RESEARCH ARTICLE

Association of IGFBP-6 Expression with Metabolic Syndrome and Adiponectin and IGF-IR Receptor Levels in Colorectal Cancer

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Abstract

Purpose: To assess IGFBP-6 expression in relation with the presence of the metabolic syndrome, adiponectin receptors (AdipoR1 and AdipoR2) and IGF-IR levels in colorectal adenocarcinoma cases. Materials and Methods: IGFBP-6 mRNA and protein levels were analyzed using real-time quantitative PCR and Western blotting in 46 patients. ELISA and flow cytometry were used for evaluation of AdipoR1, AdipoR2 and IGF-IR. Results: The results showed that IGFBP-6 mRNA expression and the IGFBP-6 content were higher in tumor tissue samples of colorectal cancer patients with tahtn without metabolic syndrome. In addition, the IGFBP-6 mRNA expression was associated with tumor invasion (tumor size) and the IGFBP-6 protein level was associated with nodal status. Positive correlations and positive nonlinear relations were found between the IGFBP-6 level and the AdipoR1 and AdipoR2 contents in colorectal cancer patients. Conclusions: The IGFBP-6 mRNA level and protein level were found to be associated with presence of metabolic syndrome. Positive correlations indicated probable cross-talk between the IGF-IR-mediated and adiponectin-mediated signaling pathways in colorectal carcinomas. IGFBP-6 may be considered as a potential biomarker associated with lymphogenous metastasis and the metabolic syndrome in colorectal cancer.

Keywords: Colorectal cancer - insulin-like growth factor binding protein - metabolic syndrome - AdipoR - IGF-IR

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Introduction

The incidence of colorectal cancer tops the list among the most common malignant tumors, and its mortality is also very high. Each year, nearly 1,000,000 new cases of colorectal cancer are diagnosed and there are 500,000 deaths from this disease in the word (Resch and Langer, 2013). Despite the advances in colorectal cancer surgery, combined treatment application with or without the targeted therapy of colorectal cancer, survival among the patients remains low. The identification of cancer progression molecular mechanisms becomes the most promising approaches for improving the prognosis of the colorectal cancer patients.

It is believed there are a great abundance of cellular processes involving in oncogenesis of colorectal cancer. Among them, the insulin-like growth factor (IGF) signaling system could play an important role. The IGF system is composed of insulin-like growth factors (IGF-I and IGF-II), their receptors (IGF-IR and IGF-II/mannose-6-phospate receptor) and six high-affinity IGF binding proteins (IGFBPs) (Firth and Baxter, 2002). It is presented as an important modulator of cancer cells proliferation, their survival, facilitating in cell motility and growth. It the widely discussed the role of IGF associated proteins in multistep process of tumor invasion and metastasis formation (Samani et al., 2007). It is known the IGF-II could acts as autocrine growth factor in some tumors and tumor cell lines. The increased IGF-II expression has been detected in some solid tumors compared with non-transformed tissues (Minniti et al., 1995). Moreover, the IGF-II has been found more frequently in cancers comparing to IGF-I, and its level is higher in transformed tissues (Yunusova et al., 2013; 2015). At present the IGFBP-6 is considered as unique peptide among the IGFBPs through its in 30 to 100-fold higher binding affinity for growth factor IGF-II comparing to IGF-I (Leng et al., 2001; Bach et al., 2013). The IGFBP-6 regulates the insulin growth factor actions and may be associated with the metabolic syndrome. In addition, the IGFBP-6 inhibits neoangiogenesis, modulates cell motility, adhesion and apoptosis in an IGF-II independent manner (Leng et al.,

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2001; Bach et al., 2013). The decreased production of IGFBP-6 in transfection colon cancer cell lines leads to stimulation of cell proliferation (Kim et al., 2002).

The emergence and progression of colorectal cancer often occurs in patients with the metabolic syndrome (Zhou et al., 2007). It is believed the effect of metabolic syndrome on tumor cells mediated by adipose tissue hormone (leptin, adiponectin) and their receptors. The adiponectin acts by binding with their receptors (AdipoR1 or AdipoR2) through the activation of AMPK, mTOR, PI3K/Akt, MAPK, PPAR-α, STAT3, and NF-kB (Sugiyama et al., 2009; Mauro et al., 2015). The anti-apoptotic and growth stimulatory actions of leptin in human colon cancer cells involve the activation of JNK mitogen activated protein kinase, JAK2, PI3kinase/Akt, and HIF-1 (Ogunwobi and Beales, 2007). It has been demonstrated the IGF-IR activation leads to the increase of growth, proliferation and survival of tumor cells, altering the expression of specific genes regulated through the activation PI3kinase/Akt and MAPK pathways. So, the IGF-dependent signaling pathway, adiponectin and leptin signaling pathways are cross-talked. Moreover, genes encoding the IGF-II, IGFBP-2, IGFBP-3 are HIF-1 target genes and the expression of many growth factors (e.g. IGF-II) are under the control of NF-ϰB (Spirina et al., 2012). There are data about association between the adiponectin and IGF-IR in breast cancer. It is noted these interactions are different in the ERα-positive and the ERα-negative breast cancer cells (Mauro et al., 2015). However, in colorectal cancers these interactions remain unclear. In a prospective case-control study, Wei et al. have showed a greater risk of colorectal cancer among patients with low plasma adiponectin levels (Ayyildiz et al., 2014). Obesity-linked down-regulation of adiponectin has been suggested to be a mechanism whereby obesity could cause insulin resistance and diabetes. The obesity decreases expression of the adiponectin receptors AdipoR1/AdipoR2, thereby reducing the adiponectin sensitivity, which leads to the insulin resistance (Zhou et al., 2007). These metabolic disturbances are typical for most colorectal cancer patients. It can be assumed that some intracellular proteins (IRS-1 - insulin receptor substrate and APPL-1 - adaptor protein, AdipoR1/AdipoR2 binding protein) and extracellular IGFBPs including IGFBP-6 will be perspective for the identifying the possible target proteins involved in the adiponectin-mediated and the IGF-IR-mediated signaling pathways in colorectal carcinomas.

Objective: The objective of this study was to investigate the expression of IGFBP-6 in colorectal adenocarcinoma in relation with clinical parameters (including with the presence or absence of the metabolic syndrome) and AdipoR1, AdipoR2 and IGF-IR levels for understanding involvement of IGFBP-6 in colorectal tumor progression and for evidence of possible association with adiponectin-mediated signaling.

Materials and Methods

Patients and tissues: The study included 46 colorectal cancer patients with Stage T2-4N0-2M0 (twenty women and twenty sixth men, mean age 61.2±1.59). All patients were treated at the Tomsk Cancer Research Institute (Tomsk, Russian Federation) from January 2012 to March 2015. The study was approved by the Local Committee for Medical Ethics and all patients provided written informed consent. The study conforms with The Code of Ethics of the Word Medical Association (Declaration of Helsinki). At the first stage all cancer patients underwent radical surgery with lymph node dissection. Standard adjuvant chemotherapy was administrated patients with presence of lymph nodes involvement (N1-2). All tumors presented as adenocarcinomas. The patients had no history of familial malignancy or other synchronous malignancy (such as ovarian cancer, prostate cancer, breast cancer). All patients with colorectal cancer, depending on the presence of the metabolic syndrome were divided into two subgroups: with metabolic syndrome (28 pts.) and without metabolic syndrome (18 pts.). The criteria for inclusion in the group with metabolic syndrome taking into account the recommendations of the International Diabetes Federation (2005) was the presence of abdominal type of obesity (waist circumference greater than 94 cm for men and 80 cm for women - at Caucasians) in combination with at least two of the four additional criteria: increase blood serum triglyceride level of more than 1.7 mmol/l or previously treated dyslipidemia; reduction in high density lipoproteins less than 1.03 mmol/L for men and 1.29 mmol/L for women; high blood pressure (systolic over 130 mm. Hg, or diastolic more than 85 mm. Hg, or hypertension therapy employed); increase in fasting blood glucose more than 5.6 mmol/L, or to identify diabetes mellitus type II. Clinical and histopathological parameters of patients are presented in the Table 1. Fresh colorectal cancer and adjacent non-tumor tissues were obtained from cancer patients during surgery. For IGFBP-6 mRNA expression study tumor and non-tumor tissues samples were immediately immersed in RNAlater (Ambion Inc., USA) and stored at -80 oC. For preparing of tissues homogenates for ELISA and Western blotting analysis fresh cancer samples were aliquot and stored at -80°C.

RNA extraction: Total RNA from 45 tumor samples was extracted using RNeasy Mini Kit (Qiagen). The total RNA concentration and quantity were determined based on the absorbance at 260 nm using a NANO DROP spectrophotometer (ThermoScientific, USA). To assess RNA integrity, RIN was measured using 2200 TapeStation Instrument and R6K ScreenTape (Agilent Technologies, Inc., Santa Clara, USA). Thermo Scientific RevertAid First Strand cDNA Synthesis Kit was used for efficient synthesis of first strand cDNA from RNA templates («Thermo Fischer Scientific», USA).

RT-qPCR: PCR was conducted in 25 µl reaction volume, containing 12.5 µl Maxima SYBR GreenqPCR Master Mix (2X) and 300 nanoM of each primers (IGFBP6 (forward primer, 5'-AGAAAGAGCTGGTGCATGATGAC-3'; reverse primer, 5'-TGCTGGATGCCTGCTCAA-3'), and GADPH (forward primer, 5'-TCTCCTCTGACTTCAACAGCGAC-3'; reverse primer, 5'-CCCTGTTGCTGTAGCCAAATTC-3'). A pre-incubation at 95°C for 10 min was to activate the Hot Start DNA polymerase and denature DNA, and was followed by 45 amplification cycles of 95°C denaturation for 10

sec, 60°C annealing for 20 sec (iCycler iQ™ , BioRad).

A ratio of specific mRNA/GADPH (GADPH as a respective control) amplification was then calculated (ΔCt vaule = CtIGFBP6 - CtGADPH), to correct for any differences in efficiency. The fold changes were calculated with the ΔΔCt method (the total ΔΔCt = fold of cancerous/normal tissue gene level), using normal tissue.

Western blotting analysis. Preparing tissue homogenates. All procedures were performed at 4°C. Frozen samples of tumor and adjacent tissues were homogenized and then resuspended in 50 mM Tris-HCl (pH 7.5), 100 mM NaCl, 1 mM EDTA, 1 mM dithiothreitol, 10% glycerol, 10 mM Na2S2O5 in the ratio 1:6 (w:v). Homogenates were centrifuged at 10,000 g for 30 min. Protein concentration in cleared homogenates was determined by the method of Lowry et al. Electrophoresis. SDS-PAGE was used, according to the method of Laemmli. The samples were incubated for 5 to 10 min in 62.5 mM Tris-HCl buffer (pH 6.8), containing 2.0% (w/v) SDS, 5.0% (v/v)/3-mercaptoethanol, 10% (v/v) glycerol, and 0.0012% Bromophenol blue. Western Blot Analysis. After SDS-PAGE, the gels were equilibrated for 10 min in 25 mM Tris and 192 mM glycine in 20% (v/v) methanol. The proteins were transferred to 0.2-/xm pore-sized PVDF membrane (GE Healthcare, UK), either at 150 mA or 100 V for 1 h using Bio-Rad Mini Trans-Blot electrophoresis cell according to the method described in the manual accompanying the unit. The PVDF membrane was incubated with monoclonal mouse anti-human anti-IGFBP-6 (at concentration 1-5 μg/ml, Abcam, UK) overnight at 4ºC, followed by three consecutive washes in 10 mM Tris-HCl buffer (pH 7.5), containing 150 mM NaCl (10 min/wash). The PVDF membrane was incubated in a 1:10000 dilution of anti-mouse antibodies for 1 h. After three more 10-min washes, the PVDF samples were incubated in Amersham ECL western blotting detection analysis system according to the method described in the manual accompanying the unit and then were exposed to ECL-films (Amersham, USA). The image analysis was performed using "ImageJ" software. Results were standardized for beta-actin levels. The content of peptides in cancer tissues was expressed in percentages to their level in non-transformed tissues, with the expression of studied proteins indicated as 100%.

ELISA: Tumor samples (100 mg) were homogenized and then resuspended in 300 lL of 50 mM Tris–HCl buffer (pH = 7.5) containing 2 mM ATP, 5 mM MgCl2, 1 mM dithiothreitol, 1 mM EDTA, and 100 mM NaCl. The homogenate was centrifuged at 10,0009g for 60 min at 4 oC as previously described [14]. The levels of adiponectin receptors (AdipoR1 and AdipoR2) were evaluated in the tumor homogenates using the ELISA kits (Human AdipoR1 and Human AdipoR2, Cusabio) in Anthos 2020 microplate reader. The protein concentration in homogenates and nuclear extracts was determined by Lowry.

Flow cytometry: Preparing cell suspension. Samples of colorectal cancer tissues were disintegrated on Becton Dickinson (BD) Medimashine System using BD "Cell Wash'' buffer. Cell suspension was filtrated through a 50 μm Syringe Filcons nylon filter. This procedure is optimal for cell suspensions from tissues due to the absence of their contamination. Number of cells was counted in BD Trucount tubes (BD, USA) by flow cytometry. Flow cytometry. Extracellular staining was performed with CD221 (IGR-IR) phycoerithrin (PE) antibody (BD). For intracellular staining cells were fixed and permeabilized using Cytofix/Cytoperm kit (BD, USA) (standard protocol). Aliquots of cell suspensions were incubated with conjugated antibodies at 200 C for 30 minutes. As primary antibody we used anti-cytokeratin 20 fluorescein isothiocyanate (FITC) (BD, Santa Cruze). Results were analyzed using the FACS Diva 6.1. software. percentage of cells specifically stained for IGF-IR was analyzed (%).

Statistical analysis: Statistical analysis was performed using Statistica 10.0 software. All data are expressed as medians with interquartile ranges or as means with standard errors. To evaluate the difference either Mann-Whitney or Kruskal-Wallis test was applied. Correlation analysis on data was carried out with Spearman Rank Correlation test. The level of significance was set at $p < 0.05$. Nonlinear regression was used to determine the contribution of error-free independent variables to the variation of observed dependent variables.

Results

The relative IGFBP-6 mRNA levels depending on depth of tumor invasion, the presence of nodal metastases, tumor Grade and presence of metabolic syndrome is shown in Table 2. The relative IGFBP-6 mRNA level in colorectal carcinomas was associated with tumor invasion (tumor size) and was higher in patients with metabolic syndrome compared to patients without metabolic disorders. The protein level of IGFBP-6 in cancer tissue measured by Western blotting as a percentage to the content of IGFBP-6 in non-transformed tissue is presented in Table 3 and Figure 1. The level of IGFBP-6 expression in colorectal cancer tissues in % of non-transformed tissue averaged 116 (57- 145) %. In 56% of cases, the IGFBP-6 level was higher in the tumor tissue compared

Table 1. Clinical and Histopathological Parameters of Colorectal Patients

Parameters	N	%
Sex:		
Male	26	56.5
Female	20	43.5
Tumor localization:		
Colon	34	73.9
Rectosigmoid junction	10	21.7
Rectum	2	4.4
Tumor invasion (T):		
T2-3N0-2M0	28	60.9
T4aN0-2M0	18	39.1
Nodal status:		
T2-4aN0M0	31	67.4
T2-4N1-2M0	15	32.6
Extracapsular lymph node invasion:		
Yes	5	33.3
No	10	66.7
Presence of metabolic syndrome:		
Yes	28	60.9
No	18	39.1

Note: N – number of patients

to normal tissue, in 44% - lower. There was only tendency to increase protein IGFBP-6 levels in patients with tumor size T4a compared to T2-3 colorectal cancer patients. Colorectal cancer patients without nodal metastases had a significantly higher level of IGFBP-6 compared to patients with nodal metastases. Thus, the relative IGFBP-6 mRNA level and IGFBP-6 expression were higher in patients with metabolic syndrome compared with non-obesity ones. In addition, the IGFBP-6 mRNA expression was associated with tumor invasion (tumor size) and the IGFBP-6 protein level was associated with nodal status.

The mean level of receptor AdipoR1 in cancer tissues was more than 2 times higher than the level of AdipoR2 - 54.4 (34.1-83.0) ng/mg of protein and 24.9 (18.1-49.0)

Table 2. Relative IGFBP-6 mRNA Levels in Colorectal Cancer: Association with Clinical and Histopathological Parameters

Parameters	Relative IGFBP-6 mRNA levels, M±m	p-value
Tumor invasion (T):		
T2-3	38.1±6.26	p<0.05
T4a	69.2±14.0	
Lymph node involvement:		
N0	49.6±8.60	>0.05
N1-2	53.1±11.1	
Tumor Grade:		
G1	53.2±8.00	>0.05
G2	43.0±6.01	
G3	37.2±15.1	
Presence of metabolic syndrome:		
Yes	59.9±7.70	p<0.05
No	34.0±7.00	

Note: M – mean, m – standard error of mean.

Table 3. Levels of IGFBP-6 in Colorectal Cancer: Association with Clinical and Histopathological Parameters

Parameters	Level of IGFBP-6 (in %), Me (25%-75%)	P
Tumor invasion (T):		
T2-3	139 (63-141)	p>0.05
T4a	152 (54-310)	
Lymph node involvement:		
N0	134 (60-250)	p<0.05
N1-2	68 (56-99)	
Tumor Grade:		
G1	137 (116-142)	p>0.05
G2	96 (54-148)	
G3	98 (47-164)	
Presence of metabolic syndrome:		
Yes	140 (67-250)	p<0.05
No	89.2 (27-134)	

Note: The results represent the Me (Q1-Q3)

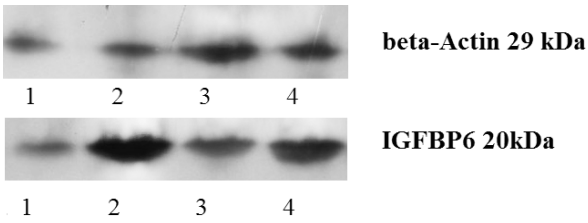

Figure 1. The IGFBP-6 Content in Colorectal Cancer Tissues. Western Blotting IGFBP6 in tumor and intact tissue of patients with colorectal cancer. Note: 1, 3 - proteins expression in the tumor; 2, 4 - expression of proteins in control tissue

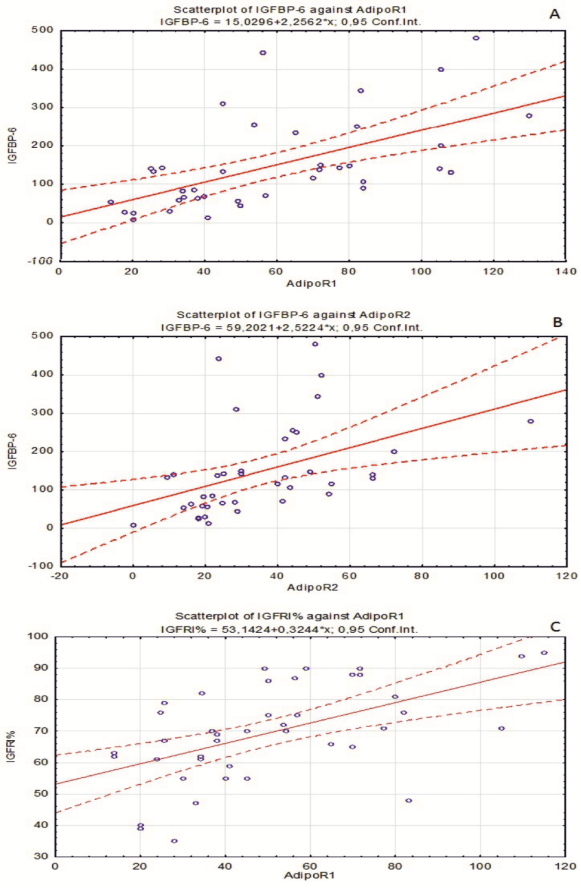

Figure 2. Scatter Plots of Correlation Analysis. (A) the IGFBP-6 content and AdipoR1 level; (B) the IGFBP-6 content and the AdipoR2 level; (C) the IGF-IR level and the AdipoR1 level in colorectal cancer samples. Solid line - trend line, dashed line - the 95% confidence interval

Table 4. Nonlinear Relations of IGFBP-6 Levels with AdipoR1 and AdipoR2 Contents in Tumors of Colorectal Cancer Patients

Regression Summary for Dependent Variable: IGFBP-6 R= 0.58039512 R2= 0.33685849 Adjusted R2= 0.30101300 p<0.00050					
	Beta	Std.Err. - of Beta	B	Std.Err. - of B	p-level
Intercept			9.076028	42.53539	0.0932205
AdipoR1	0.557268	0.239298	2.876455	1.23519	0.025442
AdipoR2	0.027654	0.239298	0.270918	2.34437	0.908625

Nonlinear regression was used to determine the contribution of error-free independent variables to the variation of observed dependent variables. The data consist of a mean given by a nonlinear function f(x,β), which are presented as standardized and unstandardized regression coefficients, their standard errors and p-level

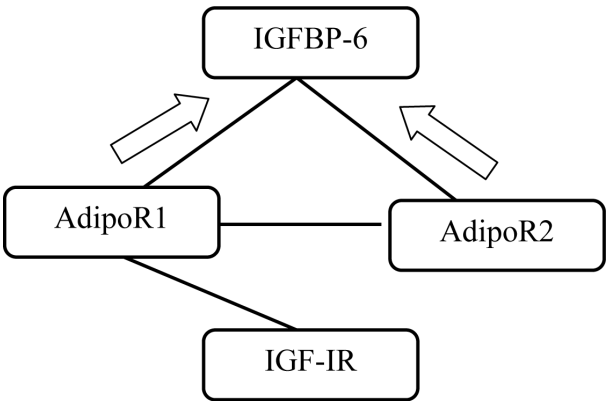

Figure 3. The Cross-talk between IGFBP-6, AdipoR1 and AdipoR2 in Colorectal Cancer. ___ : positive relationship; - ⇦ positive dependence. The scheme composed on the data of correlation analysis and data of nonlinear regression analysis of results of IGFBP-6 protein level, AdipoR1, AdipoR2 and IGF-IR expression in tumor tissues of 46 colorectal cancer patients

ng/mg of protein, respectively. The mean percentage of cytokeratin 20-positive cells stained anti-IGF-IR (CD221)+PE antibody was 71.0 (61.0-82.0). There were no differences in the levels AdipoR1, AdipoR2 and IGF-IR between colorectal patients with and without metabolic syndrome.

The results of correlation analysis as scatter plots of IGFBP-6 content according the AdipoR1 and AdipoR2 levels, as well as the IGF-IR level according the AdipoR1 level are presented in Figure 2. There were found positive correlation between the IGFBP-6 expression and the AdipoR1 content ($r = 0.58$, $p<0.05$), between the IGFBP-6 expression and the AdipoR2 level ($r=0.64$, $p <0.05$), as well as between the IGF-IR level and AdipoR1 ($r=0.64$, $p<0.05$). Significant associations were also found between the AdipoR1 and the AdipoR2 receptors ($r=0.82$, $p<0.05$). The received data were confirmed with nonlinear regression analysis. The dependence of IGFBP-6 content on the AdipoR1 and AdipoR2 levels was revealed ($p=0.00050$) (Table 4). Schematic representation of relations between IGFBP-6, AdipoR1 and AdipoR2 in colorectal cancer is presented on the Figure 3.

Discussion

Correlation analysis between relative IGFBP-6 mRNA level and IGFBP-6 expression was not revealed any of correlation. Lack of correlation between the IGFBP-6 mRNA expression and its protein level in colorectal cancer, probably could be explained by the existing multiple post-translational modifications of the IGFBP-6. Also, protein could be changed due the limited proteolysis, due the process of phosphorylation and glycosylation (Spirina et al., 2012; Ivanova et al., 2014). This could lead to the existence of multiple protein isoforms with the different binding activity to IGF-II and to glycosaminoglycans and different susceptibility to proteolysis (Marinaro et al., 2000; Ahmad et al., 2011; Bach, 2015). Both the IGFBP-6 mRNA expression and the IGFBP-6 level were higher in subgroup of colorectal patients with metabolic syndrome compared to patients without metabolic disturbance. So, the study of the IGFBP-6 content, its post-translational modifications and its clinical significance in colorectal cancer patients with metabolic syndrome is perspective for clarifying the role of IGFBP-6 in colorectal carcinogenesis.

Our findings about the association between the IGFBP-6 protein level and nodal status are in concordance with data Leng et al. (2001) and Fu et al. (2010). In these studies it is noted the IGFBP-6 protein is involved in many processes associated with the tumor growth and progression. It was shown the IGFBP-6 inhibited angiogenesis as well as promoting migration of rhabdomyosarcoma and colon cancer cell (Leng et al., 2001; Bach , 2015). Extracapsular lymph node invasion refers to the extension of cancer cells through the nodal capsule into the perinodal fatty tissue that reflects the invasiveness and aggressiveness of the primary tumor (Resch and Langer, 2013). Further research in the intratumoral relative IGFBP-6 mRNA expression and IGFBP-6 content in association with extracapsular lymph node invasion and another parameters associated with lymph node involvement would be promising to clarify the role of this protein in tumor invasion and lymphogenous metastasis in colorectal cancer. IGFBP-6 expression was decreased in a number of cancer cells and it has been postulated to act as a tumor suppressor (Bach., 2015). In our study we have shown that in colorectal cancer patients IGFBP-6 may be considered as potential biomarker associated with lymphogenous metastasis and metabolic syndrome.

According the performed analysis it was found the association between the IGFBP-6 protein level and AdipoR1, AdipoR2 and the IGF-IR levels. Previously, we have shown that in patients with colorectal cancer as opposed to endometrial cancer patients the level of the major hormones adipose tissue - leptin and adiponectin were not associated with the presence of metabolic syndrome. Also the levels of the AdipoR1, AdipoR2 and IGF-IR in cancer tissues in colorectal cancer patients were also not associated with the presence of metabolic syndrome (Yunusova et al., 2015). Therefore, during the correlation analysis we have not isolated a subgroup of patients with and without the metabolic syndrome. Apparently, the identified positive correlations between the IGFBP-6 protein level and the adiponectin receptors AdipoR1 and AdipoR2 levels and also between the IGF-IR and AdipoR1 level demonstrate the cross-talk between the IGF-IR-mediated and the adiponectin-mediated signaling pathways in colorectal carcinomas. These associations in colorectal carcinomas were revealed first. It has been shown previously association of the adiponectin and the IGF-IR expression in breast cancer depending on the ER status (Mauro et al., 2015).

Our results about AdipoR1 and AdipoR2 co-expression in colorectal carcinomas correspond to data T. Ayyildiz et al. (2014) where a significant relationship between AdipoR1-positivity and AdipoR2-positivity in tumors in colorectal cancer patients was revealed when performing immunohistochemical staining (Ayyildiz et al., 2014). There are no data about AdipoR1, AdipoR2 expression in relation with IGF-IR expression in colorectal carcinomas. But it was found that the adiponectin enhanced the

immunoprecipitation of the multiprotein complex comprising AdipoR1, AppL1, ERα, IGF-IR and c-Src proteins in the ERα positive breast cancer cell lines. The formation of this complex leads to the cSrc and MAPK pathways activation, which is abrogated in the presence of the specific RNA silencers targeting the ERα or the IGF-IR (Mauro et al., 2014). It seems the observed positive correlations of the AdipoR1 and AdipoR2 with the IGFBP-6 content cannot be considered direct. The adiponectin colon tissue level depending on serum adiponectin level can modulated the tissue level AdipoR1 and AdipoR2 but the detailed mechanisms are still unknown (Tae et al., 2014). The complexity of interpreting the association the IGFBP-6 mRNA expression/ protein level with the adiponectin and its receptors levels due to the fact that the IGF-II bioavailability is determined mainly by the IGFBP-6. But IGF-IR phosphorilated status in cancer tissues is also important for understanding of the interactions of these proteins.

It was shown the free IGF-IR is essential for the formation of a functional and stable β-cathenin-cadherin complex (Yunusova et al., 2015). The activation of the IGF-IR by binding to its ligands (IGF-I, IGF-II) and the further increase of cell motility could be explained due, apparently, a yield of IGF-IR from the protein complex and redistribution of the beta-catenin into the cytosol (Pennisi et al., 2002). The further research is needed to study the involvement of IGFBP-6 in colorectal cancer pathogenesis, including the investigation of the adiponectin detailed affecting mechanism on the IGF-IR cancer tissues status as well as studying the ratio of total IGF-RI and its phosphorylated form, on the IGFBP-6 expression and its post-translational modifications as in a patients with metabolic syndrome and without metabolic disturbances.

Thus, the IGFBP-6 mRNA expression and its protein level were higher in the patients with metabolic syndrome. It was interestingly to note the IGFBP-6 protein expression was associated with nodal status. Identified positive correlations between the expression of IGFBP-6 and levels of AdipoR1 and AdipoR2, and between the IGF-IR expression and AdipoR1 level showed the cross-talk between the IGF-IR-mediated and adiponectin-mediated signaling pathways in colorectal carcinomas. Apparently, in colorectal carcinomas IGFBP-6 may be considered as potential biomarker associated with lymphogenous metastasis and metabolic syndrome.

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References

Ahmad W, Shabbiri K, Ijaz B, et al (2011). Serine 204 phosphorylation and O-β-GlcNAC interplay of IGFBP-6 as therapeutic indicator to regulate IGF-II functions in viral mediated hepatocellular carcinoma. *Virol J*, **8**, 208.

Ayyildiz T, Dolar E, Ugras N, et al (2014). Association of adiponectin receptor (Adipo-R1/-R2) expression and colorectal cancer. *Asian Pac J Cancer Prev*, **15**, 9385-90.

Bach LA, Fu P, Yang Z (2013). Insulin-like growth factor-binding protein-6 and cancer. *Clin Sci (Lond)*, **124**, 215-29.

Bach LA (2015). Recent insight into the action of IGFBP-6. *J Cell Commun Signal*, **9**, 189-200.

Firth SM, Baxter RC (2002). Cellular actions of the insulin-like growth factor binding proteins. *Endocr Rev*, **23**, 824-54.

Ivanova EV, Kondakova IV, Spirina LV, et al (2014). Chymotrypsin-like activity of proteasomes and total calpain activity in gastric and colorectal cancer. *Bulletin Experimental Biol Med*, **157**, 781-4.

Kim EJ, Schaffer BS, Kang YH, et al (2002). Decreased production of insulin-like growth factor-binding protein (IGFBP)-6 by transfection of colon cancer cells with an antisense IGFBP-6 cDNA construct leads to stimulation of cell proliferation. *J Gastroenterol Hepatol*, **17**, 563-70.

Leng SL, Leeding KS, Whitehead RH, et al (2001). Insulin-like growth factor (IGF)-binding protein-6 inhibits IGF-II-induced but not basal proliferation and adhesion of LIM 1215 colon cancer cells. *Mol Cell Endocrinol*, **174**, 121-7.

Marinaro JA, Neumann GM, Russo VC, et al (2000). O-glycosylation of insulin-like growth factor (IGF) binding protein-6 maintains high IGF-II binding affinity by decreasing binding to glycosaminoglycans and susceptibility to proteolysis. *Eur J Biochem*, **267**, 5378-86.

Mauro L, Pellegrino M, De Amicis F, et al (2014). Evidence that estrogen receptor α interferes with adiponectin effects on breast cancer cell growth. *Cell Cycle*, **13**, 553-64.

Mauro L, Naimo GD, Riccio E, et al (2015). Cross-talk between adiponectin and IGF-RI in breast cancer. *Frontiers Oncol*, **5**, 1-8.

Minniti CP, Luan D, O'Grady C, et al (1995). Insulin-like growth factor II overexpression in myoblasts induces phenotypic changes typical of the malignant phenotype. *Cell Growth Differ*, **6**, 263-9.

Ogunwobi O, Beales IL (2007). The anti-apoptotic and growth stimulatory actions of leptin in human colon cancer cells involves activation of JNK mitogen activated protein kinase, JAK2 and PI3kinase/Akt. *Int J Colorectal Dis*, **22**, 401-9.

Pennisi PA, Barr V, Nunez NP, et al (2002). Reduced expression of insuline-like growth factor I receptors in MCF breast cancer cells leads to a more metastatic phenotype. *Cancer Res*, **62**, 6529-37.

Resch A, Langer C (2013). Lymph node staging in colorectal cancer: old controversies and recent advances. *World J Gastroenterol*, **19**, 8515-26.

Samani AA, Yakar S, LeRoith D, et al (2007). The role of the IGF system in cancer growth and metastasis: overview and recent insight. *Endocrine Reviews*, **28**, 20-47.

Spirina LV, Bochkareva NV, Kondakova IV, et al (2012). Regulation of insulin-like growth NF-ϰB proteasome system in endometrial cancer. *Molecular Biol*, **46**, 407-13.

Spirina LV, Yunusova NV, Kondakova IV, et al (2012). Association of growth factors, HIF-1 and NF-kB expression with proteasome in endometrial cancer. Mol Biol Rep, 39, 8655-62.

Sugiyama M, Takahashi H, Hosono K, et al (2009). Adiponectin inhibits colorectal cancer cell growth through the AMPK/mTOR pathway. Int J Oncol, 34(2), 339-44.

Tae CH, Kim SE, Jung SA, et al (2014). Involvement of adiponectin in early stage of colorectal carcinogenesis. *BMC Cancer*, **14**, 811.

Yunusova NV, Spirina LV, Kondakova IV, et al (2013). The relationship of metalloproteinase PAPP-A expression with the expression of growth and transcriptional factors in endometrial cancer. *Biology Bulletin*, **40**, 253-59.

Yunusova NV, Kondakova IV, Kolomiets LA, et al (2015).

The insulin-like growth factors system in ovarian cancer metastasis: the relation with p45 Ser β-catenin. *Molekulyrnaya Meditsina*, **4**, 17-22. [in Russian].

Yunusova NV, Kondakova IV, Kolomiets LA, et al (2015). Serum adipokines and their receptors in endometrial and colon cancer patients: relationship with tumor invasion and metastasis. *Vopr Onkol*, **61**, 619-23.

Zhou JR, Blackburn GL, Walker WA (2007). Symposium introduction: metabolic syndrome and the onset of cancer. *Am J Clin Nutr*, **86**, 817-9.