

Dynamic antagonism between RNA-binding protein CUGBP2 and cyclooxygenase-2-mediated prostaglandin E₂ in radiation damage

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Damage to intestinal epithelium limits the use of ionizing radiation (IR) in cancer therapy. Prostaglandins (PGs), generated through the action of cyclooxygenase-1 (COX-1) and COX-2 protect the intestinal stem cells from IR. In previous studies, we demonstrated that the RNA-binding protein CUGBP2 regulates the stability and translation of COX-2 mRNA by interacting with AU-rich sequences in 3' UTR. Here, we demonstrate a dynamic antagonistic relationship between CUGBP2 and COX-2. Both CUGBP2 and COX-2 are rapidly induced after IR in intestinal crypt epithelial cells in mice, but CUGBP2 protein expression is observed immediately and COX-2 protein expression is delayed. In contrast, administration of bacterial lipopolysaccharide induced COX-2 expression and PGE₂, resulting in the inhibition of CUGBP2 expression and radioprotection of the intestine. These effects were reversed by NS398, a COX-2-specific inhibitor, suggesting that lipopolysaccharide-mediated inhibition of CUGBP2 is a PG-dependent mechanism. Furthermore, CUGBP2 expression is higher in COX-1^{-/-} and COX-2^{-/-} mice than wild-type controls at basal conditions, which is further increased after IR.

The gastrointestinal (GI) tract is highly sensitive to ionizing radiation (IR), which results in denuding of the intestinal mucosa and ultimately leads to death (1). Patients with various cancers may be targeted with large doses of IR, which puts them at high risk of developing acute or chronic enteritis (2). Immediately after IR, intestinal crypt epithelial cells undergo apoptosis (1). At IR doses of >8 Gy, the stem cells located at the base of the crypts of Lieberkuhn are also targeted, resulting in severe GI damage (3). Although mitotic arrest occurs immediately after IR treatment, cell migration continues to occur, resulting in shrinkage of the crypts. Release from mitotic arrest occurs at ≈36 h, and the surviving stem cells divide and restore normal intestinal architecture by 2 weeks (1). At an IR dose of >15 Gy, complete denuding of the intestine occurs, resulting in death within a few days (3).

Prostaglandin E₂ (PGE₂), generated from arachidonic acid by cyclooxygenase 1 (COX-1) or COX-2, protects the intestinal epithelium from IR by increasing stem cell survival and diminishing apoptosis (4, 5). COX-1 is constitutively expressed and promotes tissue homeostasis. In contrast, COX-2 expression is induced by growth factors and tumor promoters (6–9). Transgenic overexpression of COX-2 in mammary tissue of mice results in breast cancer, suggesting that regulating COX-2 expression may have therapeutic value (10). COX-2 inhibitors enhance the antitumor effect of IR on many human cancers, including colorectal cancer (11–13).

In mice, COX-1 up-regulation occurs 84 h after IR in regenerative intestinal crypt epithelium with a concomitant increase in PGE₂ synthesis (14, 15). COX-1^{-/-}, but not COX-2^{-/-}, mice have diminished baseline intestinal PGE₂ synthesis and an impaired crypt epithelial response to IR, suggesting that COX-1 regulates intestinal stem cell survival (14, 15). In contrast,

pretreatment of mice before IR results in radioprotection due to COX-2-mediated PGE₂ (16). Recent *in vitro* studies demonstrated that IR induces COX-2 in a colon cancer cell line, but that the RNA-binding protein CUGBP2 binds to AU-rich sequences in COX-2 3' UTR and inhibits COX-2 mRNA translation (17, 18). Furthermore, IR-induced apoptosis is mediated in part through the induction of CUGBP2 expression (17).

Here, we demonstrate that CUGBP2 expression is induced in mouse intestine after whole body IR, at a time when the epithelial cells are undergoing apoptosis. COX-2 mRNA is also induced immediately after IR, but it is not translated. Basal expression of CUGBP2 is higher in both COX-1^{-/-} and COX-2^{-/-}, and up-regulated in COX-1^{-/-} mice at the time of crypt regeneration. Lipopolysaccharide (LPS) induced COX-2 expression and PGE₂ synthesis and prevented CUGBP2 expression, which was overcome with a COX-2-specific inhibitor, NS398. These observations suggest the presence of a previously undescribed negative regulatory loop between RNA-binding protein CUGBP2 and COX-2-derived PGE₂. The dynamic action of these two factors may in part regulate the intestinal response to toxic effects of radiation exposure.

Materials and Methods

Animals. COX-1^{-/-} and COX-2^{-/-} male mice in a C57BL/6 background and FVB/N mice (Taconic Farms) were maintained on a 12-h light/dark schedule and fed standard laboratory chow (19). Eight-week-old mice were irradiated in a Gammacel 19 cesium irradiator at 0.96 cGy/min and allowed to recover (15). The distal jejunum was isolated and either fixed in buffered formaldehyde for immunohistochemical analyses or snap frozen in liquid nitrogen. PGE₂ levels were measured as described (16) by using a PGE₂-specific enzyme-linked immunoassay (Cayman Chemical, Ann Arbor, MI). LPS (*Escherichia coli* K-235, Sigma) was dissolved in pyrogen-free PBS and injected i.p. at 0.5 mg/kg. dmPGE₂ (0.5 mg/kg), NS398 (1 mg/kg every 8 h), and indomethacin (1.5 mg/kg every 8 h) were injected i.p. All animal procedures were performed in accordance with Washington University Institutional Animal Care and Use Committee guidelines.

Immunohistochemistry. For immunohistochemical localization of CUGBP2 and COX-2, tissue sections were incubated with 1:200 dilution of rabbit α-CUGBP2 or α-COX-2 IgG, followed by horseradish peroxidase (HRP)-conjugated α-rabbit IgG. For HuR, mouse monoclonal α-HuR antibody (3A2, 1:100 dilution, Santa Cruz Biotechnology) followed by HRP-conjugated

Abbreviations: IR, ionizing radiation; PGE₂, prostaglandin E₂; dmPGE₂, dimethyl-PGE₂; LPS, lipopolysaccharide; COX, cyclooxygenase.

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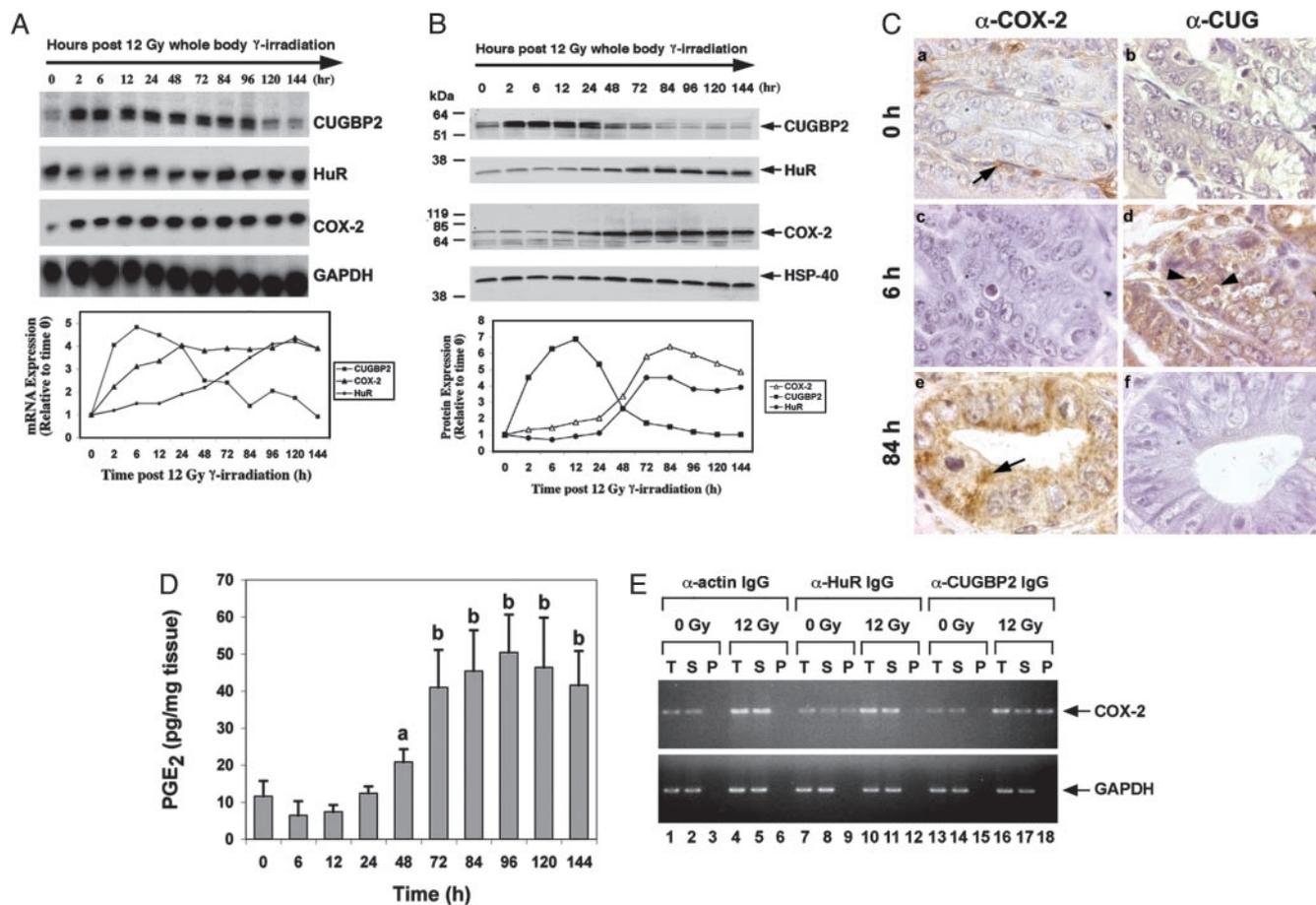


Fig. 1. Differential IR induction of CUGBP2 and COX-2 in intestinal crypt epithelial cells. (A) CUGBP2 and COX-2 mRNA are induced in the first 24 h. Mice were treated with 12-Gy whole body γ radiation and killed at the indicated times, and RNA was subjected to RNase protection analyses. Expression was normalized to GAPDH mRNA and plotted as relative to that observed at time 0 h. (B) COX-2 protein is observed when CUGBP2 expression is low and HuR expression is high. HSP-40 was used as loading control. Mobility of molecular mass markers is shown to the left. The data are presented relative to time 0 h. (C) Distal jejunum was subjected to immunohistochemistry with either α -CUGBP2 or α -COX-2 IgG. Sections stained with normal rabbit serum or without primary antibody served as controls (data not shown) (original magnification, $\times 400$). Location of staining for CUGBP2 and COX-2 is shown with arrowhead and arrow, respectively. (D) PGE₂ levels were significantly increased beginning at 48 h after IR. a, $P = 0.005$; b, $P < 0.001$. (E) CUGBP2 selectively binds COX-2 mRNA in intestinal epithelial cells of mice exposed to 12 Gy IR. T, total RNA; P, pellet; S, supernatant. Data are representative of four experiments.

α -mouse IgG was used. For apoptosis, animals were killed 6 h after IR. Apoptotic cells were also detected *in situ* by using the terminal deoxynucleotidyltransferase-mediated dUTP nick end labeling (TUNEL) assay (Boehringer Mannheim). The number of apoptotic cells per crypt as assessed with the TUNEL assay was $\approx 40\%$ higher than those assessed by morphological criteria, but the pattern when comparing test groups was identical. For crypt survival, microcolony assay was performed 3.5 days after IR (15). The mice received 120 mg/kg BrdUrd (Sigma) and 12 mg/kg fluorodeoxyuridine (Sigma) 2 h before death to label S-phase cells. Sections were incubated with 1:2,000 dilution of goat α -BrdUrd followed by HRP-conjugated α -goat IgG. Bound antibody was detected by using 3,3'-diaminobenzidine tetrahydrochloride (Vector Laboratories). For both assays, a minimum of eight complete cross sections was scored for each mouse. Western blot assays were performed with lysates by using the specific antibodies and α -HSP40 antibody (Santa Cruz Biotechnology) as control.

RNA Detection. Total RNA was isolated from the frozen tissue and subjected to RNase protection by using the RPA III Ribonuclease Protection Assay kit (Ambion, Austin, TX). ³²P-labeled probes were generated from PCR-amplified products by using gene-specific primers. For expression of transcripts in

COX-1^{-/-} and COX-2^{-/-} mice, total RNA was subjected to reverse transcriptase followed by real-time PCR analyses. As control, GAPDH expression was determined. For identification of *in vivo* RNA-protein interactions, epithelial cells from distal jejunum were isolated by using the Weiser protocol (20) and subjected to *in vivo* crosslinking as described (17). Lysates were immunoprecipitated with either affinity-purified rabbit α -CUGBP2 IgG, mouse α -HuR monoclonal IgG (Santa Cruz Biotechnology), or mouse α -actin monoclonal IgG (Santa Cruz Biotechnology). RNA isolated from the immunoprecipitates and supernatants was subjected to RT-PCR for COX-2 and GAPDH, as described (17).

Results

COX-2 mRNA Translation Is Delayed in Intestinal Epithelial Cells After IR. Studies on intestinal prostaglandin synthesis have demonstrated that PGE₂ is generated by COX-1 at 3.5 days after IR (15). Furthermore, studies with knockout mice have demonstrated that COX-2 does not play a role in IR response (14). We had previously observed that RNA-binding protein CUGBP2 binds to AU-rich sequences in the COX-2 3' UTR and inhibits COX-2 mRNA translation (17, 18). To evaluate COX-2 mRNA expression after IR, we performed RNase protection assay from distal jejunum RNA from FVB/N mice. COX-2 mRNA was

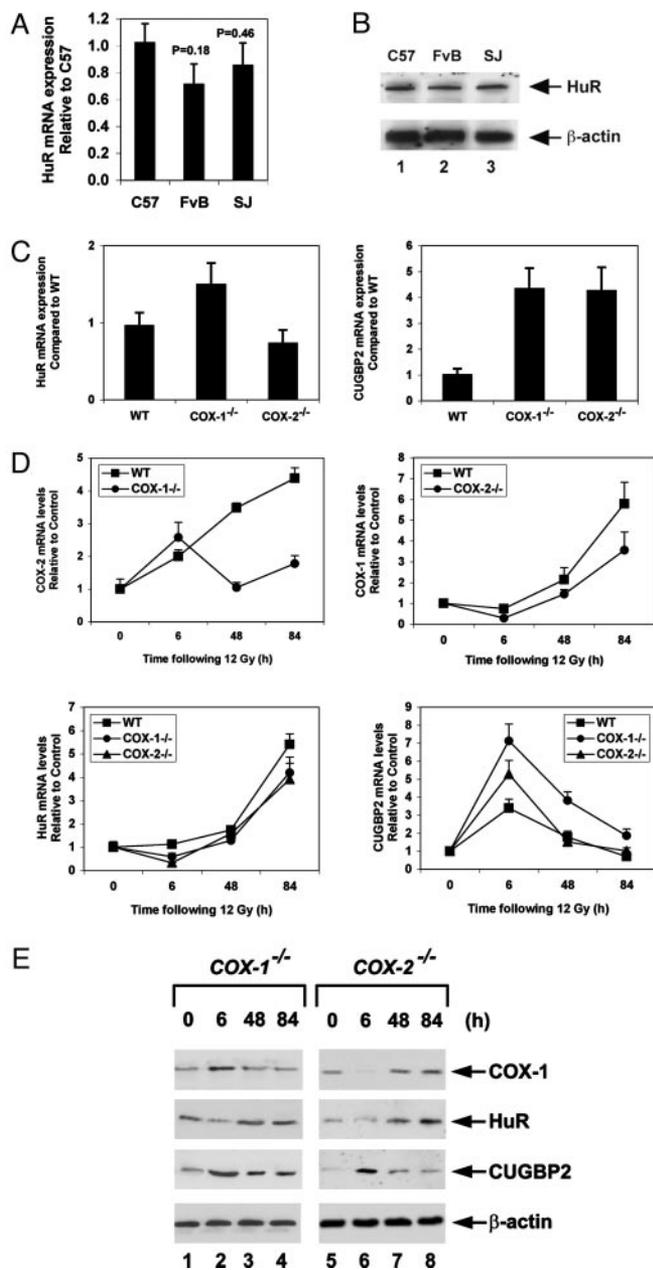


Fig. 2. CUGBP2 expression in *COX-1^{-/-}* and *COX-2^{-/-}* mice after IR. (A) Real-time PCR analyses for basal HuR levels in the distal jejunum of different strains of mice. The data are represented as relative to C57BL/6. C57, C57BL/6; FvB, FvB/N; SJ, SJL/J. (B) Western blot analysis of HuR protein levels. (C) HuR and CUGBP2 expression in the knockout mice, presented as relative to wild-type (WT) control littermates. *, $P = 0.02$; **, $P < 0.001$. (D) RNA expression in the knockout mice after IR. (E) Western blot analyses in the knockout mice after IR. Data are representative of three experiments.

induced within 2 h after IR, peaked at 24 h, and remained high until 144 h (Fig. 1A). However, Western blot analyses demonstrated a significant lag in COX-2 protein expression. There was no induction of COX-2 protein in the first 24 h (Fig. 1B). At 48 h, COX-2 protein levels were 3-fold higher than baseline (Fig. 1B). A similar COX-2 expression time course was observed in immunohistochemistry. No COX-2 protein was observed in crypt epithelial cells either at baseline or at 6 h after IR; however, significant COX-2 protein expression was observed at 84 h (Fig. 1C). We also determined PGE₂ levels in the distal jejunum after IR. Significant induction of PGE₂ was observed after 48 h. These

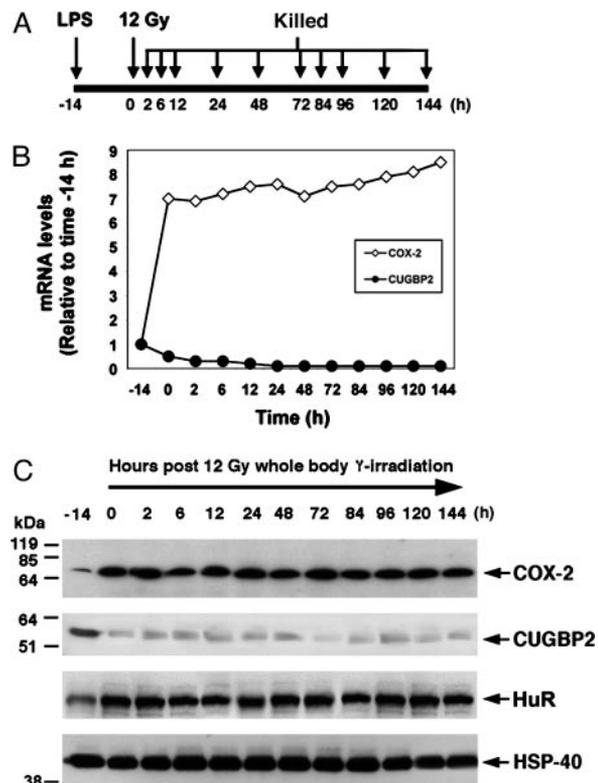


Fig. 3. COX-2-mediated PGE₂ inhibits CUGBP2 expression. (A) Schematic representation of treatment regimen. (B) Real-time PCR analysis of total RNA from isolated intestinal epithelial cells, expressed as relative to β -actin control. (C) Western blot analysis. Mobility of molecular mass markers is shown to the left. Data are representative of four experiments.

data suggest that PGE₂ production increases at a time when both COX-1 and COX-2 protein levels increase (Fig. 1D).

IR Induces CUGBP2 Expression. We next determined CUGBP2 expression in the mice. A 4-fold induction in CUGBP2 mRNA was observed at 2 h after IR in the intestine. CUGBP2 expression peaked at 6 h and returned to baseline levels at 48 h (Fig. 1A). CUGBP2 protein also increased at 2 h and returned to baseline by 48 h (Fig. 1B). Similarly, CUGBP2 was not detectable in tissue sections from either crypt or villus epithelial cells at 0 h, but its expression was visible in both nucleus and cytoplasm at 6 h after IR in the crypt epithelial cells (Fig. 1C). CUGBP2 expression continued until 24 h (data not shown). However, at 84 h, when the intestine was in the process of regeneration, CUGBP2 expression was not observed in the epithelial cells (Fig. 1C).

HuR has an opposing function with CUGBP2 with respect to COX-2 mRNA translation, although both enhance COX-2 mRNA stability (17, 21, 22). No change in HuR mRNA and protein was observed immediately after IR (Fig. 1A and B). However, increased HuR expression was observed at 48 h and continued to rise until 84 h (Fig. 1A and B).

CUGBP2 Competitively Binds to COX-2 mRNA After IR. Because both HuR and CUGBP2 interact with AU-rich sequences in COX-2 3' UTR (17), we next determined whether the lack of COX-2 mRNA translation in the first 24 h after IR was due to the effects of CUGBP2 or HuR by using a reversible crosslinking combined with immunoprecipitation and RT-PCR analysis with isolated intestinal epithelial cells. Real-time PCR analyses demonstrated 3-fold more COX-2 mRNA in extracts from irradiated cells (data not shown). In the immunoprecipitation, COX-2 and GAPDH

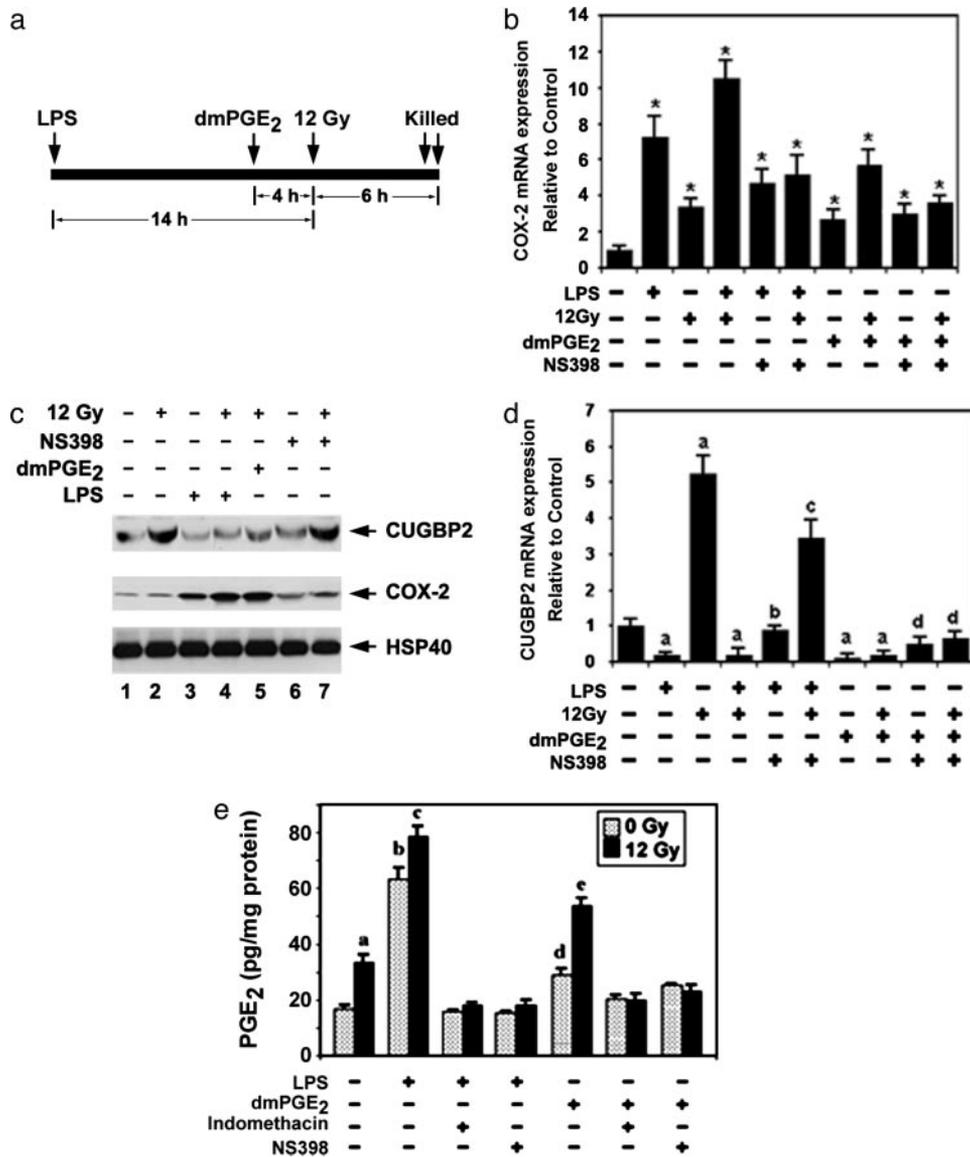


Fig. 4. PGE₂ inhibits CUGBP2 expression. (a) Schematic representation of study protocol. (b) IR induces COX-2 mRNA. Real-time PCR analysis, expressed as relative to β -actin control, is shown. *, $P < 0.001$. (c) LPS and dmPGE₂ induce COX-2 by inhibiting CUGBP2 expression. (d) PGE₂ inhibits CUGBP2 expression. Real-time PCR analysis, expressed as relative to β -actin, is shown. a, $P < 0.001$ relative to control; b, $P < 0.001$ relative to LPS alone; c, $P < 0.001$ relative to LPS and 12 Gy; d, $P = 0.008$ relative to dmPGE₂ alone. (e) LPS induces PGE₂ expression. a, $P = 0.02$ compared to control untreated; b, $P < 0.001$ compared to control untreated; c, $P = 0.008$ compared to LPS alone; d, $P < 0.001$ compared to LPS and 12 Gy; e, $P < 0.01$ compared to dmPGE₂ alone. Data are representative of three experiments.

mRNAs did not bind to actin (Fig. 1E). In unirradiated mice, COX-2 mRNA was bound by both HuR and CUGBP2, suggesting that HuR and CUGBP2 may regulate COX-2 expression under normal conditions (Fig. 1E). After IR, however, COX-2 mRNA was predominantly bound to CUGBP2, whereas the binding to HuR was significantly lower, suggesting that CUGBP2 competitively binds to COX-2 mRNA after IR (Fig. 1E). Thus, CUGBP2 appears to affect COX-2 mRNA translation *in vivo* after IR.

CUGBP2 Expression Is Induced After IR Is Prolonged in Mice Lacking the COX-1 Gene. Previous studies have demonstrated that PGE₂ produced through COX-1 promotes crypt stem cell survival and proliferation (14, 15). To establish the role of CUGBP2 and HuR in IR-mediated apoptosis and stem cell regeneration, we determined whether knockout of the COX genes affected expression of these transcripts. Because the *COX-1*^{-/-} and *COX-2*^{-/-} mice

are in the C57BL/6 background, we determined the expression of HuR in different backgrounds. Real-time PCR and Western blot analyses demonstrated that HuR levels are similar in FVB/N, SJL/J, and C57BL/6 strains of mice (Fig. 2A and B). Furthermore, no significant changes were observed in HuR expression in both *COX-1*^{-/-} and *COX-2*^{-/-} mice when compared to the wild-type C57BL/6 mice (Fig. 2C). In contrast, a significant increase in CUGBP2 levels was observed in the *COX-1*^{-/-} and *COX-2*^{-/-} mice (Fig. 2C).

To determine whether knockout of one COX gene results in compensatory induction of the other COX gene, real-time PCR and Western blot analyses were performed at various times after 12-Gy IR (19). In *COX-1*^{-/-} mice, COX-2 expression was induced to the same amount as in wild-type littermates (Fig. 2D and E). However, COX-2 expression was inhibited at 48 and 84 h in *COX-1*^{-/-} mice (Fig. 2D and E). In contrast, COX-1 was not reduced in *COX-2*^{-/-} mice after IR, but was expressed at

lower levels when compared to wild-type littermates (Fig. 2 *D* and *E*). Further studies described below demonstrated that PGE₂, generated through the actions of COX-1, regulated the expression of COX-2 during regeneration. Expression of HuR in the two knockouts was similar to that in wild-type mice. In contrast, CUGBP2 expression was significantly higher in both *COX-1*^{-/-} and *COX-2*^{-/-} mice at 6 h after IR compared with wild-type mice (Fig. 2 *D* and *E*). However, although CUGBP2 expression in wild-type mice returned to baseline levels at 48 h, its expression was higher in *COX-1*^{-/-} mice (Fig. 2 *D* and *E*). Furthermore, the number of apoptotic cells per crypt 6 h after IR was 2-fold higher in the *COX-1*^{-/-} mice when compared to wild-type or *COX-2*^{-/-} mice, as described (14). These data suggest that prostaglandins produced through both COX-1 and COX-2 may regulate CUGBP2 expression.

LPS Inhibits CUGBP2 Expression Through a Prostaglandin-Mediated Mechanism. Previous studies have demonstrated that induction of COX-2 by LPS before IR provides radioprotection to the intestine because of increased COX-2-dependent PGE₂ synthesis (16). Because CUGBP2 levels increased in mice lacking COX-2, the effect of LPS-mediated COX-2 induction on CUGBP2 expression was determined (Fig. 3*A*) (16). A 7-fold induction of COX-2 mRNA was observed after administration of LPS but before IR (Fig. 3*B*). Furthermore, IR did not affect COX-2 mRNA expression (Fig. 3*B*). COX-2 protein also increased 8-fold over baseline (Fig. 3*C*). In contrast, a 10-fold decrease in CUGBP2 mRNA and protein levels in intestinal epithelial cells was observed after LPS administration (Fig. 3*B* and *C*). Furthermore, IR did not induce CUGBP2 in the LPS-treated mice (Fig. 3*B* and *C*). These data suggest that LPS inhibits CUGBP2 expression in intestinal epithelial cells.

LPS Regulates COX-2 Expression Resulting in Increased PGE₂ Synthesis. To explore the mechanisms underlying LPS-mediated inhibition of CUGBP2, we measured the effect of LPS on COX-2-dependent prostaglandin synthesis. Mice were injected with LPS or dimethyl-PGE₂ (dmPGE₂), a synthetic stable isomer of PGE₂ before IR (Fig. 4*a*). LPS induced COX-2 mRNA, which was further induced by IR (7.2- vs. 10.3-fold) (Fig. 4*b*). dmPGE₂ also induced COX-2 mRNA expression that was further increased by IR (3.2- vs. 5.8-fold) (Fig. 4*b*). Furthermore, Western blot analysis showed that both LPS and dmPGE₂ induced COX-2 protein expression (Fig. 4*c*). Administration of NS398, a COX-2-specific inhibitor, partially suppressed LPS-mediated COX-2 induction. However, NS398 did not suppress dmPGE₂-mediated suppression of COX-2 (Fig. 4*b*).

Because LPS inhibits CUGBP2 expression, we tested the effect of NS398 on CUGBP2 levels to assess whether the inhibition is a result of COX-2-dependent dmPGE₂ synthesis. CUGBP2 expression was suppressed when either LPS or dmPGE₂ was administered before IR (Fig. 4*b* and *c*). Co-administration of NS398 with LPS resulted in increased CUGBP2 expression; however, NS398 did not affect dmPGE₂-mediated inhibition of CUGBP2 expression (Fig. 4*c* and *d*). We next assessed PGE₂ expression in the intestine after LPS and dmPGE₂ treatment. PGE₂ levels increased after LPS and dmPGE₂ treatments (3- and 1.5-fold), and were further increased after IR (Fig. 4*e*). However, no significant PGE₂ induction was observed when NS398 or indomethacin, a nonspecific COX inhibitor, were administered (Fig. 4*e*). These data suggest that LPS inhibits CUGBP2 expression through prostaglandins produced by COX-2.

LPS Is Radioprotective in Mouse Intestine Through COX-2-Mediated Prostaglandins. Because LPS and dmPGE₂ induced COX-2 but suppressed CUGBP2, we next assessed whether LPS and dmPGE₂ altered crypt epithelial cell apoptosis and stem cell

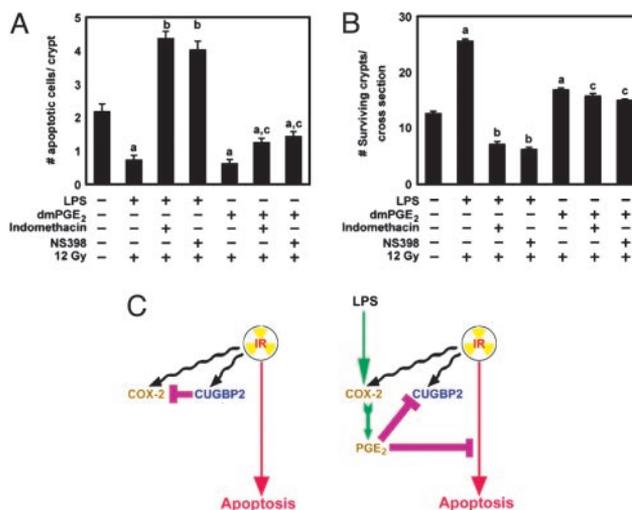


Fig. 5. LPS-mediated PGE₂ radioprotects intestine. (A) LPS or dmPGE₂ treatment before IR inhibits apoptosis. Mice received LPS or dmPGE₂ as shown in Fig. 4*a*, along with indomethacin or NS398. The number of apoptotic cells per crypt was determined after terminal deoxynucleotidyltransferase-mediated dUTP nick end labeling (TUNEL) staining. Values are mean ± SEM (*n* = 3 mice). a, *P* < 0.001 compared with control; b, *P* < 0.001 compared to LPS alone; c, *P* < 0.002 compared to dmPGE₂ alone. (B) LPS or dmPGE₂ treatment protects crypt stem cells. Two hours before death, BrdUrd was administered to the mice to label the S-phase cells. Sections were immunostained for BrdUrd and scored (*n* = 3 mice). a, *P* < 0.001 compared to radiated controls; b, *P* < 0.001 compared to LPS-treated; c, *P* = 0.1 compared to dmPGE₂-treated. (C) Schematic representation of the antagonistic activity of CUGBP2- and COX-2-dependent PGE₂ on apoptosis in intestinal epithelial cells.

survival. Both LPS and dmPGE₂ significantly inhibited crypt epithelial cell apoptosis after IR treatment (Fig. 5*A*). Apoptosis increased 8-fold in response to indomethacin or NS398 treatment, but was unaffected by treatment with dmPGE₂ (Fig. 5*A*). LPS administration also resulted in an ≈2-fold increase in crypt survival, which was not abrogated by the addition of NS398 (Fig. 5*B*). Furthermore, dmPGE₂ increased crypt survival, but the response was blunted as compared to LPS (Fig. 5*B*). These data suggest that LPS-mediated radioprotection of crypt epithelial cells was a result of COX-2-mediated PGE₂ production.

Discussion

We have previously demonstrated that CUGBP2 binds the 3' UTR of COX-2 mRNA blocking mRNA translation (17, 18). In this study, we found that CUGBP2 is rapidly induced in mouse intestinal epithelial cells after IR. In epithelial cells, CUGBP2 is expressed at very low levels and is predominantly found in the nucleus. However, after IR, the protein is present in both the nucleus and cytoplasm (17). It is interesting to note that we have observed a significant reduction in CUGBP2 at 84 h after IR, suggesting that the protein is degraded. We have provided an *in vivo* demonstration of an RNA-binding protein playing an important role in response to IR.

PGE₂ inhibits CUGBP2 expression. Both exogenous dmPGE₂ and endogenous PGE₂ blocked the induction of CUGBP2 by IR. Moreover, the IR-induced expression of CUGBP2 ends at 48 h. In *COX-1*^{-/-} mice, CUGBP2 expression continues beyond 48 h, probably because there is no endogenous PGE₂ to block CUGBP2 transcription. CUGBP2 levels are significantly higher at baseline in both *COX-1*^{-/-} and *COX-2*^{-/-} mice, suggesting that the PGE₂ produced by either source can regulate CUGBP2 expression. In contrast, the higher level of PGE₂ (60 pg/mg protein) seen after LPS administration is sufficient to totally block CUGBP2 induction by IR. It does come as a surprise that,

at baseline, *COX-2*^{-/-} mice demonstrate induction of CUGBP2, because baseline levels of COX-2 in the intestine are low and expression is observed only in subepithelial myofibroblasts (23).

CUGBP2 inhibits COX-2 mRNA translation, and PGE₂ blocks CUGBP-2 expression (17, 18). This finding suggests a dynamic antagonism between CUGBP2 and COX-2, wherein the one appearing first inhibits the expression of the other (Fig. 5C). However, the inhibitory effects may occur by distinct mechanisms. Whereas CUGBP2 modulates COX-2 expression at the level of translation, we currently do not know how PGE₂ inhibits CUGBP2 expression; this could occur at the level of transcription or mRNA degradation. In this regard, it should be noted that the 3' UTR of CUGBP2 mRNA encodes multiple AU-rich sequences.

The *in vivo* demonstration of the apoptotic affects of CUGBP2 confirms our previous *in vitro* studies where inhibition of CUGBP2 expression by silencer RNA reduced IR-mediated apoptosis of colon cancer cells in culture (17, 18). This finding raises the possibility that manipulation of CUGBP2 expression may modulate the response of the normal intestine to radiation therapy. Inhibition of CUGBP2 induction after IR should allow earlier expression of COX-2 in normal intestinal cells and, as a

consequence, diminished radiation-induced apoptosis. COX-2 is overexpressed in many tumors, including most colon cancers, resulting in increased production of PGE₂ (8, 24–26). It is possible that restoring CUGBP2 expression by preventing PGE₂ production may increase tumor sensitivity to radiation therapy. The data presented here would suggest that, in cancers producing large amounts of PGE₂, CUGBP2 expression would not be induced by radiation. This, in turn, would affect translation of those transcripts affected by CUGBP2. Many protooncogenes, cytokines, and immediate early genes, some of which regulate proliferation and/or apoptosis, encode AU-rich sequences in their 3' UTR (27). A fuller understanding of transcripts affected by CUGBP2 is required to predict the effects of manipulating CUGBP2 expression in response of tumors to radiation.

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