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TITLE: Mechanisms Underlying the Therapeutic Efficacy of Exclusive Enteral Nutrition
in Crohn's Disease

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14. ABSTRACT Crohn's disease (CD) is an inflammatory bowel disease (IBD), characterized by recurrent transmural inflammation in the colon or/and ileum. Exclusive enteral nutrition (EEN), involving oral or nasogastric tube feeding of a complete liquid diet with exclusion of normal foods, is a safe and effective treatment for active CD. However, what accounts for the benefits of EEN in CD are not clear. Our project aims to investigate the mechanisms of the benefits of EEN. We hypothesize that: 1) Transmural inflammation in CD causes mechanical stress (MS) in the inflammation and pre-inflammation sites, and the MS induces expression of certain mechanosensitive genes to contribute to the development of inflammation and bowel dysfunctions; 2) EEN treatment is beneficial in CD by preventing MS and blocking the effects of MS-induced gene expression. Accordingly, our Specific Aims are: 1) To determine if MS plays a role in the development of CD by evaluating the effects of EEN on mechanosensitive gene expression, inflammation and bowel dysfunction; 2) To investigate the mechanisms underlying the beneficial effect of EEN on gut inflammation in CD; 3) To investigate the mechanisms underlying the beneficial effect of EEN on bowel dysfunctions, especially motility dysfunction. So far, we have almost completed the study in Aim 1. We found that intracolonic instillation of TNBS induces a localized transmural inflammation (as in CD) in the distal colon, which is associated with mechanical tension in the local inflammation site and lumen distention in the bowel prior to inflammation. These changes represent significant MS in the colon, and induce marked induction of certain pro-inflammatory mediators such as osteopontin (OPN) and cyclo-oxygenase-2 (COX-2) in the inflammation and pre-inflammation sites, which contribute significantly to the development of inflammation and bowel symptoms. EEN treatment decreases feces production, and prevents inflammation-associated MS, and dramatically attenuated expression of OPN and COX-2 in the CD model, and improved inflammation score and motility function. Our study suggests that the benefit of EEN in CD may result from its action in attenuating mechanical stress in transmural inflammation in this disease.					
15. SUBJECT TERMS Inflammatory bowel disease, Crohn's disease, Exclusive enteral nutrition, Mechanical stress, Osteopontin, Cyclooxygenase-2, Yes-associated protein, Th1, Th17 immune response, Gut motility					
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1. INTRODUCTION:

Crohn's disease (CD) is an inflammatory bowel disease (IBD), characterized by recurrent transmural inflammation in the colon or/and ileum. The mechanisms of CD associated inflammation and bowel symptoms are not well understood. Among the therapeutic options, corticosteroids, immune-modulators, and biologic agents all have substantial adverse effects or limitations, whereas exclusive enteral nutrition (EEN) is safe and effective, and now a first line treatment for active CD especially in young patients. EEN involves oral or nasogastric tube feeding of a complete liquid diet with exclusion of normal foods for a defined period (usually 4 to 8 weeks). As a dietary therapy, EEN is found as equivalent as, or superior to corticosteroids in the induction of remission in children (with nearly 80% remission rate). Although EEN is widely used in Europe, Asia, and Australia, it is not well accepted in the US, partly because the exact mechanisms for its therapeutic benefits in CD are not clear. **This project aims to investigate the mechanisms of the benefits of EEN for CD.** Although mechanical stress (MS), associated with inflammatory cell infiltration, edema, fibrosis, and stenosis, are commonly encountered in CD, the role of MS in the development of inflammation and gut dysfunction is largely unrecognized. In a well-established rodent model of CD, we found that intracolonic instillation of a hapten reagent TNBS induces a localized transmural inflammation (as in CD) in the distal colon, which is associated with mechanical tension in the local inflammation site and lumen distention in the bowel prior to inflammation. These changes represent significant MS in the colon. Our preliminary studies demonstrated that MS induces marked induction of certain pro-inflammatory mediators such as osteopontin (OPN) and COX-2 in the inflammation and pre-inflammation sites, which contribute significantly to the development of inflammation and bowel symptoms. We found that EEN treatment dramatically decreases feces production, and prevents inflammation-associated MS. Our **Hypotheses** are: 1) Transmural inflammation in CD causes MS in the inflammation and pre-inflammation sites, and the MS induces expression of mechanosensitive genes OPN and COX-2 to contribute to the development of inflammation and bowel dysfunctions; 2) EEN treatment is beneficial in CD by preventing MS and blocking the effects of MS-induced gene expression. Accordingly, our **Specific Aims** are: 1) To determine if MS plays a role in the development of CD by evaluating the effects of EEN on mechanosensitive gene expression, inflammation and bowel dysfunction; 2) To investigate the mechanisms underlying the beneficial effect of EEN on gut inflammation in CD, by focusing on the role of mechanosensitive expression of OPN in Th1/Th17 immune response; 3) To investigate the mechanisms underlying the beneficial effect of EEN on bowel dysfunctions in CD, by focusing on the role of mechanosensitive expression of COX-2 in motility dysfunction. In **summary**, our project addresses the mechanism of action of EEN, the only established dietary therapy, in CD management, and thus is closely relevant to public health of US general population, our military personnel, their families and children, especially those who suffer from Crohn's disease.

2. KEYWORDS:

Inflammatory bowel disease, Crohn's disease, Exclusive enteral nutrition, Mechanical stress, Osteopontin, Cyclooxygenase-2, Yes-associated protein, T_H1 immune response, T_H17 immune response, Gut motility.

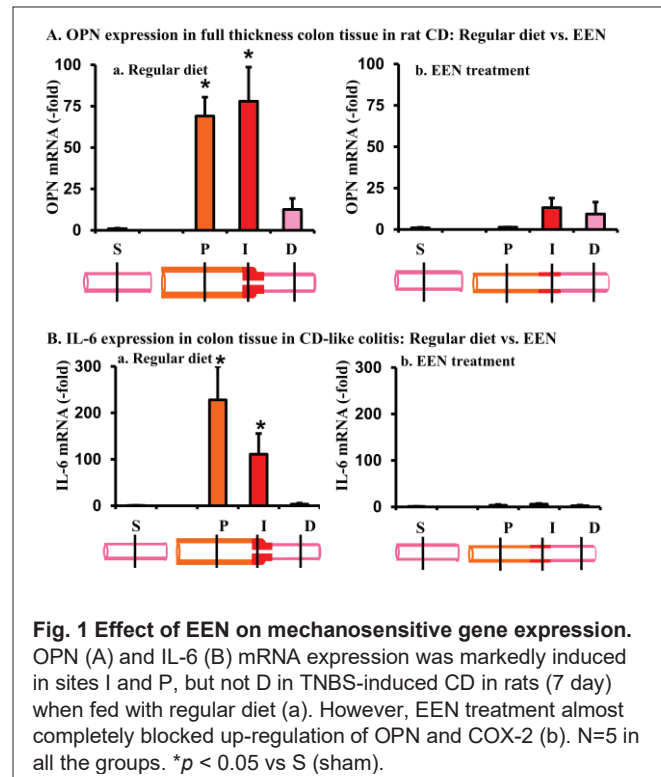
3. ACCOMPLISHMENTS:

What were the major goals of the project? The major objective of the project is to investigate the mechanisms of the benefit of exclusive enteral nutrition (EEN) for Crohn's disease (CD). Our Hypotheses are: 1) Transmural inflammation in CD causes mechanical stress (MS) in the inflammation and pre-inflammation sites, and the MS induces expression of mechanosensitive genes OPN and COX-2 to contribute to the development of inflammation and bowel dysfunctions; 2) EEN treatment is beneficial in CD by preventing MS and blocking the effects of MS-induced gene expression. Thus, the specific aims are: 1) To determine if MS plays a role in the development of CD by evaluating the effects of EEN on mechanosensitive gene expression, inflammation and bowel dysfunction; 2) To investigate the mechanisms underlying the beneficial effect of EEN on gut inflammation in CD, by focusing on the role of mechanosensitive expression of OPN in Th1/Th17 immune response; 3) To investigate the mechanisms underlying the beneficial effect of EEN on bowel dysfunctions in CD, by focusing on the role of mechanosensitive expression of COX-2 in motility dysfunction.

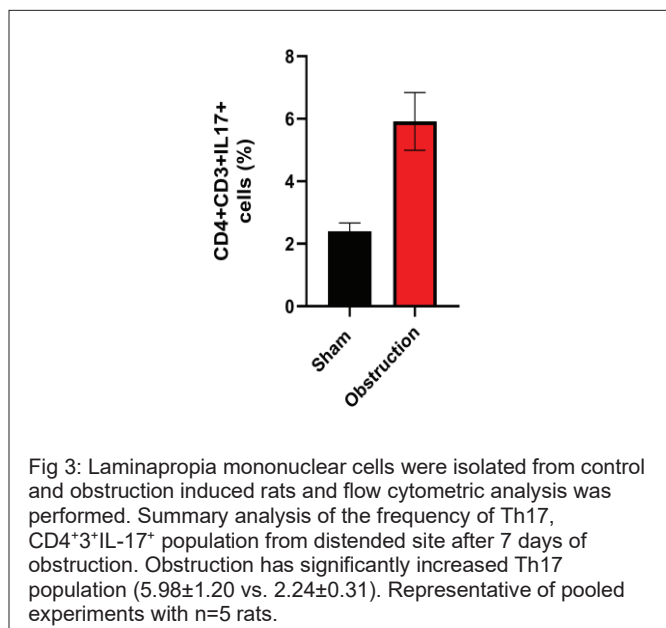
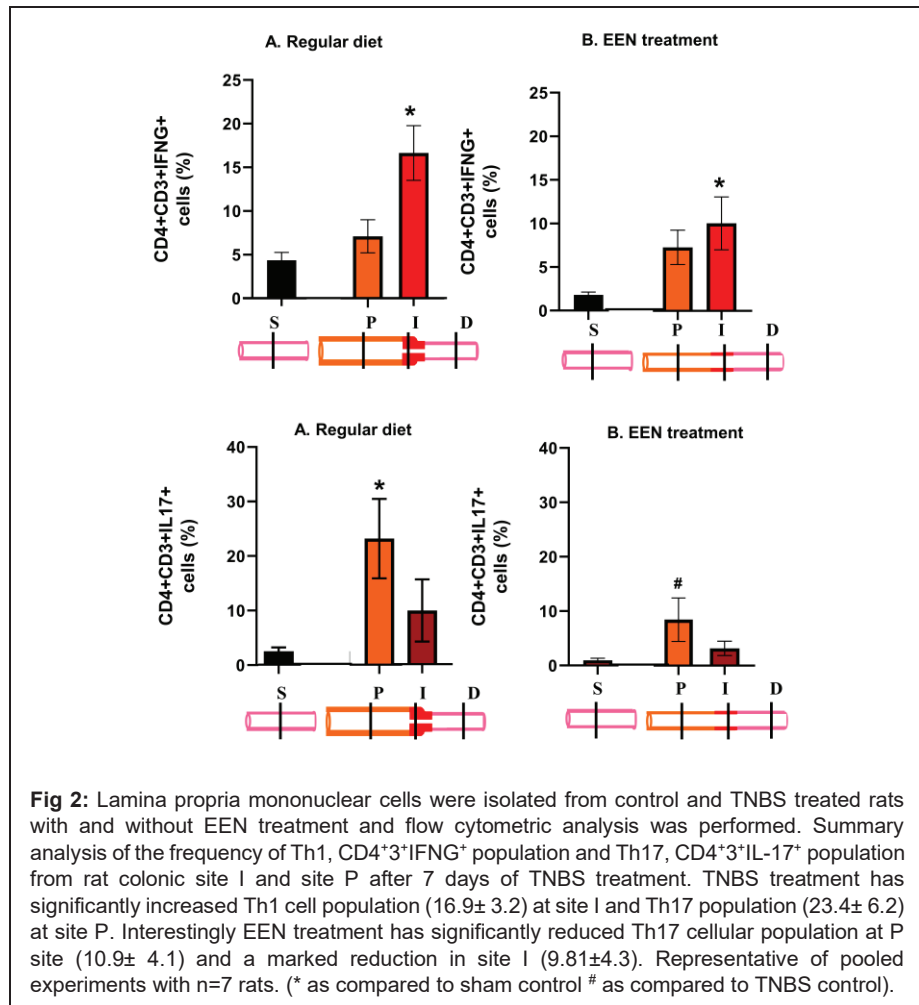
What was accomplished under these goals?

During the current reporting period (Aug. 1st, 2021 – Jul. 31st, 2022), we have worked mainly on Aim 2 and started some work of Aim 3.

1. Work on Specific Aim 2: In the established CD model in rats (TNBS induced Crohn's-like colitis), **we have determined Th1 and Th17 responses and the effect of EEN treatment on inflammatory gene expression and Th1 and Th17 responses (Major task 4 of specific aim 2 as listed in Statement of Work). We have also compared colitis-associated Th1 and Th17 response in wild-type and OPN^{-/-} mice (Major task 5 of Specific Aim 2).** Here are the main results: **1) Gene expression of osteopontin (OPN) and pro-inflammatory mediator IL-6 are significantly increased not only in the inflammation site (site I), but also the distended segment proximal to inflammation (site P) of CD rats. Interestingly, EEN treatment downregulated the expression of these genes especially at the P site (Fig 1).** Further, our in vitro mechanical stretch study of colon smooth muscle cells and in vivo mechanical obstruction model confirmed that gene expression of OPN and IL-6 are highly mechano-sensitive in the colon (last cycle and previous studies). These findings demonstrate that EEN shows its beneficial effect by eliminating MS and down-regulating MS-induced gene expression of inflammatory mediators such as IL-6 and OPN in the rodent CD model. It further suggests that MS plays a crucial role in pathogenesis of CD. **2) MS induced upregulation of pro-inflammatory mediators may be involved in maintenance of inflammation and progression of the**



disease. We investigated the effect of EEN on immune cell compartment of the rodent CD model. Immunophenotyping analysis depicted that Th1 (CD4+CD3+IFNG+ cells) immune response is prominent at I site and Th17(CD3+CD4+IL-17+ cells) immune response at P site (Fig. 2). This data revealed a close association between MS and Th17 immune response. Elimination of MS by EEN treatment significantly reduced the Th17 immune response at P site whereas there is a marked reduction in Th1 immune response at I site (Fig 2). These results were corroborated by mRNA expression levels of IL-17 and IFN γ in colon homogenates of CD model. Our findings suggest that elimination of MS by EEN administration reduced Th17 immune response. 3) We further studied this association in a gain-of-MS model (mechanical obstruction) to differentiate the effect of MS from inflammation. The results revealed a significant increase in Th17 immune response in the distended colon in the mechanical obstruction model, compared to the sham control (Fig 3). Our novel findings suggest a strong link between the mechanical stress and Th17 immunity. 4) Our on-going investigation detected increased Th1 and Th17 responses in wild-type colitis mice. We are determining if MS-induced OPN or IL-6 may be involved in the up-regulated Th1 and Th17 immune responses in the



mouse CD model. We plan to work more on this part of study in Year 3.

2. Work on Specific Aim 3: We have also started to investigate the mechanisms underlying the beneficial effect of EEN on bowel dysfunction in the CD model, especially the role of mechano-sensitive expression of COX-2 in colon motility dysfunction as proposed in Aim 3 (specifically major task 8). Here are the main findings:

1) COX-2 mRNA expression was increased 13.1($\pm$ 2.5)-fold in site I of CD rats (7 day), compared to controls (saline instillation) (Fig. 4A). Interestingly, COX-2 expression was also increased in the mechanically distended site P [16.6($\pm$ 4.3)-fold], but not in the non-distended site D. This data indicates a mechanosensitive mechanism in COX-2 expression. If mechanical distention in CD was prevented by feeding rats with EEN (clear liquid diet from *Ensure*), the increase of COX-2 mRNA expression was significantly attenuated in site I and site P (Fig. 4A). Further investigation showed that increased expression of COX-2 occurs mainly in the muscularis externae (ME) of the distended colon, particularly colonic smooth muscle cells (SMC) (Fig. 4B). 2) Inflammation led to significant suppression of colon circular muscle contractility not only in site I but also in distended site P (Fig. 5A and 5B).

However, smooth muscle contractility in site D was not significantly affected by inflammation (Fig. 5C). Moreover, clear liquid diet treatment significantly improved smooth muscle contractility in sites P and I (Fig. 5A and 5B). 4) We then determined if COX-2 is involved in the

suppression of muscle contractility in the CD model. COX-2 inhibitor NS-398 (5 mg/kg, i.p. daily)

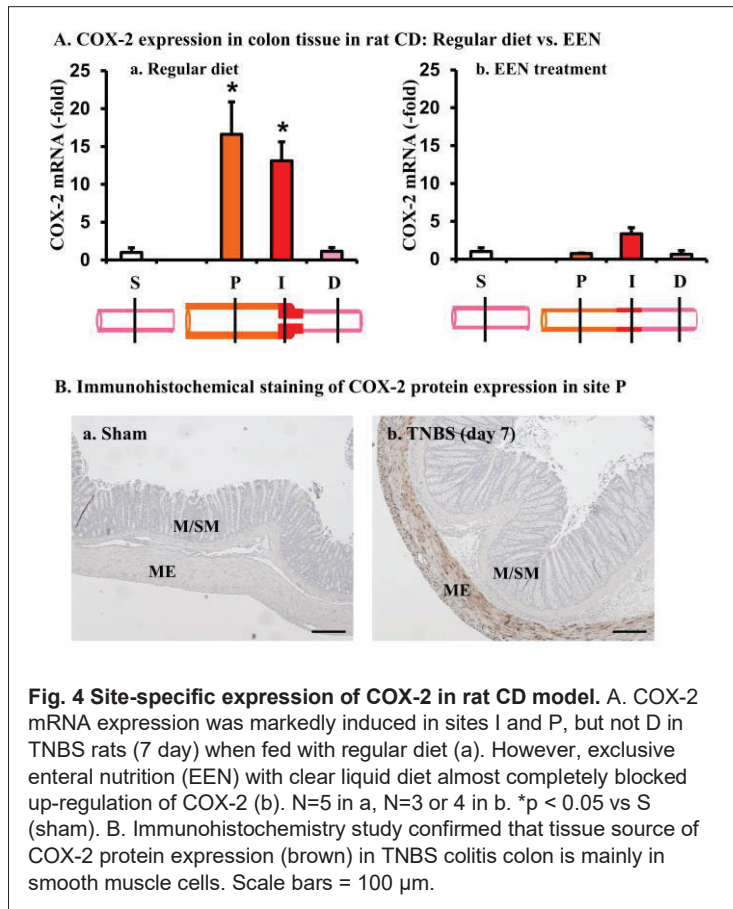


Fig. 4 Site-specific expression of COX-2 in rat CD model. A. COX-2 mRNA expression was markedly induced in sites I and P, but not D in TNBS rats (7 day) when fed with regular diet (a). However, exclusive enteral nutrition (EEN) with clear liquid diet almost completely blocked up-regulation of COX-2 (b). N=5 in a, N=3 or 4 in b. *p < 0.05 vs S (sham). B. Immunohistochemistry study confirmed that tissue source of COX-2 protein expression (brown) in TNBS colitis colon is mainly in smooth muscle cells. Scale bars = 100 μ m.

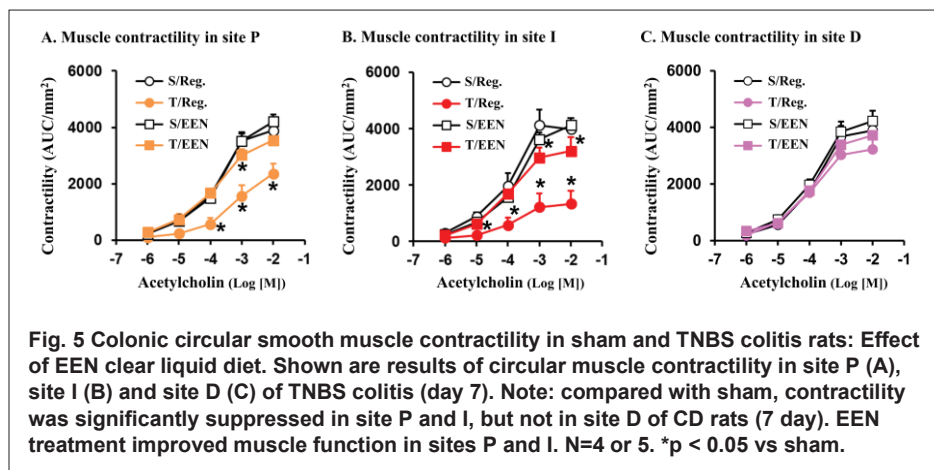


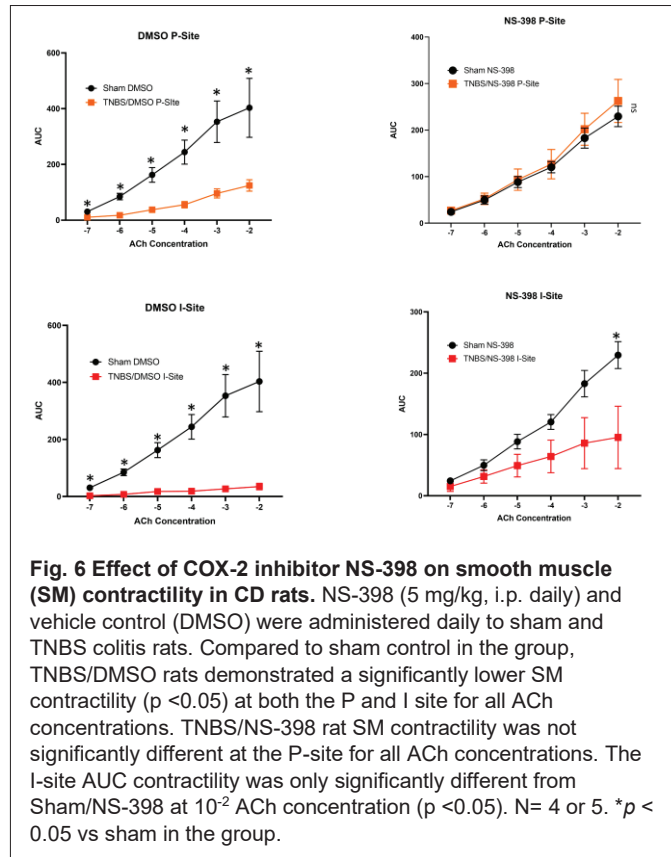
Fig. 5 Colonic circular smooth muscle contractility in sham and TNBS colitis rats: Effect of EEN clear liquid diet. Shown are results of circular muscle contractility in site P (A), site I (B) and site D (C) of TNBS colitis (day 7). Note: compared with sham, contractility was significantly suppressed in site P and I, but not in site D of CD rats (7 day). EEN treatment improved muscle function in sites P and I. N=4 or 5. *p < 0.05 vs sham.

was administered in sham and CD rats. We found that NS-398 significantly improved muscle contractility in CD rats in sites P and I (Fig. 6), suggesting that mechano-sensitive expression of COX-2 plays a crucial role in suppression of muscle contractility and motility dysfunction in the CD rats.

Some of the above results have been presented in national scientific conferences on inflammatory bowel disease (i.e. Major work 4 and 5 in the Specific Aim 2 study for Crohn's and Colitis Congress 2022) and in our publications listed in "How were the results disseminated to communities of interest" for the current funding period. The rest of the results (i.e. data in Figures 2, 3, 5 and 6) have not been published. But we plan to submit for meetings such as Crohn's and Colitis Congress 2023 and Digestive Disease Week 2023, and for publications targeting peer-reviewed scientific journals such as *Inflammatory Bowel Diseases*, *Crohn's and Colitis*, and *Cellular and Molecular Gastroenterology and Hepatology*.

What opportunities for training and professional development has the project provided?

Nothing to report.



How were the results disseminated to communities of interest?

Some of the work resulted from the Aim 1 and Aim 2 study of the project has been timely presented in (inter)national scientific meetings in two abstracts (one was presented as lecture presentation at Crohn's and Colitis 2022; and another one was recognized as abstract of distinction in the Digestive Disease Week 2022). These abstracts were published in *Inflammatory Bowel Diseases* and *Gastroenterology* [Ref #1, Ref #2].

During the current reporting period, we have published three (3) peer-reviewed articles including a full-length original research article in *Journal of Visualized Experiments*, a review article in *Frontiers in Pharmacology*, and book chapter in *Comprehensive Pharmacology*. All the three articles are very relevant to the funded DoD project and acknowledge the support of the DoD award [Ref. #3-#5]. Article #3 describes the details of the rodent Crohn's disease model and its use in studying the role of mechanical stress in Crohn's disease. Article #4 discusses the possible therapeutic targets of mechano-transcription process for gut inflammation and motility disorders in the gut. Article #5 reviews the mechanisms and pathophysiological significance of mechano-transcription especially in inflammatory and obstructive bowel disorders. The beneficial effect and possible mechanisms of action of EEN treatment have been discussed in these articles.

References (a copy of the publications are attached in Appendices):

1. Geesala R, Lin YM, Zhang K, M'Koma A, Cohn S, **Shi XZ**. NOVEL INSIGHTS INTO THE MECHANISM OF ACTION OF EXCLUSIVE ENTERAL NUTRITION IN CROHN'S DISEASE: PRE-CLINICAL STUDY IN A RODENT MODEL OF CROHN'S-LIKE COLITIS. Lecture presentation at Crohn's and Colitis Congress 2022, Las Vegas, NV. Jan. 20-22, 2022. *Inflammatory Bowel Diseases* 28 (Supplement_1), S27-S28, 2022.
2. Lin YM, Zhang K, Geesala R, Lipson K, Qiu SM, Powell DW, Cohn S, **Shi XZ**. MECHANICAL STRESS-INDUCED CONNECTIVE TISSUE GROWTH FACTOR PLAYS A CRITICAL ROLE IN INTESTINAL FIBROSIS IN A RODENT MODEL OF CROHN'S-LIKE COLITIS. Abstract of Distinction in Digestive Disease Week, May 20-24, 2022. San Diego, CA. *Gastroenterology* 162 (7), S-518, 2022.
3. Geesala R, Zhang K, Lin Y-M, Qiu SM, **Shi XZ**. A TNBS-Induced Rodent Model to Study the Pathogenic Role of Mechanical Stress in Crohn's Disease. *Journal of Visualized Experiments* 2022; doi: 10.3791/63499. Epub 2022 Mar. 1st. PMID: 35311821.
4. Geesala R, Lin Y-M, Zhang K, **Shi XZ**. Targeting Mechano-Transcription Process as Therapeutic Intervention in Gastrointestinal Disorders. *Frontiers in Pharmacology* 2021; 12: 3794 (1-25). doi:10.3389/fphar.2021.809350. PMID: 34992543. PMCID: PMC8724579.
5. **Shi XZ**. Mechano-Regulation of Gene Expression in the Gut: Implications in Pathophysiology and Therapeutic Approaches in Obstructive, Inflammatory, and Functional Bowel Disorders. In: Terry Kenakin ed. *Comprehensive Pharmacology* (1st Edition Jun. 25th, 2022). Hardcover ISBN: 9780128204726. London, UK. Elsevier, 2022.

What do you plan to do during the next reporting period to accomplish the goals?

In the next reporting period, we plan to finish the on-going work on the wild-type and OPN knockout mouse model to determine the possible role of OPN in mechanical stress-associated immune response and the effect of EEN in the mouse model, as proposed in Major Task 5 and 6 of the Statement of Work. Alternate molecules such as IL-6 will also be explored.

We also plan to work on Aim 3 study especially Major Tasks 7 and 9 to determine the association of YAP activation to mechanosensitive expression of COX-2 and the pathophysiological role of YAP and COX-2 in motility dysfunction in the CD model. The Major Task 8 is half completed in the current reporting period (role of COX-2 in motility dysfunction), and we will work on the rest of the task (involvement of YAP). But we will do our very best to complete all parts of the proposed work in the award and systemically analyze the data and disseminate the results to the communities of interest.

4. IMPACT:

What was the impact on the development of the principal discipline(s) of the project?

Our project is relevant mainly to the field of inflammatory bowel diseases especially Crohn's disease (CD). Our studies on the CD model and the effect of exclusive enteral nutrition (EEN) on expression of mechanosensitive proinflammatory genes and on inflammation, immune responses, and fibrosis in CD offer a novel explanation of the mechanism of action of EEN in Crohn's disease. Our work has been invited to present in lecture type in Crohn's and Colitis Congress and presentation of distinction

in Digestive Disease Week, the most important conferences in IBD field and digestive diseases in the world (please see references for details). We have received very positive feedback from the scientific exchanges and draw pharmaceutical companies' interests. Our publications and invited lectures on the role of mechanical stress and mechanism of action of EEN in Crohn's disease have disseminated the results to the interested scientific and pharmaceutical communities and draw public interest.

What was the impact on other disciplines?

With the development of concept of mechanical stress-induced gene expression in the gut and the novel mechanism of action of EEN to attenuate the process, we have tested the significance of EEN treatment with liquid diet in other disorders, such as obstructive bowel disorders and constipation. Our recent publications (please see references, especially ref#4 and #5) have tested the therapeutic potential of EEN in the constipation (especially opioid induced bowel dysfunction) and obstructive disorders and discussed the potential therapeutic targets derived from the mechano-transcription process for obstructive disorders and constipation in addition to IBD.

What was the impact on technology transfer?

Nothing to report.

What was the impact on society beyond science and technology?

Nothing to report.

5. CHANGES/PROBLEMS: Changes in approach and reasons; Problems or delay

As described in 3 (Accomplishments), we have some delays in completing Major Tasks 5 and 6 of the Specific Aim 2 work based on our original plan outlined in Statement of Work. This is mainly because a research scientist for the research work decided to go for a new career late in 2021. Although we acted quickly to search for a replacement, we had some difficulties finding a highly qualified researcher for the work. However, we have finally identified a candidate, and he is now waiting for his visa approval. Because of this delay, we expect that year 3 will be a busy time, and most possibly we will need a no-cost extension to fully accomplish the planned work in the award.

6. PRODUCTS:

Publications, conference papers, and presentations

Journal publications.

1. Geesala R, Zhang K, Lin Y-M, Qiu SM, **Shi XZ**. A TNBS-Induced Rodent Model to Study the Pathogenic Role of Mechanical Stress in Crohn's Disease. *Journal of Visualized Experiments* 2022; doi: 10.3791/63499. Epub 2022 Mar. 1st. PMID: 35311821.

2. Geesala R, Lin Y-M, Zhang K, **Shi XZ**. Targeting Mechano-Transcription Process as Therapeutic Intervention in Gastrointestinal Disorders. *Frontiers in Pharmacology* 2021; 12: 3794 (1-25). doi:10.3389/fphar.2021.809350. PMID: 34992543. PMCID: PMC8724579.

3. **Shi XZ**. Mechano-Regulation of Gene Expression in the Gut: Implications in Pathophysiology and Therapeutic Approaches in Obstructive, Inflammatory, and Functional Bowel Disorders. In: Terry Kenakin ed. *Comprehensive Pharmacology* (1st Edition Jun. 25th, 2022). Hardcover ISBN: 9780128204726. London, UK. Elsevier, 2022.

Other publications, conference papers, and presentations.

1. Geesala R, Lin YM, Zhang K, M'Koma A, Cohn S, **Shi XZ**. NOVEL INSIGHTS INTO THE MECHANISM OF ACTION OF EXCLUSIVE ENTERAL NUTRITION IN CROHN'S DISEASE: PRE-CLINICAL STUDY IN A RODENT MODEL OF CROHN'S-LIKE COLITIS. Lecture presentation at Crohn's and Colitis Congress 2022, Las Vegas, NV. Jan. 20-22, 2022. *Inflammatory Bowel Diseases* 28 (Supplement_1), S27-S28, 2022.

2. Lin YM, Zhang K, Geesala R, Lipson K, Qiu SM, Powell DW, Cohn S, **Shi XZ**. MECHANICAL STRESS-INDUCED CONNECTIVE TISSUE GROWTH FACTOR PLAYS A CRITICAL ROLE IN INTESTINAL FIBROSIS IN A RODENT MODEL OF CROHN'S-LIKE COLITIS. Abstract of Distinction in Digestive Disease Week, May 20-24, 2022. San Diego, CA. *Gastroenterology* 162 (7), S-518, 2022.

Other Products. Nothing to report.

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

Name:	<i>Xuan-Zheng Shi, MD, MS</i>
Project Role:	<i>PI</i>
Researcher Identifier (e.g. ORCID ID):	<i>ORCID 0000-0002-9014-853X</i>
Nearest person month worked:	<i>4.2</i>
Contribution to Project:	<i>As PI, Dr. Shi oversees the project, establishes animal models, helps on bench work, data analysis, and be responsible for paper writing and reports.</i>
Funding Support:	<i>NIH in addition to DoD.</i>

Name:	<i>You-Min Lin, MD, Ph.D</i>
Project Role:	<i>Research Scientist</i>

Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	3
Contribution to Project:	<i>Dr. Lin performed animal work, tissue processing, molecular and functional studies, statistics and data analysis.</i>
Funding Support:	<i>NIH in addition to DoD.</i>

Name:	<i>Yingzi Cong, Ph.D.</i>
Project Role:	<i>Co-Investigator</i>
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	0.24
Contribution to Project:	<i>Dr. Cong contributed to the study and analysis of Th1 and Th17 immune response.</i>
Funding Support:	NIH in addition to DoD.

Name:	<i>John Winston, Ph.D.</i>
Project Role:	<i>Co-Investigator</i>
Researcher Identifier (e.g. ORCID ID):	ORCID 0000-0003-4220-2236
Nearest person month worked:	0.6
Contribution to Project:	<i>Dr. Winston contributed to the animal model and animal protocol establishment, and performed some histology work.</i>
Funding Support:	

Name:	<i>Ramasatyaveni Geesala, Ph.D.</i>
Project Role:	<i>Post-doctoral Fellow</i>
Researcher Identifier (e.g. ORCID ID):	ORCID 0000-0002-5520-2457
Nearest person month worked:	6

Contribution to Project:	<i>Dr. Geesala has been working on the molecular work, animal work, in vitro studies, and on Th1/Th17 immune response.</i>
Funding Support:	NIH in addition to DoD.

Name:	<i>Ke Zhang, MS</i>
Project Role:	<i>Research Associate</i>
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	12
Contribution to Project:	<i>Mr. Zhang joined the lab in Jan. 2021 to help mainly on the DoD project, especially animal work, molecular, motility, and histological work.</i>
Funding Support:	

Changes in active other support

Nothing to report.

Other organization involvement

Nothing to report.

8. SPECIAL REPORTING REQUIREMENTS

Nothing to report.

9. APPENDICES:

Please see the following attachments, which acknowledged DoD award support.

- Copy of the peer-reviewed publication in *Journal of Visualized Experiments*, *Frontiers in Pharmacology*, and *Comprehensive Pharmacology*.
- Copy of two conference abstracts presented at Digestive Disease week 2021 and published in *Inflammatory Bowel Diseases* and *Gastroenterology*.

A TNBS-Induced Rodent Model to Study the Pathogenic Role of Mechanical Stress in Crohn's Disease

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Abstract

Inflammatory bowel diseases (IBD) such as Crohn's disease (CD) are chronic inflammatory disorders of the gastrointestinal tract affecting approximately 20 per 1,000,000 in Europe and USA. CD is characterized by transmural inflammation, intestinal fibrosis, and luminal stenosis. Although anti-inflammatory therapies may help control inflammation, they have no efficacy on fibrosis and stenosis in CD. The pathogenesis of CD is not well understood. Current studies focus mainly on delineating dysregulated gut immune response mechanisms. While CD-associated transmural inflammation, intestinal fibrosis, and luminal stenosis all represent mechanical stress to the gut wall, the role of mechanical stress in CD is not well defined. To determine if mechanical stress plays an independent pathogenic role in CD, a protocol of TNBS-induced CD-like colitis model in rodents has been developed. This TNBS-induced transmural inflammation and fibrosis model resembles pathological hallmarks of CD in the colon. It is induced by intracolonic instillation of TNBS into the distal colon of adult Sprague-Dawley rats. In this model, transmural inflammation leads to stenosis at the TNBS instillation site (Site I). Mechanical distention is observed in the portion proximal to the instillation site (Site P), representing mechanical stress but not visible inflammation. Colonic portion distal to inflammation (Site D) presents neither inflammation nor mechanical stress. Distinctive changes of gene expression, immune response, fibrosis, and smooth muscle growth at different sites (P, I, and D) were observed, highlighting a profound impact of mechanical stress. Therefore, this model of CD-like colitis will help us better understand CD's pathogenic mechanisms, particularly the role of mechanical stress and mechanical stress-induced gene expression in immune dysregulation, intestinal fibrosis, and tissue remodeling in CD.

Introduction

Inflammatory bowel disease (IBD), including ulcerative colitis (UC) and Crohn's disease (CD), is characterized by chronic inflammation in the gastrointestinal (GI) tract. It affects ~1-2 million Americans¹. The estimated annual costs for IBD care in the US are \$11.8 billion. Unlike UC, the CD is characterized by transmural inflammation and stricture formation^{2,3}. Stricture formation (stenosis) occurs in up to 70% of CD patients³ and may be caused by transmural inflammation (inflammatory stenosis) or intestinal fibrosis (fibrotic stenosis)^{4,5}. Intestinal fibrosis is characterized by excessive collagen deposition and other extracellular matrices (ECM) with smooth muscle cells (SMC) as one of the main mesenchymal cell types involved in the process^{3,4}. Smooth muscle hyperplasia associated with hypertrophy is another significant histological change in fibrotic stenosis in CD⁶. Although stricture formation in CD is associated with chronic inflammation, no anti-inflammatory treatment is effective, except surgical treatment^{2,6}. However, post-surgery recurrences are almost 100%, given sufficient time^{2,7}. As an inflammatory response, fibrosis and SMC hyperplasia may also develop in non-inflammatory conditions (i.e., bowel obstruction) in the gut^{8,9}; it is believed that both inflammation-dependent and independent mechanisms are involved in stricture formation^{3,4}. Given that extensive research into the inflammation-dependent mechanisms has not translated into any effective therapy for stricture formation, studies into the possible role of inflammation-independent mechanisms in intestinal fibrosis are needed.

As a non-inflammatory factor, mechanical stress (MS) associated with edema, inflammatory cell infiltration, tissue deformation, fibrosis, and stenosis^{10,11,12,13} is commonly encountered in IBD, especially CD, which is characterized

by transmural inflammation. Mechanical stress is most remarkable in stenotic CD, where stenosis (inflammatory or fibrotic) in the inflammation site presents mechanical stress in the local tissue and leads to lumen distention in the segment proximal to the obstruction site^{10,14}. Previous *in vitro* studies have demonstrated that mechanical stress alters gene expression of specific inflammatory mediators (i.e., COX-2, IL-6)^{8,14,15} and growth factors (i.e., TGF- β) in the gastrointestinal tissues, especially gut smooth muscle cells (SMC)¹⁶. Recent studies also found that the expression of specific pro-fibrotic mediators such as connective tissue growth factor (CTGF) is highly sensitive to mechanical stress^{17,18}. It was hypothesized that mechanical stress might play an independent pathogenic role in CD-associated inflammation, fibrosis, and tissue remodeling. However, the pathogenic significance of mechanical stress in gut inflammation, fibrosis, and smooth muscle hyperplasia in CD remains largely unexplored. This may be partly because inflammation is a more visible and better-studied process than mechanical stress. More importantly, there has been no well-defined animal model of IBD to distinguish the effect of mechanical stress from that of inflammation.

The current work describes a rodent model of Crohn's-like colitis induced by intracolonic injection of hapten reagent 2,4,6-trinitrobenzene sulfonic acid (TNBS)^{19,20}, which may serve the purpose to study the role of mechanical stress in CD. It was found that TNBS instillation induced a localized (~2 cm in length) transmural inflammation with lumen narrowing (stenosis) in the distal colon. The stenosis leads to marked bowel distention (mechanical stress)^{14,15} but not visible inflammation in the colonic segment proximal to the instillation site. On the contrary, the colon segment

distal to the stenosis site presents neither inflammation nor mechanical stress. Significant site-specific changes in gene expression, inflammation, fibrosis, and SMC hyperplasia were observed in the three different sites. The results suggest that mechanical stress, particularly mechanical stress-induced gene expression, may play a critical role in developing fibrosis and hyperplasia in Crohn's colitis.

Protocol

All animal experiments were conducted according to the institutional animal care and use committee of the University of Texas Medical Branch (#0907051C). Male or female Sprague-Dawley rats, ~8-9 weeks old, were used for the study.

1. Animal preparation

1. Fast rats for 24 h and treat them with laxative (bowel cleanser, see **Table of Materials**) overnight.
2. The next day, anesthetize rats using an anesthesia system (see **Table of Materials**) by exposing them to 2% isoflurane along with 1 L/min of oxygen during TNBS administration. Check for reflexes or pinch toes to confirm anesthetization.
3. Prepare fresh TNBS solution according to body weights.
NOTE: TNBS - 65 mg/kg of body weight in 250 μ L of 40% ethanol/saline was used.
4. Put rats in a supine position on the anesthesia table. To induce colitis, insert through the anus a medical-grade open-end polyurethane catheter for ~7-8 cm from the anal verge and gently instill TNBS (prepared in step 1.3) into the colon¹⁹. Administer the sham control rats with 250 μ L of saline only.

5. After instilling TNBS or saline, keep rats in supine and slightly head-down position (~30°), with the anus closed for 2 min to help TNBS distribution and avoid spills.
6. Provide rats with food and water ad libitum for 7 days and observe the rats daily for body weight, food uptakes, feces, and general health condition.

2. Tissue preparations

1. On the day of euthanasia, euthanize the rats using CO₂ inhalation and confirm euthanasia with cervical dislocation.
2. Open the rat abdomen using surgical-grade scissors and forceps.
3. Carefully remove the entire colon (above the anal canal) and transfer the colon immediately to ice-cold 1x HBSS buffer.
4. Straighten the colon in the buffer and measure the colon length using a ruler. Take nylon thread and circle around the colon to measure the external circumference of the colon segments in control and TNBS-treated rats. Take full-thickness tissues for histology.
5. Cut open the colon along the mesenteric board and clean the colon well with HBSS buffer. Assess the colon for macroscopic inflammation score based on the criteria as previously described¹⁹ with minimal modifications.
NOTE: 0 = normal mucosa; 1 = localized hyperemia but no erosions or ulcers; 2 = ulcer and stenosis (affected area < 5 mm); 3 = severe ulcer, scar, and stenosis (affected area > 5 mm).
6. Collect colonic tissue samples from site P (portion 2-3 cm before the oral margin of inflammation site), site I (inflammation site, typically 4-6 cm from the end of the

colon, where TNBS is instilled to), and site D (portion 1-2 cm distal to the aboral margin of inflammation site), respectively from TNBS-treated rats.

NOTE: Colon tissue of ~1-2 cm-long was taken from each segment. In addition, the colon tissues of 2 cm long (~4-6 cm from the end of the colon) of the saline-treated rats were taken as sham control (S) (**Figure 1**).

7. Take tissue samples from each site for full-thickness preparation, and if desired, mucosa/submucosa and muscularis externa layers, respectively, as well^{21,22}.
8. Freeze tissue samples in liquid nitrogen first before storing them at -80 °C for storage up to one year and for future purposes (i.e., RNA preparations).

3. Histopathologic assessment of gut inflammation and fibrosis

1. Fix the full-thickness colon tissues in 10% formalin for 48 h, then transfer to 70% ethanol for 24-48 h.
2. Use a microtome to cut paraffin sections of 5 µm thickness for Hematoxylin and eosin (H&E) and Masson's Trichrome stains^{6,19,23} (see **Table of Materials**), respectively.
3. Acquire and view images with an upright microscope equipped with a high-resolution camera with compatible software (see **Table of Materials**).
4. Grade inflammation and fibrosis indexes by two independent investigators, including a gastrointestinal surgical pathologist according to criteria described previously^{6,23} with modifications. See **Supplementary File 1** for the scores.
5. Measure the thickness and cell numbers of the circular and longitudinal muscle layers per cross-section in four

views of each H&E stained specimen and take the mean of the four measurements for each specimen.

4. RNA extraction and quantitative RT-PCR

1. Homogenize excised colon tissues obtained from the sham control and three sites (P, I, D) of TNBS colitis rats in the extraction reagent of an RNA extraction kit (see **Table of Materials**).
2. Isolate RNA from each sample utilizing the kit. Elute the RNA pellet in 30 µL of RNase-free water.
3. Quantify RNA concentration and check for purity using a microvolume UV-Vis spectrophotometer (see **Table of Materials**).
4. Use 1 µg of total RNA to synthesize cDNA^{21,22} using the RNA synthesis kit (see **Table of Materials**).
5. Analyze and quantify gene expression levels by performing real-time PCR with 50 ng of cDNA as a template, probes of IL-6, and CTGF using a commercial PCR kit for real-time PCR system (see **Table of Materials**).
6. Use control gene 18S rRNA to normalize the samples and quantify relative gene expression utilizing the Cq values obtained.

5. Statistical analysis

1. Utilize statistical analysis software (see **Table of Materials**) to compare sham control and TNBS colitis rats.
2. Consider p value < 0.05 to be statistically significant^{15,19}.

- To test the differences between two groups, use Student's t-test analysis and perform an ANOVA test if comparisons are more than two groups^{15,19}.

Representative Results

Macroscopic view of Crohn's-like colitis induced by intracolonic instillation of TNBS

As shown in **Figure 1**, intracolonic instillation of TNBS in rats induced a localized transmural inflammation (~2 cm in length) with thickened bowel wall and narrowed lumen (stenosis) in the site of instillation in the distal colon (**Figure 1A**). The site of TNBS instillation is referred to as site I. As a result of transmural inflammation and stenosis, both inflammation and mechanical stress are present in site I. The stenosis in site I led to marked lumen distention in the segment proximal to the site of TNBS instillation (site P) (**Figure 1**). The colon circumference was significantly increased in sites P and I, compared to sham control colon ($p < 0.05$ vs. control) (**Figure 1B**). While mechanically distended, site P did not show visible inflammation. On the contrary, the colonic segment distal to the TNBS instillation site is site D and presents neither inflammation nor mechanical distention (**Figure 1A,B**).

To help distinguish the effect of mechanical stress from inflammation, we followed a unique design in collecting tissue samples from the colon in the model described in step 2.6. Site P is the primary focus of the study, as this part is mechanically distended in the CD model. Site D is self-control, as it does not present mechanical stress. Sites P and D do not demonstrate any visible inflammation (**Figure 1**). However, the site I experience inflammation and mechanical stress (**Figure 1**).

Site-specific changes of inflammation score in sites P, I, and D in colitis rats

Criteria were developed in grading inflammation based on the macroscopic score of live tissue (0-3)¹⁹ and the microscopic score of H&E stained specimens (0-3)²³ as described with modifications. The results showed that the macroscopic score of inflammation in site I was 2.70 ± 0.20 in the TNBS treated rats (7 days after induction of inflammation), dramatically increased compared to that of sham controls (0.30 ± 0.22 , $p < 0.05$) and that of sites P (0.80 ± 0.26) and D (0.50 ± 0.22) of colitis rats (**Figure 1C**). The inflammation scores in sites P and D are not significantly increased compared to sham (**Figure 1C**). Microscopic imaging showed TNBS treatment-induced transmural inflammation in rats (**Figure 2A**). The microscopic score of inflammation in site I was 2.80 ± 0.27 in the TNBS treated rats, again significantly ($p < 0.05$, $n = 5$ each group) increased compared to that in sham controls (0.3 ± 0.2) and in sites P (1.0 ± 0.31) and D (0.80 ± 0.38) of colitis rats. The inflammation scores in sites P and D were not significantly increased compared to sham (**Figure 2C**).

Site-specific changes of fibrosis and smooth muscle hyperplasia and hypertrophy in sites P, I, and D in colitis rats

The fibrosis score was determined based on Mason's trichrome stain (**Figure 2B**) in different sites (P, I, D) (**Figure 2A**). The grading system for fibrosis is described in **Supplementary File 1**. It was found that fibrosis score is significantly increased not only in site I (2.60 ± 0.25) but also in site P (1.60 ± 0.24) of the colitis rats, compared to sham control (0.40 ± 0.25 , $p < 0.05$) (**Figure 2D**). The thickness and cell numbers of circular and longitudinal smooth muscle layers were measured in different sites in H&E stained specimens (4 views per specimen). The thickness and cell numbers of both circular and longitudinal smooth muscle layers were significantly increased in sites I and P (**Figure 2E,F**). The site D in the colitis rats does not show any

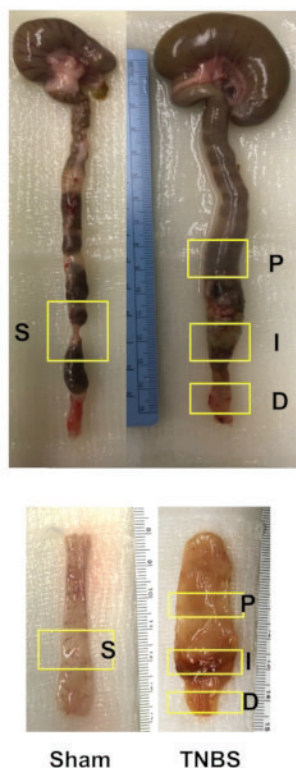
significant increase in fibrosis score, smooth muscle cell numbers, or muscle thickness (**Figure 2**).

Site-specific expression of mechano-sensitive genes in sites P, I, and D in colitis rats

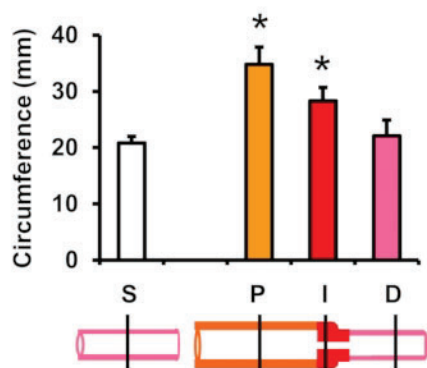
IL-6 plays a critical role in gut inflammation, as it promotes T cell differentiation, damages barrier function, and affects neuromuscular function^{8,24}. CTGF is a well-recognized pro-fibrotic mediator, as its inhibition can reverse the process of fibrosis¹⁷. More importantly, recent studies found that gene expression of IL-6 and CTGF is highly responsive to mechanical stress^{8,14,18}. The site-specific expression

of IL-6 and CTGF mRNAs were determined in the full thickness tissue of sham control and TNBS colitis rats. The mRNA expression levels of IL-6 and CTGF were significantly increased in the inflammation site (site I) compared to sham controls. In site P, where there is mechanical distention but no visible inflammation, the mRNA expression of IL-6 and CTGF was also dramatically increased compared to control rats (**Figure 3A,B**). However, IL-6 and CTGF mRNA levels in site D of colitis rats were not significantly different from that in sham controls (**Figure 3**).

A. Outlook and macroscopic view of rat colons in TNBS colitis



B. Colon circumference



C. Macroscopic inflammation score

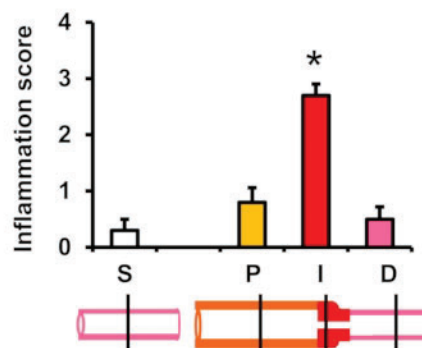


Figure 1: Rodent model of TNBS-induced CD-like colitis (7 day). (A) Outlook view of sham control and TNBS-treated colon (top) and macroscopic view of the mucosal surface of the distal colon (bottom). The yellow boxes indicate different sites of colon tissue. S, sham control; I, inflammation site; P, distended colon site proximal to inflammation site; D, non-distended site distal to inflammation. (B) Colon circumference in sham control and different sites (P, I, D) of TNBS-treated colon. (C) Macroscopic inflammation score of sham control colon and different sites of TNBS-treated colon. $n = 5$, $*p < 0.05$ vs. sham rats of the group. Bars represent SEM. [Please click here to view a larger version of this figure.](#)

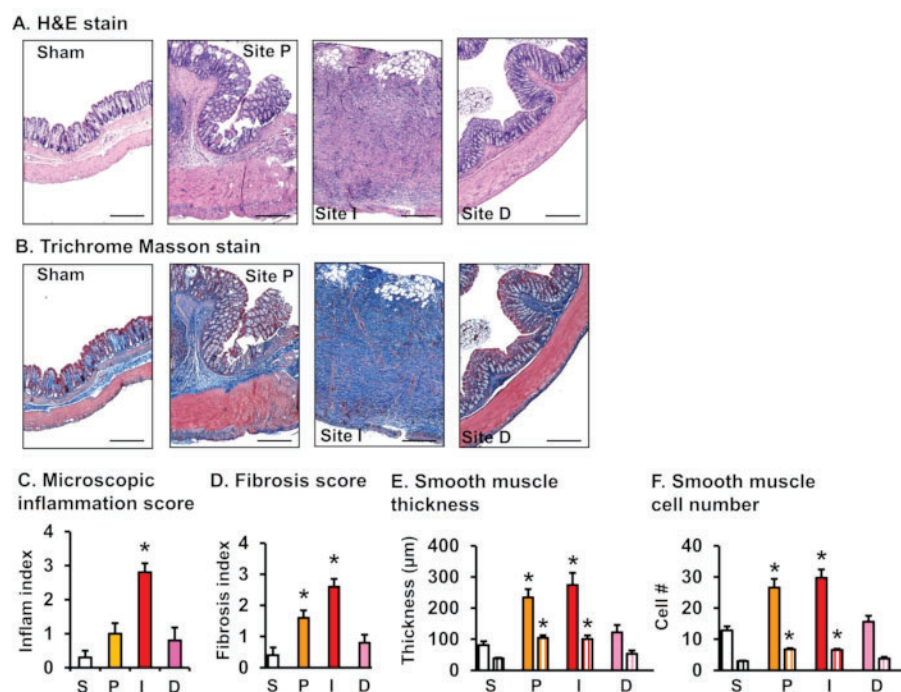


Figure 2: Histopathologic assessment. Microscopic views in H&E (A) and Masson's trichrome stains (B) showing collagen distribution in sham and different sites of colitis rats. Quantitative analysis shows increased microscopic inflammation index (C), fibrosis (D) and muscular thickness (hypertrophy) (E), and smooth muscle cell number (hyperplasia) (F) in sites I and P, but not D, in TNBS treated rats. In (E) and (F), the open bars are for the circular smooth muscle layer, and streaked bars are for the longitudinal smooth muscle layer. $n = 5$ in each group, $*p < 0.05$ vs. sham rats of the group. Bars in graphs represent SEM. Bars in (A) and (B) = 100 μ m. [Please click here to view a larger version of this figure.](#)

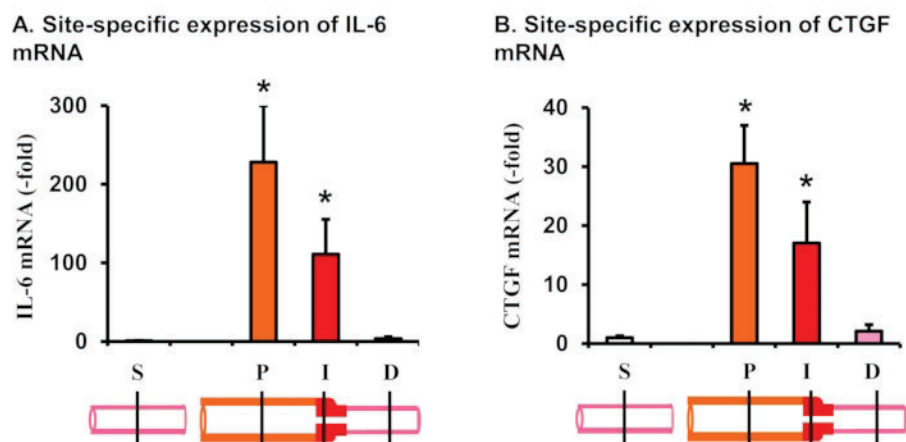


Figure 3: Site-specific expression of mechanosensitive genes (IL-6 and CTGF) in CD-like colitis. (A) Expression of IL-6 mRNA in sham control colon and different sites (P, I, and D) of TNBS-treated colitis colon. (B) Expression of CTGF mRNA in sham control colon and different sites (P, I, and D) of TNBS-treated colitis colon. $n = 4$ or 5 , $*p < 0.05$ vs. S (sham control). Bars represent SEM. [Please click here to view a larger version of this figure.](#)

Supplementary File 1: Score of inflammation and fibrosis.

[Please click here to download this File.](#)

Discussion

TNBS-induced colitis was introduced in 1989 and has been used as an experimental model of Crohn's disease since then^{19,20,23}. Significant features of this model in rodents include the development of a transmural inflammation that closely resembles the histopathological lesions developed in human Crohn's disease^{19,20}. Previous studies on the model have focused mainly on aberrant immune response in the mucosa layer in the site of visible inflammation (the site I)^{19,20,23}. Little attention has been paid to the bowel portions proximal and distal to inflammation. The present study on the inflammation site, as well as the distended proximal segment and the non-distended distal segment, reveals apparent site-specific changes in gene expression, inflammatory response, and histopathological features. The model has been revisited

to address the potential pathogenic role of mechanical stress in fibrosis and tissue remodeling in Crohn's disease.

It was found that acute inflammation is immediately developed after mucosal exposure of TNBS in ethanol and is peaked at day 3¹⁹. Transmural inflammation and inflammatory stenosis are present, which is associated with lumen distention in site P in every rat treated with TNBS. By day 7, chronic transmural inflammation is well developed in site I, as found in the current study and reported elsewhere²⁰. Meanwhile, fibrotic changes are apparent in the site, characterized by excessive collagen deposition, as seen in the present study and elsewhere²⁰. TNBS instillation in ethanol in the distal colon injures the local mucosa tissue²³ and leads to transmural inflammation in a localized area in the colon. The transmural inflammation with inflammatory infiltration, edema, and tissue deformation^{10,12,13} in the instillation site present inflammation and mechanical stress¹⁴ to the

localized area (Site I). Moreover, transmural inflammation also causes luminal stenosis in site I¹⁰. It was found that stenosis, a partial bowel obstruction, causes mechanical distention in the proximal segment (site P). However, the part distal to the instillation site is not distended (site D). As found in the macroscopic and histological scoring systems, while site I presents both inflammation and mechanical stress, sites P and D do not present inflammation. Furthermore, site P shows significantly increased circumference (and thus mechanical stress, according to the law of Laplace¹⁴), but site D does not. Therefore, a study on the site-specific changes, especially the site P, will explore the pathogenic importance of mechanical stress in the inflammation model in the gut.

It was observed that the expression of mechano-sensitive genes IL-6 and CTGF was increased in site I and in mechanically stretched site P, but not in neighboring site D, where there is no mechanical distention. To examine the hypothesis that mechanical stress may contribute to the development of fibrosis and smooth muscle hyperplasia, fibrosis scores and measured smooth muscle cell numbers and muscle thickness in sites P, I, and D were determined separately. It was found that fibrosis and SMC hyperplasia are present in sites I and P. However, fibrosis and smooth muscle hyperplasia are not detected in site D. These findings indicate that mechanical stress may play an independent pathogenic role in collagen synthesis and cell proliferation in gut inflammation. Further studies are warranted to determine if this effect may be mediated by mechanical stress-induced expression of pro-fibrotic and growth factors such as CTGF in the sites P and I.

Although the described animal model itself can be used to address mechanical stress in stenotic CD-like colitis, it has limitations in the long-term goal to fully define the

pathogenic role of mechanical stress in inflammation. For example, the effects of mechanical stress and inflammation in site I of the described model cannot be differentiated. Although it is assumed that mechanical stress is present in site I because of inflammatory infiltration, tissue deformation, stenosis, and distention, how much mechanical stress contributes to the pathological changes there cannot be determined. Further comprehensive studies using *in vitro*, *in vivo*, and *ex vivo* approaches may be needed. For example, preventing mechanical distention by feeding the colitis animals exclusively with a clear liquid diet²⁵ may help create a loss-of-mechanical distention status in the colitis model. On the other hand, induction of pure mechanical distention by obstruction band¹⁵ may help create a gain-of-mechanical stress model. Moreover, the *in vitro* mechanical stretch model in cultured cells^{14, 15} assists in the quantitative determination of the effects of mechanical stress on gene expression and function, as the mode and extent of mechanical stress can be finely controlled in the *in vitro* setting.

To prepare a reproducible model of transmural and stenotic inflammation in CD-like colitis as described in the study, TNBS at 65 mg/kg in 250 μ L of 40% ethanol must be used. The model was tested mainly in rats, male or female, 8-9 weeks old. After instillation of TNBS at the dose, transmural inflammation is consistently developed at the local instillation site. The inflammation is associated with inflammatory cells infiltration, edema, and bowel wall thickening, leading to lumen narrowing at the instillation site, resembling pathological characteristics of Crohn's disease²⁰. Pilot studies in the lab showed that TNBS at doses lower than 50 mg/kg in the same volume of 40% ethanol might cause gut inflammation but not reliable stenosis in site I. Thus, there would be no apparent mechanical distention in site P. On the other hand, TNBS at doses greater than 80 mg/kg may cause

severe inflammation and fatalities. The current protocol using TNBS at 65 mg/kg in 250 μ L of 40% ethanol hardly causes fatalities (1 in 16 rats of TNBS colitis).

It was found that bowel cleansing the day before instillation of TNBS is an important step to ensure a relatively clean colon for a reliable model of stenotic colitis. For that, rats need to fast for 24 h and give bowel cleanser overnight before TNBS treatment. It is also important to keep rats in a supine and slightly head-down position with the anus closed for 2 min after instillation of TNBS. This helps to ensure a good distribution of TNBS inside the distal colon.

In summary, it was found that intracolonic instillation of TNBS at 65 mg/kg in 250 μ L of 40% ethanol consistently induced CD-like colitis in rats. Transmural inflammation in the model is associated with stenosis at the TNBS instillation site. Mechanical distention, but not inflammation, is observed in the segment proximal to the stenosis. Neither inflammation nor mechanical stress is present in the segment distal to the stenosis. With these changes in different colon sites in a colitis rat, mechanical stress from inflammation in CD-like colitis can be distinguished.

Disclosures

The authors report no conflict of interest and have nothing to disclose.

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Targeting Mechano-Transcription Process as Therapeutic Intervention in Gastrointestinal Disorders

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Mechano-transcription is a process whereby mechanical stress alters gene expression. The gastrointestinal (GI) tract is composed of a series of hollow organs, often encountered by transient or persistent mechanical stress. Recent studies have revealed that persistent mechanical stress is present in obstructive, functional, and inflammatory disorders and alters gene transcription in these conditions. Mechano-transcription of inflammatory molecules, pain mediators, pro-fibrotic and growth factors has been shown to play a key role in the development of motility dysfunction, visceral hypersensitivity, inflammation, and fibrosis in the gut. In particular, mechanical stress-induced cyclooxygenase-2 (COX-2) and certain pro-inflammatory mediators in gut smooth muscle cells are responsible for motility dysfunction and inflammatory process. Mechano-transcription of pain mediators such as nerve growth factor (NGF) and brain-derived neurotrophic factor (BDNF) may lead to visceral hypersensitivity. Emerging evidence suggests that mechanical stress in the gut also leads to up-regulation of certain proliferative and pro-fibrotic mediators such as connective tissue growth factor (CTGF) and osteopontin (OPN), which may contribute to fibrostenotic Crohn's disease. In this review, we will discuss the pathophysiological significance of mechanical stress-induced expression of pro-inflammatory molecules, pain mediators, pro-fibrotic and growth factors in obstructive, inflammatory, and functional bowel disorders. We will also evaluate potential therapeutic targets of mechano-transcription process for the management of these disorders.

Keywords: bowel obstruction, Crohn's disease, functional bowel disorders, mechanical stress, gut motility, intestinal fibrosis, visceral pain, gene expression

INTRODUCTION

The gastrointestinal (GI) tract is subject to mechanical stimuli constantly (Shi, 2017; Sarna and Shi, 2006). Contracting forces generated by gut smooth muscle mechanically digest foods in the lumen and propel the intraluminal contents down through the GI tract (Shi and Sarna, 2005; Murthy, 2006; Karichely and Farrugia, 2007). The intraluminal contents (foods, gas, and fluids) and the movement of the contents present mechanical stress on the gut wall: i.e., pressure and shear stress (Shi, 2017). Shear stress is transient and generated at the mucosa surface tangential to the GI tract. Along with that, intraluminal pressure generates a circumferential mechanical stretch which is perpendicular to the gut wall (Figure 1A). Shear stress primarily affects mucosa and submucosa layers, and its impact on epithelial and enterochromaffin cells has been previously studied *in vitro* (Gayer and Basson, 2009; Linan-Rico et al., 2016; Beyder, 2019). Circumferential stretch, however, affects the whole gut wall including the muscularis externa, and is most remarkable in pathological conditions such as

A Mechanical stress induced by intraluminal contents



B. Mechanical stress induced by gut tissues

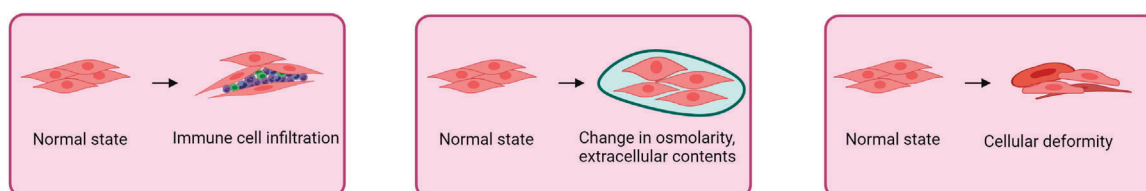


FIGURE 1 | Mechanical stress in the gut may originate from the intraluminal contents (A) or from inside the tissues (B). (A) The presence and movement of intraluminal contents create shear stress and intraluminal pressure. While shear stress is a transient force generated at the mucosa surface tangential to the GI tract, intraluminal pressure creates a circumferential mechanical stretch perpendicular to the gut wall. (B) Physical stretch and compression also exist inside the gastrointestinal tissues in pathological conditions such as in inflammation with inflammatory cell infiltrations, edema, extracellular matrix deposition, and deformation.

obstructive, inflammatory, and functional bowel disorders. Pathophysiological significance of mechanical stretch in these gastrointestinal disorders have not been reviewed comprehensively.

Under homeostasis, the intraluminal pressure in the gut is about 0 cm H₂O (Silen, 2005). Up on blockade of intraluminal contents, as in bowel obstruction, it increases up to 8–10 cm H₂O in the proximal segment of obstruction (Shikata et al., 1983; Silen, 2005). The intraluminal pressure may reach up to 30 to 60 cm H₂O during peristalsis in the obstructed segment (Shikata et al., 1983; Silen, 2005). In obstruction, overload of luminal contents (food contents, fluids and gas) at proximal segment leads to lumen distention. According to Laplace's law, the circumferential mechanical stress on the bowel wall, i.e., product of pressure and radius, will be significantly enhanced during obstruction (Russell and Welch, 1990; Shi, 2017). Many GI conditions, i.e., obstructive bowel disorders (OBD), are characterized with lumen distention, and hence mechanical stress (Shi et al., 2011; Lin et al., 2012a). OBDs are a major health concern in both adults and children (Russell and Welch, 1990; Summers, 1999; Silen, 2005). Distension of gut lumen in OBD might be due to either mechanical or functional obstruction (Russell and Welch, 1990; Summers, 1999; Silen, 2005). Functional obstruction is due to neuromuscular dysfunction, such as in achalasia, pyloric stenosis, ileus, Hirschsprung's disease, and intestinal pseudo-obstruction (Russell and Welch, 1990; Summers, 1999; Nunez et al., 2009; De Giorgio et al., 2011), whereas mechanical obstruction results from extrinsic factors such as adhesions and hernias, or intrinsic factors such as carcinoma and diverticulitis (Shi, 2017). Although circumferential mechanical stretch is most

prominent in obstructive bowel disorders, it is also well noticed in functional bowel disorders and inflammatory conditions, i.e., fecal retention, constipation, and stenotic Crohn's disease (Heredia et al., 2012; Raahave, 2015; Lin et al., 2021b).

Mechanical stress on gut tissues may not only be generated from intraluminal contents as described above, but also from gut tissues themselves (Figure 1B). In gut inflammation, for example, physical stretch and compression exist in the GI tissues, where inflammatory cell infiltrations, tissue deformation, edema, and extracellular matrix deposition (fibrosis) are all considered as mechanical stress (Cox et al., 2008; Gayer and Basson, 2009; Johnson et al., 2013; Levy Nogueira et al., 2015). Furthermore, these changes at the inflammation site may cause partial obstruction (inflammatory stenosis), leading to lumen distention in the bowel segment prior to the site of inflammation (circumferential mechanical stretch) (Katsanos et al., 2010; Shi, 2017). These changes are well represented in transmural inflammation especially stenotic Crohn's disease, where stenosis is caused not only by inflammation, but also by fibrosis-associated stricture formation (Katsanos et al., 2010; Rieder et al., 2013).

Functional and morphological changes such as dysmotility (Summers et al., 1983; Prihoda et al., 1984), visceral pain (Huang et al., 2005; Lin et al., 2017), muscular hypertrophy and hyperplasia (Gabella, 1975; Gabella, 1990), damages in the enteric nervous system and interstitial cell of Cajal (Chang et al., 2001a; Wedel et al., 2002) have been observed due to excessive mechanical stress. Interestingly, the effect of mechanical stress is very different in different tissues and cells in the gut. For example, mechanical stretch in a rat model of bowel obstruction

leads to injury and disruption in enteric nervous system and interstitial cell of Cajal network 7 days after induction of obstruction (Wu et al., 2013; Lin et al., 2014a). The similar stress conditions lead to hyperplasia of gut smooth muscle cells associated with mechanical stress-altered gene transcription, a process referred as “mechano-transcription” (Shi et al., 2011; Li et al., 2012a). Nevertheless, mechanisms underlying mechanical stress-associated dysfunctions and gene expression are not well understood.

To address the pathophysiological mechanisms, prior studies were focused on the dysfunctions several weeks after the initiation of bowel obstruction, a prototype of *in vivo* mechanical stretch (Gabella, 1975; Gabella, 1990; Chang et al., 2001b; Wedel et al., 2002; Bertoni et al., 2004; Won et al., 2006). Although this strategy may be able to document dramatic changes after obstruction, it might have ruled out some possible early cellular and molecular events, that could have led to the morphological and functional changes recorded later (Shi, 2017). Over the last decade, several investigators along with us have tested a new theory to understand bowel dysfunctions in response to mechanical stress. We hypothesized that mechanical stress regulates expression of mechanosensitive genes in the gut, and in the long run, the mechanical stress-altered gene expression may account for bowel dysfunctions (Shi et al., 2011; Li et al., 2012a; Wehner et al., 2010). Recent studies found that gut smooth muscle cells are highly sensitive to mechanical stress and contribute greatly to mechano-transcription in the GI tract (Shi et al., 2011; Shi, 2017). The cellular process transducing mechanical signals to the altered gene transcription involves mechanosensors (e.g., integrins and stretch-activated ion channels) in the plasma membrane and complex intracellular signaling pathways involving protein kinases C and D, and mitogen-activated protein kinases (MAPKs) (Li et al., 2012a; Li et al., 2012b). Mounting evidence now suggests that mechano-transcription in the GI tract plays a crucial role in motility dysfunction, visceral hypersensitivity, inflammation, and fibrosis in obstructive, inflammatory, and functional disorders.

In this review, we will discuss the effects of mechanical stress in the gut wall. Major focus will be on the process of mechano-transcription, its signal transduction mechanisms and pathophysiological significance in the gut. Furthermore, we will highlight the potential therapeutic targets of the mechano-transcription process for the treatment of obstructive, inflammatory, and functional disorders.

MECHANO-TRANSCRIPTION IN THE GASTROINTESTINAL TRACT

To gain insights into the mechanisms and significance of mechano-transcription in the gut, several *in vitro* and *in vivo* stretch models have been developed (Shi, 2017). Among these, the Flexcell system is possibly the best-established *in vitro* model to study mechanical stretch in cultured cells (Gayer and Basson, 2009; Wehner et al., 2010; Shi et al., 2011; Li et al., 2015). This system uses a computer-regulated bioreactor to apply finely controlled multi-axial static or cyclic strains through vacuum

pressure to cells cultured on flexible membrane plates. It has been used to study mechano-transcription *in vitro* in gut smooth muscle cells (SMC) (Wehner et al., 2010; Shi et al., 2011), epithelial cells (Gayer and Basson, 2009), and macrophages (Wehner et al., 2010; Docsa et al., 2020). Another *in vitro* system applies hydrostatic pressure to stretch cells (e.g., intestinal SMC) cultured on silicon membranes (Gutierrez and Perr, 1999). The amount of distension is controlled by volume of fluid added to the chamber beneath the silicon membrane.

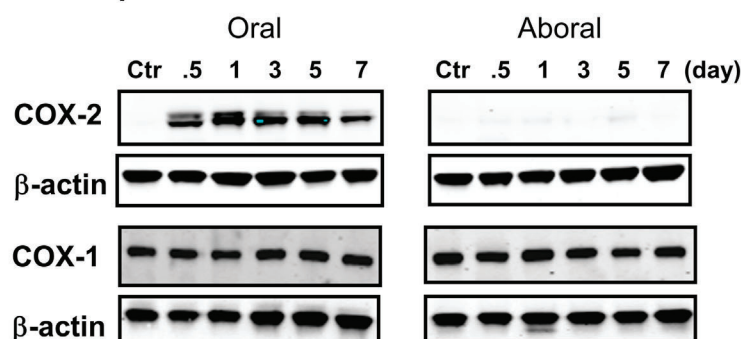
To study mechano-transcription *in vivo* in the GI tract, mechanical stretch (i.e., lumen distention) can be established by an intraluminal balloon to apply a desired pressure in the bowel (Lin et al., 2015) or surgically with an obstruction band around the studied area (esophagus, stomach, small intestine or colon) (Shi et al., 2011; Lin et al., 2012b). As mechanical bowel obstruction (BO) is the prototype obstructive disorder in the GI tract, our lab has used extensively the model of partial colon obstruction to understand mechanical regulation of gene expression and bowel functions *in vivo* (Shi et al., 2011; Li et al., 2012a; Lin et al., 2012a; Lin et al., 2017). To induce partial colon obstruction in rodents, a 3-mm wide medical grade silicon band is placed around the distal colon at about 3 cm proximal to the anus. The length of the 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 rather than complete obstruction (Shi et al., 2011). This procedure creates a significant colon distention in the segment proximal to obstruction band, and the external circumference is increased from 20 mm in control to 32–34 mm in obstruction in rats (Li et al., 2012a).

Our lab applied the *in vivo* model of partial colon obstruction to examine the scope of the effect of mechanical stress on gene expression in gut SMC utilizing a comprehensive Affymetrix cDNA array analysis (Shi et al., 2011; Shi, 2017). This study revealed that mechanical stretch dramatically altered the gene expression profile in the distended bowel segment oral to the obstruction site, compared to sham control or the non-stretch aboral segment. There is an at least 2-fold increase in the expression of 309 genes and decrease in the expression of 282 genes in stretched tissues (proximal to obstruction band), comparing to the non-stretch tissues (distal to obstruction). Gene expression of certain pro-inflammatory molecules, pain mediators, growth factors, extracellular matrix proteins, cell signaling molecules is altered by mechanical stretch. (Shi et al., 2011; Shi, 2017). This work demonstrates that mechanical stress substantially alters the gene expression profile in the gut. This altered gene expression may be one of the key mechanisms of bowel dysfunctions in conditions with lumen distention.

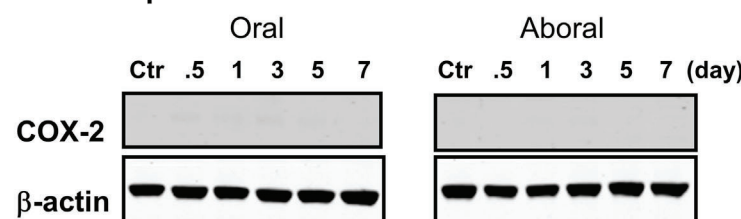
Mechano-Transcription of Prostaglandin-Endoperoxide Synthase-2 (COX-2) in Gut Smooth Muscle Cells

One of the upregulated genes detected in the micro-array study of the mechanically distended bowel is prostaglandin-endoperoxide synthase-2 (PTGS-2), also known as cyclooxygenase-2 (COX-2),

A COX expression in the colonic muscularis externa



B COX-2 expression in the colonic mucosa/submucosa



C Immunohistochemical staining of COX-2

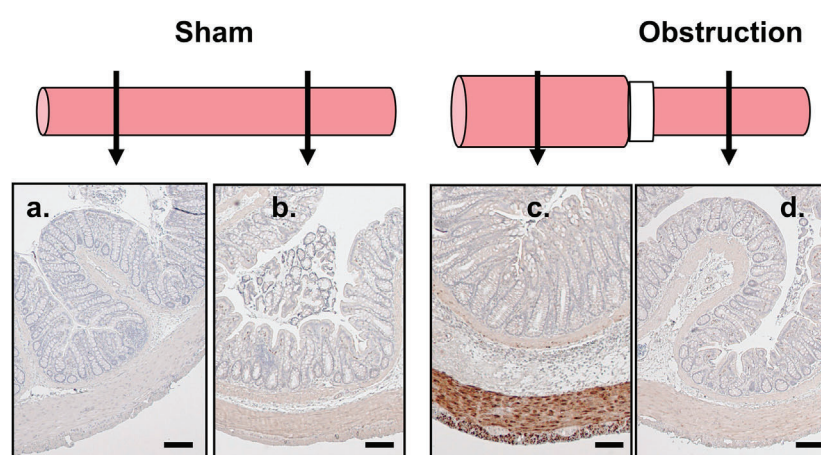
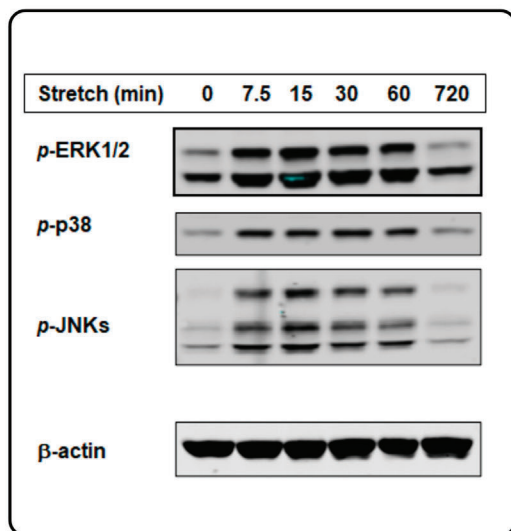


FIGURE 2 | Mechanical stress-induced up-regulation of COX-2 selectively in gut smooth muscle cells. **(A)** Western blot detected up-regulation of COX-2, but not COX-1, in the colonic muscularis externa in the oral segment, but not aboral segment, in relation to the obstruction band. **(B)** Western blot did not detect COX-2 expression in the mucosa/submucosa layer in segments either oral or aboral to the obstruction band. **(C)** Immunohistochemical study of COX-2 expression in segments oral (a, c) and aboral (b, d) colon segments in a sham operated rat (a, b) and a rat with obstruction (c, d) for 3 days. The expression of COX-2 (stained in brown) is detected only in smooth muscle cells in the distended oral segment (c). The results shown here are representative of four independent experiments. Calibration bars represent 50 μ m. Some parts of the figure are adapted with permission from **Figure 1** of Shi (2017).

which has shown a 19-fold increase in expression in the distended colon of BO rats, compared to the non-distended colonic segment aboral to obstruction. This enzyme catalyzes the major rate-limiting step in the synthesis of prostaglandins (PGs), which have profound impacts on gut inflammation, cell proliferation, motility, and visceral pain in the GI tract (Fornai et al., 2005; Krause and DuBois, 2000; Mohajer and Ma, 2000). While COX-1 is expressed constitutively in most of cell types, COX-2 is the inducible form (Krause and DuBois 2000; Mohajer and Ma,

2000). Our corroborative studies found that expression of COX-2 is increased significantly in the muscularis externa of stretched colon segment oral to obstruction, whereas its expression is not changed in the non-stretched segment aboral to obstruction (**Figure 2A**) (Shi et al., 2011). The expression of COX-1 is not altered either in the oral or aboral segment. Furthermore, immunohistochemical studies confirmed that there is an enhanced COX-2 expression in the smooth muscle cells (circular and longitudinal), but not in mucosa/submucosa,

A Mechanical stress-induced phosphorylation of MAPKs in colonic smooth muscle cells



B Intracellular signaling mechanism of mechano-transcription of COX-2 in colonic smooth muscle cells

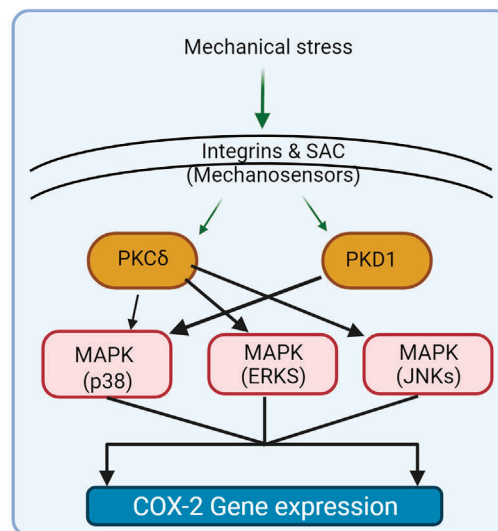


FIGURE 3 | Intracellular signaling mechanisms of mechano-transcription of COX-2 in colonic smooth muscle cells (SMC). **(A)** Mechanical stretch (18%) induced robust phosphorylation of MAPKs ERK1/2, p38, and JNKs in cultured rat colonic SMC in a time-dependent manner. **(B)** Mechanical stimulation on gut SMC is sensed by integrins and SAC (stretch-activated ion channels) at the cell membrane level. These mechano-sensors transduce mechanical signals to intracellular signaling pathways involving PKC (i.e., PKCδ), PKD (PKD1), and MAPKs (p38, ERKs, and JNKs), leading to mechano-transcription of COX-2 in colonic SMC. More specifically, PKCδ is linked to MAPKs ERKs, p38, and JNKs, whereas PKD1 is coupled to MAPK p38. Some parts of the figure are adapted with permission from **Figure 2** of Shi (2017).

or myenteric plexus (**Figures 2B,C**). These studies depict that mechano-transcription of COX-2 in bowel obstruction is a phenomenon specific to smooth muscle cells (Shi, 2017). The underlying mechanisms for selective expression of COX-2 in SMC by mechanical stretch are not yet clear. A study by Choudhury et al. (2015) has shown that mechano-transcription of COX-2 in the colon SMC was abrogated by de-polymerization of actin filament network with latrunculin B or swinholide. Mechano-transcription of COX-2 was also markedly attenuated by siRNA silencing of SMC specific α -actin (Acta2). These data indicate that SMC specific α -actin within an intact actin filament network is essential in the process whereby mechanical stress induces up-regulation of COX-2 in the colon SMC (Choudhury et al., 2015). Furthermore, Li et al. (2012a) reported that mechanical stretch potently activates mitogen-activated protein kinase (MAPK) p38 and protein kinase C (PKC), which are crucial intracellular signaling pathways involved in mechano-transcription of COX-2. However, treatments with Acta2 siRNA or latrunculin B or swinholide also restricted stretch-induced activation of p38 and PKC (Choudhury et al., 2015). Thus, an integrated smooth muscle actin network is essential for linking the mechanical signal to activation of MAPK and PKC and, thus, for mechano-transcription of COX-2.

Lin et al. (2012a) studied the effect of lumen distension on COX-2 expression in different parts of the GI tract by placing an obstruction band in the lower esophagus, pylorus, ileum, and colon. They depicted a significantly upregulated expression of

COX-2 associated with obstruction at all the sites where distention was introduced (Lin et al., 2012a). They also found that COX-2 expression was significantly elevated by lumen distension of the colon with a balloon at a pressure of 40 mmHg for 40 min. Induction of COX-2 gene expression did not occur when the pressure was set 10 mmHg or less, or when the distention period was 20 min or shorter (Lin et al., 2015). Together, these studies demonstrate that distention-induced mechano-transcription of COX-2 in the GI tract is a force- and time-dependent, and smooth muscle specific phenomenon. Furthermore, mechano-transcription is a common process in response to luminal distention throughout the GI tract (Shi, 2017).

Signal Transduction Mechanisms of Mechano-Transcription in the Gut

Mechano-transcription of COX-2 in the gut has important pathophysiological significance in obstructive conditions (Shi et al., 2011; Lin et al., 2017). We then studied the signaling mechanisms whereby mechanical stress induces gene expression of COX-2 in colonic SMC (Li et al., 2012a; Li et al., 2012b). COX-2 gene expression was increased dramatically by static mechanical stretch (18% elongation) of cultured rat colonic SMC. Phosphorylation of MAPKs including extracellular signal-regulated kinases (ERKs, i.e., ERK 1 and ERK2), p38, and c-Jun N-terminal kinases (JNKs) (Li et al., 2012a) was also markedly induced by

mechanical stress (**Figure 3A**). Treatment of the cells with MAPK inhibitors to ERK (PD98059), to p38 (SB203580) or to JNKs (SP600125) all significantly inhibited mechano-transcription of COX-2. These studies demonstrate the critical role of MAPK members in the regulation of mechano-transcription of COX-2 in colonic SMC (Li et al., 2012a; Shi, 2017).

Generally, mechanical stimuli are sensed at the cell membrane level and then get transduced into the cytoplasm and nucleus (Ruwhof and van der Laarse, 2000; Adam et al., 2004; Yamaguchi, 2004; Kanefsky et al., 2006). Among all possible mechano-sensors, integrins and stretch-activated ion channels (SACs) are two main groups which are identified in various types of cells (Hu and Sachs, 1997; Gillespie and Walker 2001; Katsumi et al., 2004; Kraichely and Farrugia, 2007). Our lab investigated whether $\alpha\text{v}\beta 3$, a major type of integrin in gut smooth muscle cells, is a mechano-sensor involved in mechano-transcription of COX-2 in rat colon SMC (Shi, 2017). Li et al. (2012a) found that stretch-induced expression of COX-2 mRNA and protein was abrogated by $\alpha\text{v}\beta 3$ inhibitor echistatin, and by neutralizing antibody against rat $\beta 3$. Further, echistatin treatment has significantly inhibited stretch-induced activation of p38, but not ERKs and JNKs. The downstream pathway of integrin signaling was found to be also important in mechano-transcription of COX-2 in gut SMC, as knockdown of integrin-linked kinase and focal adhesion kinase with specific siRNAs significantly suppressed mechano-transcription of COX-2 in the cells (Li et al., 2012a). Li et al. (2012a) further examined if SACs play a role in mechano-transcription of COX-2 in the colon SMC. General SAC inhibitor gadolinium (Ducret et al., 2010) or specific SAC blocker GsMTx-4 (Suchyna et al., 2000) dramatically attenuated mechanical stretch-induced expression of COX-2 mRNA and protein. Treatments with gadolinium or GsMTx-4 also inhibited stretch-induced phosphorylation of all MAPKs, i.e., ERKs, p38, and JNKs (Li et al., 2012a).

The potential role of protein kinase C (PKC) and protein kinase D (PKD) in mechano-transcription of COX-2 was also investigated. Mechanical stretch leads to robust activation of PKCs and PKDs in colonic SMC (Li et al., 2012b). While blockade of PKC- β or PKC- ζ could not restrict stretch-induced expression of COX-2, rottlerin, a specific PKC- δ inhibitor, almost completely inhibited stretch-induced COX-2 expression. PKD inhibitor CID755673 or siRNA silencing of PKD also significantly inhibited stretch-induced COX-2 expression. Interestingly, Rottlerin treatment attenuated mechanical stretch-induced activation of all MAPKs (ERKs, p38, and JNKs), whereas CID755673 solely restricted activation of p38 (Li et al., 2012b).

Together, recent studies have demonstrated that mechanical stress on gut SMC is sensed by integrins and SAC at the cell membrane level. Then, the mechanical signal is transduced to the cytoplasm *via* intracellular signaling molecules PKCs, PKD, and MAPKs to induce gene expression of COX-2 in colonic SMCs (Li et al., 2012a; Li et al., 2012b). Further, studies suggest that PKC- δ is linked to MAPKs ERKs, p38, and JNKs, whereas PKD is connected to MAPK p38 (Li et al., 2012a; Li et al., 2012b; Shi, 2017) (**Figure 3B**).

PATHOGENIC ROLE OF MECHANO-TRANSCRIPTION IN GASTROINTESTINAL DISORDERS

Motility Dysfunction

Motility dysfunction is well documented in obstructive conditions. Motor activity is increased immediately in the distended segment in bowel obstruction, and gradually decreased within hours (Fraser et al., 1980; Summers, 1999; Bertoni et al., 2004; Won et al., 2006). The initial phase of hyper-motility in the distended bowel is considered to be a physiological adaptation to the obstruction of food passage (Summers, 1999). However, the chronic suppression of motor activity is the biggest concern in patients with obstructive disorders, especially in chronic partial obstruction (Ripamonti and Mercadante, 2004), malignant obstruction (Ripamonti et al., 2001; Roeland and von Gunten, 2009), and functional obstruction (Thompson, 2006; Nunez et al., 2009; De Giorgio et al., 2011). The consequences of impaired motor activity include distention, nausea, vomiting, and constipation (Russell and Welch, 1990; Summers, 1999; Shi et al., 2011).

As COX-2 and COX-2-derived prostaglandins profoundly affect motility function (Krause and DuBois, 2000; Fornai et al., 2005), we investigated whether mechanical stress-induced expression of COX-2 and production of prostaglandins play a role in the sustained suppression of motility in obstruction. Our studies have revealed that smooth muscle contractility of the isolated circular muscle strips was suppressed dramatically at 24 h after obstruction, which continued through the 7-days course of obstruction (Shi et al., 2011; Li et al., 2012a). Importantly, pretreatment of the muscle strips isolated from BO rats with COX-2 inhibitor NS-398 restored muscle contractility. Moreover, PGE₂ levels were nearly 15-fold higher in the medium of BO strips than that of the sham strips, and NS-398 treatment significantly lowered the PGE₂ levels (Shi et al., 2011). Studies also revealed that colon obstruction led to marked induction of COX-2 and decrease of muscle contractility in wild-type mice (Shi et al., 2011). However, smooth muscle contractility was largely unaffected by obstruction in the COX-2 deficient mice (Shi et al., 2011). These studies indicate that stretch-induced expression of COX-2 and associated release of prostaglandins play a prominent role in the suppression of smooth muscle contractility during BO.

Recent reports suggest that induction of several other pro-inflammatory molecules by mechanical stress may also contribute to motility abnormalities (Wehner et al., 2010; Lin et al., 2014b; Docsa et al., 2020). It was discovered that mechanical stress *in vitro* in cultured gut SMCs or *in vivo* in the model of obstruction significantly induced gene expression of IL-6, chemokine (C-C motif) ligand 2 (CCL-2), iNOS, and several other pro-inflammatory mediators in the muscle cells. These molecules are known to not only play critical roles in inflammation but also lead to motility dysfunction in the gut (Lin et al., 2014b). Docsa et al. (2020) found that mechanical stress-induced chemokine (C-X-C motif) ligand 1 (CXCL-1) in macrophages suppressed intestinal smooth muscle contractility

and may account for motility dysfunction in ileus, a common functional obstruction in post-operative period. Moreover, conditioned media of stretched muscle strips induces NF- κ B activation, further increasing production of pro-inflammatory mediators. Thus, mechanical stress-induced pro-inflammatory mediators may contribute significantly to motility dysfunction in obstructive conditions.

Gut motility is subject to control by neurotransmitters and gut hormones. Mechanical stress is well known to alter release of neurotransmitters and gut hormones (Brierley and Blackshaw, 2006; Linan-Rico et al., 2016). However, it is not well known whether mechanical stress is involved in the regulation of synthesis or transcription (i.e., mechano-transcription) of gut hormones and neurotransmitters. Serotonin (5-HT) as a hormone and neurotransmitter in the gut plays an important role in mediating peristalsis in physiological status and motility dysfunction in various G.I. pathologies (Gershon and Tack, 2007; Linan-Rico et al., 2016). Lumen distention certainly increases release of 5-HT from enterochromaffin (EC) cells in the mucosa layer. The levels of 5-HT in the local gut tissues appear to be increased chronically in gut inflammation and increased 5-HT may contribute to inflammation and motility dysfunction in inflammatory conditions such as Crohn's disease and ulcerative colitis (Gershon and Tack, 2007; Linan-Rico et al., 2016; Terry and Margolis, 2017). What accounts for the chronic increase of 5-HT in the gut is not well understood. However, a recent study found that cyclic mechanical stretch *in vitro* increased transcription and activation of tryptophan hydroxylase-1 and vesicular monoamine transporter-1 and enhanced the release of 5-HT in the cultured enterochromaffin cells (Chin et al., 2012). Tryptophan hydroxylase-1 and vesicular monoamine transporter-1 are well involved in the synthesis and transport of 5-HT in the enterochromaffin cells. Given that expression of tryptophan hydroxylase-1 and vesicular monoamine transporter-1 is mechanically responsive and mechanical stress is well present in gut inflammation, it is possible that mechano-transcription may be involved in the increased production of 5-HT in GI pathologies such as inflammation. Further studies are warranted to determine if mechano-transcription plays a role in the production of other gut hormones and neurotransmitters to contribute to motility dysfunction and inflammation.

Abdominal Pain

Abdominal pain is a common complaint in obstructive conditions as well as functional bowel disorders and inflammatory bowel disease (Shi et al., 2018). Mechanical stimulation leads to instant activation of sensory nerves *via* mechanosensitive channels (Ness et al., 1990; Brierley and Blackshaw, 2006; Lin et al., 2015). This may contribute to the mechanisms involved in transmission of acute pain during early hours of obstructive conditions (Summers, Yanda, 1983; Shi et al., 2018). However, neuronal desensitization may occur upon repetitive or long-term mechanical stimulation (Slugg et al., 2000; Brierley et al., 2004). Nevertheless, chronic abdominal pain is present in obstruction (Ripamonti et al., 2001; Ripamonti and Mercadante, 2004; Roeland and von Gunten,

2009). Reports suggest that more than 90% of patients with advanced malignant obstruction have distention-associated abdominal pain (Baines et al., 1985; Ripamonti and Mercadante, 2004). Studies found that visceral sensitivity is markedly increased in chronic bowel obstruction (Huang et al., 2005; Lin et al., 2017). Visceral hypersensitivity is the main mechanism of chronic abdominal pain (Mayer and Gebhart, 1994; Bielefeldt, 2006; Azpiroz et al., 2007). We demonstrated that sensory neurons in the dorsal root ganglia (DRG) projecting to the obstructed colon in rats exhibited reduced resting membrane potential and rheobase along with increased number of action potentials (Lin et al., 2017). These studies demonstrate a highly sensitized state of the primary sensory afferents in obstruction (Lin et al., 2017). In addition, the withdrawal response to von Frey filament stimulation to the abdomen was found significantly increased in the rats with colon obstruction, suggesting referred visceral hyperalgesia (Lin et al., 2017).

Neurotrophins such as nerve growth factor (NGF) and brain-derived neurotrophic factor (BDNF) are well-known pain mediators (Bielefeldt, 2006; Pezet and McMahon, 2006). Recent studies found that expression of NGF and BDNF is highly responsive to mechanical stress (Lin et al., 2017; Fu et al., 2018). The expression of NGF mRNA and protein was significantly induced by mechanical stretch in colonic SMC *in vitro* and in bowel obstruction model (Lin et al., 2017). In the obstructed rats, the cell excitability of colon-projecting DRG neurons was augmented and the referred visceral sensitivity was increased. However, anti-NGF antibody administration largely restored the colon neuron excitability and referred visceral sensitivity in obstructed rats. Lin et al. (2017) observed that tetrodotoxin-resistant (TTX-r) Na^+ currents and TTX-r $\text{Na}_v1.8$ mRNA expression were significantly increased in colon-projecting DRG neurons in colon obstruction. However, the increased Na^+ channel activity and $\text{Na}_v1.8$ mRNA expression were attenuated by anti-NGF treatment. Therefore, mechanical stretch-induced NGF in colon SMC contributes significantly to visceral hypersensitivity in BO by sensitizing primary afferents and increasing TTX-r Na_v expression and function in the DRG neurons. Similarly, studies have revealed that expression of BDNF was also induced significantly in the colonic smooth muscle cells of the mechanically distended bowel segment in obstruction (Fu et al., 2018). The colon-projecting DRG neurons of the obstructed rats exhibited significantly reduced densities of voltage-gated K^+ channel (K_v) and transient A-type (I_A) current. These changes contributed to neuronal hyperexcitability. Anti-BDNF antibody treatment blocked these changes in neurons isolated from obstructed rats. Administration of ANA-12, an inhibitor to BDNF receptor Trk B, also blocked the changes of neuronal excitability and K_v activity, and improved referred visceral sensitivity in obstructed rats (Fu et al., 2018). Together, these studies depict the critical contribution of mechano-transcription of NGF and BDNF in gut SMC to visceral hypersensitivity and abdominal pain in obstruction.

Visceral inputs are transduced to primary sensory neurons located in DRG, which further transmit the signals to the second order neurons in the spinal cord to initiate central processing of sensory information for perception (Lin et al., 2017; Shi et al., 2018). There is evidence that mechanical distension in the gut may affect gene expression and function of sensory pathway directly and indirectly. It is found that gene expression of opioid receptors in primary sensory afferents was down-regulated by mechanical stress during bowel obstruction, and down-regulation of the anti-pain system may contribute to visceral hypersensitivity in obstruction (Hegde et al., 2020). Moreover, persistent functional and transcriptional changes may occur in neurons in the DRG and dorsal horn secondary to pro-inflammatory and pain mediators induced by mechano-transcription in the peripheral tissues after lumen distension (Lin et al., 2018 abstract). The membrane excitability of colon-specific DRG neurons remains significantly enhanced 14 days after a 7-days partial colon obstruction. The mRNA expression and channel activity of transient receptor potential cation channel subfamily V member 1 (TRPV1) are increased not only during obstruction, but 14 days after obstruction is released. The long-term change of TRPV1 gene expression may be secondary to the effect of mechanical stress-induced NGF in the obstructed colon, as NGF increases TRPV1 expression in DRG neurons (Winston et al., 2001).

Gut Inflammation

Gut inflammatory milieu involves multiple cellular and molecular processes that are mediated by cytokines, chemokines, and other inflammatory mediators (Papadakis and Targan, 2000; Stadnyk, 2002). Recent studies have shown that mechanical force is a pro-inflammatory stimulus in the gut (Wehner et al., 2010; Lin et al., 2014b; Shi, 2017). Wehner et al. (2010) reported that static mechanical stress significantly induced iNOS, COX-2, and IL-1 β gene expression levels in cultured gut SMCs and peritoneal macrophages. Mechanical stimulation also magnified lipopolysaccharide-induced iNOS and IL-1 gene expression in intestinal smooth muscle cells, and similarly COX-2 and IL-6 mRNA expression in macrophages. Lin et al. (2014b) further investigated mechano-sensitive expression of pro-inflammatory mediators in the gut with a comprehensive approach involving *in vitro*, *in vivo*, and *ex vivo* models. In the primary culture of colon smooth muscle cells, they found that static stretch significantly increased mRNA expression of iNOS, COX-2, IL-6, and CCL-2 (Lin et al., 2014b). Mechanical stretch did not have an effect on gene expression of TNF- α , IL-1 β , and IL-8. In the *in vivo* model of partial colon obstruction, the authors found that expression of iNOS, IL-6, and CCL-2 is significantly increased in a time-dependent way in the mechanically distended segment, compared to the non-distended segment of the rats with obstruction or sham control animals. The conditioned media from the stretched colon smooth muscle significantly induced translocation and phosphorylation of pro-inflammatory transcription factor NF- κ B p65, leading to increased mRNA expression of more inflammatory mediators in naïve cells. However, treatment with IL-6

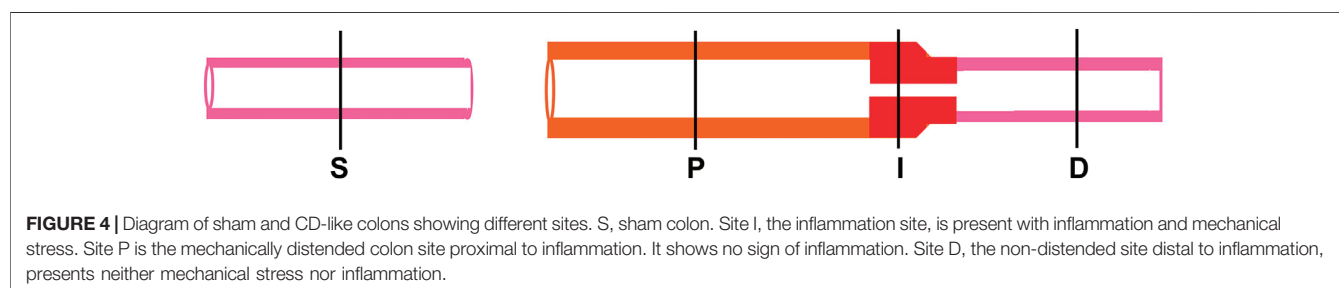
neutralizing antibody to the conditioned medium from the mechanically distended muscle showed significant reduction in the activation of NF- κ B and gene expression of inflammatory mediators, indicating a critical role of mechanical stress-induced IL-6 in the secondary activation of pro-inflammatory transcription factors (Lin et al., 2014b).

Osteopontin (OPN) is a secreted glycoprotein with many demonstrated roles in the regulation of immune response on multiple levels (Uede, 2011; Rittling and Singh, 2015). Sato et al. (2005) found that active Crohn's disease (CD) patients demonstrated significantly higher plasma OPN levels than normal or ulcerative colitis (UC) patients, and the elevated plasma OPN levels in CD patients were significantly correlated with disease activity. Further, OPN was found to facilitate production of IL-12 from lamina propria mononuclear cells and is tightly involved in the Th1 immune response in CD (Ashkar et al., 2000; Sato et al., 2005). However, the cellular source and mechanisms of increased plasma OPN in CD are not clear. We found that expression of OPN was dramatically upregulated in the mechanically distended colon in bowel obstruction (Lin and Shi, 2021). OPN level was also increased in the plasma in rats with bowel obstruction. In the rat model of stenotic Crohn's colitis, OPN expression was found increased not only at the inflammation site, but at the distended pre-inflammation site (Lin and Shi, 2021). Plasma OPN level was significantly increased in stenotic Crohn's colitis rats. However, prevention of inflammation-associated mechanical distention with liquid diet eliminated OPN expression in the pre-inflammation site and normalized plasma OPN level. These results suggest that OPN expression in Crohn's colitis is largely mediated by mechanical stress, and the plasma OPN levels in colitis are closely related to the extent of bowel distention.

Intestinal Fibrosis and Smooth Muscle Hyperplasia

Stricture formation due to tissue fibrosis and smooth muscle hyperplasia is the constant challenge in CD (Li and Kuemmerle, 2014; Latella and Rieder, 2017; Rieder et al., 2017). Stricture-associated stenosis leads to bowel obstruction and increases risks of perforation and fistula (Katsanos et al., 2010; Latella and Rieder, 2017). Conventional treatments like anti-inflammatory agents are not much effective to prevent stricture formation in CD. Although surgery provides temporary relief, the previously distended pre-stenotic region may become new sites for recurrent inflammation and fibrosis. In fact, post-surgery endoscopic or histological recurrences are almost 100%, given enough time (Rutgeerts et al., 1991; Olaison et al., 1992; Rieder et al., 2017). Release of mechanical stress by strictureplasty reduces fibrosis (Yamamoto et al., 2007; Latella and Rieder, 2017). These clinical findings indicate that mechanical stress, as an inflammation-independent factor, may play a critical role in fibrosis and stricture formation (Rieder et al., 2017; Tschumperlin et al., 2018).

In fact, there is increasing evidence that mechanical stress induces gene expression of pro-fibrotic and proliferative



mediators to contribute to intestinal fibrosis and hyperplasia in the gut (Johnson et al., 2013; Johnson et al., 2014; Lin et al., 2021a). In a rodent model of Crohn's-like colitis, Lin et al. (2021a) observed that intracolonic instillation of TNBS resulted in induction of localized transmural inflammation in the distal colon (represented as site I), with a distended colon segment (represented as site P) proximal to the inflammation site, and a non-distended segment (represented as site D) distal to the site of inflammation (Figure 4). Based on macroscopic and histological studies, site I comprises inflammation and mechanical stress, site P has mechanical stress but no visible inflammation, and site D has neither inflammation nor mechanical stress. Significant fibrosis and smooth muscle hyperplasia developed 7–21 days after TNBS instillation at sites P and I. Molecular expression analysis revealed that expression of pro-fibrotic and proliferative genes, e.g., connective tissue growth factor (CTGF) and BDNF were markedly up-regulated in both sites P and I. The increased expression of CTGF and BDNF were mainly observed in the muscularis externa. Interestingly, expression of CTGF and BDNF was not significantly upregulated in the non-distended site D, suggesting that expression of CTGF and BDNF expression is stretch-sensitive in the CD-like colitis model. However, if rats were fed exclusively with clear liquid diet, mechanical distention in sites P and I was prevented in the TNBS-treated rats. Moreover, up-regulation of CTGF and BDNF was also prevented in sites P and I. Treatment with clear liquid diet markedly improved inflammation, fibrosis, and muscle hyperplasia in the colitis rats. In the model of partial colon obstruction, where mechanical distention, but no inflammation, was induced by an obstruction band wrapped in the distal colon, the authors found that collagen production, smooth muscle cell numbers and thickness, and expression of CTGF and BDNF were all increased only in the distended segment prior to obstruction, but not in the non-distended distal colon (Lin et al., 2021a). Taken together, these studies suggest that transmural inflammation in CD-like colitis is associated with mechanical stress in the inflammation site and the distended segment proximal to inflammation, which leads to increased expression of pro-fibrotic and proliferative mediators. Mechanical stress-induced up-regulation of pro-fibrotic and proliferative mediators may contribute

significantly to the development of fibrosis and muscle hyperplasia in CD.

TARGETING MECHANO-TRANSCRIPTION PROCESS FOR THERAPEUTIC POTENTIALS IN OBSTRUCTIVE BOWEL DISORDERS

Challenges in the Management of Obstructive Bowel Disorders

Obstructions in the small and large intestines accounts for approximately 15% of hospital admissions for acute abdomen in the U.S. The management strategy for bowel obstruction varies depending on etiology and site of obstruction, and whether patients have a partial, complete or complicated obstruction, and whether they have previous abdominal surgery (Frago et al., 2014; Catena et al., 2019). Recent studies found that only 20% of bowel obstruction cases need acute surgical care, and most of patients with bowel obstruction are treated with non-operative management (Catena et al., 2019). This is particularly true for malignant bowel obstruction (MBO), which is a common complication in patients with bowel or gynecological cancers. Conservative management for BO patients includes nil per os, nasogastric suction, stenting, fluid replacement therapy, and medication treatments for various symptoms related to obstruction (Catena et al., 2019). Nasogastric drainage is generally only a temporary measure. Among medical treatments, opioid analgesics are used for abdominal pain caused by obstruction. Although they may provide certain degree of pain relief, opioid analgesics cause significant gastrointestinal adverse effects such as constipation and fecal retention, which would worsen motility dysfunction in obstruction. Anti-secretory and anti-emetics may be used to control vomiting and abdominal distention (Ripamonti et al., 2001). However, the effect of medical treatments in bowel obstruction is limited as they do not specifically address mechanical stress - the root cause of functional abnormalities such as motility dysfunction and abdominal pain in obstructive disorders.

Currently, surgical treatment for bowel obstruction is to release blockage as in mechanical obstruction or remove

constrictions as in Hirschsprung's disease (HD). In either way, the distended oral segment is kept in the gut (Williams et al., 2005; Heuckeroth, 2018). However, many patients suffer bowel dysfunctions during obstruction and even after the obstruction is surgically resolved (Di Lorenzo et al., 2000; Fevang et al., 2004; Menezes et al., 2006; Catto-Smith et al., 2007; Jarvi et al., 2010). Nearly 70% of the children with a history of HD have long-term gastrointestinal symptoms such as incontinence, constipation, abdominal distention, and enterocolitis by 10 years after pull-through surgery (Di Lorenzo et al., 2000; Catto-Smith et al., 2007). Recent reports revealed that motility dysfunction and constipation continue through adulthood in 30% of patients after surgical release of HD-associated obstruction in childhood (Jarvi et al., 2010). In the upper gut, infantile pyloric stenosis causes gastric outlet obstruction. Gastric sensory and motility functions are affected for a long time even after pyloromyotomy (Sun et al., 2000; Saps and Bonilla, 2011). Dysregulated motility is accountable for symptoms such as bloating, nausea, vomiting, and constipation. However, the reasons for the long-term bowel dysfunction even after the resolution of obstruction is not understood. There are no specific treatments for the long-term dysfunction in such cases.

Targeting Mechano-Transcription of COX-2 and Prostaglandin E Synthase for Motility Dysfunction in Bowel Obstruction and After Obstruction Is Corrected

Given that mechanical stretch induces marked expression of COX-2, and COX-2 derived prostaglandins suppress muscle contractions (Shi et al., 2011; Lin et al., 2012a), Lin et al. (2012b) studied the therapeutic and prophylactic effects of COX-2 inhibitors on smooth muscle function and gut motility in bowel obstruction in rats (Lin et al., 2012b). COX-2 inhibitor NS-398 or vehicle was administered daily pre- and post-induction of obstruction to investigate its prophylactic and therapeutic efficacies, respectively. Obstruction led to significant decrease of muscle contractility and a very slow colonic transit rate. However, prophylactic treatment with NS-398, starting before obstruction is induced, significantly improved colonic transit and muscle contractility, and attenuated fecal retention in the obstructed colon. Even with the treatment of NS-398 starting day 3 post obstruction, the muscle contractility and colonic transit still improved by day 7. The team further investigated whether inhibition of PGE₂ is beneficial in improving motility function in obstruction (Lin et al., 2012b). Four PGE₂ receptors, EP1 to EP4, were identified in the rat colonic smooth muscle cells. Although treatments with EP1 and EP3 antagonists decreased normal muscle contractility in tissues taken from sham controls, they did not improve muscle contractility in tissues taken from obstructed colon. On the other hand, the EP2 and EP4 antagonists did not significantly affect control tissue, but restored muscle contractility in obstruction (Lin et al., 2012b). These studies demonstrated that PGE₂ and its receptors EP2 and EP4 are specifically implicated in

motility dysfunction mediated by mechanical stress-induced COX-2 in obstruction. EP2 and EP4 antagonists, along with COX-2 inhibitors, may have therapeutic potential in medical treatment of motility dysfunction in bowel obstruction.

Clinical studies support the pre-clinical findings that selective inhibition of COX-2 has benefits in treating obstructive bowel disorders. Retrospective studies in large cohort of patients and clinical trials found that the use of COX-2 inhibitor celecoxib decreased the paralytic ileus rates and did not result in any significant morbidity (Wattchow et al., 2009; Raju et al., 2015). A multicenter, blinded, and randomized clinical trial study found that selective COX-2 inhibitor firocoxib, but not the nonselective COX inhibitor flunixin meglumine, is beneficial in small intestinal obstruction by strangulation in horses, as it reduced endotoxemia (Ziegler et al., 2019; Ziegler and Blikslager, 2020).

As BO-associated up-regulation of COX-2 and production of PGE₂ depend on mechano-transcription process, Li et al. further determined whether inhibition of mechano-transcription signal transduction improves motility function (Li et al., 2012a; Li et al., 2012b). Because stretch-induced expression of COX-2 depends on MAPK p38 activation in colonic SMC, Li et al. (2012a) studied the *in vivo* effects of p38 inhibitor SB203580 on COX-2 induction and motility dysfunction in obstruction. It was found that SB203580 significantly inhibited induction of COX-2 and improved colon motility in obstruction. This was associated with improvement of colon distension. These studies show that inhibition of the intestinal mechano-transcription process has therapeutic potentials for motility dysfunction in obstructive bowel disorders.

Gut motility is altered not only during BO, but for a long time even after BO is resolved (Di Lorenzo et al., 2000; Fevang et al., 2004; Jarvi et al., 2010). Recent studies suggest that mechanical stress-induced COX-2 as well as COX-2-derived PGE₂ in the distended gut SMC not just account for motility dysfunction in obstruction, but exert secondary effects on SMC even after obstruction is resolved (Lin et al., 2018). Lin et al. (2018) showed that COX-2-derived PGE₂ during BO may further increase the expression of microsomal prostaglandin E synthase-1 (mPGES-1), an important enzyme involved in the synthesis of PGE₂, in an autocrine mode. Studies *in vivo* showed that increased mPGES-1 contributes to the continuous production of PGE₂ and long-term motility dysfunction. Thus, it is crucial to recognize that once the bowel is distended during obstruction, it may never be considered as "normal" because it is the site of mechano-transcription. Comparative studies were performed using COX-2 inhibitor NS-398 and mPGES-1 inhibitor Cay 10,526 during BO and post-BO (after obstruction is corrected). Results of the study suggest that targeting COX-2 and mPGES-1 during obstruction may be an effective therapeutic strategy to treat motility dysfunction during obstruction and a prophylactic strategy to prevent long-term motility dysfunction occurring after obstruction is resolved. However, for long-term motility dysfunction after the obstruction is corrected, inhibition of mPGES-1 rather than COX-2 appears to be a better therapeutic approach (Lin et al., 2018).

Targeting Mechano-Regulation of Nociceptive Mediators and Anti-pain System for Abdominal Pain in Obstructive Bowel Disorders

Abdominal pain is the main reason for hospital visits in patients with bowel obstruction (Cappell and Batke, 2008; Gore et al., 2015). In the acute phase of obstruction (the first 12–24 h), abdominal pain may be colicky (cramping and intermittent) (Summers, 1999; Cartwright and Knudson, 2008; Gore et al., 2015). However, patients with chronic partial obstruction or pseudo-obstruction do experience persistent abdominal pain (Baines et al., 1985; Ripamonti et al., 2001; Ripamonti and Mercadante, 2004; De Giorgio et al., 2011). Currently available treatment for BO-associated pain relies on high doses of opioids (Ripamonti and Mercadante, 2004; Roeland and von Gunten, 2009). However, opioids are notorious in causing further motility dysfunction, constipation and paradoxically visceral hyperalgesia (Grunkemeier et al., 2007; Farmer et al., 2019; Lin et al., 2021b). It is imperative to identify visceral analgesics that are specific for distension-associated pain.

Recent studies have shown that mechanical stress induces marked expression of pain mediators NGF, BDNF, and COX-2 in the gut wall, which may play a crucial role in visceral hypersensitivity in experimental obstruction (Huang et al., 2005; Lin et al., 2015; Lin et al., 2017; Fu et al., 2018). Lin et al. (2017) found that inhibition of mechanical stress induced NGF by administering neutralizing antibody against NGF not only attenuated afferent neuron hyperexcitability, but also significantly improved pain behavior in rats with chronic partial colon obstruction. Fu et al. (2018) reported that BDNF is robustly induced by mechanical stress in distended bowel in obstruction, and blockade of BDNF action by administering Trk B inhibitor *in vivo* effectively inhibited BO-associated referred pain. These studies have also characterized downstream mechanisms of NGF and BDNF-mediated hyperalgesia and found that altered expression and activity of $\text{Na}_v1.8$ and K_v in the afferent nerve may account for NGF and BDNF-mediated peripheral visceral hypersensitivity (Lin et al., 2017; Fu et al., 2018). Therefore, mechanical stress-induced production of NGF and BDNF in the gut tissues and the neurotrophins-mediated Na_v and K_v activity in primary afferent nerves represent plausible therapeutic targets for distention-associated abdominal pain.

Peripheral opioid receptors as a part of anti-pain system are thought to be critical in modulating visceral pain. It is found that while mechanical distention led to up-regulation of nociceptive mediators (e.g., NGF and BDNF) in gut SMC, the same distention caused down-regulation of opioid receptors μ , δ , and κ in the sensory afferents in the colon (Hegde et al., 2018). The down-regulation of opioid receptors may contribute to visceral hyperalgesia in obstruction. Interestingly, Hegde et al. (2020) found that the abundance of gut commensal *Lactobacillus reuteri* was drastically decreased in the obstructed bowel in rats. However, when *L. reuteri* rat strains were ingested via gavage (1×10^9 colony-forming units/g daily starting 2 days before obstruction), the precision microbial therapy attenuated visceral hyperalgesia.

Treatment with *L. reuteri* also diminished hyperexcitability of the DRG neurons projecting to the distended colon. Importantly, treatment with *L. reuteri* prevented the down-regulation of opioid receptors. In addition, treatment with peripheral opioid receptor antagonist naloxone methiodide eliminated the analgesic effect of *L. reuteri* in obstruction. Thus, *L. reuteri*, via inducing opioid receptors in the gut tissues, may have therapeutic potential in preventing lumen distension-associated visceral hyperalgesia and abdominal pain in obstructive conditions (Hegde et al., 2020).

TARGETING MECHANO-TRANSCRIPTION PROCESS FOR THERAPEUTIC POTENTIALS IN INFLAMMATORY BOWEL DISEASE

Challenges in the Management of Inflammatory Bowel Disease

The pathogenic mechanisms of Crohn's disease (CD) and ulcerative colitis (UC) remain unknown, and cures are unavailable (Hendrickson et al., 2002; Sartor, 2006). Current therapies for IBD are designed to induce prolonged remission. Among the therapeutic options, 5-aminosalicylates are used mainly for mild active IBD (predominantly for UC), and for maintenance treatment in UC (Domènech, 2006). Corticosteroids are considered the mainstay for induction of remission in moderate to severe active inflammation in both UC and CD (Hendrickson et al., 2002; Domènech, 2006). Immunomodulators such as azathioprine and methotrexate are second-line treatment, mainly because of safety profile and economic costs. They are often used for maintenance therapy in both UC and CD (Domènech, 2006; Wehner et al., 2010; Kansal et al., 2013), or for patients with steroid refractoriness or dependency. The use of biologic agents, such as anti-TNF α chimeric antibody, is increasing. They are now often used as a first line therapy for moderate to severe CD and particularly useful in patients with steroid refractory disease (Ruemmele et al., 2009).

Unfortunately, most of the medical treatment options for IBD have substantial adverse effects. Corticosteroids are known for their frequent and sometimes severe side effects and their limitations include risks of infection, osteoporosis, growth retardation, poor mucosal healing, and early relapses on cessation of therapy (Sidoroff and Kolho, 2012; Cheifetz, 2013). This is especially problematic in pediatric patients, as this population of patients are prone to experience growth retardation and osteoporosis with steroid therapy (Sidoroff and Kolho, 2012). Immunomodulators increase the risk of opportunistic infections and hematologic disorders (Kandiel et al., 2005; Sidoroff and Kolho, 2012). Biologic agents targeting cytokines and adhesion molecules may lose efficacy over time, and are limited with increased vulnerability to infections, development of autoimmune disorders and even malignancy, and decreased immunogenicity of vaccinations (Kandiel et al., 2005). Thus, safe and effective therapies,

including diet-based treatments, are much needed for IBD patients (Kansal et al., 2013; Ashton et al., 2019).

Attenuation of Mechano-Transcription Process as a Novel Mechanism Underlying the Benefits of Exclusive Enteral Nutrition in Crohn's Disease

Although many dietary therapies have been explored for the management of IBD (Domènech, 2006), exclusive enteral nutrition (EEN) is the sole established dietary treatment for IBD, specifically for CD (Kansal et al., 2013; Ashton et al., 2019). EEN involves oral or nasogastric tube feeding of a complete liquid diet with exclusion of normal foods for a defined period (usually 4–8 weeks). Over the last 2 decades, EEN has emerged as a highly effective treatment for the induction of remission in CD (Levine et al., 2018; Ashton et al., 2019). As a low-risk and steroid-sparing treatment, EEN is now the first-line therapy for pediatric CD patients. The reported remission rates with EEN treatment in this population of patients are up to 80% (Swaminath et al., 2017; Levine et al., 2018; Ashton et al., 2019). Consensus guidance from several organizations in Europe and North America (i.e., ECCO, ESPGHAN, ESPEN and NASPGHAN) directs clinicians to use EEN to induce remission in young CD patients wherever possible (Critch et al., 2012; Ruemmele et al., 2014; Forbes et al., 2017). It is suggested that corticosteroids, or early immunosuppressive therapy, should be reserved for the patients where EEN is not an option (Ruemmele et al., 2014; Ashton et al., 2019). There are reports that EEN is also effective in adult CD patients (Mowat et al., 2011; Yang et al., 2017). However, the evidence for the efficacy of EEN in adult patients is weaker than in pediatric population, possibly due to practicalities of its use (compliance, tolerability, etc.) (Ashton et al., 2019). Interestingly, there seems to be no established therapeutic role for EEN in the treatment of UC, an IBD that is without transmural inflammation and stricture formation as seen in CD (Ashton et al., 2019).

With EEN treatment, patients' disease activity, mucosal healing, bowel symptoms, and nutrition status are all significantly improved (Ashton et al., 2019). Systemic inflammatory markers, such as C-reactive protein and erythrocyte sedimentation rate are normalized, and often corrected before any detectable change in nutrition status (Bannerjee et al., 2004; Grover et al., 2014). However, the exact mechanisms for the therapeutic benefits of EEN on inflammation and gut function in CD are still not well known, though several theories have been tested or postulated. Originally, elemental liquid diet (with amino acids, but no whole proteins) was first tested in CD as a means to provide nutritional support (Voitk et al., 1973). Subsequent open and randomized controlled trials confirmed the benefits of exclusive elemental liquid diet therapy in inducing remission in active CD (Axelsson and Jarnum, 1977; O'Moráin et al., 1984). Some suspected that a reduced chance of allergic reaction with the elemental liquid diet

could be a possible reason for the efficacy. However, numerous follow-up studies found that nitrogen sources of enteral feeds are not relevant to their therapeutic efficacy. EEN treatments with either elemental or whole proteins-based polymeric liquid diets are equally effective in inducing remission in active CD (Giaffer et al., 1990; Verma et al., 2000; Zachos et al., 2007).

Many studies have attempted to examine if the EEN efficacy is attributed to improved gut microbiota. (Quince et al., 2015; Ashton et al., 2016; MacLellan et al., 2017). However, as all elemental and non-elemental diets are similarly effective in inducing remission of active CD, they lead to vastly different changes of microbiome in the gut (Ashton et al., 2016; Ashton et al., 2019). With EEN treatment, normal gut commensal bacteria (i.e. *Bacteriodes*, *Prevotella*, *Enterobacteriaceae*, etc.) have been reported to both increase and decrease in relative abundances (Leach et al., 2008; Kaakoush et al., 2015; Dunn et al., 2016). For instance, early studies found that *Faecalibacterium prausnitzii*, an anti-inflammatory commensal, was increased with EEN (Sokol et al., 2008); however more recent reports have shown a reduction of its abundance over the course of treatment with EEN (Jia et al., 2010). Systemic reviews of the most recent and well-documented studies suggest a paradoxical effect of EEN that it causes a reduction of bacterial diversity and richness and changes on the microbiome usually with a dysbiosis (Gatti et al., 2017; MacLellan et al., 2017). These changes are less likely to account for the benefits of EEN in CD, because reduced bacterial diversity and richness would predispose the gut to more inflammatory changes.

Inflammation in active CD is associated with increased intestinal permeability and damaged barrier function (Levine and Wine, 2013). Whether the increased permeability is the cause, or a consequence of CD is unknown. However, Sanderson et al. (1987) reported that EEN improved the abnormal permeability after 6 weeks of EEN treatment. *In vitro* studies on enterocytes found that EEN elemental formula components such as glutamine and vitamin D3 attenuated TNF- α -induced production of IL-8 but enhanced nitric oxide production in colonic epithelial cell line HT-29 (Alhagamhmad et al., 2017). However, this effect may not be due to the direct effects of elemental components, as polymeric diet and solid foods contain these components too, especially after they undergo mechanical and chemical digestions in the GI tract. Some studies observed that EEN treatment may directly reduce production of pro-inflammatory cytokines IL-6, IL-8, and TNF- α in the intestine (Heuschkel et al., 2000; Ashton et al., 2019). However, the mechanisms for the improved intestinal permeability and reduced cytokine production are not clear (Levine and Wine, 2013).

As discussed above, mechanical stress is an inevitable pathological change in gut inflammation, especially in CD where transmural inflammation and stenosis are present. Recent studies show that mechanical stress may contribute to the development of gut inflammation and dysfunction in IBD (Wehner et al., 2010; Lin et al., 2014b; Lin and Shi, 2021). We have evidence that the benefits of EEN in CD may be due to its action to reduce mechanical stress and attenuate

mechano-transcription of pro-inflammatory mediators in the gut (Lin et al., 2021a; Lin and Shi, 2021). In the rat model of Crohn's-like colitis induced by intra-colonic instillation of TNBS, EEN treatment with liquid diet (*Ensure*, Abbott) significantly improved body weight gain and reduced inflammation and disease activity in colitis rats. TNBS instillation induced a localized transmural inflammation with thickened wall and narrowed lumen in the distal colon and caused marked lumen distention with fecal retention in the segment proximal to the inflammation site (Lin et al., 2021a; Lin and Shi, 2021). However, EEN treatment eliminated lumen distention in the inflamed colon. Our study in the control rats with no TNBS instillation found that EEN treatment dramatically reduced fecal mass and pellet size, and increased water content of the fecal pellets. We found in TNBS colitis rats that mRNA expression of pro-inflammatory mediators such as IL-6, MCP-1, OPN and COX-2 was dramatically induced in the inflammation site (site I) and the distended segment proximal to inflammation (site P), but not in the non-distended segment distal to inflammation (site D). Strikingly, EEN treatment almost completely blocked the up-regulation of pro-inflammatory gene expression, suggesting that a mechanical stress-associated mechanism is involved in colitis-associated induction of pro-inflammatory genes (Lin et al., 2021a; Lin and Shi, 2021). Among these mechano-sensitive pro-inflammatory mediators, OPN is found to contribute to the development of IBD, particularly the Th1 immune response in Crohn's disease (Ashkar et al., 2000; Sato et al., 2005). Other mechano-sensitive pro-inflammatory mediators such as IL-6, MCP-1, and COX-2 in site I and site P all are also involved in the inflammatory process in the gut (Please refer to **Section 3**). Therefore, mechanical stress in the inflammation site and the distended segment prior to inflammation may play a pathogenic role in inflammation and gut dysfunction independent of the intrinsic inflammatory process. The benefits of EEN in inducing remission of inflammation may largely depend on the effect of EEN to reduce mechanical stress and attenuate the mechano-transcription process in the inflammation site and the site prior to the site of inflammation.

Targeting Mechano-Transcription Process for Fibrosis and Smooth Muscle Hyperplasia in Crohn's Disease

Stricture formation is a hallmark of severe Crohn's disease (CD), a B2 phenotype of CD in Montreal classification (Satsangi et al., 2006). The two main pathological changes involved in stricture formation are intestinal fibrosis and smooth muscle hyperplasia (Li and Kuemmerle, 2014; Latella and Rieder, 2017; Rieder et al., 2017). Currently, there is no effective medical treatment for the debilitating complication. Although anti-inflammatory treatments may be effective for active inflammation in CD, they do not appear to be effective in preventing or treating intestinal fibrosis and muscle hypertrophy.

Increasing evidence suggests that mechanical stress induces expression of pro-fibrotic mediators and extracellular matrix (ECM) proteins in gut smooth muscle cells. Gutierrez and Perr reported that static mechanical stretch significantly enhanced expression of transforming growth factor beta-1 (TGF- β 1) mRNA and protein in intestinal smooth muscle cells. Expression of type 1 collagen mRNA and protein was also increased by mechanical stretch in these cells (Gutierrez and Perr, 1999). Recent *in vivo* studies in our lab have shown that mechanical stretch not only increases production of collagen expression and ECM deposition, but also induces robust expression of pro-fibrotic mediators such as CTGF in the colon (Lin et al., 2021a). Expression of CTGF is increased not only at the site of inflammation, but also in the distended site proximal to inflammation in TNBS-induced CD-like colitis. CTGF has long been recognized to have potent effect on cell proliferation and production of ECM proteins such as collagens. CTGF expression was found increased in CD and UC not only in the inflammation area, but also in areas of little inflammation but severe fibrosis (Dammeier et al., 1998; Di Mola et al., 2004). Our recent studies in rats have revealed that mechanical stress induced CTGF in gut SMC, which may contribute significantly to fibrosis. We inhibited CTGF by administering anti-CTGF antibody (FibroGen, San Francisco, CA). Comparing to IgG control, anti-CTGF treatment significantly attenuated fibrosis in the site of inflammation and site proximal to inflammation (Lin and Shi, unpublished observation). Our study suggests that mechanical stress-induced pro-fibrotic mediators such as CTGF may represent a potential therapeutic target in battling fibrosis in Crohn's.

While fibrosis is a well-recognized change in stricture formation in CD, recent comprehensive histopathological analysis suggests that smooth muscle hyperplasia may be the most prominent histological change in fibrostenotic stricture in CD (Chen et al., 2017; Rieder et al., 2017). Mechanisms for smooth muscle hyperplasia in CD are not well understood. BDNF is known for its neurotrophic and nociceptive effects on neurons (Boesmans et al., 2008; Al-Qudah et al., 2014; Fu et al., 2018). However, most recent studies found that BDNF potently promotes SMC proliferation (Kwapiszewska et al., 2012; Freeman et al., 2017). Clinical studies found that BDNF expression is up-regulated in IBD tissues (Li et al., 2011; Steinkamp et al., 2012). We found that BDNF expression is highly responsive to mechanical stress in gut SMC (Fu et al., 2018), and BDNF is markedly induced in the inflammation site and pre-stenotic site in the CD rats (Lin et al., 2021a), as well as in the distended bowel in mechanical obstruction (Fu et al., 2018). Exogenous BDNF leads to robust proliferation of rat colon SMC. Anti-BDNF treatment or antagonist of BDNF receptor Trk B significantly reduces gut SMC proliferation (Lin and Shi, unpublished observation). These studies suggest that mechanical stress induced BDNF plays a key role in SMC hyperplasia in a preclinical model of CD and may be considered a potential therapeutic target for prevention or treatment of SMC hyperplasia and hypertrophy in fibrostenotic CD.

TARGETING MECHANO-TRANSCRIPTION PROCESS FOR THERAPEUTIC POTENTIALS IN FUNCTIONAL BOWEL DISORDERS

Mechano-Transcription and Irritable Bowel Syndrome

Irritable bowel syndrome (IBS) is the most common and best described type of functional bowel disorders, affecting nearly 11% of the general population in the US (Lovell and Ford, 2012; Camilleri, 2014). Along with visceral pain, abdominal distention and bloating are major complaints among IBS patients (Chang et al., 2001a; Zar et al., 2002; Azpiroz and Malagelada, 2005; Shim et al., 2010). It is reported that 76% of IBS patients have abdominal bloating and 57% have abdominal distention (Chang et al., 2001b). The exact reasons of abdominal distention are not well understood. However, current evidence suggests that it may be ascribed to excessive gas accumulation and impaired gas transit in the gut (Koide et al., 2000; Serra et al., 2001; Hernando-Harder et al., 2010). Intraluminal retention of fluid and solid contents and altered gut microflora are also considered possible reasons for bloating and abdominal distention (Chang et al., 2001a; Agrawal et al., 2009; Sweetser et al., 2012). Some investigators found that ineffective evacuation, resulting in fecal retention in the colon and rectum, may well contribute to abdominal distention in IBS patients (Sweetser et al., 2012; Camilleri, 2014; Raahave and Jensen, 2021). Thus, abdominal distention in IBS is largely due to luminal retention of gas, liquid, or solid contents in the GI tract. The luminal retention of gas, liquid, and solid contents clearly represent mechanical stress to the gut wall [Lin et al., 2015].

As abdominal pain and distention are co-present in nearly 70% of IBS patients (Chang et al., 2001b), several groups have tried to determine whether distention contributes to abdominal pain. Earlier studies have focused on the immediate effect of repetitive distensions in the distal colon to noxious pressure levels (Ness et al., 1990; Gschossmann et al., 2001; Million et al., 2006). In general, a pressure is considered noxious when it is greater than 40 mmHg (Ness et al., 1990). In both humans and rats, repetitive colonic tonic distention (i.e., 60 mmHg for 10 min) resulted in visceral hypersensitivity (Ness et al., 1990; Gschossmann et al., 2001; Million et al., 2006). Moreover, luminal distention at noxious pressure (60 mmHg) induced changes in neuropeptide expression and ERK 1/2 activation in the dorsal horn (Lu et al., 2005; Million et al., 2006). These data suggest that colon distention at noxious pressure may induce acute visceral hypersensitivity *via* a central sensitization mechanism (Munakata et al., 1997; Lin et al., 2015; Shi et al., 2018).

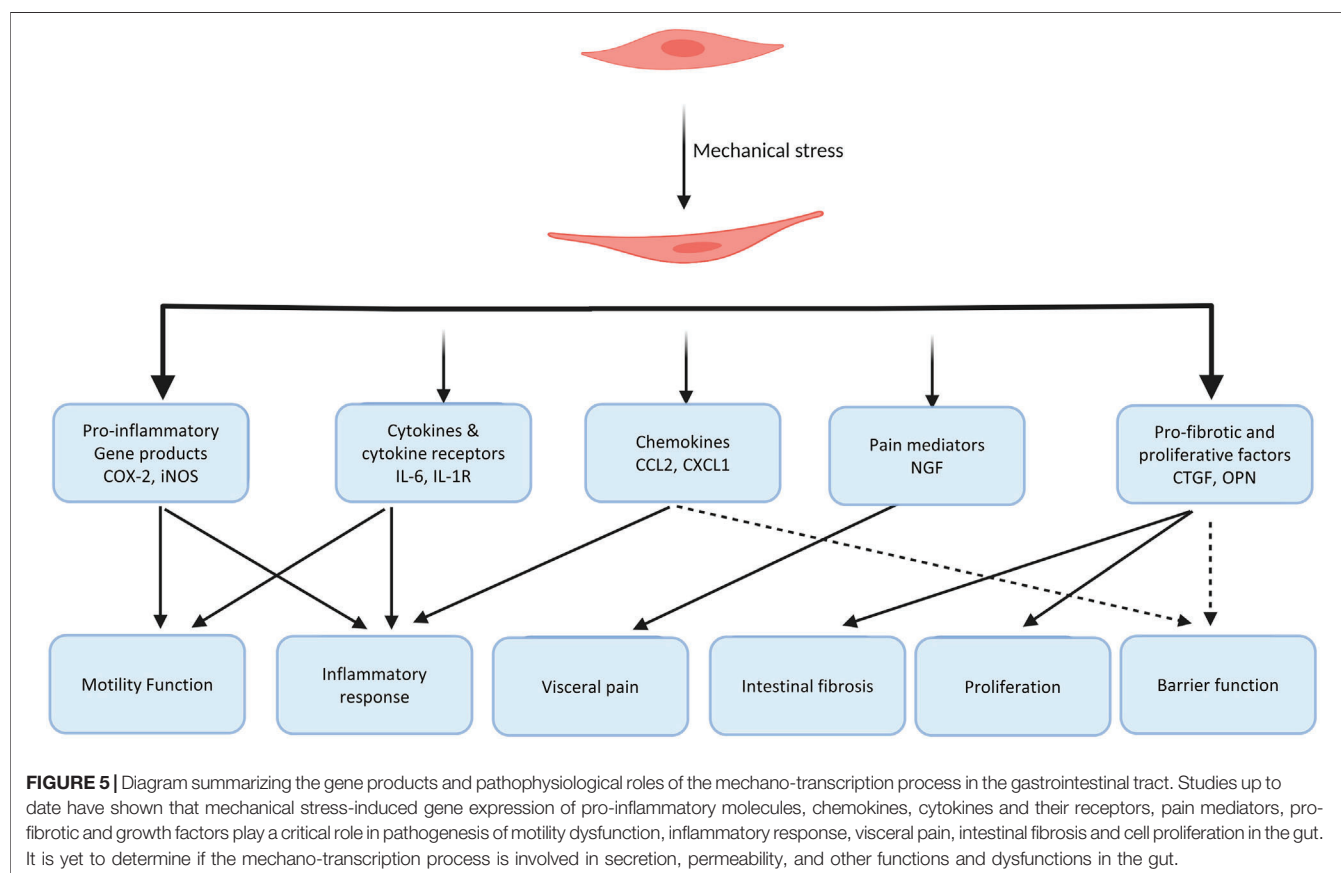
Further studies found that colon distention may also contribute to chronic visceral hypersensitivity (Al-Chaer et al., 2000; Lin et al., 2015). Al-Chaer found that repetitive colon distention (60 mmHg) with a balloon in rats during neonatal stage led to visceral hypersensitivity detectable in adult stage and suggested that peripheral sensitization may be involved (Al-Chaer et al., 2000). Lin et al. (2015) tested colon distention in

adult rats and found that tonic distention of the distal colon with a balloon at sub-noxious levels (20–40 mmHg) for 40 min led to significantly increased visceral sensitivity. The state of visceral hypersensitivity remained for at least 3 days. Electrophysiological studies showed that excitability of colon projecting sensory neurons in the dorsal root ganglia was significantly enhanced. Interestingly, they found that the sub-noxious mechanical distention induced expression of COX-2 and increased release of PGE₂ in colonic muscularis externae, but not in the mucosa layer (Lin et al., 2015). Importantly, treatment with COX-2 inhibitor NS-398 abolished distention-induced production of PGE₂, and significantly attenuated visceral hypersensitivity. These reports demonstrate that mechanical distention-induced production of inflammatory mediators, e.g., PGE₂, contributes to visceral hypersensitivity in “functional” bowel disorders with lumen distention. PGE₂ is found significantly increased in diarrhea-dominant IBS patients in the mucosa samples obtained by biopsy (Grabauskas et al., 2015), and is found to be the main cause of visceral hypersensitivity in IBS patients and animal models (Grabauskas et al., 2020). It is not known whether PGE₂ is increased in IBS patients in the smooth muscle layer, which is the very site for mechanical stress-induced increase of COX-2 and PGE₂. This is mainly because the smooth muscle layer is not accessible with a conventional biopsy. Nevertheless, COX-2 expression and PGE₂ production were found significantly increased in smooth muscle layer of the colon obtained in surgery in patients with chronic constipation (Cong et al., 2007).

Several other mechanosensitive pain mediators like NGF and BDNF were found to be substantially increased in the bowel tissues of IBS patients compared to normal subjects (Yu et al., 2012; Dothel et al., 2015). These mediators are known to contribute to visceral hypersensitivity and abdominal pain in IBS (Dothel et al., 2015; Coelho et al., 2019). These studies did not specify the cause(s) for the increased expression of NGF and BDNF in the IBS tissues. However, mounting evidence suggests that expression of NGF and BDNF in the gut is highly inducible by mechanical stress, i.e., lumen distention (Lin et al., 2017; Fu et al., 2018). Thus, targeting mechano-transcription of inflammatory and pain mediators may have great therapeutic potentials for IBS (Shi et al., 2018).

Mechano-Transcription and Other Functional Bowel Disorders

Among other functional bowel disorders, chronic constipation and fecal retention may present the most significant mechanical stress in the distal colon and rectum (Bharucha et al., 2006; Heredia et al., 2012; Raahave, 2015; Lin et al., 2021b). In chronic functional constipation, slowed colonic transit and decreased frequency of bowel movement directly lead to fecal accumulation or impaction in the distal bowel (Heredia et al., 2012; Lin et al., 2021b). As a separate entity of functional bowel disorders, fecal retention may be functional (voluntary withholding of stool) or due to obstructed defecation (Raahave, 2015; Raahave and Jensen, 2021). In either of the conditions, increased fecal accumulation in the distal bowel imposes apparent



mechanical stretch to the bowel tissues, and thus may lead to mechano-transcription of select bioactive mediators (Lin et al., 2021b). A *Gastroenterology* study found that COX-2 expression and PGE₂ production were significantly increased in the colonic muscularis externa tissues in patients with slow transit constipation (Cong et al., 2007). COX-2 expression was also found to be significantly up-regulated in the distended colon in slow transit constipation generated experimentally by outlet obstruction (Heredia et al., 2012), or by opioid-induced constipation (Lin et al., 2021b). Increased inhibitory prostaglandins such as PGE₂ were found to be responsible for the diminished contractility in slow transit constipation in human (Liu et al., 2009) and in animal models (Heredia et al., 2012; Lin et al., 2021b). In fact, administration of COX-2 inhibitor NS-398 was found to be effective to improve bowel movement in constipation in chronic morphine treatment (Lin et al., 2021b). Fecal retention in the rodent model of opioid-induced bowel dysfunction also leads to significant increase of pain mediator NGF. Interventional studies suggest that the mechanical stress-induced pain mediators such as NGF may account for paradoxical hyperalgesia and narcotic bowel syndrome in chronic morphine treatment (Lin et al., 2021b).

Mechanical stress is also present in functional bowel disorders in the upper GI tract. In gastroparesis, for instance, slowed gastric emptying rate leads to increased retention of foods in the stomach as demonstrated in gastroparesis patients (Azpiroz et al., 2014; Steinsvik et al., 2021). When full thickness gastric tissues were available for studies, several groups reported that COX-2 expression was increased in the gastric tissues in animal models of gastroparesis induced by glucagon (Chen et al., 2017), lipopolysaccharide (West et al., 2006), or stress (Li et al., 2016). Partial obstruction of the gastric outlet leads to marked gastric retention and distention in the stomach, which is associated with dramatic induction of COX-2 (Lin et al., 2012a). Moreover, *in vivo* administration of COX-2 inhibitors improved gastric motor function in gastroparesis models (Lin et al., 2012a; Chen et al., 2017).

These studies suggest that mechanical stress-induced expression of COX-2 and production of prostaglandins may represent common potential targets for “functional” disorders with luminal distention such as constipation, fecal retention, and gastroparesis. Currently available medical treatments for these disorders rely on prokinetics or secretagogues to arbitrarily stimulate smooth muscle contractions or increase mucosal secretion (Bharucha et al., 2006; Sharma and Rao, 2017). Long

term efficacy of these treatments has been compromised. However, inhibition of mechano-transcription process represents a promising therapeutic strategy, as it targets mechanical stress—perhaps the root cause of bowel dysfunctions in these disorders.

CONCLUSION

Mechanical stress, i.e., shear, stretch, and compression, is a common phenomenon encountered in the GI tract. Excessive mechanical stress is the root cause of obstructive conditions and is constitutively present in inflammatory conditions and some functional bowel disorders. Different tissues and cells in the GI tract respond differently to mechanical stress. In this review, we have focused on the recent discoveries of mechanical stress-altered gene expression, i.e., mechano-transcription, in the gut. Numerous *in vitro* and *in vivo* studies have demonstrated that mechanical stress profoundly alters gene expression in the GI tract especially smooth muscle cells. Mechano-transcription of pro-inflammatory molecules, pain mediators, fibrogenic and growth factors plays a critical role in motility dysfunction, abdominal pain, inflammation, fibrosis, and hyperplasia in various gastrointestinal disorders (Figure 5). We have shown pre-clinical and clinical evidence that mechanical stress-induced COX-2 and other pro-inflammatory mediators in gut smooth muscle cells account for motility dysfunction and inflammatory process in obstructive, inflammatory and some functional disorders. Mechanical stress-induced up-regulation of pain mediators such as nerve growth factor and brain-derived neurotrophic factor, and down-regulation of opioid receptors in the

peripheral tissues may lead to visceral hypersensitivity. Emerging evidence suggests that mechanical stress in the gut also leads to up-regulation of certain genes encoding pro-fibrotic and proliferative mediators such as connective tissue growth factor and osteopontin, which may contribute to fibrostenotic Crohn's disease. It is yet to determine whether the mechano-transcription process is involved in secretion, permeability, and other pathophysiological changes in the GI tract. Taken together, the studies over the last decade suggest that the mechano-transcription process may represent novel therapeutic targets for the management of obstructive, inflammatory, and functional bowel disorders. The strategy to target mechano-transcription process has a unique advantage, as it addresses one of the root causes (mechanical stress) of these disorders. Further studies of these targets are warranted before therapeutics can be developed for clinical use.

AUTHOR CONTRIBUTIONS

RG and X-ZS drafted the original article. RG, Y-ML, KZ, and X-ZS reviewed and revised the article. X-ZS and RG finalized the article. All have reviewed the final version.

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5.09 Mechano-Regulation of Gene Expression in the Gut: Implications in Pathophysiology and Therapeutic Approaches in Obstructive, Inflammatory, and Functional Bowel Disorders

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Glossary

Gut motility Ability of the gastrointestinal tract to mix and propel intraluminal contents by contraction and relaxation of the smooth muscles.

Hyperplasia Increase in the number of cells.

Hypertrophy Increase in size of cells or tissue mass.

Intestinal fibrosis Excessive accumulation of connective tissue in the intestinal wall.

Mechanical stress in the gut Mechanical force acting on gut tissues (per unit area), manifested mainly as shear, stretch, and compression.

Mechano-transcription Process by which mechanical stress alters gene transcription in the cells.

Mechano-transduction Signaling process by which mechanical stress leads to cellular, molecular and genetic responses in the cells.

Visceral hypersensitivity A state of increased nociceptive sensation experienced in visceral organs.

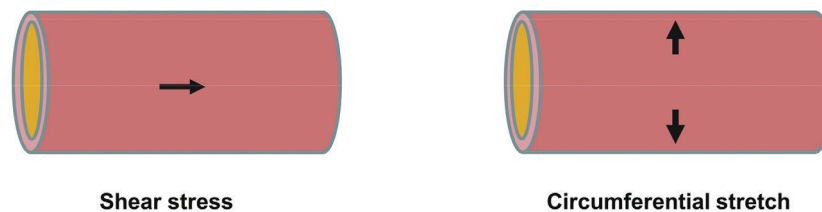
Visceral pain Nociception arising from neural sources in deep organs of the body, i.e. heart, airways, gastrointestinal tract, pancreas.

5.09.1 Introduction: Mechanical stress in the gastrointestinal tract

The gastrointestinal (GI) tract is constantly subjected to mechanical stimuli (Shi, 2017; Sarna and Shi, 2006). On the one hand, contracting forces generated by gut smooth muscle mechanically digest foods in the lumen and move the intraluminal contents down through the GI tract (Sarna and Shi, 2006; Murthy, 2006; Kraichely and Farrugia, 2007). On the other hand, the presence and movement of intraluminal contents (foods, gas, and fluids) create mechanical stress (MS) on the gut wall: i.e. shear stress and pressure (Shi, 2017). Shear stress is a transient force generated at the mucosa surface tangential to the GI tract, whereas intraluminal pressure creates a circumferential mechanical stretch perpendicular to the gut wall (Fig. 1). As the shear stress is generated at the mucosa surface, it mainly affects mucosa and submucosa layers especially in physiological processes, i.e. digestion and absorption. The impact of shear stress on epithelial cells and enterochromaffin cells have been reviewed previously (Gayer and Basson, 2009; Linan-Rico et al., 2016; Beyder, 2019). In contrast, circumferential stretch may influence whole gut wall including smooth muscle cells (SMC), enteric nervous system (ENS), and interstitial cells of Cajal (ICC) in the deeper muscularis externa layer. Circumferential mechanical stretch is most remarkable in pathological conditions such as obstructive, inflammatory, and functional bowel disorders. However, the impact of mechanical stretch on bowel functions in these disorders have not been discussed comprehensively.

Under homeostasis, the intraluminal pressure in the intestine is nearly 0 cmH₂O (Summers, 1999; Silen, 2005). However, if the movement of intraluminal contents is blocked, as in bowel obstruction, the intraluminal pressure increases to 8–10 cmH₂O in the segment proximal to obstruction (Silen, 2005; Shikata et al., 1983). When peristalsis occurs, the intraluminal pressure may reach 30 to 60 cmH₂O in the obstructed bowel (Silen, 2005; Shikata et al., 1983). Obstruction also leads to overload of luminal contents (food contents, fluids and gas) proximal to the occlusion, leading to lumen distention. Thus, the circumferential mechanical tension on the gut wall (product of pressure and radius) will be greatly increased in obstruction, according to Laplace's law (Shi, 2017; Russell and Welch, 1990). Unfortunately, many GI conditions are associated with obstruction and lumen distention, hence mechanical stress. These conditions are classified as obstructive bowel disorders (OBD) (Shi et al., 2011; Lin et al., 2012b), a major health concern in both adults and children (Summers, 1999; Silen, 2005; Russell and Welch, 1990). Lumen distention in OBD may be due to mechanical or functional obstruction (Summers, 1999; Silen, 2005; Russell and Welch, 1990). Functional obstruction results from neuromuscular dysfunction, such as in achalasia, pyloric stenosis, ileus, intestinal pseudo-obstruction, and Hirschsprung's disease (Summers, 1999; Russell and Welch, 1990; De Giorgio et al., 2011; Nunez et al., 2009). Mechanical obstruction may originate extrinsic to the intestine, e.g., adhesions and hernias, or intrinsic to the intestine, e.g., carcinoma and diverticulitis. Adhesions and hernias are the most common causes of small bowel mechanical obstruction, whereas carcinoma and sigmoid diverticulitis

(A) Mechanical stress originating from the intraluminal contents



(B) Mechanical stress originating within the gut tissues

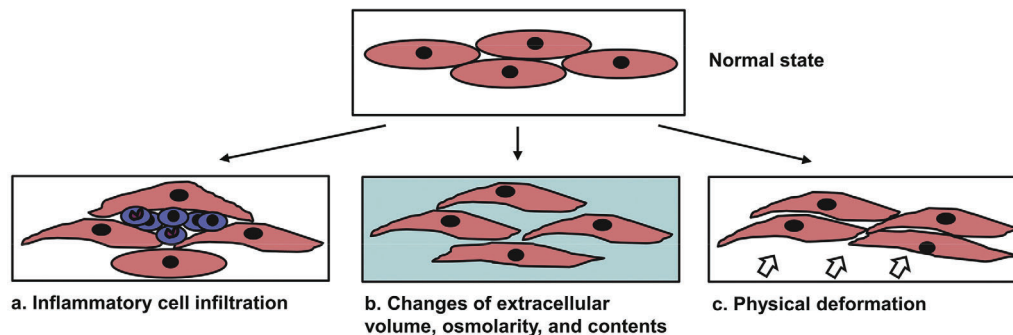


Fig. 1 Mechanical stress in the gut may originate from the intraluminal contents (A) or from inside the tissues (B). (A) The presence and movement of intraluminal contents create shear stress and pressure. Shear stress is a transient force generated at the mucosa surface tangential to the GI tract, whereas intraluminal pressure creates a circumferential mechanical stretch perpendicular to the gut wall. (B) Physical stretch and compression exist inside the gastrointestinal tissues in physiological state (i.e. transient muscle contraction and relaxation), or pathological conditions such as in inflammation with inflammatory cell infiltrations, edema, extracellular matrix deposition, and deformation.

constitute most of the causes for colonic obstruction (Summers, 1999; Silen, 2005; Russell and Welch, 1990). Mechanical bowel obstruction (BO) is the most common OBD, and accounts for more than 300,000 hospital admissions per year in the US alone (Silen, 2005; Russell and Welch, 1990; Milenkovic et al., 2006). The aggregate cost for hospital stays in BO is more than \$2.7 billion annually, topping all other GI conditions (Milenkovic et al., 2006).

Although circumferential mechanical stretch is most prominent in obstructive bowel disorders, it is also well present in functional and inflammatory disorders. Distention of the distal colon and rectum due to fecal accumulation is apparent in functional disorders such as fecal retention and chronic constipation (Raahave, 2015; Heredia et al., 2012; Lin et al., 2021a). Abnormal gas transit, lumen distention, and fecal retention all have been reported in irritable bowel syndrome (Koide et al., 2000; Serra et al., 2001; Raahave and Jensen, 2021). In the upper GI tract, gastric retention and distention associated with slow gastric emptying rate are pathological characteristics in gastroparesis (Azpiroz et al., 2014; Steinsvik et al., 2021).

Mechanical stress on gut tissues may not only originate from the intraluminal contents, but also from inside the tissues themselves (Fig. 1). In gut inflammation, physical stretch and compression exist in the GI tissues, where inflammatory cell infiltrations, tissue deformation, edema, and extracellular matrix deposition (fibrosis) are all considered as mechanical stress (Gayer and Basson, 2009; Levy Nogueira et al., 2015; Cox Jr et al., 2008; Johnson et al., 2013). Furthermore, these changes at the inflammation site may cause partial obstruction (inflammatory stenosis), leading to lumen distention in the bowel segment prior to the site of inflammation (circumferential mechanical stretch) (Shi, 2017; Katsanos et al., 2010). These changes are well represented in transmural inflammation especially stenotic Crohn's disease, where stenosis is caused not only by inflammation, but also by fibrosis-associated stricture formation (Katsanos et al., 2010; Rieder et al., 2013).

Morphological and functional changes in response to excessive mechanical stress in the GI tract have been well documented (Summers, 1999; Thompson, 2006). These include motility dysfunction (Summers et al., 1983a; Prihoda et al., 1984), visceral hypersensitivity (Huang and Hanani, 2005; Lin et al., 2017), muscle hypertrophy (Cabella, 1975, 1990), and injuries in the ENS and ICC (Wedel et al., 2002; Chang et al., 2001a). However, different cells may respond to mechanical stress very differently. For example, mechanical stretch in a rat model of bowel obstruction leads to injury and disruption in ENS and ICC network 7 days after induction of obstruction (Wu et al., 2013; Lin et al., 2014a). The same stress for the same time, however, leads to hyperplasia of gut smooth muscle cells associated with mechanical stress-altered gene transcription, a process referred as "mechano-transcription" (Shi et al., 2011; Li et al., 2012a). The functional and morphological changes are responsible for symptoms such as nausea, vomiting, dysmotility, abdominal distention, pain, and constipation in obstructive conditions (Shi, 2017; Summers, 1999; Russell and Welch, 1990). However, mechanisms underlying mechanical stress-associated dysfunctions are not well understood.

To address the pathophysiological mechanisms, most of the early studies focused on the dysfunctions several weeks after the introduction of bowel obstruction, a prototype of in vivo mechanical stretch (Cabella, 1975, 1990; Wedel et al., 2002; Chang et al., 2001a; Bertoni et al., 2004; Won et al., 2006). Although this approach may be able to document dramatic changes after obstruction, it would have missed some important early molecular events, which could eventually lead to the latent functional and morphological changes. Over the last decade, we and others have tested a new theory in the understanding of bowel dysfunctions in response to mechanical stress. We hypothesized that mechanical stress regulates expression of mechanosensitive genes in the gut, and in the long run, the process of mechano-transcription may account for bowel dysfunctions (Shi et al., 2011; Li et al., 2012a; Wehner et al., 2010). Mounting evidence now suggests that mechano-transcription plays a crucial role in motility dysfunction, visceral hypersensitivity, inflammation, and fibrosis in obstructive, inflammatory, and functional disorders.

In this chapter, we will first review the effects of mechanical stress in the gut wall. We will focus on the process of mechano-transcription, its signal transduction mechanisms and pathophysiological significance in the gut. In addition, we will highlight the potential therapeutic targets of the mechano-transcription process for the management of obstructive, inflammatory, and functional disorders.

5.09.2 Mechano-transcription in the gastrointestinal tract

5.09.2.1 Mechano-regulation of gene expression in the gut

To examine mechanical stress-induced gene expression in the gut, in vitro and in vivo stretch models have been developed. The Flexcell system is a well-established in vitro model to study mechanical stretch in cultured cells (Gayer and Basson, 2009; Shi et al., 2011; Li et al., 2012a; Wehner et al., 2010). In this system, the computer-regulated bioreactor applies finely controlled multi-axial static or cyclic strains through vacuum pressure to cells cultured on flexible membrane plates. Applying this in vitro model of mechanical stretch, several labs tested the effect of mechanical stretch on pro-inflammatory gene expression in gut smooth muscle cells (Shi et al., 2011; Wehner et al., 2010). This system has also been used to test the effect of mechanical stress in other cells such as intestinal epithelial cells and macrophages (Gayer and Basson, 2009; Wehner et al., 2010; Docsa et al., 2020).

Mechanical bowel obstruction (BO) is the prototype of obstructive disorders in the GI tract. We have used an in vivo model of partial colon obstruction to investigate mechanical regulation of gene expression and bowel functions (Shi et al., 2011; Lin et al., 2012b, 2017; Li et al., 2012a). To induce partial colon obstruction in rats, a 3-mm wide medical grade silicon band is placed around the distal colon at about 3 cm proximal to the anus. The length of the 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 significant partial rather than complete obstruction (Shi et al., 2011). This model is unique, as one can compare not only the obstructed animal with sham operated control animal, but also tissues from the opposite sides of the obstruction in each animal with obstruction: the

mechanically stretched oral segment and the non-stretched aboral segment. By using this *in vivo* model, one can also compare the biology of different tissues and cells of the gut wall under mechanical stretch or not.

To better understand the scope of the biological impacts of mechanical stretch on gene expression in gut SMC, we used the *in vivo* model of partial colon obstruction to screen for mechanosensitive genes in a comprehensive Affymetrix cDNA array analysis (Shi, 2017; Shi et al., 2011). In that study, the colon segments oral and aboral to the obstruction site from three rats were collected after obstruction for 1 day. As a control, the mid colon from sham operated rats was also obtained. Total RNA was isolated from the colonic muscularis externa of the animals, reverse transcribed and hybridized with 28,700 candidate genes (each duplicate). We found that mechanical stretch dramatically altered the gene expression profile in the distended bowel segment oral to the obstruction site, compared to sham control or the non-stretch aboral segment (Fig. 2). The mRNA levels of 309 genes were increased more than twofold, whereas that of 282 genes was decreased more than twofold in the mechanically stretched tissues, comparing to the non-stretch tissues (aboral to obstruction). The gene expression profile in the colon tissue of sham controls was very similar to the non-stretch segment in BO rats. Overall, we identified several major groups of genes whose expression is altered by mechanical stretch, including those encoding certain pro-inflammatory mediators, growth factors, pain mediators, adhesion molecules, extracellular matrix proteins, and some cell signaling proteins (Shi, 2017; Shi et al., 2011). This work demonstrates that mechanical stretch substantially alters the gene expression profile in the gut muscle. This altered gene expression may underlie the mechanisms of bowel dysfunctions in obstructive conditions.

5.09.2.2 Mechano-transcription of COX-2 gene in gut smooth muscle cells

One of the upregulated genes detected in the mechanically distended bowel is prostaglandin-endoperoxide synthase-2, also known as cyclooxygenase-2 (COX-2). In fact, the array study detected a 19-fold increase of COX-2 mRNA expression in the distended colon of BO rats, compared to the non-distended colon segment aboral to the site of obstruction. The enzyme cyclooxygenase catalyzes the major rate-limiting step of the synthesis of prostaglandins (PGs), which have profound impacts on gut inflammation, cell proliferation, motility, and visceral pain in the GI tract (Fornai et al., 2005; Krause and DuBois, 2000; Mohajer and Ma, 2000). While COX-1 is considered a constitutive isoform of COX in most cell types, COX-2 is the inducible form (Krause and DuBois, 2000; Mohajer and Ma, 2000). Our corroborative studies found that the levels of COX-2 mRNA and protein in the muscularis externa were dramatically increased in the stretched colon segment oral to obstruction, but not in the non-stretch aboral segment (Fig. 3) (Shi et al., 2011). Compared with the sham controls, COX-2 mRNA and protein levels increased more than 20-fold at 12 and 24 h in the stretched oral segment and remained up-regulated in the following 7 days of BO. To the contrary, COX-1, a constitutively expressed cyclooxygenase, was not significantly altered by obstruction at any of the time points (Shi et al., 2011). We found that COX-2 gene expression was induced only in the muscle layer, but not in the mucosa or submucosa. Further immunohistochemical studies in the full-thickness colon specimens confirmed that the increased COX-2 expression occurs selectively in the circular and longitudinal SMC, but not in the mucosa, submucosa, or myenteric plexus (Fig. 3). These studies suggest that mechano-transcription of COX-2 in bowel obstruction is a smooth muscle specific phenomenon.

The reasons for the selective expression of COX-2 in SMC by mechanical stretch are not well understood. However, Choudhury et al. found that mechano-transcription of COX-2 in colonic SMC was blocked by de-polymerization of actin filaments with latrunculin B or swinholide (Choudhury et al., 2015). Mechano-transcription of COX-2 was also markedly attenuated by siRNA silence of

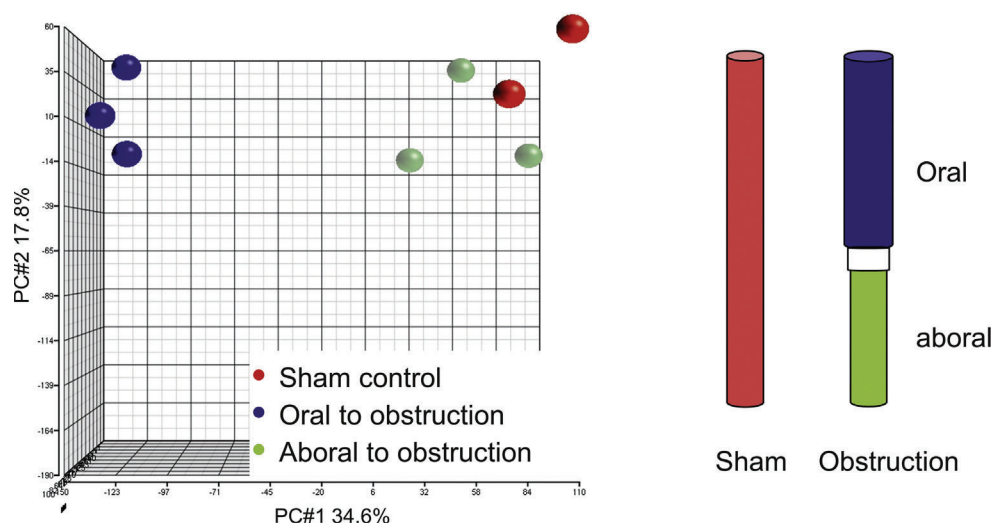


Fig. 2 Venn analysis of Affymetrix cDNA array results to compare gene expression profiles in colon muscularis externa tissues oral (blue circles) and aboral (in green) to the obstruction band in three obstruction rats. Tissue samples from two sham control rats (in red) were taken as a reference. The right panel is a diagram showing where the colon tissue samples were taken from.

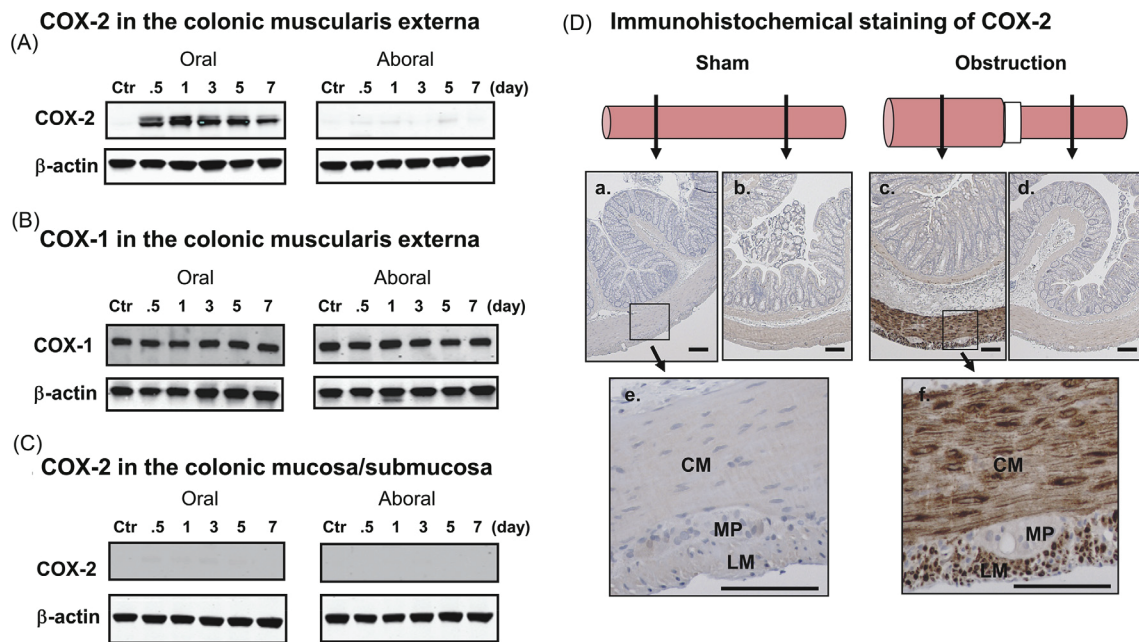


Fig. 3 Mechanical stress-induced up-regulation of COX-2 selectively in gut smooth muscle cells. (A) Western blot detection of COX-2 expression in the colonic muscularis externa in segments oral (left) and aboral (right) to the obstruction band. (B) Western blot detection of COX-1 in the colonic muscularis externa in segments oral (left) and aboral (right) to the obstruction band. (C) Western blot detection of COX-2 in the mucosa/submucosa layer in segments oral (left) and aboral (right) to the obstruction band. (D) Immunohistochemical staining of COX-2 expression in segments oral (a, c) and aboral (b, d) colon segments in a sham operated rat (a, b) and a rat with obstruction (c, d) for 3 days. Note that COX-2 (stained in brown) is detected only in smooth muscle cells in distended oral segment (e and f). The results were representative of four independent experiments. Calibration bars represent 50 μ m. CM, circular muscle; LM, longitudinal muscle; MP, myenteric plexus. Some parts of the figure are adapted with permission from Fig. 1 of Shi XZ (2017) Mechanical regulation of gene expression in gut smooth muscle cells. *Frontiers in Physiology* 8: 1000. doi: 10.3389/fphys.2017.01000. eCollection 2017.

smooth muscle specific α -actin (Acta2) (Choudhury et al., 2015). These data indicate that smooth muscle specific α -actin with an intact actin filament network plays a critical role in mechanical stress-induced up-regulation of COX-2 in colonic SMC. Furthermore, Li et al. reported that mechanical stretch potently activates mitogen-activated protein kinase (MAPK) p38 and protein kinase C (PKC), which are important components of signaling cascade in mechano-transcription of COX-2 (Li et al., 2012b). However, treatments with Acta2 siRNA or latrunculin B or swinholide also blocked stretch-induced activation of p38 and PKC (Choudhury et al., 2015). Thus, an intact smooth muscle actin network is required for linking the mechanical signal to activation of MAPK and PKC and, thus, for mechano-transcription of COX-2. These studies may partly explain why mechano-transcription of COX-2 is a smooth muscle specific mechanism in BO.

Lin et al. studied the outcomes of lumen distension on COX-2 expression in different parts of the GI tract by placing the obstruction band in the lower esophagus, pyloric sphincter, ileum, and colon, respectively. They found that obstruction associated expression of COX-2 mRNA and protein was up-regulated dramatically in all the sites where distention was induced (Lin et al., 2012b). Moreover, when lumen distention of the colon was induced with a balloon at a pressure of 40 mmHg for 40 min, the distended segment demonstrated a significant up-regulation of COX-2. This did not occur when the pressure was set 10 mmHg or less, or when the distention period was 20 min or shorter (Lin et al., 2015). Together, these studies suggest that obstruction-induced mechano-transcription of COX-2 in the GI tract is a force- and time-dependent, and smooth muscle-specific phenomenon. Furthermore, mechano-transcription is a common mechanism in response to lumen distention throughout the GI tract.

5.09.2.3 Signal transduction mechanisms of mechano-transcription in the gut

COX-2 and COX-2-derived prostaglandins have profound impacts in the G.I. tract (Krause and DuBois, 2000; Mohajer and Ma, 2000). Mechano-transcription of COX-2 in the gut has important pathophysiological significance in obstructive conditions (Shi et al., 2011; Lin et al., 2017). We then investigated the signaling mechanisms linking mechanical stretch to induction of COX-2 gene expression in colonic SMC (Li et al., 2012a,b). Static mechanical stretch of cultured rat colonic SMC at 18% elongation robustly increased expression of COX-2 mRNA and protein. Stretch also markedly induced phosphorylation of MAPKs including extracellular signal-regulated kinases (ERKs, i.e. ERK 1 and ERK2), MAPK p38, and c-Jun N-terminal kinases (JNKs) (Li et al., 2012a). The phosphorylation of MAPKs was most significant during early time points at 5 min and lasted for at least 30 min. Treatment of the cells with MAPK inhibitor PD98059, p38 inhibitor SB203580 or JNKs inhibitor SP600125 all significantly inhibited MT

of COX-2. These studies depict that all three major MAPK members are involved in the regulation of mechano-transcription of COX-2 in colonic SMC (Li et al., 2012a).

Mechanical signals must be sensed at the cell membrane level before it is transduced into the cytoplasm and nucleus (Adam et al., 2004; Kanefsky et al., 2006; Ruwhof and van der Laarse, 2000; Yamaguchi, 2004). Integrins and stretch-activated ion channels (SACs) are two major groups of mechanosensors identified in various types of cells (Kraichely and Farrugia, 2007; Gillespie and Walker, 2001; Hu and Sachs, 1997; Katsumi et al., 2004). As $\alpha v\beta 3$ is the major type of integrins in gut SMC (Kuemmerle, 2006), we investigated whether $\alpha v\beta 3$ is a mechano-sensor involved in MT of COX-2 in rat colonic SMC. It was found that the stretch-induced up-regulation of COX-2 mRNA and protein was almost completely inhibited by treatment with echistatin, a specific inhibitor to $\alpha v\beta 3$, or by neutralizing antibody against rat $\beta 3$ (Li et al., 2012a). Further study showed that stretch-induced phosphorylation of p38, but not ERKs and JNKs, was significantly inhibited by echistatin treatment. The downstream pathway of integrin signaling was also found to be involved in mechano-transcription of COX-2 in gut SMC, as gene knockdown of integrin-linked kinase (ILK) and focal adhesion kinase (FAK) with specific siRNAs significantly suppressed mechano-transcription of COX-2 (Li et al., 2012a). We further examined if SACs play a role in mechano-transcription of COX-2 (Li et al., 2012a). General SAC inhibitor gadolinium (Ducret et al., 2010) or specific SAC blocker GsMTx-4 (Suchyna et al., 2000) significantly inhibited stretch-induced expression of COX-2 mRNA and protein (Li et al., 2012a). Treatments with gadolinium or GsMTx-4 also inhibited stretch-induced activation of all MAPKs ERK1, ERK2, p38, and JNKs.

Subsequent studies found that mechanical stretch also activated protein kinase C (PKC) and protein kinase D (PKD) in colonic SMC (Li et al., 2012b). Inhibition of PKC β or PKC ζ did not significantly block stretch-induced expression of COX-2. However, the PKC δ inhibitor rottlerin almost completely blocked stretch-induced COX-2 expression. PKD inhibitor CID755673 or PKD siRNA also significantly inhibited stretch-induced COX-2 expression. Interestingly, Rottlerin treatment inhibited stretch-induced activation of all MAPKs ERKs, p38, and JNKs, whereas CID755673 solely blocked activation of p38 (Li et al., 2012b).

Together, these studies have demonstrated that mechanical stretch on gut SMC is sensed by integrins and SAC at the cell membrane level. Then, these mechano-sensors transduce mechanical stimulus to the intracellular signaling pathways involving PKCs, PKD, and MAPKs to induce gene transcription of COX-2 in colonic SMCs (Li et al., 2012a,b). Further studies suggest that PKC δ is coupled to MAPKs ERKs, p38, and JNKs, whereas PKD is coupled to MAPK p38 (Li et al., 2012a,b; Shi, 2017) (Fig. 4).

5.09.3 Pathophysiological significance of mechano-transcription in gastrointestinal disorders

5.09.3.1 Pathophysiological role of mechano-transcription in motility dysfunction

Motility dysfunction has long been documented in obstructive conditions. Earlier in vivo studies found that gut motor activity increased in the distended oral segment immediately after induction of obstruction (Summers, 1999; Prihoda et al., 1984). However, the motor activity gradually decreased within hours after initiation of obstruction (Summers, 1999; Bertoni et al., 2004; Won et al., 2006; Fraser et al., 1980). The early phase of hyper-motility oral to the obstruction may result from neuronal mechanisms similar to the physiological peristalsis reflex (Summers, 1999; Prihoda et al., 1984; Grider, 1989). According to the

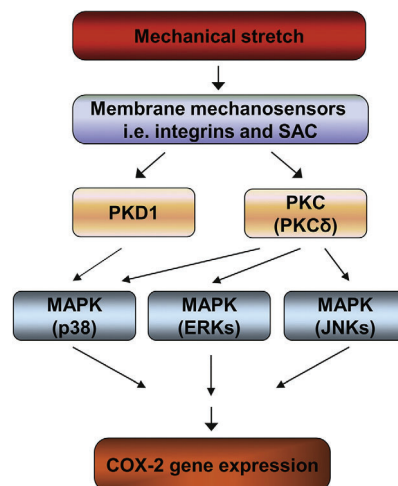


Fig. 4 Cell signaling mechanisms of mechano-transcription of COX-2 in colonic smooth muscle cells (SMC). Mechanical stretch on gut SMC is sensed by integrins and SAC (stretch-activated ion channels) at the cell membrane level. The mechano-sensors transduce mechanical stimulus to intracellular signaling pathways involving PKC (i.e. PKC δ), PKD (PKD1), and MAPKs (p38, ERKs, and JNKs) to induce mechano-transcription of COX-2 in colonic SMC. More specifically, PKC δ is coupled to MAPKs ERKs, p38, and JNKs, whereas PKD1 is coupled to MAPK p38. Some parts of the figure are adapted with permission from Fig. 2 of Shi XZ (2017) Mechanical regulation of gene expression in gut smooth muscle cells.

Frontiers in Physiology 8: 1000. doi: 10.3389/fphys.2017.01000. eCollection 2017.

peristalsis reflex theory, food chyme stimulates mechano-receptors in the gut wall to initiate neuronal regulations of ascending excitation and descending inhibition (Grider, 1989). While the initial phase of hyper-motility in the obstructed bowel is a physiological adaptation, the sustained long-term suppression of motor activity is the most troublesome to patients with obstructive disorders. This is particularly true in chronic partial obstruction (as in malignant obstruction) (Ripamonti and Mercadante, 2004; Ripamonti et al., 2001; Roeland and von Gunten, 2009) and functional obstruction (i.e. chronic intestinal pseudo-obstruction) (De Giorgio et al., 2011; Nunez et al., 2009; Thompson, 2006). The impaired motor activity leads to distention, nausea, vomiting, and constipation (Summers, 1999; Russell and Welch, 1990; Shi et al., 2011).

As mechanical stress upregulates expression of COX-2, and COX-2-derived prostaglandins profoundly affect motility function (Fornai et al., 2005; Krause and DuBois, 2000), we tested the hypothesis that mechano-transcription of COX-2 may contribute to the sustained hypo-motility in obstruction. Our studies have revealed that smooth muscle contractile response of the isolated circular muscle strips was suppressed dramatically at 24 h after obstruction, and this continued through the 7-day course of obstruction (Shi et al., 2011; Li et al., 2012a). However, incubation of the muscle strips isolated from BO rats with COX-2 inhibitor NS-398 significantly restored muscle contractility. Importantly, PGE₂ levels were nearly 15-fold higher in the medium of BO strips than that of the sham strips, and NS-398 treatment significantly lowered the PGE₂ levels (Shi et al., 2011). We further tested the effects of partial colon obstruction in mice and observed that obstruction led to marked upregulation of COX-2 and suppression of muscle contractility in wild-type mice (Shi et al., 2011). However, BO associated suppression of smooth muscle contractility was largely attenuated in the COX-2 deficient mice (Shi et al., 2011). These studies indicate that stretch-induced expression of COX-2 and subsequent release of prostaglandins play a critical role in suppressing smooth muscle contractile function during BO.

While mechano-transcription of COX-2 is observed to be a prominent pathway involved in motility dysfunction in BO, recent reports suggest that induction of several other inflammatory genes by mechanical stress may also contribute to motility abnormalities (Wehner et al., 2010; Docsa et al., 2020; Lin et al., 2014b). It was discovered that in vitro mechanical stretch of gut SMCs or in vivo obstruction significantly induced expression of IL-6, MCP-1, iNOS, and other pro-inflammatory mediators in the muscle cells. These mediators are known to not only play critical roles in inflammation but also affect motility function in the gut (Lin et al., 2014b). Docsa et al. found that mechanical stress-induced CXCL-1 in macrophages suppressed intestinal smooth muscle contractility and may account for motility dysfunction in ileus, a common functional obstruction in post-operative period (Docsa et al., 2020). Moreover, media collected from stretched muscle strips induces activation of transcription factor NF- κ B, further increasing production of pro-inflammatory mediators. Thus, these mechanical stress-induced inflammatory mediators, like COX-2-derived prostaglandins, may contribute significantly to motility dysfunction in obstructive conditions.

5.09.3.2 Pathophysiological role of mechano-transcription in abdominal pain

Abdominal pain is a common complaint in obstructive conditions as well as functional bowel disorders and inflammatory bowel disease. Mechanical stretch and other forms of mechanical stress are well present in these disorders (See Section 5.09.1). The role of mechanical stress, particularly mechanical stress-altered up-regulation of pain mediators and down-regulation of anti-pain system, in the pathophysiology of abdominal pain have been investigated over the last few years. Mechanical stimulation may lead to immediate activation of sensory nerves directly via mechanosensitive channels (Lin et al., 2015; Ness et al., 1990; Brierley and Blackshaw, 2006). This may be one of the mechanisms involved in transmission of acute pain during early hours of obstructive conditions (Summers et al., 1983b; Shi et al., 2018). However, repetitive or long-term mechanical stimulation of neurons may cause desensitization (Slugg et al., 2000; Brierley et al., 2004). Nevertheless, chronic abdominal pain is present in obstruction (Ripamonti and Mercadante, 2004; Ripamonti et al., 2001; Roeland and von Gunten, 2009). It is reported that 92% of patients with advanced malignant obstruction have distention-associated abdominal pain (Ripamonti and Mercadante, 2004; Baines et al., 1985). Recent preclinical studies found that visceral sensitivity is markedly increased in chronic BO (Huang and Hanani, 2005; Lin et al., 2017). Visceral hypersensitivity is a well-recognized mechanism for abdominal pain (Azpiroz et al., 2007; Bielefeldt, 2006; Mayer and Gebhart, 1994). Huang and Hanani (2005) reported that the firing threshold of primary afferent neurons was decreased, and that sensory threshold to abdominal stimulation was also reduced in mice with intestinal obstruction. Our study in the rat model of partial colon obstruction demonstrated that the primary sensory neurons projecting to the distended colon were hyper-excited with decreased resting membrane potential and rheobase, and increased number of action potentials, suggesting a highly sensitized state of the primary sensory neurons in obstruction (Lin et al., 2017).

Recent studies have attempted to elucidate the mechanisms underlying visceral hypersensitivity in obstructive conditions. Neurotrophins such as nerve growth factor (NGF) and brain-derived neurotrophic factor (BDNF) are well-recognized pain mediators (Bielefeldt, 2006; Pezet and McMahon, 2006). In the rat model of BO, Lin et al. found that the expression of NGF mRNA and protein was dramatically increased in colonic SMC in the distended colon oral to obstruction, but not in the segment distal to the site of obstruction (Lin et al., 2017). Mechanical stretch in vitro led to robust expression of NGF mRNA and protein in colonic SMC. In obstructed rats, the cell excitability of colon-projecting neurons in the dorsal root ganglia (DRG) was significantly enhanced. Moreover, the withdrawal response to von Frey filament stimulation markedly increased in BO rats. Treatment with anti-NGF antibody attenuated colon neuron hyper-excitability and referred hypersensitivity in BO rats. Furthermore, obstruction led to a significant increase of tetrodotoxin-resistant (TTX-r) Na⁺ currents and up-regulation of mRNA expression of TTX-r Na_v1.8, but not TTX-sensitive Na_v1.6 and Na_v1.7 in colon-projecting sensory neurons. These changes were abolished by anti-NGF treatment (Lin et al., 2017). Therefore, stretch-induced NGF in colon SMC, acting on TTX-r Na⁺ channels in sensory afferents, may play an important role in visceral hypersensitivity in BO.

Expression of BDNF at both mRNA and protein levels was also increased dramatically in the colonic smooth muscle of the oral distended segment, but not in the non-dilated segment aboral to obstruction (Fu et al., 2018). There was an eightfold increase of BDNF mRNA expression in colonic smooth muscle on day 3 in BO. Compared with sham controls, colon neurons of the BO rats demonstrated significantly reduced densities of total K^+ current and the transient inactivating A-type (I_A) current, but not the delayed rectifier current (I_K). In parallel, treatment with anti-BDNF antibody restored total and I_A currents of neurons from BO rats, and partially attenuated colon neuron hyper-excitability in obstruction. Administration of ANA-12, an inhibitor to BDNF receptor Trk B, blocked BO-associated changes of neuronal excitability, Kv activity and gene expression in obstruction. This is associated with improved withdrawal response to the stimulation of von Frey filaments to the abdominal wall (Fu et al., 2018). These studies suggest that mechano-transcription of BDNF in gut SMC contributes to visceral hypersensitivity in BO mainly by reducing A-type K^+ currents in the primary sensory afferents.

Visceral sensitivity is mediated not only by the nociceptive pathways, but also by the anti-nociceptive systems (Hughes et al., 2016). As the primary anti-nociceptive system, endogenous opioids and their receptors are distributed widely throughout the body, including peripheral sensory nerves of the gut (Hughes et al., 2016). There are at least three types of opioid receptors: μ , δ , and κ receptors (MOR-1, DOR-1, and KOR-1, respectively) (Hughes et al., 2016, 2014). Recent study by Hegde et al. found that mechanical stress in chronic bowel obstruction dramatically decreased expression of opioid receptors in the distended colon (Hegde et al., 2020). The mRNA expression levels of all three opioid receptors (MOR, KOR, and DOR) in colonic muscularis externa were markedly downregulated in obstructed rats to 4–14% of that in sham controls. The mechanical stress-induced down-regulation of opioid receptors occur mainly in colon-specific primary afferent nerves of the distended colon. As forced up-regulation of opioid receptors in the colon partially attenuated visceral hypersensitivity, the authors suggest that down-regulation of the opioid receptors may contribute to visceral hypersensitivity in chronic obstruction (Hegde et al., 2020).

5.09.3.3 Pathophysiological role of mechano-transcription in gut inflammation

Inflammatory response in the GI tract involves numerous cellular and molecular events that are orchestrated by cytokines, chemokines, and other inflammatory mediators such as prostaglandins, nitric oxide and cell surface adhesion molecules (Papadakis and Targan, 2000; Stadnyk, 2002). These pro-inflammatory mediators can be secreted by inflammatory cells or non-inflammatory cells such as epithelial cells, myofibroblasts, and smooth muscle cells in the gut (Stadnyk, 2002; Powell et al., 1999; Shi and Sarna, 2005). Although gut inflammation and infections are the most visible causes of production of pro-inflammatory mediators, recent studies have shown that mechanical force is a potent pro-inflammatory stimulus in the gut (Wehner et al., 2010; Lin et al., 2014b).

Wehner et al. reported that static mechanical strain significantly induced iNOS, COX-2, and IL-1 β gene expression levels in cultured intestinal smooth muscle cells and peritoneal macrophages (Wehner et al., 2010). Mechanical stimulation also amplified LPS-induced iNOS and IL-1 gene expression in intestinal smooth muscle cells, and similarly COX-2 and IL-6 mRNA expression in macrophages. Lin et al. further investigated mechano-sensitive expression of pro-inflammatory mediators in vitro, in vivo, and ex vivo in primary culture of colon smooth muscle cells and found that static stretch significantly induced mRNA expression of iNOS, COX-2, IL-6, and MCP-1 (Lin et al., 2014b). Gene expression of TNF- α , IL-1 β , and IL-8 was not significantly affected by mechanical stretch. In an in vivo model of partial colon obstruction, they found that gene expression of iNOS, IL-6, and MCP-1 is also significantly increased in a time-dependent manner in the mechanically distended proximal segment, compared to the distal segments or sham control animals. The conditioned medium from the muscle strips of the stretched proximal segment, but not the distal segment or control, significantly induced translocation and phosphorylation of pro-inflammatory transcription factor NF- κ B p65 leading to increased mRNA expression of more inflammatory mediators in naïve cells. However, treatment of the conditioned medium from the proximal segment with neutralizing antibody against IL-6 significantly attenuated the activation of NF- κ B and gene expression of inflammatory mediators, indicating a critical role of mechanical stress-induced IL-6 in the secondary activation of pro-inflammatory transcription factors. These studies demonstrate that mechanical stress upregulates gene expression of certain cytokines, chemokines, and inflammatory mediators i.e. iNOS, COX-2, IL-6, and MCP-1 in gut smooth muscle cells and other cells in the intestine. The mechanical stress-induced mediators are well recognized in inflammatory response. MCP-1 is a potent chemo-attractant recruiting lymphocytes, monocytes and other inflammatory cells into the local site (MacDermott, 1999; Gijssbers et al., 2006). IL-6 is a well-defined pro-inflammatory mediator involved in immune cell activation and differentiation and plays a key role in the development of inflammatory bowel disease (Papadakis and Targan, 2000; Mudter and Neurath, 2007; Pawłowska-Kamieniak et al., 2021). Needless to say, iNOS and the COX-2/prostaglandin E synthase/PGE₂ axes are among the best-described pathways implicated in inflammatory regulation (Krause and DuBois, 2000; Mohajer and Ma, 2000; Mancini and Di Battista, 2011).

Recent studies have tested if mechanosensitive expression of certain pro-inflammatory mediators has a role in immune response in Crohn's disease (CD). Osteopontin (OPN) is a secreted glycoprotein with many demonstrated roles in the regulation of immune response on multiple levels (Uede, 2011; Rittling and Singh, 2015). Sato et al. found that active CD patients demonstrated significantly higher plasma OPN levels than normal or UC patients, and the elevated plasma OPN levels in CD patients were significantly correlated with disease activity (Sato et al., 2005). These findings were later confirmed by other groups (Agnholt et al., 2007; Komine-Aizawa et al., 2015). Further, OPN was found to facilitate production of IL-12 from lamina propria mononuclear cells (LPMC) and is closely involved in the Th1 immune response in CD (Sato et al., 2005; Ashkar et al., 2000). However, the cellular source and mechanisms of increased plasma OPN in CD are not clear. We found that expression of OPN was dramatically upregulated in the mechanically distended colon in bowel obstruction (Lin and Shi, 2021). OPN level was also increased in the plasma in

rats with bowel obstruction. In the rat model of stenotic Crohn's colitis, OPN expression was found increased not only at the inflammation site, but at the distended pre-inflammation site (Lin and Shi, 2021). Plasma OPN level was significantly increased in stenotic Crohn's colitis rats. However, prevention of inflammation-associated obstruction with liquid diet eliminated OPN expression in the pre-inflammation site and normalized plasma OPN level. These results suggest that OPN expression in Crohn's colitis is largely regulated by mechanical stress, and the plasma OPN levels in colitis are closely related to the extent of bowel distention.

5.09.3.4 Pathophysiological role of mechano-transcription in intestinal fibrosis

Stricture formation due to tissue fibrosis and smooth muscle hyperplasia is the most common complication in Crohn's disease (CD) (Latella and Rieder, 2017; Rieder et al., 2017; Li and Kuemmerle, 2014). Stricture-associated stenosis leads to bowel obstruction and increases risks of perforation and fistula (Katsanos et al., 2010; Latella and Rieder, 2017). Although stricture formation in CD is associated with chronic inflammation, anti-inflammatory treatment is not effective to prevent it. Even though surgery may temporarily relieve obstruction in stenotic CD, the previously distended pre-stenotic region may become new site of recurrent inflammation and fibrosis. In fact, post-surgery endoscopic or histological recurrences are almost 100%, given enough time (Rieder et al., 2017; Olaison et al., 1992; Rutgeerts et al., 1991). Thus, inflammation-independent mechanisms may play a substantial role in fibrosis and hypertrophy in CD (Rieder et al., 2017; Zhao et al., 2020). As stated above, mechanical stress associated with tissue deformation, edema, fibrosis, and obstruction (Levy Nogueira et al., 2015; Cox Jr et al., 2008; Johnson et al., 2013; Katsanos et al., 2010) are commonly encountered in IBD, especially CD. Release of mechanical stress by strictureplasty reduces fibrosis (Latella and Rieder, 2017; Yamamoto et al., 2007). These clinical findings indicate that mechanical stress, as an inflammation-independent factor, may play a critical role in fibrosis and stricture formation (Rieder et al., 2017; Tschumperlin et al., 2018).

There is increasing evidence that mechanical stress induces gene expression of pro-fibrotic and proliferative mediators to contribute to intestinal fibrosis and hyperplasia in the gut (Johnson et al., 2013, 2014; Lin et al., 2021b). Johnson et al. tested the effect of mechanical property of the cellular environment in cultured human colonic fibroblasts (Ccd-18co cells). They found that growth of cells on a stiff matrix increased cell proliferation, induced fibrogenic gene expression, decreased matrix metalloproteinase, and proinflammatory gene expression, and was associated with nuclear localization of the transcriptional cofactor MRTF-A (Johnson et al., 2013, 2014). In a rodent model of Crohn's disease, Lin et al. (Lin et al., 2021b) found that intracolonic instillation of TNBS induced localized transmural inflammation (~2 cm length) in the distal colon (site I), with a distended colon segment (site P) prior to, and a non-distended segment (site D) distal to the site of inflammation. Macroscopic and histological studies found that site I encompasses inflammation and mechanical stress, site P has mechanical stress but no visible inflammation, and site D has neither inflammation nor mechanical stress (Fig. 5). Significant fibrosis and muscle hyperplasia were detected 7 days after TNBS treatment, not only in site I (inflammation site), but also in site P (distended segment proximal to inflammation). Molecular expression analysis revealed that expression of pro-fibrotic and proliferative genes, e.g., connective tissue growth factor (CTGF) and brain-derived neurotrophic factor (BDNF) were markedly up-regulated in both sites P and I. The increased CTGF and BDNF were mainly observed in the muscularis externa. Interestingly, there was no up-regulation of CTGF and BDNF in the non-distended site D, suggesting a mechanosensitive mechanism of CTGF and BDNF expression in the CD model. However, if mechanical distention was prevented by feeding rats exclusively with clear liquid diet (Fig. 5), expression of CTGF and BDNF was completely prevented in site P, and dramatically attenuated in site I. Inflammation, fibrosis, and hyperplasia were significantly improved with liquid diet treatment. In a separate model, where only mechanical distention but no inflammation was induced with an obstruction band in the distal colon, the authors found that collagen deposition, muscle hyperplasia, and expression of CTGF and BDNF were present only in the distended segment prior to obstruction, but not in the segment distal to obstruction (Lin et al., 2021b). Taken together, these studies suggest that transmural inflammation in the CD model causes mechanical stress at the inflammation site and in the distended segment proximal to inflammation and induces expression of pro-fibrotic and proliferative mediators. Mechanical stress-induced up-regulation of pro-fibrotic and proliferative mediators may play a critical role in the development of fibrosis and muscle hyperplasia in CD.

The rodent model of CD mimics well the clinical observations that significant fibrosis, muscular hyperplasia, and pro-fibrotic gene expression were present not only in the stenotic region, but in the seemingly "normal" tissue proximal to stenosis (Chen et al., 2017; Warnaar et al., 2006). Most recent studies found that these events in the pre-stenotic region are highly associated with an increased risk of post-operative recurrence (Hammoudi, et al., 2020). Studies focusing on the distended pre-stenotic segment may help to explain why recurrence occurs so often in the pre-stenotic region, and also have surgical implications on how to approach a stenotic CD, i.e. to what extent the resection should be. These findings support clinical practice to undergo a pan-colectomy for stenotic Crohn's colitis because even if the colonic stricture is in the distal colon, the proximal bowel may have already suffered significant mechanical stress-induced lesions, especially in the deeper muscularis externa which would not be accessible by endoscopy or conventional biopsy.

5.09.4 Mechano-transcription process as potential therapeutic targets for obstructive bowel disorders

5.09.4.1 Treatment strategy and challenges in the management of obstructive bowel disorders

Small and large bowel obstructions are responsible for approximately 15% of hospital admissions for acute abdomen in the United States. The management strategy for bowel obstruction varies depending on etiology and site of obstruction, and whether patients

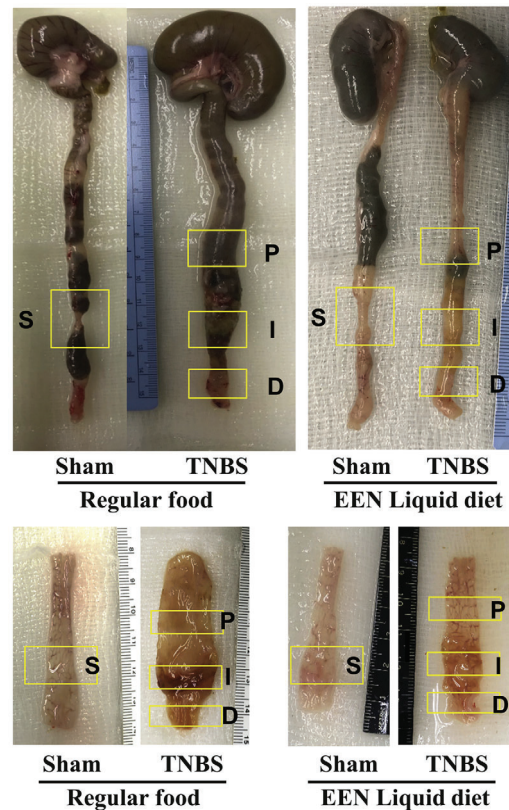


Fig. 5 TNBS-induced Crohn's-like colitis in rats and the effect of exclusive enteral nutrition with liquid diet. Rats were treated with intracolonic instillation of saline (sham control) or TNBS (65 mg/kg in 250 μ L of 40% ethanol) into the distal colon ($\sim$ 7 cm from the anus) and were fed with either regular pellet diet (*left*) or liquid diet (*right*). Rats were euthanized 7 days after the treatment. EEN relieves lumen distention in TNBS treated rats (7 days). The *top panel* shows the outlooks of the colons, and the *bottom panel* shows the macroscopic view of the distal colons. While TNBS induced obstructive colitis in the regular food group, liquid diet treatment prevents colon distention associated with inflammation. The *yellow boxes* indicate different sites of colon tissues taken for the study. S, sham control; I, inflammation site where TNBS was instilled into; P, distended colon site proximal to the inflammation site; D, no-distended site distal to inflammation.

have a partial, complete or complicated obstruction, and whether they have previous abdominal surgery (Frago et al., 2014; Catena et al., 2019). Overall, only 20% of bowel obstruction cases need acute surgical care such as when obstruction is complete or complicated with ischemia, perforation, or other severe emergencies (Catena et al., 2019). Most bowel obstruction cases are treated with a non-operative conservative management. This is particularly true for malignant bowel obstruction (MBO). MBO is a common and distressing outcome especially in patients with bowel or gynecological cancers. Although surgery may be a treatment choice for some selected patients with MBO, it is not undertaken routinely in patients known to have poor prognostic criteria for surgical intervention such as intra-abdominal carcinomatosis, poor performance status, and massive ascites (Ripamonti et al., 2001; Frago et al., 2014).

Conservative management for BO patients includes nil per os, nasogastric suction, stenting, fluid replacement therapy, and medication treatments for various symptoms related to obstruction (Catena et al., 2019). Nasogastric drainage is generally only a temporary measure. Self-expanding metallic stents are an option in malignant obstruction of the gastric outlet, small bowel and colon. Among medical treatments, opioid analgesics are used for abdominal pain caused by obstruction. Although they may provide certain degree of pain relief, opioid analgesics cause significant gastrointestinal adverse effects such as constipation and fecal retention, which would worsen motility dysfunction in obstruction. Anti-secretory and anti-emetics may be used to control vomiting and abdominal distention (Ripamonti et al., 2001). However, the effect of medical treatments in bowel obstruction is limited as they do not specifically address mechanical stress—the root cause of functional abnormalities such as motility dysfunction and abdominal pain.

Surgical approach for obstruction is to release blockage as in mechanical BO or remove constriction as in Hirschsprung's disease (HD), but to leave alone the distended oral segment in the gut (Heuckeroth, 2018; Williams et al., 2005). However, many patients suffer motility dysfunction not only during obstruction, but after the obstruction is surgically resolved (Menezes et al., 2006; Catto-Smith et al., 2007; Di Lorenzo et al., 2000; Jarvi et al., 2010; Fevang et al., 2004). Among children with a history of HD, 50–70% suffer long-term gastrointestinal (GI) symptoms such as constipation, incontinence, abdominal distention, and enterocolitis in the first 5 to 10 years after pull-through surgery (Catto-Smith et al., 2007; Di Lorenzo et al., 2000). The latest data showed that gut

dysfunction continues into adulthood after surgical release of obstruction in childhood, as 30% of HD patients (9% in control) were reported to suffer from constipation and motility dysfunction when they became adults (mean age of 43) (Jarvi et al., 2010). In the upper gut, pyloromyotomy to correct infantile pyloric stenosis has also shown long-term effects on gastric sensory and motor functions (Saps and Bonilla, 2011; Sun et al., 2000). The tonic and phasic contractions in the stomach are impaired for more than 10 years after surgical resolution of the outlet obstruction (Sun et al., 2000). Motility dysfunction is responsible for symptoms such as bloating, nausea, vomiting, and constipation. However, the reasons for the long-term bowel dysfunction even after the release of obstruction is not known. There are no specific treatments for the long-term dysfunction in such cases. It is imperative to elucidate the underlying mechanisms of long-term motility dysfunction after obstruction, so that therapeutic targets may be identified.

5.09.4.2 Targeting mechano-transcription of COX-2 and prostaglandin E synthase for motility dysfunction in bowel obstruction and after obstruction is resolved

In the non-operative management of bowel obstruction especially chronic partial obstruction, the focus is to relieve obstructive symptoms such as vomiting, bloating, abdominal distention and pain. The preponderance of these symptoms is due to motility dysfunction, i.e. suppressed muscle contractility and abnormally slow transit. To withdraw drugs inhibitory to motility has always been the practice in managing bowel obstruction (Summers, 1999). Prokinetics (motor-stimulating drugs) have been tried in conservative management of obstructive disorders (Summers, 1999; De Giorgio et al., 2004). In fact, neostigmine has been used effectively in the medical management of acute intestinal or colonic pseudo-obstruction (De Giorgio et al., 2004; De Giorgio and Knowles, 2009). However, the use of other prokinetics in treating motility dysfunction is disappointing (Summers, 1999; De Giorgio et al., 2004), possibly because the mechanisms of motility dysfunction in bowel obstruction is rooted in mechanical stretch, and current prokinetics do not target mechano-transduction or mechano-transcription processes.

Given that mechanical stretch induces marked expression of COX-2, and COX-2 derived prostaglandins suppress muscle contractions (Shi et al., 2011; Lin et al., 2012b), Lin et al. studied the therapeutic and prophylactic effects of COX-2 inhibitors on smooth muscle function and gut motility in bowel obstruction in rats (Lin et al., 2012a). Daily administration of COX-2 inhibitor NS-398 or vehicle was started before or after the induction of obstruction to investigate its prophylactic and therapeutic effects, respectively. They found that smooth muscle contractility was significantly suppressed, and colonic transit rate was much slower in obstruction. Prophylactic treatment with NS-398 significantly prevented the impairments of colonic transit and smooth muscle contractility and attenuated fecal retention in the obstructed colon. When NS-398 was administered therapeutically 3 days after the initiation of obstruction, the muscle contractility and colonic transit still improved by day 7. The study also depicted a marked increase in COX-2 expression and production of PGE₂ in the distended colon and found that exogenous PGE₂ decreased colonic smooth muscle contractility. The team further investigated whether inhibition of PGE₂ is beneficial in improving motility function in OB (Lin et al., 2012a). All four PGE₂ receptors, E-prostanoid receptor types (EP1 to EP4), were detected in the rat colonic muscularis externa. Treatments with EP1 and EP3 antagonists suppressed muscle contractility in control tissue but did not improve contractility in obstruction tissue. On the contrary, the EP2 and EP4 antagonists did not affect control tissue but significantly restored muscle contractility in obstruction. Thus, PGE₂ and its receptors EP2 and EP4 are specifically involved in motility dysfunction mediated by mechanical stress-induced COX-2 in obstruction. EP2 and EP4 antagonists may have therapeutic potential in medical treatment of motility dysfunction in obstruction.

Clinical studies support the pre-clinical findings that selective inhibition of COX-2 has benefits in treating obstructive bowel disorders. Retrospective studies in large cohort of patients and clinical trials found that the use of COX-2 inhibitor celecoxib decreased the paralytic ileus rates and did not cause any significant morbidity (Raju et al., 2015; Wattchow et al., 2009). A multicenter, blinded, and randomized clinical trial study found that selective COX-2 inhibitor firocoxib, but not the nonselective COX inhibitor flunixin meglumine, is beneficial in small intestinal strangulating obstruction in horses, as it reduced endotoxemia (Ziegler et al., 2019; Ziegler and Blikslager, 2020).

As BO-associated up-regulation of COX-2 and production of PGE₂ depend on mechano-transcription process, Li et al. further determined whether inhibition of mechano-transcription signal transduction improves motility function (Li et al., 2012a,b). Because stretch-induced expression of COX-2 depends on MAPK p38 activation in colonic SMC, Li et al. studied the in vivo effects of p38 inhibitor SB203580 on COX-2 induction and motility dysfunction in obstruction. It was found that SB203580 significantly attenuated induction of COX-2 and improved colon motility in obstruction (Li et al., 2012a). This was associated with improvement of colon distension. These studies show that inhibition of the intestinal mechano-transcription process has therapeutic potentials for motility dysfunction in obstructive bowel disorders.

Gut motility is altered not only during BO, but for a long time even after BO is resolved (Di Lorenzo et al., 2000; Jarvi et al., 2010; Fevang et al., 2004). Recent studies suggest that mechano-transcription of COX-2 and COX-2-derived PGE₂ in the SMC of the distended bowel segment may not only account for motility dysfunction during obstruction but exert secondary effects on SMC to contribute to gut dysfunction even after obstruction is resolved (Lin et al., 2018). Lin et al. showed that COX-2-derived PGE₂ during BO may further up-regulate the expression of microsomal prostaglandin E synthase-1 (mPGES-1) in an autocrine mode (Lin et al., 2018). Administering mPGES-1 inhibitors in vivo showed that increased mPGES-1 contributes to the continuous production of PGE₂ and long-term suppression of motility dysfunction in the post-BO state. Thus, the once distended bowel segment during obstruction may not be considered as “normal” because it is the site of mechano-transcription. Comparative studies using COX-2 inhibitor NS-398 and mPGES-1 inhibitor Cay 10526 during BO and in post-BO implied that inhibition of both COX-2 and

mPGES-1 during obstruction may be effective as a therapeutic strategy to treat the motility dysfunction during obstruction, and also as a prophylactic strategy to prevent long-term motility dysfunction occurring after obstruction is resolved. Once the obstruction is resolved, inhibition of mPGES-1 rather than COX-2, may be a better therapeutic approach for motility dysfunction (Lin et al., 2018).

5.09.4.3 Targeting mechano-transcription process for abdominal pain in obstructive bowel disorders

Abdominal pain is the primary reason for hospital visits in bowel obstruction. In fact, mechanical BO composes 15% of all emergency admissions for abdominal pain (Cappell and Batke, 2008; Gore et al., 2015). Abdominal pain may be the only prominent symptom in patients with functional obstruction, i.e. intestinal pseudo-obstruction (De Giorgio et al., 2011; Downes et al., 2018). In the acute phase of BO (i.e., the first 12–24 h), abdominal pain is usually colicky (cramping and intermittent). The pain may increase, peak, and subside periodically (Summers, 1999; Gore et al., 2015; Cartwright and Knudson, 2008). However, patients with chronic partial obstruction may experience persistent abdominal pain (Ripamonti and Mercadante, 2004; Ripamonti et al., 2001). Among patients with malignant obstruction, 92% have distention-associated chronic abdominal pain (Baines et al., 1985). Current analgesic management for BO-associated pain relies mainly on high doses of opioids (Ripamonti and Mercadante, 2004; Roeland and von Gunten, 2009). However, opioids are notorious in causing further bowel dysfunction, such as constipation and narcotic bowel syndrome (Lin et al., 2021a; Farmer et al., 2019; Grunkemeier et al., 2007). It is imperative to identify therapeutic targets specifically for distention-associated pain among patients with OBD.

As discussed in Section 5.09.3.3, visceral sensitivity is significantly increased in chronic obstruction in the small intestine or colon (Huang and Hanani, 2005; Lin et al., 2017; Shi et al., 2018). We and others have shown that mechano-transcription of pain mediators NGF, BDNF, and COX-2 in the gut wall play a critical role in visceral hypersensitivity in experimental BO (Huang and Hanani, 2005; Lin et al., 2017, 2015; Fu et al., 2018). Lin et al found that inhibition of mechanical stress-induced NGF by administering neutralizing antibody against NGF not only attenuated afferent neuron hyperexcitability, but also significantly improved pain behavior in rats with chronic partial colon obstruction (Lin et al., 2017). Fu et al reported that BDNF is robustly induced by mechanical stress in distended bowel in obstruction, and blockade of BDNF action by administering Trk B inhibitor in vivo effectively inhibited BO-associated referred pain (Fu et al., 2018). These studies have also characterized downstream mechanisms of NGF and BDNF-mediated hyperalgesia and found that altered expression and activity of $\text{Na}_v1.8$ and K_v in the afferent nerve may account for NGF and BDNF-mediated peripheral visceral hypersensitivity (Lin et al., 2017; Fu et al., 2018). Therefore, mechanical stress-induced production of NGF and BDNF in the gut tissues and the neurotrophins-mediated Na_v and K_v activity in primary afferent nerves may represent potential therapeutic targets for distention-associated abdominal pain.

Gut microbiota composition and diversity are altered significantly in bowel obstruction (Hegde et al., 2018). Hegde et al. found that partial colon obstruction led to decreased abundance of gut commensal *Lactobacillus reuteri* in the obstructed bowel in rats (Hegde et al., 2018). Interestingly, when *L. reuteri* rat strains were ingested via gavage (1×10^9 colony-forming units/g daily starting 2 days before OB), the precision microbial therapy significantly improved food intake and body weight in OB rats and attenuated referred visceral hyperalgesia measured by the withdrawal response to von Frey filament applications to the abdomen. Treatment with *L. reuteri* also attenuated hyperexcitability of the dorsal root ganglia neurons projecting to the distended colon. While the expression of μ , δ , and κ opioid receptors was significantly downregulated in colonic muscularis externae and sensory neurons in OB rats (Hegde et al., 2020), *L. reuteri* treatment prevented the loss of opioid receptors. Furthermore, administration of peripheral opioid receptor antagonist naloxone methiodide abolished the analgesic effect of *L. reuteri* in OB. Thus, *L. reuteri* may have therapeutic potential in preventing lumen distension-associated visceral hypersensitivity in obstructive conditions by inducing opioid receptors in the gut tissues (Hegde et al., 2020).

5.09.5 Mechano-transcription process as potential therapeutic targets for inflammatory bowel disease

5.09.5.1 Treatment options and challenges in the management of inflammatory bowel disease

Inflammatory bowel disease (IBD), including Crohn's disease (CD) and ulcerative colitis (UC), is defined by chronic inflammation of the gastrointestinal tract with frequent disease relapses and remissions (Sartor, 2006; Hendrickson et al., 2002). The pathogenic mechanisms of CD and UC remain unknown, and cures are unavailable (Hendrickson et al., 2002). Current therapies are designed to induce prolonged remission. Among the therapeutic options, 5-aminosalicylates are used mainly for mild active IBD (predominantly for UC), and for maintenance treatment in UC (Domènech, 2006). Corticosteroids are considered the mainstay for induction of remission in moderate to severe active inflammation in both UC and CD (Hendrickson et al., 2002; Domènech, 2006). Immunomodulators such as azathioprine and methotrexate are second-line treatment, mainly because of safety profile and economic costs. They are often used for maintenance therapy in both UC and CD (Domènech, 2006; Kansal et al., 2013), or for patients with steroid refractoriness or dependency. The use of biologic agents, such as anti-TNF α chimeric antibody, is increasing. They are now often used as a first line therapy for moderate to severe CD and particularly useful in patients with steroid refractory disease (Ruemmele et al., 2009).

Unfortunately, most of the medical treatment options for IBD have substantial adverse effects. Corticosteroids are known for their frequent and sometimes severe side effects and are limited in their use by risks of infection, osteoporosis, growth retardation, poor mucosal healing, and early relapses on cessation of therapy (Cheifetz, 2013; Sidoroff and Kolho, 2012). This is especially

problematic in pediatric patients, as this population of patients may experience significant growth retardation and osteoporosis with steroid therapy (Sidoroff and Kolho, 2012). Immunomodulators increase the risk of opportunistic infections and hematologic disorders (Sidoroff and Kolho, 2012; Kandel et al., 2005). Biologic agents targeting cytokines and adhesion molecules may lose efficacy over time, and are limited with increased vulnerability to infections, development of autoimmune disorders and even malignancy, and decreased immunogenicity of vaccinations (Kandel et al., 2005). Thus, safe and effective therapies, including diet-based treatments, are much needed for IBD patients (Kansal et al., 2013; Ashton et al., 2019).

5.09.5.2 Attenuation of mechano-transcription process as a novel mechanism underlying the benefits of exclusive enteral nutrition in Crohn's disease

Many dietary therapies have been explored for the management of IBD (Domènech, 2006). However, exclusive enteral nutrition (EEN) is the only established dietary treatment for IBD, specifically for CD (Kansal et al., 2013; Ashton et al., 2019). EEN involves oral or nasogastric tube feeding of a complete liquid diet with exclusion of normal foods for a defined period (usually 4 to 8 weeks). Over the last two decades, EEN has emerged as a highly effective treatment for the induction of remission in CD (Ashton et al., 2019; Levine et al., 2018). A low-risk and steroid-sparing treatment, EEN is now the first-line therapy for pediatric CD patients as the reported remission rates in this population of patients are up to 80% (Ashton et al., 2019; Levine et al., 2018; Swaminath et al., 2017). Consensus guidance from several organizations in Europe and North America (i.e. ECCO, ESPGHAN, ESPEN and NASPGHAN) directs clinicians to use EEN to induce remission in young CD patients wherever possible (Ruemmele et al., 2014; Forbes et al., 2017; Critch et al., 2012). It is suggested that corticosteroids, or early immunosuppressive therapy, should be reserved for the patients where EEN is not an option (Ashton et al., 2019; Ruemmele et al., 2014). There are reports that EEN is also effective in adult CD patients (Mowat et al., 2011; Yang et al., 2017). However, the evidence for the efficacy of EEN in adult patients is weaker than in pediatric population, possibly due to practicalities of its use (compliance, tolerability, etc.) (Ashton et al., 2019). Interestingly, there seems to be no established therapeutic role for EEN in the treatment of UC, an IBD that is without transmural inflammation and stricture formation as seen in CD (Ashton et al., 2019).

With EEN treatment, patients' disease activity, mucosal healing, bowel symptoms, and nutrition status are all significantly improved (Ashton et al., 2019). Systemic inflammatory markers, such as C-reactive protein and erythrocyte sedimentation rate are normalized, and often corrected before any detectable change in nutrition status (Bannerjee et al., 2004; Grover et al., 2014). However, the exact mechanisms for the therapeutic benefits of EEN on inflammation and gut function in CD are still not well known, though several theories have been tested or postulated. Originally, elemental liquid diet (with amino acids, but no whole proteins) was first tested in CD as a means to provide nutritional support (Voitk et al., 1973). Subsequent open and randomized controlled trials confirmed the benefits of exclusive elemental liquid diet therapy in inducing remission in active CD (Axelsson and Jamum, 1977; O'Moráin et al., 1984). These early findings raised a question: Is reduced chance of allergic reaction a possible reason for the efficacy of the elemental liquid diet? However, numerous studies in the following years found that nitrogen sources of enteral feeds are not relevant to their therapeutic efficacy as EEN treatments with either elemental or whole proteins-based polymeric liquid diets are equally effective in inducing remission in active CD (Giaffer et al., 1990; Verma et al., 2000; Zachos et al., 2007).

As IBD may be associated with intestinal microbiota dysbiosis in the gut, it is not illogical to postulate that the benefits of EEN in the CD treatment might be due to its ability to correct dysbiosis in IBD (Ashton et al., 2016). In fact, many studies have examined the impact of EEN on gut microbiome, especially the broad shifts in bacteria taxa, the changes of microbiome diversity, and alterations of specific bacterial species (Ashton et al., 2016; Quince et al., 2015; MacLellan et al., 2017). Although all elemental and non-elemental diets are similarly effective in inducing remission of active CD, different forms, or types, of EEN lead to vastly different changes of microbiome in the gut (Ashton et al., 2016, 2019). Microbiota changes within specific taxa are reported, but with huge variations in the changes observed (Gatti et al., 2017). Normal gut commensal bacteria (i.e. *Bacteriodes*, *Prevotella*, *Enterobacteriaceae*, etc.) have been reported to both increase and decrease in relative abundances (Leach et al., 2008; Kaakoush et al., 2015; Dunn et al., 2016). The role of specific bacteria has also been explored. For instance, early studies found that *Faecalibacterium prausnitzii*, an anti-inflammatory commensal, was increased with EEN (Sokol et al., 2008); however more recent reports have demonstrated a reduction in its abundance over the course of treatment with EEN (Jia et al., 2010). Systemic reviews of the most recent and well-documented studies suggest a paradoxical effect of EEN that it causes a reduction of bacterial diversity and richness and changes on the microbiome usually with a dysbiosis (MacLellan et al., 2017; Gatti et al., 2017). These changes are less likely to account for the benefits of EEN in CD, because reduced bacterial diversity and richness would predispose the gut to more inflammatory changes.

Inflammation in active CD is linked to increased intestinal permeability with a damaged barrier function (Levine and Wine, 2013). Whether the increased permeability is the cause, or a consequence of CD is unknown. However, Sanderson et al. reported that EEN improved the abnormal permeability after 6 weeks of EEN (Sanderson et al., 1987). In vitro studies on enterocytes found that EEN elemental formula components such as glutamine and vitamin D3 attenuated TNF- α -induced production of IL-8, but enhanced nitric oxide production in colonic epithelial cell line HT-29 (Alhagahmad et al., 2017). However, this may not be due to the direct effects of elemental components, as polymeric diet and solid foods contain these components too, especially after they undergo mechanical and chemical digestions in the GI tract. Some studies observed that EEN treatment may directly reduce production of pro-inflammatory cytokines IL-6, IL-8, and TNF- α in the intestine (Ashton et al., 2019; Heuschkel et al., 2000). However, the mechanisms for the improved intestinal permeability and reduced cytokine production are not clear (Levine and Wine, 2013).

As discussed in [Sections 5.09.3.3 and 5.09.3.4](#), mechanical stress is an inevitable pathological change in gut inflammation, especially in CD where transmural inflammation and stenosis are present. Recent studies show that mechanical stress may contribute to the development of gut inflammation and dysfunction in IBD ([Wehner et al., 2010](#); [Lin et al., 2014b](#); [Lin and Shi, 2021](#)). We have evidence that the benefits of EEN in CD may depend on its action to reduce mechanical stress and attenuate mechano-transcription of pro-inflammatory mediators in the gut ([Lin and Shi, 2021](#); [Lin et al., 2021b](#)). In the rat model of Crohn's-like colitis induced by intra-colonic instillation of TNBS, EEN treatment with liquid diet (*Ensure*, Abbott) significantly improved body weight gain and reduced inflammation and disease activity in colitis rats. TNBS instillation induced a localized transmural inflammation with thickened wall and narrowed lumen in the distal colon and caused marked lumen distention with fecal retention in the segment proximal to the inflammation site ([Fig. 5](#)) ([Lin and Shi, 2021](#); [Lin et al., 2021b](#)). However, EEN treatment eliminated lumen distention in the inflamed colon. Our study in the control rats with no TNBS instillation found that EEN treatment dramatically reduced fecal mass and pellet size, and increased water content of the fecal pellets. We found in TNBS colitis rats (day 7) that mRNA expression of pro-inflammatory mediators such as IL-6, MCP-1, OPN and COX-2 was dramatically induced in the inflammation site (site I) and the distended segment proximal to inflammation (site P), but not in the non-distended segment distal to inflammation (site D). Strikingly, EEN treatment almost completely blocked the up-regulation of pro-inflammatory gene expression, suggesting that a mechanical stress-associated mechanism is involved in colitis-associated induction of pro-inflammatory genes ([Lin and Shi, 2021](#); [Lin et al., 2021b](#)). OPN is found to contribute to the development of IBD, particularly the Th1 immune response in Crohn's disease ([Sato et al., 2005](#); [Ashkar et al., 2000](#)). Other mechanical stress-induced pro-inflammatory mediators such as IL-6, MCP-1, and COX-2 in site I and site P all are also involved in the inflammatory process in the gut (Please refer to [Section 5.09.3.3](#)). Therefore, mechanical stress in the inflammation site and the distended segment prior to inflammation may play a pathogenic role in inflammation and gut dysfunction independent of the intrinsic inflammatory process. The benefits of EEN in inducing remission of inflammation may largely depend on the effect of EEN to reduce mechanical stress and attenuate the mechano-transcription process in the inflammation site and the site proximal to inflammation.

5.09.5.3 Targeting mechano-transcription process for fibrosis and smooth muscle hyperplasia in Crohn's disease

Stricture formation is a hallmark of severe Crohn's disease (CD), a B2 phenotype of CD in Montreal classification ([Satsangi et al., 2006](#)). The two main pathological changes involved in stricture formation are intestinal fibrosis and smooth muscle hyperplasia ([Latella and Rieder, 2017](#); [Rieder et al., 2017](#); [Li and Kuemmerle, 2014](#)). Currently, there is no effective medical treatment for the debilitating complication. Although anti-inflammatory treatments may be effective for active inflammation in CD, they do not appear to be effective in preventing or treating intestinal fibrosis and muscle hypertrophy. Surgical procedures to release stenosis and related obstructive symptoms become inevitable in many patients with B2 type of CD.

Intestinal fibrosis is characterized by excessive deposition of collagens and other extracellular matrix (ECM) molecules with gut smooth muscle cells as one of the main mesenchymal cell sources of these molecules ([Latella and Rieder, 2017](#); [Rieder et al., 2017](#)). Earlier in vitro studies found that mechanical stress induces expression of pro-fibrotic mediators in gut smooth muscle cells ([Gutierrez and Perr, 1999](#)). Gutierrez and Perr reported that static mechanical stretch significantly increased expression of transforming growth factor beta-1 (TGF- β 1) mRNA and protein in intestinal smooth muscle cells. Expression of type 1 collagen mRNA and protein was also increased by mechanical stretch in these cells ([Gutierrez and Perr, 1999](#)). Recent in vivo studies in our lab have shown that mechanical stretch not only increases production of collagen expression and ECM deposition, but also induces robust expression of pro-fibrotic mediators such as CTGF in the colon ([Lin et al., 2021b](#)). Expression of CTGF is increased not only at the site of inflammation, where TNBS is instilled to, but also in the distended site proximal to inflammation. CTGF has long been recognized to have potent effect on cell proliferation and production of ECM proteins such as collagens. CTGF expression was found increased in CD and UC not only in the inflammation area, but also in areas of little inflammation but severe fibrosis ([Dammeier et al., 1998](#); [Di Mola et al., 2004](#)). TGF- β 1 has been the only known mediator to induce CTGF in the gut ([Latella and Rieder, 2017](#); [Li and Kuemmerle, 2014](#); [Dammeier et al., 1998](#)). However, anti-TGF treatment does not improve fibrosis. Our recent studies in rats have revealed that mechanical stress-induced CTGF in gut SMC contributes to fibrosis. We inhibited mechanical stress-induced CTGF production by administering anti-CTGF antibody (FibroGen, San Francisco, CA), and this significantly attenuated fibrosis in the site of inflammation and site proximal to inflammation in rats (Lin and Shi, unpublished observation). Our study suggests that mechanical stress-induced pro-fibrotic mediators such as CTGF may represent a potential therapeutic target in battling fibrosis in Crohn's.

Gut smooth muscle hyperplasia (increased SMC number) and associated muscular hypertrophy (increased muscle mass) have long been known to play a critical role in stricture formation. Recent comprehensive histopathological analysis suggests that SMC hyperplasia may be the most prominent histological change in fibrostenotic stricture in CD ([Rieder et al., 2017](#); [Chen et al., 2017](#)). No medical treatment is identified to target smooth muscle hyperplasia, as the mechanisms for smooth muscle hyperplasia in CD are not well understood. BDNF is known for its neurotrophic and nociceptive effects on neurons ([Fu et al., 2018](#); [Boesmans et al., 2008](#); [Al-Qudah et al., 2014](#)). However, most recent studies found that BDNF potently promotes SMC proliferation ([Freeman et al., 2017](#); [Kwapiszewska et al., 2012](#)). Clinical studies found that BDNF expression is increased in IBD tissues ([Steinkamp et al., 2012](#); [Li et al., 2011](#)). We found that BDNF expression is highly responsive to mechanical stress in gut SMC ([Fu et al., 2018](#)), and BDNF is markedly induced in the inflammation site and pre-stenotic site in the CD rats ([Lin et al., 2021b](#)), as well as in the distended bowel in mechanical obstruction ([Fu et al., 2018](#)). Exogenous BDNF leads to robust proliferation of rat colon SMC. Anti-BDNF treatment or antagonist of BDNF receptor Trk B significantly reduces gut SMC proliferation (Lin and Shi, unpublished observation). These

studies suggest that mechanical stress-induced BDNF plays a critical role in SMC hyperplasia in a preclinical model of CD, and may be considered a potential therapeutic target for prevention or treatment of SMC hyperplasia and hypertrophy in fibrostenotic CD.

5.09.6 Mechano-transcription process as potential therapeutic targets for functional bowel disorders

5.09.6.1 Mechano-transcription and irritable bowel syndrome

Irritable bowel syndrome (IBS) is the most common and best described type of functional bowel disorders, affecting ~11% of the general population in the United States (Camilleri, 2014; Lovell and Ford, 2012). Although visceral pain is the most prominent symptom in IBS, abdominal distention and bloating are other major complaints among IBS patients (Azpiroz and Malagelada, 2005; Chang et al., 2001b; Shim et al., 2010; Zar et al., 2002). Based on a comprehensive bowel symptom questionnaire, 76% of IBS patients reported abdominal bloating and 57% had abdominal distention (Chang et al., 2001b). Objective measurements with an abdominal inductance plethysmography found that 60% of constipation-predominant IBS patients demonstrated measurable abdominal distention (Houghton et al., 2006). The exact reasons of abdominal distention are not well understood. However, current evidence suggests that it may be attributed to excessive gas accumulation and impaired gas transit in the intestine and colon (Koide et al., 2000; Serra et al., 2001; Hernando-Harder et al., 2010). Other postulated mechanisms include intraluminal retention of fluid and solid contents and altered gut microflora (Chang et al., 2001b; Agrawal et al., 2009; Sweetser et al., 2012). Some investigators found that ineffective evacuation, resulting in fecal retention in the colon and rectum, may also contribute to abdominal distention in IBS (Raahave and Jensen, 2021; Camilleri, 2014; Sweetser et al., 2012). Thus, abdominal distention in IBS is largely due to luminal retention of gas, liquid, or solid contents in the GI tract. The luminal retention of the gas, liquid, and solid contents clearly represent mechanical stress to the gut wall.

As abdominal pain and distention are co-present in nearly 70% of IBS patients (Chang et al., 2001b), several groups have tried to determine whether distention modulates visceral sensitivity. Earlier studies have focused on the acute effect of repetitive distensions in the distal colon to noxious pressure levels (Gschossmann et al., 2001; Million et al., 2006; Ness et al., 1990). A noxious pressure is generally considered greater than 40 mmHg (Ness et al., 1990). It was found that in naive rats that colonic tonic distention at 60 mmHg for 10 min followed by a series of phasic distentions for another 10 min increased visceromotor response (VMR) (Gschossmann et al., 2001; Million et al., 2006). This result is similar to the findings in humans that repetitive colonic distention (60 mmHg, 30 s) for 10 times at 4-min intervals resulted in visceral hypersensitivity and hyperalgesia in normal subjects (Ness et al., 1990). Moreover, Munakata et al. (Munakata et al., 1997) found that repetitive distention of the sigmoid colon at 60 mmHg for 10 min led to rectal hypersensitivity and hyperalgesia in IBS patients. Luminal distention at noxious pressure (60 mmHg) induced changes in neuropeptide expression and ERK 1/2 activation in the dorsal horn (Million et al., 2006; Lu et al., 2005). These data suggest that colon distention at noxious pressure may induce acute visceral hypersensitivity via a central sensitization mechanism (Munakata et al., 1997).

Further, Al-Chaer et al. tested if colon distention might contribute to chronic visceral hypersensitivity (Al-Chaer et al., 2000). They found that repetitive colon distention (60 mmHg) with a balloon in rats during neonatal stage led to visceral hypersensitivity detectable in adult stage, suggesting that peripheral sensitization may be involved (Al-Chaer et al., 2000). Lin et al. tested colon distention in adult rats and found that sub-noxious tonic distention of the distal colon with a balloon at 20–40 mmHg for 40 min led to significantly increased visceral sensitivity (Lin et al., 2015). The visceral hypersensitivity persisted for at least 3 days. Electrophysiological studies found that the excitability of colon projecting sensory neurons in the dorsal root ganglia was markedly augmented. Interestingly, they found that the sub-noxious distention for 40 min induced COX-2 expression and PGE₂ production in colonic smooth muscle, but not in the mucosa layer (Lin et al., 2015). Nevertheless, administration of COX-2 inhibitor NS-398 blocked distention-induced production of PGE₂, and significantly attenuated visceral hypersensitivity (Lin et al., 2015). These data thus suggest that mechanical distention-induced production of inflammatory mediators, e.g., PGE₂, contributes to visceral hypersensitivity in conditions with lumen distention. Other mechanosensitive pain mediators like NGF and BDNF were found to be substantially increased in the bowel tissues of IBS patients compared to normal subjects (Yu et al., 2012; Dothel et al., 2015). These mediators are known to contribute to visceral hypersensitivity and abdominal pain in IBS (Dothel et al., 2015; Coelho et al., 2019). Thus, targeting mechano-transcription of inflammatory and pain mediators may have therapeutic potential for IBS.

5.09.6.2 Mechano-transcription and other functional bowel disorders

Among other functional bowel disorders, chronic constipation and fecal retention may present the most significant mechanical stress in the distal colon and rectum (Raahave, 2015; Heredia et al., 2012; Lin et al., 2021a; Bharucha et al., 2006). In chronic constipation, slowed colonic transit and decreased frequency of bowel movement directly lead to fecal accumulation or impaction in the distal bowel (Heredia et al., 2012; Lin et al., 2021a). As a separate entity of functional bowel disorders, fecal retention may be functional (voluntary withholding of stool) or due to obstructed defecation (Raahave, 2015; Raahave and Jensen, 2021). In either of the conditions, increased fecal accumulation in the distal bowel represents apparent mechanical stretch to the bowel tissues, and thus may lead to mechano-transcription of select bioactive mediators (Lin et al., 2021a). A *Gastroenterology* study found that COX-2 expression and PGE₂ production were significantly increased in the colonic muscularis externa tissues in patients with

slow transit constipation (Cong et al., 2007). COX-2 expression was also found to be significantly up-regulated in the distended colon in slow transit constipation generated experimentally by outlet obstruction (Heredia et al., 2012), or by opioid-induced constipation (Lin et al., 2021a). Increased inhibitory prostaglandins such as PGE₂ were found to be responsible for the diminished contractility in slow transit constipation in human (Liu et al., 2009) and in animal models (Heredia et al., 2012; Lin et al., 2021a). In fact, administration of COX-2 inhibitor NS-398 was found to be effective to improve bowel movement in chronic constipation in morphine treatment (Lin et al., 2021a).

In the upper GI tract, slowed gastric emptying rate leads to increased retention of foods in the stomach as seen in gastroparesis patients (Azpiroz et al., 2014; Steinsvik et al., 2021). The increased retention of intra-gastric contents presents mechanical stress in the gastric tissues and could induce mechano-transcription process. When full thickness gastric tissues were available for studies, several groups reported that COX-2 expression was increased in the gastric tissues in animal models of gastroparesis induced by glucagon (Xu and Chen, 2007), lipopolysaccharide (West et al., 2006), or stress (Li et al., 2016). Moreover, in vivo administration of COX-2 inhibitors improved gastric motor function in the glucagon-induced gastroparesis model (Xu and Chen, 2007).

These studies suggest that mechanical stress-induced expression of COX-2 and production of prostaglandins may represent common potential targets for “functional” disorders with luminal distention such as constipation, fecal retention, and gastroparesis. Current medical treatment for these disorders relies on prokinetics or secretagogues to arbitrarily stimulate smooth muscle contractions or increase mucosal secretion (Bharucha et al., 2006; Sharma and Rao, 2017). These treatments are not very effective over time. On the contrary, inhibition of mechano-transcription process represents a promising therapeutic strategy aiming to target mechanical stress—perhaps the root cause of bowel dysfunctions in these disorders.

5.09.7 Conclusions

Mechanical stress, i.e. shear, stretch, and compression, is commonly encountered in the GI tract. Excessive mechanical stress is the root cause of obstructive conditions and is constitutively present in inflammatory conditions and some functional bowel disorders. Different tissues and cells in the GI tract respond differently to mechanical stress. In this chapter, we have focused on the recent discoveries of mechanical stress-altered gene expression in the gut, i.e. mechano-transcription. Numerous in vitro and in vivo studies have demonstrated that mechanical stress profoundly alters gene expression in the gut especially gut smooth muscle cells. Mechano-transcription of pro-inflammatory molecules, pain mediators, fibrogenic and growth factors plays a critical role in motility dysfunction, abdominal pain, inflammation, fibrosis, and hyperplasia in various gastrointestinal disorders (Fig. 6). We have shown pre-clinical and clinical evidence that mechanical stress-induced COX-2 and other pro-inflammatory mediators in gut smooth muscle cells account for motility dysfunction and inflammatory process in obstructive, inflammatory and some functional disorders. Mechanical stress-induced up-regulation of pain mediators such as nerve growth factor and brain-derived neurotrophic factor, and down-regulation of opioid receptors in the peripheral tissues may lead to visceral hypersensitivity. Emerging evidence suggests that mechanical stress in the gut also leads to up-regulation of certain genes encoding pro-fibrotic and proliferative mediators such as connective tissue growth factor and osteopontin, which may contribute to fibrostenotic Crohn’s disease. It is yet to determine whether the mechano-transcription process is involved in secretion, permeability, and other pathophysiological changes in the GI tract. Taken together, the studies over the last decade suggest that the mechano-transcription process may represent novel therapeutic targets for the management of obstructive, inflammatory, and functional bowel disorders. The strategy to target mechano-transcription process has a unique advantage, as it addresses one of the root causes (mechanical stress) of these disorders. However, further studies of these targets are warranted before therapeutics can be developed for clinical use.

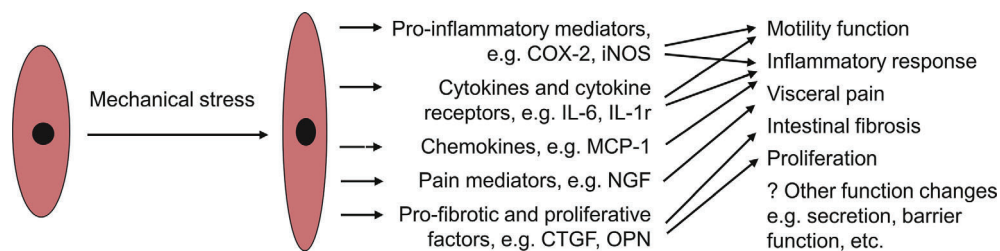


Fig. 6 Diagram summarizing the gene products and pathophysiological roles of the mechano-transcription process in the gastrointestinal tract. Studies up to date have shown that mechanical stress-induced gene expression of pro-inflammatory mediators, chemokines, cytokines and their receptors, pain mediators, pro-fibrotic and growth factors play a critical role in pathogenesis of motility dysfunction, inflammatory response, visceral pain, intestinal fibrosis and cell proliferation in the gut. It is yet to determine if the mechano-transcription process is involved in secretion, permeability, and other functions and dysfunctions in the gut.

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See Also: 5.08: Abdominal Pain

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offered to IBD patients or is not comprehensive and lacks practical resources to implement and sustain the modification. Up to 70% of IBD patients self-prescribe dietary restrictions with demonstrated risk of nutrient deficiencies. There is currently no comprehensive IBD dietary intervention presented in a customizable, easy-to-follow protocol format. To meet this need, we developed and tested a full-featured online version of an effective IBD dietary intervention. **METHOD:** 'My SCD Protocol' is a comprehensive, customizable protocol based on the specific carbohydrate diet (SCD). The online, self-paced protocol provides educational resources, self-assessment tools, and step-by-step instructions to start and advance through SCD. Ingredients are presented in 4 ordered Stages (Elimination, Reintroduction, Maintenance, Expansion) to help methodically expand tolerable ingredients. Stage-specific curated recipes support implementation and offer variety and personalization. Patients self-report symptoms, meals, and progress using a HIPAA-compliant mobile app. Entries are accessible in real-time by the patient's care team. The protocol currently holds 304 SCD ingredients and 164 recipes. Patients (n=87) referred to Modulla Health IBD Nutrition Clinic, or seeking support directly, implemented My SCD Protocol based on preference and ingredient tolerance. Symptoms were self-reported using the accompanying app. Interim results are reported here. **RESULTS:** Patients successfully accessed and personalized My SCD Protocol. Table 1 summarizes patient profiles. Table 2 presents self-reported symptom changes, showing improvements in all categories except urgency and appearance of mucus. Additional outcomes, including fecal calprotectin, will be reported. **DISCUSSION:** SCD has been shown to induce clinical response and biochemical remission in adult and pediatric IBD patients. SCD is either self-implemented by patients using a trial-and-error approach, or is dietitian-supervised, primarily using the 'legal' SCD ingredient list and little else. SCD was implemented in a clinical trial setting using a predefined, prescriptive meal model with little room to personalize diet (DINE-CD). My SCD Protocol is the first comprehensive protocol to help implement, personalize, and safely advance through SCD. Patients reliably accessed the educational videos, staged ingredients, matching recipe libraries, and logging tools using mobile and web tools. We show self-reported symptom improvements during the first 21 days of the protocol leading to longer-term adherence to diet modification. Care teams who recommend My SCD Protocol to patients can conveniently and continuously monitor patient progress in real-time and supervise patients throughout the intervention.

Table 1. Summary of patient profiles

Variable	CD	UC
IBD	54%	44%
Disease duration, years	12.6 ± 13.4	8.6 ± 9.38
	Male	Female
Sex	44%	56%
	Yes	No
Previously seen RD or nutritionist	40%	60%
Already following a special diet	43%	57%
Lost weight in past 12 months	60%	40%
IBD Medications	78%	22%
Prolonged use of antacids/PPIs	18%	82%
Frequent antibiotics use	24%	76%
Long term antibiotics use	15%	85%

Table 2. Patients reporting changes to symptoms with dietary intervention

	Baseline	Intervention*	Change
Patients reporting no symptoms			
Heartburn	47%	60%	+ 28%
Nausea	57%	76%	+ 33%
Urgency	27%	30%	+ 14%
Diarrhea	28%	35%	+ 24%
Constipation	58%	61%	+ 5%
Diarrhea + Constipation	57%	68%	+ 20%
GI Bleeding	43%	58%	+ 35%
Joint Pain	34%	46%	+ 33%
Back Pain	31%	55%	+ 75%
Patients reporting improvement in symptoms			
Vomiting	7%	22%	+ 218%
Belching	7%	39%	+ 466%
Bloating/Pressure	15%	38%	+ 151%
Flatulence	18%	37%	+ 105%
Abdominal Cramping	24%	30%	+ 26%
Urgency	30%	30%	+ 1%
Diarrhea	22%	30%	+ 39%
Diarrhea + Constipation	7%	9%	+ 36%
Mucus in stool	23%	23%	+ 1%
Shiny oil drops in stool	10%	13%	+ 30%
Body Pain	13%	22%	+ 68%
Fatigue	22%	30%	+ 39%
Anxiety / Mental Distress	20%	27%	+ 36%

* Average intervention period = 21 days

NOVEL INSIGHTS INTO THE MECHANISM OF ACTION OF EXCLUSIVE ENTERAL NUTRITION IN CROHN'S DISEASE: PRE-CLINICAL STUDY IN A RODENT MODEL OF CROHN'S-LIKE COLITIS

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BACKGROUND AND AIMS: Exclusive enteral nutrition (EEN) is the only established dietary therapy for Crohn's disease (CD). However, mechanisms of action of EEN in CD are not clear. CD is characterized by recurrent transmural inflammation in the gut. Mechanical stress, associated with inflammatory cell infiltration, edema, fibrosis, and obstruction, is inevitably present in CD tissue. We hypothesized that transmural inflammation causes mechanical stress, which induces expression of mechanosensitive genes such as IL-6 and COX-2 in the gut, and that EEN treatment attenuates inflammation by alleviating the effect of mechanical stress-induced expression of proinflammatory genes in CD. **METHODS:** CD-like colitis was induced by intracolonic instillation of TNBS (65 mg/kg in 250 μ L of 40% ethanol) into the distal colon of 8~10 weeks old SD rats. Control rats were treated with intracolonic saline instillation. We fed the control and colitis rats with either normal pellet food or EEN with Ensure liquid diet ad lib. Rats were euthanized 7 days after treatments. **RESULTS:** (1) TNBS colitis rats presented decreased body weight, increased disease activity and inflammation index of the colon in pellet food treated rats, which were dramatically improved by EEN treatment. (2) EEN did not significantly affect body weight in control rats, though it dramatically reduced fecal weight by 84.5($\pm$ 4.3)%, and increased fecal water content by 29.8($\pm$ 1.3)%. (3) In the solid food fed rats, TNBS treatment induced a localized transmural inflammation with narrowed



lumen in the site of instillation (site I) (Figure 1). This led to a distended colon segment (site P) prior to site I, and a non-distended segment (site D) distal to site I. Expression of IL-6 and COX-2 mRNAs was increased significantly ($p < 0.01$) by $111(\pm 44)$ and $13.1(\pm 2.5)$ -fold in site I of CD rats, compared to controls. Importantly, the IL-6 and COX-2 mRNA expression was also significantly increased in the distended site P [$228(\pm 72)$ and $16.6(\pm 4.3)$ -fold], but not in site D (Figure 2). The increased expression of IL-6 and COX-2 occurred mainly in the colonic smooth muscle cells (SMC). (4) However, EEN treatment with Ensure Regular prevented lumen distention in colitis rats. The increase of IL-6 and COX-2 expression in colitis was attenuated drastically in sites I and P (Figure 2). Treatment with other types of liquid diet (Ensure Clear or rodent liquid diet) had similar effects. (5) Expression of IL-6 and COX-2 was highly inducible by mechanical stretch in cultured colonic SMC in vitro and by lumen distention in colon obstruction. **CONCLUSIONS:** Mechanical stress plays a critical role in gut inflammation by inducing expression of mechanosensitive proinflammatory mediators in CD-like colitis. EEN treatment reduces inflammation in CD by alleviating the effects of mechanical stress on expression of genes such as IL-6 and COX-2.

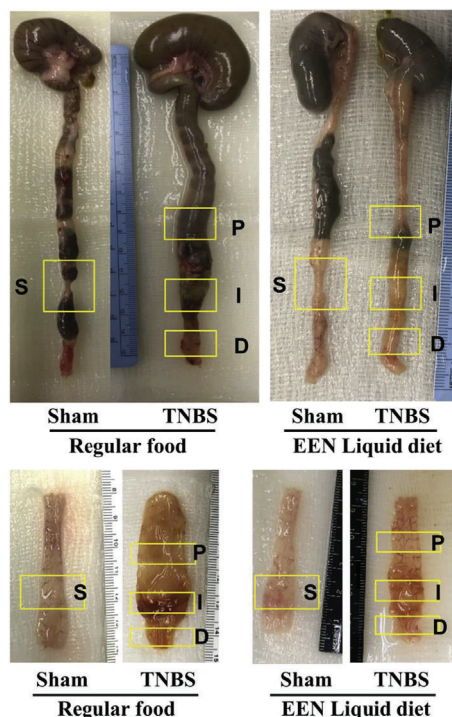


Fig. 1 EEN relieves lumen distention in TNBS colitis rats (7 day). Outlook view of colons (top) and macroscopic view of distal colons (bottom). The yellow boxes indicate different sites of colon tissue for the study. S, sham control; I, site of inflammation where TNBS was instilled to; P, colon segment proximal to inflammation site; D, colon segment distal to inflammation.

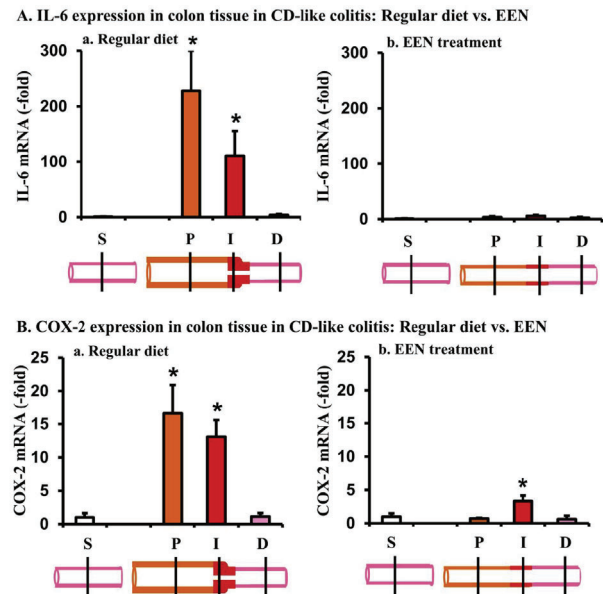


Fig. 2 Effect of EEN on mechanosensitive gene expression. Expression of IL-6 (A) and COX-2 (C) mRNAs was markedly increased in sites I and P, but not D in TNBS rats (7 day) when rats were fed with regular diet (a). However, EEN treatment almost completely blocked up-regulation of IL-6 and COX-2 (b). N = 5 in a, N = 4 in b. * $p < 0.05$ vs S (sham).

SELECTIVELY IMPROVEMENT OF THE RESPONSE OF IMMUNE CELLS TO OPPORTUNISTIC MICROORGANISMS: AN INNOVATIVE PROPOSAL FOR IBD FROM AN ANCIENT GRAIN

Sergio Pumarola Segura

BACKGROUND: Inflammatory Bowel Disease (IBD) results from an unbalanced immune response to the gut microbiome. There is a lack of proposals that integrate and may balance the multiple factors (Immunity, microbiome and diet) that together condition the onset and evolution of the disease. D-fagomine is a small molecule, naturally present in buckwheat grain. It is a monosaccharide mimetic showing glycosidase enzyme inhibition properties. Clinical studies have shown its efficacy to control postprandial glycemic response. Subsequent research “in vitro” had also shown D-fagomine to inhibit the fimbriae adhesion and biofilm formation of some proteobacteria, including ie *E. coli* or *Salmonella*. **ORIGINAL AIM:** The aim of the study was to confirm the effects of D-fagomine on the adhesion to human gut mucosa using adherent-invasive *E. coli* (AIEC) that is a microorganism usually linked to IBD. **METHOD:** Ex vivo human resected colonic mucosa was cultured (4 h) with AIEC inoculum and D-fagomine at different range of doses (0-5000 ppm). The amount of *E. coli* in tissue and supernatant was quantified, measuring also TNF-alfa and LDH. **RESULTS:** It was confirmed the inhibition of AIEC adhesion to the colonic mucosa in a direct dose-response relationship. Totally unexpected, D-fagomine was found to modulate TNF-alfa in an apparent double sigmoid curve with the max activity in the range of 50 ppm (Figure 1). This curve corresponds to an activator of the innate immune cells with mechanism related to complement activation, (ie mannose, betaglucan). Finally D-fagomine was helping the mucosa to

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MECHANICAL STRESS-INDUCED CONNECTIVE TISSUE GROWTH FACTOR PLAYS A CRITICAL ROLE IN INTESTINAL FIBROSIS IN A RODENT MODEL OF CROHN'S-LIKE COLITIS

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Background and Aims: Crohn's disease (CD) is an inflammatory bowel disease characterized by transmural inflammation and intestinal fibrosis. Mechanisms of fibrosis in CD are not well understood. Anti-inflammatory treatments have not exhibited efficacy in CD fibrosis. Transmural inflammation in CD is associated with edema, inflammatory cell infiltration, stenosis, and distention, which represent mechanical stress (MS) to the bowel wall. We hypothesize that MS, a non-inflammatory factor, induces gene expression of pro-fibrotic mediators such as connective tissue growth factor (CTGF), which may contribute to fibrosis in CD. **Methods:** A rodent model of CD was induced by intracolonic instillation of TNBS (65 mg/kg in 250 μ L of 40% ethanol) to the distal colon. Control rats were treated with saline. To determine the potential role of CTGF in fibrosis, mouse CTGF antibody FG-3149 and corresponding IgG (supplied by FibroGen, CA) were administered i.p. at 40 mg/kg twice a week, starting on the day TNBS was instilled. **Results:** (1) TNBS treatment induced a localized transmural inflammation in the distal colon (site I), with a distended colon segment (site P) prior to inflammation. We detected significant fibrosis 7 to 21 days after TNBS treatment not only in site I, but also in site P in CD rats. CTGF mRNA expression increased 30($\pm$ 6.5) and 17($\pm$ 7.0)-fold in sites P and I, respectively, but not in the segment distal to the inflammation site in CD. Further study detected CTGF expression mainly in the smooth muscle. When rats were fed exclusively with clear liquid diet (Ensure) to prevent mechanical distention in colitis, expression of CTGF in sites P and I was blocked, suggesting that CTGF expression is induced by mechanical stress. Colon distention via mechanical obstruction also led to robust expression of CTGF. (2) Compared to IgG controls (N=8), FG-3149 treatment (7 day) improved disease activity in CD rats and significantly ($p=0.002$, N=7) mitigated fibrosis index in site I and had a trend to reduce fibrosis in site P ($p=0.091$, N=7). (3) The total collagen levels (μ g/cm) were significantly increased in both sites in CD rats. FG-3149 treatment significantly attenuated collagen level in site I ($p=0.005$) and had a trend to decrease it in site P ($p=0.058$). (4) Expression of collagen 1 α 1, 1 α 2, and 3 α mRNAs in site I was significantly increased in CD rats when treated with control IgG. FG-3149 treatment exhibited consistent trends towards normalizing expression of all three collagen mRNAs. **Conclusions:** Transmural inflammation causes mechano-sensitive expression of CTGF in the colon in CD-like rats. Administration with CTGF antibody significantly improved disease activity and fibrosis score, and decreased collagen levels in CD rats. This data suggests that mechanical stress, by up-regulating pro-fibrotic mediators, may play a key pathogenic role in fibrosis in CD.

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EXPRESSION OF ERBB RECEPTORS AND LIGANDS IS ALTERED BY INTESTINAL IRRADIATION INJURY IN MICE

Stephanie King, Mark R. Frey

Background: The ErbB receptor family regulates proliferation and growth of intestinal epithelia. Wild-type ileal crypts treated with neuregulin (NRG)-1 or NRG-4 have enhanced enteroid formation. Previous studies demonstrated these receptors are critical for protection against injury and inflammation in the intestinal mucosa. *ErbB3* knockout in the intestinal epithelium leads to precocious Paneth cell maturation, increased ER stress and expression of inflammatory mediators, and increased expression of the intestinal stem cell markers, while *ErbB4* knockout in the intestinal epithelium produces no overt defect. However, expression of *ErbB4* is induced in *Il10* knockout mice and expression of the ErbB4 ligand, *Nrg4* is decreased in IBD patient biopsies. We hypothesized ErbB4 expression is responsive to intestinal injury and inflammation, while ErbB3 is repressed early post-irradiation during regeneration. **Methods:** Adult wild-type mice were irradiated (12 Gy) and sacrificed at days 1, 3, and 5 post-irradiation. Mucosal scrapings of the ileum, proximal and distal colon were collected for gene expression and protein analysis. Quantification of NRG4 was determined by ELISA. Ileal enteroids from *ErbB3* floxed and knockout mice were irradiated (2 Gy) and immediately treated with NRG-1 (100 ng/mL) or NRG-4 (100 ng/mL) until harvested. Enteroids were harvested at days 1, 3, and 5 post-irradiation. **Results:** Expression of *Lgr5* was significantly decreased following irradiation injury in all intestinal regions. *ErbB3* expression decreased steadily following irradiation. In contrast, *ErbB4* expression increased over time in the ileum and distal colon. *Nrg1* expression was increased in all intestinal sections. *Nrg4* expression in the ileum decreased, while there was a significant increase in its expression in the proximal colon at day 3 post-irradiation. However, *Nrg4* expression was significantly decreased in the proximal colon when normalized to *Cdh1* (E-cadherin). NRG4 protein was significantly increased in proximal colon at day 5 post-irradiation. Similar to WT mice, *ErbB3* expression decreased following irradiation in ileal enteroids and was unaffected by NRG-4 treatment. However, NRG-1 treatment increased *ErbB3* expression, peaking at day 3 post-irradiation. **Conclusions:** We observed that irradiation injury increased *ErbB4* expression, confirming that *ErbB4* is induced following intestinal injury and inflammation. Expression of *ErbB3* decreased following irradiation. Changes in expression of *Nrg4* appears to be both region and cell type specific and correlated with protein levels in the proximal colon, while *Nrg1* expression is consistently increased after irradiation. Altered expression of these receptors and their ligands may be important in the regenerative response following intestinal injury.

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LPS INDUCED CASPASE-1 INHIBITED BY EPI-GALLOCATECHIN GALLATE (EGCG), REDUCES INTERLEUKIN-1 FAMILY CYTOKINES.

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Epigallocatechin-3-gallate (EGCG), a bioactive green tea polyphenol, exerts potent anti-inflammatory/cytokines modulation effects by inhibiting signaling events and gene expression. It has promising therapeutic effects for inflammatory bowel diseases (IBD). The multimodal mechanism has yet to be fully elucidated. This study evaluated downstream events from EGCG-induced Caspase-1 inhibition, which imparts a global effect on several IL-1 family cytokines produced by human epithelial Caco-2 cells (e.g., IL-1 β and IL-18, IL-33, IL-35) plus closely related Type 1 cytokines (TNF- α and INF- γ). IL-1 family cytokines and their ligands play a significant role in gut homeostasis and inflammation via the caspase-1-activating multiprotein complex NLRP3 Inflammasome. **Methods:** Caco-2 cells (ATCC) grew under normal conditions in RPMI-1640 medium+10% fetal calf serum. Viability assessed by MTT assay. 80% confluent monolayers (1x10⁶ cells/well) were treated either with EGCG alone or 10 ng/mL lipopolysaccharide (LPS) plus increasing concentrations of EGCG (0, 2.5, 5.0, 7.5, 10 ng/ μ L) then incubated for additional 24 hrs. Caspase-1 activity inhibition was quantitatively measured by Promega kit. Catalytically active caspase-1 activation governs processing and release of IL-1 family cytokines (IL-1 β , IL-18, IL-33 and IL-35). EGCG inhibition of cytokine production measured by ELISA (R&D Systems). In addition, tight-junction protein effects on ZO-1, occludin, and Claudin isoforms (C-1, C-4, C-8) measured by Western blot. Target cytokine expression measured by real-time PCR. **Statistical analysis:** Data expressed as means $\pm$ SD (standard deviation). Statistical significance of differences between treatment and control groups determined by Student t test. Differences considered statistically significant when $p<0.05$. **Results:** The inhibitory effect of EGCG on caspase-1 activity followed a dose dependent curve based on concentration of EGCG (0-10 ng/ μ L). Since LPS induces caspase-1-dependent IL-1 β processing in mammalian cell line, the inhibitory effect was highly significant when 80% confluent Caco-2 cells are primed with LPS prior to adding EGCG. LPS increased caspase-1 activation by 160% over cells without LPS. Dose-dependent caspase-1 inhibition (1-10 μ g/mL) reduced LPS stimulated activity by 82%, 61%, 42%, and 64% at (2.5, 5.0, 7.5, and 10 μ g/mL) respectively. LPS-primed human Caco-2 cells strongly enhanced IL-1 RNA message, while EGCG reduced stimulated message by 82%, 75%, 68% and 70%. EGCG increased tight junction proteins (TJPs) occludin (164%) and ZO-1 (132%) and claudin-1 (182%), claudin-2 (154%) and claudin-8 (144%). Pre-treatment incubation with LPS doubled TJP production. **Conclusion:** EGCG benefits inflammation by reducing IL-1 family cytokine production, specifically inhibiting caspase-1 activation, and modulating clinically relevant claudin production.

Sul1145

MATRIX METALLOPROTEINASE (MMP-9) INDUCED INCREASE IN INTESTINAL EPITHELIAL TIGHT JUNCTION BARRIER IS MEDIATED BY EGFR ACTIVATION

Rana Al-Sadi, Mohammad Haque, Jessica Engers, Thomas Y. Ma

Background: The defective intestinal tight junction (TJ) barrier has a key pathogenic factor in Crohn's disease (CD) and other inflammatory conditions of the gut. Clinical studies in CD patients have shown that a persistent increase in intestinal permeability is predictive of poor clinical outcome. Matrix metalloproteinase-9 (MMP-9) has been shown to be markedly increased in intestinal tissues of patients with CD and it has been postulated that MMP-9 plays an important role on the pathogenesis of intestinal inflammation in CD. Our recent studies suggested that MMP-9 at clinically relevant concentrations causes an increase in intestinal epithelial TJ permeability *in-vitro* and *in-vivo* by inducing intracellular signaling pathways via activating distinct basolateral membrane receptors. However, the specific protein receptors and the intracellular signaling cascades involved have not been fully identified. One of the potential protein receptors that might be a target of MMP-9 on the basolateral membrane of the intestinal epithelial cells is the epidermal growth factor receptor (EGFR), which has been shown to have opposing roles in the pathophysiology of CD. **Aims:** Therefore, our aims in this study were to examine the effect of MMP-9 on EGFR activation and delineate the role of EGFR in MMP-9 induced increase in intestinal epithelial TJ permeability by using Caco-2 monolayers as an *in-vitro* model system. **Results:** MMP-9 (400 ng/mL) caused a time-dependent increase in EGFR activation as assessed by EGFR phosphorylation, and by *in-vitro* kinase activity measurement of EGFR in filter-grown Caco-2 monolayers. Inhibition of EGFR by pharmacologic inhibitor, PD 153035, prevented the MMP-9 induced increase in Caco-2 TJ permeability measured by trans-epithelial resistance (TER) and mucosal-to-serosal flux of a paracellular marker dextran 10kd. MMP-9 caused a decrease in the TJ protein occludin expression. Inhibition of EGFR by PD 153035 prevented the MMP-9 induced degradation of occludin expression. In addition, the effect of EGFR activation by a known activator, NSC 228155 25, in the absence of MMP-9, caused an increase in Caco-2 TER, dextran 10 kd flux and a decrease in occludin expression. **Conclusion:** Our data showed that MMP-9 induced increase in intestinal epithelial TJ permeability is mediated in part by the activation of EGFR and the subsequent degradation of occludin expression. Identifying the specific membrane receptors that mediate the MMP-9 induced increase in intestinal TJ permeability could be important in developing future therapeutic strategies in IBD.