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Cancer Research

Persistent Cyclooxygenase-2 Inhibition Downregulates NF-κB, Resulting in Chronic Intestinal Inflammation in the Min/+ Mouse Model of Colon Tumorigenesis

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Abstract

Cyclooxygenase-2 (COX-2) inhibition prevents adenoma formation in humans and mouse models of colon cancer. The selective COX-2 inhibitor celecoxib reduces COX-2 and prostaglandin E_2 (PGE_2) expression and adenomas in the intestine of Min/+ mice after treatment for several weeks, but prolonged treatment increases PGE_2 production, resulting in drug-resistant tumor formation and transforming growth factor β (TGFβ)–dependent intestinal fibrosis. In this study, we examined pathways that regulate COX-2 expression and suppress chronic intestinal inflammation. We show that NF-κB signaling was inhibited in the ileum of Min/+ mice receiving long-term treatment with celecoxib. This effect was associated with inhibition of TGFβ-associated kinase-1 and IκB kinase α/β activities and reduced expression of the Toll-like receptor (TLR) 2 and TLR4 that enhance colonic barrier function. Additionally, we observed reduced activities of protein kinases c-Jun NH_2-terminal kinase 1 and protein kinase A and transcription factor cyclic AMP–responsive element binding protein, regulators of COX-2 expression, which cross-talk with NF-κB. In ileum subjected to long-term celecoxib treatment, we noted relatively higher expression of COX-2, vascular endothelial growth factor, and interleukin-1β in Paneth cells, whereas NF-κB and COX-2 were more strongly expressed by an expanded population of stromal myofibroblasts. Our findings argue that celecoxib resistance is an acquired adaptation to changes in the crypt microenvironment that is associated with chronic intestinal inflammation and impaired acute wound-healing responsiveness. *Cancer Res; 70(11); 4433–42. ©2010 AACR.*

Introduction

Chronic inflammation promotes tumorigenesis by altering the intestinal microenvironment in ways that both activate the stroma and enhance epithelial cell growth and survival. Cyclooxygenase-2 (COX-2) and its downstream prostaglandin, prostaglandin E_2 (PGE_2), are driving factors in colorectal cancer (CRC) and inflammatory bowel disease (IBD; ref. 1). COX-2 inhibition by nonsteroidal anti-inflammatory drugs (NSAID) effectively prevents the formation of precancerous adenomas in humans (2).

We showed that responses to celecoxib in the intestine change with the duration of treatment. Using the Apc-deficient Min/+ mouse, a CRC model, we reported that resistance occurred with long-term treatment (3). Short-term (3 wk) celecoxib treatment of these mice inhibited intestinal adenoma formation, COX-2 expression, and PGE_2 production, but long-term (5 mo) treatment accelerated tumor growth and progression. Importantly, high levels of PGE_2 and COX-2 expression recurred in tumors and nontumor mucosa of long-term–treated mice. Recently, we showed that long-term celecoxib treatment increased the number of COX-2–expressing myofibroblasts in the ileum and resulted in transforming growth factor β (TGFβ)–mediated intestinal fibrosis resembling patients with and rodent models of IBD (4).

The transcription factor NF-κB regulates inflammation and plays a role in CRC promotion (5). NF-κB signaling is activated by bacterial products, such as lipopolysaccharides (LPS), and inflammatory cytokines, and its transcriptional targets include the genes encoding COX-2, intercellular adhesion molecule-1 (ICAM-1), vascular endothelial growth factor (VEGF), and inflammatory cytokines interleukin-1β (IL-1β) and tumor necrosis factor α (TNFα). NF-κB signaling is needed to orchestrate wound-healing responses to acute intestinal injury, and its long-term inhibition is associated with chronic intestinal inflammation (6, 7). NF-κB signaling positively regulates the expression of Toll-like receptors (TLR), which, when activated, promote signals required for innate immunity (8). Several studies showed the relevance of TLRs to COX-2 expression. For instance, LPS-induced TLR4 activation caused NSAID-suppressible, NF-κB–dependent expression of COX-2 in enterocytes (8). In addition, several lines of evidence link TLR signaling to intestinal tumorigenesis.

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TLR signaling in response to gut microbial flora modulated the number of tumors in Min/+ mice (9). Signal transduction from TLRs depends on the NF-κB upstream adaptor MyD88, and TLR signaling-deficient *MyD88*$^{-/-}$Min/+ mice developed fewer intestinal tumors with reduced COX-2 expression than *MyD88*$^{+/+}$Min/+ littermates (10). Importantly, under conditions of tissue injury, MyD88 function was required to position COX-2–expressing mesenchymal cells around the base of crypts in the colon to stimulate enterocyte proliferation (11).

NF-κB is integrated with other pathways and activated by inflammatory cytokines during wound healing to allow proper temporal sequence and eventual termination of responses. For instance, receptor binding of the cytokines TNFα, IL-1β, and TGFβ activates TGFβ-associated kinase-1 (TAK-1) and subsequently NF-κB (12). Showing pathway cross-activation, TGFβ enhanced IL-1β–mediated TAK-1 activity and IL-1β–activated canonical TGFβ signaling (13). Consistent with the requirement of TLR signaling and NF-κB activity for homeostasis, inducible TAK-1 ablation in enterocytes caused colitis (14). Whereas in certain contexts TGFβ induces NF-κB–dependent COX-2 expression, it inhibits COX-2 activity in others (15). Moreover, PGE_2 both stimulates inflammation and promotes wound-healing resolution. PGE_2 elicits this negative feedback by autocrine signals initiated from its G protein–coupled receptors (EP2/4) that modulate intracellular cyclic AMP (cAMP; ref. 16). In these circumstances, PGE_2 induces cAMP-dependent and protein kinase A (PKA)–dependent inhibition of NF-κB and TGFβ signaling that limits inflammation (17, 18).

Intestinal wound-healing and inflammatory responses also involve cross-talk between COX-2 and Wnt signaling, a crucial pathway for enterocyte proliferation and tumorigenesis (19). Increased Wnt signaling stimulates enterocyte proliferation, allowing rapid coverage of surface lesions (20). PKA activates the transcription factor cAMP-responsive element binding protein (CREB), which positively regulates COX-2 expression (21) and also activates certain Wnt target genes (22). PGE_2 stimulates Wnt signaling downstream of the epidermal growth factor receptor (EGFR); activity of this receptor tyrosine kinase is constitutively increased in the Min/+ mucosa relative to *Apc*$^{+/+}$ littermates (23). Finally, the mitogen-activated protein kinases (MAPK) c-Jun NH_2-terminal kinases (JNK), which phosphorylate certain transcription factors, also promote COX-2 expression (24). After specific MAPK kinase (MKK)–mediated activation, JNKs and other MAPKs cross-talk extensively with the NF-κB pathway.

In the intestine of Min/+ mice, celecoxib resistance likely is not intrinsic but rather is a reversible adaptation to targeted therapy (25). Therefore, acquired resistance should alter the microenvironment of the intestinal crypt composed of epithelial and mesenchymal cells. COX-2 expression in this modified niche by any or all of its cell types then should exceed the bioavailable drug concentration. Here, we asked what effects long-term COX-2 inhibition produced in pathways that regulate inducible COX-2 expression. We expected to find changes in NF-κB activity and other pathways known to promote COX-2 expression and chronic intestinal inflammation. A better understanding of the biology underlying this type of drug resistance will inform the design of safer and more effective CRC chemoprevention strategies.

Materials and Methods

Materials

C57BL/6J-Min/+ mice were purchased from The Jackson Laboratory. Ten mice per treatment group were fed AIN-76A diet (Research Diets) with/without celecoxib (1,500 ppm) for timed intervals (3, 4). Antibodies are listed in Supplementary Table S1.

Immunohistochemical analyses

Immunohistochemistry used standard techniques (3); for CD34, IL-1β, TLR2, TLR4, and VEGF, all solutions were prepared in Antibody Diluent (Invitrogen). Immunohistochemistry of control and treatment groups was performed in parallel. Experiments were repeated using tissues from three different mice of each treatment group. Quantitative analysis and cell counting of immunohistochemistry used only well-oriented crypt-villus units from nontumor ileum of three different mice per treatment group and were performed by an investigator blinded to treatment status. Twelve high-powered fields (120 crypt-villus units) for von Willebrand factor–positive (vWF$^+$) cell counting were analyzed per treatment group. For MPO$^+$ cell counting, 50 crypt-villus units were analyzed per treatment group. For comparison of the mean number of NF-κB p65$^+$ myofibroblasts, simultaneous immunohistochemistry for vimentin$^+$ and p65$^+$ cells was performed on serial sections, and doubly positive cells were counted in 50 crypt-villus units. Statistical evaluations used an unpaired Student's *t* test.

Immunoblotting

Ileum of Min/+ mice untreated or treated with celecoxib for 4 days, 3 weeks, or 5 months was processed; lysates were prepared; and immunoblottings were performed as described (26). Total cell lysates from scraped small bowel mucosa mainly reflect expression patterns in enterocytes because they outnumber stromal cells by >100-fold (26). Immunoblottings were replicated three times. All experiments were repeated using tissues from two different mice of each treatment group. For NF-κB, ImageJ software from the NIH determined relative band intensities (27). Data were pooled and quantitatively analyzed from three independently performed experiments for total versus active NF-κB p65. Band intensity was normalized to the internal β-actin control and then compared relative to the untreated control as a fold difference using the unpaired Student's *t* test ($P < 0.05$ was considered statistically significant).

Results

Long-term celecoxib treatment suppressed TLR–TAK-1–NF-κB signaling in the ileum of Min/+ mice

NF-κB signaling in enterocytes shortly after injury induces the expression of cytokines that recruit myeloid cells from the circulation to initiate healing (7). To learn the effect of celecoxib treatment on NF-κB expression and

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activity, immunoblotting analyses were performed using lysates prepared from mucosa of the nontumor ileum of Min/+ mice that were untreated versus celecoxib treated (4 d, 3 wk, and 5 mo). Expression of total NF-κB p65 protein (filled columns) and its active form, NF-κB p65-p-Ser^{536} (hatched columns), increased after 4 days of treatment but declined thereafter to amounts lower than were found in untreated controls (Fig. 1A, left). Importantly, NF-κB activity was significantly lower in animals treated long term (P = 0.006). The active forms of IκB kinase (IKK) α/β kinases phosphorylate the NF-κB inhibitor IκBα, causing its turnover. Expression of these kinases was elevated after 4 days but decreased after 3 weeks. Sustained inhibition of NF-κB p65 was confirmed by the reduced active IKKα/β levels in the long-term–treated lysate relative to the untreated one. After 4-day treatment, IκBα expression was reduced, consistent with an initial NF-κB signaling induction. IκBα expression was increased in the long-term–treated sample to a level that resembled the untreated control. Confirming that IκBα expression in the untreated lysate was similar in the long-term–treated one, the amount of NF-κB p65 protein was not significantly different (P = 0.06).

Expression of ICAM-1 is NF-κB inducible in enterocytes *in vivo* (7); the level of this proangiogenic protein was increased after 4-day treatment (Fig. 1B). NF-κB activity triggered by chemical wounding is known to cause signal transducer and activator of transcription 3 (STAT3) activation (STAT3-p-Y705), stimulating enterocyte proliferation and survival (7). In our model, active STAT3 was increased in 4-day–treated Min/+ ileum relative to the untreated control but was decreased in samples from 3-week– and 5-month–treated mice (Fig. 1B). Consistent with STAT3 activation, its transcriptional target, suppressor of cytokine signaling 3, was induced after 4-day treatment (Fig. 1B). NF-κB signaling in enterocytes immediately after injury also induces the expression of cytokines that recruit myeloid cells (macrophages and neutrophils) from the circulation to initiate healing (7). We used an antibody for myeloperoxidase (MPO), a myeloid cell-specific marker to identify these cells by immunohistochemistry. We found that MPO$^+$ cells were significantly increased in the lamina propria of Min/+ mice treated with celecoxib for 4 days relative to the control (Fig. 1C).

Recently, we reported that the number of COX-2–expressing myofibroblasts was increased in ileum of Min/+ mice treated long term with celecoxib (4). Immunohistochemistry of serial sections for NF-κB p65 and vimentin, a myofibroblast-specific marker, was performed on ileum of Min/+ mice treated with and without celecoxib. Doubly positive cells were counted. As observed previously, treatment of Min/+ mice for 3 weeks significantly reduced the number of NF-κB p65$^+$ myofibroblasts in the lamina propria, whereas long-term treatment increased their number (Fig. 1D). The nuclear expression of NF-κB p65 was not observed in crypt enterocytes by this method.

Active TAK-1 (TAK-1-p-Thr$^{180/187}$) phosphorylates IKKβ, both stimulating NF-κB signaling and protecting enterocytes against chronic inflammation (12, 14). Immunoblottings showed that levels of total TAK-1 protein were invariant with respect to treatment duration. However, levels of active TAK-1 were inversely associated with the duration of celecoxib treatment. As expected, TAK-1-p-Thr$^{180/187}$ levels correlated with those of IKKα/β-p-Ser$^{176/180}$ (Fig. 2A). TAK-1-p-Ser^{412} is a different isoform associated with a cAMP-PKA–dependent pathway (28). Relative to the untreated control, TAK-1-p-Ser^{412} expression also was decreased with lengthened treatment time, suggesting a corresponding decrease in PKA activity.

TLR4, TLR2, and NOD2 expression is NF-κB dependent and enhances enterocyte barrier function (8, 10). Immunoblotting analysis of these receptors in the tissue lysates showed that 3-week and long-term drug exposures reduced TLR4 and TLR2 expression (Fig. 2B), whereas NOD2 expression was unchanged. Immunohistochemistry of serially sectioned ileum showed differential staining of crypt base enterocytes for TLR4 but low overall staining of the entire crypt-villus unit for TLR2. Consistent with these results, twice as much protein was needed to produce band intensities for TLR2 that were similar to that of TLR4, indicating that Min/+ enterocytes contain a lower level of TLR2 compared with TLR4. In contrast, however, more stromal cells were positively stained for TLR2 at all treatment times relative to TLR4. The complete treatment series are shown in Supplementary Figs. S1 and S2. These results suggest that innate immunity was reduced in long-term–treated Min/+ mice.

Long-term celecoxib treatment increased microvessel density, the number of CD34$^+$ vessels in the submucosa, and VEGF expression in Paneth cells

Angiogenesis is an essential feature of intestinal inflammation, and short-term and celecoxib treatment is known to produce antiangiogenic effects (29, 30). As further evidence for chronic inflammation following long-term celecoxib treatment, we examined the effect of celecoxib treatment duration on angiogenesis in the ileum of Min/+ mice. Using antibody for the endothelial cell marker vWF to perform immunohistochemistry, we counted the microvessel density (MVD) in the ileum of study animals (Fig. 3A). MVD was significantly reduced after 3-week treatment relative to untreated mice but was increased after 5-month treatment. This result correlated the inhibition of angiogenesis by short-term celecoxib treatment with previously observed inhibition of COX-2 and PGE_2 expression and confirmed the opposite effects after long-term treatment (3).

To confirm and extend this result, we performed immunohistochemistry for CD34, an endothelial progenitor cell marker of new blood vessels. Overall CD34 staining of the mucosa was low in short-term–treated but strong in long-term–treated Min/+ mice (Fig. 3B). Low-magnification images best showed the staining intensity differences. Arrows in the high-magnification images indicate CD34$^-$ vessels of short-term–treated and CD34$^+$ vessels of long-term–treated Min/+ ileum. These results suggest that chronic exposure to celecoxib promoted dynamic vessel remodeling. The entire treatment series is shown in Supplementary Fig. S3.

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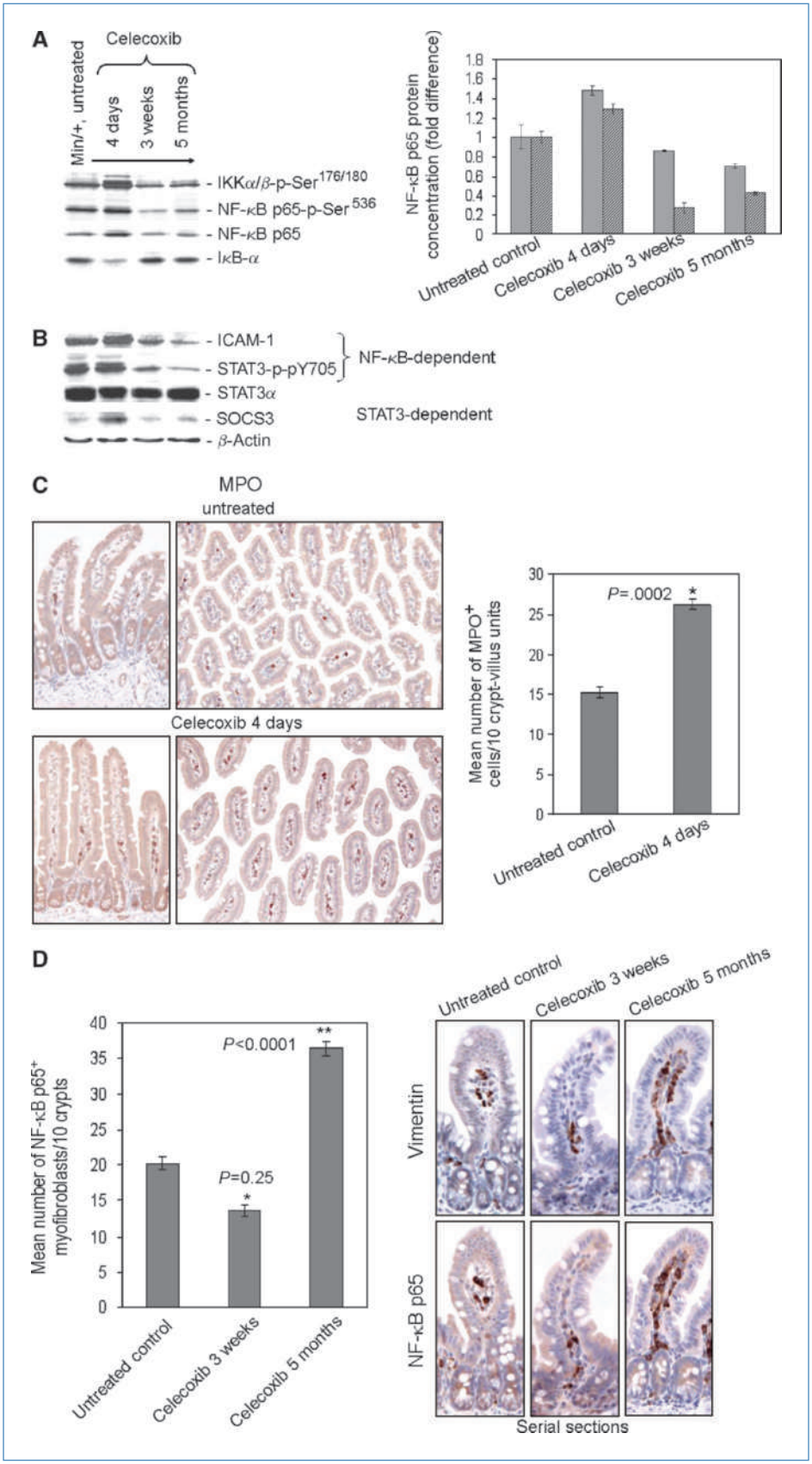

Figure 1. Long-term celecoxib treatment suppressed NF-κB activity in enterocytes, a condition associated with chronic intestinal inflammation. A, immunoblottings used lysates prepared as detailed (37) from Min/+ mice that were untreated or treated with celecoxib for 4 d, 3 wk, or 5 mo. The antibodies used in this study are listed in Supplementary Table S1. In this and subsequent immunoblottings, the protein concentration of each sample was the same except where indicated. β-Actin served as a control for the uniformity of sample loading. Immunoblottings of total versus phosphorylated proteins were performed in parallel. Right, fold difference at each treatment time for NF-κB p65 and p65-p-Ser^{536}. Filled columns, total NF-κB; hatched columns, active phospho-NF-κB. Bars, SE. *P* values for NF-κB p65: untreated versus 4 d, $P = 0.0194$; 4 d versus 3 wk, $P = 0.0002$; 3 wk versus 5 mo, $P = 0.0030$; and untreated versus 5 mo, $P = 0.06$. *P* values for NF-κB p65-p-Ser^{356}: untreated versus 4 d, $P = 0.0207$; 4 d versus 3 wk, $P = 0.0001$; 3 wk versus 5 mo, $P = 0.0363$; and untreated versus 5 mo, $P = 0.0006$. B, immunoblottings of NF-κB and STAT3 downstream signaling targets. C, representative photomicrographs of immunohistochemistry for MPO using sectioned ileum from Min/+ mice untreated or treated with celecoxib for 4 d. The number of myeloid cells (MPO$^+$ or F4/80$^+$) was significantly reduced at subsequent treatment times (data not shown; ref. 4). D, representative photomicrographs of serial sections of ileum from Min/+ mice untreated or treated with celecoxib for indicated times were immunostained for vimentin and p65 to show the expression and location of NF-κB$^+$ in myofibroblasts.

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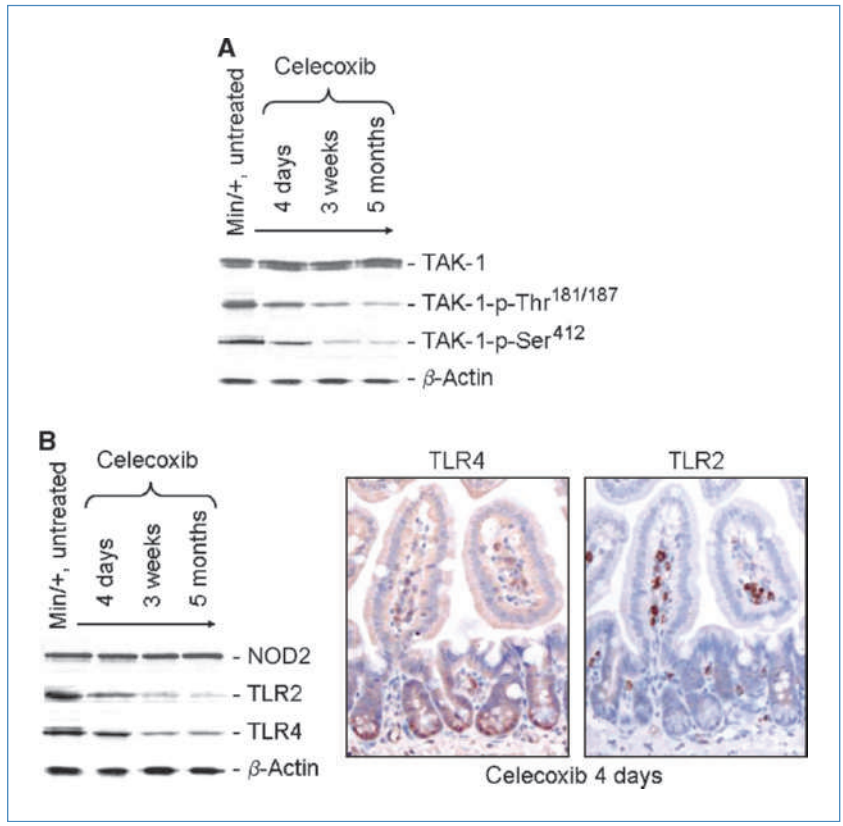

Figure 2. NF-κB inhibition on long-term celecoxib exposure was associated with reduced TLR2 and TLR4 expression and TAK-1 activity. A, immunoblottings examined treatment time-dependent changes in expression of TAK-1 versus its active isoforms. B, left, immunoblotting analysis is shown of treatment time-dependent changes in TLR2 and TLR4 but not in NOD2 expression; right, representative photomicrographs of serial sections of ileum from Min/+ mice treated with celecoxib for 4 d that were immunostained separately for TLR4 and TLR2.

VEGF is a key regulator of angiogenesis. Immunohistochemistry for VEGF expression in untreated and celecoxib-treated ileum of Min/+ mice revealed positively stained enterocytes at the base of crypts. Tissue sections are shown in two orientations. As in the case of TLR4 (Fig. 3C), VEGF-positive cells had the appearance and localization of Paneth cells.

The number of Paneth cells and their expression of IL-1β and COX-2 were modulated by the duration of celecoxib treatment

Residing subjacent to the submucosa, Paneth cells are appropriately placed to modulate angiogenesis and were shown to secrete other proangiogenic factors (31, 32). We therefore examined the effect of celecoxib treatment time on these secretory enterocytes. Using immunohistochemistry to identify the Paneth cell marker lysozyme, we counted the relative number of positively stained cells (Fig. 4A) in treated and untreated tissues. Consistent with the known antiangiogenic effect of celecoxib, the proangiogenic role of Paneth cells, and our MVD data (Fig. 3A), treatment for 3 weeks reduced the number of Paneth cells, whereas long-term treatment increased their number relative to the control.

IL-1β, a potent inflammatory cytokine, is inducible by NF-κB and can perpetuate NF-κB activation via autocrine signaling. Production of active IL-1β requires processing in lysosomes for secretion. Immunohistochemistry showed that IL-1β was expressed in the secretory granules of Paneth cells, implying that this cytokine is released into the lumen and acts on enterocytes (Paneth cells, stem cells, and progenitors) at the base of crypts (Fig. 4B, left). Immunoblotting analysis also showed that expression of mature 17-kDa IL-1β was increased by all celecoxib treatments relative to the untreated control (Fig. 4B, right). Consistent with the initial induction of NF-κB activity (Fig. 1A), overall IL-1β expression in ileum was highest in the 4-day treatment lysate. Finally, we found that COX-2 was also expressed in Paneth cells of long-term–treated Min/+ mice. Figure 4C shows 3-week– and 5-month–treated ileum cut in two orientations. By immunohistochemistry, COX-2 was more highly expressed in Paneth cells (green arrows) and stromal cells from long-term–treated mice when compared with those treated short term. These results indicate that Paneth cells of Min/+ mice contribute to celecoxib resistance and modulate inflammation and angiogenesis.

Long-term celecoxib treatment suppressed alternate pathways that cross-talk with NF-κB to activate COX-2 expression

JNK1 and JNK2 phosphorylate transcription factors, are activated by a Ras-Rac1-MKK4 pathway, and stimulate COX-2 expression (33). By immunoblotting analysis, we evaluated the relative expression of active MKK4 (MKK4-p-Ser^{80}) and JNK1/2 (JNK-p-Thr^{183}/Tyr^{185}) when compared with total MKK4 and JNK1, respectively (Fig. 5A). The activity of MKK4 was strongly reduced in the mucosa of Min/+ mice after 5-month treatment but not the steady-state expression

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of this protein. Overall expression of 42-kDa JNK1 declined slightly with treatment duration; activity of this kinase was reduced more severely. We did not pursue the possibility of COX-2 expression via MKK7-JNK2 activity because of the low intensity of the 54-kDa JNK2 band.

CREB is a transcription factor that promotes COX-2 expression (21). CREB transactivation requires phosphorylation of Ser^{133} by PKA or other kinases. Immunoblotting analysis of total CREB versus CREB-p-Ser^{133} levels in treatment samples showed that activity of this regulator declined in parallel with

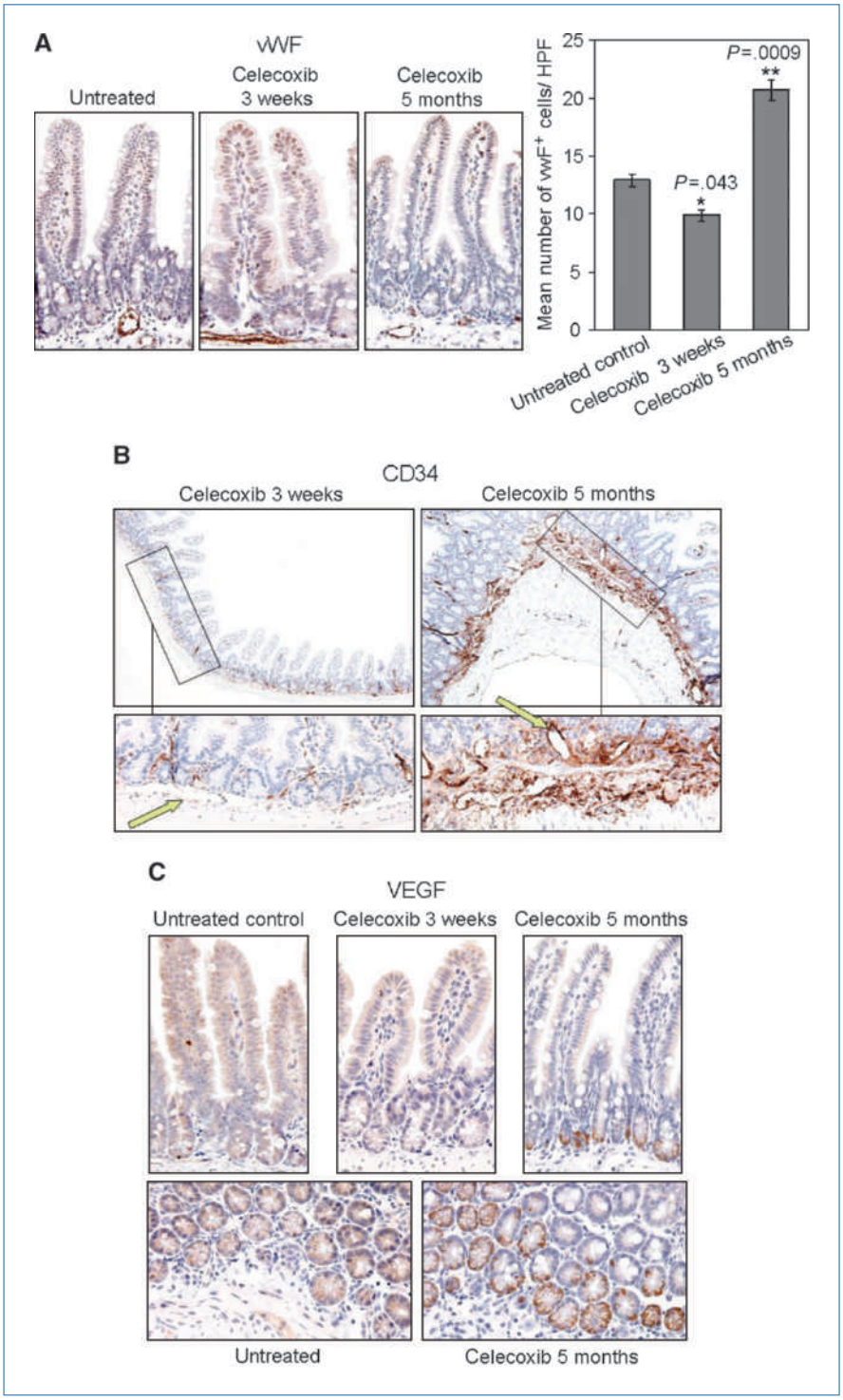

Figure 3. Long-term celecoxib treatment increased MVD, the number of $CD34^+$ vessels in the submucosa, and VEGF expression in Paneth cells. A, left, representative photomicrographs of sectioned ileum from Min/+ mice untreated and treated for indicated times that were immunostained for vWF; right, mean number of vWF^+ cells per high-power magnification (×40) field in crypt-villus units. $P = 0.0432$, untreated versus 3 wk; $P = 0.0009$, untreated versus 5 mo; $P < 0.0001$, 3 wk versus 5 mo. B, representative photomicrographs of sectioned ileum from Min/+ mice treated with celecoxib for indicated times that were immunostained for CD34 at original magnifications of ×10 (top) and ×40 (bottom). Green arrows point to blood vessels in the submucosa. C, top, representative photomicrographs of sectioned ileum from Min/+ mice untreated and treated for indicated times show crypt-villus units that were immunostained for VEGF; bottom, images of ileum cross-sectioned at the base of crypts at ×40 show positive staining for VEGF in Paneth cells of Min/+ mice that were untreated or treated with celecoxib for 5 mo.

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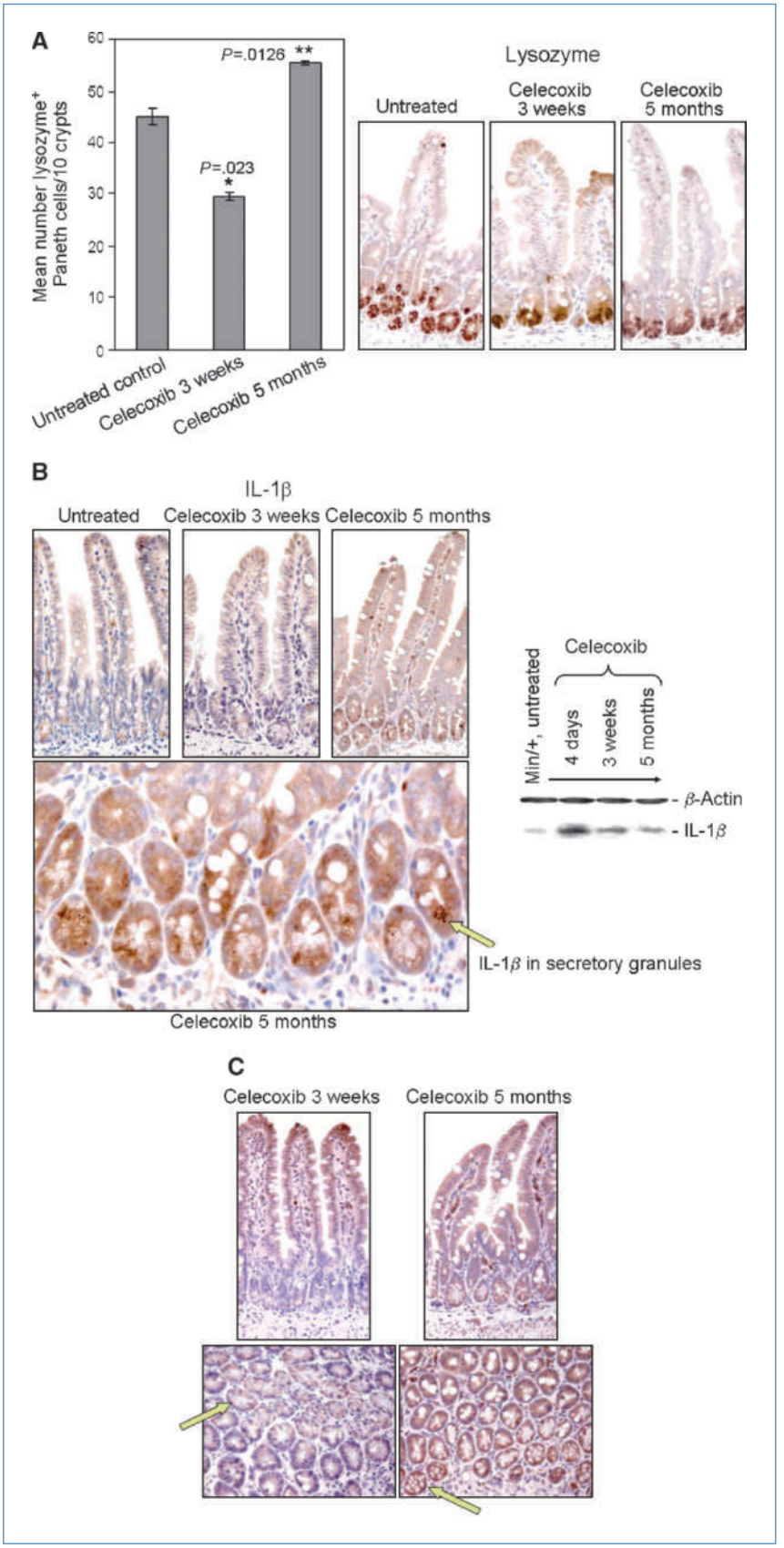

Figure 4. Paneth cell number and IL-1β and COX-2 expression were modulated by the duration of celecoxib treatment. A, right, representative photomicrographs of sectioned ileum from Min/+ mice untreated or treated for indicated times that were immunostained for lysozyme; left, mean number of Paneth cells/10 crypt length of ileum versus the duration of treatment time. B, top, left, representative photomicrographs of ileum from Min/+ mice untreated or treated for indicated times that were immunostained for IL-1β; bottom, ×40 magnification image of ileum from Min/+ treated with celecoxib for 5 mo; an arrow points to IL-1β in the secretory granules of Paneth cells. Left, immunoblotting analysis of IL-1β expression in lysates of ileum from Min/+ mice untreated or treated for indicated times. C, representative photomicrographs of ileum from Min/+ mice untreated or treated for indicated times that were immunostained for COX-2; arrows indicate COX-2 expression, or the lack of its expression, in Paneth cells.

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its overall expression (Fig. 5B). Because levels of PKA-targeted TAK-1-p-Ser^{412} were reduced in long-term–treated versus untreated Min/+ mice (Fig. 2A), and PKA is a positive regulator of CREB activity, we expected that celecoxib resistance would be associated with PKA inhibition. Of the three PKA catalytic subunits, PKAβ was the predominant isoform expressed in our mucosal lysates (data not shown). Active PKAβ cat-p-Thr^{198} was reduced in mice treated with celecoxib for 3 weeks and longer. This result associated short-term celecoxib-mediated COX-2 and PGE_2 inhibition with a reduction of PKA activity and downstream NF-κB signaling, suggesting cross-talk between these pathways. Because the level of PGE_2 was increased but PKA activity remained low after the 5-month treatment, chronic exposure of enterocytes to celecoxib may have altered calcium signaling, as suggested previously (16). Taken together, celecoxib resistance was associated with inhibition of pathways that stimulate COX-2 expression in most enterocytes but not in Paneth cells or in stromal myofibroblasts. Moreover, associated with chronic intestinal inflammation produced by long-term celecoxib treatment was the suppression of signaling necessary for acute wound healing.

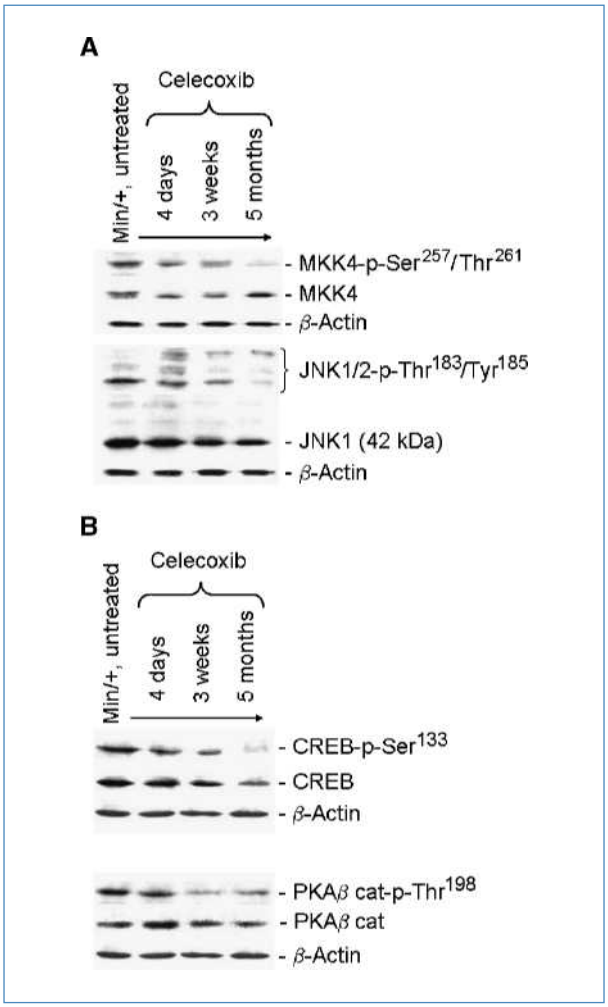

Figure 5. Long-term celecoxib treatment was associated with decreased PKA-CREB and MKK4-JNK1 activities, pathways that cross-talk with NF-κB. A, immunoblotting of the relative expression of total MKK4 versus its active phosphorylated isoform and of total JNK1 versus its active 42-kDa phosphorylated isoform. B, immunoblotting of the relative expression of total CREB versus its active phosphorylated isoform and of total PKAβ catalytic subunit versus its active phosphorylated isoform.

Discussion

These data add to our previous findings, supporting the hypothesis that short-term administration of celecoxib in Min/+ mice is anti-inflammatory and tumor suppressive, whereas long-term administration results in chronic inflammation and tumor promotion (3, 4). We showed that resistance to the antitumor effects of celecoxib in Min/+ mice evolved by adaptations that altered enterocyte-stromal interactions and extracellular matrix (ECM) composition (3, 4). Our present work revealed that NF-κB activation in small bowel enterocytes of Min/+ mice occurred rapidly (4 d) after initiating celecoxib treatment and induced a cellular program of NF-κB–dependent wound healing (Fig. 1A–C). In contrast, long-term celecoxib treatment inhibited NF-κB signaling in enterocytes, a condition associated with chronic intestinal inflammation.

As indicated by the expression of target gene products (VEGF, COX-2, and IL-1β), NF-κB signaling was upregulated in Paneth cells and myofibroblasts of long-term–treated mice (Figs. 1A and D and 4B and C). It is reasonable to propose that Paneth cells and myofibroblasts produced the >2-fold increase in COX-2 expression in the mucosa of long-term–treated Min/+ mice compared with controls to overcome the concentration of bioavailable drug in the stem/progenitor cell compartment (3). The close apposition of myofibroblasts expressing COX-2 may also produce a physical barrier limiting celecoxib bioavailability in the stem cell niche. In addition, it is plausible that celecoxib resistance is limited to the crypt base because this is where COX-2 cooperates with Wnt signaling. For instance, stem, progenitor, and Paneth cells maintain high levels of stabilized β-catenin to affect Wnt signaling, and this oncoprotein can promote *COX-2* gene transcription and stabilize its mRNA (34, 35). Furthermore, PGE_2 stimulates enterocyte proliferation by transactivating EGFR (19, 23), and COX-2 enhances Wnt signaling by increasing integrin-dependent adhesion to ECM (36, 37).

The increased number of Paneth cells in the celecoxib-resistant Min/+ mucosa likely reflects enhanced survival of these long-lived cells (Fig. 4A). Several other observations support a role for Paneth cells in this chronic inflammation model. Paneth cells regulate the number and type of commensal microbes and limit pathogen contact with the mucosa (38). These enterocytes exclusively express matrix metalloproteinase 7, an enzyme that both promotes tumorigenesis and inhibits chronic intestinal inflammation (39, 40). Germline mutations predisposing to IBD modulate NF-κB signaling in Paneth cells, as well as their relative number, survival, and expression of inflammatory cytokines (41). To induce the expression of defensins, Paneth cells activate NF-κB via a

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TLR2-NOD2-RIP2 pathway (42). The importance of this pathway for the expression of defensins and survival signaling was confirmed by the hypersensitivity to chemically induce colitis displayed in mice bearing a conditional deletion of NF-κB p65 in enterocytes (43). Finally, Paneth cells regulate angiogenesis in the intestine during development and inflammation by secreting β-defensins and angiogenins (31, 32).

NSAIDs, including celecoxib, produce COX-2–independent effects that are likely modulated by the duration of treatment time. For instance, genome expression and proteome analyses identified numerous changes in mRNA expression of nontumor mucosa from familial adenomatous polyposis (FAP) patients treated with celecoxib for 1 year (44). COX-2–independent pharmacologic effects were reported, including inhibition of 5-lipoxygenase and phosphodiesterase activities; suppression of the latter caused increased intracellular levels of cyclic nucleotides and protein kinase G activity (45, 46). Interestingly, IKKβ is a secondary pharmacologic target of celecoxib (47). Here, we identified TAK-1 as the most upstream kinase in the NF-κB pathway inhibited by long-term celecoxib exposure (Fig. 2A). We showed that IL-1β was more highly expressed in celecoxib long-term–treated relative to untreated Min/+ mice (Fig. 4B). IL-1β overexpression was associated with chronic intestinal inflammation after prolonged treatment of mice with the selective IKKβ inhibitor ML120B (6). In addition, we found that long-term celecoxib treatment inhibited IKKβ activity (Fig. 1A), a result consistent with the essential role of enterocyte-intrinsic IKKβ expression in maintaining intestinal homeostasis (48).

We do not yet know the extent to which the adaptive processes here are present in FAP patients treated long term with NSAIDs for prevention of colorectal adenomas. In addition, the relationship of this response to germline *Apc* mutation is not yet known, and studies of long-term NSAID administration in animals that are wild-type at the *Apc* locus are under way. Based on the results presented here, it may be possible to maximize chemoprevention effectiveness while limiting toxicity by using lower NSAID doses or by providing drug holidays. Chemoprevention might also be achieved by alternating NSAIDs such as celecoxib with other agents. For example, statins inhibit isoprenoid synthesis, particularly geranyl geranylpyrophosphate, thereby inhibiting RhoA and its downstream kinase, ROCK (49). Statins also are direct extracellular integrin antagonists (50). The statin simvastatin showed anti-inflammatory effects in animal models of chemically induced colitis and tumor formation and inhibited RhoA and induced NF-κB activities in doxorubicin-treated colon cancer cells (51). Because we previously showed that enterocytes of Min/+ mice constitutively maintain increased RhoA activity, alternating cycles of celecoxib and simvastatin may inhibit acquired resistance to celecoxib in this model. A selective focal adhesion kinase (FAK) inhibitor may produce a similar effect. For example, in nontransformed cells, FAK activity is strictly integrin adhesion dependent. If celecoxib resistance is associated with TGFβ signaling downstream of integrin-ECM engagement, then a FAK inhibitor such as PF-562,271 may enhance chemoprevention efficacy in combination with celecoxib.

In conclusion, the tissue-specific effects of chemopreventive NSAIDs such as celecoxib change over time. In association with an altered intestinal microenvironment, acquired resistance may occur in nontumor IBD mucosa or premalignant adenomas, just as in invasive cancers (25). Signaling pathway cross-activation may promote drug resistance (13). The more selective the drug and the longer the treatment time, the greater will be the pressure driving resistance. This view is consistent with the model of dynamic reciprocity, which posits that the microenvironment, especially the ECM, exerts an influence on gene expression in epithelial cells. Importantly, this type of drug resistance is reversible. Our results emphasize that more comprehensive knowledge of stromal-epithelial interactions, signaling cross-talk, and negative feedback mechanisms operating during different stages of inflammation and tumorigenesis is needed to develop safe and effective CRC chemoprevention regimens.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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References

1. Rhodes JM, Campbell BJ. Inflammation and colorectal cancer: IBD-associated and sporadic cancer compared. Trends Mol Med 2002;8:10–6.
2. Bertagnolli MM. Chemoprevention of colorectal cancer with cyclooxygenase-2 inhibitors: two steps forward, one step back. Lancet Oncol 2007;8:439–43.
3. Carothers AM, Moran AE, Cho NL, et al. Changes in the antitumor response in C57BL/6J-Min/+ mice during long-term administration of a selective cyclooxygenase-2 inhibitor. Cancer Res 2006;66:6432–8.
4. Davids JS, Carothers AM, Damas BC, Bertagnolli MM. Chronic cyclooxygenase-2 inhibition promotes myofibroblasts-associated intestinal fibrosis. Cancer Prev Res 2010;3:348–58.
5. Karin M. Nuclear factor-κB in cancer development and progression. Nature 2006;441:431–6.
6. Greten FR, Arkan MC, Bollrath J, et al. NF-κB is a negative regulator of IL-1β secretion as revealed by genetic and pharmacological inhibition of IKKβ. Cell 2007;130:918–31.
7. Eckmann L, Nebelsiek T, Fingerle AA, et al. Opposing functions of IKKβ during acute and chronic intestinal inflammation. Proc Natl Acad Sci U S A 2008;105:15058–63.
8. Fukata M, Abreu MT. Role of Toll-like receptors in gastrointestinal malignancies. Oncogene 2008;27:234–43.
9. Newman JV, Kosaka T, Sheppard BJ, Fox JG, Schauer DB. Bacterial infection promotes colon tumorigenesis in *Apc*$^{Min/+}$ mice. J Infect Dis 2001;184:227–30.

Published OnlineFirst May 18, 2010; DOI: 10.1158/0008-5472.CAN-09-4289

10. Rakoff-Nahous S, Medzhitov R. Regulation of spontaneous intestinal tumorigenesis through the adaptor protein MyD88. Science 2007; 317:124–7.
11. Brown SL, Riehl TE, Walker MR, et al. Myd88-dependent positioning of Ptgs2-expressing stromal cells maintains colonic epithelial proliferation during injury. J Clin Invest 2007;117:258–69.
12. Shim J-H, Xiao C, Paschal AE, et al. TAK1, but not TAB1 or TAB2, plays an essential role in multiple signaling pathways *in vivo*. Genes Dev 2005:2668–81.
13. Lu T, Tian L, Han Y, Vogelbaum M, Stark GR. Dose-dependent cross-talk between the transforming growth factor-β and interleukin-1 signaling pathways. Proc Natl Acad Sci U S A 2007;104:4365–70.
14. Kajino-Sakamoto R, Inagaki M, Lippert E, et al. Enterocyte-derived TAK1 signaling prevents epithelium apoptosis and the development of ileitis and colitis. J Immunol 2008;181:1143–52.
15. Yan M, Rerko RM, Platzer P, et al. 15-Hydroxyprostaglandin dehydrogenase, a Cox-2 oncogene antagonist is a TGFβ-induced suppressor of human gastrointestinal cancers. Proc Natl Acad Sci U S A 2004;101:17468–73.
16. Hansen-Petrik MB, McEntee MF, Jull B, Shi H, Zemel MB, Whelan J. Prostaglandin E_2 protects intestinal tumors from nonsteroidal anti-inflammatory drug-induced regression in $Apc^{Min/+}$ mice. Cancer Res 2002;62:403–8.
17. Wall EA, Zavzavadjian JR, Chang MS, et al. Suppression of LPS-induced TNF-α production in macrophages by cAMP is mediated by PKA-AKAP95-p105. Sci Signal 2009;2:ra28.
18. Schiller M, Dennler S, Anderegg U, et al. Increased cAMP levels modulate transforming growth factor-β (TGF-β)/SMAD-induced expression of extracellular matrix components and other key fibroblast effector functions. J Biol Chem 2010;285:409–21.
19. Buchanan FG, DuBois RN. Connecting Cox-2 and Wnt in cancer. Cancer Cell 2006;9:6–8.
20. Seno H, Miyoshi H, Brown SL, Geske MJ, Colonna M, Stappenbeck TS. Efficient colonic mucosal wound repair requires Trem2 signaling. Proc Natl Acad Sci U S A 2009;106:256–61.
21. Tang Q, Chen W, Gonzales MS, Finch J, Inoue H, Bowden GT. Role of cyclic AMP response element in the UVB induction of cyclooxygenase-2 transcription in human keratinocytes. Oncogene 2001;20: 5164–72.
22. Chen AE, Ginty DD, Fan CM. Protein kinase A signaling via CREB controls myogenesis induced by Wnt proteins. Nature 2005;433: 317–22.
23. Moran AE, Hunt DH, Javid SH, Redston M, Carothers AM, Bertagnolli MM. Apc deficiency is associated with increased Egfr activity in the intestinal enterocytes and adenomas of C57BL/6J-Min/+ mice. J Biol Chem 2004;279:43261–72.
24. Davis RJ. Signal transduction by the JNK group of MAP kinases. Cell 2000;103:239–52.
25. Berger G, Hannahan D. Modes of resistance to anti-angiogenic therapy. Nat Rev Cancer 2008;8:592–603.
26. Carothers AM, Melstrom KA, Mueller JD, Weyant MJ, Bertagnolli MM. Progressive changes in adherens junction structure during intestinal adenoma formation in *Apc* mutant mice. J Biol Chem 2001; 276:39094–102.
27. National Institutes of Health. Image Processing and Analysis in Java Program (ImageJ). Available from: http://rsbweb.nih.gov/ij/.
28. Kobayashi Y, Mizoguchi T, Take I, Kuihara S, Udagawa N, Takahashi N. Prostaglandin E_2 enhances osteoclastic differentiation of precursor cells through protein kinase A-dependent phosphorylation of TAK-1. J Biol Chem 2005;280:11395–403.
29. Sartor RB. Mechanisms of disease: pathogenesis of Crohn's disease and ulcerative colitis. Nat Clin Pract Gastroenterol Hepatol 2006;3: 390–407.
30. Masferrer JL, Leahy KM, Koki AT, et al. Antiangiogenic and antitumor activities of cyclooxygenase-2 inhibitors. Cancer Res 2000; 60:1306–11.
31. Stappenbeck TS, Hooper LV, Gordon JI. Developmental regulation of intestinal angiogenesis by indigenous microbes via Paneth cells. Proc Natl Acad Sci U S A 2002;99:15451–5.
32. Crabtree B, Holloway DE, Baker MD, Acharya KR, Subramanian V. Biological and structural features of murine angiogenin-4, an angiogenic protein. Biochemistry 2007;46:2431–43.
33. Guan Z, Buckman SY, Miller BW, Springer LD, Morrison AR. Interleukin-1β-induced cyclooxygenase-2 expression requires activation of both c-Jun NH_2-terminal kinase and p38 MAPK signal pathways in rat renal mesangial cells. J Biol Chem 1998;273:28670–6.
34. Araki Y, Okamura S, Perwez Hussain S, et al. Regulation of cyclooxygenase-2 expression by the Wnt and Ras pathways. Cancer Res 2005;65:728–34.
35. Lee HK, Jeong S. β-Catenin stabilizes cyclooxygenase-2 mRNA by interacting with AU-rich elements of 3′-UTR. Nucleic Acid Res 2006; 34:5705–14.
36. Zaric J, Rüegg CJ. Integrin-mediated adhesion and soluble ligand binding stabilize COX-2 protein levels in endothelial cells by inducing expression and preventing degradation. J Biol Chem 2005;280: 1077–85.
37. Yazawa K, Tsuno NH, Kitayama J, et al. Selective inhibition of cylooxygenase-2 inhibits colon cancer cell adhesion to extracellular matrix by expression of β1 integrin. Cancer Sci 2005;96:93–9.
38. Vaishnava S, Behrendt CL, Ismail AS, Eckmann L, Hooper LV. Paneth cells directly sense gut commensals and maintain homeostasis at the intestinal host-microbial interface. Proc Natl Acad Sci U S A 2008;105:20858–63.
39. Wilson CL, Heppner KJ, Labosky PA, Hogan BL, Matrisian LM. Intestinal tumorigenesis is suppressed in mice lacking the metalloproteinase matrilysin. Proc Natl Acad Sci U S A 1997;94: 1402–7.
40. Wilson CL, Ouellett AJ, Satchell DP, et al. Regulation of intestinal α-defensin activation by the metalloproteinase matrilysin in innate host defense. Science 1999;286:113–7.
41. Kaser A, Blumberg RS. Paneth cells and inflammation dance together in Crohn's disease. Cell Res 2008;18:1160–2.
42. Kobayashi KS, Chamaillard M, Ogura Y, et al. Nod2-dependent regulation of innate and adaptive immunity in the intestinal tract. Science 2005;307:731–4.
43. Steinbrecher KA, Harmel-Laws E, Sitcheran R, Baldwin AS. Loss of epithelial RelA results in deregulated intestinal proliferative/apoptotic homeostasis and susceptibility to inflammation. J Immunol 2008; 180:2588–99.
44. Glebov OK, Rodriquez LM, Lynch P, et al. Celecoxib treatment alters the gene expression profile of normal colonic mucosa. Cancer Epidemiol Biomarkers Prev 2006;15:1382–91.
45. Maier TJ, Tausch L, Hoernig M, et al. Celecoxib inhibits 5-lipoxygenase. Biochem Pharmacol 2008;76:862–72.
46. Soh JW, Kazi JU, Li H, Thompson WJ, Weinstein IB. Celecoxib-induced growth inhibition in SW480 colon cancer cells is associated with activation of protein kinase G. Mol Carcinog 2008;47: 519–25.
47. Sethi G, Tergaonkar V. Potential pharmacological control of the NF-κB pathway. Trends Pharmacol Sci 2009;30:313–21.
48. Zaph C, Troy AE, Taylor BC, et al. Epithelial-cell-intrinsic IKK-β expression regulates intestinal immune homeostasis. Nature 2007; 446:552–6.
49. Zhou Q, Liao JK. Rho kinase: an important mediator of atherosclerosis and vascular disease. Curr Pharm Des 2009;15:3108–15.
50. Weltz-Schmidt G, Welzenbach K, Brinkmann V, et al. Statins selectively inhibit leukocyte function antigen-1 by binding to a novel regulatory integrin site. Nature Med 2001;7:687–92.
51. Riganti C, Doublier S, Costamagna C, et al. Activation of nuclear factor-κB pathway by simvastatin and RhoA silencing increases doxorubicin cytotoxicity in human colon cancer HT29 cells. Mol Pharmacol 2008;74:476–84.

Cancer Research

Correction: Online Publication Dates for *Cancer Research* April 15, 2010 Articles

The following articles in the April 15, 2010 issue of *Cancer Research* were published with an online publication date of April 6, 2010 listed, but were actually published online on April 13, 2010:

Garmy-Susini B, Avraamides CJ, Schmid MC, Foubert P, Ellies LG, Barnes L, Feral C, Papayannopoulou T, Lowy A, Blair SL, Cheresh D, Ginsberg M, Varner JA. Integrin $\alpha4\beta1$ signaling is required for lymphangiogenesis and tumor metastasis. Cancer Res 2010;70:3042–51. Published OnlineFirst April 13, 2010. doi:10.1158/0008-5472.CAN-09-3761.

Vincent J, Mignot G, Chalmin F, Ladoire S, Bruchard M, Chevriaux A, Martin F, Apetoh L, Rébé C, Ghiringhelli F. 5-Fluorouracil selectively kills tumor-associated myeloid-derived suppressor cells resulting in enhanced T cell-dependent antitumor immunity. Cancer Res 2010;70:3052–61. Published OnlineFirst April 13, 2010. doi:10.1158/0008-5472.CAN-09-3690.

Nagasaka T, Rhees J, Kloor M, Gebert J, Naomoto Y, Boland CR, Goel A. Somatic hypermethylation of *MSH2* is a frequent event in Lynch syndrome colorectal cancers. Cancer Res 2010;70:3098–108. Published OnlineFirst April 13, 2010. doi:10.1158/0008-5472.CAN-09-3290.

He X, Ota T, Liu P, Su C, Chien J, Shridhar V. Downregulation of HtrA1 promotes resistance to anoikis and peritoneal dissemination of ovarian cancer cells. Cancer Res 2010;70:3109–18. Published OnlineFirst April 13, 2010. doi:10.1158/0008-5472.CAN-09-3557.

Fiorentino M, Judson G, Penney K, Flavin R, Stark J, Fiore C, Fall K, Martin N, Ma J, Sinnott J, Giovannucci E, Stampfer M, Sesso HD, Kantoff PW, Finn S, Loda M, Mucci L. Immunohistochemical expression of BRCA1 and lethal prostate cancer. Cancer Res 2010;70:3136–9. Published OnlineFirst April 13, 2010. doi:10.1158/0008-5472.CAN-09-4100.

Veronese A, Lupini L, Consiglio J, Visone R, Ferracin M, Fornari F, Zanesi N, Alder H, D'Elia G, Gramantieri L, Bolondi L, Lanza G, Querzoli P, Angioni A, Croce CM, Negrini M. Oncogenic role of *miR-483-3p* at the *IGF2/483* locus. Cancer Res 2010;70:3140–9. Published OnlineFirst April 13, 2010. doi:10.1158/0008-5472.CAN-09-4456.

Lu W, Zhang G, Zhang R, Flores LG II, Huang Q, Gelovani JG, Li C. Tumor site–specific silencing of *NF-κB p65* by targeted hollow gold nanosphere–mediated photothermal transfection. Cancer Res 2010;70:3177–88. Published OnlineFirst April 13, 2010. doi:10.1158/0008-5472.CAN-09-3379.

Geng H, Rademacher BL, Pittsenbarger J, Huang C-Y, Harvey CT, Lafortune MC, Myrthue A, Garzotto M, Nelson PS, Beer TM, Qian DZ. ID1 enhances docetaxel cytotoxicity in prostate cancer cells through inhibition of p21. Cancer Res 2010;70:3239–48. Published OnlineFirst April 13, 2010. doi:10.1158/0008-5472.CAN-09-3186.

Yoo BK, Chen D, Su Z-z, Gredler R, Yoo J, Shah K, Fisher PB, Sarkar D. Molecular mechanism of chemoresistance by astrocyte elevated gene-1. Cancer Res 2010;70:3249–58. Published OnlineFirst April 13, 2010. doi:10.1158/0008-5472.CAN-09-4009.

Lu ZH, Shvartsman MB, Lee AY, Shao JM, Murray MM, Kladney RD, Fan D, Krajewski S, Chiang GG, Mills GB, Arbeit JM. Mammalian target of rapamycin activator RHEB is frequently overexpressed in human carcinomas and is critical and sufficient for skin epithelial carcinogenesis. Cancer Res 2010;70:3287–98. Published OnlineFirst April 13, 2010. doi:10.1158/0008-5472.CAN-09-3467.

Hattermann K, Held-Feindt J, Lucius R, Müerköster SS, Penfold MET, Schall TJ, Mentlein R. The chemokine receptor CXCR7 is highly expressed in human glioma cells and mediates antiapoptotic effects. Cancer Res 2010;70:3299–308. Published OnlineFirst April 13, 2010. doi:10.1158/0008-5472.CAN-09-3642.

Nadiminty N, Lou W, Sun M, Chen J, Yue J, Kung H-J, Evans CP, Zhou Q, Gao AC. Aberrant activation of the androgen receptor by NF-κB2/p52 in prostate cancer cells. Cancer Res 2010;70:3309–19. Published OnlineFirst April 13, 2010. doi:10.1158/0008-5472.CAN-09-3703.

Acu ID, Liu T, Suino-Powell K, Mooney SM, D'Assoro AB, Rowland N, Muotri AR, Correa RG, Niu Y, Kumar R, Salisbury JL. Coordination of centrosome homeostasis and DNA repair is intact in MCF-7 and disrupted in MDA-MB 231 breast cancer cells. Cancer Res 2010;70:3320–8. Published OnlineFirst April 13, 2010. doi:10.1158/0008-5472.CAN-09-3800.

McFarlane C, Kelvin AA, de la Vega M, Govender U, Scott CJ, Burrows JF, Johnston JA. The deubiquitinating enzyme USP17 is highly expressed in tumor biopsies, is cell cycle regulated, and is required for G_1-S progression. Cancer Res 2010;70:3329–39. Published OnlineFirst April 13, 2010. doi:10.1158/0008-5472.CAN-09-4152.

Dudka AA, Sweet SMM, Heath JK. Signal transducers and activators of transcription-3 binding to the fibroblast growth factor receptor is activated by receptor amplification. Cancer Res 2010;70:3391–401. Published OnlineFirst April 13, 2010. doi:10.1158/0008-5472.CAN-09-3033.

Cho SY, Xu M, Roboz J, Lu M, Mascarenhas J, Hoffman R. The effect of CXCL12 processing on $CD34^+$ cell migration in myeloproliferative neoplasms. Cancer Res 2010;70:3402–10. Published OnlineFirst April 13, 2010. doi:10.1158/0008-5472.CAN-09-3977.

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Persistent Cyclooxygenase-2 Inhibition Downregulates NF-κB, Resulting in Chronic Intestinal Inflammation in the Min/+ Mouse Model of Colon Tumorigenesis

Adelaide M. Carothers, Jennifer S. Davids, Beatrice C. Damas, et al.

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