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Original Research

BCL-3 and β-catenin signaling and tumor staging in colorectal cancer

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Abstract: Colorectal cancer (CBC) is the third most common cancer in men and the second most common cancer in women in the world, as well as the second leading cause of death among the world's cancers. In this study, to identify the genes involved in the CRC clinical outcomes, the expression of B cell leukemia/lymphoma 3 (*BCL-3*) gene in patients with colorectal cancer was investigated along with serum level of β-catenin. In this study, samples of 23 patients with colorectal cancer were prepared. mRNA was extracted and then cDNA was prepared for evaluation of PCR. Then, with specific primers for the BCL3, a semi-quantitative RT-PCR test was performed. The enzyme-linked immunosorbent assay (ELISA) was used to assess the serum level of β-catenin. In this study, 23 men with an average age of years were included. BCL-3 expression in tumor tissue was significantly higher than its level in healthy tissue (P=0.021). BCL-3 expression at stage 0 of the tumor was significantly lower than at all other stages (P<0.05), and the comparison between the rest of the CRC stages was not significant (P>0.05). The median of serum β-catenin levels in the patients was 32.83 (22.45, 46.09). There was no significant difference in the amount of serum β-catenin between all 5 stages of the disease and in terms of lymph node metastasis. There were no relationships between age, BMI, smoking history, familial CRC history, and BCL-3 or β-catenin serum levels (P>0.05). In this study, the expression of the BCL-3 gene in tumor specimens was high. It can be said that the BCL-3 gene can act as one of the genes involved in colorectal cancer, along with some genes such as β-catenin. While BCL-3 was associated with a higher stage of CRC, β-catenin didn't show such a relationship with CRC.

Key words: BCL-3; β-catenin; Colorectal cancer.

Introduction

Colorectal cancer (CRC) is the third most prevalent deadly cancer. According to statistics, the CRC is responsible for 19,000 deaths in the UK, 85,000 in Europe, and 61,000 in the United States each year (1). Early identification, elimination, or surgery is necessary for the treatment. Colorectal cancer is one of the most leading causes of death in men and also women in industrial cities (1). Many etiological factors are linked with CRC formation and progression. One of the most studied biological pathways through the pathogenesis of CRC is WNT signaling (2).

Various factors including the WNT (Wingless and Int-1) signal pathway, impair the capacity to apoptosis of cancerous stem cells (2). Beta-catenin is a central or primary molecule in the intracellular messaging pathway of WNT that was previously known to be involved in the carcinogenesis of a variety of gastrointestinal cancers, including gastric and colon cancer (3).

Previous studies on colon cancer have shown a link between the emergence of nuclear beta-catenins and cancerous cell formation, and poor survival in patients with CRC. According to research, the role of beta-catenins in the colon and rectal cancer alone cannot be explained by the pathway of WNT messengers. This protein can play an alternative role in differentiating cancer cells (4). It should be noted that based on the results of a study by Wanitsuwan et al. in 2008, the overexpression of beta-catenins as a nucleus accumulator is a very important factor in predicting colorectal cancer outcomes (5). This pathway also plays a significant role in the growth of body tissues during fetal and postpartum periods (4). The signal pathway of the Wnt family acts by secreted glycolipoprotein which is one of the essential pathways that leads the cell proliferation, and polarization and tissue homeostasis. As a consequence, defects in the Wnt system are sometimes linked with congenital malformations, tumors, and other human diseases. This signal pathway contains proteins that relay signals and messages to the cell through cellular receptors (4, 5). B cell leukemia/lymphoma 3 (*Bcl3*) is a factor that binds to β-catenin and may affect Wnt/β-catenin/T-cell factor (TCF) signaling (6). BCL-3 was first described in a subset of chronic lymphocytic leukemia B cells and has since been documented in other cancers of the blood, bone marrow, and lymph nodes and is correlated with poor prognosis of these cancers (7). In fact, abnormal activity of BCL-3 was found without disruption in various subgroups of non-Hodgkin's and Hodgkin's lymphomas (7). Emerging data indicate that BCL-3 is essential in the development and growth of tumors; BCL-3 mRNA proteins in breast tumors, nasopharyngeal cancer, endometrial cancer, and colorectal cancer are reported. It was known to be associated with the stem cells function in cancer growth (8, 9). While

there is close contact between BCL-3 and β-catenin and the potential association of them with the stem cells, we conducted this study to evaluate the mentioned associations in a clinical view.

Materials and Methods

In this study, patients who referred from 2017 to 2018 with a definitive diagnosis of colorectal cancer. First, according to previous studies and the opinion of experts, a checklist consisting of demographic and clinical information was prepared. The inclusion criteria were to have access to the patient's specimen, a medical record, and at least one examination by a specialist. Subjects with incomplete medical records were excluded from the study, and finally, 23 people entered the study and were examined. Individual and clinical characteristics of the patient including sex, age, marital status, history of smoking and drug use, family history of cancer, body mass index, type of first treatment, tumor stage, tumor grade, site of cancer, and recurrence of the disease have been collected.

Collection and maintenance of samples

In this case study, 23 tissue samples from people with colorectal cancer and 53 tissue samples adjacent to the tumor (control) were collected between 2017 and 2018. To comply with the rules of bioethics, written consent was obtained from all patients before starting the work, and also the clinical information of patients was recorded. All studies complied with the Helsinki Declaration of Principles. Written informed consent from the donors has been obtained for all procedures.

Complete RNA from colorectal cancer tissue and controls were isolated using Trizol (Invitrogen, Carlsbad, CA). Then the extracted RNAs were kept in -70 degrees Celsius until the next steps. Concentration and purity of RNAs were evaluated with the Nanodrop device and to confirm the quality of the samples agars gel electrophoresis was used. cDNA was synthesized by the Revert Aid First Strand cDNA method based on the manufacturer instructions (K1622 kit, Thermo Fisher, USA). Accompanied by 1 microliter dNTP and 1 microliter Random Hexaimer (40pg/ml) and 1 microliter Oligo dT was poured into the microtube and brought to a volume of 10 µl with water, then the tube was put for 5 minutes at -70 C. immediately put it in the ice, then 1 microliter of Reverse Transcriptase enzyme was added to each sample with 0.5 micro-liter of Riblock, 4 microliters of 5X buffer, and finally water. The synthesized cDNA was used for PCR. Pre-developed Taqman assay probes (Applied Biosystems ABI, Foster City, CA) were used for the study of Bcl-3 expression. All analyzes were performed in triplicate from two to five independent cell stimulation experiments in the ABI Prism 7000 Sequence Detection System. The slides were blocked for 30 minutes at room temperature at 3% BSA in PBS after methanol fixation and subsequent washing in phosphate-buffered saline (PBS) and stained with anti-Bcl-3 rabbit or pre-immune serum. The slides were reprobated with an anti-rabbit goat FITC-labelled antibody after washings PBS. The slides were mounted in ProLong Anti-Fade reagent containing 4′,6-diamidino-2-phenylindole (Molecular Samples, Eugene, OR) after subsequent washing with PBS and evaluated with an Olympus BX41 microscope (Olympus, Melville, NY) at an original magnification of 400 (10).

A commercially accessible enzyme-linked immunosorbent assay kit (ELISA) (CUSABIO, Wuhan, China) was used to test the serum β-catenin amount in all samples, as directed by the supplier. The CEA values were measured using the "ECLIA" immunoassay of the electrochemiluminescence (11).

Results

In the present study, 23 patients with CRC tumors were examined, all of whom were male. The mean age of our patients was 64.37 ± 8.14. The average BMI of patients was 23.98 ± 2.13 in the study. In our study, 8 subjects (34.78%) had a history of smoking, and a family history of CRC was positive for 3 subjects (13.04%). In terms of Tumor stage, patients were evaluated at 5 levels of TNM: 0, I, II, III and IV. 12 Individuals (8.69%) were at 0 stages, stage I was seen in 6 subjects, and stages II and III each in 2 persons (8.69%); Number of Patients with Level IV Tumors was in 1 person (4.34%). In our study, the mean tumor size was 5.37 ± 1.46. Also, 7 people (30.43%) had Lymph node metastasis (Table 1).

The TNM staging system is a system that uses TNM to refer to the number and spread of cancer in patients. T means the size of the tumor and the spread of cancer to nearby tissues; N means the spread of cancer to nearby lymph nodes; and M means the metastasis.

In the present study, the amount of BCL-3 relative expression in healthy tissue and tumor tissue was exa-

Table 1. Patient's characteristics.

Variables	Patients (n=23)
Age, years, mean ± STD	64.37±8.14
BMI, mean ± STD	23.98±2.13
Smoking history, n (%)	8(34.78)
Family history, n (%)	3(13.04)
Tumor stage, n (%) 0	12(52.17)
I	6(26.08)
II	2(8.69)
III	2(8.69)
IV	1(4.34)
Tumor size, cm, mean ± STD	5.37±1.46
Lymph node metastasis, n (%)	7(30.43)

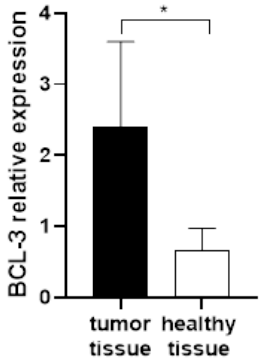

Figure 1. BCL-3 relative expression in healthy tissue and tumor tissue.

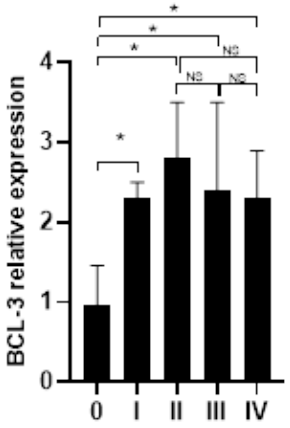

Figure 2. BCL-3 relative expression in different CRC stages.

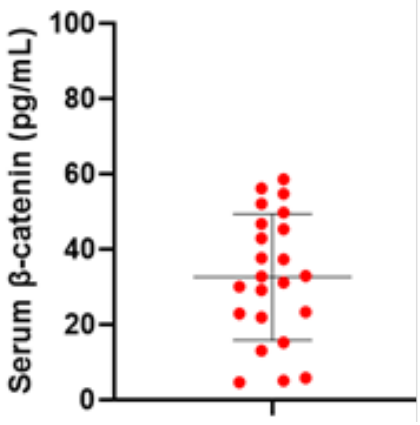

Figure 3. Serum β-catenin levels.

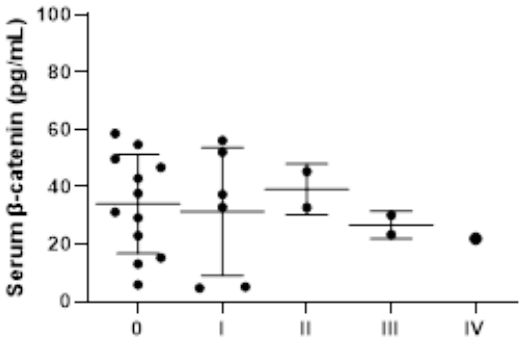

Figure 4. Serum β-catenin levels in different CRC stages.

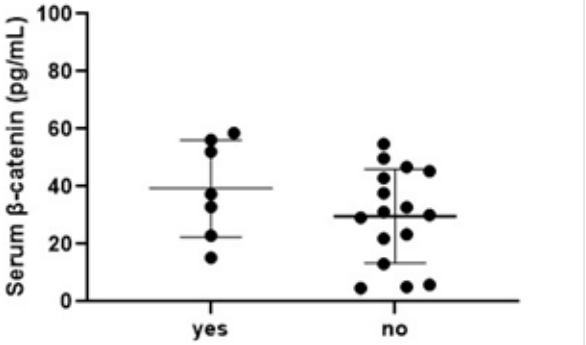

Figure 5. Serum β-catenin level based on lymph metastasis.

mined, and the results of our study showed that the amount of BCL-3 in tumor tissue was significantly higher than its level in healthy tissue (P=0.021) (Figure 1). In this study, we looked at the level of BCL-3 expression at different tumor stages and finally found that the level of BCL-3 expression at stage 0 of the tumor was significantly lower than at all other levels (P<0.05), and the comparison between the rest of the CRC stages was not significant (P>0.05) (Figure 2). Also, in the present study, we examined the level of serum β-catenin in all those present in the study. The results of our study showed that the median of serum β-catenin in the patients was 32.83 (22.45, 46.09) (Figure 3). The results of a study on the level of serum β-catenin in all 5 stages of colorectal cancer showed that there was no significant difference in the amount of serum β-catenin between all 5 stages of the disease (Figure 4). We also compared serum β-catenin in patients with Lymph node metastasis and patients without Lymph node metastasis. Finally, our results showed that serum β-catenin levels didn't have any significant difference between subjects with Lymph node metastasis and subjects without Lymph node metastasis (Figure 5). There were no relationships between age, BMI, smoking history, familial CRC history, and BCL-3 or β-catenin serum levels (P>0.05).

Discussion

In the present study, the TNM staging system was used as one of the main outcomes to compare with biological and genetic factors. The TNM name scheme is used in assessing the therapeutic stage of cancer. T stands for Tumor which indicates the degree to which tissue is damaged. N stands for a node which shows that the disease has spread to the lymph nodes or not. M stands for metastases and shows that cancer has spread to other areas of the body (12).

The stage of the disease shows how far cancer has spread in the body and is one of the most important factors in deciding on the type of treatment and its success rate. As a result, depending on the location of the colon or rectal lesion and the stage of the disease, different types of treatment are given to patients (12). As previous studies had evaluated the relationship of the BCL-3 gene and β-catenin (9); its clinical association was not yet demonstrated and this was the first attempt to evaluate this hypothesis in real patients. So cancer staging was a good study target. Our study showed that BCL-3 expression in tumor tissue was significantly higher than its level in healthy tissue. BCL-3 expression at stage 0 of the tumor was significantly lower than at all other stages. A comparison between the rest of the CRC stages was not significant. While BCL-3 expression was significantly higher in higher stages of CRC, Serum β-catenin didn't show such a relationship. This shows that BCL-3 may use another pathway than effecting β-catenin in cancer progression.

Given that BCL-3 is expressed in a subset of colorectal cancers and is associated with poor prognosis, and Wnt pathways are disrupted in the vast majority of colorectal tumors. Also, it was found that the survival of mutant stem cells was enhanced by NF-kB signaling which is being regulated by Wnt pathways (13). Chronic lymphocytic leukemia protein 3 B-cell / lymphoma (BCL-3) is strongly expressed in a subset of CRC that has recently been shown to prevent apoptosis and cause tumor growth (14). BCL-3 is an unusual member of the Kappa B (IBB) protein-inhibiting family and has been

shown to transcribe NF-κB target genes by binding to sub-dimeric p50 or p52 subunits through the domain. (15, 16). However, early experiments demonstrated the interaction between BCL-3 and β-catenin and their effect on stem cell genes of colorectal tumors; this showed that BCL-3 plays an important role in enhancing the stem cell potential of colorectal cancer cells. The regulation of BCL-3 mediators by a specific subset of intestinal stem cells and Wnt target genes increases proliferation as the suppression of BCL-3 expression inhibits total beta-catenin. Many of the proteins that interact with βcatenin to regulate its transcription activity are regulated by Wnt pathways (13-15).

Binding of BCL-3 to β-catenin increases the expression of Cyclin DL-C-my-CD44 in colon carcinoma. It is thought to be due to the fact that on chromosome one (1q21), the genes RAB25, CKSIB, PDZK1, and MUCT are located, which themselves are overexpressed in colorectal cancer (17). BCL-3 shows a small increase in normal tissue, but in colon carcinoma, it has an increase of about 65%.

The product of the CTNNB1 gene is the β-catenin protein, which is a cadmium binding protein and is involved in some intercellular pathways (3). But an important role of this protein is to activate cell transcription, often when the protein forms a complex in the cell nucleus with the TCF/LEF family of T lymphocyte cell factor / lymphoid-induced factor (18).

The amount of beta-catenin in cell cytosol increases through the waterfall of the Wnt transduction signal pathway in the protein complex, including APC, Axin, and GSK-3β, or serine glycogen kinase synthetase. In the mutation of the CTNNB1 gene, an important part of the GSK-3β serine/threonine enzyme gets altered, which is the location of the beta-catenin protein, resulting in the accumulation of the beta-catenin + TCF/LEF protein complex in the nucleus of dysplastic cells (19).

The role of mutations is well demonstrated in CRC. Carmen et al. in 2001 and Nelson in 1997 interpreted the colonic cancer inductive effects of 1 and 2 dimethylhydrazine and the expression of the nuclear beta-catenin protein (17, 18). According to their study, 1 and 2 dimethylhydrazines induce mutations in the gene that produces a beta-catenin protein to induce colon carcinoma in rats. They stated that colonic carcinoma was caused mainly by gene mutations, especially genes that were involved in DNA repair and replication. Among these genes, we can name the APC gene or adenomatosis polyposis gypsy, in which 80-85% of cases of colonic carcinoma are related to the mentioned gene mutation (20-22).

Therefore, according to the results of the present study, while it cannot be stated that the occurrence of serum beta-catenin can be associated with colon carcinoma CRC and as we didn't have control healthy patients to compare with our patients in term of the serum levels of this protein, it may be necessary to conduct additional studies with precise assumptions and express quantitative and qualitative variables to pave the way for other research activities.

Research conducted by Puvvada et al. (23) identified a correlation between BCL-3 expression and clinical results for colorectal cancer. A significant association has been documented between nuclear BCL-3 expression and poor prognosis of CRC. Another recent research by Liu et al. reports that BCL-3 plays a significant role in stabilizing C-MYC proteins by ERK activation. Unregulated C-MYC is of special significance for colorectal cancer (24, 25). Moreover, Taheri et al. found that the DNA methylation status of the miR-200c/141 cluster could be used as a progression marker from benign polyps to colorectal cancer (26). Dysregulated KDR and FLT1 gene expression in colorectal cancer patients was found and was concluded that the combination of FLT1 and KDR is a good predictive biomarker to discriminate malignant from non-malignant tissues in colorectal cancer (27).

However, given these important findings, there are few investigations on how BCL-3 expression may lead to cancer of the colon in CRC patients. BCL-3 is a powerful residual factor in colorectal cancer, especially in the sense of stress associated with the microenvironment of the tumor. BCL-3 / NF- κB complexes serve as a new activator of the AKT anti-type signaling pathway implicated in the molecular pathogenesis of several human malignancies, including colorectal cancers (13). In order to the regulation of BCL-3 expression, new technologies such as gene editing (28) can be used.

In this study, the expression of the BCL-3 gene in tumor specimens was high. It can be said that the BCL-3 gene can act as one of the genes involved in colorectal cancer, along with some genes such as β-catenin. While BCL-3 was associated with a higher stage of CRC, β-catenin didn't show such a relationship with CRC. Among the limitations of this study are the following: Problems of coordination with hospitals for tissue sampling and patients' poor condition to fill out a questionnaire that led to samples being excluded from the study. Therefore, it is recommended that similar studies be performed on a larger number of tissue samples, and on genes involved in the Wnt pathway so that its valuable results can be applied to the community health system.

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