Prostaglandin E\textsubscript{2} (PGE\textsubscript{2}) can stimulate tumor progression by modulating several proneoplastic pathways, including proliferation, angiogenesis, cell migration, invasion, and apoptosis. Although steady-state tissue levels of PGE\textsubscript{2} stem from relative rates of biosynthesis and breakdown, most reports examining PGE\textsubscript{2} have focused solely on the cyclooxygenase-dependent formation of this bioactive lipid. Enzymatic degradation of PGE\textsubscript{2} involves the NAD\textsuperscript{+}-dependent 15-hydroxyprostaglandin dehydrogenase (15-PGDH). The present study examined a range of normal tissues in the human and mouse and found high levels of 15-PGDH in the large intestine. By contrast, the expression of 15-PGDH is decreased in several colorectal carcinoma cell lines and in other human malignancies such as breast and lung carcinomas. Consistent with these findings, we observe diminished 15-Pgdh expression in Apc\textsuperscript{min−/−} mouse adenomas. Enzymatic activity of 15-PGDH correlates with expression levels and the genetic disruption of 15-Pgdh completely blocks production of the urinary PGE\textsubscript{2} metabolite. Finally, 15-PGDH expression and activity are significantly down-regulated in human colorectal carcinomas relative to matched normal tissue. In summary, these results suggest a novel tumor suppressive role for 15-PGDH due to loss of expression during colorectal tumor progression.

Multistep carcinogenesis unfolds as stochastic mutations and epigenetic changes accumulate within individual cells. In concert with stromal influences, those cells that develop the correct combination of mutations in tumor suppressor genes and oncogenes become fully transformed (1). As an example, the dysregulation of balanced prostaglandin metabolism in vivo contributes to carcinogenesis. Numerous reports have demonstrated the increased expression of cyclooxygenase-2 (COX-2)\textsuperscript{1} in a variety of human malignancies (2, 3), and higher COX-2 expression correlates with a poor clinical outcome (4). Furthermore, targeted overexpression of COX-2 in mouse mammary tissue leads directly to the development of breast carcinomas in transgenic mice (5). Elevated levels of COX-2-derived prostaglandin E\textsubscript{2} (PGE\textsubscript{2}) are associated with resistance to programmed cell death (6) as well as stimulation of cell migration, cell proliferation, and angiogenesis (7). Although steady-state tissue levels of PGE\textsubscript{2} depend on relative rates of biosynthesis and breakdown, virtually all reports examining the role of PGE\textsubscript{2} in physiology and disease have focused solely on the cyclooxygenase-dependent formation of this bioactive lipid. A plausible complementary pathway yielding increased local levels of PGE\textsubscript{2} in cancer involves reduced degradation of PGE\textsubscript{2} by NAD\textsuperscript{+}-dependent 15-hydroxyprostaglandin dehydrogenase (15-PGDH).

15-PGDH catalyzes the rate-limiting step of prostaglandin catabolism (8). The human gene is located on chromosome 4 and encodes a 29-kDa protein that oxidizes the 15(S)-hydroxyl group of prostaglandins to yield inactive 15-keto metabolites (9). Genetic deletion of 15-Pgdh leads to increased tissue levels of PGE\textsubscript{2} (10). Although previous studies on the distribution and activity of 15-PGDH have focused primarily on parturition and uterine biology, recent data suggest that 15-PGDH plays a role in carcinogenesis (11, 12). Although 15-PGDH may promote certain androgen-sensitive prostate cancers (13), preliminary reports on medullary thyroid and transitional bladder cancers suggest that the loss of 15-PGDH expression contributes to malignancy (14, 15). These conflicting reports are intriguing, and although no mechanism has been demonstrated in vivo that explains its down-regulation, a variety of compounds that modulate the expression and activity of 15-PGDH in vitro have been reported, including the hypoglycemic thiazolidinediones (16) as well as NSAIDs and PPAR\textgamma agonists (17).

To more completely understand prostaglandin function in epithelial biology, our laboratory sought to identify pathways complementary to COX-2 by which PGE\textsubscript{2} might accumulate and contribute to colorectal carcinogenesis. The present study examines the hypothesis that decreased expression of 15-PGDH correlates with colorectal tumor formation. We present data suggesting that 15-PGDH is down-regulated in malignant disease relative to normal tissue and that epider-
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Malignant growth factor (EGF) and indomethacin can modulate 15-PGDH expression in colorectal carcinoma cells. We also provide functional data both in vitro and in vivo showing that 15-PGDH expression levels correlate with enzymatic activity and production of the urinary PGE2 metabolite (PGE-M). Here we report the first indication that decreased catabolism of PGE2 may regulate tumor formation in the intestine. These data are suggestive of a previously unrecognized pathway in colorectal carcinogenesis, whereby elevated PGE2 levels derive in part from reduced expression and activity of 15-PGDH. Ultimately, this novel observation may shed light on the adverse clinical outcomes of patients with high levels of tumor-derived cyclooxygenase-2.

EXPERIMENTAL PROCEDURES

Reagents—EGF was purchased from Sigma, and indomethacin, cigitazone, and GW9662 were purchased from Cayman Chemical (Ann Arbor, MI). Erlotinib (Tarceva®) was obtained from Genentech (San Francisco, CA). First Choice mouse and human Northern blots were purchased from Ambion (Austin, TX). The Cancer Profiling Array II was purchased from BD Biosciences. Antibody to Cox-2 (160107) was purchased from Research Biologicals (Manassas, VA). cDNA for human 15-PGDH was a kind gift from Dr. Hsin-Hsiung Tai (University of Kentucky, Lexington, KY). The Northern blot probe for Cox-2 was generously provided by Dr. Sanjoy Das (Vanderbilt University, Nashville, TN), and that for 15-Pgdh was a kind gift from Dr. Beverly Koller (University of North Carolina, Chapel Hill, NC). The Northern probe for 15-PGDH was obtained by Spel digestion of IMAGE clone ID 3838799 from the American Type Culture Collection (Manassas, VA).

Animals and Cell Culture—C57BL/6 and C57BL/6-Apcmin/− mice were obtained from Jackson Laboratory (Bar Harbor, ME). C57BL/6-Pgdh−/− mice were a generous gift from Dr. Beverly Koller. The mice were housed and fed with standard mouse diet in the Animal Care Facility according to National Institutes of Health and institutional guidelines for laboratory animals. LS-174T, HCT-15, HCT-116, DLD-1, HT-29, CaCo-2, Colo 201, and SW480 cells were purchased from the American Type Culture Collection, and HCA-7 cells were a generous gift from Dr. Susan Kirkland. LS-174T, HCT-15, HCT-116, HCA-7, and HT-29 cells were maintained in McCoy’s 5A medium containing 10% fetal bovine serum, 100 units/ml penicillin, and 100 μg/ml streptomycin in a 5% CO2 atmosphere. SW480, Colo 201, and DLD-1 cells were maintained in RPMI 1640 medium containing 10% fetal bovine serum, 100 units/ml penicillin, and 100 μg/ml streptomycin in a 5% CO2 atmosphere. CaCo-2 cells were maintained in minimum essential medium, and LoVo cells were maintained in Ham’s F-12K medium containing 10% fetal bovine serum, 100 units/ml penicillin, and 100 μg/ml streptomycin in a 5% CO2 atmosphere.

Northern Blotting—Total cellular RNA was isolated from cells by TRIzol Reagent (Life Technologies, Carlsbad, CA) following the manufacturer’s protocol. 5 μg of total RNA was fractionated with a MOPS-formaldehyde agarose gel and transferred to Hybond N1 membrane (Amerham Biosciences). Following UV cross-linking, the blots were prehybridized for 30 min at 42 °C in Hybrisol I (Intergen Company, Purchase, NY) and then hybridized with 32P-labeled cDNA in the same buffer at 42 °C, washed, and subjected to autoradiography.

Western Blotting—Cells were washed with phosphate-buffered saline and lysed with radioimmune precipitation assay buffer (50 mM Tris-HCl, pH 7.4, 150 mM NaCl, 1 mM EDTA, 1% Triton X-100, 1% sodium deoxycholate, 0.1% SDS, and protease inhibitors from Roche Applied Science). Protein concentrations were measured using Bio-Rad reagent. Proteins were then separated on precast 4–20% SDS polyacrylamide gels (Invitrogen) and transferred to nitrocellulose membranes. Proteins were then separated on precast 4–20% SDS polyacrylamide gels (Invitrogen) and transferred to nitrocellulose membranes. Membranes were blocked in 5% milk in Tris-buffered saline, 0.1% Tween 20 and incubated with primary antibody (15-PGDH, 1/15,000 and β-actin, 1/2,000) overnight at 4 °C. The membranes were then treated with horseradish peroxidase-conjugated secondary antibody and developed using an ECL kit (Amerham Biosciences).

15-PGDH Activity Assay—15-PGDH activity was assayed by measuring the production of 15(S)-15-[3H]HPGE2 to glutamate through coupling 15-PGDH and glutamate dehydrogenase as described previously (18). Briefly, the reaction mixture contained 5 μM NH4Cl, 1 μM α-ketoglutarate, 1 μM NAD+, 1 mM 15(S)-15-[3H]HPGE2, 100 μg of glutamate dehydrogenase, and crude enzyme extract in a final volume of 1 ml of 50 mM Tris-HCl, pH 7.5. The reaction was allowed to proceed for 10 min at 37 °C and was terminated by the addition of 0.3 ml of 3% aqueous charcoal suspension. Supernatant radioactivity following centrifugation (1000 × g, 5 min) was determined by liquid scintillation counting. Calculation of oxidized PGE2 levels was based on the assumption that no kinetic isotope effect was involved in the oxidation of the 15(S)-hydroxy group of 15(S)-15-[3H]HPGE2.

Light Chromatography and Mass Spectrometry—The major urinary metabolite of PGE2, 11-oxo-hydroxy-9,15-dioxo-2,3,4,5-tetranor-prostane-1,20-dioic acid (PGE-M), was measured in urine as described previously (19). Briefly, PGE-M contained in 400 μl of mouse urine was derivatized using methoxime HCl (16% w/v) in a 1.5 s sodium acetate solution, diluted in water, and adjusted to pH 5 with acetic acid. Samples were purified by extraction using a C18 SepPak, after which 12.4 ng of O-H6)methoxime PGE-M internal standard was added. Samples were dried under nitrogen, resuspended in 50 μl of mobile phase A (95:4.9:0.1 (v/v/v) 5 mM ammonium acetate:acetonitrile:acetic acid), and analyzed by liquid chromatography tandem mass spectrometry as described previously (19).

RESULTS

Heterogeneous Expression of 15-PGDH with Highest Levels in the Large Intestine—As a preliminary study, we examined a wide range of tissue types to identify the physiologic expression pattern of 15-PGDH. 15-PGDH is known to be ubiquitously expressed in several organs in mammals (8), and total RNA was analyzed by Northern blot for tissues from human (Fig. 1A) and mouse (Fig. 1B). In humans, we found the highest expression levels in the large intestine (Fig. 1A), but elevated 15-PGDH mRNA was also detected in the lung, the liver, and the small intestine (Fig. 1, A and B). The lungs and liver are known to play an important role in prostaglandin metabolism in vivo (20), but this is the first report suggesting that 15-PGDH plays a role in the intestine.

These findings prompted us to determine whether the relative expression of 15-PGDH is altered in normal versus malignant tissues. A preliminary analysis of matched normal/tumor samples from multiple human tumors revealed that 15-PGDH was dysregulated in a wide range of human cancers, including those of the breast, stomach, lung, skin, kidney, small intestine, pancreas, liver, and colon (Fig. 1C). We chose to focus our attention initially on the role of 15-PGDH in colorectal cancer because of our long term focus on understanding prostaglandin function in intestinal biology.

Down-regulation of 15-PGDH Expression in Colorectal Carcinoma Cells—To assess the expression pattern and modulation of 15-PGDH in cultured colorectal carcinoma cells, we evaluated the following cell lines: HCT-15, HCT-116, HCA-7, HT-29, DLD-1, SW480, and LS-174T (Fig. 2A). Examination of the additional colorectal carcinoma cell lines CaCo-2, Colo 201, and LoVo revealed that only LoVo cells expressed 15-PGDH (data not shown). Most colorectal carcinoma cells (7 of 10) examined in this study displayed little or no 15-PGDH mRNA, although certain cell lines retained varying levels of 15-PGDH expression, including HCT-15 and HCA-7. These results are consistent with our in vivo findings that most colorectal carcinomas have significant reductions in 15-PGDH levels (see Fig. 4).

Four representative lines were employed in the following experiments: HCT-15, HCT-116, HCA-7, and LS-174T. This panel of cells was selected to span a full range of colorectal carcinoma lines with regard to 15-PGDH expression and endogenous prostaglandin levels. HCT-15 and HCT-116 cells do not express appreciable amounts of cyclooxygenase and have high (HCT-15) and low (HCT-116) endogenous levels of 15-PGDH.
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PGDH expression, respectively. LS-174T cells express low levels of 15-PGDH and cyclooxygenase but have been shown to be extremely sensitive to exogenous PGE₂ treatment (21). Finally, HCA-7 cells have high levels of COX-2 and endogenous prostaglandins as well as moderate levels of 15-PGDH.

To study the regulation of 15-PGDH expression in vitro, we focused initially on indomethacin and cigitazone, compounds reported to modulate 15-PGDH in other experimental models. Indomethacin is a widely used NSAID with a number of pharmacologic activities beyond the inhibition of COX-1 and COX-2 activity. Previous reports indicate that indomethacin induces 15-PGDH expression in certain contexts (14, 22, 23), and our results suggested that 10 μM indomethacin could induce 15-PGDH expression in LS-174T cells (p = 0.035) yet have no apparent effect in HCA-7, HCT-15, and HCT-116 cells (Fig. 2B). Quantitation of 15-PGDH data by densitometry supports these findings.

While evaluating additional mediators that can modulate the expression of 15-PGDH, we examined the PPARγ agonist cigitazone with and without pretreatment with an irreversible PPARγ-specific antagonist, GW9662. Although previous reports indicate that cigitazone is a potent antagonist of 15-PGDH enzymatic activity with an IC₅₀ of 2.7 μM (17), we did not see a significant effect of this PPARγ ligand on 15-PGDH expression in our studies (data not shown). To evaluate whether 15-PGDH may serve as a tumor suppressor gene, we sought to identify known oncogenic pathways that could alter expression of 15-PGDH.

EGF is an established mediator of proliferation in a variety of human cancers. In addition, signal transduction pathways downstream from the EP4 PGE₂ receptor have been shown to transactivate the EGFR (EGFR) (24, 25). We sought to identify whether stimulation or blockade of the EGFR (ErbB1) could modulate expression of 15-PGDH. Consistent with a tumor-suppressor role for 15-PGDH, EGF down-regulated 15-PGDH protein in HCT-15 (p = 0.013) and HCA-7 (p = 0.008) cells relative to untreated controls. Conversely, the inhibition of this pathway with the EGFR-specific tyrosine kinase inhibitor (erlotinib) led to the increased expression of 15-PGDH in both HCT-15 (p = 0.016) and HCA-7 (p = 0.070) (Fig. 2C). Pretreatment with erlotinib for 1 h prior to the addition of EGF largely blocked the down-regulation of 15-PGDH compared with EGF treatment alone (Fig. 2C). To our knowledge, this is the first observation that stimulation of the EGFR signaling cascade can down-regulate prostaglandin catabolism.

Modulation of 15-PGDH Expression and Activity in Vivo—Coordinate regulation of increased PGE₂ production and inhibition of PGE₂ degradation may increase local tissue levels of PGE₂. The relevance of this dysregulated state to carcinogenesis in vivo is emphasized by our recent study indicating that treatment of ApcMin+/− mice with PGE₂ significantly accelerates adenoma growth (26). Thus, we next evaluated 15-Pgdh levels in ApcMin+/− adenomas to see whether decreased catabolism could provide a partial explanation for the stimulatory effect of exogenous PGE₂ on adenoma growth in vivo. Northern and Western analysis of C57BL/6 and C57BL/6-ApcMin+/− mouse intestine showed that wild-type C57BL/6 mice exhibited strong expression of 15-Pgdh mRNA (Fig. 3A). The situation was reversed in adenomas of 15-week-old ApcMin+/− mice, in which the expression of Pgdh was markedly reduced (Fig. 3A). Interestingly, intestinal mucosa with a microscopically normal appearance maintained the expression of 15-Pgdh in these mice. By contrast, both Cox-2 mRNA and protein levels were low in the normal mucosa but significantly increased within the polyp microenvironment (Fig. 3, A and B). This finding is very interesting in light of the recent report that basal EGFR activity is increased in ApcMin+/− intestinal adenomas (27), combined with our current observation that the activation of EGFR lowers 15-PGDH expression.

Functional Analysis of 15-PGDH in Vitro and in Vivo—The modulation of 15-PGDH expression by EGF naturally raised questions regarding the functional significance of these changes. To assess whether 15-PGDH protein levels correlate with enzymatic catabolism of PGE₂, 15-PGDH activity was assayed in vitro as described previously (18). The treatment of HCT-15 cells with EGF (100 ng/ml) for 24 h decreased 15-PGDH enzymatic activity by 59% relative to untreated control cells (p < 0.001) (Fig. 3C). This reduction mirrors the decrease in protein levels under these conditions and provides evidence that protein expression and enzymatic activity of 15-PGDH correlate in vitro.

Recent reports indicate that genetic disruption of 15-Pgdh in the mouse results in increased tissue levels of PGE₂ (10). To determine how the loss of 15-PGDH expression affects PGE₂ catabolism in vivo, we measured PGE-M, the major urinary metabolite of PGE₂ (19). Interestingly, analysis of urine collected from wild-type C57BL/6 and C57BL/6-Pgdh−/− mice showed that PGE-M is absent in urine collected from Pgdh−/− mice compared with control animals (p < 0.001) (Fig. 3D). This provides strong evidence for the hypothesis that loss of 15-PGDH expression alone can directly disrupt catabolism of PGE₂ and contribute to elevated levels of bioactive PGE₂.

FIG. 1. 15-PGDH expression in normal and pathologic tissues. FirstBlot membranes blotted with RNA from normal human (A) and mouse (B) tissues were probed for expression of 15-PGDH. Each blot contains 2 μg of poly(A) RNA/lane. C, a Cancer Profiling Array II was hybridized with a 15-PGDH cDNA probe and examined for differential expression in paired normal (N) and tumor (T) samples from the following tissues: breast (n = 10), colon (n = 10), stomach (n = 10), lung (n = 10), skin (n = 10), kidney (n = 9), small intestine (n = 7), pancreas (n = 7), and liver (n = 3). Nine cancer cell lines were included as follows (from top to bottom): HeLa (cervical carcinoma), Daudi (Burkitt’s lymphoma), K562 (CML), HL60 (promyelocytic leukemia), G361 (melanoma), A549 (lung carcinoma), MOLT4 (ALL), SW480 (colorectal carcinoma), and Raji (Burkitt’s lymphoma).
Finally, to extend our in vivo studies and assess the biological relevance of these observations, we examined clinical samples of human colorectal carcinomas. A comparison of human colon cancers and matched normal tissue revealed greatly reduced expression of 15-PGDH in malignant tissue relative to normal colonic mucosa. Northern and Western blotting indicated that 15-PGDH levels were decreased in 85% of the 23 pairs of colon carcinoma samples compared with adjacent normal mucosa (Fig. 4A). To assess the functional significance of these findings, 15-PGDH enzymatic activity was compared in eight paired human colorectal cancer tissues and matched normal mucosa. 15-PGDH activity was found to reflect changes in expression levels ($p = 0.019$) (Fig. 4B). These data support the hypothesis that 15-PGDH expression and activity are down-regulated in colorectal cancer.

DISCUSSION

PGE$_2$ levels are elevated significantly at sites of inflammation and malignancy; these findings are often attributed to the increased expression of the inducible cyclooxygenase isoenzyme COX-2. The well studied functions of COX-2-derived PGE$_2$ in malignant and metastatic disease indicate a role in modulation of apoptosis, stimulation of angiogenesis, and promotion of tumor invasion (6, 7). A large body of evidence has revealed a 40–50% reduction in risk and mortality from colorectal cancer in individuals taking NSAIDs regularly, either in the context of sporadic colorectal cancers or in familial adenoma-
atous polyposis. Both conditions are associated with high levels of PGE2, and the protective effects of NSAIDs are due, at least in part, to inhibition of cyclooxygenase-dependent formation of PGE2 (28–30).

While virtually all studies have focused on pathologic overproduction by COX-2, steady-state levels of PGE2 depend on the relative rates of biosynthesis and breakdown. Reduced degradation of PGE2 by the rate-limiting catabolic enzyme 15-PGDH provides a potential alternative mechanism for the local accumulation of PGE2. The role of 15-PGDH in malignancy has not been addressed adequately. To increase our understanding of prostaglandin function in carcinogenesis, the present study sought to test the hypothesis that dysregulation of 15-PGDH is associated with colorectal tumorigenesis. We employ a variety of approaches to support our contention that 15-PGDH is down-regulated in colorectal cancer both in vitro and in vivo. Several human colorectal carcinoma cell lines, Apc\textsuperscript{Min}\textsuperscript{+/-} mice, and human sporadic colorectal cancer samples show decreased 15-PGDH expression and activity relative to matched normal tissues.

These findings naturally raise questions regarding the regulation of 15-PGDH expression at the molecular level. Rao et al. (11) report that inhibition of 15-PGDH activity by the isoflavonoid genistein increases tumor burden in azoxymethane-treated rats. Although previous studies show induction of 15-PGDH by indomethacin in certain contexts (14, 22, 23), we only observed induction of 15-PGDH by indomethacin in one of four colorectal carcinoma cell lines (LS-174T). Examination of additional cell lines and further testing in vivo are necessary to elucidate whether indomethacin regulates 15-PGDH in the intestine. Conversely, the inhibition of 15-PGDH expression and activity by EGF suggests a previously unknown mechanism by which EGFR ligands positively regulate local PGE2 levels. While ErbB2 is known to induce COX-2 (31), the current study suggests that EGF can decrease the expression of 15-PGDH in colonocytes. Our findings with EGF in HCT-15 and HCA-7 are complemented by data showing induction of 15-PGDH following inhibition of EGFR tyrosine kinase activity with erlotinib. Although the majority of EGFR studies focus on activation of intracellular signaling cascades, recent data from Lu et al. (32) support the view that EGF-induced negative regulation of gene function can promote tumor formation.

The loss of 15-PGDH expression in colorectal carcinomas is quite intriguing in light of previous reports indicating that up-regulation of EGFR occurs in 60–80% of colorectal cancers (33–35). The expression of EGFR is known to be associated with poor survival in patients with colorectal cancer (36), and recent studies indicate that inhibitors of EGFR signaling have clinically significant activity when given to patients with colorectal cancer who are refractory to other treatment (37). As several anticancer drugs that target EGFR signaling are currently being developed, our present results may help to explain...
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the clinical efficacy of these new agents. These data may also be quite significant when coupled with our original report (38) that COX-2 is an EGF/transforming growth factor-α-inducible gene in intestinal epithelial cells, with robust enhancement of expression from 23 individual human colorectal cancer tissues and matched normal mucosa. Equal amounts of RNA and protein were analyzed for 15-PGDH expression. N, normal mucosa; T, tumor tissue. B, determination of 15-PGDH activity is shown in eight human colorectal cancer tissues and matched normal mucosa.

A.

![Image](http://www.jbc.org/content/276/28/3176.figure4)

**FIG. 4.** Loss of 15-PGDH expression and activity in human colorectal cancer tissues. A, total RNA and protein were isolated from 23 individual human colorectal cancer tissues and matched normal mucosa. Equal amounts of RNA and protein were analyzed for 15-PGDH expression. N, normal mucosa; T, tumor tissue. B, determination of 15-PGDH activity is shown in eight human colorectal cancer tissues and matched normal mucosa.

Data presented in this paper indicate that the regulation of local PGE₂ levels in human colorectal cancers is complex, involving both increased expression of COX-2 and loss of 15-PGDH. Although 15-PGDH is known to play an important role in physiologic processes such as parturition through modulation of local prostaglandin levels, our data show that 15-PGDH may inhibit cellular transformation by metabolizing local PGE₂ produced at the tumor site. Coordinate regulation of increased PGE₂ production and inhibition of PGE₂ degradation lead to elevated levels of PGE₂, and the relevance of this dysregulated state to carcinogenesis in vivo is emphasized by our recent study (26) indicating that treatment of Apc<sup>Min−/−</sup> mice with PGE₂ significantly accelerates adenoma growth.

Apc<sup>Min−/−</sup> mice develop intestinal adenomas spontaneously and have elevated levels of PGE₂ at 15 weeks that correlate closely with multiplicity of intestinal polyps (40). Previously attributed solely to elevated COX-2 activity, our data in 15-week-old Apc<sup>Min−/−</sup> mice suggest that loss of 15-Pgdh also correlates with tumor progression in this setting. Given that the loss of 15-Pgdh decreases PGE₂ catabolism, our data also support the hypothesis that 15-Pgdh serves an important homeostatic function involving degradation of PGE₂. Loss of enzyme expression specifically within the tumor microenvironment coincides with adenoma formation in 15-week-old Apc<sup>Min−/−</sup> mice, a process that can be accelerated by treatment with exogenous PGE₂ (26).

In summary, this study examines 15-PGDH expression in both normal and tumor tissues of the mouse and human intestine, with preliminary analysis of 15-PGDH regulation in vitro. We show that 15-PGDH mRNA and protein levels are significantly reduced in human colorectal cancers relative to normal mucosa. A similar decrease in 15-PGDH expression is found specifically within the tumor microenvironment of intestinal adenomas in 15-week-old Apc<sup>Min−/−</sup> mice. Loss of 15-PGDH expression correlates with reduced enzymatic activity in vitro and in vivo. We also show that the loss of 15-PGDH function in vivo markedly decreases metabolic inactivation of PGE₂. Taken together, our data provide the first evidence that reduced catabolism of PGE₂ may promote colorectal tumorigenesis. These preliminary findings provide a novel framework for further investigation of the regulation of 15-PGDH expression and function in vivo.

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REFERENCES

1. Sabichi, A. L., Demierre, M. F., Hawk, E. T., Lerman, C. E., and Lippman, S. M. (2003) Cancer Res. 63, 5649–5655
2. Subbaramaiah, K., and Dannenberg, A. J. (2003) Trends Pharmacol. Sci. 24, 96–102
3. DuBois, R. N. (2003) Prog. Exp. Tumor Res. 37, 124–137
4. Pai, R., Soreghan, B., Szabo, I. L., Pavelka, M., Baatar, D., and Tarnawski, A. S. (2002) Prostaglandins Leukotrienes Essent. Fatty Acids 67, 461–465
5. Tai, H. H., Ensor, C. M., Tong, M., Zhou, H., and Yan, F. (2002) Prostaglandins Other Lipid Mediat. 68–69, 483–493
6. Frenkian, M., Delage-Mouroux, R., Pidoux, E., Jullienne, A., and Rousseau-Merck, M. F. (1997) Hum. Genet. 99, 279–281
7. Coggins, K. G., Labour, A., Coughlin, S. M., Audoly, L., Coffman, T. M., and Koller, B. H. (2002) Nat. Med. 8, 91–92
8. Rozic, J. G., Chakraborty, C., and Lala, P. K. (2001) Int. J. Cancer 93, 497–506
9. Rao, C. V., Wang, C. X., Simi, B., Lubet, R., Kelloff, G., Steele, V., and Reddy, B. S. (1994) Prostate Cancer Res. 58, 362–366
10. Frenkian, M., Segond, C., Jullienne, A., and Lausson, S. (2001) Endocrinology 145, 2141–2147
11. Cho, H., and Tai, H. H. (2002) Biochem. Biophys. Res. Commun. 297, 76–81
12. Rozic, J. G., and Lala, P. K. (2001) Prostate Cancer Res. 11, 20–25
Down-regulation of 15-PGDH in Colorectal Cancer

31. Vadlamudi, R., Mandal, M., Adam, L., Steinbach, G., Mendelsohn, J., and Kumar, R. (1999) *Oncogene* **18**, 305–314
32. Lu, Z., Ghosh, S., Wang, Z., and Hunter, T. (2003) *Cancer Cell* **4**, 499–515
33. Messa, C., Russo, F., Caruso, M. G., and Di Leo, A. (1998) *Acta Oncol.* **37**, 285–289
34. Porebska, I., Harlozinska, A., and Bojarowski, T. (2000) *Tumour Biol.* **21**, 105–115
35. Salomon, D. S., Brandt, R., Ciardiello, F., and Normanno, N. (1995) *Crit. Rev. Oncol. Hematol.* **19**, 183–232
36. Mayer, A., Takimoto, M., Fritz, E., Schellander, G., Kofler, K., and Ludwig, H. (1993) *Cancer* **71**, 2454–2460
37. Cunningham, D., Humblet, Y., Siena, S., Khayat, D., Bleiberg, H., Santoro, A., Bets, D., Mueser, M., Harstrick, A., Verslype, C., Chau, I., and Van Cutsem, E. (2004) *N. Engl. J. Med.* **351**, 337–345
38. DuBois, R. N., Tsujii, M., Bishop, P., Awad, J. A., Makita, K., and Lanahan, A. (1994) *Am. J. Physiol.* **266**, G822–G827
39. Nandy, A., Jenatschke, S., Hartung, B., Milde-Langosch, K., Bamberger, A. M., and Gellersen, B. (2003) *J. Mol. Endocrinol.* **31**, 105–121
40. Kettunen, H. L., Kettunen, A. S., and Rautonen, N. E. (2003) *Cancer Res.* **63**, 5136–5142
15-Hydroxyprostaglandin Dehydrogenase Is Down-regulated in Colorectal Cancer

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