Journal of Cellular Biochemistry Supplement 34:23–27 (2000)

Implications of Low COX-2 Expression in Colorectal Neoplasms With Defective DNA Mismatch Repair

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Abstract Most colorectal adenomas and carcinomas arise in the setting of chromosomal instability and progressive loss of heterozygosity. Approximately 15–20% of colorectal neoplasms arise through a distinct genetic pathway characterized by microsatellite instability (MSI) and frequent loss of expression of one of the DNA mismatch repair enzymes, most often hMLH1 or hMSH2. These distinct genetic pathways are reflected by differences in tumor histopathology, distribution in the colon, prognosis, and dwell time required for progression from adenoma to carcinoma. The molecular and clinical distinctions between these tumors suggest that they are biologically distinct and may respond differently to therapeutic and chemopreventive agents. Recently, we showed that expression of a putative chemopreventive target, COX-2, is significantly reduced in colorectal cancers with defective mismatch repair as assessed by MSI and absent staining for hMLH1 and/or hMSH2. The mechanisms responsible for low COX-2 expression in tumors with MSI remain unknown, but they may be linked to molecular events giving rise to MSI tumors. Although the clinical implications of these observations are unknown, the presence of MSI should be considered an important variable when assessing the efficacy of COX-2 inhibitors in chemoprevention trials. J. Cell. Biochem. Suppl. 34:23–27, 2000.

Key words: colorectal adenoma; MSI; low COX-2 expression

Colorectal cancer is the second leading cause of cancer deaths in the United States [American Cancer Society, 1996]. Because available drug therapies for colorectal cancer are largely ineffective, strategies to reduce colorectal cancer mortality and mobidity are increasingly focused toward prevention. While periodic endoscopic removal of precancerous colorectal adenomas is of proven benefit, drugs that prevent formation of adenomas may eventually provide a noninvasive and safe alternative. In this regard, nonsteroidal anti-inflammatory drugs (NSAIDs) show considerable promise. They effectively reduce polyp burden in cases of familial adenomatous polyposis (FAP) [Giardiello et al., 1993] and reduce adenoma incidence and colon cancer mortality in the general population [Thun et al., 1991; Logan et al., 1993]. However, these drugs induce gastrointestinal injury and bleeding in a significant percentage of patients, prompting a search for safer alternatives.

CYCLOOXYGENASE-2 AND COLORECTAL CANCER

NSAIDs inhibit cyclooxygenase-1 (COX-1) and cyclooxygenase-2 (COX-2), which both catalyze the conversion of arachidonic acid to prostaglandins [Donnelly and Hawkey, 1997]. Inhibition of cyclooxygenase-1 mediates the adverse side effects of NSAIDs, including gastrointestinal injury and platelet inhibition. By contrast, the chemopreventive effects of NSAIDs may be largely related to inhibition of cyclooxygenase-2 (COX-2). Knockout of the COX-2 gene significantly suppresses adenoma formation in $APC^{+/-}$ mice, a mouse model of FAP that lack one of the two copies of the adenomatous polyposis coli (APC) gene [Oshima et al., 1996]. Similarly, novel selective COX-2 inhibitors suppress polyp formation in $APC^{+/-}$ mice [Roy and Lu, 1997; Oshima et al., 1996] and in azoxymethane-treated rats [Kawamori et al., 1998]. These observations suggest that inhibitors of COX-2 may eventually prove themselves as safe and

Contract grant sponsor: National Institutes of Health; Contract grant number: R01 CA 68535; Contract grant number: R29 CA 71974; Contract grant number: R01 DK47294; Contract grant sponsor: Abbott Laboratories.

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Received 2 September 1998; Accepted 3 September 1998

effective chemopreventative agents for colorectal adenomas in humans.

COX-2 is ordinarily expressed at very low levels in the colonic mucosa. However, approximately 50% of adenomas and 80% of carcinomas of the colorectum in humans express high levels of COX-2 mRNA and protein [Eberhart et al., 1994; Rigas et al., 1993]. COX-2 levels are also increased in FAP-associated adenomas in humans [Giardiello et al., 1997]. Similarly, COX-2 levels are increased in colonic tumors azoxymethane-treated rats [DuBois et al., 1996a] and in $APC^{+/-}$ mice [Williams et al., 1996b]. COX-2 activity in implicated is cell growth, survival, and angiogenesis [DuBois et al., 1996b; Chan et al., 1998; Tsujii et al., 1998]. Its expression in colorectal neoplasms may therefore offer a survival advantage. The molecular events responsible for elevated COX-2 expression in colorectal neoplasms, however, remain unknown.

Colorectal neoplasms arise through at least two distinct genetic mechanisms. The majority (80%) involves chromosomal instability (CIN), characterized by loss of heterozygosity [Kinzler and Vogelstein, 1996]. Functional loss of both APC alleles is among the earliest and most common molecular events seen in CIN tumors [Polakis, 1997]. As many as 15–20% of sporadic colorectal neoplasms, however, arise through an alternative mechanism involving defective DNA mismatch repair [Thibodeau et al., 1993; Marra and Boland, 1995; Eshleman and Markowitz, 1995]. Defective DNA mismatch repair is characterized by microsatellite instability mediated by alterations in one or more of a family of DNA mismatch repair enzymes. The predominant enzymes affected in sporadic colon cancers are *hMSH2* and *hMLH1* [Liu et al., 1995, 1996; Papadopoulos and Lindblom, 1997]. Compared with most sporadic colorectal cancers, those with MSI exhibit improved prognosis [Thibodeau et al., 1993; Bubb et al., 1996; Lothe et al., 1993; Cawkwell et al., 1995; Lukish et al., 1998; Sankila et al., 1996], right-sided predominance, and distinct histologic features (cribiform or signet cell histology, and/or numerous tumor infiltrating lymphocytes) [Kim et al., 1994; Ruschoff et al., 1997; Risio et al., 1996; Krishna et al., 1997a,b]. These same features are characteristic of colorectal cancers in hereditary nonpolyposis colon cancer (HNPCC), which arise in the setting of germline mutations affecting DNA mismatch repair enzymes [Peltomaki and Vasen, 1997].

LOW COX-2 LEVELS IN TUMORS WITH DEFECTIVE DNA MISMATCH REPAIR

The molecular, clinical, and pathological distinctions between CIN and MSI tumors prompted us to determine if these two groups of colorectal cancers differ in their expression of COX-2 (manuscript in review). We examined 53 tumors with no instability (MSS) and 41 tumors with high levels of MSI (MSI-H) affecting >39% of a minimum of 7 tested loci. Our results of blinded scoring of immunohistochemical levels of COX-2 in these tumors are summarized in Table I. COX-2 protein levels were significantly lower in MSI tumors than in those without MSI. We speculate that this biological distinction may have implications related to the efficacy of COX-2 inhibitors for chemoprevention of colorectal neoplasms that arise in the setting of defective DNA mismatch repair.

The difference in expression of COX-2 between MSI and CIN tumors remains unexplained but may be related to underlying differences in their molecular genesis. COX-2 is tightly regulated at the level of transcription [Inoue et al., 1995] and mRNA stability [Gou et al., 1998]. Nuclear extracts of colorectal cancer cell lines that overexpress COX-2 transactivate reporter genes under the control of the COX-2 promoter [Kutchera et al., 1996], suggesting that transcriptional activation is an important determinant of COX-2 overexpression in colorectal neoplasms. Like most early response genes, COX-2 transcription is rapidly induced by a variety of signals, including those stimulated

TABLE I. COX-2 Scores Among MSS and MSI Tumors*

		MSI group	
COX-2 score	Total N	MSS N(%)	MSI N(%)
0	25	8 (28)	17 (61)
1	36	18 (43)	18 (43)
2	18	14 (67)	4 (19)
3	16	14 (88)	2 (12)

MSI, microsatellite instability; MSS, microsatellite stability.

*Values represent the numbers and percentages of tumors within each COX-2 score that fell into MSS and MSI groups. Highly significant trends in the proportion of COX-2 scores were detected between MSS and MSI groups using Mantel-Haenszel χ^2 test for linear trend ($P = 0.001$).

by phorbol esters, transforming growth factor-β (TGF-β), interleukins and epidermal growth factor receptor (EGFR) ligands [Sheng et al., 1997; Williams, DuBois, 1996a]. Both TGF-β [Derynck et al., 1987] and EGFR ligands [Ciardiello et al., 1991] are frequently expressed by colorectal adenomas and cancers and therefore could stimulate constitutive COX-2 expression through autocrine mechanisms.

COX-2 levels increase coincidentally with loss of the wild-type APC allele during adenoma development in $APC^{+/-}$ mice, suggesting a direct role of APC loss in COX-2 overexpression [Oshima et al., 1996]. This also could be related to transcriptional activation. APC loss is associated with formation of β-catenin/TCF complexes and transactivation of reporters under the control of Tcf response elements [Korinek et al., 1997; Morin et al., 1997]. Coincidentally, the COX-2 promoter is reported to contain TCF-like response elements [Williams et al., 1997]. These observations raise the interesting possibility that TCF/β-catenin complexes induced by APC loss may transcriptionally regulate COX-2.

Reduced levels of COX-2 in MSI tumors may be due to suppression of signals that normal stimulate COX-2 induction. For example, MSI tumors frequently have mutations at an instability locus within the TGFβII receptor gene resulting in a truncated and nonfunctioning TGFβII receptor [Parsons et al., 1995; Markowitz et al., 1995]. Loss of a receptor for TGF-β would effectively prevent this potential autocrine mechanism of COX-2 induction. Alternatively, epigenic mechanisms that influence the activity of the COX-2 promoter could be involved. For example, MSI tumors frequently show hypermethylation of promoters, which appears to be responsible for reduced expression of several tumor suppressor genes, including DNA mismatch repair enzymes hMLH1 and hMSH2 observed in many MSI tumors [Ahuja et al., 1997; Kane et al., 1997; Myohanen et al., 1998; Cunningham et al., 1998]. To date, however, the methylation state of the COX-2 promoter in MSI tumors remains unknown. Finally, mechanistic links between APC loss and COX-2 expression, if they exist, may not play a role in tumors that arise through defective DNA mismatch repair, which have a lower incidence of APC mutations [Olschwang et al., 1997].

The clinical significance of reduced COX-2 expression in MSI tumors is unknown. COX-2 activity may provide survival advantage for early neoplasms by promoting cell growth, survival, and angiogenesis [DuBois et al., 1996b; Chan et al., 1998; Tsujii et al., 1998]. It would appear that MSI colorectal cancers develop independent of COX-2 expression. However, reduced COX-2 expression in colorectal cancers with MSI does not necessarily reflect events during precancerous adenoma stages, and therefore, may have no bearing on the preventive efficacy of COX-2 inhibitors for adenomas. Finally, the absence of immunohistochemically detectable COX-2 does not exclude the possibility that COX-2 activity may be sufficient to support the growth and survival of these tumors.

Despite these caveats, our observations raise an important issue related to the use of COX-2 inhibitors in patients whose tumors arise in the setting of defective DNA mismatch repair, such as those with HNPCC. Our observations encourage analysis of MSI as an important variable that may influence efficacy of selective COX-2 inhibitors in future chemoprevention studies.

ACKNOWLEDGMENTS

The work described herein represents collaborative efforts of members of the laboratories of Dr. Stephen N. Thibodeau (Mayo Clinic, Rochester, MN), Dr. Raymond N. DuBois (Vanderbilt University, Nashville, TN), and Dr. William E. Karnes, Jr. (Mayo Clinic, Rochester, MN). This work was supported by NIH grants R01 CA 68535 (to S.N.T.), R29 CA 71974 (to W.E.K.) and RO1 DK47294 (to R.N.D.) and by a research grant from Abbott Laboratories (to R.N.D.).

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