



## Role of Molecular Markers (P53, EGFR AND VEGF) in Prognostication of Carcinoma Rectum

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### Abstract

*Colorectal cancer is the third most common cancer worldwide. Surgery remains the primary determinant of cure in patients with localized rectal cancer and for patients with invasive tumors neo-adjuvant chemoradiotherapy has been utilized to promote tumor regression. EGFR, VEGF and p53 are among the markers currently of interest as potential predictors of pathologic response, prognosis and recurrence-free survival in rectal cancer. In this study we assess the prognostic value of p53, VEGF and EGFR and predictive value of these molecular markers in assessing the overall outcome in cases of carcinoma rectum. Biopsy proven patients of carcinoma rectum (stage I to stage IV) were included in the study. Patients were treated according to standard protocols. Patients were followed for response to CRT, disease free survival and overall outcome. Our study shows over expression of p53, VEGF and EGFR are associated with poor response from CRT, poor outcome and short survival in carcinoma rectum. These findings were statistically significant for VEGF and EGFR (not significant for p53). We conclude that study with larger sample size and longer follow up may establish these markers as independent predictor of overall outcome in patient of carcinoma rectum.*

**Keywords:** Epidermal growth factor receptor (EGFR), vascular endothelial growth factor (VEGF), p53, colorectal cancer (CRC) and chemoradiotherapy (CRT).

### Introduction

Colorectal cancer is the third most common cancer worldwide while significant geographical, racial and ethnic variations exists in its incidence rate and pattern<sup>1,2,3</sup>. Globally, cancer of rectum and anus constitutes more than 40 percent of the CRC cases, and its incidence peaks between the age of 60 and 70 year<sup>4</sup>. The incidence of rectal

cancer in India is lower than that in the western countries, and it is the tenth leading cancer in India<sup>5</sup>. Several individual studies on Indian patients have consistently documented a relatively high proportion of young age rectal cancer (RC), with a mean age of around 40-45 years<sup>6</sup>.

Surgery remains the primary determinant of cure in patients with localized rectal cancer, and total

mesorectal excision (TME) is now widely accepted as standard of care. For patients with invasive tumors, neo-adjuvant chemoradiotherapy (CRT) has been utilized to promote tumor regression. The widespread implementation of neo-adjuvant radiotherapy with chemotherapy (CRT) has reduced local recurrence rate to less than 10% from 25% to 40%<sup>7</sup>.

In addition to TNM stage, several other tumor related features have been identified as essential or important prognostic factors. Epidermal growth factor receptor (EGFR), vascular endothelial growth factor (VEGF) and p53 are among the immunohistochemical protein markers currently of interest as potential predictors of pathologic response, prognosis and recurrence-free survival in rectal cancer.

The epidermal growth factor receptor (EGFR) plays an important role in tumor genesis and tumor progression of colorectal cancer (CRC). Vascular endothelial growth factor (VEGF) is a signal protein produced by cells that stimulates vasculogenesis and angiogenesis. In CRC, VEGF expression detected by immunohistochemistry has been linked with tumour aggressiveness and overall survival<sup>8</sup>.

The p53 gene is a tumor suppressor gene. If a person inherits only one functional copy of the p53 gene from their parents, they are predisposed to cancer and usually develop several independent tumors in a variety of tissues in early adulthood<sup>9</sup>. In this study we assess the prognostic value of p53, VEGF and EGFR in cases of carcinoma rectum. We studied the predictive value of these molecular markers in assessing the overall outcome.

### Material and Methods

Biopsy proven patients of carcinoma rectum (stage I to stage IV) were included in the study. Tissue samples for immunohistochemistry of molecular marker were taken. Stage I patients were treated by surgery. Patients with locally advanced disease (stage II and stage III) were given radiation therapy (1.8-2Gyx25-28cycle)

with 5FU based chemotherapy. The response to neo-adjuvant chemo-radiotherapy was assessed using RECIST criteria (table 1). Following CRT patients were planned for surgery. Stage IV patients were offered palliative treatment.

**Table 1:** RECIST criteria

Complete response(CR)	Disappearance of all target lesions. Any pathological lymph nodes(whether target or non-target) must have reduction in short axis to <10mm
Partial response(PR)	At least a 30% decrease in the sum of diameters of target lesions,taking as reference the baseline sum diameters.
Progressive disease(PD)	At least 20%increase in the sum of diameters of target lesions, taking as reference the smallest sum on study. In addition to the relative increase of 20%, sum must also demonstrate an absolute increase of at least 5mm(appearance of one or more new lesions is also considered progression).
Stable disease(SD)	Neither sufficient shrinkage to qualify for PR nor sufficient increase to qualify for PD, taking as reference the smallest sum diameters while on study.

Immunohistochemistry for the molecular markers was carried out on formalin fixed, paraffin embedded serial sections cut at 3-4 microns and dried at 60°C overnight. Counter staining of the slides was performed with Hematoxylin. The positive cells expressing VEGF/EGFR/p53 positivity were assessed for cytoplasmic staining at higher magnification (20X).

Patients were followed for response to CRT, disease free survival and overall outcome. Minimum follow up time was two years. The level of molecular markers was correlated with the outcome. Categorical variables were presented in number and percentage. Continuous variables were presented as mean and standard deviation. Quantitative variables were compared using unpaired t-test between two groups. Qualitative variables were compared using chi-square test/fishers exact test as appropriate. A p value of <0.05 was considered statistically significant. The data were entered in MS EXCEL spread sheet and analysis was done using statistical package for social sciences (SPSS) VERSION 16.0.

## Results

Sixty patients were included in study. Four patients had early disease (stage I) and they were operated upon. Forty four patients had locally advanced disease (T3,T4 and node positive). These patients were given neo-adjuvant radiotherapy and chemotherapy. Twenty four patients had good response of chemo-radiotherapy and were operated four to five weeks later. Twenty patients had poor response to neo-adjuvant chemo-radiotherapy. Twelve of these patients were operated. In eight patients diversion

colostomy was done as the disease was inoperable. In 4 patients low anterior resection was performed, two of them developed recurrence one year after surgery. Twelve patients had stage 4 disease and were offered palliative treatment. In this study over-expression of VEGF and EGFR was associated with poor response from neoadjuvant CRT (table 2), short survival and poor outcome (table 3) and findings were statistically significant. Over expression of p53 is associated with poor outcome and short survival but statistically not significant.

**Table 2:**

Markers	RESPONSE TO ADJUVANT CRT				P value
	Good (N=24)		Poor (N=20)		
	Mean	Std. Deviation	Mean	Std. Deviation	
Marker P53	17.24	20.12	28.09	30.50	0.1648
Marker VEGF	47.67	29.05	68.08	28.62	0.0243*
Marker EGFR	38.41	28.19	57.61	28.16	0.0297*

**Table 3:**

Markers	OUTCOME				P value
	Good (N=36)		Poor (N=24)		
	Mean	Std. Deviation	Mean	Std. Deviation	
Marker P53	17.80	27.30	27.89	36.56	0.226
Marker VEGF	41.17	28.43	65.39	29.60	0.0024*
Marker EGFR	36.50	27.76	56.06	29.19	0.011*

## Discussion

Over expression of p53 is associated with poor outcome and short survival in our study but the findings were statistically not significant.

Luderer LA et al studied isolated expression of p53 and study shows than over expression of p53 had poor outcome and short survival<sup>10</sup>. In the studies of Z S Seng et al and Christine Rebischung et al over expression of p53 shows poor outcome and shorter survival<sup>11,12</sup>. Ruud Wiggenraad et al and N Scott et al studied that there was not significant relationship between p53 expression and survival in carcinoma rectum patients.<sup>13,14</sup>

Over expression of VEGF was associated with poor outcome and short survival in our study and findings were statistically significant.

In studies of Zlobec I et al and S. Cascinu et al patients with VEGF over expression shows poor outcome<sup>9,15</sup>. Over expression of VEGF was associated with nearly two times increase risk of death in study of Yibaina et al.<sup>16</sup>

Over expression of EGFR was associated with poor outcome and short survival in our study and findings were statistically significant.

Study of Zlobec and Lugli showed over expression of EGFR leads to overall poor outcome<sup>9</sup>. In the studies of Giralt et al and Luderer LA et al over expression of EGFR associated with short survival and poor outcome.<sup>17,10</sup>

Findings of our study shows that VEGF and EGFR are independent predictive factors of outcome in patients of carcinoma rectum.

### Conclusion

Our study shows over expression of p53, VEGF and EGFR are associated with poor response from CRT poor outcome and short survival in carcinoma rectum. These findings were statistically significant for VEGF and EGFR (not significant for p53).

We conclude that study with larger sample size and longer follow up may establish these markers as independent predictor of overall outcome in patient of carcinoma rectum.

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