Cyclooxygenase-2 Expression in Rectal Cancer Predicts Poor Prognosis for Patients Receiving Postoperative Chemoradiation

Jun Won Kim

Department of Medicine

The Graduate School, Yonsei University

Cyclooxygenase-2 Expression in Rectal Cancer Predicts Poor Prognosis for Patients Receiving Postoperative Chemoradiation

Directed by Professor Ki Chang Keum

The Master's Thesis submitted to the Department of Medicine, the Graduate School of Yonsei University in partial fulfillment of the requirements for the degree of Master of Medical Science

Jun Won Kim

December 2010

This certifies that the Master's Thesis of
Jun Won Kim is approved.

Thesis Supervisor: Ki Chang Keum

Won Ho Kim

Sun-Young Rha

The Graduate School
Yonsei University

December 2010

ACKNOWLEDGEMENTS

I have a special debt of gratitude to Professor Ki Chang Keum for his knowledge, guidance and continuous support for the completion of this thesis. I am also very grateful to Professor Won Ho Kim and Professor Sun-Young Rha for all the support and advice, which improved the quality of this work. I would like to extend my gratitude to Professor Yong Bae Kim who provided continuous teaching in developing research techniques and writing skills from the beginning of the research. I also would like to thank other members of the Department of Radiation Oncology, including Professors Gwi Eon Kim, Chang Ok Suh, Jinsil Seong, Jae Ho Cho, Ik Jae Lee, and Woong Sub Koom and my fellow residents. Finally, I would like to thank my wife, Anna Kim, and two sons, Joseph and Daniel, who gave me joy and meaning of life, and my parents and family in Canada who gave me continuous support and encouragement when I needed them the most.

Jun Won Kim, M.D.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

ABSTRACT

Cyclooxygenase-2 Expression in Rectal Cancer Predicts Poor Prognosis for Patients Receiving Postoperative Chemoradiation

Jun Won Kim

Department of Medicine
The Graduate School, Yonsei University

(Directed by Professor Ki Chang Keum)

Purpose: The objective of this retrospective study was to determine the role of cyclooxygenase-2 (COX-2) expression in pre-irradiation rectal cancer specimens as an indicator of prognosis for patients undergoing postoperative chemoradiation.

Patients and Methods: We performed immunohistochemical (IHC) study with COX-2 antibody on the pre-irradiation surgical specimen obtained from 72 patients who were treated with postoperative chemoradiation for locally advanced rectal cancer between 2000 and 2001. IHC scores were assigned based on both intensity and extent of staining, and patients were divided into two groups: a COX-2 positive group (IHC score $\geq$ 5, n = 27) and a COX-2 negative group (IHC score < 5, n = 45). Clinicopathologic parameters, patterns of failure and survival rates were compared between the two groups. Univariate and multivariate analyses were performed to determine the prognostic factors influencing patient survival.

Results: Median follow-up was 65 months. COX-2 overexpression was observed in 37.5% of patients. Locoregional failure rates were higher in the COX-2 positive group (25.9% vs. 6.7%, p = 0.02) as well as distant metastasis (48.1% vs. 17.8%, p = 0.006) in comparison with the COX-2 negative group. The 5-year disease free survival (DFS) and overall survival (OS) rates were lower for the COX-2 positive group compared with the COX-2 negative group (DFS: 46.8% vs. 76.7%, p = 0.019 / OS: 54.1 % vs. 85.9%, p = 0.026). Univariate and multivariate analyses of DFS and OS showed that COX-2 overexpression was an independent prognostic factor that surpassed other well-known clinicopathologic parameters.

Conclusions: COX-2 overexpression in pre-RT surgical specimen was strongly associated with a higher rate of treatment failure and can be used as a potent molecular risk factor predicting poor prognosis for locally advanced rectal cancer patients receiving postoperative chemoradiation.

Key words: Cyclooxygenase-2, rectal cancer, prognostic factor, adjuvant pelvic chemoradiation

Cyclooxygenase-2 Expression in Rectal Cancer Predicts Poor Prognosis fir Patients Receiving Postoperative Chemoradiation

Jun Won Kim

Department of Medicine
The Graduate School, Yonsei University

(Directed by Professor Ki Chang Keum)

I. INTRODUCTION

In Korea, the annual incidence rate of colorectal cancer (CRC) increased by 57% between 1999 and 2007, and CRC is now the third most common cancer in men and fourth most common cancer in women.[1] Despite the effort to increase the early detection rate through screening process and development of multi-modality treatment, long-term outcome of CRC has not significantly changed, and the 5-year overall survival (OS) rate remains at approximately 70%.[2] For locally advanced rectal cancer, total mesorectal excision (TME) is the mainstay of treatment, and addition of preoperative chemoradiation has helped reduce the incidence of local recurrence without further survival benefit.[3] Molecular markers such as epidermal growth factor receptor (EGFR), vascular endothelial growth factor (VEGF), and cyclooxygenase-2 (COX-2) have been studied to measure tumor responses to chemoradiation, but the value of these molecular markers in predicting patient survival remains controversial.[4,5]

COX-2 is a key enzyme in the conversion of arachidonic acids into

prostaglandins and other eicosanoids. Two isoforms of COX have been characterized. COX-1 is constitutively expressed in most tissues and controls normal physiologic functions, whereas COX-2 is undetectable in most normal tissues and is induced by proinflammatory and mitogenic stimuli increasing the synthesis of prostaglandins in inflamed and neoplastic tissues during the early stages of cell differentiation or replication.[6,7] COX-2 is upregulated in many human malignancies, including cancers of the lung,[8] breast,[9] pancreas,[10] uterine cervix,[11] nasal type NK-T cell lymphoma,[12] and colorectum.[13] COX-2 is involved in multiple stages of cancer lifecycle. It promotes carcinogenesis and tumor proliferation, inhibits cell apoptosis, and regulates angiogenesis and tumor surveillance through host immune responses.[14-16]

Numerous studies suggest the role COX-2 as a molecular prognostic factor for CRC patients, but the results have been inconsistent.[17-19] COX-2 levels and subsequent production of prostaglandin, particularly PGE2, increased during radiotherapy,[20-23] and COX-2 inhibitors synergized with chemotherapy and radiotherapy in several preclinical models.[24-26] Recent studies demonstrated a prognostic role of COX-2 in rectal cancer patients treated with preoperative chemoradiation[27-29] with an emphasis on the induction of resistance to apoptosis and tumor response.[29,30] However, investigation on post-RT resection specimens has general limitations that the level of molecular markers is likely to have changed from the pretreatment level, no tumor can be assessed after a complete response to chemoradiation, and tumor sampling can be problematic after a high degree of tumor regression.

COX-2 may influence survival of patients treated with postoperative chemoradiation through mechanisms that are different from those in the preoperative chemoradiation setting. Surgical specimens from patients who received no preoperative radiotherapy or chemotherapy provide an adequate ground for molecular studies evaluating initial COX-2 level and its impact on patient survival. The objective of this study was to determine whether COX-2

expression in pre-RT surgical specimen had any influence on the prognosis of rectal cancer patients treated with definitive surgery and adjuvant chemoradiation.

II. METHODS AND MATERIALS

1. Patients and Treatment Protocol

We reviewed 110 patients with stages II-IV adenocarcinoma of the rectum who were treated with definitive surgery (TME) and postoperative concurrent chemoradiation at the Department of Radiation Oncology, Severance Hospital, Yonsei University Health System (Seoul, Korea) between 2000 and 2001. Among them, 38 patients were excluded from analysis, because their paraffin embedded tissue blocks were unavailable, leaving 72 patients eligible for the study. Clinical staging and histologic classification of the rectal cancer for each patient were based on the tumor, node, metastasis (TNM) system of the American Joint Committee on Cancer, 6^{th} edition. All patients received pretreatment computed tomography scans or magnetic resonance imaging (MRI) for staging purposes. Indications for adjuvant radiotherapy and concurrent chemotherapy included patients with high-risk factors such as T3 or T4 tumor, positive lymph nodes or positive resection margin after definitive surgery. All patients underwent adjuvant chemoradiation as recommended by the National Institute of Health Consensus Conference in 1990.[31] Chemotherapy based on 5-FU (450 mg/m^2 for 5 days) and leucovorin (20 mg/m^2 for 5 days) was intravenously given each month with six cycles. External whole pelvis irradiation was performed after the second chemotherapy treatment and consisted of 54 Gy delivered (with a 6 MV/10 MV dual photon linear accelerator) in 30 fractions, five times per week, with individually shaped portals and using a three-field technique.

2. Tissue Array Block

The recipient blocks were made with purified agar in 3.8 x 2.2 x 0.5 cm frames. Holes measuring 2 mm in diameter were made on the recipient blocks using a core needle, and the agar core was discarded. The donor blocks

were prepared after a thorough evaluation of the hematoxylin and eosin-stained slides. Representative tumor tissues obtained from the matching donor blocks were transplanted to the recipient blocks using a 2-mm core needle. The recipient blocks were framed in the mold, which was used to frame the conventional paraffin blocks. Subsequently, paraffin was added to the frame. Consecutive 4-μm-thick sections were cut from the recipient blocks using an adhesive-coated slide system (Instrumedics Inc., NJ) to support the cohesion of the 2-mm array elements on the glass.

3. Immunohistochemistry

Four-μm-thick tissue sections were cut from the formalin fixed, paraffin embedded tissue blocks, dewaxed in xylene, rehydrated in graded ethanol solutions, rinsed in phosphate buffered saline (PBS) for 5 minutes, and then immersed in 0.3% hydrogen peroxide in methanol for 30 minutes to block endogenous peroxidase. For antigen retrieval, the sections were microwaved in a 0.01 mol/L sodium citrate-buffered saline, pH 6.0, for 30 minutes at 95 ℃. The slides were then rinsed in PBS for 5 minutes and blocked with a solution of 10% normal rabbit serum in PBS at room temperature for 10 minutes. They were then incubated at 4 ℃ overnight with the COX-2 primary antibodies from Lab Vision (Fremont, CA) at dilutions of 1:1500. The antibody was soaked in citrate buffer (pH 6.0) and preheated using a microwave for 10 minutes, based on the unmasking effect of the masked epitope. Tissues were incubated with biotinylated horse antimouse secondary antibodies diluted to 1:500 (Vector Laboratories, Burlingame, CA) followed by extensive washes in avidin-biotin peroxidase complex (1:25 dilution). Diaminobenzene was used as the chromogen, and hematoxylin was used as the nuclear counterstain. The entire tissue section was scanned to assign the scores for COX-2 expression. The staining intensity was scored as 0 (negative), 1 (weak), 2 (medium), and 3 (strong). The extent of staining was scored as 0 (0%), 1 (1 to 25%), 2 (26 to

50%), 3 (51 to 75%), and 4 (76 to 100%), according to the percentages of the positive staining areas in relation to the whole carcinoma area or entire section for the normal samples. The sum of the intensity and extent scores was used as the final IHC scores (0 to 7) for COX-2. To rule out the possibility of interpersonal bias, the results were interpreted by one investigator who was blinded to the clinical outcome.

4. Statistical Analysis

The clinical profile and patterns of treatment failure in both groups were compared using a chi-square test. The disease free survival (DFS) and overall actuarial survival (OS) rates were calculated using the Kaplan–Meier method, and the log-rank test was used to compare the rates between COX-2 expression groups. Univariate analysis was used to define the prognostic factors for survival. The relative importance of the covariates in determining prognostic factors also was assessed by using a multivariate Cox proportional hazards model. *P* values ≤ 0.05 were considered significant.

II. RESULTS

1. Immunohistochemical Findings

The patterns of staining for the COX-2 enzyme exhibited marked intratumoral heterogeneity in both intensity and extent, ranging from tumors with few weakly positive cells to tumors with apparent overexpression. COX-2 immunoreactivity was mainly detected in the cytoplasm of the epithelial cells and vascular endothelial cells, but rarely in stromal cells which are probably fibroblasts and mononuclear cells (Figure 1). A strong correlation was observed between the staining intensity and extent ($p = 0.0001$). Using the median staining score of 4 as the cutoff value, patients were divided into s COX-2 positive group (IHC score $\geq$ 5) and a COX-2 negative group (IHC score < 5). Overexpression of COX-2 was detected in 27 patients (37.5%).

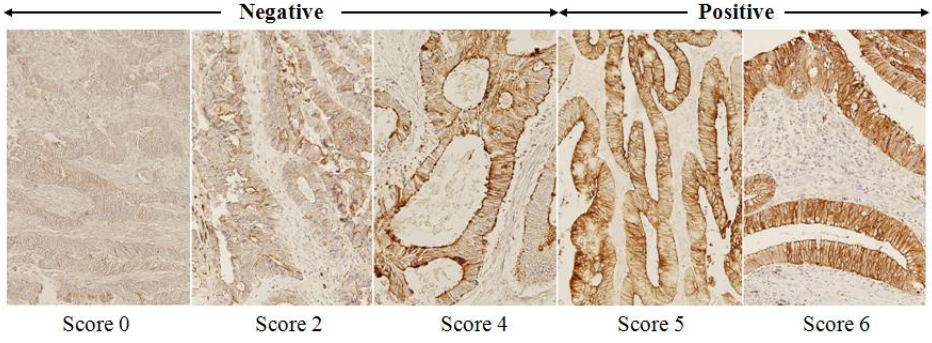

Figure 1. Representative immunohistochemical features are shown in photomicrographs from patients with rectal cancer. Cyclooxygenase-2 (Cox-2) staining was observed dominantly in epithelial cells but rarely in stromal cells. The pattern of COX-2 staining was mostly cytoplasmic reactivity.

2. Clinical Profiles

Clinicopathological profiles of the patients are listed in Table 1. The median follow-up was 65 months, and the median age was 59 years. Most patients received TME through low anterior resection (89%). Four patients were diagnosed with isolated liver metastasis and received curative resection. An effort was made to determine a correlation between COX-2 expression and several clinical parameters. No significant difference was found in types of surgery, tumor size, overall stage or status of lymphovascular invasion between the two groups. Although patients in the COX-2 positive group showed a tendency to have a higher incidence of pathologic N2 disease (≥ four pelvic lymph nodes), the difference was not statistically significant.

3. Patterns of Treatment Failure

There were a total of 24 recurrences among all patients at the time of this analysis: 15 patients (56%) in the COX-2 positive group and nine patients (20%) in the COX-2 negative group ($p = 0.002$). Figure 2 illustrates the patterns of treatment failure of the two groups. The COX-2 positive group had local failures in three patients, distant failures in nine patients, both local and distant failures in two patients, and local, regional, and distant failures combined in one patient. In the COX-2 negative group, two patients had both local failure and hematogenous metastasis, one patient suffered regional failure, and six patients were diagnosed with hematogenous metastases. The locoregional failure rate in the COX-2 positive group was significantly higher than the COX-2 negative group (22% vs. 7%, $p = 0.022$). The rate of hematogenous metastases was also significantly higher in the COX-2 positive group compared with the COX-2 negative group (18% vs. 44%, $p = 0.006$).

Table 1. Patient Characteristics

Clinical factors	COX-2 (+)		COX-2 (-)		Total		P value
	No	%	No	%	No	%	
Patients	27	37.5	45	62.5	72	100	
F/U (mo)	12-114	median 65	3-108	median 65	3-114	median 65	
Age (year)	45-73	median 63	23-76	median 59	23-76	median 59	
Gender							0.547
Male	18	66.7	33	73.3	51	70.8	
Female	9	33.3	12	26.7	21	29.2	
Surgery							0.331
LAR	25	92.6	39	86.7	64	88.9	
APR	2	7.4	6	13.3	8	11.1	
Tumor size							0.951
< 5cm	11	40.7	18	40.0	29	40.3	
≥ 5cm	16	59.3	27	60.0	43	59.7	
N stage							0.094
pN0	11	40.7	18	40.0	29	40.3	
pN1	4	14.8	16	35.6	20	27.8	
pN2	12	44.5	11	24.4	23	31.9	
Stage							0.856
II	11	40.7	18	40.0	29	40.3	
III	14	51.9	25	55.6	39	54.2	
IV	2	7.4	2	4.4	4	5.6	
LV invasion	7	25.9	7	15.6	14	19.4	0.282
Lateral RM (+)	0	0	5	11.1	5	6.9	0.073

Abbreviations: F/U = follow-up; LAR = low anterior resection; APR = abdominoperineal resection; LN = lymph node; LV = lymphovascular; RM = resection margin; COX = cyclooxygenase; NS = not significant

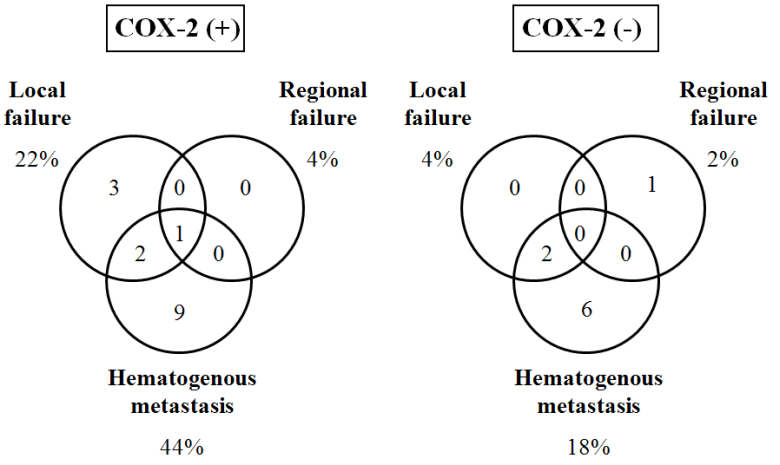

Figure 2. Diagrams showing the difference in failure patterns between the cyclooxygenase-2 (COX-2) positive group and the COX-2 negative group.

4. Survival and Prognostic Factors

With a median follow-up of 65 months, the 5-year DFS and OS rates for all patients who were treated with surgical resection and adjuvant chemoradiation were 64% and 72%, respectively. The COX-2 positive group had a poorer prognosis than the COX-2 negative group. The 5-year DFS rate was 47% (95% CI, 49–83%) for patients in the COX-2 positive group and 77% (95% CI, 75–99%) for patients in the COX-2 negative group, and the difference was significant (p = 0.023; log-rank test). The 5-year OS rate was 54% (95% CI, 62–91%) in the COX-2 positive patients and 86% (95% CI, 85–103%) in the COX-2 negative patients (p = 0.030; log-rank test). The DFS and OS curves for the two groups are shown in Figure 3. Because the prognosis of the patients was correlated with several clinicopathologic variables, we investigated the influence of COX-2 expression on patient survival by using Cox regression analyses. In the initial univariate analysis for the DFS and OS rates, the pathologic N2 disease, overall stage, and COX-2 overexpression were important

prognostic factors (Table 2), but only COX-2 over-expression was found as an independent prognostic factor in the multivariate analysis (Table 3).

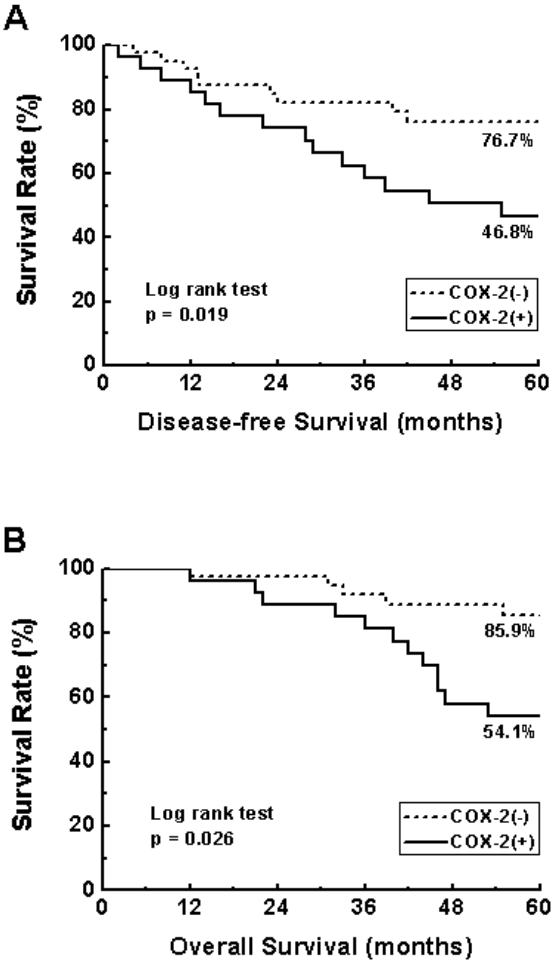

Figure 3. Comparison of the overall survival (A) and disease free survival (B) rates in patients according to their cyclooxygenase-2 (COX-2) expression level (Kaplan–Meier method). The COX-2 positive group (n = 27 patients; solid lines) had a poorer prognosis compared with the COX-2 negative group (n = 45 patients; dotted lines).

Table 2. Univariate Analysis of the Prognostic Factors

Prognostic variables	No. of patients	5-yr DFS		P value	5-yr OS		P value
		%	95%CI		%	95%CI	
Age (yrs)							
<60	36	62.9	65-95	0.871	71.1	76-101	0.912
≥60	36	65.6	65-93		73.5	74-98	
Tumor (cm)							
< 5	29	58.3	60-90	0.640	72.2	73-96	0.664
≥5	43	68.8	69-98		71.8	78-102	
LNs							
pN0, N1	49	72.6	78-102	0.019	78.4	85-105	0.046
pN2	23	46.0	43-84		58.7	60-93	
Stage							
II	29	78.6	78-108	0.050	84.4	87-111	0.045
III, IV	43	54.1	58-87		64.0	70-94	
Lat. RM							
Negative	67	63.7	70-92	0.783	72.6	80-98	0.970
Positive	5	66.7	32-103		66.7	46-100	
COX-2							
Negative	45	76.7	76-99	0.019	85.9	85-103	0.026
Positive	27	46.8	49-83		54.1	62-92	

Abbreviations: DFS = disease free survival; OS = overall survival; LN = lymph node, RM = resection margin, COX = cyclooxygenase; CI = confidence interval; NS = not significant

Table 3. Multivariate Analysis of the Prognostic Factors

Prognostic variables	5-yr DFS		P value	5-yr OS		P value
	RR	95%CI		RR	95%CI	
COX-2 (positive vs. negative)	2.5	1.1-6.1	0.038	2.9	1.1-7.3	0.026
pN2 (N2 vs. N0-1)	1.5	0.5-4.1	0.456	1.2	0.4-3.4	0.773
Stage (III & IV vs. II)	2.0	0.6-6.3	0.256	2.7	0.8-9.1	0.120

Abbreviations: DFS = disease free survival; OS = overall survival; RR = relative risk; COX = cyclooxygenase; CI = confidence interval; NS = not significant

IV. DISCUSSION

In our study, the rate of COX-2 overexpression in locally advanced rectal cancer patients was 37.5%. Patients in the COX-2 positive group showed a significantly higher rate of treatment failure after definitive surgery and adjunctive chemoradiation compared with those in the COX-2 negative group. DFS and OS of the COX-2 positive group were also significantly lower than the COX-2 negative group. In univariate and multivariate analyses, COX-2 overexpression was an independent prognostic factor for both DFS and OS. A number of known prognostic factors for rectal cancer, including tumor size, lymph node metastasis, overall stage, and positive resection margin, were included in the prognostic factor analysis, and COX-2 overexpression demonstrated the strongest correlation with treatment failure and patient survival.

The clinical implication of increased COX-2 expression has been evaluated in a large number of studies of CRC although the results have been inconsistent.[17-19] Considering the distinct differences in tumor biology, treatment approach, recurrence pattern, and metastatic behavior, it is unfortunate that many studies make no distinction between rectal cancer and more proximal colon cancer. It is well documented that COX-2 is upregulated by chemotherapy and radiotherapy and induces treatment resistance.[26,32] With inclusion of chemoradiation to the standard treatment of locally advanced rectal cancer, COX-2 has become an important prognostic factor for treatment outcome. Recent studies reported that elevation of COX-2 expression in irradiated tumors was associated with poor prognosis in rectal cancer patients undergoing preoperative chemoradiation.[27-29] Bouzourene et al. showed that a large majority of negative tumor biopsies expressed COX-2 protein after radiotherapy and that COX-2 was significantly associated with local recurrence, suggesting the role of COX-2 as a mediator of radioresistance.[27]

COX-2 is considered an immediate early response gene,[33] and mechanisms through which COX-2 influences prognosis of rectal cancer patients treated with preoperative chemoradiation may be associated with increased resistance to apoptosis and tumor regression. In a retrospective study involving 30 rectal cancer patients undergoing chemoradiation and surgery, Min et al. pointed out that patients with COX-2 overexpression in pretreatment biopsy were highly resistant to treatment, resulting in poorer tumor regression and histopathologic nodal downstaging after chemoradiation.[29] Using tumor samples from the Dutch TME trial, de Heer et al. compared patients treated by RT (5 x 5 Gy in 5 days) followed by TME with patients treated by TME alone and discovered that an increased level of COX-2 expression in irradiated rectal cancer specimens was associated with reduced levels of tumor apoptosis, higher distant recurrence rates, and shorter DFS and OS in the RT + TME group. It was pointed out that the interval between the short-term radiotherapy and surgery could be sufficient for a change in COX-2 activity and subsequent prostaglandin production to influence the clinical behavior of the tumor.[28] COX-2 is known to induce Bcl-2 expression and is associated with apoptosis resistance.[14] Moreover, de Bruin et al. showed that intrinsic apoptosis is a prognostic factor for local recurrence in rectal cancer by IHC evaluation of M30.[34]

Few studies have assessed correlation between COX-2 level in pretreatment biopsy of locally advanced rectal cancer and treatment outcome of radiotherapy. Database search found no report of clinical studies evaluating the role of COX-2 in the prognosis of rectal cancer patients undergoing postoperative chemoradiation. In the study by de Heer et al., COX-2 level in no-preoperative-RT arm showed poor correlation with treatment failure and patient survival, suggesting non-irradiated rectal cancer is not a useful discriminant determining the response to therapy or prognosis. However, our study showed that increased COX-2 levels in pre-RT surgical specimens were strongly correlated with locoregional and distant failures as well as patient

survival, suggesting COX-2 level in non-irradiated tumor can be considered a prognostic factor. The mechanisms through which COX-2 influences prognosis of patients undergoing postoperative chemoradiation have not been established yet. Elevated COX-2 expression has shown to alter the invasive and metastatic potential of cancer cells.[35] COX-2 expression and prostaglandin production induce cell-surface glycosyltransferases and type 1 sialyl Lewis antigens, leading to enhanced tumor cell adhesion to endothelial cells, and animal studies reported that COX-2 inhibition prevented the formation of distant metastases.[36] Moreover, the immunosuppressive effect of increased prostaglandin production may allow circulating tumor cells to escape the host antitumor response and metastasize.[16] It is not very likely that these events will take place during the short interval between preoperative radiation and surgery, but rather play a key role in deciding the outcome of patients treated with surgery and postoperative chemoradiation which require significantly longer treatment time.

COX-2 inhibitors have potential of being sensitizers to chemotherapy and radiotherapy for human malignancies; several animal models showed that treatment with a COX-2 inhibitor greatly enhanced the intrinsic tumor cell radiosensitivity without markedly affecting the radioresponsiveness of normal tissues.[25,37] The improved safety profile of selective COX-2 inhibitors such as rofecoxib, celecoxib, valdecoxib and etoricoxib makes it attractive to consider their adjuvant use in rectal cancer patients undergoing chemoradiation either preoperatively or postoperatively. However, rofecoxib (Vioxx) was withdrawn from the market in 2004 due to the increased cardiovascular risks observed in the APPROVe (Adenomatous Polyp Prevention on Vioxx) study,[38] and the use of valdecoxib (Bextra) was also suspended in 2005. Although celecoxib showed no increased risk of cardiovascular thrombotic events in the CLASS (Celecoxib Long Term Arthritis Safety Study) trial,[39] a phase 2 trial showed that addition of celecoxib to chemoradiation for rectal cancer was not feasible due to a high incidence of unexpected skin rash.[40] The results of our study suggest that

COX-2 inhibitors may be beneficial throughout the course of multi-modal treatment, and investigation on the synergistic role of COX-2 in cancer treatment must continue after establishing safety of these drugs.

This is the first report demonstrating a significant correlation between COX-2 overexpression in pre-RT surgical specimens and poor outcome of postoperative chemoradiation for rectal cancer. We suggest that COX-2 induces radioresistance not only in the early stage of tumor progression but also throughout the course of treatment by increasing metastatic potential and interfering with tumor surveillance. COX-2 expression can be used as a useful molecular risk factor in rectal cancer patients requiring adjuvant chemoradiation and may help individualize treatment plans for these patients. Addition of COX-2 inhibitors may benefit patients undergoing surgical resection by improving the outcome of adjuvant treatment.

.

REFERENCES

1. Ministry for Health WaFA. Annual Report of cancer incidence (2007), cancer prevalence (2007) and survival (1993-2007) in Korea. 2009. p.136.
2. Ratto C, Sofo L, Ippoliti M, Merico M, Doglietto GB, Crucitti F. Prognostic factors in colorectal cancer. Literature review for clinical application. Diseases of the Colon & Rectum 1998;41:1033-49.
3. Sauer R, Becker H, Hohenberger W, Rdel C, Wittekind C, Fietkau R, et al. Preoperative versus postoperative chemoradiotherapy for rectal cancer. The New England journal of medicine 2004;351:1731-40.
4. Dannenberg AJ, Lippman SM, Mann JR, Subbaramaiah K, DuBois RN. Cyclooxygenase-2 and epidermal growth factor receptor: pharmacologic targets for chemoprevention. Journal of clinical oncology 2005;23:254-66.
5. Willett CG, Kozin SV, Duda DG, di Tomaso E, Kozak KR, Boucher Y, et al. Combined vascular endothelial growth factor-targeted therapy and radiotherapy for rectal cancer: theory and clinical practice. Seminars in oncology 2006;33:S35-S40.
6. Smith WL, Langenbach R. Why there are two cyclooxygenase isozymes. Journal of Clinical Investigation 2001;107:1491-5.
7. Dannenberg AJ, Altorki NK, Boyle JO, Dang C, Howe LR, Weksler BB, et al. Cyclo-oxygenase 2: a pharmacological target for the prevention of cancer. The lancet oncology 2001;2:544-51.
8. Wolff H, Saukkonen K, Anttila S, Karjalainen A, Vainio H, Ristimki A. Expression of cyclooxygenase-2 in human lung carcinoma. Cancer research 1998;58:4997-5001.
9. Soslow RA, Dannenberg AJ, Rush D, Woerner BM, Khan KN, Masferrer J, et al. COX-2 is expressed in human pulmonary, colonic,

and mammary tumors. Cancer 2000;89:2637-45.

10. Molina MA, Sitja-Arnau M, Lemoine MG, Frazier ML, Sinicrope FA. Increased cyclooxygenase-2 expression in human pancreatic carcinomas and cell lines: growth inhibition by nonsteroidal anti-inflammatory drugs. Cancer research 1999;59:4356-62.
11. Kim YB, Kim GE, Cho NH, Pyo HR, Shim SJ, Chang SK, et al. Overexpression of cyclooxygenase-2 is associated with a poor prognosis in patients with squamous cell carcinoma of the uterine cervix treated with radiation and concurrent chemotherapy. Cancer 2002;95:531-9.
12. Shim SJ, Yang W, Shin E, Koom WS, Kim YB, Cho JH, et al. Clinical significance of cyclooxygenase-2 expression in extranodal natural killer (NK)/T-cell lymphoma, nasal type. International journal of radiation oncology, biology, physics 2007;67:31-8.
13. Eberhart CE, Coffey RJ, Radhika A, Giardiello FM, Ferrenbach S, DuBois RN. Up-regulation of cyclooxygenase 2 gene expression in human colorectal adenomas and adenocarcinomas. Gastroenterology 1994;107:1183-8.
14. Tsujii M, DuBois RN. Alterations in cellular adhesion and apoptosis in epithelial cells overexpressing prostaglandin endoperoxide synthase 2. Cell 1995;83:493-501.
15. Tsujii M, Kawano S, Tsuji S, Sawaoka H, Hori M, DuBois RN. Cyclooxygenase regulates angiogenesis induced by colon cancer cells. Cell 1998;93:705-16.
16. Stolina M, Sharma S, Lin Y, Dohadwala M, Gardner B, Luo J, et al. Specific inhibition of cyclooxygenase 2 restores antitumor reactivity by altering the balance of IL-10 and IL-12 synthesis. The journal of immunology 2000;164:361-70.
17. Tuynman JB, Peppelenbosch MP, Richel DJ. COX-2 inhibition as a tool

to treat and prevent colorectal cancer. Critical reviews in oncology/hematology 2004;52:81-101.

18. Fux R, Schwab M, Thon K, Gleiter CH, Fritz P. Cyclooxygenase-2 expression in human colorectal cancer is unrelated to overall patient survival. Clinical cancer research 2005;11:4754-60.

19. Soumaoro LT, Uetake H, Higuchi T, Takagi Y, Enomoto M, Sugihara K. Cyclooxygenase-2 expression: a significant prognostic indicator for patients with colorectal cancer. Clinical cancer research 2004;10:8465-71.

20. Davis TW, O'Neal JM, Pagel MD, Zweifel BS, Mehta P, Heuvelman DM, et al. Synergy between celecoxib and radiotherapy results from inhibition of cyclooxygenase-2-derived prostaglandin E2, a survival factor for tumor and associated vasculature. Cancer research 2004;64:279-85.

21. Williams CS, Tsujii M, Reese J, Dey SK, DuBois RN. Host cyclooxygenase-2 modulates carcinoma growth. Journal of Clinical Investigation 2000;105:1589-94.

22. Eldor A, Vlodavsky I, HyAm E, Atzmon R, Fuks Z. The effect of radiation on prostacyclin (PGI2) production by cultured endothelial cells. Prostaglandins 1983;25:263-79.

23. Fosslien E. Review: molecular pathology of cyclooxygenase-2 in cancer-induced angiogenesis. Annals of clinical & laboratory science 2001;31:325-48.

24. Davis TW, Hunter N, Trifan OC, Milas L, Masferrer JL. COX-2 inhibitors as radiosensitizing agents for cancer therapy. American journal of clinical oncology: cancer clinical trials 2003;26:S58-S61.

25. Kishi K, Petersen S, Petersen C, Hunter N, Mason K, Masferrer JL, et al. Preferential enhancement of tumor radioresponse by a cyclooxygenase-2 inhibitor. Cancer research 2000;60:1326-31.

26. Milas L. Cyclooxygenase-2 (COX-2) enzyme inhibitors and radiotherapy: preclinical basis. American journal of clinical oncology: cancer clinical trials 2003;26:S66-S9.
27. Bouzourene H, Yan P, Sandmeier D, Zouhair A, Matter M, Vuilleumier H, et al. The role of COX-2 in rectal cancer treated with preoperative radiotherapy. Virchows Archiv 2008;452:499-505.
28. de Heer P, Gosens MJ, de Bruin EC, Dekker-Ensink NG, Putter H, Marijnen CA, et al. Cyclooxygenase 2 expression in rectal cancer is of prognostic significance in patients receiving preoperative radiotherapy. Clinical cancer research 2007;13:2955-60.
29. Min BS, Choi YJ, Pyo HR, Kim H, Seong J, Chung HC, et al. Cyclooxygenase-2 expression in pretreatment biopsy as a predictor of tumor responses after preoperative chemoradiation in rectal cancer. Archives of surgery 2008;143:1091-7.
30. Rdel C, Grabenbauer G, Papadopoulos T, Bigalke M, Gnther K, Schick C, et al. Apoptosis as a cellular predictor for histopathologic response to neoadjuvant radiochemotherapy in patients with rectal cancer. International journal of radiation oncology, biology, physics 2002;52:294-303.
31. NIH consensus conference. Adjuvant therapy for patients with colon and rectal cancer. JAMA 1990;264:1444-50.
32. Nakata E, Mason KA, Hunter N, Husain A, Raju U, Liao Z, et al. Potentiation of tumor response to radiation or chemoradiation by selective cyclooxygenase-2 enzyme inhibitors. International journal of radiation oncology, biology, physics 2004;58:369-75.
33. Xie WL, Chipman JG, Robertson DL, Erikson RL, Simmons DL. Expression of a mitogen-responsive gene encoding prostaglandin synthase is regulated by mRNA splicing. Proceedings of the National Academy of Sciences of the United States of America 1991;88:2692-6.

34. de Bruin EC, van de Velde CJ, van de Pas S, Nagtegaal ID, van Krieken JH, Gosens MJ, et al. Prognostic value of apoptosis in rectal cancer patients of the dutch total mesorectal excision trial: radiotherapy is redundant in intrinsically high-apoptotic tumors. Clinical cancer research 2006;12:6432-6.

35. Tsujii M, Kawano S, DuBois RN. Cyclooxygenase-2 expression in human colon cancer cells increases metastatic potential. Proceedings of the National Academy of Sciences of the United States of America 1997;94:3336-40.

36. Kakiuchi Y, Tsuji S, Tsujii M, Murata H, Kawai N, Yasumaru M, et al. Cyclooxygenase-2 activity altered the cell-surface carbohydrate antigens on colon cancer cells and enhanced liver metastasis. Cancer research 2002;62:1567-72.

37. Milas L, Hunter N, Furuta Y, Nishiguchi I, Runkel S. Antitumour effects of indomethacin alone and in combination with radiotherapy: role of inhibition of tumour angiogenesis. International journal of radiation biology 1991;60:65-70.

38. Bresalier RS, Sandler RS, Quan H, Bolognese JA, Oxenius B, Horgan K, et al. Cardiovascular events associated with rofecoxib in a colorectal adenoma chemoprevention trial. The New England journal of medicine 2005;352:1092-102.

39. Fitzgerald GA. Coxibs and cardiovascular disease. The New England journal of medicine 2004;351:1709-11.

40. Jakobsen A, Mortensen JP, Bisgaard C, Lindebjerg J, Rafaelsen SR, Bendtsen VO. A COX-2 inhibitor combined with chemoradiation of locally advanced rectal cancer: a phase II trial. International Journal of Colorectal Disease 2008;23:251-5.

ABSTRACT (IN KOREAN)

수술 후 항암화학방사선 치료를 받은 직장암환자에서 Cyclooxygenase-2 발현의 임상적 유용성

<지도교수 금기창>

연세대학교 대학원 의학과

김준원

목적: 근치적 수술 시행 후 얻은 국소진행성 직장암 조직에서의 cyclooxygenase-2 (COX-2)의 발현 정도가 수술 후 항암화학방사선치료 시행 후 환자의 예후에 미치는 영향을 분석하고자 후향적 연구를 시행하였다.

재료 및 방법: 2000년부터 2001년까지 국소진행성 직장암으로 근치적 수술과 수술 후 항암화학방사선치료를 시행한 72명의 환자의 수술 조직을 대상으로 COX-2에 대한 면역조직화학 염색 (IHC stain)을 실시하였다. 염색의 강도 (intensity)와 범위 (extent)를 조합하여 IHC score를 정의하였고 27명의 COX-2 양성군 (IHC score ≥5)과 45명의 COX-2 음성군 (IHC score <5)으로 구분하였다. 두 군에 속한 환자들간의 임상 및 조직학적 인자, 재발양상, 생존율을 비교하였고 환자의 생존에 영향을 미치는 예후인자를 일변량 및 다변량 분석을 통해 확인하였다.

결과: 추적관찰기간의 중앙값은 65개월이었다. COX-2 과발현은

37.5%의 환자에서 관찰되었다. 국소재발율 (25.9% 대 66.7%, p = 0.02)과 원격전이율 (48.1% 대 17.8%, p = 0.006)에서 모두 COX-2 양성군이 음성군보다 높은 것으로 확인되었다. 5년 무병생존율 (46.8% 대 76.7%, p = 0.019)과 전체 생존율 (54.1% 대 85.9%, p = 0.026)에 대해서 COX-2 양성군이 음성군에 비해 유의하게 낮은 것으로 확인되었다. 일변량 분석과 다변량 분석을 시행하여 COX-2 과발현이 다른 알려진 인자들에 비해 생존율에 더 밀접한 연관성을 가진 독립된 예후인자임을 확인할 수 있었다.

결론: 본 연구를 통해 근치적 수술로 얻은 국소진행성 직장암 조직에서의 COX-2 과발현이 수술 후 항암화학방사선요법 시행 후 치료실패와 유의한 연관성을 보였고 환자의 예후에 대한 예측인자로 사용할 수 있을 것으로 사료된다.

핵심되는 말: COX-2, 직장암, 예후, 수술후 항암화학방사선 치료