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Correlation of Cyclooxygenase 2 (Cox-2) Presentation and Inflammatory Cells Infiltration in Colorectal Cancer: A Histopathological and Immunohistochemical Study

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Abstract: Colorectal cancer is the third most common cancer in the world and the second most common cause of cancer related death. Cox-2 has been reported to be significantly increased in up to 85% of human sporadic colorectal carcinoma. Little is known about interaction between Cox-2 and inflammatory cells of colorectal carcinoma. In all cases, archival H and E slides of the primary tumor were retrieved and reviewed to confirm pathological facture and to select suitable tissue blocks for Immunohistochemical analysis. The expression of Cox-2 was positive (grade 3-7) in 71.3% of tumors and associated with neutrophils, eosinophil, mast cell, macrophages and CD3+ lymphocytes ($p = 0.001$). Correlation of Cox-2 expression and CD8 lymphocytes was not significant ($p = 0.569$). 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 of Cox-2 expression and mast cells, neutrophils, eosinophils, macrophages, lymphocyte (CD3+). The only exception was CD8 positive lymphocytes.

Key words: Cerrical spine, traoma, surgical decompression, cancer, inflammatory cells

INTRODUCTION

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

Cycloaxygenase-2 (Cox-2) is needed for production of prostaglandins and other eicosanoids in inflammation site (Juan, 2004). NSAIDS decrease the inflammation by inhibition this enzyme-expression of Cox-2 related to carcinogenesis and their inhibitors have antitumoral effect (Hla and Neilson, 1992; Statton and Alberts, 2002; Masfercer *et al*., 2000). 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).

Little is known about interaction between Cox-2 and inflammatory cells of colorectal carcinoma (Zhang and Sun, 2002). Therefore, we aimed to determine whether Cox-2 expression can predict intratumoral specific T-cells and nonspecific inflammatory reaction.

MATERIALS AND METHODS

Patients and tissue samples: A total of 150 patients had undergone surgical resections for primary sporadic colorectal carcinoma at the Department of surgery, Imam hospital, mazandaran university of medical science and shafa hospital (Sari, Iran), between January 2000 and December 2005 was included in this study. None of them had a history of hereditary colon cancer syndromes. There was no preoperative chemotherapy or radiotherapy.

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

Immunohistochemical staining: A universal immunoenzyme polymer method was used for immunostaining. Three micrometer thick sections were cut from formalin-fixed, paraffin-embedded tissue blocks and mounted on poly-lysine coated slides, dewaxed in

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xylene and rehydrated through graded series of ethanol. After deparaffinization, antigen retrieval treatment was performed at 121°C (autoclave) for 5 min in 10 mmol L^{-1} sodium citrate buffer (PH 6.0) then treated with 3% hydrogen peroxidase in methanol solution. Nonspecific bindings were blocked by treating slides with 10% normal sheep serum for 10 min. Thereafter, the slides were incubated with mouse monoclonal antibodies against human Cox-2 (dilution 1:250:DAKO Co Denmark) for 2 h at room temperature. Next slides were incubated with labeled polymer (DAKO Co) in room temperature for 30 min. Color development was done 0.02% diaminobenzidine tetrahydrochloride (DAKO Co) and 0.06% hydrogen peroxide in 50 mmol L^{-1} Tris HCL (PH 7.6) for 5 min. Finally, the slides were counterstained with 1% meyers hematoxylin.

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

Various inflammatory cells was assessed by following antibodies: anti-CD3 (Anti-human T cell, 1:1600, DAKO CO., Denmark) anti CD 8 (1: 3200, DAKO, Denmark), anti: CD68 (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).

Evaluation of staining: All sections were scored blind by 2 investigators (F.N. and K.E.) under a light microscope and in cases of occasional scoring discrepancy, consensus was always achieved after discussion of findings. For Cox-2, the entire tissue section was scanned to assign the scores. The staining intensity was scored as o (negative), 1 (weak), 2 (medium) and 3 (strong). Extent of staining was scored as O (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 studiesc (Soumaoro *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 inffammatory 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 (F.N and K.E) performed this determination. This was done by calculating stepwise the mean number of inflammatory cells for the total of HPFs, until the difference between successive means became negligibly small in a few cases with pour agreement, the cases were reviewed using multiheaded microscope. It was decided to count 15 HPFs for every cell type.

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

The scoring was performed within the tumor (intratumoral infiltrating cells, present in the stroma of the tumor). 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: All statistical analysis were carried out with SPSS software. The correlation between expression of Cox-2 and various inflammatory cells were assessed with X2-mthods and bivariate pearsons correlation analysis. At $p<0.05$, difference were considered statistically significant.

RESULTS

The allocation of staining score of Cox-2 and inflammatory cells by the two investigators was concordant in > 95% of samples in addition, as we stated in materialas and methods, the discrepant cases were reevaluated and scored by consensus.

The expression of Cox-2 was positive (grade 3-7) in 71.3% of tumors and associated with neutrophils, eosinophil, mast cell, macrophages and CD3 lymphocytes ($p = 0.001$) correlation of Cox-2 expression and CD8 lymphocytes was not significant ($p = 0.569$).

Table 2: Correlation of Cox-2 expression and inflammatory cells severity of inflammation (%)

Cell type	None/few	Moderate	Many
Neutrophils			
Cox-2 positive	15	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
Macrophages			
Cox-2 positive	15.9	48.6	35.8
Cox-negative	69.8	30.2	0
T cells (CD3)			
Cox-2 positive	58.9	39.3	1.8
Cox-negative	32.6	34.9	32.5
CD8 T cells			
Cox-2 positive	71	28	1
Cox-negative	65	35	0

The association of Cox -2 expression and inflammatory cells in shown in Table 2.

The most severe inflammation associated with positive Cox-2 was mast cell infiltrate (59.8%) and least severe inflammation was CD3 lymphocytes (1.9 %). The most severe inflammation associated with negative Cox-2 was macrophages (69.8%) and least severe inflammation was esoinophils (32.6%). CD3 lymphocytes show increase severity of inflammation when the expression of Cox-2 is negative.

DISCUSSION

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 of Cox-2 expression and mast cells, neutrophils, eosinophils, macrophages, lymphocyte (CD3). The only exception was CD8 positive lymphocytes. It is may be due to independent role of anti tumoral effect of this inflammatory cell positive expression of Cox-2 in tumor cells is related with most severe infiltrate of mast cells and least severity of infiltrate in lymphocytes. as we known lymphocytes has known role of anti tumoral effect. This study showed that expression of Cox-2 has a reverse relationship with lymphocytes infiltrate. Probably Cox-2 expression keep away the lymphocytes from tumor cells and then tumor cells growth and metastases are increased. on the other hand expression of Cox-2 and increased infiltrate of other inflammatory cells of this study stimulate production of cytokines and growth factors can provide a growth stimulating. microenvironment for tumor cells. At this point, it is considered that expression of Cox-2 produce a stimulating microenvironment for tumor cells to growth and metastases. Interestingly, we showed a significant correlation between negative expression of Cox-2 and increase severity of CD3 lymphocytes, in contrast with other inflammatory cells. This can be the cause of better prognosis of Cox-2 negative tumor cells, due to increased CD3 lymphocytes with their known anti tumoral effect.

CONCLUSION

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

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