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Review

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Emerging role of protein modification in inflammatory bowel disease

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Abstract: The onset of inflammatory bowel disease (IBD) involves many factors, including environmental parameters, microorganisms, and the immune system. Although research on IBD continues to expand, the specific pathogenesis mechanism is still unclear. Protein modification refers to chemical modification after protein biosynthesis, also known as post-translational modification (PTM), which causes changes in the properties and functions of proteins. Since proteins can be modified in different ways, such as acetylation, methylation, and phosphorylation, the functions of proteins in different modified states will also be different. Transitions between different states of protein or changes in modification sites can regulate protein properties and functions. Such modifications like neddylation, sumoylation, glycosylation, and acetylation can activate or inhibit various signaling pathways (e.g., nuclear factor-κB (NF-κB), extracellular signal-regulated kinase (ERK), and protein kinase B (AKT)) by changing the intestinal flora, regulating immune cells, modulating the release of cytokines such as interleukin-1β (IL-1β), tumor necrosis factor-α (TNF-α), and interferon-γ (IFN-γ), and ultimately leading to the maintenance of the stability of the intestinal epithelial barrier. In this review, we focus on the current understanding of PTM and describe its regulatory role in the pathogenesis of IBD.

Key words: Inflammatory bowel disease (IBD); Protein modification; Neddylation; Sumoylation; Glycosylation; Acetylation

1 Introduction

Inflammatory bowel disease (IBD) is a chronic inflammatory disease with a range of underlying causes. At present, most scholars consider that IBD is mainly caused by the imbalance of a combination of environmental, genetic, and innate immunity factors (Baumgart and Carding, 2007; Ananthakrishnan, 2015). The study has shown that the pathogenesis of IBD mainly involves damage to the mucosal barrier and the luminal microbiota, which ultimately leads to a persistent imbalance of the intestinal immune system (Schreiner et al., 2019). The imbalance of microflora coupled with chronic irritation as an immune response results in the decrease of mucosal permeability. Exposed bacterial Toll-like receptor (TLR) ligands directly activate antigens of innate and T cell immune responses. The dysfunction of regulatory T (Treg) cells and antigen-presenting cells (APCs) also leads to reduced tolerance to microbial antigens or induces a cross-reactive autoimmune response between the host and microbial antigens (Singh et al., 2012). Signaling pathway transcription factors (such as nuclear factor-κB (NF-κB)) have been recognized as major regulators of inflammation and immune homeostasis (Mitchell and Carmody, 2018). The proper folding and post-translational modification (PTM) of proteins are essential for appropriate function. Misfolded and aggregated proteins can cause cellular stress and death (Wang and Kaufman, 2016). Unfolded protein response (UPR) is a highly conserved stress response that enables cells to deal with the endoplasmic reticulum (ER) stress imposed by secretory demands associated with environmental forces. Under these conditions, the role of UPR in

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immunity and inflammation is becoming more recognized, including the development, differentiation, function, and survival of immune cells, which are crucial for maintaining intestinal epithelial homeostasis (Grootjans et al., 2016).

Protein modification refers to the chemical modification of proteins after biosynthesis, which is also known as PTM, and includes the addition of groups (such as methylation, acetylation, glycosylation, and phosphorylation), the covalent coupling of small peptides or proteins (such as ubiquitination, neddylation, and sumoylation), and chemical changes to amino acids (such as citrullination) (Wang and Wang, 2019). PTM is not always carried out on de novo synthesized proteins, but it can rapidly and reversibly change the functional relevance of the proteome, thereby achieving tight control and quick response to inflammation (Ehrentraut and Colgan, 2012). In this review, we focus on the research progress on protein modification, particularly neddylation, sumoylation, glycosylation, and acetylation, as documented in IBD.

2 Neddylation

Ubiquitination is an important PTM in eukaryotes (Haglund and Dikic, 2005). The complex formed by ubiquitinated protein and substrate is easily recognized by the proteasome, and facilitates the recruitment of proteins to the signal platform (Tsukamoto, 2016; Glorian et al., 2017). There are multiple lysine residues in the ubiquitin molecule, which interact with specific substrates in an enzyme cascade reaction to promote substrate degradation or promote protein entry into corresponding signaling pathways. As a PTM, neddylation combines the ubiquitinated protein, the neural precursor cell expressed developmentally downregulated protein 8 (NEDD8), with its target substrate protein, which in turn regulates various cellular processes (Song et al., 2016). The difference between ubiquitination and neddylation is that the former participates in most biological processes in the organism, while the latter has only few target substrates (Rabut and Peter, 2008).

One of the most studied ubiquitin-like molecules, NEDD8, is widely expressed in adult tissues as a conserved nuclear protein (Kamitani et al., 1997; Hori et al., 1999; Kim et al., 2013). The most specific substrate proteins for neddylation belong to the cullin family. There are eight members of the cullin family in the human genome, including cullin 1–3, 4A, 4B, 5, 7, and 9, also known as Parkin-like cytoplasmic proteins (PARCs) (Pan et al., 2004). In addition to cullin proteins, there are several other neddylation target proteins, such as murine double microsome 2 (Mdm2), von Hippel-Lindau tumor suppressor protein (pVHL), human antigen R (HuR), epidermal growth factor receptor (EGFR), tumor suppressor p53 (TP53), ribosomal protein (RP), liver kinase B1 (LKB1), and protein kinase B (PK/AKT) (Stickle et al., 2004; Xirodimas et al., 2004, 2008; Oved et al., 2006; Embade et al., 2012; Barbier-Torres et al., 2015).

NEDD8 is continuously catalyzed by the E1 enzyme (a heterodimer of NEDD8-activating enzyme E1 subunit 1 (NAE1) and ubiquitin-like modifier activating enzyme 3 (UBA3)), ubiquitin-conjugating enzyme 12 (UBC12), and NEDD8 E3 ligase, and finally combines with the corresponding substrate protein to form a unique complex structure. NEDD8 bound to the substrate cannot degrade the protein but can regulate the conformation, stability, positioning, and function of the substrate protein (Zhao et al., 2014). This process of protein modification is called neddylation. The function of neddylation has the following main aspects (Enchev et al., 2015): (1) regulating the interaction between proteins; (2) regulating the activity of transcription factors to control cell cycle and proliferation; (3) antagonizing ubiquitination.

Ubiquitin is upregulated in inflamed tissues, such as in the case of colitis mice, and is related to the mammalian target of rapamycin (mTOR) signaling pathway (Fujimoto et al., 2017). Studies have found that human umbilical cord mesenchymal stem cell (hucMSC)-derived exosomes reduce ubiquitin protein expression and inhibit the activation of NF-κB and mTOR (Wu et al., 2018). Epithelial barrier dysfunction is the hallmark of mucosal inflammatory diseases, including IBD, which leads to the transfer of its infiltrate to the serous membrane (Koch and Nusrat, 2012). The destruction of the epithelial barrier may be related to the increased apoptosis of cells by activating caspase 3 (Nava et al., 2010). Once caspase 3 is activated, it can initiate an irreversible apoptotic cascade, leading to DNA fragmentation and eventually increased cell death (Porter and Jänicke, 1999). The study has proved that the stimulation of intestinal inflammation

significantly enhances the caspase 3-dependent apoptosis response by relying on neddylation, which has a negative impact on the integrity of the mucosal barrier (Ehrentraut et al., 2016). Sentrin-specific protease 8 (SENP8) is essential for regulating NF-κB-mediated inflammation (Ehrentraut et al., 2013). In human tissue samples from patients with active IBD, it was observed that regardless of the type of colitis (ulcerative colitis (UC) or Crohn's disease (CD)), the messenger RNA (mRNA) level of *SENP8* was significantly lower than that of healthy people (Ehrentraut et al., 2016).

The NAE plays an important role in neddylation because it makes activated NEDD8 easy to combine with the E2 enzyme (Soucy et al., 2009). MLN4924 is a representative NAE inhibitor, which can exert a good inhibitory effect on NAE, thereby regulating the function and expression of the target protein (Godbersen et al., 2014). Through affecting the substrate protein of neddylation, MLN4924 has potential effects of causing DNA damage, regulating the conduction of transcription factors, and inducing cancer cell apoptosis (Milhollen et al., 2010; Han et al., 2016). In mice treated with lipopolysaccharide (LPS) pretreated with MLN4924, pro-inflammatory cytokines such as interleukin-1β (IL-1β), interferon-γ (IFN-γ), and tumor necrosis factor-α (TNF-α) were downregulated, indicating that MLN4924 also has anti-inflammatory effects via inhibiting cullin 1 neddylation (Ehrentraut et al., 2013). Cullin neddylation results in the activation of cullin-RING ligase (CRL), the largest family of E3 ubiquitin ligases, which is responsible for the ubiquitination and degradation of many key signal transduction/regulatory proteins (Zhao and Sun, 2013). Our previous study has revealed that exosomes derived from hucMSCs can inhibit cullin 1 neddylation through microRNA-326 (miR-326)-targeted NEDD8, thereby alleviating IBD in mice (Wang et al., 2020). Other studies have proved that the deterioration of inhibitor of NF-κB (IκB) mainly depends on neddylation, and blocking cullin activation results in the accumulation of many CRL substrates (including IκB), thus inhibiting NF-κB activity (Muniandy et al., 2018; Schwechheimer, 2018). In addition, since the microenvironment of inflammatory lesions is hypoxic, large amounts of adenine nucleotide metabolites are produced (Colgan and Taylor, 2010; Kominsky et al., 2010). The extracellular accumulation of adenosine leads to the accumulation of cullin 1 and the inhibition of NF-κB activity (Khoury et al., 2007). The hypoxia inducible factor-1 (HIF-1) protein is one of the main regulators of oxygen homeostasis. It coordinates the metabolism of adenine nucleotides and regulates adenosine signaling. Low concentrations of MLN4924 promote HIF signal transduction and inhibit NF-κB signal transduction (Curtis et al., 2015a). The inhibition of cullin 2 neddylation was demonstrated to regulate HIF to alleviate mucosal inflammation (Curtis et al., 2015b).

Other research findings indicate that the inhibition of NF-κB activation related to intestinal symbiotic bacteria depends on cullin de-neddylation (Neish et al., 2000). For instance, it was proved that symbiotic bacteria can affect the esterification state of cullin 1 by producing reactive oxygen species (ROS), and that when co-cultured with commensal bacteria, epithelial cells would react with the increase of ROS, leading to transient and reversible dendriticization of cullin 1 and the inhibition of NF-κB signaling pathway (Kumar et al., 2007). Neddylation in IBD is described in Fig. 1.

3 Sumoylation

Sumoylation is a reversible and dynamic PTM, which involves the formation of an isopeptide bond between the carboxy-terminal glycine (Gly)-residue of small ubiquitin-like modifier protein (SUMO) and the lysine (Lys)-side chain of the receptor protein. As a crucial molecule, SUMO participates in the regulation of DNA damage repair, immune response, and cell apoptosis (Han et al., 2018). Three SUMO paralogs (SUMO1, SUMO2, and SUMO3) exist in mammals (Hay, 2005). In the sumoylation-binding process, SUMO is activated via the E1 enzyme (a heterodimer of SUMO-activating enzyme subunit 1 (SAE1) and SAE2), and then transferred to the E2-conjugating enzyme, UBC9, and finally activated by E3 ligase (protein inhibitor of activated signal transducer and activator of transcription (STAT) (PIAS)) during its transfer to the substrate. Once SUMO is coupled to the substrate, it can be uncoupled by different SUMO isopeptidases called whistle protein-specific proteases (SENP1–3 and SENP5–7), thereby regulating the level of protein sumoylation (Flotho and Melchior, 2013).

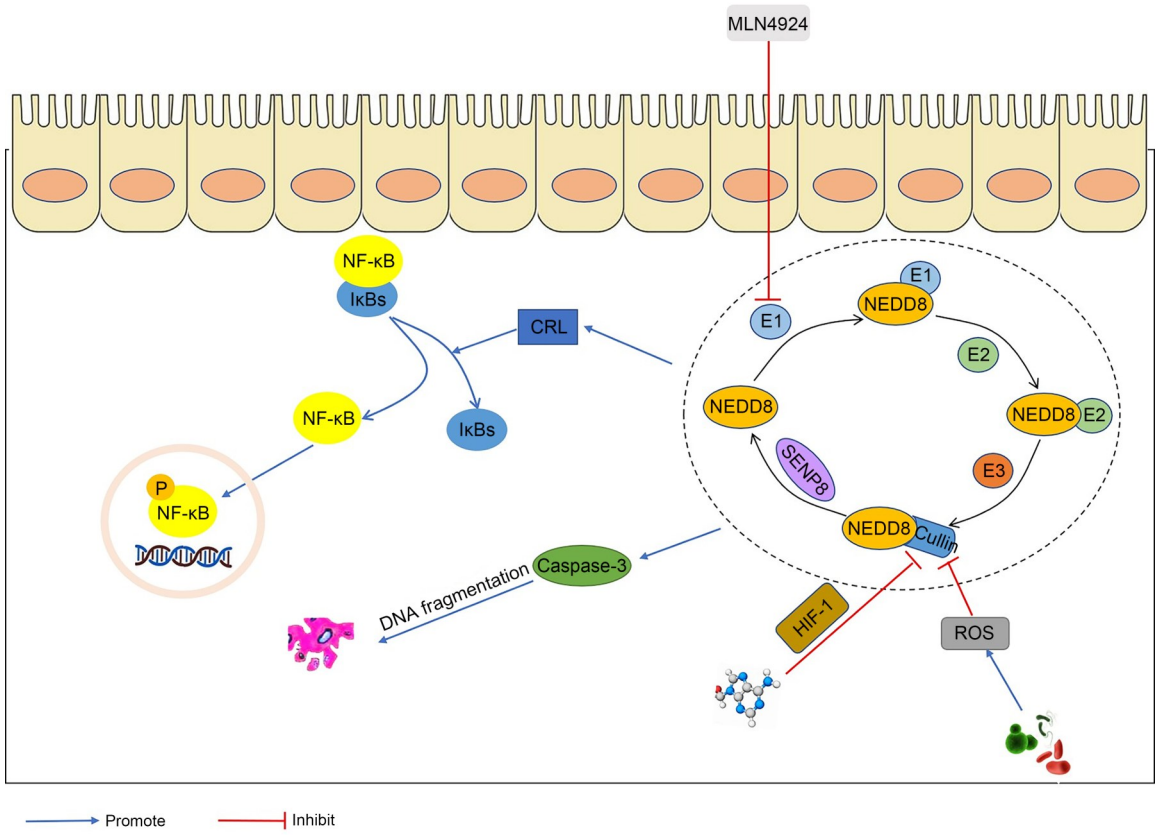

Fig. 1 Neddylation in IBD. MLN4924 prevents neddylation by inhibiting the E1 enzyme (NAE); a hypoxic microenvironment triggers the accumulation of adenine nucleotide metabolites, which cause the increase of cullin 1 and subsequent inhibition of the binding of cullin 1 to NEDD8; HIF-1 regulates oxygen homeostasis and adenosine signal transduction, and inhibits neddylation to relieve mucosal inflammation; ROS produced by bacteria control the esterification state of cullin 1, and inhibit the NF-κB signaling pathway by inhibiting neddylation. IBD: inflammatory bowel disease; NEDD8: neural precursor cell expressed developmentally downregulated protein 8; NAE: NEDD8-activating enzyme E1; HIF-1: hypoxia inducible factor-1; ROS: reactive oxygen species; SENP8: sentrin-specific protease 8; NF-κB: nuclear factor-κB; IκB: inhibitor of NF-κB; CRL: cullin-RING ligase; P: phosphorylated.

The uncoupling of SUMO from the protein substrate of SUMO acylation is also a key step towards maintaining the balance of SUMO signal during the SUMO acylation process, which is called de-sumoylation (Verma et al., 2018). The imbalance between sumoylation and de-sumoylation is related to the occurrence and development of various diseases (Yang et al., 2017). Studies have shown that modulators related to the inflammatory cascade are affected by changes in the sumoylation function (Ribet et al., 2010; Flotho and Melchior, 2013; Verma et al., 2015). The molecules involved include RelA, which is a component of the main regulatory NF-κB and its repressor (IκB-α) (Mabb and Miyamoto, 2007). Mitogen-activated protein kinase (MAPK) signaling is another important signaling pathway for amino acids and intestinal inflammation (Coskun et al., 2011). MAPK signals in mammals are mainly composed of extracellular signal-regulated kinase (ERK), c-Jun N-terminal kinase (JNK), and p38 MAPK as pathway mediators, which play an important role in cell growth, proliferation, differentiation, and inflammation (Docena et al., 2010; Raza et al., 2017; You et al., 2017). The metabolism of cellular ROS is stably regulated by various proteins involved in the redox mechanism of phosphoinositide 3-kinase (PI3K)/AKT signaling. AKT activates the IκB kinase (IKK), thereby stimulating p38 MAPK in IL-1β receptor signal transduction (Tokuhira et al., 2015). Yavvari et al. (2019) demonstrated that SUMO3 ligase and the encoding plasmid DNA to target sumoylation through nanogel oral delivery of PIAS inhibited NF-κB-mediated inflammatory signals to achieve the effect of treating IBD. Mustfa et al. (2017) reported that the sumoylation of intestinal epithelial

cells (IECs) can inhibit the activity of major regulatory factors including serine-threonine kinase AKT1, thereby reducing the severity of IBD.

Evidence showed that SUMO participates in the regulation of host proteins of innate immunity, thus promoting the regulation process of interferon production during viral infection (Saul et al., 2015; Xia et al., 2015; Hannoun et al., 2016; Liu et al., 2016). Other study has also indicated that increased levels of nitric oxide (NO) after pathogen recognition can promote the S-nitrosylation of SUMO-conjugating enzyme 1 (SCE1) in cysteine 139 (Cys139), thereby inhibiting the activity of SCE1 and UBC9. The S-nitrosylation of SCE1 on Cys139 enables NO to drive immune activation by alleviating the inhibitory effect mediated by SUMO1/2 and reducing pathogen susceptibility (Skelly et al., 2019). The retinoic acid receptor (RAR)-related orphan receptor-γt (ROR-γt) mediating IL-17 transcription dysregulation is the core of the pathogenesis of several inflammatory diseases, while the molecular mechanism that controls the activity of ROR-γt transcription factors in IL-17 regulation has not been fully clarified (Honda and Littman, 2016; Veldhoen, 2017). It has been documented that the SUMO conjugation enzyme UBC9 interacts with the conserved GKAE motif in ROR-γt to induce the sumoylation of ROR-γt and inhibit IL-17 expression. The sumoylation of ROR-γt helps histone deacetylase 2 (HDAC2) bind to the IL-17 promoter and consequently inhibits its transcription to relieve colitis (Singh et al., 2018).

Sumoylation is essential for maintaining the dynamic balance of intestinal flora, barrier integrity, and inflammation control during bacterial invasion (Demarque et al., 2011; Fritah et al., 2014). The intestinal mucosa of patients with CD allows the abnormal attachment of invasive *Escherichia coli* (AIEC), which adheres to and invades IECs, survives in macrophages, and induces inflammation. According to Dalmasso et al. (2019), infection with the AIEC LF82 reference strain significantly reduced the level of SUMO-coupled protein in human intestinal epithelial T84 cells and manipulated host sumoylation to prevent the autophagy reaction from replicating in cells. Furthermore, Singh et al. (2018) showed that T helper 17 (Th17) cells expressing sumoylation-deficient ROR-γt are highly vulnerable to *E. coli* after being transferred to *Rag1*$^{-/-}$ mice. *Shigella flexneri*, a pathogen causing bacillary dysentery, invades the human colon epithelium and causes massive inflammation and destruction (Perdomo et al., 1994). Research by Fritah et al. (2014) indicated that the SUMO pathway is an important part of the host's innate protection, as it reduces the invasion of the intestinal epithelium by shigellosis and the pro-inflammatory transcriptional response of the intestine through sumoylation. Sumoylation in IBD is described in Fig. 2.

4 Glycosylation

Glycosylation comprises the transfer of sugar to protein under the action of glycosyltransferase and the formation of glycosidic bonds with amino acid residues on the protein. Proteins undergo glycosylation to form glycoproteins. Glycosylation, that can modulate the physiological properties of small molecules and peptides, has specific effects such as improved metabolic stability, membrane permeability, biodistribution, and ligand–target interactions (Wadzinski et al., 2018). According to the type of glycoside chain, the common types of protein glycosylation are O-glycosidic bond and N-glycosidic bond. In O-glycosylation, the hydroxyl groups of serine, threonine, hydroxylysine, and hydroxyproline are used as the connection points to form an O-glycosidic bond, which is one of the most abundant and diverse types of protein PTM. O-glycans regulate the structure, stability, and function of proteins, and play a universal and highly specific role in most biological processes (Joshi et al., 2018). In the case of N-glycosylation, the amide group of asparagine, the α-amino group of the N-terminal amino acid, and the ω-amino group of lysine or arginine are used as the connection points to form an N-glycosidic bond. It was reported that more than 50% of all eukaryotic proteins are N-glycosylated. Such bonds also play an important role in a series of biological events, such as protein folding and quality control (Tamir and Eichler, 2017).

Glycans regulate cellular and humoral immune responses, including the assembly of major histocompatibility complex (MHC) antigens and human leukocyte antigen (HLA), the regulation of immune receptor clusters, endocytosis, receptor signaling, and immunoglobulin function (Demetriou et al., 2001; Grigorian et al., 2009, 2012; Ryan and Cobb, 2012; Johnson et al., 2013; Wolfert and Boons, 2013; Pereira et al., 2018). They also control the development, activation,

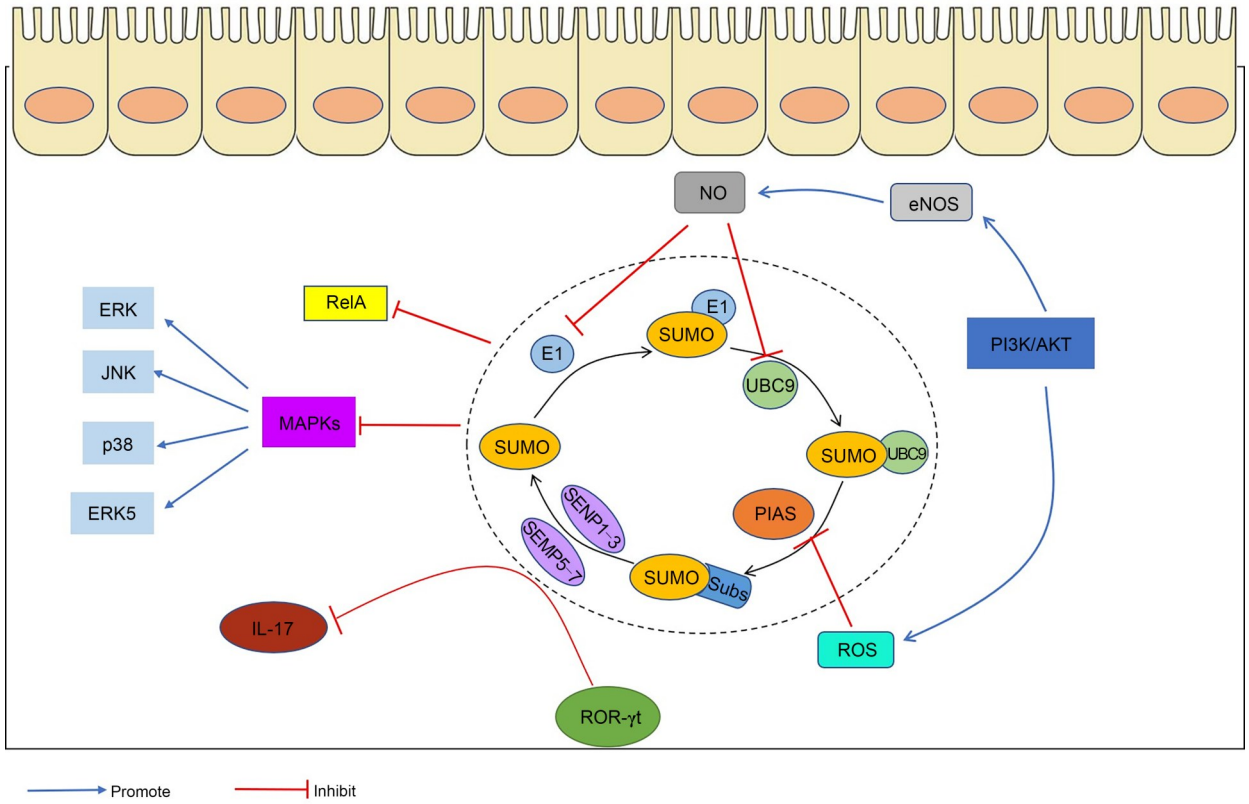

Fig. 2 Sumoylation in IBD. The redox process of PI3K/AKT signaling promotes the release of ROS and NO. NO inhibits the enzyme activity of SCE1 and UBC9 by regulating the S-nitrosylation of SUMO E2 enzyme in Cys139; ROS inhibit PIAS activity and the binding of SUMO and substrates; the sumoylation of ROR-γt inhibits the expression of IL-17 and relieves IBD. The dynamic balance between sumoylation and desumoylation regulates a series of inflammatory cascades. IBD: inflammatory bowel disease; PI3K: phosphoinositide 3-kinase; AKT: protein kinase B; ROS: reactive oxygen species; NO: nitric oxide; SUMO: small ubiquitin-like modifier protein; SCE1: SUMO-conjugating E2 enzyme 1; UBC9: ubiquitin-conjugating enzyme 9; Cys139: cysteine 139; PIAS: protein inhibitor of activated signal transducer and activator of transcription (STAT); ROR-γt: retinoic acid receptor (RAR)-related orphan receptor-γt; IL-17: interleukin-17; ERK: extracellular signal-regulated kinase; JNK: c-Jun N-terminal kinase; MAPK: mitogen-activated protein kinase; eNOS: endothelial nitric oxide synthase; SENP: sentrin-specific protease.

signal transduction, differentiation, and proliferation of T cells and the selection of thymocytes. The participation of N-glycosylation in the pathogenesis of inflammatory diseases has been reported to be an early event (Mkhikian et al., 2011). The intestinal T cells of IBD patients and healthy people express different glycoprotein profiles, essentially showing that IBD patients have low expression of N-glycans and high expression of core fucosylation. These changes increase T cell-mediated intestinal immune response (Dias et al., 2014; Fujii et al., 2016; Dias et al., 2018). Grigorian et al. (2012) reported that IL-2 and IL-7 alter the expression of Golgi branching enzyme, which controls N-glycosylation in T cells and regulates the inflammatory environment. T cells in mice expressing human fucosyltransferase 2 (FUT2) have genetic defects and abnormal N-glycosylation levels, which can promote the development of colitis by regulating T cells (Brown et al., 2004). As a key glycosyl hydrolase, α-mannosidase II (α-MII) plays a pivotal role in the maturation of N-glycans. IECs establish a first-line intestinal barrier to regulate immunity, and selectively express α-MII. In the absence of the mannosidase α class 2A member 1 (*MAN2A1*) gene encoding α-MII, the maturation of N-glycans in IECs is inhibited, the sensitivity to dextran sulfate (DSS)-induced colitis is reduced, and neutrophil infiltration in the colonic mucosa is attenuated by the downregulated expression level of neutrophil chemotactic factor (Suzuki et al., 2018). Similar to phosphorylation, glycosylation has been shown to play a key role in regulating the balance between the soluble and filamentous

insoluble state of keratin. Keratin 18 (K18) glycosylation can protect IECs from damage (Srikanth et al., 2010).

As formerly stated, the pathogenesis of IBD involves many factors, including host genetics, immune disorders, and changes in the intestinal flora (Sugihara et al., 2019). Abnormal glycosylation significantly changes the function of key proteins in the intestinal niche, and glycosylation is very important for fine-tuning intestinal processes to ensure homeostasis. A disruption of this homeostasis process potentially leads to IBD (Hanić et al., 2019). Emerging evidence suggests that IBD is associated with the increased expression of truncated O-glycans and changes in the structure of terminal glycans. Other factors like *IBD* gene, incorrect positioning of glycosyltransferase, altered expression of glycosyltransferase and glycosidase, as well as malnutrition lead to changes in glycogen (Sommer et al., 2014). These glycan changes destroy the mucus layer, glycan–lectin interaction, host–microbe interaction, and mucosal immunity, and ultimately lead to the occurrence of IBD. Epithelin is particularly important in regulating the intestinal flora through providing bacterial ligands and nutrients, and ultimately determining the spatial organization of gut microbiome (Kudelka et al., 2020). The glycan sugar chains that cover the cell surface are also called glycocalyxes. These mediate the interaction between intestinal cells and the microbiota, including influencing intestinal homeostasis and mucosal immune response (Verhelst et al., 2020). Fluctuations in the glycocalyx or insufficient dietary glycan content may lead to malnutrition, and in turn lead to IBD and possible colon cancer (Hibberd et al., 2017). The study has also established that individuals lacking FUT2 have altered gut microbial composition and increased sensitivity to infection and inflammation (Rausch et al., 2011). Changes in mucin glycosylation increase the sensitivity of core 1- or core 3-derived O-glycans to colitis and raise the permeability of the intestinal epithelium, leading to bacterial infiltration and pathogen growth (Sommer et al., 2014). Moreover, it has been reported that symbiotic bacteria (such as Bacteroides) can induce epithelial fucosylation (Goto et al., 2014). The fragment crystallizable (Fc)-glycosylation of immunoglobulin G (IgG) affects its effector function, and the galactosylation level of IgG in UC or CD patients is lower than that of healthy individuals (Šimurina et al., 2018). The decreased glycosylation observed in the intestinal mucus of patients with IBD indicates that the lack of galactosylation of IgG on the inner mucus layer may lead to increased contact between bacteria and the epithelium, triggering inflammation (Theodoratou et al., 2014). Galactosylation in IBD is described in Fig. 3.

5 Acetylation

Reversible protein acetylation is the main regulatory mechanism that controls protein function. The first biologically functional protein acetylation to be studied was the acetylation of histones (Baeza et al., 2016). In the past 15 years, an increasing number of studies have shown that protein acetylation-related enzymes, including histone acetyltransferases (HATs), HDACs, and acetyllysine-binding proteins, participate in many cell functions other than transcription regulation (Verdin and Ott, 2015).

Evidence showed that histone acetylation is related to the immune pathway (Shakespear et al., 2011), and epigenetic regulators of histone modification can regulate the gene-specific expression of T cell differentiation during inflammation (Foster and Medzhitov, 2009; Wei et al., 2009; Rodriguez et al., 2015). IL-17A produced by Th17 cells plays a role as a pro-inflammatory cytokine in the development of IBD. In colon tissues lacking Mof (males absent on the first; responsible for the acetylation of histone H4 at lysine 16 (H4K16)), a series of key genes related to Th17 genes (including *IL-17A*, *IL-22*, *ROR-γt*, *ROR-α*, *STAT3*, transforming growth factor-β1 (*TGF-β1*), and *IL-6*) were significantly downregulated and the NF-κB signaling pathway was inhibited, thereby alleviating DSS-induced colitis in mice (Yang et al., 2018). IL1-β is a pro-inflammatory cytokine that can act as a mediator of inflammatory cell infiltration and mucosal barrier destruction in intestinal inflammation (Weber et al., 2010). Zhong et al. (2018) found that neonates with colitis expressed high levels of norepinephrine and epinephrine, which promoted histone acetylation at the *IL-1β* gene promoter, leading to abnormally increased IL-1β expression levels. TGF-β1, as an immunosuppressive cytokine, can transmit negative signals in many immune cells (Letterio and Roberts, 1998). Studies have shown reduced activity of TGF-β1

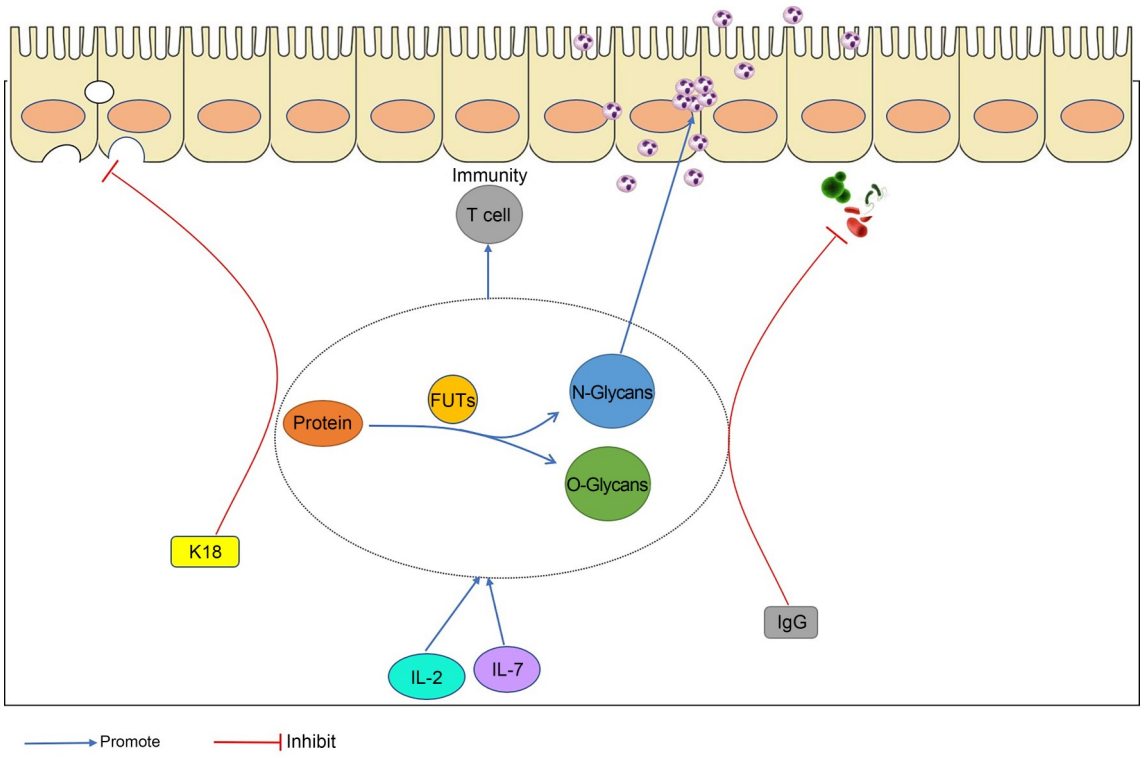

Fig. 3 Glycosylation in IBD. In early inflammation, cytokines such as IL-2 and IL-7 modulate N-glycosylation to change the immune response of T cells; excessive N-glycosylation in intestinal epithelial cells promotes the infiltration of neutrophils; K18 glycosylation maintains the steady state of keratin between soluble and filamentous insoluble states and protects intestinal epithelial cells from damage; IgG galactosylation inhibits the increase of contact between bacteria and epithelium, thereby reducing inflammation. IBD: inflammatory bowel disease; IL: interleukin; K18: keratin 18; IgG: immunoglobulin G; FUT: fucosyltransferase.

in IBD patients, which was related to the increase in the level of recombinant mothers against decapentaplegic homolog 7 (SMAD7). SMAD7 is an intracellular protein that binds to the TGF-β1 receptor and inhibits the signal transduction induced by TGF-β1 (Monteleone et al., 2001). In the mucosa of IBD patients, SMAD7 is highly acetylated on lysine residues, and ubiquitin-mediated protein degradation is inhibited (Monteleone et al., 2004). SMAD7 is also regulated by P300 (a protein with inherent acetyltransferase activity) (Monteleone et al., 2005). Research by Sedda et al. (2018) showed that SMAD7 and sirtuin 1 (SIRT1) have a negative regulatory effect on each other and amplify inflammatory signals in the intestine. SIRT1 is a type III nicotinamide adenine dinucleotide positive (NAD^+)-dependent deacetylase, which mainly participates in a variety of cell biological functions through the deacetylation of certain non-histone and histone proteins. The study has indicated that SIRT1 can inhibit inflammation and regulate immune cells (He et al., 2017). It also promotes the proteosomal degradation of various intracellular proteins mediated by ubiquitin and triggers anti-inflammatory signals. The low expression of SIRT1 in IBD patients leads to persistent inflammation (Caruso et al., 2014). Research by Melhem et al. (2016) proved that after DSS-induced colitis in rats, the expression of SIRT1 decreased and the degree of acetylation of heat shock factor protein 1 (HSF1) increased. According to reports, histone acetylation is related to the transcriptional activity of inflammatory cytokines (Ventham et al., 2013; Meng et al., 2016). HDAC2 inhibits the transcription of IL-6 and other inflammatory factors through histone deacetylation (Zhang et al., 2015). According to Yin et al. (2018), matrix metalloproteinase (MMP) increases the expression of HDACs, which leads to the inhibition of histone H3/H4 acetylation and TLR-4 pathway-related signaling pathways, the decreased secretion of pro-inflammatory cytokines, and the increase of anti-inflammatory cytokines.

Certain types of keratin in epithelial cells provide strength and integrity. The main keratins expressed by IECs are K8, K18, and K19. The solubility of keratin is regulated and its degradation is inhibited through acetylation. A large number of acetylation sites were reported in K8. Majumdar et al. (2012) found that mice lacking K8 were more likely to develop colitis. Dierckx et al. (2019) revealed that glycoprotein acetylation (GlycA) levels in patients with active IBD are significantly higher than those in healthy controls (Dierckx et al., 2019).

As a multifactorial chronic disease, IBD usually relates to changes in the composition and function of the intestinal flora. The elimination of pathogenic microbiota has been widely applied in the treatment of IBD (Grimm and Riedel, 2016). Studies have shown that *Lactobacillus plantarum* (LP) can act as a probiotic to enhance the intestinal epithelial barrier (Qin et al., 2009; Liu et al., 2010, 2011b). LP can significantly reduce the damage of IECs caused by enteropathogenic *E. coli* (EPEC) (Liu et al., 2011a). Thermogenic bacteria usually produce butyrate through carbohydrate fermentation or amino acid degradation. Butyrate plays an important protective role in the intestinal homeostasis of adaptive and innate immunity. It was reported that butyrate can increase the acetylation of histones, thereby regulating the activation of Treg and reducing the activation of NF-κB (Silva et al., 2018). *Freundella prausnitzii* produces butyrate and reduces Th17 differentiation by inhibiting the metabolism of HDAC3 and cellular-myelocytomatosis viral oncogene (c-Myc) in T cells to reduce colitis (Zhang et al., 2019). Acetylation in IBD is described in Fig. 4.

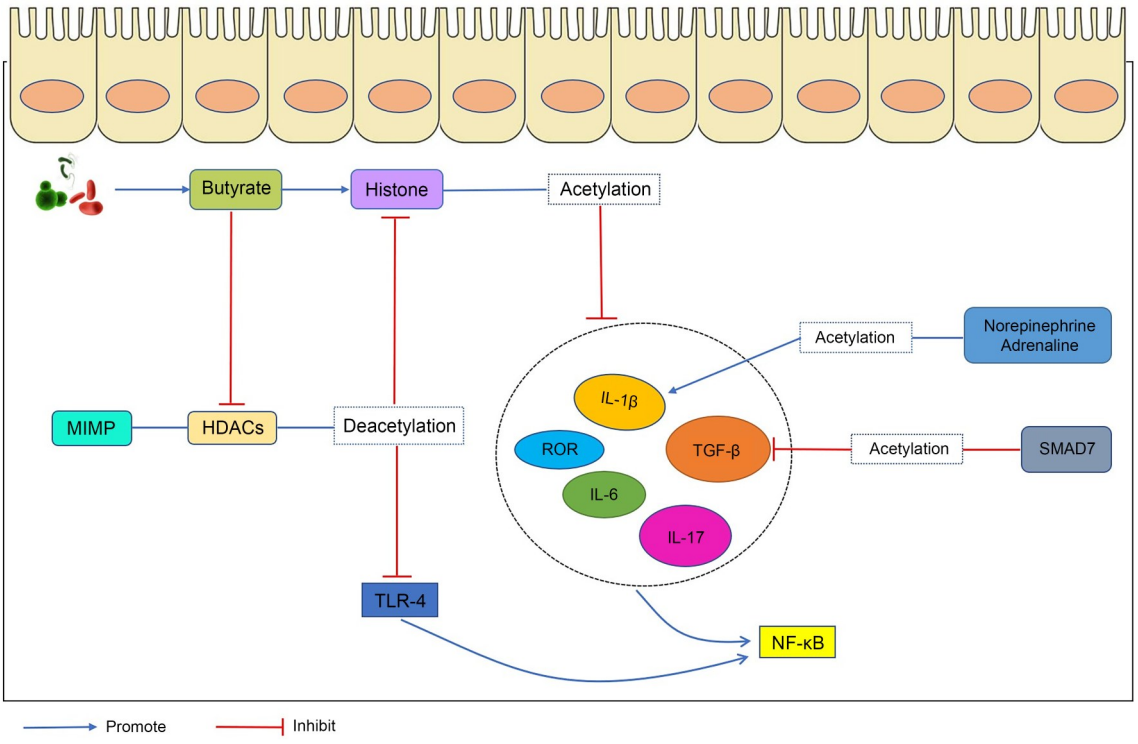

Fig. 4 Acetylation in IBD. Butyrate produced by bacteria increases the acetylation of histones, which in turn regulates T cells. A series of Th17-related genes are significantly downregulated, and the NF-κB signaling pathway is inhibited, thereby alleviating IBD; MIMP increases the expression of HDACs, inhibiting histone acetylation, the transcription of IL-6 and other inflammatory factors, and TLR-4 related signaling pathways. Norepinephrine and adrenaline promote the acetylation of histones at the *IL-1β* gene promoter, leading to an abnormal increase in IL-1β expression; SMAD7 is highly acetylated on lysine residues and ubiquitin-mediated protein degradation is inhibited; SMAD7 binds to the TGF-β 1 receptor and inhibits its signal transduction. IBD: inflammatory bowel disease; Th17: T helper 17; NF-κB: nuclear factor-κB; MIMP: micro integral membrane protein; HDAC: histone deacetylase; IL: interleukin; TLR-4: Toll-like receptor-4; SMAD7: recombinant mothers against decapentaplegic homolog 7; ROR: retinoic acid receptor (RAR)-related orphan receptor; TGF-β: transforming growth factor-β.

In summary, the ultimate effects of PTMs are to change the structure and function of the substrate proteins. Proteins are widely expressed in mammalian tissues and receive various stimulation signals both in vivo and in vitro. Under normal circumstances, protein modification (glycosylation or acetylation) plays a central role in maintaining the correct transcription, translation, and function of proteins. Under special pathological stimuli, however, over- or undermodification can cause cells (immune cells, tissue cells, etc.) to have abnormal function, which in turn activates a series of signaling pathways. Although the several PTMs introduced in this review differ in the substrate-ubiquitin-like molecule binding process, and the directly activated target molecules are also different, their final outcomes during the occurrence of IBD are the destruction of the intestinal epithelial barrier, the changed permeability of IEC, the promotion of the infiltration of inflammatory factors and immune cells, and the induction of classic inflammatory signaling pathways, such as NF-κB. At the same time, the IEC barrier is weakened and the invasion of intestinal bacteria is intensified, leading to the imbalance of the microbial environment and the dominance of pathogenic bacteria, further aggravating the development of IBD. The relationships and differences between the roles of PTMs in the occurrence of IBD are listed in Table 1 and Fig. 5.

6 Conclusions

We can find that regardless of the kind of modification, once the protein structure and function change, the permeability of the intestinal epithelium will also be different. The homeostasis of the intestinal microenvironment is subsequently destroyed paving the way for invading bacteria, which, to an extent, also aggravates the infiltration of immune cells, and recruits various inflammatory factors. To a certain degree, we can infer that these protein modifications play a combined role in IBD. IBD is an intestinal disease

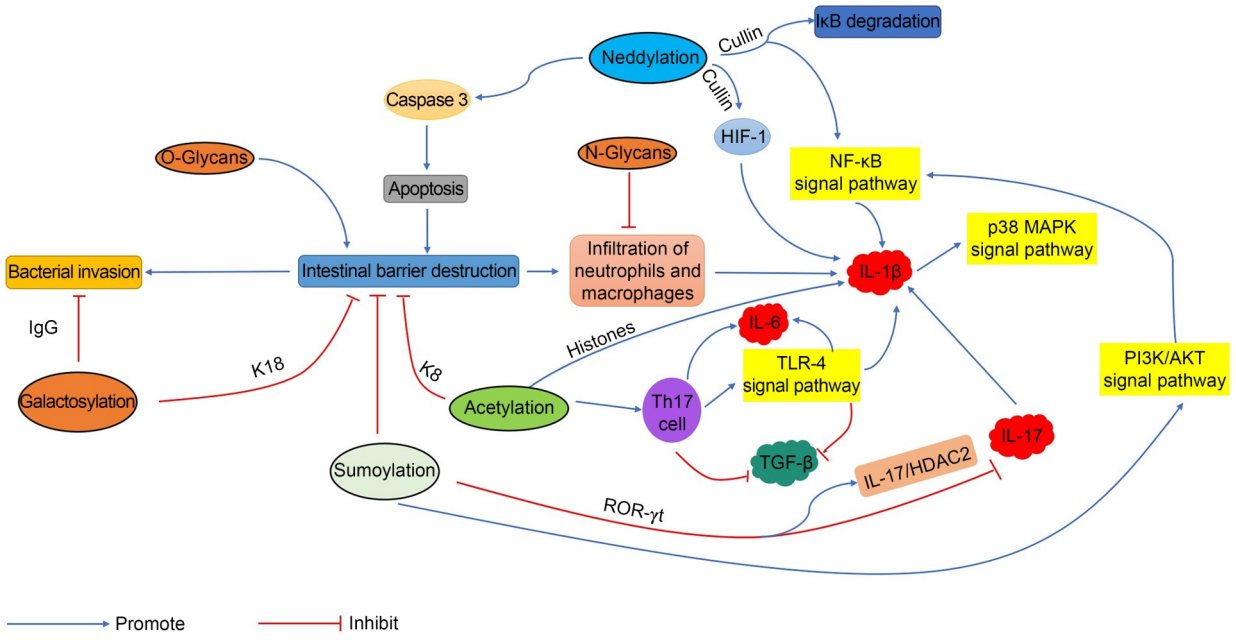

Fig. 5 Roles of PTMs in the occurrence of IBD. O-Glycosylation and acetylation inhibit the destruction of the intestinal epithelial barrier and reduce the invasion of bacteria. N-Glycosylation exhibits the opposite effect. In addition to causing apoptosis mediated by the caspase 3 cascade, the most important role of neddylation is to activate the NF-κB signaling pathway and promote the expression of IL-1β. ROR-γt undergoes sumoylation, which is combined with acetylation, helps IL-17 bind to HDAC2, and inhibits the expression of IL-17 and other inflammatory factors. PTM: post-translational modification; IBD: inflammatory bowel disease; NF-κB: nuclear factor-κB; IL: interleukin; ROR-γt: retinoic acid receptor (RAR)-related orphan receptor-γt; HDAC2: histone deacetylase 2; IgG: immunoglobulin G; K18: keratin 18; Th17: T helper 17; HIF-1: hypoxia inducible factor-1; TLR-4: Toll-like receptor-4; TGF-β: transforming growth factor-β; IκB: inhibitor of NF-κB; MAPK: mitogen-activated protein kinase; PI3K: phosphoinositide 3-kinase; AKT: protein kinase B.

Table 1 Differences in the involvement of various protein modifications in the pathological process of IBD

PTM	Modification molecule	Substrates	Stimulus signals	Related enzymes	Main effects	Pathogenesis involved in IBD
Neddylation	NEDD8	Cullin 1–3, 4A, 4B, 5, 7, 9; Mdm2, PVHL, HuR, EGFR, p53	ROS, HIF-1	NAE1, UBA3, UBC12, CRL	Prevents protein degradation; regulates protein conformational stability and positioning; regulates the activity of transcription factors; antagonizes ubiquitination	mTOR and NF-κB signaling pathways; cascade reaction mediated by caspase 3
Sumoylation	SUMO	ROR-γt	ROS, NO	SAE1/SAE2, UBC9, PIAS, SENP	DNA damage repair; immune response; apoptosis	NF-κB, MAPKs, PI3K/AKT signaling pathway; maintaining the dynamic balance of intestinal flora and bacterial invasion
Glycosylation	O-Glycosidic bond, N-Glycosidic bond	K18, IgG		FUT2, α-MII	Mediates cellular and humoral immune response	T cell-mediated intestinal immune response; regulating the homeostasis of intestinal flora
Acetylation	Acetyl	SMAD7, HSF1, K8, K18, K19	Butyric acid	HAT, HDAC	Transcriptional regulation; regulates T cell differentiation gene expression	NF-κB, TLR-4 signal pathway; inflammatory factor secretion

IBD: inflammatory bowel disease; PTM: post-translational modification; NEDD8: neural precursor cell expressed developmentally downregulated protein 8; SUMO: small ubiquitin-like modifier protein; Mdm2: murine double microsome 2; PVHL: von Hippel-Lindau tumor suppressor protein; HuR: human antigen R; EGFR: epidermal growth factor receptor; ROR-γt: retinoic acid receptor (RAR)-related orphan receptor-γt; K18: keratin 18; IgG: immunoglobulin G; SMAD7: recombinant mothers against decapentaplegic homolog 7; HSF1: heat shock factor protein 1; ROS: reactive oxygen species; HIF-1: hypoxia inducible factor-1; NO: nitric oxide; NAE1: NEDD8-activating enzyme E1 subunit 1; UBA3: ubiquitin-like modifier activating enzyme 3; UBC: ubiquitin-conjugating enzyme; CRL: cullin-RING ligase; SAE: SUMO-activating enzyme; PIAS: protein inhibitor of activated signal transducer and activator of transcription; SENP: sentrin-specific protease; FUT2: fucosyltransferase 2; HAT: histone acetyltransferase; HDAC: histone deacetylase; mTOR: mammalian target of rapamycin; NF-κB: nuclear factor-κB; MAPK: mitogen-activated protein kinase; PI3K: phosphoinositide 3-kinase; AKT: protein kinase B; TLR-4: Toll-like receptor-4.

that has been studied more frequently, and its mechanism has been explored from many aspects. There are different types of protein structures and functions that are widely implicated in the treatment of diseases. In the quest to find effective treatments for IBD, researchers have modified existing proteins and studied their therapeutic utilities. In addition to genetic engineering, one of the most researched areas in this regard is the change of the properties and biological characteristics of proteins through chemical modification. The pursuit of a more in-depth understanding of protein modifications in the immune system, microbial environment, and other components of the inflamed intestinal environment has become a new and promising direction for potential effective IBD therapies.

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Author contributions

Gaoying WANG determined the topic of the article, proposed this program, and wrote the article. Jintao YUAN summarized and drew diagram of the mechanism. Dickson Kofi Wiredu OCANSEY modified the language of the article. Ji LUO, Xu ZHANG, Hui QIAN, Wenrong XU, and Fei MAO collected the literature. Fei MAO guided the article and gave opinions. All authors have read and approved the final manuscript, and therefore, have full access to all the data in the study and take responsibility for the integrity and security of the data.

Compliance with ethics guidelines

Gaoying WANG, Jintao YUAN, Ji LUO, Dickson Kofi Wiredu OCANSEY, Xu ZHANG, Hui QIAN, Wenrong XU, and Fei MAO declare that they have no conflict of interest.

This article does not contain any studies with human or animal subjects performed by any of the authors.

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