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

Authors
Ganesh Chandra Yadav¹, Arshad Ahmad²*, Abhishek Kumar³, Abhinav Arun Sonkar⁴, Suresh Kumar⁵, Vijay Kumar⁶
Department of General Surgery, King Georges Medical University, Uttar Pradesh, Lucknow, India-226003
*Corresponding Author
Dr Arshad Ahmad
Associate Professor, Department of Surgery, King George’s Medical University, Lucknow, India-226003
Email: arshadahmadkgmu@gmail.com

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 pattern¹,²,³. 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 year⁴. The incidence of rectal cancer in India is lower than that in the western countries, and it is the tenth leading cancer in India⁵. 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 years⁶. 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%.

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 tumor aggressiveness and overall survival.

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.

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 (1.8-2Gy×25-28cycle) 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. In the studies of Z S Seng et al and Christine Rebischung et al over expression of p53 shows poor outcome and shorter survival. Ruud Wigenraad et al and N Scott et al studied that there was not significant relationship between p53 expression and survival in carcinoma rectum patients. 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. Over expression of VEGF was associated with nearly two times increase risk of death in study of Yibaina et al. 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. In the studies of Giralt et al and Luderer LA et al over expression of EGFR associated with short survival and poor outcome.
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.

**Bibliography**
1. World Cancer Research Fund and American Institute for Cancer Research Food, Nutrition, Physical Activity, and the Prevention of Cancer: A Global Perspective. Washington, DC: American Institute for Cancer Research; 2007.
2. Center MM, Jemal A, Smith RA, Ward E. Worldwide variations in colorectal cancer. CA Cancer J Clin. 2009;59:366–78. [PubMed: 19897840]
3. Center MM, Jemal A, Ward E. International trends in colorectal cancer incidence rates. Cancer Epidemiol Biomarkers Prev.2009;18:1688–94. [PubMed: 19505900]
4. Virk R, Gill S, Yoshida E, Radley S, Salh B. Racial differences in the incidence of colorectal cancer. Can J Gastroenterol. 2010;2:47–51. [PMC-ID: PMC2830637][PubMed: 20186356]
5. Three-years report of Population Based Cancer Registries 2006-2008 (Detailed Tabulations of Individual Registries Data). National Cancer Registry Programme (Indian Council of Medical Research), Bangalore November. 2010. [accessed on December 27, 2012]. Available from: http://www.PBCR_2006_2008.aspx
6. Nath J, Wigley C, Keighley MR, Perakath B. Rectal cancer in young adults: a series of 102 patients at a tertiary care centre in India. Colorectal Dis. 2009;11:475–9.
7. Koukourakis GV, Role of radiation therapy in neoadjuvant era in patients with locally advanced rectal cancer, world J Gastrointest Oncol 2012 Dec 15;4(12): 230-7.
8. Nozue M, Isaka N, Fukao K. Overexpression of vascular endothelial growth factor after preoperative radiation therapy for rectal cancer. Oncol Rep 2001;8:1247–9.
9. Zlobec I, Vuong T, Compton CC, Lugli A, Michel RP, Hayashi S, Jass JR. Combined analysis of VEGF and EGFR predicts complete tumour response in rectal cancer treated with preoperative radiotherapy. Br J Cancer. 2008 Jan 29;98(2): 450-6.
10. Luderer LA, Lustosa SAS, Silva SEM, Denadai MVA, Afonso Jr RJ, et al. (2015) Significance of a Biomarkers Immunohistochemistry Panel for Survival Prognostic in Patients with Sporadic Colorectal Cancer. Ann Clin Pathol 3(2): 1050.
11. Z S Zeng, A S Sarkis, Z F Zhang, D S Klimstra, E Charytonowicz, J G Guillem, C Cordon-Cardo and A M Cohen: p53 nuclear overexpression: an independent predictor of survival in lymph node-positive colorectal cancer patients.
12. Rebischung C, Gérard JP, Gayet J, Thomas G, Hamelin R, Laurent-Puig P. Prognostic value of P53 mutations in rectal carcinoma. Int J Cancer. 2002 Jul 10;100(2):131-5S
13. Ruud Wiggenraad., Reinder Tamminga, Paul Blok., Remigio Rouse. The Prognostic Significance of P53 Expression for Survival and Local Control in Rectal Carcinoma Treated with Surgery and Postoperative Radiotherapy. International
14. N. Scott, A. Hale, M. Deakin, P. Hand, F.A. Adab, C. Hall, G.T. Williams, J.B. Elder: A histopathological assessment of the response of rectal adenocarcinoma to combination chemo-radiotherapy: relationship to apoptotic activity, p53 and bcl-2 expression.

15. S Cascinu, F Graziano, V Catalano, M P Staccioli: An analysis of p53, BAX and vascular endothelial growth factor expression in node-positive rectal cancer. Relationships with tumour recurrence and event-free survival of patients treated with adjuvant chemoradiation. Br J Cancer. 2002 Mar 4;86(5):744-9.

16. Yibaina Wang, Xiaoping Yao, Jie Ge, Fulan Hu and Yashuang Zhao et al: Can Vascular Endothelial Growth Factor and Microvessel Density Be Used as prognostic Biomarkers for Colorectal Cancer? A systematic Review and Meta-Analysis. Sci W Jor Volume 2014.

17. Giralt J, Navalpotro B, Hermosilla E, et al. Prognostic significance of vascular endothelial growth factor and cyclooxygenase-2 in patients with rectal cancer treated with preoperative radiotherapy. Oncology 2007;71:312–9.