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Correlation of Cyclooxygenase 2 Expression and Inflammatory Cells Infiltration in Colorectal Cancer

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Abstract: This study want to determine correlation of cyclooxygenans 2 (Cox-2) expression and inflammatory reaction in colorectal carcinoma. Archival H and E slides of 150 patients with primary colorectal carcinoma were reviewed to confirm pathological feature and to select suitable tissue blocks for immunohistochemical staining with mouse monoclonal antibody against human Cox-2 and various inflammatory cells. After scoring, statistical analysis were carried out with SPSS software, χ^2 methods and bivariate pearson correlation analysis. The expression of Cox-2 (grade 3 to 7) in 71.3% of patients associated with mast cells, neutrophils, eosinophils, macrophages, CD^{3+} lymphocytes infiltration was significant ($p = 0.001$). Correlation of Cox-2 expression associated with CD^{8+} lymphocytes infiltration was not significant ($p = 0.569$). Also CD^{3+} lymphocytes show severe infliltration when the expression of Cox-2 is negative ($p<0.05$). The main purpose of this study was to evaluate the interaction between the Cox-2 expression and inflammatory cells infiltration. This study showed that there is a close relationship of Cox-2 expression and mast cells, neutrophils, eosinophils, macrophages and CD^{3+} lymphocytes. The only exception was CD^{8+} lymphocytes. It is may be due to independent role of anti tumoral effect of this inflammatory cells.

Key words: Carcinoma, immunohistochemical, mast cell

INTRODUCTION

The cyclooxygenase (Cox) enzyme catalyze a key step in the conversion of arachidonate to PGH2, the immediate substrate for a series of cell specific prostaglandin and thromboxane synthases (Christofer *et al*., 1999).

There are two Cox isoforms, which differ mainly in their pattern of expression (Christofer *et al*., 1999).

Cox-1 is expressed in most tissues, whereas Cox-2 usually is absent , but is induced in numerous physiologic stimuli (Christofer *et al*., 1999). Cycloaxygenase-2 (Cox-2) is needed for production of prostaglandins and other eicosanoids in inflammation site (Christofer *et al*., 1999). Cox-2 is overexpressed in 50% of benign polyps and 80-85% of adenocarcinoma (Christofer *et al*., 1999).

Colorectal cancer is the third most common cancer in the world and the second most common cause of cancer related death (Rosai, 2004).

Cox-2 has been reported to be significantly increased in up to 85% of human sporadic colorectal carcinoma (Soumaoro *et al*., 2004). The infiltration of inflammatory cells in cancer tissue is considered an important aspect of the host response in cancer (Gao *et al*., 2005). The inflammatory response can have dual effects in the progression of cancer. On one side inflammatory cells are a prognostic good sign probably by maintaining control due to elimination of tumor cells, while on the other side production of cytokines and growth factors can provide a growth stimulating microenvironment for tumor cells (Nagtegaal *et al*., 2001). Cox-2 expression is correlated with increased growth and metastases of tumor cells (Klintrup *et al*., 2005; Yao *et al*., 2005). Epidemiologic, animal and human data indicate that NSAIDS, inhibitors of Cox-2, decrease inflammation and are chemopreventive for colon cancer (Hla and Neilson, 1992; Statton and Albers, 2002; Masfercer *et al*., 2000).

Little is known about interaction between Cox-2 and inflammatory cells of colorectal carcinoma (Zhang and Sun, 2002). Therefore , we aimed to determine correlation between Cox-2 expression and inflammatory reaction in colon cancer.

MATERIALS AND METHODS

In this study 150 patients had undergone surgical resections for primary sporadic colorectal carcinoma at the Department of Surgery, Imam and Shafa hospitals (Sari, Iran), between January 2000 and December 2005 were included. None of them had a history of hereditary colon cancer syndromes. There was no preoperative chemotherapy or radiotherapy.

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In all cases, archival H and E slides of the primary tumor were reviewed to confirm pathological feature and to select suitable tissue blocks for immunohistochemical analysis.

A universal immunoenzyme polymer method was used for immunostaining with mouse monoclonal antibody against human Cox-2 (dilution 1:250:DAKO Co., Denmark).

As a negative control for Cox-2 tissue sections were treated with normal serum instead of each primary antibody.

Various inflammatory cells was assessed by following antibodies: anti-CD 3 (Anti-human T cell, 1:1600, DAKO CO., Denmark) anti CD 8 (1 : 3200 , DAKO, Denmark), anti: CD 68 (Antihuman macrophage, 1:6400, DAKO Co., Denmark), anti human mast cell tryptase (1:3200, DAKO, Denmark), elastase (Anti human neutrophil elastase, 1:800, DAKO Co., Denmark), EG-2 (Anti-human ECP/EPX, 1:1000, Pharmacia Upjohn, Uppsala, Sweden).

All sections were scored blind by two investigators under a light microscope. For Cox-2, the entire tissue section was scanned to assign the scores. The staining intensity was scored as 0 (negative), 1 (weak), 2 (medium) and 3 (strong). Extent of staining was scored as 0 (6%), 1 (1-25%), 2 (26-50%), 3 (51-75%) and 4 (76-100%), according to the percentages of the positive staining area in relation to the whole carcinoma area or entire section for the normal sample. The sum of intensity and extent score was used as the final staining score (0-7) for Cox-2. This relatively simple, reproducible scoring method that gives highly concordant results between independent evaluators has been used in past studies (Soumoaro *et al*., 2004).

For the purpose of statistical evaluation, tumors having a final staining score of≥3 were considered to be positive for scoring of various inflammatory cells the running mean method was used to determine the minimum number of High Power Fields (HPFs) to be scored for the result to be reliable. Two independent investigators performed this determination.

Necrotic areas were avoided after analysis of the distribution of the numbers of inflammatory cells, the count were divided into three categories: none/few, moderate and many, based on the distribution of the numbers of cells in the study population (Table 1).

Statistical analysis were carried out with SPSS software. The correlation between Cox-2 expression and various inflammatory cells were assessed with χ^2 methods and bivariate pearson correlation analysis. At $p<0.05$, difference were considered statistically significant.

Table 1 : Categorical arrangement for different cell type

Cell type	Cell mm^{-2}
Eosinophils	
None/few	0-10
Moderate	11-50
Many	>50
Neutrophils	
None/few	0-5
Moderate	6-50
Many	>50
Mast cell	
None/few	0-5
Moderate	6-50
Many	>50
Macrophages	
None/few	0-50
Moderate	51-150
Many	>150
T cell (CD 3)	
None/few	0-55
Moderate	56-105
Many	>105
T cell (CD 8)	
None/few	0-15
Moderate	16-75
Many	>75

RESULTS AND DISCUSSION

The expression of Cox-2 in 71.3% of patients associated with neutrophils, eosinophils, mast cells, macrophages and CD 3 lymphocytes was significant ($p = 0.001$). Correlation of Cox-2 expression and CD^{8+} lymphocytes was not significant ($p = 0.569$).

The most severe inflammation associated with positive Cox-2 was for infiltration of mast cells (59.8%) and least severe inflammation was for infiltration of CD^{3+} lymphocytes (1.9%). The most severe inflammation associated with negative Cox-2 was for infiltration of macrophages (69.8%) and least severe inflammation was for infiltration of eosinophils (32.6%). CD^{3+} lymphocytes show increase severity of inflammation when the expression of Cox-2 is negative (Table 2).

The main purpose of this study was to evaluate the interaction between the Cox-2 expression and inflammatory cells.

We showed that there is a close relationship between Cox-2 expression and infiltration of mast cells, neutrophils, eosinophils, macrophages, CD^{3+} lymphocytes.

The only exception was CD^{8+} lymphocytes. It is may be due to independent role of anti tumoral effect of this inflammatory cells.

Cox-2 expression in tumor cells is related with severe infiltration of mast cells and least severe infiltration of lymphocytes.

This study showed that expression of Cox-2 has a opposite relationship with lymphocytes infiltration. Probably Cox-2 expression keep away the lymphocytes from tumor cells and then growth and metastases of tumor cells increase on the other hand, increased infiltration of

Table 2: Correlation of Cox-2 expression and inflammatory cells

	Severity of inflammation (%)		
Cell type	None/few	Moderate	Many
Neutrophils			
Cox-2 positive	15.0	62.6	22.4
Cox-negative	65.1	32.6	2.3
Eosinophils			
Cox-2 positive	60.7	27.1	12.2
Cox-negative	32.6	65.1	2.3
Mast cells			
Cox-2 positive	2.8	37.4	59.8
Cox-negative	67.4	32.6	0.0
Macrophages			
Cox-2 positive	15.9	48.6	35.8
Cox-negative	69.8	30.2	0.0
T cells (CD3)			
Cox-2 positive	58.9	39.3	1.8
Cox-negative	32.6	34.9	32.5
T cells (CD 8)			
Cox-2 positive	71.0	28.0	1.0
Cox-negative	65.0	35.0	0.0

other inflammatory cells is may be by production of cytokines and growth factors create microenvironment for tumor cells growth.

Interestingly, we showed a significant correlation between negative expression of Cox-2 and severe infiltration of CD^{3+} lymphocytes, in contrast with other inflammatory cells.

This can be the cause of better prognosis of Cox-2 negative tumor cells.

Finally, we conclude that present study can clarified cause of anti tumoral effect of Cox-2 inhibitors.

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