Detection of PRL-3 protein in the preoperative serum of patients with colorectal cancer

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ABSTRACT

Purpose: The aim of study was to detect PRL-3 protein in sera of patients with colorectal cancer.
Methods: The study group consisted of 22 patients with colorectal carcinoma and 10 healthy controls. The serum concentration of PRL-3 protein was evaluated by the ELISA method.
Results: In the study group with colorectal cancer, the level of PRL-3 protein in preoperative sera was negative (<0.115ng/ml) in 7 cases while the mean value of PRL-3 concentration was 0.616ng/ml (range 0.206-2.072ng/ml) in 15 patients. No statistically significant differences between the study group and healthy controls were observed. Our data showed that increase in level of PRL-3 protein in sera of patients with colorectal cancer is associated with greater tumor size ($p<0.05$).
Conclusions: PRL-3 protein was found to be present in sera of patients with colorectal cancer. However, our study indicates lack of clinical usefulness of determining the levels of PRL-3 in the sera of patients with colorectal cancer.
Key words: colorectal cancer, PRL-3, serum.

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INTRODUCTION

Numerous research on carcinogenesis in colorectal cancer have been presented PRL-3 protein as one of the major poor prognostic marker for patients but also as a promising target for the cancer treatment. PRL-3 protein (phosphatase of regenerating liver-3) belongs to the family of protein tyrosine phosphatases, which play an important role in regulating physiological and pathological cell processes. Specifically, it is involved in reconstructing of the cytoskeleton, regulating adhesion and cell cycle of the cancer cells, and epithelial-mesenchymal transition. Through these mechanisms PRL-3 protein participates in invasion, migration, metastasis and angiogenesis. Studies indicate the PRL-3 protein takes part especially in metastases of colon cancer [1].

The family of PRL phosphatases consist of three proteins (PRL-1, PRL-2, PRL-3), all of which have a unique C-terminal prenylation motif. They show 75% homology in amino acid sequence and have a molecular weight of about 20kDa [2]. The gene responsible for encoding PRL-3 protein is located at chromosome 8q24.3 [3]. Human PRL-3 molecule is composed of 173 amino acids and is a monomer with a complex structure. Enzyme active side is marked by a signature sequence HCXXGXXR where Cys104 is the enzymatic nucleophile and Arg110 coordinates with the phosphate group on phosohotyrosine [4]. In contrast to other phosphatases, catalytic core of PRL-3 does not contain serine or threonine, important for the catalytic activity of the other phosphatases [5]. PRL-3 molecule contains C-terminal consensus sequence for prenylation (CAAX motif) which is responsible for farnesylation of the protein. As a result, it can be found in the membranes and intracellular structures when is prenylated and in the nucleus as the non-prenylated form [6-8]. In healthy subjects, PRL-3 protein is detected in cardiomyocytes, skeletal muscles and to a certain degree in the pancreas [2, 3]. In colorectal cancer, PRL-3 protein is found in a small percentage of primary tumors and in almost all metastases [1]. The cellular localization of PRL-3 protein was closely associated with its function in tumor metastases [9].

The aim of study was to detect PRL-3 protein in sera of patients with colorectal cancer.

MATERIALS AND METHODS

Patients

The study group consisted of 22 patients diagnosed with colorectal cancer (13 men and 9 women) treated surgically in the 2nd Department of General and Gastroenterological Surgery in the Medical University of Białystok in 2007-2009. The pathological diagnosis confirmed colorectal cancer and classified as adenocarcinoma type without mucin in 20/22 of patients and adenocarcinoma with mucous component in 2/22 of patients. The investigated tumors were classified as moderately (20/22 of patients) and poorly differentiated (2/22 of patients). According to TNM classification, tumor infiltrated a submucosa (pT1) in 1 case, muscle layer (pT2) in 2 cases and subserosa (pT3) in 19 cases. The metastases to local lymph nodes were observed in 9/22 of cases and the presence of metastases to distant organs was noted in 7/22 of cases.

Study material consisted of serum samples obtained from both the blood of the patients with colorectal carcinoma collected prior to the surgery.

Blood serum was stored at -80^0C immediately after centrifugation until the assay was performed.

The control group consisted of 10 healthy volunteers (5 males and 5 females aged 49-80).

The study received the approval of the local Bioethics Committee. All the participants received information about method and purpose of study and signed consent forms prior to the examination.

ELISA method

To determine the concentration of PRL-3 protein in serum it was used commercially available ELISA kit (Uscn Life Science Inc.) according to the manufacturer's instructions. The sera were slowly defrosted to room temperature. The detectable concentration of PRL-3 protein range from 0.312-20ng/mL.

Statistics

Statistical analysis was conducted based on the STATISTICA 8.0 program. In order to compare the two groups, the U Mann-Whitney test was used. Correlations between the protein level in serum and clinical-pathological parameters were calculated by the Spearman's correlation coefficient tests. The level of significance was <0.05.

RESULTS

Serum PRL-3 levels in colorectal cancer and control patients

In the control group, 4 cases had the levels of PRL-3 protein below minimum value indicated by kit (<0.115ng/ml) and those results were defined as a level 0 (absent protein) whereas the concentration of PRL-3 protein ranged from 0.263-1.158ng/ml) in 6 cases.

In the study group with colorectal cancer, the level of protein in preoperative sera was negative (<0.115ng/ml) in 7 cases while the mean

value of PRL-3 concentration was 0.616ng/ml (range 0.206-2.072ng/ml) in 15 patients. No statistically significant differences between the study group and healthy controls were observed (Tab. 1).

Correlation between serum PRL-3 levels and clinicopathological parameters in colorectal cancer patients

No correlations of preoperative serum PRL-3 levels in colorectal cancer patients with age, gender, tumor localization, histological type, stage, local lymph node involvement, distant organs metastases and Duke's classification were observed. However, our data showed that increase in level of PRL-3 protein in sera of patients with colorectal cancer is associated with greater tumor size (p<0.05) (Tab.2).

Table 1. Serum PRL-3 levels in patients with colorectal cancer and control group.

		PRL-3 (ng/ml)				
	N	Mean	Median	SD	Range	*p* value
Normal	10	0.251	0.036	0.383	0-.158	NS
Tumor	22	0.616	0.500	0.637	0-.072	

Mann-Whitney U-test. NS – non significant.

Table 2. Correlations between serum PRL-3 levels and clinicopathological parameters in colorectal cancer patients.

		PRL-3 (ng/ml)			
Parameter		N	Mean	Coefficient	*p* value
Age	≤60	9	0.581	– 0.099	NS
	>60	13	0.640		
Gender	Male	13	0.501	– 0.772	NS
	Female	9	0.782		
Localization	Colon	14	0.622	0.067	NS
	Rectum	8	0.606		
Adenocarcinoma type	Nonmucinous	20	0.646	– 0.570	NS
	Mucinous	2	0.312		
Grade of malignancies	Poorly differentiated	20	0.616	0.455	NS
	Moderately differentiated	2	0.615		
pT stage	1	1	0.891	– 0.759	NS
	2	2	0.539		
	3	19	0.610		
Lymph node metastasis	Absent	13	0.466	0.983	NS
	Present	9	0.833		
Distant metastasis	Absent Present	15 7	0.642 0.560	– 0.385	NS
Duke stage	A B C D	2 7 3 10	0.862 0.354 0.879 0.671	0.019	NS
Tumor size	< 5cm	12	0.421	0.511	<0.05
	≥ 5 cm	10	0.727		

Spearman's correlation coefficient test. NS – non significant

DISCUSSION

Since it has been demonstrated that the PRL-3 protein is significantly involved in the development of cancer, it began to see it as a possible target in anti-cancer treatment. It was also observed that PRL-3 knockdown inhibits the tumor growth through reducing the potential for its expansion and the activation of apoptosis. It reduces the lymph node involvement too [10].

Therefore, it has been started to explore the inhibitors of PRL-3 protein such as TGFβ, PCBP1 [11], thienopryridone [12], curcumin [13], methanolic extract of the roots of Rubia Akane [14] as possible attractive targets for cancer therapy. However, antibody therapy gives the best hope for cancer patients. Guo et al. [9] presented the

treatment of a mouse model of chimeric antibodies which blocked the intracellular PRL-3 protein. They observed that those antibodies specifically reduce the metastases formation related to the expression of PRL-3 protein. Since the research on therapy associated with PRL-3 protein has been improved, an attempt was made to select the patients too. The mechanism of this protein overexpression is still not understood so Ooki et al. [15] evaluated the *PRL-3* gene amplification in patients with gastric cancer based on the assessment of *HER2* gene status. They demonstrated that gene amplification occurred in 20% of cases with the positive expression of PRL-3 protein in tumour tissue. Moreover, they stated that the assessment of the presence of PRL-3 expression, but not the level of expression, may have a significant potential for the classification of PRL-3-linked therapy. A similar research should be carried out on the group of patients with colorectal cancer.

In our study, we tried to detect the extracellular presence of PRL-3 protein in serum. While it has been proved that PRL-3 protein presents in sera of patients with colorectal cancer, we did not observe a significant differences between the levels of this protein compare to sera of healthy patients. However, the question is if the presence of PRL-3 in serum may influence on antibody therapy.

The level of PRL-3 protein was not found to correlate with clinicopathological parameters, except tumor size. In cases of tumor greater than 5 cm, an increase in the level of PRL-3 protein was observed. Nevertheless, we cannot establish the source of PRL-3 protein.

CONCLUSIONS

1. PRL-3 protein was fund to be present in sera of patients with colorectal cancer.
2. However our study indicates lack of clinical usefulness of determining the levels of PRL-3 in the sera of patients with colorectal cancer.

Conflicts of interest

The authors declare no conflict of interest.

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