may serve as an important predictor of pathologic complete response to preoperative treatment. In the present study, EGFR$^+$ expression was associated with a lack of pathologic complete response. Therefore, we concluded that analysis of EGFR expression may be helpful in determining a subgroup of high-risk patients requiring more therapeutic modalities, such as monoclonal antibodies or tyrosine kinase inhibitors, against this receptor.

References

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