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Research Article

Role of CXCL5 and PDCD-4 as Prognostic Biomarkers in Patients with Colorectal Cancer

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ABSTRACT

The objective of this study was to assess serum CXCL5 and PDCD-4 levels in patients with colorectal cancer (CRC) to evaluate their value as prognostic markers and their correlation with clinicopathologic features. The study was conducted on 103 colorectal cancer patients divided into two groups according to stage of disease (early [groupI] and late [group II]) beside 50 healthy controls. The levels of CXCL5 and PDCD-4 were measured in sera of all patients before and after treatment and healthy controls using enzyme-linked immunosorbent assay kit (ELISA). Our results showed that serum CXCL5 was significantly elevated in cancer patients in comparison to controls and this increase decreased after treatment, the elevation correlated positively with stage and progression of disease. Serum PDCD-4 was significantly elevated in early stage group [group I] in comparison to controls while in late stage group [group II] its level was significantly low in comparison to controls and group I. It correlated negatively with stage and progression of disease.

In conclusion; Serum CXCL5 level was increased in CRC patients and it correlated positively with progression of disease so it could serve as a predictive marker for CRC patient prognosis while PDCD-4 decreased with progression of tumor and correlated negatively with stage, so it can be used as prognostic marker. Also, elevation of PDCD-4 expression is a promising strategy for cancer therapeutics. Since PDCD-4 expression is frequently downregulated in cancer cells, the compounds to suppress these actions will be valuable agents for cancer therapeutics.

Keywords; CXCL5, PDCD-4, colorectal cancer

INTRODUCTION

Colorectal cancer (CRC) is the third most commonly diagnosed cancer worldwide, with almost 1.4 million new cases in 2012[1]. In Egypt; it is one of the most common malignant neoplasms and the third leading cause of death. It represents about 6% of cancers in Egypt (after bladder, breast carcinoma and lymphoma) with high male predominance 3:1and more than 1/3 under age 45 years. It is rectal in 51% of the cases. The number of new cases is therefore projected to rise dramatically with the aging of the population that is currently occurring in nearly every country and region. As a result of improved screening, earlier detection, and increased treatment efficacy, relative survival from CRC can be improved markedly[2]. Although of the improved therapeutic agents; metastases, especially to liver and lung, are the main causes of morbidity and mortality in case of cancer colon[3].

Metastasis development is a complex process involving many biochemical and immunological changes, including overexpression of growth factors and cytokines[4]. It was suggested that chemokines, a family of cytokines, are involved with human malignancy progression and metastases happens due chemokine-receptor interactions; for example chemokine receptor CXCR4 is not expressed on normal breast epithelia but is frequently expressed on breast cancer cells which have tendency to metastasize to lymph node, lung and liver , sites where ligand CXCL12 is expressed, subsequent reports have revealed that chemokines facilitate progression of various human cancers and are partly associated with organ-specific metastasis[5,6]. Also, recently, studies have reported that CXC-ligand 5 (CXCL5) is involved in the development of several human cancers[7].

Programmed cell death 4 (PDCD-4), a newly identified tumor suppressor and originally identified as a neoplastic transformation inhibitor, has been demonstrated to inhibit tumor promoter-induced neoplastic transformation. The human PDCD-4 gene is localized in chromosome 10q24[8]. It is expressed in small duct epithelial cells of the normal mammary gland, normal human lung tissue and senescent human fibroblasts[9]. It was commonly lost in lung cancer and such a loss was correlated with higher histological grade, disease stage and poor prognosis. PDCD-4 expression is attenuated with progression in human tumors of the colon, prostate and breast[10].

The aim of work was to assess serum CXCL5 and PDCD-4 levels in patients with colorectal cancer (CRC) to evaluate their correlation with clinicopathologic features and prognosis.

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Table 1: Clinico-pathological characteristics of the studied colorectal cancer patients and controls

Variables	Patients	controls
Numbers	103	50
Gender		
Male	64	30
Female	39	20
Age (years)		
<60	43	22
>60	60	28
TNM classification		
T. stage		
T1,T2	70	-
T3,T4	33	-
LN metastasis		
-ve	62	-
+ve	41	-
Distant metastasis		
-ve	85	-
+ve	18	-
CEA ng/ml		
<5	75	50
>5	28	-

Table 2: Serum level of CXCL5 and PDCD-4 in healthy controls, early stage (Group I) and late stage colorectal cancer patients (Group II) before and after treatment

	CXCL5 (ng/ml)	PDCD-4 (ng/ml)
Controls	0.58±0.18	4.37±0.73
Group I		
Before	1.402±0.38 *§	9.08±2.2*§
treatment	0.7±0.33	4.71±0.61
After treatment		
Group II		
Before	3.33±0.92*§	2.98±0.78*
treatment	0.92±0.19*	3.91±0.52§
After treatment		

*P< 0.05 significant in comparison to controls
§ P<0.05 significant in comparison between groups before treatment and after treatment

MATERIALS AND METHODS

This study was conducted on 103 patients with colorectal cancer, their age ranged between 42-68 years beside 50 healthy persons served as control group. All patients were classified according to UICC stage classifications using resected specimens[11]. The patients were classified according to TNM stage classifications (stages I, II, III and IV). Stages I and II concerned as early stage (group I) and stages III and IV concerned as late stage (group II). Blood samples were obtained by venipuncture before and after treatment. Each sample was centrifuged at 3000g for 5 min then frozen at -80°C until analysis. The study was conducted after the approval of research ethical committee and informed written consents were obtained from all participants before serum collection.

-Serum CXCL5 levels was assayed by enzyme-linked immunosorbent assay (ELISA), Quantikine Human ENA-78; R&D Systems, Minneapolis, MN) in accordance with the manufacturer's instructions.

- Apoptotic marker in serum was carried out using the assay employed the quantitative sandwich enzyme immunoassay technique. The kits (Glory Science Co., Ltd, USA) use double – antibody sandwich enzyme- linked immunosorbent assay (ELISA) to assay the level of Human Programmed cell death protein 4 (PDCD-4).

Statistical analysis

Analysis of data was done IBM computer using SPSS Version 17; quantitative data were presented using the mean and standard deviation. Qualitative data were presented using the frequency and percentage. Comparison of quantitative data was done using independent T test (Unpaired Student T-test). Qualitative data were compared using the Chi - square test. Paired Student T-test was used to compare between related samples. Pearson correlation (Linear Correlation Coefficient) was done to estimate the correlation between quantitative data.

Table 3: Correlation between CXCL5, PDCD-4 levels and different stages of colorectal cancer

Parameters	Different stages	
	Pearson correlation	P value
CXCL5 (ng/ml)	0.8329*	P<0.05
PDCD-4 (ng/ml)	-0.692*	P<0.05

*P<0.05 significant

RESULTS

Table (1) shows the clinicopathological characteristics of the studied colorectal cancer patients and the healthy controls.

Table (2) shows the serum level of CXCL5 and PDCD-4 in healthy controls, early stage (Group I) and late stage colorectal cancer patients (Group II) before and after treatment. CXCL5 serum levels were significantly increased (P<0.05) in early stage group and highly significantly increased in late stage group compared to control group. PDCD-4 serum levels were significantly increased in early stage group before therapy, after therapy compared to the control group and increased in early stages before therapy compared to early stages after therapy and control group. In the late stage PDCD-4 was significantly decreased before treatment and after treatment compared to control group (P value<0.05).

Table (3) shows the correlation between CXCL5, PDCD-4 levels and different stages of colorectal cancer. CXCL5 shows positive correlation with cancer colon stages while PDCD-4 shows negative correlation with cancer colon stages (Figure 1 and 2).

DISCUSSION

The prognosis of advanced colorectal cancer is generally poor. New therapeutic strategies are needed to improve the outcome of patients with locally advanced or metastatic cancer. In this setting, the knowledge of prognostic determinants might be important for treatment planning[2]. In this study serum CXCL5 and PDCD-4 levels in patients

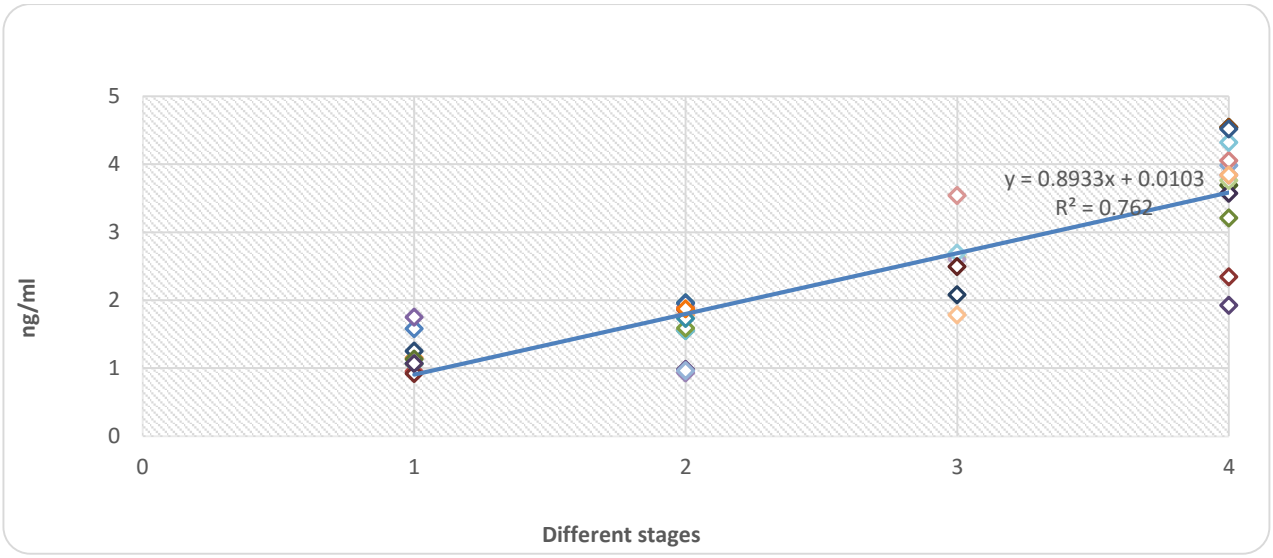

Figure 1: Scatter plot showing correlation between serum CXCL5 levels and different stages of colorectal cancer

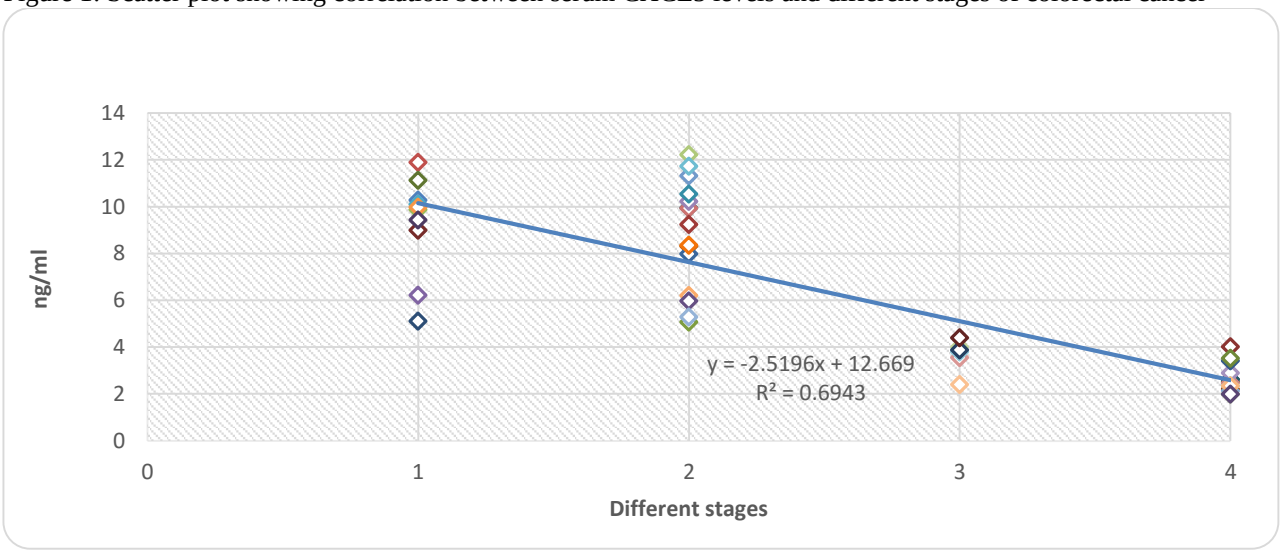

Figure 2: Scatter plot showing correlation between serum PDCD-4 levels and different stages of colorectal cancer

with colorectal cancer were assessed to evaluate their correlation with clinicopathologic features and prognosis.

Serum levels of CXCL5 in our study were significantly increased according to stage and correlated with progression of disease. These results were in accordance with Kawamura et al[12] and Matsushita et al[13] who stated that preoperative serum CXCL5 could serve as a novel predictive marker for prognosis determination of CRC patients. Several reports have demonstrated relevance of CXCL5 expression and CRC. High expression of CXCL5 was significantly associated with better prognosis for CRC patients possibly due to CD8+ T-cell infiltration caused by CXCL5 promoting a tumour-specific immune response, as reported previously for other chemokines in CRC. Thus, CXCL5 may play a favourable role in peritumoural loci[14,15,16].

It has been reported that chemokines expressed in metastatic target organs and their receptors on tumour cells could play crucial roles in establishment of organ-specific metastasis, such as CXCL12/CXCR4[17,18]. Kawamura et al[12] have demonstrated CXCL5 expression in the liver and CXCR2 expression in primary CRC, suggesting these may be specifically involved in CRC liver metastasis. In addition, they detected significant correlation between serum CXCL5 and liver metastasis. Serum chemokine might be related to organ-specific metastasis. It has been found that chemokines play a role during physiological processes by mediating lymphoid tissue organogenesis, lymphocyte homing and hematopoiesis[19]. In addition, chemokines are also involved in pathological processes including microbial infections, autoimmune diseases and inflammatory bowel diseases (IBD), as well as tumor growth and metastasis[20]. Indeed, besides their role in chemoattraction, chemokines possess other functions related to cell survival and proliferation and to stimulation or inhibition of angiogenesis. Chemokines modulate tumor behavior by three important mechanisms: regulation of angiogenesis, activation of a tumor-specific immune response and direct stimulation of tumor proliferation in an autocrine or paracrine fashion[21].

In this study serum levels of PDCD-4 were significantly increased in early stages of CRC but decreased

significantly in late stages of disease, it correlated negatively with the disease progression. As increased cell death (PDCD-4) in early stage may be an attempt to limit the expansion of the tumor cell population, several studies have linked the rate of apoptosis with the proliferative rate of adenomas and carcinomas, yielding conflicting results. PDCD-4 is up-regulated on induction of apoptosis and down-regulated in certain aggressive tumours including lung, breast, colon, brain, and prostate cancers[22]. Also the results were in agreement with Leupold et al[23]; Yang et al[24] who demonstrated that PDCD-4 regulates tumor invasion and metastasis as it overexpressed in early stages of disease and downregulated as metastasis occurs. For instance, ectopic expression of PDCD-4 cDNA suppressed invasion in colon carcinoma. Knockdown of PDCD-4 expression promoted invasion in colon HT29 and GEO cells as well as in breast cancer MCF-7 and T47D cells[25]. In agreement with these in vitro studies, knockdown of PDCD-4 in colon tumor cells also promotes metastasis when injected into nude mice.

In addition, KO of PDCD-4 in mice induces lymphomas with frequent metastasis[26]. A detailed mechanism of inhibiting invasion and metastasis by PDCD-4 was studied in colon tumor cells. Knockdown of PDCD-4 in the colon HT29 and GEO cells was shown to decrease expression of epithelial proteins (α-catenin, γ-catenin, and E-cadherin) and increase expression of mesenchymal proteins (N-cadherin and fibronectin), suggesting that knockdown of PDCD-4 leads to EMT[27].

Loss of PDCD-4 mRNA expression was suggested as an indicator for tumor progression and prognostic marker in cancer colon [28]. Low PDCD-4 expression was also associated with poor prognosis. Further reports showed an inverse correlation of PDCD-4 protein expression with advancing tumor stages and proposed loss of PDCD-4 protein as a negative prognostic marker for carcinoma. Thus, loss of PDCD-4 emerges as a promising diagnostic marker of tumor progression and appears to contain prognostic value for various tumor types[29].

CONCLUSION

Serum CXCL5 level was increased in CRC patients and it correlated positively with progression of disease so preoperative serum CXCL5 could serve as a novel predictive marker for prognosis of CRC patients. While PDCD-4 decreased with progression of tumor and correlated negatively with stage, so it can be used as prognostic marker. Also, elevation of PDCD-4 expression is a promising strategy for cancer therapeutics. Since PDCD-4 expression is frequently downregulated in cancer cells, the compounds to suppress these actions will be valuable agents for cancer therapeutics.

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