RESEARCH COMMUNICATION

Lack of Diagnostic Potential of Dickkopf-1 in Colon and Rectum Cancers

Hilal Oguz Soydinc[1*], Derya Duranyildiz[1], Hakan Camlica[2], Ethem Nezih Oral[3], Vildan Yasasever[1]

Abstract

The Wnt/b-catenin signalling pathway plays crucial roles in development and its aberrant activation is an initial and crucial event in the majority of colon cancers. The *Dickkopf-1* (*Dkk-1*) gene encodes an extracellular Wnt inhibitor that blocks the formation of signalling receptor complexes at the plasma membrane. Here, we report the serum levels of Dkk1 in colorectal cancer patients without any therapy. The levels were determined by enzyme-linked immunosorbent assay (ELISA) in 135 colon and 160 rectum cancer patients, as well as 90 healthy subjects. Data analyses were performed using SPSS software (SPSS 16, Chicago, IL). There were no significant differences among the groups for Dkk-1 (p=0.363). In conclusion, the present study did not confirm that serum Dkk-1 levels could have any diagnostic potential in colon and rectum cancers.

Keywords: Colorectal cancer - Dickkopf-1 - lack of diagnostic potential - Turkey

Asian Pacific J Cancer Prev, **12**, 3187-3189

Introduction

Colorectal cancer (CRC) is the third most common cancer diagnosed among men and women and the third leading cause of cancer mortality. Colorectal cancer, if detected early, is associated with a high survival rate. However many patients are still diagnosed with advanced disease (Jemal et al., 2002). A number of key oncogenes and tumor suppressor genes have been proposed to drive progression from healthy colonic epithelia to malignant tumors, including members of the Wnt/beta-catenin pathway.

The Wnt–b-catenin signalling pathway plays crucial roles in development and its aberrant activation is an initial and crucial event in the majority of colon cancers. The Wnt–b-catenin pathway is aberrantly activated in most colon cancers and a proportion of other carcinomas by mutation in adenomatous polyposis coli, or less frequently (Aguilera et al., 2007).

Dickkopfs (Dkks) are secreted antagonists of Wnt signaling pathway. Activated Wnt signal pathway, characterized by the stabilization of beta-catenin, plays an important role in most gastrointestinal cancers (Maehata et al., 2008). Dickkopf-1 (Dkk-1) gene encodes an extracellular Wnt inhibitor that blocks the formation of signalling receptor complexes at the plasma membrane (Aguilera et al., 2007). Some Wnt antagonists (such as Dkk-1) act as tumor suppressors and the loss of them may contribute to cancer development. Wnt signalling dysregulation has been implicated in cancer, including colon and gastric cancer. Initiation of Wnt signalling is modulated by soluble Wnt antagonists (sWAs), including soluble frizzled related proteins, dickkopf proteins, and Wnt inhibitory factor-1 (Byun et al., 2005).

Recent cell culture study findings have indicated that Dkk-1 and its The Wnt/beta-catenin pathway play a critical role in cell transformation and tumorogenesis. Although, Dkk-1 expression have been shown in colorectal cancer cell lines, there is no study showing a significant difference in serum Dkk-1 levels in gastrointestinal cancer patients. Here, we report the serum levels of Dkk-1 in colorectal cancer patients without any therapy.

Materials and Methods

135 colon and 160 rectum cancer patients with pathologically verified colorectal cancer, consecutively admitted to the Istanbul University, Oncology Institute during two- year period, January 2007 to December 2009 were investigated in this study. Our study is on human materials and has been approved by the relevant institutional ethics committee. The protocol was consistent with the Declaration of Helsinki (2000). Informed consent was obtained from all patients.

Serum samples were obtained on first admission before any type of treatment (radiotherapy or / and chemotherapy) was given. Staging was performed on a pathological basis according to the American Joint Committee on Cancer (AJCC).

Blood samples were obtained from the 295 cancer patients (median age 59 years) and 90 healthy controls (median age 50 years) who where blood donors

*[1]Basic Oncology, [2]Preventive Oncology, Biostatistics and Epidemiology, [3]Radiation Oncology, Istanbul University Oncology Institute, Istanbul, Turkey *For correspondence: hilaloguz@yahoo.com*

Table 1. Statistical Data

	Colon Patients (n=135) x ± sd; m (min-max)	Rectum Patients (n=160) x ± sd; m (min-max)	Controls (n=90) x ± sd; m (min-max)
Dkk-1 (ng/mL)	29.8 ± 3.09 29.3 (23.2 -34.2)	28.9 ± 6.6 31.3 (10.0 -35.2)	28.8 ± 4.8 31.2 (15.2 -34)

undergoing regular physical and laboratory examinations, by venipuncture and clotted at the room temperature. The sera were collected following centrifugation and frozen immediately at - 20° C pending analysis.

Dkk-1 (Titer Zyme EIA, Assay Design, Inc., Ann Arbor, MI, USA) levels were measured by solid phase enzyme – linked immunosorbent assay (ELISA). The amounts of Dkk-1 were quantitated by an automated ELISA reader (Rayto, RT-1904C Chemistry Analyzer). The results were expressed as nanograms per milliliter (ng /mL).

Data analysis was performed by using SPSS software (SPSS 16, Chicago, IL,USA). The report design was adopted from the standards for reporting diagnostic accuracy (STARD) group (Bossuyt et al., 2004). Nonparametric Mann Whitney U test was used to compare the median rank for statistical significance. p values < 0.05 were considered to be significant. Cut-off values were calculated using by ROC curves, which plot the ROC curve corresponding to the sensitivity and specificity of the test.

Results

Descriptive statistics and the Dkk-1 levels of patients with colorectal cancer and the control group are shown in Table 1. The serum Dkk-1 levels were not high in patients with colorectal cancer than in the control group. There were not significant differences between the groups for Dkk-1 (p=0.363) (Table 1).

To determine the cut-off values and sensitivity and specificity of serum Dkk-1 test in the patients we used receiver operating characteristic (ROC) curves. The cut-off value was chosen according to the ROC curve coordinate points and cut-off point for serum Dkk-1 was equal to its mean value. The sensitivities and specificities determined from the ROC curves at cut-off level of 29.36 ng / mL were 59.3 % and 50 % for serum Dkk-1 respectively (AUC : 62.4 ± 2.8%).

Discussion

Homeostasis of the intestinal epithelium is strongly dependent on the balance existing between cell proliferation, cell cycle arrest, and cell migration. The Wnt signalling pathway is one important regulator of gastrointestinal stem cell proliferation and homeostasis (Byun et al., 2005).

Some genes involved in embryogenesis and fetal development are re-activated in tumors and may be implicated in the neoplasia process. The expression of some of these proteins can be exploited as tumor markers, serves in diagnosis, prognosis and in monitoring of relapse or treatment effectiveness. Specifically, very few secreted tumor markers are available for the management of common cancers (Forget et al., 2007).

Wnt proteins influence many aspects such as embryonic development and tumorigenesis, and their activities are regulated by several secreted antagonists. Dickkopfs (Dkks) are secreted antagonists of Wnt signaling pathway. Activated Wnt signal pathway, characterized by the stabilization of beta-catenin, plays an important role in most gastrointestinal cancers. Down-regulation of the Dkks and Krm2 associated to promoter hypermethylation was frequently involved in gastrointestinal tumorigenesis. Hypermethylated Dkks could be a marker for screening gastrointestinal cancer (Maehata et al., 2008).

To our knowledge, this is the first report on serum Dkk-1 levels in colorectal cancer. Previous studies on dkk-1 have been limited to cell lines or tissue, and therefore we discuss our results in comparison with these findings. The most striking feature of this study was the serum Dkk-1 levels were equal in patients with colon and rectum cancer and the control group. The sensitivity (59.3%) and specificity (50%) of serum Dkk-1 was low in patients, because of these results Dkk-1 is not used as a marker in the diagnosis of colorectal cancer.

Dkk-1 expression level was decreased in human colon cancers, mesothelioma, and malignant melanoma, suggesting that Dkk-1 acts as a tumor suppressor gene in these neoplasms. The Wnt/beta-catenin pathway was down-regulated by the induction of Dkk-1 expression, a mechanism that is lost in a subset of colon cancers (Maehata et al., 2008).

González-Sancho et al. found that Dkk-1 expression decreases in human colon tumors, which suggests that Dkk-1 acts as a tumor suppressor gene in this neoplasia. This data indicate that the Wnt/beta-catenin pathway is downregulated by the induction of Dkk-1 expression, a mechanism that is lost in colon cancer (González-Sancho et al., 2005).

However, to evaluate the role of soluble Wnt antagonists in upper (gastric) and lower (colon) gastrointestinal tract tumorigenesis, Dkk1 expression was evaluated on normal and malignant human gastric and colon tissues and no expression of Dkk1 was seen in the normal or malignant gastric or the normal or malignant colonic tissues (Byun et al., 2005). In our study, we observed that the serum Dkk-1 levels were same levels in patients with colorectal cancer (m=31.2 ng/mL) compared to controls (m=31.2 ng/mL).

In conclusion, the present study does not show and confirm that serum Dkk-1 levels could have a diagnostic property in colorectal cancer and it is not necessary to use as markers in the diagnosis of colorectal cancer.

Acknowledgement

The present work was supported by the Research Fund of Istanbul University. Project No: BYPS-11-16/271106.

References

Aguilera O, Peña C, García JM, et al (2007) The Wnt

antagonist DICKKOPF-1 gene is induced by 1alpha,25-dihydroxyvitamin D3 associated to the differentiation of human colon cancer cells. *Carcinogenesis*, **28**, 1877-84.

Bossuyt PM, Reitsma JB, Bruns DE, et al (2004). Towards complete and accurate reporting of studies of diagnostic accuracy: the STARD initiative. *Fam Pract*, **21**, 4-10.

Byun T, Karimi M, Marsh JL, et al (2005). Expression of secreted Wnt antagonists in gastrointestinal tissues: potential role in stem cell homeostasis. *J Clin Pathol*, **58**, 515-9.

Forget MA, Turcotte S, Beauseigle D, et al (2007). The Wnt pathway regulator DKK1 is preferentially expressed in hormone-resistant breast tumours and in some common cancer types. *Br J Cancer*, **96**, 646-53.

González-Sancho JM, Aguilera O, García JM, et al (2005). The Wnt antagonist DICKKOPF-1 gene is a downstream target of beta-catenin/TCF and is downregulated in human colon cancer. *Oncogene*, **24**, 1098-103.

Jemal A, Thomas A, Murray T, et al (2002). Cancer statistics. *CA Cancer J Clin*, **52**, 23-47.

Maehata T, Taniguchi H, Yamamoto H, et al (2008). Transcriptional silencing of dickkopf gene family by CpG island hypermethylation in human gastrointestinal cancer. *World J Gastroenterol*, **14**, 2702-14.