Cellular mechanism of mechanotranscription in colonic smooth muscle cells

Feng Li, You-Min Lin, Sushil K. Sarna, and Xuan-Zheng Shi

Division of Gastroenterology, Department of Internal Medicine, University of Texas Medical Branch, Galveston, Texas

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Mechanical stretch induces expression of cyclooxygenase-2 (COX-2) in gut smooth muscle cells (SMCs). The stretch-induced expression of COX-2 plays a critical role in motility dysfunction in bowel obstruction. As smooth muscle contractility in obstruction was significantly improved by pharmacological inhibition of COX-2 and in COX-2 gene-deficient mice (36). However, little is known concerning the mechanism whereby a mechanical force is transduced into a biochemical response leading to gene expression (mechanotranscription) in gut SMCs.

A series of intracellular signaling cascades is involved in mechanotranscription (1, 13, 21, 29, 44). First, the mechanical signal must be sensed at the cell membrane level before it is transduced into the cytoplasm and nucleus for gene expression. Integrins and stretch-activated ion channels (SACs) are two major groups of mechanosensors identified in different cells (8, 10, 11, 15). Integrins link extracellular matrix proteins to intracellular signaling through adaptor molecules, such as integrin-linked kinase (ILK) and focal adhesion kinase (FAK) at the site of membrane-associated focal adhesion (15). These focal adhesion molecules are coupled to the actin cytoskeleton and to various signaling molecules including MAPKs. Another major class of mechanosensors is SACs, which are found in many different types of cells including gut SMCs (10, 17). SACs can open rapidly in response to mechanical stretch and amplify signals by permitting the entry of ions such as Na$^+$ and Ca$^{2+}$ (10, 11, 17). The influx of these ions may participate in the activation of various cell signaling cascades including MAPKs (44). Consequently, activation of these intermediate molecules may lead to gene expression and functional changes in the mechanically stretched cells. MAPKs are known intracellular signaling components involved in the response to growth factors and inflammatory mediators in the gut (42, 43). However, it is not known whether mechanical stretch activates MAPKs, and if so, whether they are involved in mechanotranscription in gut SMCs.

In the present study, we investigated the signaling mechanism linking mechanical stretch to induction of COX-2 gene expression in colonic SMCs in vitro. Our hypothesis was that mechanical stretch-induced gene expression of COX-2 in colonic SMCs depends on both integrin- and SAC-mediated mechanosensing mechanisms and a MAPK-dependent intracellular signaling pathway. We also wished to determine whether inhibition of the mechanotranscription signaling pathway has therapeutic effects in restoring motility function in bowel obstruction in rats.

MATERIALS AND METHODS

Isolation and culture of rat colonic circular SMCs. Male Sprague-Dawley rats weighing 180–250 g were obtained from Harlan Sprague Dawley (Indianapolis, IN). The Institutional Animal Care and Use Committee at the University of Texas Medical Branch approved all procedures performed on the animals.

Address for reprint requests and other correspondence: X.-Z. (Peter) Shi, Dept. of Internal Medicine, Division of Gastroenterology, The Univ. of Texas Medical Branch, 301 Univ. Blvd., Basic Science Bldg. 4.106, Galveston, TX 77555-0655 (e-mail: xushi@utmb.edu).

THE GASTROINTESTINAL (GI) tract is a lengthy hollow organ system and is constantly subjected to mechanical forces. Although mechanical forces are important regulators of GI physiology such as motility function (33), responses of cells to abnormal mechanical stretch may contribute to functional changes in conditions such as bowel obstruction (36). Lumen distension-associated mechanical stretch is apparent in bowel obstruction and other obstructive bowel disorders (OBDs) such as achalasia, pyloric stenosis, pseudo-obstruction, and idiopathic mega-colon (20, 26, 28). The motility function in OBDs is impaired, resulting in symptoms such as nausea, bloating, abdominal pain, and constipation (28). The mechanism for the impaired motility function in these conditions is largely unknown. Recently we reported that lumen distension in bowel obstruction markedly increased expression of cyclooxygenase-2 (COX-2) in gut smooth muscle cells (SMCs). The stretch-induced expression of COX-2 plays a critical role in motility dysfunction in bowel obstruction, as smooth muscle contractility in obstruction was significantly improved by pharmacological inhibition of COX-2 and in COX-2 gene-deficient mice (36). However, little is known concerning the mechanism whereby a mechanical force is transduced into a biochemical response leading to gene expression (mechanotranscription) in gut SMCs.

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Mechanotranscription Signaling in the Colon

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Rats were killed with CO2 inhalation, and the distal colons of ~5 cm in length were isolated. The mucosal/submucosal and muscularis externa layers were separated by microdissection as described previously (37–39). Rat colonic circular SMCs (RCCSMCs) were isolated as described previously (39). In brief, the circular muscle tissue (0.5 × 0.5 cm2) was washed three times, each 5 min in sterile Hank’s solution. The tissue pieces were then incubated in Hank’s buffer with 1.5 mg/ml collagenase (type II, 319 U/mg; Worthington, Freehold, NJ) and 1.0 mg/ml soybean trypsin inhibitor (Sigma-Aldrich) for 45 min at 31°C. After incubation in fresh buffer without digestion enzymes for another 45 min, the spontaneously dispersed cells were collected and cultured in DMEM supplemented with 10% FBS in the presence of 100 U/ml of penicillin G, 100 μg/ml streptomycin sulfate, and 0.25 μg/ml amphotericin B (Invitrogen). The culture medium was changed every 3 days. Immunofluorescence staining showed that more than 95% of the cultured cells stained for smooth muscle-specific α-actin (36–38).

Primary culture was allowed to grow for 8–10 days until confluent. The cells were then seeded at 8 × 105 cells/well in six-well BioFlex culture plates coated with type I collagen (Flexcell, Hillsborough, NC) and allowed to grow to ~80% confluence before being subjected to DMEM/1% FBS for 24 h prior to stretch (36).

Transfection of FAK and ILK siRNAs in RCCSMCs. Rat FAK siRNA corresponding to the coding sequence was synthesized by Dharmacon (Chicago, IL). The rat ILK1 siRNA and scrambled siRNA were purchased from Cell Signaling Technology (Cambridge, MA). Cells were cultured in BioFlex culture plates to 60–80% confluence at the time of transfection. A premixed combination of 200 pmol siRNA and 5 μl of Lipofectamine 2000 (Invitrogen, Carlsbad, CA) in serum-free medium were added to each well of the six-well plate. The cells were incubated at 37°C in a CO2 incubator for 48 h before stretch.

Mechanical stretch in the primary culture of RCCSMC. RCCSMCs were mechanically stretched using FX-4000 Tension Plus System (Flexcell, Hillsborough, NC) as described previously (36). This computer-regulated bioreactor system applies multiaxial strain to the cells cultured on flexible membrane plates through vacuum pressure. Cells incubated in parallel under identical conditions but without exposure to stretch served as controls. Cells were subjected to static stretch at 18% elongation in all the experiments, unless specified otherwise. In the experiments involving treatments of pharmacological inhibitors or neutralizing antibodies, the inhibitors/antibodies or vehicle/serum controls were added 30 min prior to mechanical stretch. The concentrations of the inhibitors or antibodies were determined in the preliminary studies, and represent the minimal concentrations to completely block stretch-induced activation of individual kinases or gene expression.

Mechanical stretch of colonic circular muscle strips. Distal colon was cleansed and cut open along the mesenteric border in fresh Krebs buffer bubbled with 5% CO2/95% O2. The mucosa and submucosa layers were trimmed off with a sharp scissors. The colonic muscularis externa was cut into strips, 3 mm wide and 10 mm long, with the long axis along the circular muscle orientation. The tissue pieces were washed in sterile Hank’s medium supplemented with antibiotics three times, 5 min each. The strips were placed flat in DMEM in silicon elastomer-bottomed plates with pins at each end without stretching, and the length of the strips was measured as the original length. The muscle strips were stretched to 130% of their original length with pins at each end (36) and were called stretched strips. The nonstretch controls were treated the same way, except that the strips were kept in their original length without stretch. In some experiments involving treatments of pharmacological inhibitors, the inhibitors or vehicles were added 30 min before the strips were treated with mechanical stretch.

Protein extraction and Western blot analysis. To extract whole cell proteins from cultured cells, the cells were lysed directly on the plate by adding 0.35 ml of lysis buffer (1% Nonidet P40, 250 mMol NaCl, 50 mMol/l HEPES pH 7.4, 1 mMol/l EGTA, 5 mMol/l sodium pyrophosphate, supplemented with a cocktail protease inhibitor, and 50 mMol/l Na2VO3 freshly prepared before use). To ensure complete cell lysis, cells were kept on ice for 15 min, followed by centrifugation at 14,000 g at 4°C. Aliquots were stored at −80°C.

To extract whole cell proteins from muscle strips, the tissue was crushed into small particles in a custom mortar in liquid nitrogen followed by homogenization in the lysis buffer at 4°C.

Western blot analysis was carried out to detect specific protein bands as described previously (36–39). Equal quantities (15 μg) of protein samples were loaded and run on premaid 4–12% Bis-Tris SDS-PAGE (Invitrogen). The following primary antibodies were used in the study for incubation overnight at 4°C: primary antibodies to COX-2 (1:1,000; Cayman Chemical, Ann Arbor, ML), and to FAK, ILK, phospho-ERK1/2, phospho-JNK, phospho-p38, and phosphor-MAPKAPK-2 (1:1,000; Cell Signaling Technology). β-actin (1:5,000; Sigma, St. Louis, MO) was used as loading control. Bound antibody was coated with peroxidase-conjugated secondary antibody RDiye 800-conjugated anti-mouse IgG (Rockland, Gilbertsville, PA), or Alexa Fluor 680 goat anti-rabbit IgG (Invitrogen), diluted 1:10,000 in blocking solution for 40 min. After washing, the proteins were detected using Odyssey Infrared Imaging System (LI-COR Biosciences, Lincoln, NE).

RNA preparation and real-time PCR. Total RNA samples were extracted from cells or tissues by using the Qiagen RNeasy kit (Qiagen, Valencia, CA), and were reverse-transcribed by the SuperScript III first-strand synthesis system (Invitrogen). Real-time quantitative PCR was performed by using the Applied Biosystems 7000 real-time PCR system (Foster City, CA) with 40 ng cDNA for the target gene COX-2 (ID: Rn00568225_m1, TaqMan) as described previously (36). The amount of target was obtained by normalization to endogenous reference (18S rRNA).

Administration of mechanotranscription blocker in rats and COX-2-deficient mice with partial colon obstruction. Partial colon obstruction was prepared in rats (Harlan Sprague Dawley), and in wild-type and homozygous COX-2-deficient mice (B6;129P2- Ptgs2tm1Unc; Tacronic, Germantown, NY) by following procedures as previously described (36). Animals were anesthetized with 1.5–2% isoflurane inhalation by an E-Z anesthesia vaporizer (Palmer, PA). Partial colon obstruction was induced by placing a 3-mm wide medical grade silicon band around the distal colon at 2–3 cm proximal to the anus. The size of the silicon band (20–21 mm for rat; 10 mm for mice) is 1–2 mm longer than the outer circumference of the colon when the colon segment is filled with a fecal pellet, allowing a partial obstruction. The sham control rats underwent the same surgical procedure except that the band was removed immediately after implantation.

Animal groups were randomly assigned to include sham and obstruction each with vehicle or p38 inhibitor SB203580 (Cayman Chemical, Ann Arbor, MI) at 25 mg/kg ip daily (7). Treatment with SB203580 was started 1 h before the induction of obstruction. Once started, the inhibitors or vehicle controls were given daily until the time of death.

Animals were killed 3 days after operation. Rat distal colon of 5-cm length was taken out with the two openings closed with hemostats. The circumference of the distal colon and the weight of the fecal content in the 5-cm-long segment were measured. The rat and mouse colons were cut open and the colonic muscularis externa was taken for biochemical and molecular measurements and for contractility determination.

Measurements of colonic circular muscle contractility in muscle bath. Colonic smooth muscle strips (3 mm × 10 mm) were mounted along the circular muscle orientation in individual muscle baths (Radnoti Glass, Monrovia, CA) filled with 10 ml carbogenated Krebs solution at 37°C. The contractile activity was recorded as previously described (36, 39) with Grass isometric force transducers and amplifiers connected to Biopac data acquisition system (Biopac Systems, Goleta, CA). The muscle strips were equilibrated in the muscle bath
under 1 g tension for 60 min at 37°C before they were tested for contractile response to ACh. Preliminary studies found that the force tension curves of the muscle strips (using force range from 0.25 to 2.5 g) obtained from sham and obstruction (3 days) were similar in terms of percentage changes relative to the maximal response in both sham and obstruction tissues. The smooth muscle contractility was determined by obtaining concentration-response curves to ACh (10^-6 to 10^-2 M) in the muscle bath. The strips were left to equilibrate for at least 15 min between the tests of different concentrations of ACh. The contractile response of circular muscle strips was quantified as the increase in area under contractions during 4 min after addition of ACh to the bath, over the baseline area under contractions during 4 min before the addition of ACh.

**Statistical analysis.** All data are presented as means ± SE. ANOVA with nonrepeated measures (by Student-Newman-Keuls test) was used for multiple comparisons, and Student’s t-test was used for comparison of two means. In each case, P < 0.05 was considered statistically significant.

**RESULTS**

**Static stretch induces expression of COX-2 mRNA and protein in primary culture of RCCSMCs.** Both cyclic and static modes of stretch induced gene expression of COX-2 in RCCSMCs. To mimic sustained distension in bowel obstruction, we chose to stretch the cultured colonic SMCs in vitro with a static mode rather than a cyclic mode in the present study. Static stretch of RCCSMCs at 18% elongation increased the expression of COX-2 mRNA and protein in a time-dependent manner (Fig. 1). Preliminary studies showed that stretch-induced expression of COX-2 mRNA peaked at 3 h after the start of stretch, and COX-2 protein peaked after 24 h. When the cells were stretched for 5 min and harvested 3 h after the start of stretch, the COX-2 mRNA expression increased by 2.0 ± 0.8-fold compared with nonstretch control. The COX-2 mRNA expression increased by 5.4 ± 0.3-fold when the cells were stretched for 1 h and harvested 3 h after the start of the stretch (all P < 0.01, n = 6).

The expression of COX-2 protein was also significantly induced by static stretch (Fig. 1). The COX-2 protein expression increased 3.1 ± 0.4-fold (P < 0.01, n = 6) in the cells stretched for 1 h and harvested 24 h later.

**Mechanical stretch activates MAPKs ERK1/2, p38, and JNKs in the RCCSMCs.** Western blot analysis showed that mechanical stretch markedly induces phosphorylation of MAPKs ERK1/2, p38, and JNKs in a time-dependent manner (Fig. 2). The phosphorylation of MAPKs was significantly increased in as early as 5 min, lasted for at least 30 min, and returned to almost the nonstretch level by 60 min. At the 15-min time point, the phosphorylation levels of ERKs, p38, and JNKs increased by 6.9 ± 1.3-, 6.6 ± 0.8- and 3.8 ± 0.8-fold, respectively, compared with nonstretch controls (all P < 0.05, n = 3 or 4).

**Effects of ERKs, p38, and JNK inhibitors on stretch-induced expression of COX-2 in RCCSMCs.** We then determined whether MAPKs play a role in the expression of stretch-induced COX-2 in RCCSMCs. When the cells were treated with ERKs inhibitor PD98059 (20 μM), the stretch-induced expression of COX-2 mRNA and protein were almost completely blocked (P < 0.01, n = 4 to 6) (Fig. 2). Treatment of the cells with p38 inhibitor SB203580 (10 μM) or JNKs inhibitor SP600125 (10 μM) (Fig. 2) also significantly inhibited stretch-induced expression of COX-2 mRNA level by 70.7 ± 4.9% and 66.2 ± 6.0%, respectively (P < 0.01, n = 6). The COX-2 protein expression was also markedly inhibited by SB203580 and SP600125 (n = 6). This data suggests that all three MAPK members tested in our study are involved in the regulation of stretch-induced COX-2 gene expression in RCCSMCs.

**Integrins as a mechanosensor in the process of stretch-induced expression of COX-2 in RCCSMC.** As integrin α5β3 is the major type of integrins in gut SMCs (18), we determined
whether α1β3 is involved in the regulation of stretch-induced COX-2 expression in RCCSMCs. As shown in Fig. 3, the stretch-induced expression of COX-2 mRNA and protein were almost completely inhibited \( (P < 0.05, n = 6) \) when treated with a specific inhibitor to α1β3 echistatin \((400 \, \text{nM}, P < 0.05, n = 4)\) or a neutralizing antibody against rat β3 \((10 \, \mu\text{g/ml}, P < 0.05, n = 3)\). Further Western blot analysis results showed that stretch-induced phosphorylation of ERKs and JNKs were not affected in the presence of echistatin. However, the p38 phosphorylation was significantly inhibited by echistatin (Fig. 3).

To determine whether the common pathway of integrin signaling is involved in the stretch-induced expression of COX-2 in RCCSMCs, the integrin adaptor molecules ILK and FAK were knocked down by respective RNAi transfections (Fig. 4). When ILK gene expression was knocked down with ILK RNAi (Fig. 4), the stretch-induced COX-2 mRNA expression was suppressed partially but significantly, compared with scrambled RNAi treatment \( (P < 0.05, n = 6) \). Studies showed that stretch-induced expression of COX-2 mRNA was also suppressed in the FAK RNAi-treated cells by 71.7 ± 2.0\%, compared with the scrambled RNAi controls \( (P < 0.05, n = 4) \). Western blot analysis showed that the expression of ILK and FAK was markedly decreased by transfections of cells with RNAi to ILK and FAK, respectively (Fig. 4).

Stretch-activated ion channels as a mechanosensor in the process of stretch-induced expression of COX-2 in RCCSMC. To examine whether SACs play a role in the stretch-induced expression of COX-2 in RCCSMCs, a general SAC inhibitor...
gadolinium (5) and a more specific SAC blocker GsMTx-4 (2, 40) were used in the study. As shown in Fig. 5, the stretch-induced expression of COX-2 mRNA was inhibited by 81.4 ± 5.5% in the presence of gadolinium (0.1 mM) compared with controls (P < 0.05, n = 4). Stretch-induced COX-2 protein expression was also markedly inhibited. Treatment of cells with gadolinium inhibited stretch-induced activation of all MAPKs ERK1/2, p38, and JNKs (Fig. 5).

The spider peptide toxin GsMTx-4 (0.5 μM) also dramatically inhibited stretch-induced COX-2 expression in RCCSMCs. The stretch-induced expression of COX-2 mRNA was inhibited by 44.1 ± 11.3% with GsMTx-4 treatment compared with controls (P < 0.05, n = 4 to 6) (Fig. 5). Consistent with the effects of gadolinium, GsMTx-4 treatment also significantly inhibited stretch-induced activation of all MAPKs ERKs, p38, and JNKs (P < 0.05, n = 3) (Fig. 5).

**Static stretch in colonic smooth muscle strips and effect of mechanotranscription blocker.** As in the primary culture of colonic SMCs, sustained stretch of colonic muscle strip to 130% of its original length induced phosphorylation of ERKs, p38, and JNKs in a time-dependent manner (Fig. 6A). The phosphorylation level peaked at 15 min. Mechanical stretch of muscle strips induced the expression of COX-2 mRNA and protein by 10.0 ± 2.4-fold, and 6.9 ± 2.1-fold, respectively, in 48 h.

We found that the colonic circular muscle contractile response to ACh was significantly suppressed in the stretched muscle strips compared with nonstretch controls (Fig. 6B). Moreover, stretch-induced impairment of muscle contractility was blocked by pretreatment of the strips with p38 inhibitor SB203580 (10 μM). SB203580 treatment almost completely inhibited stretch-induced activity of p38 (at 15 min) and...
induction of COX-2 expression (at 48 h) in the muscle strips (Fig. 6C).

Blockade of mechanotranscription by p38 inhibitor improved motility function in obstruction in vivo. We further determined whether inhibition of mechanotranscription signaling pathway will improve motility function in vivo in a rat model of partial colon obstruction. Consistent with previous studies (36), the colonic circular muscle contractile response to ACh was significantly suppressed in rats with colon obstruction compared with sham-operated controls (Fig. 7). However, when SB203580 (25 mg/kg) was administered, the colonic smooth muscle contractility was not statistically different between sham and obstruction rats. Western blot analysis showed that p38 activation and COX-2 expression were robustly induced in the obstructed colon. However, both p38 activation and COX-2 expression were attenuated by SB203580 treatment (Fig. 7).

We also measured the weight of the intraluminal fecal contents and the colon circumference in the 5 cm-long colon segment oral to obstruction at the time of death, as an indication of colonic distension and motor activity in vivo. Obstruction led to massive accumulation of intraluminal contents in the distal colon as a result of colon obstruction and dysmotility (Fig. 7). The weight of intraluminal content increased from 0.69 ± 0.17 g in sham to 2.64 ± 0.20 g in obstruction on day 3 (P < 0.05, n = 3 or 4). SB203580 treatment did not affect this value in sham rats, but it markedly decreased the intraluminal accumulation in the obstructed colon to 1.92 ± 0.16 g. The circumference of distal colon in sham control was 20.3 ± 0.33 mm, when filled with pellets. It increased to 31.3 ± 0.88 mm in obstruction with vehicle control (Fig. 7). However, the colon circumference decreased to 26.7 ± 0.88 mm when the obstruction rats were treated with daily SB203580 (P < 0.05 vs. obstruction, n = 3). This data indicates that the colonic lumen distension and motor function in obstruction were significantly improved with SB203580 treatment.

Administration of p38 inhibitor in obstruction in wild-type and COX-2-deficient mice. To determine whether SB203580 has any effect other than inhibition of mechanotranscription of COX-2, wild-type, and COX-2-deficient mice in sham and obstruction were given daily administration of SB203580 (25 mg/kg). Obstruction-associated suppression of colonic smooth muscle contractility was markedly attenuated in the COX-2-deficient mouse (Fig. 8A). SB203580 treatment improved colonic contractility in obstruction in the wild-type mice, but did not significantly increase contractile response in the COX-2-deficient animals (Fig. 8, B–D). Western blot analysis showed that obstruction-induced expression of COX-2 was completely abolished in the COX-2-deficient mice, and markedly attenuated by SB203580 treatment (Fig. 8E).

DISCUSSION

We have shown that lumen distension leads to a marked induction of COX-2 gene expression in the colonic SMCs, and the stretch-induced COX-2 plays a critical role in pathophysiology of motility dysfunction in bowel obstruction (36). Administration of COX-2 inhibitors significantly improved motility function in animals with colon obstruction (26). However, the use of COX-2 inhibitors may lead to numerous adverse effects in humans (3). It is important to uncover the signaling mechanism linking mechanical distension to the induction of COX-2 gene expression in gut SMCs, as an understanding of this pathway may help to design safe and effective therapeutic treatments toward motility dysfunction in OBDs.

The mechanotranscription signaling mechanism is a complicated cellular process, and is cell-type specific. The mode, force, and duration of stretch also affect the intracellular signaling pathways (8, 21, 22, 29, 44). To mimic sustained mechanical distension in obstruction in vivo, we used a static stretch mode in the primary culture of colonic SMCs and in the colonic muscle strips in vitro. This is in contrast to the studies in respiratory and cardiovascular systems, in which a cyclic stretch mode is applied most of the times (13, 22). Our studies showed that 18% static stretch of the cultured colonic SMCs or 30% sustained stretch in the colonic smooth muscle strips consistently led to marked induction of COX-2 mRNA and protein in vitro. It is noteworthy that static stretch of cells for
as short as 5 min significantly induced expression of COX-2. These data suggest that mechanotranscription is a potent regulatory mechanism of gene expression in gut SMCs.

MAPKs are a group of serine/threonine-specific protein kinases, which respond to mitogens and inflammatory mediators. It is reported that MAPKs play an important role in GI motility dysfunction in inflammatory response (9, 12, 42). We found in the present study that MAPKs ERKs, p38, and JNKs are all markedly activated by mechanical stretch in RCCSMCs. Moreover, inhibition of MAPKs with specific inhibitors to ERKs, p38, and JNKs significantly attenuated stretch-induced expression of COX-2. Our study therefore demonstrates that stretch-activated MAPKs play a critical role in mechanotranscription signaling in gut SMCs.

SACs are present in gut SMCs and activated by mechanical stretch (17). However, it is not known whether SACs play a role in regulation of gene expression in gut SMCs. We found that inhibition of SAC with either gadolinium or GsMTx-4 significantly attenuated stretch-induced expression of COX-2 in RCCSMCs. Furthermore, SAC blockers markedly inhibit stretch-induced activation of MAPKs ERKs, p38, and JNKs in RCCSMCs. This data demonstrates that SAC activation is a prerequisite for phosphorylation of MAPKs in response to stretch, and SAC is an important component of the mechanotranscription signaling cascade upstream to activation of MAPKs in RCCSMCs. How SACs regulate MAPKs activation is not known. However, as known regulators of MAPK activation, receptor tyrosine kinase and protein kinase C are also activated by mechanical stretch (16) (F. Li and X.-Z. Shi, unpublished observation), it is possible that SACs-associated ionic influx may well regulate receptor tyrosin kinase, protein kinase C, and MAPK kinases to further influence MAPKs activation.

Integrins are not only the main receptors to connect the cytoskeleton to the extracellular matrix (6), but also regulate signaling pathways inside of the cells (34, 35). They are positioned to transduce mechanical stress to cellular response. We found that RNAi knockdown of integrin linked FAK and ILK signifi-

Fig. 5. Role of stretch-activated ion channels (SACs) in stretch-induced COX-2 gene expression and MAPKs activation. A: effect of SAC inhibitor gadolinium. Cells were treated with vehicle or gadolinium (0.1 mM) 30 min before being stretched for 1 h. For detection of COX-2 gene expression, RNA samples were taken 3 h after the start of stretch, and proteins taken in 24 h. For detection of phosphorylation of MAPKs, cells were stretched for 15 min and harvested immediately. *P < 0.05 vs. nonstretch (ns) in the group. #P < 0.05 vs. stretch (str) in the control group. B: effect of SAC inhibitor GsMTx-4. Cells were treated with vehicle control or GsMTx-4 (0.5 μM) for 30 min prior to stretch. Cells were harvested for detections of COX-2 protein (n = 4), COX-2 mRNA (n = 6), and phosphorylated ERKs, p38, and JNKs (n = 3) as described in A. *P < 0.05 vs. ns in the group. #P < 0.05 vs. str in the control group.
significantly inhibited stretch-induced COX-2 gene expression. As reported elsewhere, \( \alpha_\beta_3 \) is the major integrin type in gut SMCs (18). We found that treatment of RCCSMC with echistatin, a specific inhibitor to integrin \( \alpha_\beta_3 \), or inhibition of \( \beta_3 \) with neutralizing antibody significantly attenuated stretch-induced COX-2 expression. Echistatin treatment inhibited stretch-activated p38, but not ERKs and JNKs of the MAPK family in RCCSMCs. This data demonstrates that integrins are another class of important mechanosensors in the colonic SMCs, which transduce mechanical signals to intracellular intermediate molecules such as MAPKs in a distinctively different mechanism from that of SAC-mediated pathways. However, MAPKs appear to be a crucial component in the mechanotranscription signaling cascade as both integrin- and SAC-mediated pathways converge to MAPKs. Inhibition of individual MAPKs ERKs, p38, or JNKs drastically attenuated stretch-induced expression of COX-2 gene.

To block p38 activation in RCCSMCs in response to static stretch in vitro, we chose the prototype p38 inhibitor SB203580 (4). Inhibition of p38 activity attenuated stretch-induced COX-2 mRNA expression by > 70%. Although stretch-induced activation of MAPKs is relatively transient, p38 activation in the colonic SMCs in obstruction lasted longer than other MAPKs (F. Lin and X.-Z. Shi, unpublished observation). In addition, p38 is involved in stretch-induced expression of COX-2 by both SAC- and integrin-mediated pathways. We then tested p38 inhibitor SB203580 as a representative blocker of mechanotranscription for therapeutic potentials in conditions with sustained lumen distension. Treatment of colonic muscle strips with SB203580 markedly alleviated stretch-induced impairment of muscle contractility. When administered in vivo, SB203580 significantly improved smooth muscle contractility in the obstructed colon. The in vivo motor function was also significantly improved with SB203580 treatment, as the colonic fecal accumulation was decreased and the lumen distension was relieved in obstruction with SB203580 treatment. Interestingly, SB203580 administration did not further improve muscle contractility in obstruction in the COX-2-deficient mice. This indicates that SB203580 improved motility function in obstruction mainly through its effect to inhibit mechanotranscription of COX-2. Overall, our data suggest that inhibition of mechanotranscription has therapeutic potentials in the management of motility dysfunction in OBDs. It is noteworthy that SB203580 itself may have liver toxicity in the humans (4). However, novel p38 inhibitors are under development for improved safety (4, 25). Furthermore, SB203580 was used in the present study only as a representative agent to block mechanotranscription signaling in the colon. Conceptually, any agent with an inhibitory effect on the mechanotranscription signaling molecules (e.g., SACs, integrin, ERKs, and JNKs) may have therapeutic potentials in OBDs.

Current medical treatments for chronic motility dysfunctions in OBDs and other GI conditions depend on prokinetic agents to augment smooth muscle contractions (14, 31). Cholin-
Ergic agents stimulate smooth muscle contraction directly by acting on muscarinic receptors on gut SMCs (e.g., bethanechol) or by increasing the stay of excitatory neurotransmitter acetylcholine at the neuromuscular junction (e.g., neostigmine) (19, 31). The benzamidazole derivatives such as metoclopramide and domperidone, and 5-HT receptor modulators such as cisapride and tegaserod all stimulate gut SMC contractions mainly through increased release of acetylcholine from enteric nervous system (24, 27, 30). Finally, erythromycin and other motilides may mimic motilin effect to increase acetylcholine release or act on SMC directly to stimulate muscle contractions (32). As we now know, COX-2 expression is markedly induced in OBDs. The stretch-induced COX-2 and COX-2-derived prostaglandins have potent impacts on smooth muscle contractility (23, 36). Therefore, COX-2 inhibitors and mechanotranscription modulators may prove to be an important therapeutic approach in the treatment of motility dysfunction particularly in OBDs, complementary to the contraction-stimulating prokinetics. If inhibitory mechanisms, such as increased expression of COX-2 and secretion of prostaglandins dominate the pathological conditions of motility dysfunction such as in lumen distension or intestinal stasis, the use of prokinetics alone may not be very effective. Even though prokinetics may have temporarily augmented smooth muscle contractions, the inhibitory mechanism remains and eventually prevails. This may, at least partly, explain why current prokinetics are not very effective in the management of motility disorders (14, 41).

In summary, we found that static stretch markedly induces expression of COX-2 gene and activates MAPKs ERKs, p38 and JNKs in the RCCSMCs. Treatments with ERKs inhibitor PD98059, p38 inhibitor SB203580, and JNKs inhibitor SP600125 significantly attenuated stretch-induced COX-2 expression. Our study also identified that SACs and integrins are the upstream mechanosensors, which couple mechanical

Fig. 7. Effects of in vivo administration of p38 inhibitor SB203580 in rats. A: effect of administration of SB203580 on colonic circular smooth muscle contractility in rats with sham operation or obstruction (OB) for 3 days. Contractility was normalized by cross section area of each muscle strip, and presented as percentage of the maximal response in sham control rats, veh, Vehicle. B: effect of in vivo treatment with SB203580 on phosphorylation of p38 substrate MAPKAPK-2 and expression of COX-2 in colonic smooth muscle in obstruction (3 days). C: effect of in vivo administration of SB203580 on colonic circumference. D: effect of SB203580 on intraluminal fecal content in the colon. Daily administration of vehicle (20% DMSO) or SB203580 (25 mg/kg in 200 µl ip) was started 30 min before the operation. Sham-operated or obstructed rats with vehicle or SB203580 treatment were killed 3 days later for determinations of circular muscle contractility, COX-2 expression, outer circumferences, and intraluminal fecal content in the 5 cm colon segment oral to the obstruction band. N = 3 or 4 for each group. *P < 0.05 vs. sham in the group. #P < 0.05 vs. OB in vehicle control group.
stretch to the activation of MAPKs and induction of COX-2 gene expression. Further functional studies demonstrate that inhibition of the mechanotranscription signaling process with SB203580 blocked p38 phosphorylation and COX-2 induction and significantly restored motility function in obstruction. We therefore conclude that mechanical stretch induces expression of COX-2 in RCCSMCs through mechanosensors SACs and integrins and an intracellular mechanism involving MAPKs ERKs, p38, and JNKs. Inhibition of the mechanotranscription signaling pathway holds therapeutic potentials in the management of motility dysfunction in OBDs.

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AUTHOR CONTRIBUTIONS


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