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Original article

# Evaluating the expression level of co-stimulatory molecules CD 80 and CD 86 in different types of colon polyps

N. Peyravian [a], E. Gharib [a], A. Moradi [b], M. Mobahat [a], P. Tarban [a], P. Azimzadeh [c], E. Nazemalhosseini-Mojarad [c,*], H. Asadzadeh Aghdaei [a]

[a] *Basic and molecular epidemiology of Gastroentestinal disorders Research center, Research Institute for Gastroenterology and Liver Diseases, Shahid Beheshti University of Medical Sciences, Tehran, Iran*
[b] *Department of Pathology, Shohada Hospital, Shahid Beheshti University of Medical Sciences, Tehran, Iran*
[c] *Gastroenterology and Liver Disease Research center, Research Institute for Gastroenterology and Liver Diseases, Shahid Beheshti University of Medical Sciences, Tehran, Iran*

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ABSTRACT

*Purpose of the study.* – Co-stimulatory molecules CD80 and CD86 are the members of B7 family, which stimulate the T lymphocytes in response to the malignant colon polyps. However, the expression of these molecules is depressed in cancers. In the present study, the transcription levels of *CD80* and *CD86* genes in the colon polyps (Precancerous lesions) and its association with the clinical features were examined.
*Patients and methods.* – Forty-nine biopsies samples from patients with the colorectal polyps and 10 healthy subjects were collected by the colonoscopy. Questionnaires including clinical and demographic data were filled for all cases. Using Real-time PCR, the mucosal mRNA expression levels of *CD80* and *CD86* genes were quantified.
*Results.* – Adenoma and hyperplastic polyps were reported in 69.3 and 30.7 percent of 49 patients, respectively. Unlike hyperplastic polyps, the expression of CD86 was increased in adenoma polyps compared to controls (RQ = 2.75 vs. 0.837, respectively). The data from CD80 showed noticeable reduction about 0.31 and 0.11 in adenoma and hyperplastic polyps, respectively, in response to control group (RQ = 0.729). Also, analyzing colon and rectum polyps depicted a marked increment in CD86 level, in contrast to CD80.
*Conclusion.* – Examining the mRNA expression levels of *CD80* and *CD86* genes between colon polyps with the rectal polyps shows that the enhanced level of CD86 in adenoma samples could be considered as a valuable biomarker for distinguishing the adenoma from hyperplastic polyps and the masses located in the colon from the rectum.



## 1. Introduction

Cancer cells are different from the normal cells by the expression of tumor-specific antigens, which potentially make them susceptible to the host natural immunity [1]. In cancer microenvironment, the cells recruit molecules to repress the immune responses or active the survival cascades by association with the tumor-infiltrating immune cells such as T- lymphocytes and macrophages [2,3].

Cell-mediated immune responses against the tumors are conducted through the T-cell activation. Effective activation of the T-cells requires engagement of the T-cell receptor with the MHC-peptide complex, in parallel with the exposure of co-stimulatory B7 molecules B7.1 (CD 80) and B7.2 (CD86) on the antigen-presenting cells (APC) such as macrophages, dendritic cells, and B- lymphocytes [1,4]. CD28 is a primary co-stimulatory signal receptor on the T CD4+ cells' membrane that binds to the CD80 and CD86 ligands. The impaired co-stimulatory pathway in cancer cells may lead to the failure in creation of an effective anti-tumor immunity response. Previous studies indicate that the CD80 and CD86 have distinct functions. Whereas APC activation is

* Corresponding author at: Gastroenterology and Liver Disease Research center, Research Institute for Gastroenterology and Liver Diseases, Shahid Beheshti University of Medical Sciences, P.O. Box: 19857-17411, Yeman Street, Chamran Expressway, Tehran, Iran.
*E-mail addresses:* nazemalhosseini@sbmu.ac.ir, ehsanmojarad@gmail.com (E. Nazemalhosseini-Mojarad).

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followed by an increased in the CD80 expression, CD86 is constitutively expressed by the resting APC cells [5–7].

Colorectal cancer (CRC) is mainly arising from the noncancerous adenoma or polyps (abnormal growth) that develop on the lining of the colon or rectum. Today, the epithelial polyps are categorized as either adenomatous or hyperplastic (HPs) [8]. Adenomatous polyps are considered as the precursor lesions whereas the hyperplastic polyps are defined as the benign innocuous lesions without malignant potential. Although the biological properties of the hyperplastic polyps are not well characterized, yet studies identify the HP polyps as a potent inducer in cancer progression [9,10].

Various mucosal epithelial cells, specially the intestinal epithelial cells as a non-specific APCs, display the class II MHC molecules, constitutively. These cells are supposed to express the co-stimulatory molecules, CD80 or CD86 and as result activate the local CD4+ T lymphocytes [11]. Many lines of evidence suggest that, the absence of the B7 co-stimulatory molecules suppress the ability of the epithelial cells to activate the T cells [12]. However, some studies support this hypothesis that low levels of the CD80 and CD 86 are expressed in a series of cell lines derived from the human carcinomas, including some with the colorectal origins [13]. Hence, in this study we were aimed to evaluate the expression levels of co-stimulatory molecules (CD80 and CD86) in different types of the colon polyps specially the adenoma and hyperplastic samples.

## 2. Patients and methods

### 2.1. Patients

In this study we recruited forty-nine patients who had been diagnosed with the colorectal polyps in the Research Institute for Gastroenterology and Liver Diseases, and 10 healthy subjects, as controls. The Diagnosis of the colorectal polyp was based on the findings from the colonoscopy and histopathology. Control subjects were selected as healthy people without any abnormal colonoscopy results and negative family history for gastrointestinal diseases.

After obtaining the informed consents from all the participating subjects; the patients and controls were interviewed to obtain information on demographic and clinical data. Tissue samples of polyps were taken from the different regions of the colon during polypectomy or colonoscopy. The characteristics of these patients are summarized in Table 1.

Each biopsy specimen was divided into two parts: one was transferred via liquid nitrogen and stored at -80 °C for molecular analysis and the other was fixed in 10% formalin solution for histological analysis. The histopathological analysis was confirmed via a hospital pathology report. This study was conducted under the approval of the ethics committee (No.2014/770) of the gastroenterology and liver disease research center, Shahid Beheshti University of medical sciences (Tehran, Iran).

**Table 1**
Demographic and clinical characteristics of the cases.

| Characteristics | Variable | Frequency, *n* (%) |
|---|---|---|
| Sex | Male | 27 (55.1) |
| | Female | 22 (44.9) |
| Smoking | Yes | 8 (16.3) |
| | No | 41 (83.7) |
| Alcohol | Yes | 5 (10.3) |
| | No | 44 (89.7) |
| Diabetes | Yes | 7 (14.3) |
| | No | 52 (85.7) |
| Blood per rectum | Yes | 5 (10.2) |
| | No | 44 (89.8) |
| Diarrhea | Yes | 4 (8.2) |
| | No | 45 (91.8) |
| Constipation | Yes | 6 (12.2) |
| | No | 43 (87.8) |
| Abdominal pain | Yes | 7 (14.3) |
| | No | 42 (85.7) |
| Weight loss | Yes | 4 (8.2) |
| | No | 45 (91.8) |
| Family history | Yes | 11 (21.4) |
| | No | 38 (77.6) |
| IBD | No | 43 (87.8) |
| | Ulcerative colitis | 4 (8.1) |
| | Crohn disease | 2 (4.8) |
| Total | | 49 (100) |

### 2.2. Immunohistochemistry (IHC) and evaluation of staining

To analysis the composition of immune cells, IHC test was performed on the 3 to 5 mm thick slices of formalin-fixed, paraffin-embedded tissues that prepared as described previously [14].

For deparaffinization, the slides were incubated in 37 °C for 24 h and then washed with xylol (100%), ethanol (100%, 85% and 75%) and distilled water, respectively. After deparaffinization, slides were incubated in the solution of 10% H2O2 and methanol in the ratio of 1:9, for 15 min and subsequently washed with the distilled water. Next, slides were treated in the 10 mM citrate buffer solution (pH = 6) and microwaved with 800 W for 24 min and washed with the Tris-buffer saline (TBS). After treating with the blocking serum for 15 min, the slides were immunostained with monoclonal mouse anti-human antibodies including CD3+ (T-cells), CD20+ (B-cells) and CD68+ (Macrophages and Monocytes), for 45 min and later washed with TBS. Later, by treating with the Envision + visualization system (Dako) for 30 min followed by DAB as the chromogen substrate for 10 min, the bound primary antibody was visualized. Finally, slides were washed with the distilled water, dehydrated with the alcohol and stained in the hematoxylin. All the slides were independently checked twice by the light microscopy. Analysis of CD markers expressions was performed using a quantitative scale. The mean values were estimated through the scanning of the entire tissue sections of all samples, using a two graded scale (negative, $< 5\%$); (positive, $> 5\%$). Nuclear β-catenin was monitored on the standard sections from the colon carcinomas tissues.

### 2.3. RNA isolation

Total RNA was extracted from the frozen colonic polyp tissues using the QIAamp RNA Mini Kit (Qiagen, Hilden, Germany). The RNA concentration was quantified via the spectrophotometry (Thermo nanodrop 2000, spectrophotometer Uv/vis Reader), and the integrity of the samples were assessed by electrophoresis on 1% agarose gel (FMC Bio Product, Rockland, ME, USA) containing ethidium bromide.

### 2.4. Reverse transcription

Total RNA was reverse-transcribed with the RevertAid ™ Reverse Transcriptase kit and random hexamer primers (Thermo Fisher Scientific, USA). First total RNA was incubated with the random hexamer primers according at 65 °C for 5 min, then 6 μl of the mixture was added to 15 μl reaction solution containing 4 μl of 5 × reaction buffer, 2 μl of dNTPs (10 mM), 1 μl of RiboLock RNase Inhibitor (20 U/μl), 1 μl of Revert Aid RT (200 U/μl) and 7 μl of nuclease-free water.

The reaction was run in a Perkin–Elmer Gene Amp PCR System 2400. The reverse transcription reaction was performed at 25 °C for 5 min, at 42 °C for 60 min and at 70 °C for 5 min. The prepared cDNA was stored at -70 °C.

### 2.5. Real time polymerase chain reaction (PCR)

Quantitative real-time reverse transcription-PCR was carried out on an ABI Step One RT-PCR thermal cycler (ABI Stepone, NY, USA) using a SYBR Premix Ex Taq II kit (TaKaRa Biotechnology). Primers for CD80/CD86 were designed using the Primer Express software version 3.0.1 (Applied Bio-systems, CA). The primers used in the present study were as follows:CD80 (112 bp) forward: CATCTGACGAGGGCACATAC, reverse: GGTGTAGGGAAGTCAGCTTTG; CD86 (155 bp) forward: CAACGGAATTAG GAAGAC, reverse: CTCTGTATGCAAGTTTCC; Beta-2-microglobulin (β–2-microglobulin) (86 bp) forward: 5'_TGCTGTCTCCATGTTTGATGTATCT_3', reverse: 5'_TCTCT GCTCCCCACCTCTAAG_3'.

The reaction was performed in a 20 μL final volume containing 0.8 μl of each primer, 2 μL of cDNA template, 10 μL of SYBR Premix Ex Taq II (Tli RNaseH Plus) (2 × ), 0.4 μL of ROX Reference Dye (50 × ) and 6 μL of sterile distilled water. The thermal profile for the PCR was 95 °C for 30 s, followed by 40 cycles of 95 °C for 5 s and 60 °C for 34 s. Beta-2-microglobulin (β–2-microglobulin) mRNA was utilized to calculate the relative abundance of mRNA transcripts. Each measurement was performed in triplicate. Relative mRNA abundance was determined by normalizing to β–2-microglobulin using the 2-ΔΔCT method [15].

### 2.6. Statistical analyses

Data are expressed as the mean ± S.D. (Standard division), and the statistical analysis was executed using the SPSS software (version 13). The associations between the different types of polyps with categorical variables or demographic and clinical characteristics were evaluated by the Chi-square tests. Also, the analysis of continuous variables was performed with T-test and One-way analysis of variance (ANOVA) followed by an appropriate post-hoc test. Moreover, by using the Fisher's exact test, the frequency data were examined. Statistical significance was achieved when $P < 0.05$.

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