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**New insights into the interplay between intestinal flora and bile acids in inflammatory bowel disease**

the interplay between intestinal flora and bile acids in inflammatory bowel disease

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## **Abstract**

Intestinal flora plays a key role in nutrient absorption, metabolism and immune defense, and is considered to be the cornerstone of maintaining the health of human hosts. Bile acids synthesized in the liver can not only promote the absorption of fat-soluble substances in the intestine, but also directly or indirectly affect the structure and function of intestinal flora. Under the action of intestinal flora, bile acids can be converted into secondary bile acids, which can be reabsorbed back to the liver through the enterohepatic circulation. The complex dialogue mechanism between intestinal flora and bile acids is involved in the development of intestinal inflammation such as inflammatory bowel disease (IBD). In this review, the effects of intestinal flora, bile acids and their interactions on IBD and the progress of treatment were reviewed.

**Key Words:** Intestinal flora; bile acids; Inflammatory bowel disease; Fecal microbiota transplantation; prebiotics

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**Core Tip:** With the increase of economic level and the improvement of people's living standard, the incidence of IBD in China is gradually increasing, causing a heavy burden to the society. The pathogenesis of IBD is related to genetics, environment, intestinal microecology and immunity, but the specific biological mechanism is still unclear. As an important part of intestinal microecology, intestinal flora can directly affect intestinal environmental homeostasis and participate in bile acid metabolism, while the abnormal bile acid metabolism also affects the quality and quantity of intestinal flora, and both of them are involved in the occurrence and development of intestinal inflammation.

## INTRODUCTION

Inflammatory bowel disease (IBD) is a chronic relapsing disease, including Crohn's disease (CD) and ulcerative colitis (UC), which has become a public health problem worldwide. With changes in diet and lifestyle, the incidence of IBD is rising rapidly worldwide. The composition of intestinal flora is considered to be the main driver of intestinal immune dysfunction in IBD, but this concept has not been fully proven<sup>[1]</sup>. Bile acids are steroid molecules produced by interaction between the host and gut flora. It is one of the largest bioactive substances found in mammals and acts on the G protein and nuclear receptor families<sup>[2]</sup>. In this review, we reviewed the effects of intestinal flora, bile acid receptors and their interactions on IBD and the progress of its treatment.

## INTESTINAL FLORA AND IBD

IBD patients were found to have intestinal microbiota imbalance, which was mainly characterized by decreased intestinal microbiota diversity. The anti-inflammatory bacteria in feces of IBD patients, such as *Faecalibacterium prausnitzii* and *Roseburia*, have decreased. In the intestinal mucus layer, *Roseburia* can convert acetate to butyrate and produce secondary bile acids, which may have anti-inflammatory effects<sup>[3]</sup>. The proportion of *Bacteroides Fragilis* in IBD patients also decreased significantly. The polysaccharide A produced by The bacterium induces the development of CD4<sup>+</sup> T cells and the anti-inflammatory function of regulatory T cells (Treg)<sup>[4]</sup>. A recent study found that the use of short-term antibiotics at an early age increased the susceptibility of mice to colitis induced by Dextran sulfate sodium (DSS), suggesting that the imbalance of intestinal flora is closely related to the incidence of IBD<sup>[5]</sup>.

The intestinal flora metabolizes to produce many bioactive molecules that interact with the host. The typical representatives are short chain fatty acids (SCFAs), which mainly include acetic acid, propionic acid and butyric acid. These bioactive molecules not only serve as energy for intestinal epithelial cells, but also increase the secretion of anti-inflammatory cytokines such as interleukin (IL-10) and the number of Treg cells by activating the G-protein-coupled bile acid receptor Gpbar1 (TGR5) on intestinal cells

and immune cells<sup>[6]</sup>. It can reduce tissue inflammation and maintain the stability of intestinal mucosal barrier function. Studies have shown that butyrate can promote the recovery of intestinal barrier function and accelerate the repair of intestinal epithelial cell injury through synaptopoptin, while the loss of bacterial flora blocks the expression of synaptopoptin and increases the sensitivity to colitis and intestinal permeability in mice<sup>[7]</sup>.

Dietary tryptophan can be metabolized by intestinal flora into metabolites such as indoleacetic acid, indole3-acetaldehyde, indole3-aldehydes, indole3-acrylics and indole3-propionic acid, thus acting as ligands of aromatic hydrocarbon receptors, which are closely related to the pathogenesis of IBD<sup>[8]</sup>.

Indoles, indoles propionic acid and indoles acrylic acid bind to progesterone X receptors, thereby reducing intestinal permeability and affecting mucosal homeostasis<sup>[9]</sup>. Indoleformaldehyde secretes IL-22 by activating aromatic hydrocarbon receptors on intestinal immune cells. Indole-3-propionic acid protects mice from DSS-induced colitis by binding to aromatic hydrocarbon receptors to produce IL-10<sup>[10]</sup>. Therefore, intestinal flora disorder can disrupt immune regulation and promote inflammation through its metabolites.

### **BILE ACID METABOLISM**

Bile Acid (BA) is an important compound and structural component in human and animal Bile, and the liver is the main site of Bile Acid formation<sup>[11]</sup>. Bile acid synthesis by the great influence of diet, the body from free cholic acid, the primary bile acids combined with secondary free cholic acid and bile acid composition, type of free bile acid is by chenodeoxycholic acid (CDCA) and cholic acid (CA), primary bile acid combination type of cows sulfonated goose deoxycholic acid and ammonia goose deoxycholic acid, taurocholic acid as well as the composition of gca, Secondary free cholic acid consists of cholic acid and deoxycholic acid<sup>[12]</sup>. Bile acids are a kind of important host-derived compounds, which have many important physiological functions and effects on the host and its intestinal flora. Bile acids are metabolites of cholesterol, and the transformation of bile acids requires the help of intestinal

microflora<sup>[13]</sup>. The classical pathway and the alternative pathway are two pathways of bile acid synthesis. It is regulated by cholesterol 7 $\alpha$  -hydroxylase (CYP7A1), sterol 12 $\alpha$  -hydroxylase (CYP8B1), cholesterol 27 $\alpha$  -hydroxylase (CYP27A1) and other enzymes related to bile acid synthesis<sup>[14]</sup>. It has been confirmed that in the classical pathway of bile acid synthesis, cholesterol in human liver is catalyzed by CYP7A1 to produce 7 $\alpha$  -hydroxyl cholesterol, and then is catalyzed by 3 $\beta$  -hydroxyl -  $\delta$  5-C27-steroid dehydrogenase (3 $\beta$ -HSD), CYP8B1 and CYP27A1. 7 $\alpha$  -hydroxyl cholesterol is catalyzed to produce <sup>10</sup> primary bile acids, free cholic acid (CA) and goose deoxycholic acid (CDCA)<sup>[15]</sup>.

In the alternative pathway, cholesterol is catalyzed by CYP27A1 to produce 27 $\alpha$  -hydroxyl cholesterol, followed by deoxycholic acid in response to CYP7B1<sup>[16]</sup>. The free cholic acid then binds to glycine and taurine by its own amide bond to form conjugated bile acids which enter the intestinal tract of the body<sup>[17]</sup>. Taurocholic acid forms deoxycholic acid (DCA) after hydroxy release by intestinal bacteria. In the metabolism of goose deoxycholic acid, goose deoxycholic acid is a combination of glycine and goose deoxycholic acid<sup>[18]</sup>. The intestinal bacteria of the body hydrolyze goose deoxycholic acid and dehydroxyl to form stone cholic acid (LCA)<sup>[19]</sup>. Goose deoxycholic acid can also react with taurine to form taurocholic acid. Cholesterol 7 $\alpha$  -hydroxylase is a rate-limiting enzyme of bile acid synthesis in the body, and its activity can regulate the rate of bile acid synthesis in the process of bile acid synthesis, which has been proved in an experimental study<sup>[20]</sup>. Rats in the experimental group were fed bile acid, and the activity of 7 $\alpha$  -hydroxylase was decreased and the rate of bile acid synthesis was also significantly decreased in the experimental group compared with normal rats<sup>[21]</sup>. After bile acid synthesis by bile salt export pump into the gall bladder stores, when the body after eating, in the gallbladder bile into the intestine to help the body absorb the lipid in food, the bile acid level in the body is not fixed, it is in the steady state environment, in the terminal ileum 95% bile acid enterohepatic circulation will be absorbed by weight, over 5% of conjugated bile acid excretion of excrement and urine, Limited bile acids are reused through the enterohepatic circulatory system<sup>[22]</sup>. This process, called bile acid

enterohepatic circulation, occurs about six times a day in the body. Most bile acids are reabsorbed at the terminal ileum via the ASBT (apical membrane sodium-dependent bile salt transporter) of intestinal cells, where bile salts are transported from the intestinal epithelial cells to the basal outer membrane side into the blood with the help of intestinal bile acid proteins<sup>[23]</sup>.

## BILE ACID AND IBD

Repeated stimulation of intestinal epithelial cells with high concentration of bile acids is an important risk factor for the pathogenesis of IBD, which will destroy host material metabolism and signal transduction<sup>[24]</sup>. In rats with colitis induced by Trinitrobenzenesulfonic acid (TNBS), apical sodium-dependent bile acid transporter, ASBT expression decreased. When intestinal inflammation occurs, the intestinal barrier is damaged, which leads to the reduction of ASBT expression, and finally the destruction of enterohepatic circulation leads to the accumulation of bile acids in the intestinal mucosa, and the intestinal inflammation is aggravated. In IBD patients, ileal inflammation blocks hepatoenteric circulation of bile acids, leading to reduced ileal reabsorption, which may be due to inhibition of ASBT promoter expression by inflammatory cytokines, thus increasing fecal bile acids<sup>[25]</sup>.

Hepatic bile acid synthesis is regulated by the FXR-FGF15/19 signaling pathway. Activation of this signaling pathway reduces the expression of enzymes related to hepatic bile acid synthesis and reduces bile acid synthesis<sup>[26]</sup>. Activation of FXR can improve colon inflammation, protect intestinal inflammation, reduce intestinal permeability, and reduce goblet cell extinction. The activation of FXR can also inhibit the secretion of TNF- $\alpha$ , interferon (IFN)- $\gamma$ , IL-17 and other inflammatory cytokines in the mucosal cells of IBD patients, and up-regulate the expression of anti-inflammatory factor IL-10 in the intestinal tract<sup>[27]</sup>. Therefore, compared with the healthy control group, the enterohepatic circulation of IBD patients is blocked, the negative regulatory pathway of intrahepatic bile acid synthesis is reduced, and the total amount of bile acids in the intestinal lumen is increased, leading to intestinal inflammation<sup>[28]</sup>.



## BILE ACID-ACTIVATED RECEPTORS IN IBD

<sup>1</sup> Bile acid receptors mainly consist of G protein-coupled receptor 5 (TGR5), Farnesoid X receptor (FXR), and pregnane X receptor (PREGNane X) Constitutive Androstane receptor (CAR), Vitamin D receptor (VDR), Constitutive Androstane receptor (PXR), It has the functions of regulating <sup>1</sup> bile acid metabolism, glucose utilization, fatty acid synthesis and oxidation, energy homeostasis balance, immune cell function, nerve activity and so on.

### G protein-coupled receptor 5 (TGR5) and IBD

TGR5 is a membrane receptor containing seven transmembrane regions. TGR5 mRNA expression was found in almost all human and rodent tissues, especially in gallbladder, ileum and colon<sup>[29]</sup>. Lithocholic acid (LCA) was the most effective in TGR5 stimulation. The rest were <sup>15</sup> deoxycholic acid (DCA), chenodeoxycholic acid (CDCA) and cholic acid (CA). Activation of TGR5 can trigger the elevation of cyclic adenosine monophosphate (C-AMP) or epidermal factor growth The activation of receptor (EGFR)- sarcoma (SRC) kinase affects the physiological state of cells<sup>[30]</sup>.

IBD is caused by an overactive immune response to intestinal antigens. TGR5 deletion has been found to exacerbate intestinal inflammation in DSS - induced colitis mice<sup>[31]</sup>. There was no significant difference in TGR5 expression in colonic mucosa between patients with ulcerative colitis (UC) and the control group<sup>[32]</sup>. However, a recent study showed that TGR5 expression was significantly elevated in the colonic mucosa of children with UC, and was concentrated in lamina propria phagocytes<sup>[33]</sup>. Tgr5-specific activation of macrophages isolated from the intestines of patients with Crohn's disease (CD) significantly inhibited the production of TNF- $\alpha$  in macrophages, suggesting that the TGR5 signaling pathway may play an immunomodulatory role in IBD<sup>[34]</sup>.

TGR5 is highly expressed in mononuclear macrophages, and intestinal macrophages, as the main source of cytokines, play an important role in immune



homeostasis<sup>[35]</sup>. Polarization of macrophages is generally divided into two types, M1, which promotes inflammation, and M2, which suppresses inflammation. Rather than inducing macrophage activation to either phenotype alone, bile acid-activated TGR5 induces "mixed phenotype" macrophages, where an elevated IL-10/IL-12 ratio indicates the dominance of the immunosuppressive M2 phenotype<sup>[36]</sup>. TGR5 specific activation can reduce the production of pro-inflammatory cytokines such as IL-6, IL-1 $\beta$  and TNF- $\alpha$  in THP1 cells, and TGR5 activation can inhibit the secretion of inflammatory cytokines in intestinal macrophages in a dose-dependent manner<sup>[37]</sup>. In terms of mechanism, TGR5 activates c AMP and EGFR-Src kinase pathways in response to bile acids. On the one hand, bile acid-activated TGR5 mediated the activation of CAMP, which further activated PKA, up-regulated the expression and activity of CAMP binding element, and finally inhibited the translocation of NF- $\kappa$ B into the nucleus through a series of steps<sup>[38]</sup>. Meanwhile, the expression of anti-inflammatory factor IL-10 was significantly increased after the activation of CAMP binding element<sup>[39]</sup>. On the other hand, in M1-type macrophages, TGR5-dependent EGFR trans-activated SRC kinase activation leads to NF- $\kappa$ B activation through downstream protein kinase C, and increased expression of pro-inflammatory cytokines IL-1 $\beta$ , IL-6, and TNF- $\alpha$ <sup>[40]</sup>. In summary, bile acid-TGR5 signal transduction regulates a complex balance between pro-inflammatory and anti-inflammatory cytokines in the gut.

### **Farnesol receptor (FXR) and IBD**

A nuclear receptor superfamily member, farnesol receptor (FXR), with bile acid ligand activity was first identified in a 1995 study of rat liver C DNA<sup>[41]</sup>. FXR mainly exists in the intestine, liver and kidney, especially in the ileum, colon and liver, and is involved in the regulation of a large number of physiological activities of the human body. In addition to regulating bile acid metabolism and transport, FXR also plays a key role in regulating lipid and glucose homeostasis, inflammatory response, and barrier function<sup>[42]</sup>.

Bile acids can be classified according to their affinity for binding FXR in vitro. CDCA has the highest excitatory effect on FXR, followed by CA, DCA and LCA<sup>[43]</sup>.

Compared with their natural forms, the sugar-taurosulfo-conjugated forms of CDCA, DCA and LCA are more effective agonists. Among the synthesized FXR agonists, GW4064 selectively excites FXR with high affinity, which is widely used in experimental studies<sup>[44]</sup>.

FXR plays an important role in the development and progression of IBD. Early colon cell tests showed that FXR gene knockout mice were more likely to develop severe intestinal inflammation than wild-type mice, suggesting that intestinal FXR could reduce intestinal inflammation<sup>[45]</sup>. It has been found that activation of intestinal FXR can inhibit NF- $\kappa$ B activation and reduce intestinal inflammation through multiple pathways<sup>[46]</sup>. FXR attenuates the translocation of NF- $\kappa$ B subunit P65, thereby inhibiting NF- $\kappa$ B transcription, reducing the gene expression of pro-inflammatory factor IL-8, and alleviating intestinal inflammation<sup>[47]</sup>. Activation of intestinal FXR expression can inhibit intestinal <sup>14</sup> toll-like receptor 4-myeloid differentiation factor 88 (TLR4/MyD88) signaling pathway, thereby down-regulating NF- $\kappa$ B expression and alleviating intestinal inflammation<sup>[48]</sup>. In addition, activation of FXR can up-regulate the expression of il-10, an anti-inflammatory factor in the intestinal tract, thus exerting an anti-inflammatory effect. It is concluded that activation of intestinal FXR can reduce intestinal inflammation and play a protective role in IBD intestine, and FXR is expected to become a drug target for IBD treatment<sup>[49]</sup>. It is important to note that FXR has different functions in different tissues, and currently there are no intestinal FXR specific agonists. Therefore, when FXR is used as a treatment for IBD, it may activate hepatic FXR and cause adverse reactions<sup>[50]</sup>.

Intestinal bile acid accumulation can cause the proliferation and apoptosis of intestinal epithelial cells, leading to IBD. FXR can regulate bile acid synthesis and reabsorption to maintain intestinal bile acid homeostasis<sup>[51]</sup>. On the one hand, FXR can regulate the expression of fibroblast growth factor (FGF), thereby inhibiting the expression of CYP7A1 and reducing bile acid synthesis<sup>[52]</sup>. On the other hand, intestinal FXR also promotes the expression of organic solute transporter alpha-beta (OST $\alpha/\beta$ ), <sup>11</sup> Inhibit the expression of apical sodium dependent bile acid transporter (ASBT) in ileum,

thus promoting the excretion of intestinal bile acid and alleviating the injury of intestinal bile acid<sup>[53]</sup>. Therefore, intestinal FXR has a protective effect on the intestinal tract of IBD and can be used as a therapeutic target for IBD. As a synthetic FXR agonist, GS-9674 alleviates cholestatic intestinal injury by activating FXR in intestinal epithelial cells to up-regulate FGF19 expression<sup>[54]</sup>. Based on 6- $\alpha$ -ethyl-chenodeoxycholic acid (6-ECDCA), which is mainly used for the treatment of cholestatic diseases, the 6-ETHYL-chenodeoxycholic acid (6-ECDCA) can activate FXR, regulate the expression of OST $\alpha/\beta$  and ASBT, and improve the intestinal cholestasis<sup>[55]</sup>. However, there is no clinical trial of 6-ECDCA as a treatment for IBD, but with the deepening of basic research, it is expected to become a treatment for IBD targeting FXR.

### **Pregnane X receptor (PXR) and IBD**

PXR is an important member of the nuclear receptor superfamily and is mainly expressed in colon and liver. Studies have shown that PXR plays an important role in maintaining intestinal homeostasis, and its gene deletion leads to an increased risk of IBD<sup>[56]</sup>. Moreover, PXR not only participates in intestinal immune response by regulating inflammatory signaling pathways, but also can receive endogenous signals to regulate intestinal homeostasis, so it is expected to become a new therapeutic target.

Excessive inflammatory response is the most prominent feature of IBD. NF- $\kappa$ B is the most classic inflammatory signaling pathway, and when activated, it releases a large number of inflammatory factors, exacerbating IBD<sup>[57]</sup>. The nuclear receptor PXR is an upstream regulatory factor of NF- $\kappa$ B, and can regulate NF- $\kappa$ B through PXR to reduce intestinal inflammation. We found that compared with wild-type mice, NF- $\kappa$ B was activated in the colon of PXR knockout mice, resulting in the release of a large number of inflammatory factors (such as TNF- $\alpha$ , IL-6, etc.), and increased intestinal inflammation<sup>[58]</sup>. It is speculated that PXR gene deletion may activate NF- $\kappa$ B pathway and increase intestinal inflammation. Activation of PXR receptor can inhibit NF- $\kappa$ B expression in the intestinal tract, thereby reducing the level of downstream inflammatory factors and reducing intestinal inflammation<sup>[59]</sup>. PXR protects the intestine by regulating NF- $\kappa$ B signaling. In addition, PXR also regulates non-classical

inflammatory pathways, such as transforming growth factor (TGF- $\beta$ 1) expression, which plays a role in reducing intestinal inflammation. Therefore, PXR is considered as one of the most promising targets for IBD treatment<sup>[60]</sup>.

Intestinal mucosal barrier is an important physical barrier to prevent toxic substances from invading the intestine, maintaining intestinal mucosal homeostasis and avoiding intestinal injury. When the intestinal permeability is increased, the intestinal mucosal barrier function is reduced, which can directly lead to the occurrence or exacerbation of IBD symptoms. Increased intestinal permeability in IBD patients is closely related to the abnormal expression of Myosin light-chain kinase (MLCK) and C-Jun n-terminal kinase 1/2 (JNK1/2)<sup>[61]</sup>. However, nuclear receptor PXR can reduce intestinal permeability by down-regulating the expression of MLCK and JNK1/2, and play a role in maintaining intestinal mucosal barrier function. It was found that MLCK expression and myosin II light chain phosphorylation level in colon tissue of IBD patients were significantly increased, and intestinal permeability was increased<sup>[62]</sup>. Pregnenolone 16- $\alpha$  carbonitrile (PCN), a PXR agonist, can inhibit MLCK and myosin II light chain phosphorylation, reduce the permeability of the intestinal barrier, and avoid intestinal injury. The up-regulation of JNK1/2 expression in intestinal cells of IBD patients increases intestinal permeability, while PCN can down-regulate intestinal JNK1/2 expression by inducing GADD45 $\beta$  protein transcription, reducing intestinal permeability and avoiding toxin invasion<sup>[63]</sup>. Therefore, PXR can maintain intestinal mucosal barrier function, and its ligand can be used to treat IBD. However, PXR receptor agonist PCN is only used in animal experimental studies, and has not been used for clinical treatment. The study of intestinal protective mechanism of PXR will promote the application of PXR agonists in clinical treatment<sup>[64]</sup>.

In human body, metabolic enzymes and transporters are highly expressed in the intestine, among which metabolic enzymes are mainly involved in the detoxification process of intestinal toxic substances, such as CYP3A4 and CYP3A11<sup>[65]</sup>. Transporters are mainly involved in the excretion of intestinal cytotoxic substances, such as P-glycoprotein (P-GP)<sup>[66]</sup>. Studies have shown that the reduced expression of metabolic

enzymes and transporters involved in the metabolism of heterogenic substances in the intestine of IBD patients leads to the accumulation of intestinal toxins, and PXR is an upstream regulatory factor of multiple metabolic enzymes and transporters, which can regulate their expression to play a detoxification role<sup>[67]</sup>. Activation of PXR can significantly up-regulate the expression of CYP3A4 gene in wild-type mice, thus improving the symptoms of abdominal pain and diarrhea. The expression level of P-GP in colon tissues of IBD mice prepared by DSS was down-regulated, and the poison was accumulated in intestine. PXR could reduce the accumulation of poison by regulating the expression of P-GP<sup>[68]</sup>. PXR can up-regulate the expression of drug metabolism enzymes and transporters to eliminate intestinal toxicity, and has a protective effect on IBD intestinal tract. Tanshinone II A, the active ingredient of *Salvia miltiorrhiza* in labiaceae, is A highly active PXR agonist, which mainly upregulates the expression of PXR to increase the expression of downstream metabolic enzymes and transporters, thereby promoting intestinal toxin metabolism and efflux, and improving the symptoms of IBD<sup>[69]</sup>. PXR agonists speed up the metabolism of other drugs in the body, reducing the potential for adverse reactions to these drugs. However, large-scale activation of PXR can up-regulate the expression of metabolic enzymes and transporters, and then affect the metabolism of other drugs, leading to decreased efficacy and even induced drug interactions, which may limit the clinical application of PXR agonists in the treatment of IBD<sup>[70]</sup>. It can be seen from the above that the protective effect of PXR on the intestinal tract of IBD has been preliminarily confirmed. Based on its protective mechanism, PXR can be used as a target for drug therapy of IBD, providing a new perspective for innovative drug research and IBD treatment.

### **Constitutive Androstane receptor (CAR) and IBD**

CAR is a nuclear receptor for steroidal hormones, which is mostly expressed in intestinal epithelial cells. Although the protective mechanism of CAR against IBD is not fully understood, there is increasing evidence that it also plays a key role in regulating intestinal inflammation and protecting the intestinal mucosal barrier<sup>[71]</sup>. Biopsies of the intestinal mucosa of IBD patients showed that CAR gene expression was strongly



associated with intestinal inflammation levels. In IBD mice, intestinal mucosal barrier was disrupted, and the activation of p38MAP kinase by CAR agonist CITCO enhanced IEC cell migration and accelerated intestinal mucosal healing<sup>[72]</sup>. In addition, CAR significantly regulates metabolic enzymes and transporters located in the intestine, and protects the intestine from toxic interference by inducing the expression of metabolic enzymes and transporters<sup>[73]</sup>. These results suggest that CAR, like PXR and FXR, can play a protective role in IBD by reducing intestinal inflammation and maintaining intestinal mucosal homeostasis, but the specific mechanism remains to be studied<sup>[74]</sup>. In conclusion, CAR is also a promising drug target for IBD treatment, and further study of its protective mechanism against IBD can provide reference for drug development targeting CAR.

#### **Vitamin D receptor (VDR) and IBD**

<sup>12</sup> Vitamin D receptor (VDR) is a member of the nuclear hormone receptor superfamily, which exists in all the target tissues of vitamin D<sub>3</sub>(VD<sub>3</sub>), such as intestinal tract and liver<sup>[75]</sup>. VDR, as an important nuclear transcription factor, intervenes in many downstream genes through specific binding with ligands. Studies have confirmed that VDR gene polymorphism is associated with the risk of IBD, and there are differences in VDR genotypes among different genders and populations<sup>[76]</sup>. Human proteomics shows that VDR is highly expressed in the normal small intestine and colon, but reduced intestinal VDR expression and impaired VD/VDR signaling pathway were observed in patients with CD and UC<sup>[77]</sup>. Therefore, intestinal VDR <sup>1</sup> plays an important role in the occurrence and development of IBD.

Loss of VDR **in** intestinal epithelial cells leads to activation of NF- $\kappa$ B signaling, which promotes production of pro-inflammatory cytokines<sup>[78]</sup>. A genome-wide association analysis showed that VDR binds to 42 disease-associated single nucleotide polymorphisms (SNPs), of which one-third significantly affect transcription factor NF- $\kappa$ B binding and gene regulation. Immunoprecipitation results suggested that VDR had a protein-protein interaction with IKK $\beta$  upstream of NF- $\kappa$ B<sup>[79]</sup>. VDR inhibited ser-177

phosphorylation of IKK $\beta$  by binding to IKK $\beta$ , thereby inhibiting NF- $\kappa$ B activation and IL-6 elevation induced by TNF- $\alpha$ , and improving intestinal inflammation<sup>[80]</sup>.

A meta-analysis shows that variations in the VDR gene significantly affect the human gut microbiome<sup>[81]</sup>. It was found that the protective effect of probiotics on IBD depends on the epithelial VDR signaling pathway. In the normal intestinal flora of mice, the distribution and abundance of bacteria in the intestinal epithelium after VDR knockout were significantly changed, mainly manifested as increased abundance of *Bacteroides fragilis* in mice with VDR deletion<sup>[82]</sup>. In addition, intestinal epithelial VDR deletion exacerbated the intestinal inflammatory damage caused by sodium glucan sulfate modeling in mice, while the intestinal epithelial VDR deletion mice and wild-type control mice were reared in the same cage for modeling, this difference in intestinal inflammation caused by different genotypes disappeared<sup>[83]</sup>. This indicates that VDR deficiency causes intestinal flora disorder and aggravates the occurrence and development of IBD. Another study showed that defective VDR expression in intestinal Paneth cells leads to reduced lysozyme secretion, impaired antimicrobial activity of pathogenic bacteria, and thus increased inflammatory response<sup>[84]</sup>. Other studies have found that lack of VD in the diet of mice can cause intestinal microflora disorder, mainly manifested in increased abundance of *Helicobacter hepaticus* (H.h) and decreased abundance of probiotics *Akkermansia muciniphila*<sup>[85]</sup>. Therefore, VDR genes may play an important role in homeostasis and signal transduction between the microbiome and host in intestinal inflammation.

Some studies have speculated that metabolites of intestinal flora regulate intestinal immune responses in a VDR dependent manner<sup>[86]</sup>. Butyrate is a short-chain fatty acid produced by intestinal microorganisms. 2% sodium butyrate in drinking water increased intestinal VDR expression and inhibited inflammation in mice with colitis<sup>[87]</sup>. In addition, secondary bile acids and shicholic acids produced by intestinal flora metabolism inhibit Th cell immune response by activating VDR of CD4+Th cells, thereby reducing IFN- $\gamma$  and IL-2 production in intestinal inflammation<sup>[88]</sup>. In conclusion,



VDR related basic studies provide many new ideas and explanations for the mechanism of intestinal flora in IBD.

### **Sphingosine-1-Phosphate Receptor 2 (S1PR2) and IBD**

Sphingosine-1 (S1P) is an active sphingosine-1 that participates in the regulation of various cell functions under physiological and pathological conditions<sup>[89]</sup>. S1P can function directly as intracellular signaling molecules or extracellular by activating 5 G protein-coupled receptors (GPCRs). S1P has been shown to be a key regulator of proliferation, migration, and survival of many cell types. The expression of 5 S1PRs was different in different tissues or organs. All five sphingosine-1-phosphate receptors (S1PRs) were detected in the human intestine, but the expression levels of S1PRs were different<sup>[90]</sup>. It has been reported that S1P regulates the expression of e-cadherin by activating S1PR2 to enhance intestinal epithelial cell barrier function. It has also been reported that S1P reduces intestinal epithelial cell apoptosis through the Akt dependent pathway<sup>[91-92]</sup>. These studies suggest that S1P and its receptor can promote intestinal epithelial cell proliferation and enhance epithelial cell barrier function, and play a protective role in intestinal mucosal barrier.

### **Retinoid-related orphan receptor gamma (ROR $\gamma$ T) and IBD**

Retinoid-related orphan receptor gamma (ROR $\gamma$  T) is a specific transcription factor controlling Th17 cell differentiation. Regulatory T cells (Treg cells) are from the same source as Th17 cells, and they are closely related<sup>[93]</sup>. Treg cells play an important role in maintaining the body's immune tolerance state and the stability of internal environment, and preventing the occurrence of autoimmune diseases. Th17 cells, a new type of CD4<sup>+</sup> cell subpopulation discovered in 2003, play a pro-inflammatory role mainly by secreting cytokines such as IL-17, IL-22 and IL-21<sup>[94]</sup>. ROR $\gamma$  T is a transcriptional activator that plays a key role in the differentiation of Th17 cells. Inhibition of ROR $\gamma$  T expression can inhibit the differentiation of non-sensitized T cells into Th17 cells<sup>[95]</sup>. It has been found that ROR $\gamma$  T directs the differentiation of proinflammatory Th17 cells and regulates the production of IL-17 in peripheral blood<sup>[96]</sup>. Therefore, it is reasonable to believe that ROR $\gamma$  T can be used as an important target for

the treatment of autoimmune and inflammatory diseases. Treg cells are newly discovered T cell subsets that negatively regulate the body's immune response, and their immune regulatory function is closely related to the continuous expression of Foxp3<sup>[97]</sup>. Foxp3 is considered to be a key transcription factor and specific marker of Treg cells, which can regulate the expression and function of multiple genes after binding to chromosomes, thus controlling the development and function of Treg cells<sup>[98]</sup>. In vitro studies have shown that transforming growth factor  $\beta$  (TGF- $\beta$ ) can inhibit ROR $\gamma$ T function and promote Treg differentiation by inducing Foxp3 expression, and the full-length Foxp3 subtype can bind to ROR $\gamma$  T to inhibit ROR $\gamma$  T function<sup>[99]</sup>. In the presence of pro-inflammatory cytokines, Foxp3 levels decreased and ROR $\gamma$  T levels increased, ultimately promoting Th17 cell differentiation. In a mouse model of colitis, ROR $\gamma$  T binding reduced IL-17 production and Th17 cell count and reduced intestinal inflammation<sup>[100]</sup>. Studies have shown that Th17 lymphocytes are involved in the pathogenesis of CD and UC. Increased IL-17 expression in mucosa and serum of IBD patients was associated with increased ROR $\gamma$  T expression and Th17 cell number<sup>[101]</sup>. Therefore, Th17 and Treg cells antagonize each other functionally and are closely related in differentiation. Under normal circumstances, they maintain a relative balance, which is beneficial to maintain the immune stability of the body<sup>[102]</sup>. At present, the relationship between Th17/Treg cell imbalance and disease occurrence and development has become the focus of people's attention.

## INTERACTION BETWEEN INTESTINAL FLORA AND BILE ACIDS

### Intestinal flora and bile acid synthesis

Intestinal flora can further modify the synthetic bile acids to form a series of intestinal bile acid metabolites. These metabolites can act as important signaling molecules to regulate cholesterol metabolism and energy balance of the host through bile acid receptors<sup>[103]</sup>. The involvement of intestinal flora in the synthesis of bile acids increases the diversity of bile acids and the hydrophobicity of bile acid pools, which is conducive to bile acid excretion<sup>[104]</sup>. The modification of bile acids by intestinal flora

mainly includes early uncoupling, dehydrogenation, dehydroxylation and differential isomerization of bile acids. Bile salt-hydrolases (BSHs) produced by intestinal bacteria catalyze bile salt-hydrolases (BSHs), and then uncouple bile c-24 with n-acetyl amino bonds bound to amino acids to form free bile acids<sup>[105]</sup>. Studies have found that there are many bacteria in the intestinal tract of the organism that can produce bile acid salinase, such as bifidobacterium, Lactobacillus, Bacteroides, Listeria and Clostridium have bile acid salinase activity<sup>[106]</sup>. 7 $\alpha$  -hydroxyl dehydrogenation occurs in free bile acids under the catalytic action of Clostridium and Clostridium, and hydroxyl steroid dehydrogenase (HSDH) produced by intestinal microflora such as Clostridium, Eubacter, Ruminococcus, Bacteroidetes and Digestive streptococcus dehydrogenases at the positions of C-3, C-7 and C-12. Secondary bile acids deoxycholic acid (DCA) and shicholic acid (LCA) were then produced, **as shown in Figure 1.** **Increased LEVELS of DCA have been associated with obesity and cancer in mice,** further **supporting the important role of bile acid conversion** in the intestinal flora in host metabolism<sup>[107]</sup>.

Metabolome study found that in C57BL/6 mice, under the action of intestinal microflora on bile acid dehydroxylation and decoupling, the primary bile acid gradually decreased and the secondary bile acid gradually increased during the continuation process from small intestine to large intestine<sup>[108]</sup>. Compared with Specific pathogen-free (SPF) mice fed a normal rich-diet diet, The changes of bile acid components in feces of SPF mice fed with minimal chemical diet and Germ free (GF) mice fed with normal diet were detected by mass spectrometry. Levels of liver-derived taurine conjugated primary bile acids in the intestinal tract of the minimal pathogen-free mice were significantly decreased compared with those in the RICH-diet SPF mice, while they were increased in the RICH-diet GF mice<sup>[109]</sup>. The results indicate that diet can directly control the hepatic synthesis of bile acids, and the intestinal flora mainly controls the modification process of bile acids in the intestine.

As a potential regulator of gut microbiota composition and host metabolism, microbial HSDH may open up new pathways for how the microbiota regulates signaling pathways in the host.

## THE EFFECT OF INTESTINAL FLORA ON BILE ACIDS VIA FXR

Study method of alcohol receptor is closely related to the metabolism of bile acid synthesis of highly expressed in the organs, such as the liver, small intestine, bile acid synthesis of organisms play a regulatory role of bile acid in the bile acid, goose deoxycholic acid and lithocholic acid and deoxycholic acid is liver alcohol receptor agonist, 7 alpha hydroxylase (CYP7A1) is the promoter of bile acid synthesis<sup>[110]</sup>. In the liver, bile acid-activated FXR induces the expression of a small heterodimer partner (SHP) that binds to liver receptor homologous protein -1 (LRH-1), thereby inhibiting Cyp7a1 gene expression. In addition to local effects in the liver, FXR is also activated by bile acids in the distal ileum. FXR induces expression of FGF15(FGF19 in humans) in the ileum. So farnesol receptor-fibroblast growth factor 15/19(FGF15/19) signaling pathway plays an important role in bile acid synthesis. In the study of Lactobacillus rhamnosus GG(LGG) on BDL mice, it was found that compared with the sham operation group, In BDL mice, the content of deoxycholic acid (deoxycholic acid is a strong agonist of FXR) and the concentration of T-αMCA and T-βMCA (MCA is an antagonist of FXR) were decreased, and the mRNA expression of CYP7A1 and FGF15 in BDL mice were increased<sup>[111]</sup>. The bile acid content and the size of total bile acid pool in liver were significantly increased, and the bile acid content and total bile acid pool size were significantly decreased after LGG treatment. At the same time, it was found that the mRNA expression level of FXR target gene SHP and FGF15 were significantly decreased in the ileum of BDL mice, while LGG could inhibit the decrease of FGF15 protein level<sup>[112]</sup>.

This confirms that in BDL mice, LGG treatment-mediated reduction in bile acid synthesis is achieved through upregulation of the intestinal FXR-FGF15 signaling pathway<sup>[113]</sup>. Other studies confirm the bile acid levels of traditional breeding mice, and the germ-free mice raised in bile acid levels, may be due to the traditional breeding mice intestinal microbial flora make mice reduced levels of MCA, activation of FXR,

make FGF15 higher expression, thus inhibiting the activity of CYP7A1 to inhibit the synthesis of bile acid<sup>[114]</sup>.

It was found that after fecal microbiota transplanta-tion (FMT) of sterile mice received fecal microbiota transplanta-tion (FMT), the expression of FXR in intestinal epithelium was up-regulated, and FXR further induced the expression of FGF15, thereby inhibiting the activities of CYP7A1, CYP8B1 and other enzymes. Thus inhibiting the synthesis of bile acids<sup>[115]</sup>. The expression of FGF15 in ileum was inhibited by antibiotics, and the expression level and activity of CYP7a1 in liver increased significantly, resulting in bile acid synthesis. *Parabacteroides distasonis* was used to treat obese mice. It was found that *Parabacteroides distasonis* can hydrolyse a variety of conjugated bile acids, convert primary bile acids into secondary bile acids (LCA, UDCA, etc.), and produce a large amount of succinic acid<sup>[116]</sup>. LCA and other secondary cholic acids increased the level of FGF15 in serum and colon, and decreased the level of Cyp7a1 in liver by activating the intestinal FXR signaling pathway. UDCA can repair intestinal wall integrity and succinic acid can improve host sugar metabolism disorder<sup>[117]</sup>.

G protein-coupled bile acid receptor 5(TGR5) can also be activated by intestinal flora to inhibit bile acid synthesis. TGR5 is a G protein-coupled receptor (GPCR), and it has been found that compared with WT mice, the bile acid pool size of mice lacking the TGR5 gene in a high-fat diet decreased by 21% to 25%, and body fat accumulation increased, and body mass increased<sup>[118]</sup>. Intestinal bacteria can also induce the expression of cardiac transcription factor 4(GATA4) in intestinal epithelial cells by stimulating them continuously, and inhibit the expression of ABST, resulting in reduced bile acid reabsorption in the terminal ileum<sup>[119]</sup>.

In conclusion, intestinal flora not only participates in the processes of bile acid decoupling, dehydrogenation and dehydroxylation, but also negatively regulates bile acid synthesis through the FXR-FGF15/19 pathway.

## **INTESTINAL FLORA PARTICIPATES IN THE REGULATION OF NORMAL METABOLISM OF BILE ACIDS**



The metabolism of bile acids in the body is mediated by intestinal flora. The whole metabolic process of bile acids synthesized in liver cells is regulated by intestinal flora. The intestinal flora in patients with gallstones is unbalanced and the metabolism of bile acids is also in disorder, which may be because the imbalance of intestinal flora in the body affects the hepatoenteric circulation of bile acids in the body and causes the metabolic disorder of bile acids and cholesterol. BSHs produced by *bifidobacterium*, *Clostridium*, *Lactobacillus*, *Listeria*, *enterococcus*, *bacteroidetes* and other bacteria in the intestinal tract of the body can reduce the production of cholesterol in serum<sup>[120]</sup>. BSHs is mainly involved in the uncoupling of conjugated bile acids to form free bile acids in the body. When intestinal flora in the body is unbalanced, BSH activity increases and free bile acid content increases, which then activates the NEGATIVE feedback regulation system of FXR-FGF15/19 bile acid, resulting in reduced bile acid synthesis content and over-saturated cholesterol<sup>[121]</sup>. If it is not dissolved effectively by bile acids, it will remain as a deposit, slowly turning into a stone state. In addition, *Lactobacillus* and *bifidobacterium* in intestinal flora also has the ability of removing cholesterol, mainly through the intake to the cholesterol assimilation or binding to the cell or and bile acid form coprecipitation<sup>[122]</sup>, some intestinal bacteria also can produce cholesterol reductase, catalytic cholesterol into insoluble prostaglandins, and turn it into the feces. Other studies have confirmed that intestinal flora mediates normal metabolism of bile acids. In the study of liver cancer, antibiotics can increase the Natural killer T cell (NKT) in mouse liver, and CXCL16, a chemokine expressed by hepatic sinusoid endothelial cells, can inhibit the growth of liver tumors by regulating hepatic NKT cells<sup>[123]</sup>. The primary bile acids in liver can promote the expression of CXCL16, while the secondary bile acids can inhibit the expression of CXCL16. When mice were treated with vancomycin (an antibiotic), vancomycin eliminated gram-positive bacteria (including those involved in primary bile acid conversion) from their intestines and induced the accumulation of hepatic NKT cells, thereby inhibiting the development of liver cancer<sup>[124]</sup>. At the same time, vancomycin-treated mice were fed with secondary bile acids or *Clostridium*

bacteria that colonized and transformed primary bile acids, and the accumulation of NKT cells in the liver was reduced and the anti-tumor effect was reduced<sup>[125]</sup>.

Studies have shown that in patients with ulcerative colitis (UC), the levels of secondary bile acids (deoxycholic acid and stone cholic acid) in the intestinal tract are reduced, and rumen bacteria and other bacteria that convert primary bile acids into secondary bile acids are also reduced<sup>[126]</sup>. Supplementation of secondary bile acids with G-protein-coupled receptor for Bile Acids (TGR5) improved intestinal inflammation in mice with colitis. In the enterohepatic circulation with normal enteral nutrition, bile acids activate the enterofarnicol receptor (FXR), triggering the release of FGF19 into the portal vein circulation<sup>[127]</sup>. FGF19 regulates the synthesis of intrahepatic bile acids through enteral nutrition. This signaling pathway is impaired in patients with total venous nutrition (TPN), and studies have shown a decrease in serum FGF19 levels in subjects receiving TPN. Due to intestinal dysfunction, the intestinal microbiota in TPN patients is severely altered. Changes in intestinal flora can affect patients' immune response and promote endotoxin secretion, thus negatively affecting liver function, suggesting that intestinal flora affects the related bile acid signaling pathway in the treatment of TPN<sup>[128]</sup>.

### **BILE ACIDS AFFECT THE COMPOSITION OF INTESTINAL FLORA**

The regulation between intestinal flora and bile acid metabolism is bidirectional, intestinal flora can participate in the synthesis and normal metabolism of bile acid, and bile acid can in turn regulate the composition of intestinal flora. The effects of bile acids on intestinal flora include damage to bacterial cell membrane, destruction of bacterial amino acids, nucleotides and carbohydrate metabolism, activation of innate immune genes in the small intestine to change the composition of intestinal flora and affect body metabolism<sup>[129]</sup>. The size and diversity of bile acid pools can affect the intestinal flora of the body. Studies on colorectal cancer patients found higher concentrations of *Clostridium 7 $\alpha$* -dehydroxy in feces, which can promote the production of secondary bile acids. High levels of *clostridium 7 $\alpha$* -dehydroxy increase the content of secondary



bile acids in the intestinal tract, leading to an imbalance of intestinal microflora that promotes the development of colorectal cancer (CRC)<sup>[130]</sup>. High-fat diet can cause the imbalance of intestinal flora in mice. When adding ursodeoxycholic acid into the diet of high-fat diet mice, it was found that the intestinal flora in mice restored to the similar level as normal mice (for example, the contents of *Faecalis* and *Ackmanniella* increased, while the contents of *Spironella* and *ruminococcus* decreased)<sup>[131]</sup>. The effects of bile acids on the composition of intestinal flora can also be mediated by FXR. When mice were fed a high-fat diet, the levels of T- $\beta$ MCA in FXR deficient mice increased and the abundance of *Firmicutes* increased while the abundance of *Bacteroidetes* decreased compared with the control mice. It is possible that the FXR-mediated high-fat diet altered the bile acid pool in mice, leading to changes in gut microbiota<sup>[132]</sup>.

Bile acids can also change the composition of intestinal flora by inhibiting the growth of intestinal bacteria, and the antimicrobial activity of non-conjugated bile acids is stronger than conjugated bile acids, and the sensitivity of gram-positive bacteria to bile acids is stronger than gram-negative bacteria<sup>[133]</sup>. It was found that the synthesis of bile acid in rats with liver cirrhosis was lower than that in healthy rats, and the total bacterial content in ileum and bacterial translocation rate were increased<sup>[134]</sup>. After bile acid injection, the bacterial quantity in ileum of cirrhotic rats returned to healthy level and the bacterial translocation rate decreased. Obecholic acid (OCA) is a bile acid derivative that activates FXR to inhibit endogenous bile acid synthesis. When healthy subjects were given doses of OCA, they found increased levels of gram-positive bacteria in their small intestines, such as *Lactococcus lactis*, *Lactobacillus casei* and *Streptococcus thermophilus*, while normal levels of bile acid inhibited the growth of these bacteria. When healthy mice were fed OCA, the bile acid content in their small intestine decreased, while the content of firmicide bacteria, mainly gram-positive bacteria, increased, suggesting that OCA can inhibit bile acid synthesis through activation of FXR and thus alter the intestinal microflora<sup>[135-137]</sup>.

## **INTESTINAL FLORA, BILE ACID METABOLISM AND IBD**

### **Probiotics and prebiotics:**

Exogenous supplementation of probiotics to regulate bile acids to prevent or treat diseases has been demonstrated in metabolic diseases, such as hypercholesterolemia or obesity<sup>[138]</sup>. Probiotics can relieve the clinical symptoms of IBD patients to different degrees. Probiotic mixture VSL#3 can significantly reduce cryptitis, and *Clostridium butyricum* MIYAIRI is also better than placebo in clinical efficacy, but its exact efficacy needs to be further studied<sup>[139]</sup>. Bile acids levels are reduced in IBD patients and experimental enteritis animals<sup>[130]</sup>. However, the improvement of enteritis symptoms by exogenous *Clostridium scindens* supplementation has only been demonstrated in animals, and clinical studies on strains that regulate BSH or 7 $\alpha$  dehydroxylase in a targeted way are lacking<sup>[140]</sup>.

### **Fecal Microbiota Transplantation**

Fecal microbiota transplantation (FMT) is a process in which feces from healthy people are transferred to patients, and it was first used to treat patients with recurrent *Clostridium difficile* infection. Recent studies have shown that FMT can significantly improve the composition of bile acids in the gut of patients with *C. difficile*, increase the content of secondary bile acids and prevent *C. difficile* colonization<sup>[141]</sup>. Because of its apparent efficacy in treating recurrent *C. difficile* infection, it has been applied to other intestinal diseases, such as IBD, IBS, and pancreatitis. In IBD studies, FMT has shown significant efficacy in inducing remission of UC. A study of UC in children showed that the gut microbiota and metabolome of FMT responders were significantly more similar to those of healthy people<sup>[141]</sup>.

### **Antibiotics**

Studies have found that antibiotics on deoxycholic acid induced inflammation of the intestinal protective, may significantly reduced intestinal flora diversity and broad-

spectrum antibiotics, reduced intestinal tract has 7 alpha to hydroxylation enzyme bacteria, lead to waste source of primary bile acid dominate in the host, and the source of intestinal flora secondary bile acid decreased<sup>[140]</sup>. However, the choice of antibiotics is also important. In a 12-weeks clinical study, the nonabsorbable antibiotic rifaximin showed higher remission rates in patients with active CD. <sup>4</sup> Given that different antibiotics have different effects on bile acid concentration and composition as well as IBD, antibiotic and patient selection will be important in evaluating antibiotic efficacy against IBD in the future<sup>[141]</sup>.

## **CONCLUSION**

Changes in lifestyle and diet have contributed to the increasing incidence of IBD. High fat diet not only changes the characteristics of intestinal flora, but also affects the metabolism of bile acids in intestinal lumen. Therefore, studies focusing on bile acids and gut microbiota have attracted much attention in digestive diseases. Characteristic changes in the gut microbiota in IBD patients affect the composition of the bile acid pool. Secondary bile acids, as anti-inflammatory factors, may be non-invasive biomarkers in mucosal healing. The emergence of novel metabolomics has revealed the bacterial species that transform bile acids and the mechanism of signaling pathways that regulate the development of IBD disease. The interaction between gut microbiota and bile acids represents a promising new therapeutic approach for IBD. Some animal studies have shown the important value of the gut microbial-bile acid axis. However, there is no clear evidence of a similar effect in clinical practice, and further clinical studies are needed to verify it.

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