Original Research Article

Predictive Value of VEGF, EGFR and p53 in Locally Advanced Carcinoma Rectum Treated with Neoadjuvant Chemoradiotherapy

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Abstract

Introduction: Colorectal cancer is a major cause of morbidity and mortality throughout the world. The widespread implementation of neoadjuvant radiotherapy and chemotherapy (CRT) has reduced local recurrence rates from 25% to 40% to less than 10%. However, many patients undergo radiotherapy and chemotherapy for resistant cancers, thus incurring side-effects without benefit. Expression of particular genes at molecular level can be related to response or resistance to systemic therapies. In this study we have assessed the predictive value of VEGF, EGFR and p53 in predicting the response to neoadjuvant CRT in locally advanced rectal cancers. *Material and Methods:* Patients with locally advanced carcinoma rectum (stage II and III) were included in the study. Following completion of neoadjuvant CRT patients were evaluated to assess the response of CRT. The response of CRT was assessed using RECIST criteria. The response to CRT was correlated with the level of molecular markers. *Results:* Total 35 patients were included in the study. Over expression of VEGF, EGFR and p53 was associated with poor response to neo-adjuvant chemo-radiotherapy. The findings were statistically significant in case of VEGF and EGFR. *Conclusion:* We conclude that molecular markers (VEGF, EGFR and p53) may become tools for selection of patients suitable for chemoradiation in carcinoma rectum. However, more studies are needed with larger sample size and longer follow up for establishing molecular markers as predictors of response to chemoradiation and overall outcome in patients of carcinoma rectum.

Keywords: Rectal Carcinoma; Neoadjuvant Chemoradiotherapy.

Introduction

Colorectal cancer is a major cause of morbidity and mortality throughout the world [1]. It is the third most common cancer worldwide and the fourth most common cause of death [2]. 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 [3,4]. For, patients with locally advanced rectal cancer (LARC), neoadjuvant therapy has been utilized to promote tumour regression. The widespread implementation of neoadjuvant radiotherapy and chemotherapy (CRT) has reduced local recurrence rates from 25% to 40% to less than 10% [5].

However, many patients undergo radiotherapy and chemotherapy for resistant cancers, thus incurring side-effects without benefit. The ability to predict complete pathologic response or sensitivity to radiation before treatment would have a significant impact on the selection of patients for preoperative CRT. Microarray technology has led to a series of promising results through tissue gene expression profiling of different malignancies [6]. Expression of particular genes at molecular level can be related to response or resistance to systemic therapies. VEGF (vascular endothelial growth factor), EGFR (epidermal growth factor receptor) and p53are among the most promising immunohistochemical prognostic indicators in CRC. VEGF is recognized as a potent pro-angiogenic factor whose downstream signalling events include

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endothelial cell proliferation, migration and vascular permeability [7]. EGFR plays an important role in tumorigenesis and tumor progression of colorectal cancer [8]. Over-expression of EGFR and VEGF has been linked to poor prognosis in the majority of studies [9-11]. The p53 is a tumour suppressor gene, its activity stops the formation of tumours [12].

In this study we have assessed the predictive value of VEGF, EGFR and p53 in predicting the response to chemo-radiotherapy in locally advanced rectal cancers.

Material and Methods

Biopsy proven patients of locally advanced carcinoma rectum (stage II and III) were included in the study. Preoperative staging was performed according to the International Union against Cancer Classification and carried out by magnetic resonance imaging and CT scan. Patients with metastasis (stage IV) and small T1-2 tumours with favourable features (stage I) were excluded from the study. Patients who had already received CRT were not included in the study.

Tissue samples for immunohistochemistry of molecular markers were taken. Immunohisto-chemistry for the molecular markers was carried out on formalin fixed, paraffin embedded serial sections cut at 3-4 microns and dried at 60ÚC overnight. The slides were incubated with primary antibody (DAKO, Denmark) for 1 hr and secondary antibody (HRP-DAKO, Denmark) for 30 minutes. 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).

For multivariate analysis, the cut-off scores were determined to be 50% for p53, 20% for VEGF and EGFR. Tumours with scores above the obtained cut-off values were considered positive for the marker [11].

Patients were given radiation therapy (1.8-2Gyx25-28cycle) with 5FU based chemotherapy. Following completion of CRT patients were evaluated to assess the response of CRT. The response of CRT was assessed using RECIST criteria (Table 1).

The response to CRT was correlated with the level of molecular markers. Categorical variables were presented in number and percentage and 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 spreadsheet and analysis was done using statistical package for social sciences (SPSS) VERSION 16.0.

Results

Total 35 patients were included in the study. Of these 12 patients had Stage II and 23 patients had stage III disease (Table 2) Following CRT in stage II disease, 9 patients had good response (complete or partial response) and 3 patients had poor response (stable or progressive disease). In patients of stage II disease, 16 Patients had good response and 7 patients had poor response to neo-adjuvant chemoradiotherapy.

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.

Table 2: Results

Markers	(N=35)					
	Stage III (23)		P value	Stage II (12)		P value
	Good response (16)	Poor response (7)		Good response (9)	Poor response (3)	
P53	16.7±18.3	22.3±17.6	0.502	17.2±15.4	23.6±19.3	0.567
VEGF	38.6±25.6	66.0±26.7	0.029	33.3±23.3	58.5±19.5	0.925
EGFR	39.8±22.3	52.1±26.3	0.049	37.5±24.5	50.1±22.2	0.453

Overall, 25 patients had good response to CRT and 10 patients showed poor response to neoadjuvant CRT. (Table 3) Over-expression of VEGF and EGFR was associated with poor response to neo-adjuvant chemo-radiotherapy and findings were statistically significant. Over-expression of p53 was also associated with poor response to neo-adjuvant chemo-radiotherapy but the findings were statistically not significant.

Our findings indicate that VEGF and EGFR are independent predictive factors and their combined analysis is highly predictive of response to neo-adjuvant chemo-radiotherapy in locally advanced carcinoma rectum. However, the multivariate analysis of the markers did not yield any significant outcome.

Discussion

For locally advance rectal cancer (LARC), neoadjuvant chemo-radiotherapy (CRT) significantly improves local control and reduces toxicity profiles

Table 3: Results

| Markers | Locally advanced carcinoma rectum (n=35) | | P value |
	Good response (n=25)	Poor response(N=10)	
P53	16.7±5.3	18.3±7.8	0.487
VEGF	28.6±16.3	46.3±26.1	0.020
EGFR	33.3±13.6	53.4±23.3	0.003

compared with postoperative CRT but with similar survival rates [13]. Furthermore the ability to achieve pathological down staging, or a complete pathologic response, after neo-adjuvant CRT is correlated with improved survival, decreased local recurrence and a higher rate of sphincter-preservation [14]. Approximately 40-60% of LARC patients treated with neo-adjuvant CRT achieve some degree of pathologic response [14]. However, there is no effective method of predicting which patient will respond to neo-adjuvant CRT. Prospective identification of patients who have a higher likelihood of responding to preoperative CRT could be important in decreasing treatment morbidity and improving survival and local control in LARC [14]. Recent studies have evaluated the potential of genetic biomarkers to predict outcome in LARC treated with neo-adjuvant CRT. [15,16]

In our study over expression of VEGF and EGFR was associated with poor response to neoadjuvant chemo-radiotherapy and findings are statistically significant. In a study by Zlobec I, overexpression of VEGF correlated with poor outcome [11]. In a study by S. Cascinu, patients with VEGF positive tumour showed worse event free survival than VEGF negative patients and poor response to neo-adjuvant chemo-radiotherapy [18]. In a study of Yibaina et al over expression of VEGF correlates with nearly 2 times increase risk of death [19].

In the study of Zlobec and Lugli, over-expression of EGFR correlated with poor overall outcome [10]. In the study of Giralt et al., over-expression of EGFR correlated with poor response to chemo-radiotherapy [6]. In the study of Luderer LA, patients with over-expression of EGFR had shorter overall time of survival [19].

In our study over-expression of p53 was associated with poor response to neoadjuvant chemo-radiotherapy, however the correlation is statistically not significant. In the study of Luderer LA et al isolated expression of p53 showed that patients with overexpression had shorter survival [19]. In a study by Z S Seng over-expression of p53 correlated with poor survival [17]. In the study of Christine Rebischung, presence of p53 over-expression was associated with significantly shorter survival [20]. In the study of Ruud Wiggenraad and N Scott, no significant relationship was found between p53 and survival [15,16].

In multivariate analysis by Zlobec et al VEGF positive and EGFR negative expression was associated with lack of complete tumour regression in more than 94% of cases and a 12 fold decreased odds of response compared with EGFR positive and VEGF negative tumours [11].

Conclusion

In our study Over expression of VEGF, EGFR & p53 were associated with poor response to neo-adjuvant chemo-radiotherapy. The findings were statistically significant for VEGF and EGFR but not significant for p53.

However, we conclude that more studies are needed with larger sample size and longer follow up for establishing molecular markers as predictors of response to chemo-radiotherapy and overall outcome in patients of carcinoma rectum.

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