RESEARCH ARTICLE

Analysis of PTEN, VEGF, HER2 and P53 Status in Determining Colorectal Cancer Benefit from Bevacizumab Therapy

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Abstract

Background: No factor has thus far been identified to predict the efficacy of bevacizumab therapy for colorectal cancer. We here therefore studied PTEN, VEGF, HER2 and p53 by immunohistochemistry as possible prognostic and predictive factors. Materials and Methods: A total of 34 retrospectively collected tumor samples were evaluated, all from patients receiving bevacizumab-based regimens. VEGF-A, PTEN, HER2, p53 were assessed and data was compared with clinicopathologic characteristics of patients and the bevacizumab response rate. Results: In this study, the median age of the 34 metastatic colorectal cancer patients was 55.5 (24-75), twelve (35.3%) being women and 22 (64.7%) men. PTEN, VEGF, HER2, p53 expressions were compared with bevacizumab response and other chacteristics of disease. Statistical significant differences were not found between bevacizumab response rates and different expression levels of VEGF, PTEN, HER2 and p53 (respectively p=0.256, p=0.832, p=0.189, p=0.131). However, a survival difference was noted in the VEGF expression negative group (median OS:55 months; 95%CI, 22-88 months) (p=0.01). There was no statistically significant OS difference in other groups (PTEN p=0.6, HER2 p=0.189, p53 p=0.13). Conclusions: We did not find any predictive factor for BV therapy in our study. VEGF negative expression could be an important prognostic factor in metastatic colorectal carcinoma.

Keywords: Bevacizumab - VEGF - PTEN - HER2-neu - p53 - colorectal cancer

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Introduction

Colorectal carcinoma is the third common cause of cancer related mortality in the world (Jemal et al., 2008). Targeted biologic agents has increased the overall median survival in metastatic colorectal carcinoma(m CRC) to 23.5 months (Van Cutsem et al., 2011). Kirsten –ras(KRAS) mutation in the epidermal growth factor receptor (EGFR) pathway has changed the chemotherapy to a more personalized therapy, tailored approach with respect to the use EGFR monoclonal antibodies (Wong et al., 2008). Different biomarkers were assessed in numerous studies, any predictive biomarker for bevacizumab has not been identified (Asghar et al., 2010).

PTEN is a tumor suppressor protein that regulates the activity of PI3K/AKT by converting PIP3 back to PIP2. PTEN loss leads to AKT-mediated hyperphosphorlylation that protects cells from apoptosis (Christos et al., 2004). Vascular endothelial growth factor is a heparin binding peptide that acts as a potent angiogenic factor that induces endothelial cell proliferation, increases vascular permeability, promotes the extravasation of proteins from tumor vessels (Asghar et al., 2010). HER2 is the member of EGFR family. Overexpression of EGFR and HER2 has often been associated with malignant transformation (Wei et al., 2011). All of these biomarkers are related tumor growth. The clinical benefit of bevacizumab in metastatic colorectal is independent of KRAS and BRAF mutational status (Asghar et al., 2010). Different biomarkers were analysed for bevacizumab therapy (Asghar et al., 2010). Despite numerous studies, an equivalent predictive biomarker for bevacizumabhas not been identified (Asghar et al., 2010). PTEN loss, VEGF, HER2 are responsible for tumor angiogenesis, cell proliferation, apoptosis regulation by activitation by different pathways (Asghar et al., 2010; Cacheux et al., 2011). The primary function of the p53 protein is as a tumour suppressor (Christos et al., 2004; Munro et al., 2005) (Figure 1).

In the guidance of this functions of these biomarkers we analysed PTEN, VEGF, HER2 and p53 expressions by immunohistochemistry for determining BV response in metastatic colorectal carcinoma.

Materials and Methods

Patients with a diagnosis of histologically proven colorectal carcinoma between 2000-2009 years were eligible for our study. Ninety patients were analysed

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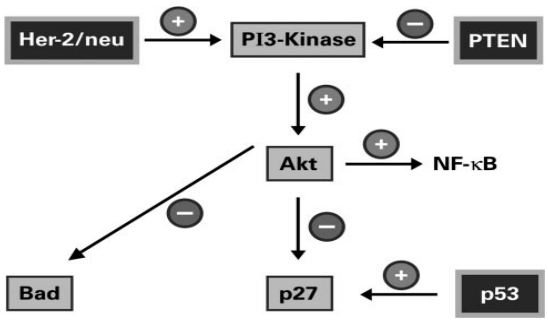

Figure 1. Important Cellular Mediators Cancer Growth. PTEN and HER-2/neu regulate the phosphatidylinositol 3_-kinase (PI3-Kinase)/Akt signaling pathway, which, in turn, regulates the activity of important mediators of proliferation, survival, and apoptosis (Christos et al., 2004)

retrospectively and We collected 34 tumor samples from the patients with colorectal carcinoma that treated in Cukurova University Medical Faculty. The other patients were excluded due to incomplete data. All of the patients received bevacizumab in combination with FOLFIRI or FOLFOX. VEGF, PTEN, HER2, p53 status were determined by immunohistochemistry (IHC) before treatment. Tumor Node Metastasis staging system was used to staging th e patients. Response was evaluated by RECIST criteria. VEGF, PTEN, HER2, p53 expression rates in colorectal carcinoma in Table 1.

VEGF staining quantity was scored as follows: no staining is 0, 1-10% of cells stained are scored as 1, 11-50% as 2, 51-80% as 3 and 81-100% as 4. Staining intensity was scored on a scale of 0-3 where 0 is no staining, 1 is weak, 2 is moderate and 3 is strong. The staining intensity score and staining quantity score were multiplied to give the immunohistochemical scor (IHS). An IHS of 9-12 was considered strong immunoreactivity; 5-8 moderate, 1-4 weak and 0 was considered as negative (Perrone et al., 2006). PTEN, p53, Staining of cells was scored as negative if <10%, +1 if 10-50% and +2 if >50% of slide's area was stained positive (Sarmadi et al., 2009).

HER2-neu, a score of 0 was given to those specimens showing no staining, or membrane staining in <10% of the tumor cells. A score of 1+ was given to a specimen showing faint or barely perceptible membrane staining in >10% of tumor cells. A score of 2+ was given to specimens showing weak to moderate complete membrane staining in >10% of tumor cells. A score of 3+ was given to specimens showing strong complete membrane staining in >10% of tumor cells (Ahwon et al., 2007).

Statistical analysis

Data was compared with clinicopathologic characteristics of patients. Khi2 test was used for categoric measurements between groups. Progression free survival was analyzed using Kaplan-Meier plots and log rank test. Data are reported as mean S.D.±, median (range) or proportions. Estimates of overall survival with associated 95%CIs were obtained using the Kaplan–Meier method. The calculations were performed using SPSS 16.0 for Windows (SPSS, Chicago, IL, USA).

Results

Thirthy four CRCs analyzed in this study: 12 (64.7%) patients were woman and 22 (35.3%) patients were man. Median age was 55.5 (24-75). Five patients (14.7 %), were Stage II, 10 (29.4%) patients were Stage III, 19 (55.9 %) were Stage IV at the time of diagnosis. All patients were metastatic and treated with bevacizumab-based regimens at the time of inclusion to the study.

The correlation between expression levels of HER2, VEGF, PTEN, p53 were analysed. Statistically significant difference was not found between expressions.

Time to progresssion was 15±6.8 months in patients. Time to progression in VEGF negative expression group was 16.4±7.9 months and 14.3±6.2 months in VEGF expression positive group (p=0.53). Time to progression in PTEN negative expression group was 14±7.5 months and 15±6.4 months in PTEN expression positive group (p=0.392). Time to progression in HER2 negative expression group was 14.6±7.1 months and 17±4.5 months in HER2 expression positive group (p=0.53). Time to progression in p53 negative expression group was 16.2±8.5 months and 13.9±4.8 months in p53 expression positive group (p=0.392). Statistically significant difference was not found between time to progression and VEGF, PTEN, HER2, p53 expression.

VEGF, PTEN, HER2 and p53 expressions were divided into two groups in subgroup analysis. Negative expression was clasified as negative and other all levels of expressions were classified as positive group (Figure 2).

In this subgroup analysis stastically significant difference was not found between VEGF, PTEN, HER2

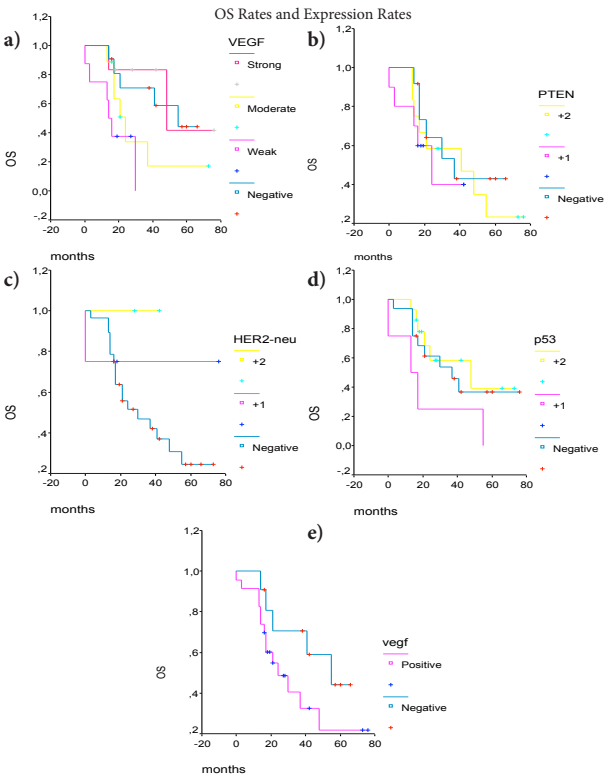

Figure 2. Expression and OS (Overall Survival). a) VEGF, b) PTEN, c) HER2-neu, d) p53 and e) VEGF

Table 1. VEGF, PTEN, HER2, p53 Expression Rates in Colorectal Carcinoma

	Expressin	No (%)
VEGF	Negative	11 (32.4)
	Weak	8 (23.5)
	Moderate	9 (26.5)
	Strong	6 (17.6)
PTEN	Negative	12 (35.3)
	1 (+)	10 (29.4)
	2 (+)	12 (35.3)
HER2	Negative	28 (82.4)
	1 (+)	4 (11.8)
	2 (+)	2 (5.9)
p53	Negativie	16 (47.1)
	1 (+)	4 (11.8)
	2 (+)	14 (41.2)

Table 2. Impact of VEGF, PTEN, HER2, p53 Expression to Response Rate of Bevacizumab Containing Regimens

		Responder No (%)	Non-responder No (%)	p
Bevacizumab containing regimens response status				
VEGF:	Negative	4 (28.6)	7 (35)	0.256
	Weak	3 (21.4)	5 (25)	
	Moderate	6 (42.9)	3 (15)	
	Strong	1 (7.1)	5 (25)	
PTEN:	Negative	5 (35.7)	7 (35)	0.832
	1+	4 (28.6)	6 (30)	
	2+	5 (35.7)	7 (35)	
HER2:	Negative	11 (78.6)	17 (85)	0.189
	1+	3 (21.4)	1 (5)	
	2+	0 (0)	2 (10)	
P53:	Negative	4 (28.6)	12 (60)	0.131
	1+	3 (21.4)	1 (5)	
	2+	7 (50.0)	7 (35)	

Table 3. Association between LVI, PNI, Differantiatioan and VEGF, PTEN, p53

	Lenfovasculer Invasion (p)	Perineural Invasion (p)	Differantiation (p)
VEGF	0.32	0.255	0.48
PTEN	0.15	0.228	0.64
HER2	0.8	0.16	0.3
p53	0.5	0.268	0.455

and p53 expressions and BV response rate (respectively p=0,256, p=0,832, p=0,189, p=0,131) (Table 2).

Perineural invasion, lenfovasculer invasion and differentiation of the tumor specimens were compared with expression of VEGF, PTEN, HER2 and p53. Statistically significant difference was not found (Table 3).

Discussion

Biologic targeted therapies are so named because their activity is restricted to impeding one or several pathways within tumor cells. Decisions regarding treatment options were dependent on performance status, age, organ function and previous treatment decision in the past (Asghar et al., 2010). Nowadays Kras mutation in the EGFR pathway is predictive for Cetuximab therapy. KRAS mutation has changed the approach from universal chemotherapy to a more personalized. Multiple factors, including relatively low response rates and high costs for targeted agents, are driving the search to identify further biomarkers within the EGFR/Ras/Raf/Mek/Erk and PTEN/PI3K/AKT signaling pathways. Bevacizumab (BV) targets the angiogenesis needed for tumor growth by inhibition of the vascular endothelium growth factor VEGF pathway (Cacheux et al., 2011). Any predictive biomarker has been identified. We aimed to analyse some biomarkers that can predict BV response in this study (Asghar et al., 2010).

PTEN loss, VEGF, HER2 expressions are responsible for tumor angiogenesis, cell proliferation, apoptosis regulation by activitation by different pathways (Asghar et al., 2010; Cacheux et al., 2011). The primary function of the p53 protein is as a tumour suppressor (Munro et al., 2005).

Numerous retrospective studies showed the prognostic value of VEGF expression in CRC. High VEGF expression in tumor tissue indicated a shorter relapse free survival and overall survival was reported by Des Guetz et al. (2006).

No conclusive evidence yet exists that VEGF is a predictive biomarker of efficacy for antiangiogenic therapy (Asghar et al., 2010). Pretreatment VEGF levels revealed no significant relationship between clinic response and time to progression (Longo et al., 2007).We didn't find any correlation between VEGF expression and BV response but statistically significant difference was found OS between negative expression of VEGF in our study. Jalbăet al. (2011) showed VEGF-A expression as a poor prognostic marker for CRC in their study (Jalbă et al., 2011). In other study; there was found no correlation between treatment response and VEGF expression within the tumour tissue (Pohl et al., 2011).

In our study we found longer overall survival rates with negative VEGF expression but we didn't find any correlation between BV treatment response and VEGF expression like other studies.

PTEN expression rate was found as 30-40% in sporadic breast, lung, and colorectal cancer (Hawkes et al., 2010). We found in the rate of 35% PTEN loss in m CRC. Colakoglu et al. (2008) have reported PTEN expression and its correlation with the prognostic factors in CRC. PTEN expression showed a negative correlation with young age, female sex, and left-sided (distal) tumors. On multivariate analysis, low PTEN expression (PTEN loss) was noted as an independent parameter for local recurrence (P=0.024) in their study (Colakoglu et al., 2008). We analysed PTEN as prognostic and predictive biomarker in m CRC. We didn't find any correlation between PTEN expression and BV response and the other parametres (LVI, PNI, differentiation). OS difference was not found between PTEN, VEGF, HER2, p53 expression rates in our study.

Zhou et al. (2004) reported that inactivation of PTEN gene and over-expression of VEGF contribute to the neovascularization and progression of gastric cancer. PTEN related angiogenesis might be attributed to its up-regulation of VEGF expression for their report. PTEN and VEGF could be used as the markers reflecting

the biologic behaviors of tumor and viable targets in therapeutic approaches to inhibit angiogenesis of gastric cancers (Zhou et al., 2004). In our study we didn't find any correlation between VEGF and PTEN expression.

Herreros-Villanueva et al. (2011) have demonstrated compelling evidence supporting HER2 gene amplification in colorectal carcinoma. These data appear to speculate that some patients who present HER2 gene amplification could respond to anti-HER2 therapies, such as Trastuzumab.

In our study 82.4%patients had negative HER2 expression. None of the patients had 3(+) strong expression. In the rate of 17.6% 1(+) and 2(+) HER2 expression were determined in our study. We analysed HER2 expression as prognostic and predictive factor in m CRC. We didn't find any corelation between HER2 expression and BV response rate. There wasn't statistically signifacant difference between OS and HER2 expression. Correlation was not found between VEGF expression and HER2 expression. VEGF is one of the most potent inducers of angiogenesis where as HER2/neu has been implicated in the regulation of VEGF. HER2 overexpression is correlated with VEGF expression in breast cancer (Yen et al., 2000; 2002; Yang et al., 2002) but we didn't find any corelation in this study.

HER2 expression led to translational up regulation of VEGF and increased angiogenesis through ERK, PI3K/Akt, mTOR and p70S6K (Yen et al., 2000).

Previous studies demonstrating that the expression of p53 in tumor tissues was associated with the reduced disease-free interval with anti-VEGF monoclonal antibody (Klos et al., 2006; Murad et al., 2007; Li et al., 2011).

The primary function of the p53 protein is as a tumour suppressor. It can induce temporary cell cycle arrest, permitting time for repair of any DNA damage; it can induce apoptosis; and it can impose a permanent block on any future attempts at cell division (Lohrum et al., 2001).

The results from the studies on patients treated with potentially curative surgery again suggest that abnormalities of p53 may have no significant impact upon the response of colorectal cancer to chemotherapy (Lohrum et al., 2001).

We didn't find any correlation between p53 expression and BV response.Statistically significant difference was not found between difference expression rates of p53 and OS. P53 is not associated with poor prognosis in our study

VEGF is a key player in tumor angiogenesis and the target for the monoclonal antibody (MoAb) bevacizumab, which is currently licensed for use in mCRC. Despite numerous studies, an equivalent predictive biomarker for bevacizumab has not been identified. Preclinical work indicates that inhibition of the insulin growth factor receptor (IGFR) pathway stops cellular transformation and tumor regression, thus identifying this pathway as a strong potential target for anticancer drug development and the identification of novel biomarkers. The molecules KRAS, BRAF, NRAS, PTEN, PIP3, VEGF, IGF-1R, and IGF binding protein 3 are discussed. Currently, KRAS is the only biomarker used in clinical practice for mCRC. Pending the results of ongoing and future studies, additional biomarkers will be tested, tailoring our approach to targeted therapy in mCRC (Asghar et al., 2010).

Targeted MoAbs to VEGFR and EGFR are well-established therapies for the treatment of colorectal cancer. The costs and toxicities associated with these novel treatments are not insignificant, and therefore molecular markers that predict treatment efficacy are needed to individualize the therapy administered to each patient (Chua et al., 2009).

We didn't find any correlation between BV response and different biomarkers expression like the literature. VEGF and HER2 expressions rates were correlated each other for our study. VEGF negative expression is associated higher OS rate.

In conclusion, BV is important for treatment in m CRC. We aimed to analyse predictive biomarker for BV treatment. In our study we didn't find any correlation between BV treatment and VEGF, PTEN, HER2 and p53 expresion, we need prospective study.

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