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Hypoxia related growth factors and p53 in preoperative sera from patients with colorectal cancer – evaluation of the prognostic significance of these agents

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Abstract

Background: Insulin-like growth factor-I (IGF-I) and vascular endothelial growth factor (VEGF) belong to a group of hypoxia related proteins. IGF-I induces expression of VEGF and decomposes wild type p53 in cancer cell lines. The goal of our study was to evaluate serum IGF-I, VEGF and p53 with respect to overall and disease free survival of patients with colorectal cancer (CRC) patients compared with healthy volunteers.
Methods: Preoperative blood samples from 125 patients with CRC and 16 healthy volunteers were examined using ELISA for serum IGF-I, p53 and VEGF concentrations.
Results: Concentrations of p53 and VEGF were significantly higher in CRC patients than in controls ($p<0.0006$ and $p<0.0001$, respectively). IGF-I was not statistically different between both groups. Serum IGF-I showed negative correlation with p53 in CRC patients ($p<0.04$, $r=-0.193$). IGF-I and VEGF showed negative correlation in poorly differentiated cancers (G3) ($p<0.03$, $r=-0.339$). Patients with VEGF concentrations that were above average for the cancer population survived for a shorter period of time ($p=0.065$ in evaluation of overall survival and 0.071 in estimation of disease-free survival during a 3-year follow-up) compared with patients with serum VEGF lower than the highest values seen in controls.
Conclusions: Comparisons between serum IGF-I and p53 appear to confirm the metabolism of p53 by IGF-I. Serum VEGF showed prognostic significance in our study. Serum concentrations of IGF-I and VEGF did not show positive correlation, as expected due to IGF-I induction of VEGF in malignant colon cell lines. Clin Chem Lab Med 2009;47:1439–45.

Keywords: colorectal cancer; insulin-like growth factor-I; p53; serum; vascular endothelial growth factor.

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Introduction

Insulin-like growth factor-I (IGF-I) interacts with vascular endothelial growth factor (VEGF) and p53 (1, 2). Both IGF-I and VEGF belong to a group of hypoxia related proteins. Studies of IGF-I are important due to its therapeutic implications in the management of cancer. Particularly, anti-IGF-I agents such as specific antibodies limits the development of hepatic metastases of human colorectal cancer (CRC) (3). CRC is associated with a wide array of various proteins. For example, IGF-I, VEGF and p53 impact tumor growth (4). VEGF is induced by IGF-I CRC cell lines (1, 5). Neoplastic development is restricted by the blood supply to new tumor. VEGF is a major agent that is responsible for development of new capillaries and their differentiation into venules and arterioles in the process of angiogenesis. VEGF has proven to be a valuable prognostic marker for tumor progression, and its role has also been elucidated in colorectal carcinoma (6, 7). Additionally, VEGF was shown to correlate with acute-phase proteins in patients with gastroesophageal cancer (8). Furthermore, preoperative and postoperative serum concentrations of VEGF increase in patients with CRC compared with healthy volunteers (7, 9). These are increased even greater in patients with metastases from CRC (9). In addition, preoperative concentrations were assumed to correspond with disease-specific and disease-free survival (9). There is evidence for synergy of IGF-I and VEGF in the development of the vascular network that correlates with serum concentrations of IGF-I and VEGF in diabetic disorders (10) and polycystic ovary syndrome (11). However, it should be stressed that increases in IGF-I are not always associated with the release of VEGF into circulation, as exemplified by conditions such as acromegaly. In the course of this illness, there was no upregulation of plasma VEGF despite increases in IGF-I (12). In addition, IGF-I degrades wild type p53 in one particular type of NIH-3T3 cells (2). In a

previous study, we showed a close link between preoperative concentrations of p53 and VEGF in sera from patients with CRC (13). As an inhibitor of uncontrolled proliferation, p53 can be considered a good guardian of the human genome. p53 has an indispensable function in repairing cellular defects. In the event that repairs fail, p53 induces apoptosis of abnormal cells. However, p53 cannot be detected in the sera of patients with certain cancers such as lung cancer (14). Overexpression of p53 occurs during progression from benign adenoma to CRC, with a prevalence for left-sided colon cancers (15, 16). Given these findings, our task was to reveal the relationship between IGF-I and VEGF or p53 in sera collected preoperatively from patients with CRC.

Materials and methods

Group of patients

Preoperative blood samples were obtained consecutively from 125 patients with CRC (51 females and 74 males) who qualified for tumor resection. All subjects were citizens of the north-eastern region of Poland. Patients did not have any prior radiotherapy or chemotherapy prior to collection of samples. None of them was reported to suffer from diabetes mellitus. The average age of the entire patient population was 66 years. Patients were divided into those aged >60 years (95 patients) and those 60 years of age or less (30 individuals). Conventional histopathological parameters (including American Joint Committee on Cancer/International Union Against Cancer Tumor Node Metastasis stage (AJCC/UICC TNM stage), tumor type and grade of histological differentiation (G) were assessed independently by two pathologists. According to guidelines of the World Health Organization (WHO), the primary tumors of 81 patients were classified as moderately differentiated cancers (histological differentiation grade (G2) and 44 individuals were determined to be poorly differentiated (G3). A diagnosis of adenocarcinoma (adc) was established in 106 of the cases and the other 19 tumors were mucinous adenocarcinomas (muc adc). We divided patients into two groups according to tumor location: 58 cases were found in the rectum and 67 cancers were located in the colon. We also divided all subjects into two groups according to depth of tumor invasion of intestinal wall (pT) classification: there were 10 subjects with pT1 or pT2 (pT1+pT2) (shallowly invading tumors confined to the submucosa or muscularis propria). One hundred and fifteen primary cancers were classified as pT3 or pT4 (pT3+pT4) (deeply invading tumors that extended through the muscularis propria). The tumor had metastasized to local lymph nodes in 61 cases (N+), while 64 patients were node negative (N–) (Table 1). Blood samples from controls were obtained from 16 healthy volunteers. There was no marked variation in protein concentrations in the control group to warrant further investigation. Half of the healthy volunteers were over 60 years of age. There were equal numbers of women and men in the control group. This study was performed in agreement with the ethical standards in the latest revision of Declaration of Helsinki from 2004 (the approval by the Ethical Committee for studies on animals and humans at Medical University of Bialystok). All the subjects gave their informed consent prior to their inclusion in the study.

ELISA analysis – evaluation of serum levels of the proteins

The serum concentrations of p53, VEGF and IGF-I were measured using ELISA. We used serum for the evaluation of VEGF, although there are numerous reports concerning more precise measurement of this protein in plasma. However, the other proteins were measured in sera. Thus, we decided to be uniform in our investigation with respect to examination of single body fluid. However, plasma VEGF is more representative of circulating VEGF since VEGF concentrations increase due to secretion of VEGF from platelets that are activated during preparation of serum (17, 18). Although VEGF concentrations have been reported to be much lower in plasma compared with serum in patients with CRC, serum concentrations do correlate with plasma concentrations (17, 18). Therefore, this was appropriate for statistical analysis to compare serum VEGF concentrations with other serum proteins because the proportions of VEGF do not change with respect to the type of body fluid examined (17, 18). The ELISA methods employed mouse monoclonal antibody against IGF-I (human IGF-I immunoassay, Catalog Number DG100, Qantikine R&D Systems, Inc. Minneapolis, USA), sheep antibodies against both mutant and wild type of p53 (p53 pan ELISA, Cat. Nr.1 828 789 Roche Diagnostics GmbH, Roche Molecular Biochemicals, Mannheim, Germany) and biotinylated rabbit anti-human VEGF polyclonal antibodies (human VEGF ELISA kit CatNo C-64406, PromoKine, PromoCell GmbH, Heidelberg, Germany). Dilution factors used were in accordance with the manufacturer's instructions. We followed the manufacturer's protocol for measurement of serum p53 (Roche Diagnostics, Germany). Samples were allowed to clot, centrifuged and diluted. For measurement of VEGF, samples were diluted in standard as recommended by the manufacturer (PromoCell GmbH, Heidelberg, Germany). For IGF-I, serum samples were pretreated with blue dye and an acid solution which required taking this dilution factor into consideration (R&D Systems, Inc. Minneapolis, USA). ELISA procedures were performed separately for each protein using different plates. The biotin-labeled antibody that was attached to streptavidin-covered microtiter plates bound the p53 molecules in 100 μL serum. This was followed by the addition of a peroxidase labeled polyclonal sheep antibody against p53 (700 μL of anti-p53-POD prediluted, Roche Diagnostics, Germany). The antibody detected both mutant and wild type p53. VEGF molecules (using 25 μL of its solution) reacted with biotinylated rabbit anti-human VEGF polyclonal antibody. Fifty μL of pretreated serum was incubated with mouse monoclonal antibody against IGF-I coated onto the wells of a microtiter plate. The wells were washed several times. Different types of peroxidase dyed p53 and IGF-I reacted with substrate – tetramethylbenzidine (TMB) (200 μL of substrate solution). For VEGF, a red product (formazan) appeared after dephosphorylation of NADPH to NADH. Color reactions were inhibited with stop solutions. The absorbances were evaluated using appropriate calibration curves and converted to p53, VEGF and IGF-I concentrations. Measurements were performed in duplicate.

Statistical analysis

Correlations were examined for bivariate associations using Spearman's rank correlation. p-Values <0.05 were considered to be statistically significant. Highly significant correlation was considered in cases where coefficients of correlation (r) had values of 0.5 or greater. Kaplan-Meier analysis was performed to investigate overall survival rates

Table 1 Clinical and pathological characteristics of patients.

Groups of patients	n	%
N		
(–)	64	51.2
(+)	61	48.8
G		
2	81	64.8
3	44	35.2
pT		
pT1+pT2	115	92
pT3+pT4	10	8
HP type		
Adc	106	84.8
Adc muc	19	15.2
Gender		
Males	74	59.2
Females	51	40.8
Age		
≤60 years	30	24
>60 years	95	76
Site		
Rectum	58	46.4
Colon	67	53.6

N, lymph node involvement; G, grading of cancer differentiation; pT, depth of cancer invasion of intestinal wall; HP type, histopathological type; adc, adenocarcinoma; adc muc, mucinous adenocarcinoma.

of patients with higher and lower serum concentrations of the proteins measured. The control mean concentrations of proteins were used for classification of cases as low or high in the estimation of overall survival. This was performed for the 36 patients that expired by the end of this study.

Kaplan-Meier analysis was also used to evaluate statistical significance in disease-free survival rates of patients with higher and lower serum levels the measured proteins compared with the mean values in the cancer population during a 3-year period. The mean concentrations of proteins in controls were used as cut-off thresholds for establishing cases with low or high values in the estimation of disease-free survival.

Results

Comparison of serum p53, IGF-I and VEGF between CRC patients and control group

Concentrations of p53 and VEGF were significantly higher in the serum of patients with CRC compared with controls ($p<0.0006$ and $p<0.0001$, respectively). IGF-I did not differ significantly between both groups ($p>0.05$).

Separate comparison of serum p53, IGF-I and VEGF in CRC patients

CRC patients who were 60 years or age of younger had significantly higher concentrations of IGF-I compared with patients over 60 years of age ($p<0.0001$). Sera from male patients with CRC contained more IGF-I, on average, than females ($p<0.003$). There was no relationship between the proteins investigated with respect to node involvement (N), depth of tumor invasion of intestinal wall (pT), grading (G), location of primary CRC, histological type of cancer. Comparisons of p53 or VEGF concentrations were not significant between groups according to age and gender (Table 2).

Comparison of IGF-I to VEGF in CRC groups of different clinicopathologic features

IGF-I did not correlate with VEGF in sera of CRC patients and in the subgoups of lymph node status, pT stage, tumor grade, histological type, age and location of tumor.

Poorly differentiated cancers (G3) were associated with a trend toward highly significant negative correlation between concentrations of IGF-I and VEGF ($p<0.03$, $r=-0.339$) but no statistical significance in patients with G2 tumors (Table 2).

Linkages of IGF-I and p53 in CRC groups of different clinicopathologic features

Serum values of p53 and IGF-I correlated negatively for the entire group of CRC patients ($p<0.04$, $r=-0.193$) and individuals with pT3 or pT4 ($p<0.05$, $r=-0.187$). There was an trend towards negative correlation but without statistical significance in pT1 or pT2 tumors ($r=-0.546$, $p>0.05$). Comparison of serum p53 and IGF-I was statistically significant but failed to display a negative correlation in CRC patients with adc ($r=-0.205$, $p<0.04$). Subjects with mucinous carcinoma did not show any linkage of this kind. A non-significant relationship was seen between p53 and IGF-I in sera from male patients ($r=-0.230$, $p=0.050$), and no relationship seen in female patients with CRC. Concentrations of p53 and IGF-I showed negative correlation but no statistical significance between p53 and IGF-I in CRC patients' lymph node involvement ($r=-0.242$, $p=0.065$), in CRC subjects more than 60 years of age ($r=-0.202$, $p=0.067$) and in individuals with colon tumors ($r=-0.233$, $p=0.064$). There was no significant relationship between p53 to IGF-I in groups of N– patients, younger individuals or those with rectal tumors (Table 2).

Survival of patients in groups of low and high levels of studied proteins

For survival rates of patients grouped according to low or high serum concentrations of the proteins studied, we noted that patients with VEGF concentrations above average for the cancer population had shorter survival, achieving almost statistical significance ($p=0.065$ in evaluation of overall survival) compared with patients with VEGF concentrations below the mean (~6 pg/mL in healthy controls) (Figure 1). We also evaluated disease-free survival between patients with VEGF concentrations lower or higher than mean serum values seen in the cancer population during 3 years and 9 months of follow-up ($p=0.071$) (Figure 2). Similar analyses were performed for p53 and IGF-I with no trend toward statistical significance. No statistical difference was found in the evaluation of differences in survival between groups with increased or low marker concentrations

Table 2 Comparison between IGF-I and other proteins from sera obtained preoperatively from patients with CRC. Spearmann's correlation test.

Groups of patients	Mean levels and SD			IGF-I and VEGF		IGF-I and p53	
	IGF-I	VEGF	p53	p-Value	r	p-Value	r
Control group	81.47±37.38	5.93±1.15	16.75±18.44	NS	−0.393	NS	0.020
All CRC patients	79.99±37.4	128.35±146.0	181.39±382.95	NS	−0.141	<0.04	−0.193
N							
(−)	83.3±39.57	113.47±124.46	144.13±312.69	NS	−0.042	NS	−0.123
(+)	76.51±34.96	144.22±165.63	220.54±444.46	NS	−0.218	0.065	−0.242
G							
2	77.56±28.65	115.73±116.91	161.90±276.09	NS	0.085	NS	−0.171
3	85.76±49.56	188.75±193.12	220.26±532.45	<0.03	−0.339	NS	−0.232
pT							
pT1+pT2	65.07±13.15	158.52±171.34	219.67±285.54	NS	−0.433	NS	−0.546
pT3+pT4	81.28±38.55	125.7±144.2	178.31±390.56	NS	−0.129	<0.05	−0.187
HP type							
Adc	75.84±29.69	131.30±149.23	198.36±410.06	NS	−0.080	<0.04	−0.205
Adc muc	103.13±61.84	112.04±129.49	84.28±120.68	NS	−0.340	NS	−0.228
Gender							
Males	86.14±31.50	127.89±146.25	170.32±323.13	NS	−0.077	<0.05	−0.230
Females	71.05±43.38	129.03±147.23	198.23±462.60	NS	−0.216	NS	−0.161
Age							
≤60 years	105.33±47.46	106.66±115.26	66.10±78.16	NS	−0.149	NS	−0.034
>60 years	72.31±29.78	138.15±158.78	226.11±450.04	NS	−0.128	0.067	−0.202
Site							
Rectum	77.74±30.40	94.95±117.87	152.63±289.38	NS	−0.155	NS	−0.112
Colon	81.93±42.67	157.70±162.21	207±451.10	NS	−0.156	0.067	−0.233

CRC, colorectal cancer; SD, standard deviation; IGF-I, insulin-like growth factor-I; VEGF, vascular endothelial growth factor; NS, not significant; N, lymph node involvement; G, grading of cancer differentiation; pT, depth of cancer invasion of intestinal wall; HP type, histopathological type; adc, adenocarcinoma; adc muc, mucinous adenocarcinoma.

with respect to gender, younger or older patients, G3, G2 tumors, pT1+2, pT3+4 cancers and groups of other different clinical and pathological features. There were no differences in survival time for IGF-1 or p53 with respect to different age groups. Median values and interquartile range (IQR) of proteins are presented in Table 3. Receiver operating characteristic (ROC) curve analysis did not identify survival on the basis of VEGF, IGF-1 or p53 concentrations (data not shown).

Discussion

Increases in circulating IGF-I predict development of colon cancer (19). Growth of murine, cecum adcs was

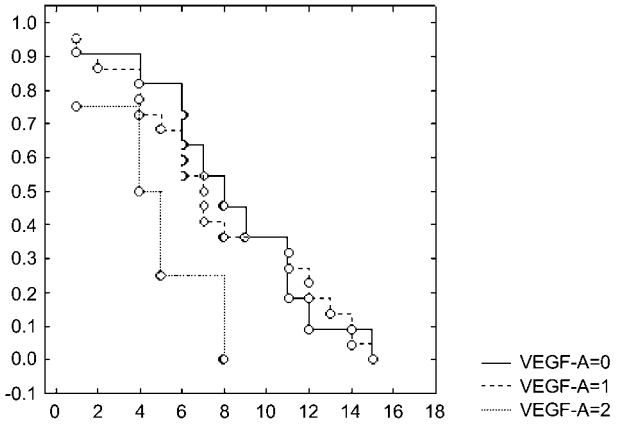

Figure 1 Kaplan-Meier plot showing overall survival. p=0.065 between group VEGF-A=2 and VEGF-A=0. VEGF-A=2, higher concentrations of VEGF compared with the average concentration of VEGF in all cancer patients; VEGF-A=1, VEGF in cancer patients greater than mean concentrations of VEGF seen in controls, but less than average VEGF concentrations seen in all cancer patients; VEGF-A=0, VEGF concentrations in cancer patients less than the mean VEGF concentration seen in controls; y-axis, cumulative number of survivors; x-axis, survival time in trimesters.

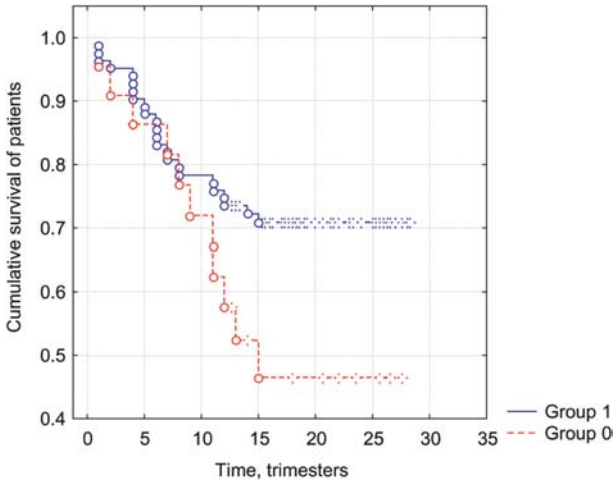

Figure 2 Kaplan-Meier plot showing disease-free survival. p=0.071. Group 0, lower serum concentrations of VEGF compared with the mean concentrations seen in all patients with cancer. Group 1, higher serum concentrations of VEGF compared with the mean concentrations seen in all patients with cancer. y-axis, cumulative number of survivors; x-axis, survival time in trimesters.

Table 3 Median values and IQR of proteins in sera obtained preoperatively from patients with CRC.

Groups of patients	IGF-I		VEGF		p53	
	Median	IQR	Median	IQR	Median	IQR
Control group	80.9	32.375	6	0	3	32.25
All of CRC patients	74.7	44.65	71.2	182.25	52	131.5
N						
(–)	75.6	41.225	65.3	174.3	48	79
(+)	69.7	49.3	79.5	178.8	58	187
G						
2	73.1	33.725	65.9	196.475	51.5	144
3	77.75	61.775	78.9	167.775	51.5	96.5
pT						
pT1+pT2	69.8	19.8	77.1	204.25	163	306.5
pT3+pT4	75.05	47.9	70	180.45	51.5	113
HP type						
Adc	73	44	73.6	194.1	52	141
Adc muc	100.8	60.35	59.95	128.2	36	86.75
Gender						
Males	78.8	39.9	73.6	159.8	51	141.5
Females	63.85	34.375	59.4	203.4	53	117
Age						
≤60 years	99.5	56.3	66.5	145.6	46	62
>60 years	69.9	37.3	73.6	201.2	52	182
Site						
Rectum	74.7	39.25	52.3	120.8	52	122
Colon	74.7	49.15	84.25	162.8	50	138

IQR, interquartile range; CRC, colorectal cancer; IGF-I, insulin-like growth factor-I; VEGF, vascular endothelial growth factor; N, lymph node involvement; G, grading of cancer differentiation; pT, depth of cancer invasion of intestinal wall; HP type, histopathological type; adc, adenocarcinoma; adc muc, mucinous adenocarcinoma.

more extensive in control mice than in liver-specific IGF-I-deficient (LID) mice due to administration of recombinant human IGF-I (19). In particular, the speed of neoplastic enlargement was slower in LID mice. Similarly, liver metastases from colon cancer were less frequent in LID mice. Furthermore, most relevant to our study, tumor immunoreactivity for VEGF and vessel density correlated with serum IGF-I concentrations (19). Transplantation of carcinomatous fragments resulted in development of cancer in smaller number of LID mice in comparison with a homologous amount of tumors in control mice. IGF-I forces neoplastic progression via exertion of VEGF synthesis in human CRC (1, 5). However, our findings contradict any positive relationship between IGF-I and VEGF in humans. The influence of IGF-I on colonic tumor growth was suggested to be due to multiple factors (4, 19). Studies of cultured cell lines were the first to show linkage between IGF-I stimulation and VEGF expression in human CRC (4). IGF-I stimulated transcription of VEGF mRNA five-fold in COLO 205 cells. IGF-I also augmented stability of VEGF mRNA after exposure to actinomycin. Striking mismatches were revealed by experiments using a monoclonal antibody against type I IGF receptor (αIR3). The antibody significantly impaired the capability to promote VEGF mRNA synthesis. However, it caused tyrosine phosphorylation of the β-subunit of the IGF-I receptor. Most likely, cell proliferation and VEGF expression are maintained via different pathways that are turned on by IGF-I (4). In addition, we reported a significant positive correlation between IGF-I and Bax or Bak in previous work (20). That would suggest that IGF-I could counteract survival of malignant cells. We did not find any linkage between VEGF and IGF-I in sera from patients with CRC. However, grade G3 was associated with a negative trend between IGF-I and VEGF concentrations. Thus, the relationship between IGF-I and VEGF during tumor growth is not clear and suggests that further studies are needed.

It is widely known that CRCs develop faster and more aggressively if they occur early in a life (21). However, hereditary non-polyposis colorectal cancers (HNPCC) cancer, found in relatively younger patients (mean age of 45), is associated with overall improved survival compared with CRC in the elderly (22, 23). Our dataset included 30 patients 60 years of age or less. However, all but four patients were above 45 years of age. Thus, they do not fit to characteristics for HNPCC patients. Evaluation of microsatellite instability status would help classifying these patients into the HNPCC group. However, after closer analysis of the patient's age, such an approach would only produce data on mismatch repair genes in few patients and the results would not be statistically sound. Although a high-frequency of MSI (MSI-H) was reported to characterize most HNPCC cases, MSI-H was also found in non-HNPCC cases (24).

Our data showing significantly increased serum IGF-I values can underlie the acceleration of tumor growth in younger patients. As IGF-I induces anabolic processes, it is not surprising that IGF-I significantly increases in the sera of men with CRC that usually develop greater muscle mass compared with women with CRC. It has been reported that low caloric diet and physical effort could greatly decrease serum IGF-I

in rodents and significantly slow down development of neoplasms in p53 null homozygous mice and p53 heterozygous mice (25).

If 4-nitroquinoline 1-oxide damaged DNA, IGF-I switched on extranuclear transport and a proteasomic mdm2-dependent decomposition of p53 in the cytoplasm of NIH-3T3 cells that overexpressed IGF-IRs (NWTb3 cells). IGF-I indirectly abolished p53-dependent induction of p21WAF1 that stopped the cell cycle in G1 phase. The downregulation of p53 did not preserve all DNA abnormalities in descendant cells since IGF-I activated an *ERCC-1* gene which mediated repairs to DNA damage (26, 27). Overexpression of p53 was usually observed when this protein was inactive due to mutations (28). This mutant p53 could be a target for an increased degradation of this protein by IGF-I. Therefore, IGF-I dependent upregulation of VEGF expression4 might be reversed as wild-type p53 decreases VEGF expression (29). However, mutant p53 upregulated VEGF (30, 31). This could explain the trend toward a highly significant negative correlation between IGF-I and VEGF. Concerning knockout of p53 action, it is not surprising that IGF-I accelerated oncogenesis of murine mammary tumors (27). IGF-I prolonged the life span of cells via enhancement of Bcl-2 production and increases in the Bcl-2-to-Bax protein ratio, which is a consequence of mdm2-dependent decreases in p53 activity (32). The statistically significant differences that indicated negative correlation between serum IGF-I and p53 agreed with previously reported IGF-I dependent attenuation of p53. In a previous study (13), we inferred that there is some correlation between IGF-I and p53 in colorectal cancer. In the current paper, we present a detailed comparison of IGF-I with p53 in patients with CRC. In cancer patients, p53 was commonly mutated and this mutant protein induced overexpression of IGF-IR on malignant cells (33). Therefore, even decreased concentrations of IGF-I in serum was enough to stimulate malignant cells by IGF-IR due to upregulation of the receptor.

Regarding the impact of these proteins on the prognosis of CRC, it is well known that VEGF is a well-established indicator of poor survival. VEGF measured in preoperative serum was a significant predictor of tumor recurrence in patients with CRC (34). We demonstrated that CRC patients with higher serum VEGF survived for shorter periods of time compared with patients with VEGF concentrations that were lower than the highest value for the control group. To our knowledge, this comparison is the first to be observed in patients with CRC. Our results are consistent with other publications that reported on the negative prognostic significance of VEGF in CRC (35, 36).

The tendency toward negative correlation between serum concentrations of IGF-I and p53 seemed to confirm indirectly, in association with certain clinicopathological features, experiments on cell lines that revealed IGF-I dependent attenuation of p53. Serum concentrations of IGF-I and VEGF did not show positive correlation in cases of poorly differentiated tumors (G3), and instead revealed a negative relationship. In our opinion, this report adds further information concerning the role of IGF-I. Further studies to determine the nature of IGF-I in CRC are warranted (37). In CRC, therapies are more effective if they are combined (38). Studies have shown the role that IGF-I and VEGF play in therapy (3, 38). Our study showed the negative impact of VEGF on the prognosis of CRC and justifies clinical application of VEGF antibodies. However, our work did not show prognostic significance for IGF-I and does not support use of IGF-1 antagonists for the management of CRC.

Conflict of interest statement

All the authors declare they have no conflict of interest.

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