

## Review Article

# Role of Rho kinase signal pathway in inflammatory bowel disease

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**Abstract:** Inflammatory Bowel Disease (IBD) is nonspecific inflammation in the intestinal track, including Ulcerative Colitis (UC) and Crohn's disease (CD). The incidence of IBD has increased significantly, with its numerous rising up to five million globally, more than 1,700,000 in China. Pathological character of IBD is the inflammation of intestinal mucosa and intestinal fibrosis. Although the pathogenesis of the disease has not yet been fully clarified, some evidence suggests that excessive intestinal inflammation reaction, intestinal barrier impairment and abnormal immune response can initiate IBD. As research continues, some of them have provided new insights toward understanding of Rho kinase signal pathway function at the occurrence and development of IBD. This review aims to summarize the general principles of Rho kinase signal pathway in the pathological procedure of IBD.

**Keywords:** Rho/Rho kinase, IBD, intestinal barrier, intestinal fibrosis

### Rho/Rho kinase signal pathway

The Rho/Rho kinase signal pathway is ubiquitously expressed across the species, ranging from yeast to human. Rho kinase is a subgroup of the Ras superfamily of 20-to 30-KD GTP-binding proteins that have been shown to regulate a wide spectrum of cellular functions. In Rho family, RhoA, Rac and Cdc42, which are regarded as the core molecules that induce stress fibers to form and regulate cellular adherence by reconstructing cytoskeleton in response to extracellular growth factors, are deeply researched [1]. ROCK (Rho-associated kinase), which belongs to the member of the family of serine/threonine protein, is an acknowledgeable important signal molecule in the downstream of the Rho kinase signal pathway. It mainly includes two types: ROCK1 and ROCK2 [2]. ROCK1 is widely expressed in various tissues except the central nervous system; ROCK2 is mainly expressed in the central nervous system. Like all members of the Ras superfamily, the Rho kinase functions as molecular switches, cycling between an inactive GDP-bound form and an active GTP-bound form [3]. Extracellular signals trigger the activity of Rho, which then binds to Rho kinase and change GDP-Rho into GTP-Rho. Activated Rho

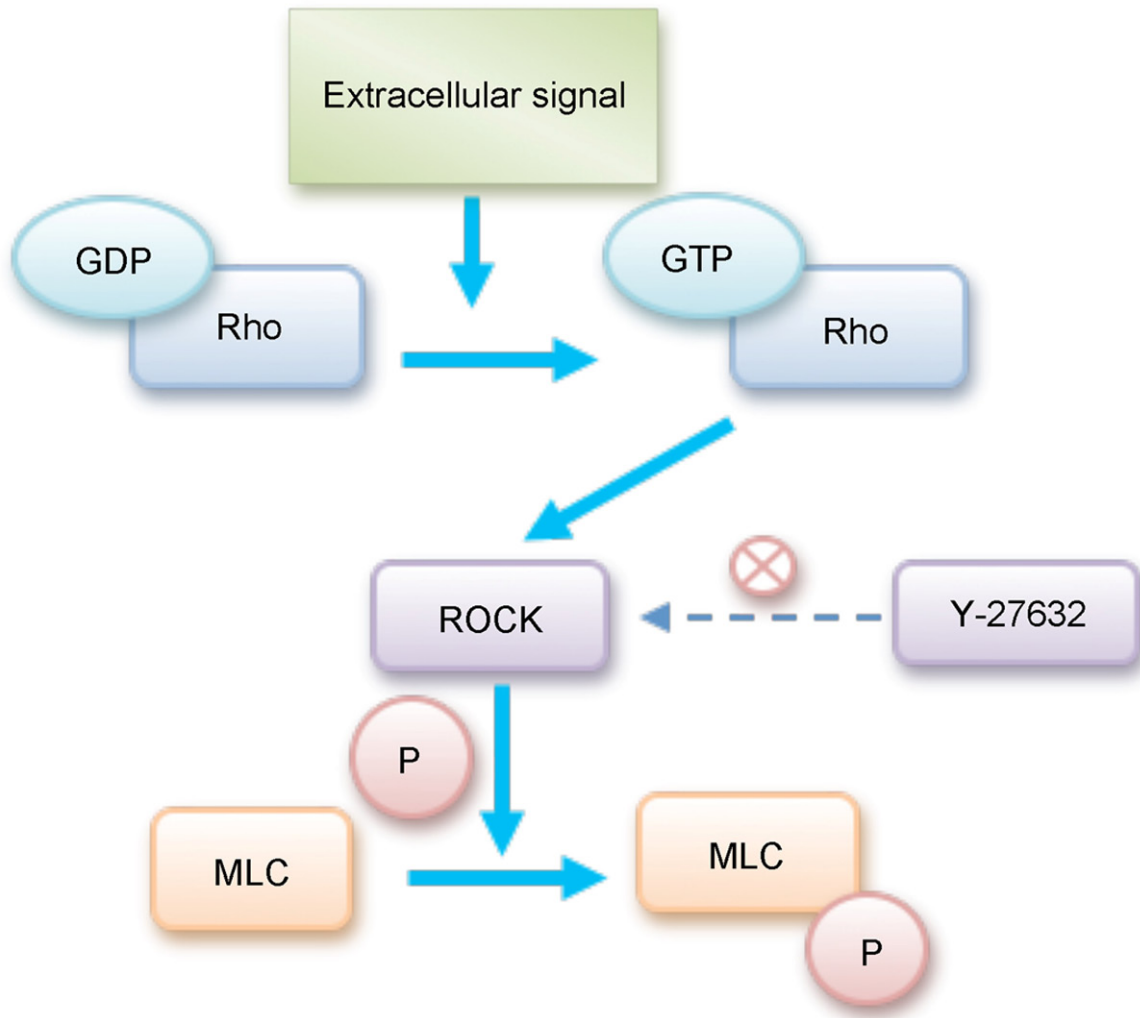
kinase conducts signals to ROCK and phosphorylates the myosin light chain (MLC), leading to an accumulation of the phosphorylated form of MLC (pMLC), and then changing the cytoskeleton. Y-27632 is the unique inhibitor of ROCK that can inhibit the cell biology behavior induced by Rho signal pathway [4] (**Figure 1**). Numerous basic experiments suggest that Rho kinase signal pathway plays a critical role in diseases' occurrence and development, such as heart disease [5], pancreas fibrosis [6] and pulmonary hypertension [7]. As research continues, it is believed that Rho kinase is related to intestinal diseases, IBD is one of them.

### Intestinal barrier damage and immune activation in IBD

#### *Intestinal epithelial integrity and immune isolation*

Intestinal barrier is composed of three layers—the slime layer, the epithelial layer, and the basement membrane, which produces a complete biological barrier that isolates bacteria and antigens of the intestinal tract from immune cells in order to avoid immune response [8, 9]. Of the three layers, cellular junction (**Figure 2**; [10]) which consists of tight junction (TJ), adher-

## Rho kinase signal pathway in inflammatory bowel disease



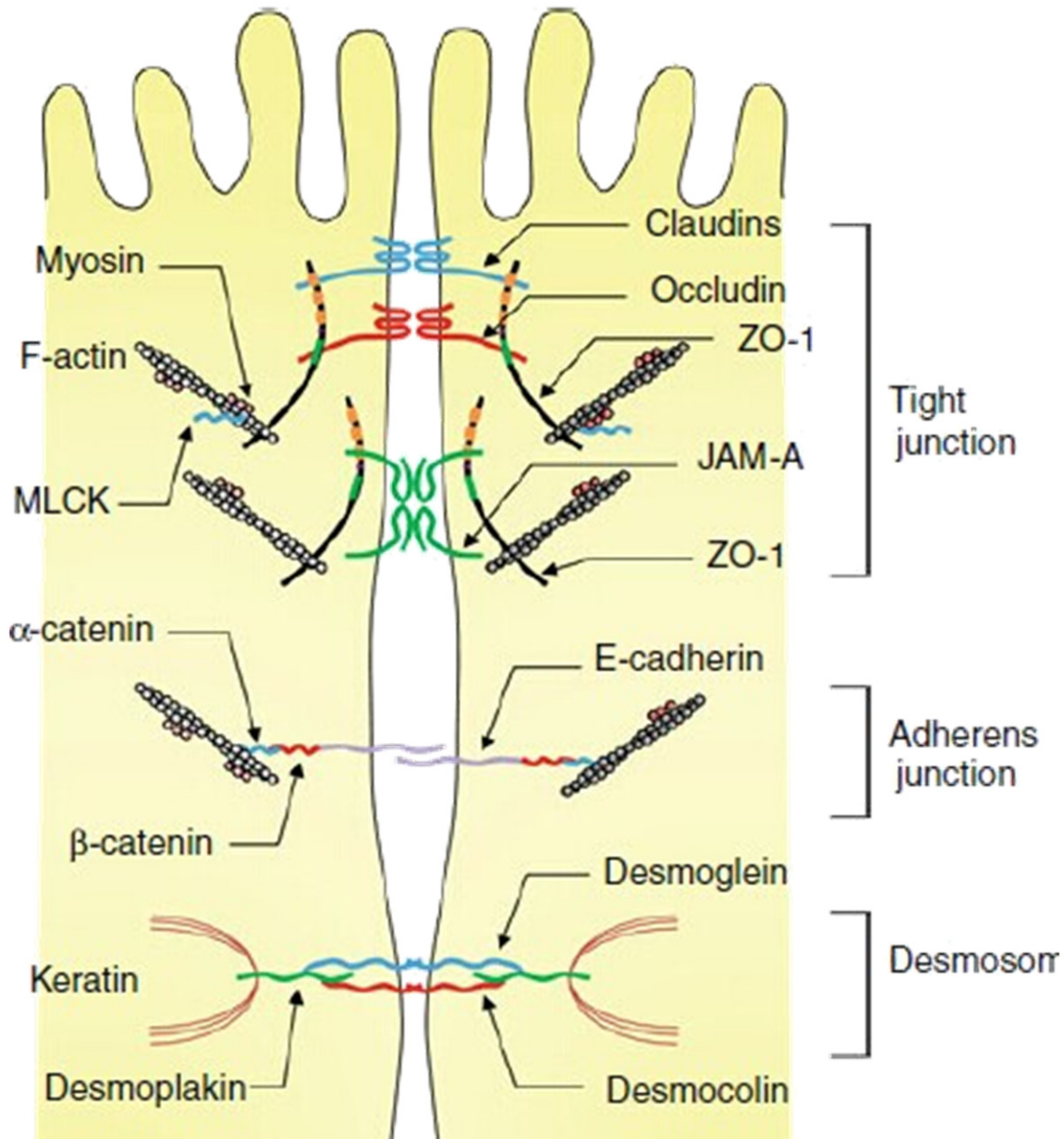
**Figure 1.** Regulation between ROCK and MLC. Extracellular signals initiate the activity of Rho, which then binds to Rho kinase and change GDP-Rho into GTP-Rho. Activated Rho kinase phosphorylates MLC, leading to an accumulation of pMLC. With the increasing of pMLC, it enhances stress fibrosis formation and rearranges F-actin cytoskeleton.

ens junction (AJ) and desmosome in mucosa layer is the critical one. TJ is a multiple protein complex located at the apical ends of the lateral membrane of intestinal epithelial cells in keeping intestinal barriers integrality [10]. The TJ complex consists of transmembrane proteins [11-13] such as occluding, claudin, junction associated molecule (JAM) and intracellular proteins [14] such as zonula occludens (ZO). The transmembrane proteins interact with the intracellular proteins, which in turn create a permselective barrier in preventing bacteria and antigens from activating immune cells located in laminae propria. Simultaneously, TJ also plays an important role in intercellular substance transportation and maintenance of intestinal epithelial differentiation and recov-

ery. Alteration of intestinal tight junction is regulated by myosin light chain (MLC) [15]. ROCK, myosin light chain kinase (MLCK) and other phosphorylated kinase can phosphorylate MLC to promote tight junction proteins (including transmembrane proteins and intracellular proteins) depredated in cells, ultimately inducing intestinal barrier damage and mucosal permeability impairment [16]. In addition, ROCK can also cause the contraction of F-actin cytoskeleton to regulate intestinal barrier integrity [17].

### *Rho kinase signal pathway in intestinal barrier damage and immune activation*

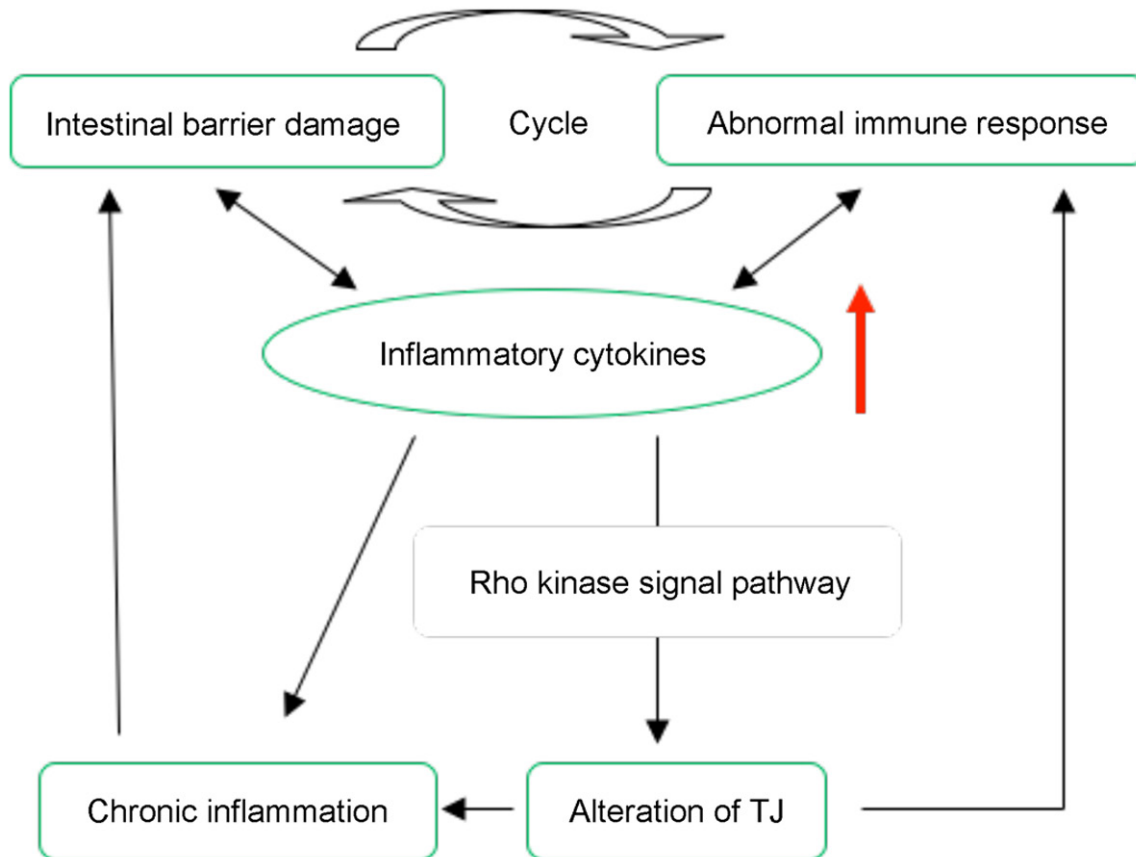
Accumulating evidence from basic science and clinical studies demonstrates three essential



**Figure 2.** Cellular junctions in intestinal epithelium. It consists of TJ, AJ and desmosome. The TJ complex comprises transmembrane proteins such as occluding, claudin, JAM and intracellular proteins like ZO. The MLCK and F-actin can be regulated by Rho kinase.

factors for IBD pathogenesis: disruptions of the intestinal barrier, exposure of the luminal content to mucosal immune cells and an abnormal immune response [18-21] (**Figure 3**). In IBD patients, it is suggested that expression of TJ proteins decreased obviously, but there was no difference between non-inflammatory intestinal tissue in IBD patients and healthy patients, indicating that abnormal expression of TJ proteins mainly located in inflammatory intestinal tissues [22]. Therefore, excessive inflammatory

response in the intestinal tract destroys the expression and distribution of tight junction proteins. In CD, we can find that numerous inflammatory cytokines express in intestinal mucosa cells [23-31], including TNF- $\alpha$ ,  $\gamma$ -IFN, Interleukin-1 (IL-1), Interleukin-1 $\beta$  (IL-1 $\beta$ ), Interleukin-13 (IL-13). All of cytokines regarded as extracellular signals influence the expression of tight junction proteins, thus interfering cellular junction structure and changing intestinal integrity via Rho kinase mediated F-actin cyto-



**Figure 3.** Essential factors for IBD pathogenesis involves Rho signal pathway. Disruptions of the intestinal barrier result in luminal content exposure to mucosal immune cells and activate abnormal immune response, which cause pro-inflammatory cytokines increase. Simultaneously, it can damage the structure of TJ via Rho signal pathway that induced chronic inflammation.

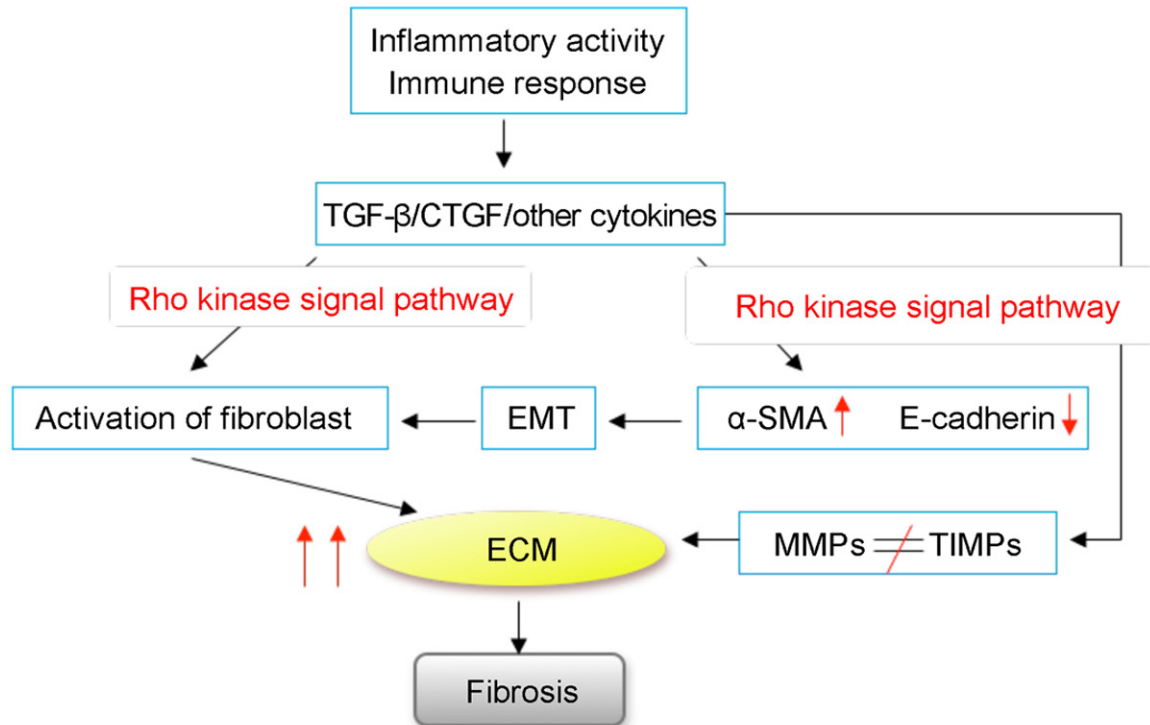
skeleton regulation. Simultaneously, increased expression of  $\gamma$ -IFN [25] in IBD not only can inhibit the expression of TJ associated proteins like Occludin, Claudin-1/4, but increase the regulative proteins of TJ, such as ROCK, MLCK, which can make TJ proteins depredated [32]. TNF- $\alpha$  can also coordinate with IFN- $\gamma$  to increase TJ regulative proteins of intestinal mucosa to change the intestinal barrier [33]. Furthermore, Segain's experiment [34] provided the direct evidence that increased activation of RhoA was found in inflamed colonic mucosa of patients with CD and of rats with 2, 4, 6-trinitrobenzene sulfonic acid-induced colitis. Oral administration of Y-27632 in rats significantly reduced the colonic inflammation via nuclear factor kappa B inhibition. It is clarified that radiation-induced enteropathy is associated with microvascular injury and epithelial barrier dysfunction [35]. Rho kinase activity constituted an important signaling mechanism in leucocyte-platelet-

endothelium interaction, formation of cytokinase and reactive oxygen species, neutrophil accumulation. We deduce that this mechanism may have a similar effect in IBD, but more long-term studies are needed.

### Rho kinase signal pathway in intestinal fibrosis of IBD

#### *Intestinal fibrosis in IBD*

In the pathological procedure of IBD, the intestinal mucosa expresses reduplicated damage and recovery with the repeated stimulation of inflammation, which activates intestinal stromal cells that can produce ECM mediated intestinal fibrosis. Histopathological observation indicates that ECM in UC mainly located in rectal mucosa and submucosa, but ECM in CD can be observed in the all intestinal wall [36]. As the present study suggests, the pathogene-



**Figure 4.** Fibrosis in IBD involves Rho signal pathway. Misbalance of ECM induced by MMPs, TIMPs and activation of fibroblast can be induced by Rho signal pathway. This procedure is activated by the primitive inflammatory response.

sis of fibrosis in IBD involves cellular and molecular mechanism [36-39]. The cellular mechanism mainly includes the excessive activation of intestinal stromal cells, fibroblast migration and epithelial mesenchymal transition (EMT). The molecular aspects include the misbalance of ECM; increasing of TGF- $\beta$ , connective tissue growth factors (CTGFs) [40, 41] and other cytokines. Above these mechanisms jointly promote the ECM deposition and lead to intestinal wall fibrosis. If fibrous hyperplasia sustained, the progress will eventually result in intestinal deformation, narrow lumen, and even cause intestinal obstruction and other severe complications [42].

#### *Rho kinase signal pathway in intestinal fibrosis of IBD*

Based on the related research about the cellular and molecular mechanism of the fibrosis, it is shown that the Rho kinase signal pathway makes sense (Figure 4). Misbalance of ECM is the direct origin of fibrosis, and matrix metalloproteinases (MMPs) and tissue inhibitors of metalloproteinases (TIMPs) are the crucial factors to maintain normal metabolism of ECM in

cells, and Rho/ROCK-dependent actin-cytoskeleton reorganization influences MMPs and TIMPs [43]. In endoscopic biopsy specimens of CD patients, the level of MMPs and its inhibitor TIMPs' mRNA increased definitely, the level of MMP-2, MMP-4 and TIMP-1 dramatically increased, and the expression of MMP-1's, MMP-3's mRNA are 15 times than normal tissues [44, 45]. It is also indicated that the activity of TIMP-1 in myofibroblast of CD patients is increased, which can inhibit the increased activity of MMP and result in misbalance of ECM, leading to fast fibrosis reaction [44]. Rho/ROCK caused by abnormal regulation of MMPs/TIMPs controls ECM imbalance, which plays an important part in the development of IBD intestinal fibrosis. IBD's intestinal wall inflammation activity showed high expression of TGF- $\beta$  and receptor in the upstream was the starting factors during the process of ECM's production. Initiating factors accelerate the GTP-Rho into GDP-Rho transformation, acting on the downstream of the cytoskeleton contraction induced by ROCK activated fibroblasts, and secrete a large number of ECM [46]. Simultaneously, the increased level of TGF- $\beta$  is associated with the expression of CTGF, jointly promoting fiber with

the Rho kinase signal pathway [40]. In the study of radiation-induced enteritis, Bourcier [47] found an obvious increase in the intestinal smooth muscle cells and tissues in the coding Rho/ROCK signaling pathway protein gene expression. Its unique inhibitor Y-27632 can specially inhibit the activation of fibroblast, which may be relevant to nuclear factor-kappa B (NF- $\kappa$ B). But all of these are not definitely observed in IBD in vivo and vitro. So, we may put more emphasis on the Rho signal pathway involved in the mechanism of IBD.

Another pathway inducing intestinal fibrosis is EMT, which is considered to be the crucial mechanism of cell transition [48]. Especially, concrete expression can be embodied in: depolymerization of cellular junction; cytoskeleton reorganization; expression of cell adhesion molecules like E-cadherin reduced; cell polarity disappeared; mesenchymal markers such as alpha smooth muscle protein ( $\alpha$ -SMA), type I collagen enzyme and other proteins expression increased; cell migration. EMT has a pervasive effect on organ's fibrosis. It has been indicated that epithelial cells of different organs can be changed into myofibroblasts and fibroblasts phenotype which cause the tissue fibrosis, such as alveolar epithelial cells [49] and tubular epithelial cells [50]. Studies on kidney fibrosis suggest that the occurrence of EMT can be also induced by TGF- $\beta$  [51]. Precisely, in vivo, TGF- $\beta$ 1 secreted by IMF functions as a signal molecule in the upstream increases  $\alpha$ -smooth muscle actin ( $\alpha$ -SMA), reconfigures actin stress fibers and stimulates actin-myosin contraction in the cell body, finally resulting in EMT. In CD's patients, the level of TGF- $\beta$ 1, TGF- $\beta$ 2 is apparently elevated in intestinal myofibroblast [52], accordingly, we confer that TGF- $\beta$  induced EMT in IBD is possibly existed. Identification of EMT as a novel source of fibrosis in the intestinal tract, Flier SN [53] showed that intestinal epithelium detected the feature of mesenchymal cells on the CD model mice induced by TNBS, which prompt EMT happened. At the same time, they found in the intestinal wall sample CD's patient increased  $\alpha$ -SMA expression and E-cadherin expression decreased, but it did not appear in the normal intestinal tissue. As noted above, these can be regarded as EMT's marker in cell biology. This evidence may support our inference that EMT is a cause of activated fibroblasts and existed in IBD. But for the Rho signal

pathway mediated EMT and the occurrence of fibrosis in IBD is still in the exploratory stage, the relevant experimental studies do not illustrate a clear idea of its specific mechanism, which remains to be further exploration and discovery.

### Conclusion

Based on the findings described above, it is evident that the Rho kinase signal pathway is involved in the three essential starting segments in the chronic pathogenic procedure of IBD: disruption of the intestinal barrier, exposure of the luminal contents to mucosal immune cells and an abnormal immune response. Furthermore, reduplicate intestinal tract inflammation not only damages the permeability and integrity of intestinal mucosa barrier, but disrupts the balance between MMPs and TIMPs and initiating EMT. The above procedures also can be controlled by Rho kinase signal pathway. Evidence from basic science also revealed that ROCK special inhibitor Y-27632 made sense in improving inflammatory action and fibrosis in experimental model. Thus, studies on the Rho kinase signal pathway should be extremely useful for the precise mechanism of IBD in vivo. In addition, as research moves along, specific inhibition of Rho kinase signal pathway may be a promising novel approach for the treatment of patients with IBD, which effectively functions as preventing inflammatory action, promoting the recovery of intestinal mucosa, reducing fibrosis and improving prognosis.

### Disclosure of conflict of interest

None.

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