New insights on IL-36 in intestinal inflammation and colorectal cancer (Review)

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Abstract. Interleukin (IL)-36 is a member of the IL-1 superfamily, which includes three receptor agonists and one antagonist and exhibits a familial feature of inflammatory regulation. Distributed among various tissues, such as the skin, lung, gut and joints, the mechanism of IL-36 has been most completely investigated in the skin and has been used in clinical treatment of generalized pustular psoriasis. Meanwhile, the role of IL-36 in the intestine has also been under scrutiny and has been shown to be involved in the regulation of various intestinal diseases. Inflammatory bowel disease and colorectal cancer are the most predominant inflammatory and neoplastic diseases of the intestine, and multiple studies have identified a complex role for IL-36 in both of them. Indeed, inhibiting IL-36 signaling is currently regarded as a promising therapeutic approach. Therefore, the present review briefly describes the composition and expression of IL-36 and focuses on the role of IL-36 in intestinal inflammation and colorectal cancer. The targeted therapies that are currently being developed for the IL-36 receptor are also discussed.

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Abbreviations: IL-36, interleukin 36; IL-36R, IL-36 receptor; IL-1RAcP, IL-1 receptor accessory protein; GPP, generalized pustular psoriasis; MyD88, myeloid differentiation factor 88; MAPK, mitogen-activated protein kinase; NF-κB, Nuclear factor-κB; CRC, Colorectal cancer; IBD, inflammatory bowel disease; UC, ulcerative colitis; CD, Crohn's disease; DSS, dextran sodium sulfate; STAT: signal transducer and activator of transcription; Lcn2, lipocalin 2; ECM, extracellular matrix; PI3K, phosphatidylinositol 3 kinase; Akt, protein kinase B; TLS, tertiary lymphoid structures; DC, dendritic cell; PD, programmed cell death

Key words: interleukin-36, inflammatory bowel disease, fibrosis, colorectal cancer

Contents

1. Introduction

Interleukin (IL)-36 is a group of cytokines that contains three receptor agonists IL-36α, IL-36β IL-36γ and an antagonist IL-36 receptor a (IL-36Ra). The IL-36 genome, located at human genome 2Q14, was first discovered by genome screening around the year 2000 (1,2). It is thought to be a homologue of IL-1 cytokines because of its similarity to IL-1 in conserved sequence and predicted structure (3). Thus, the IL-36 cytokine family was originally named as IL-1F6, IL-1F8, IL-1F9 and IL-1F5, before being formally unified in 2011 (4,5). IL-36α, IL-36β and IL-36γ are agonistic ligands of IL-36R, while IL-36Ra is an inhibitory ligand. All of them require protease shearing to be active (6,7). Upon binding to the IL-36 functional receptor complex, which consists of the IL-36R and the IL-1 receptor accessory protein (IL-1RAcP), IL-36 agonists activate the mitogen-activated protein kinase (MAPK) and nuclear factor-κB (NF-κB) signaling pathways and then regulate the downstream immune and inflammatory responses (8-10). IL-36Ra, as a receptor antagonist, hinders dimerization of the IL-36R/IL-1RAcP complex by inhibiting IL-1RAcP recruitment, hence suppressing downstream inflammatory signaling (11). IL-36 ligands and IL-36R have been shown to be broadly expressed in human tissues, especially in mucosal barrier regions such as the skin, lung and intestine (8). Its role in the etiology of skin inflammation, in particular, has been successfully developed as a therapeutic target and has been approved by the Food and Drug Administration (FDA) for the treatment of generalized pustular psoriasis after phase III clinical trials (12).

The role of IL-36 in the gut is complicated. Several previous studies in inflammatory diseases have shown a key role for IL-36 in regulating inflammation, particularly in chronic

inflammation as a pro-inflammatory factor that exacerbates inflammation (13,14). In chronic intestinal inflammation, IL-36 is not only involved in intestinal inflammation but also promotes the development of intestinal fibrosis (15). However, it also has a protective role and may exert a protective function in acute intestinal inflammation by promoting healing after mucosal injury (16). Cancer is closely associated with inflammation and the mechanism of action of IL-36 has been identified in many types of cancer (17,18). In colorectal cancer (CRC), IL-36α and IL-36γ have been more extensively studied, and their expression levels appear to be used as biomarkers for predicting colorectal cancer prognosis (19). Available evidence suggests that IL-36α has antitumor effects, whereas IL-36γ has a more complex role, both in promoting antitumor immune responses, such as modulating tumor-infiltrating immune cells, and inducing genes involved in the IL-17/IL-23 axis to promote colorectal cancer cell proliferation (20,21). The present review briefly summarizes the regulation of IL-36 expression and function, discusses the role of IL-36 in intestinal inflammation and colorectal cancer and the latest research progress in the development of targeted IL-36 signaling therapy.

2. Expression and function of IL-36

IL-36 is a novel cytokine subfamily of the IL-1 superfamily. Based on the gene sequences and binding receptors, this family also contains IL-1, IL-18 and IL-33 and has been shown to have a significant role in the regulation of immunity and inflammation (22). Because of its near proximity and similar conserved sequence to the IL-1 genome, IL-36 has been extensively researched in numerous immunological disorders and inflammatory diseases (1,23-26). IL-36 has been found in a variety of human tissues, such as the skin, lung, intestine and synovium, and can be expressed by a variety of cells, including epithelial cells such as keratinocyte, bronchial and intestinal epithelial cells, as well as immune cells such as neutrophils, monocyte macrophages and T cells (27,28). Under physiological conditions, the production of IL-36 in human tissues is low; however, under inflammatory conditions, the expression of IL-36 is greatly elevated after proinflammatory cytokine stimulation or signal activation by Toll-like receptors, particularly in the epithelial cells of the barrier tissues of the skin, respiratory tract and digestive tract, where they are in close contact with external pathogens (29-31). It is therefore hypothesized that IL-36 is specialized in the regulation of mucosal innate and adaptive immunity. When IL-36 signaling is activated in cells, it can activate a variety of immune cells, epithelial cells, fibroblasts and keratinocytes in an autocrine or paracrine manner and induce the expression of various cytokines and chemokines, thus promoting the restoration of mucosal homeostasis and epithelial repair, as well as inducing the development of inflammatory responses and even promoting the progression of fibrosis and malignant tumors (21,22,27,32). In addition, as an inflammatory regulator, IL-36 also participates in the host immune response to bacterial and viral infections (33-36).

IL-36 cytokines must be processed with neutrophil-derived proteases before they are activated. After truncating the N-terminal with different enzymes, the affinity of IL-36 ligand to IL-36R can be magnified by several hundred times (6,10). Cathepsin G and elastase can activate IL-36α, whereas IL-36β and IL-36γ are activated by cathepsin G and elastase respectively (6). Similarly, in order to have an active antagonistic form, IL-36Ra must be cleaved by elastase (7). IL-36R is a 575-amino-acid multi-domain transmembrane receptor with high sequence homology with IL-1R that is comprised of three immunoglobulin-like extracellular domains and an intracellular Toll-IL-1-receptor domain (36). The helper protein, IL-1RAcP, is structurally similar to IL-36R and is a critical co-receptor shared by family members of IL-1 that is significantly engaged in cell signaling transmission (36,37). IL-36Ra is a natural IL-36R antagonist encoded by the IL-36RN gene. The fact that IL-36Ra binds to IL-36R with greater affinity and slower off-rate compared with the IL-36 agonist reveals that activation of the IL-36 signaling pathway has a strong self-negative regulation (38). Thus, IL-36 activation will be out of control and worsen the inflammatory response when IL-36Ra is mutated or deleted, as has been shown in patients with GPP (39). This is a severe and potentially life-threatening pustular skin condition caused by congenital deletion of the IL-36RN gene (40). Furthermore, IL-38 has also been revealed to be an antagonist of IL-36, exerting comparable biological activity to IL-36Ra by binding to IL-36R (41). This may be associated with its chromosomal location and gene sequence structure that is similar to that of IL-36Ra. This is why numerous studies often classify IL-38 as part of the IL-36 family for discussion (23,42-44).

IL-36 activators bind to the receptor complex formed by IL-36R and IL-1RAcP and then recruit the intracellular signaling molecules myeloid differentiation factor 88 (Myd88), IL-1R-related kinase and tumor necrosis factor receptor-related factor 6 to activate the NF-κB and MAPK signaling pathways (10). This, in turn, promotes the nuclear translocation and activation of activator protein 1 and NF-κB, thereby regulating the transcription and expression of downstream pro-inflammatory genes (8-10,45). MyD88 is essential for IL-36 signaling. A gene expression sequencing analysis of primary keratinocytes has revealed that silencing MyD88 via CRISPS/Cas9 inhibits IL-36 responsiveness in epithelial cells (Fig. 1) (46).

3. Role of IL-36 in chronic intestinal inflammation

Inflammatory bowel disease (IBD) is a chronic non-specific inflammatory disorder of the gastrointestinal tract that is frequently divided into Crohn's disease (CD) and Ulcerative colitis (UC) based on disease features. The pathophysiology of IBD is not yet fully determined, and the available evidence attributes its etiology to a variety of factors, including genetic, environmental, intestinal flora and immune (35). Immune attributes have been thoroughly researched as therapeutic targets for IBD and have been successfully applied in clinical therapy with remarkable efficacy (36). IL-36, an emerging inflammatory factor, is significantly dysregulated in patients with IBD and mice with colitis and is engaged in intestinal homeostasis and inflammation (43).

In the inflamed colon of patients with active IBD, mRNA expression of IL-36 cytokines is elevated compared with non-inflamed and normal mucosal tissues, especially in the colonic mucosa of patients with active UC, where IL-36α and IL-36γ expression is significantly upregulated and correlated

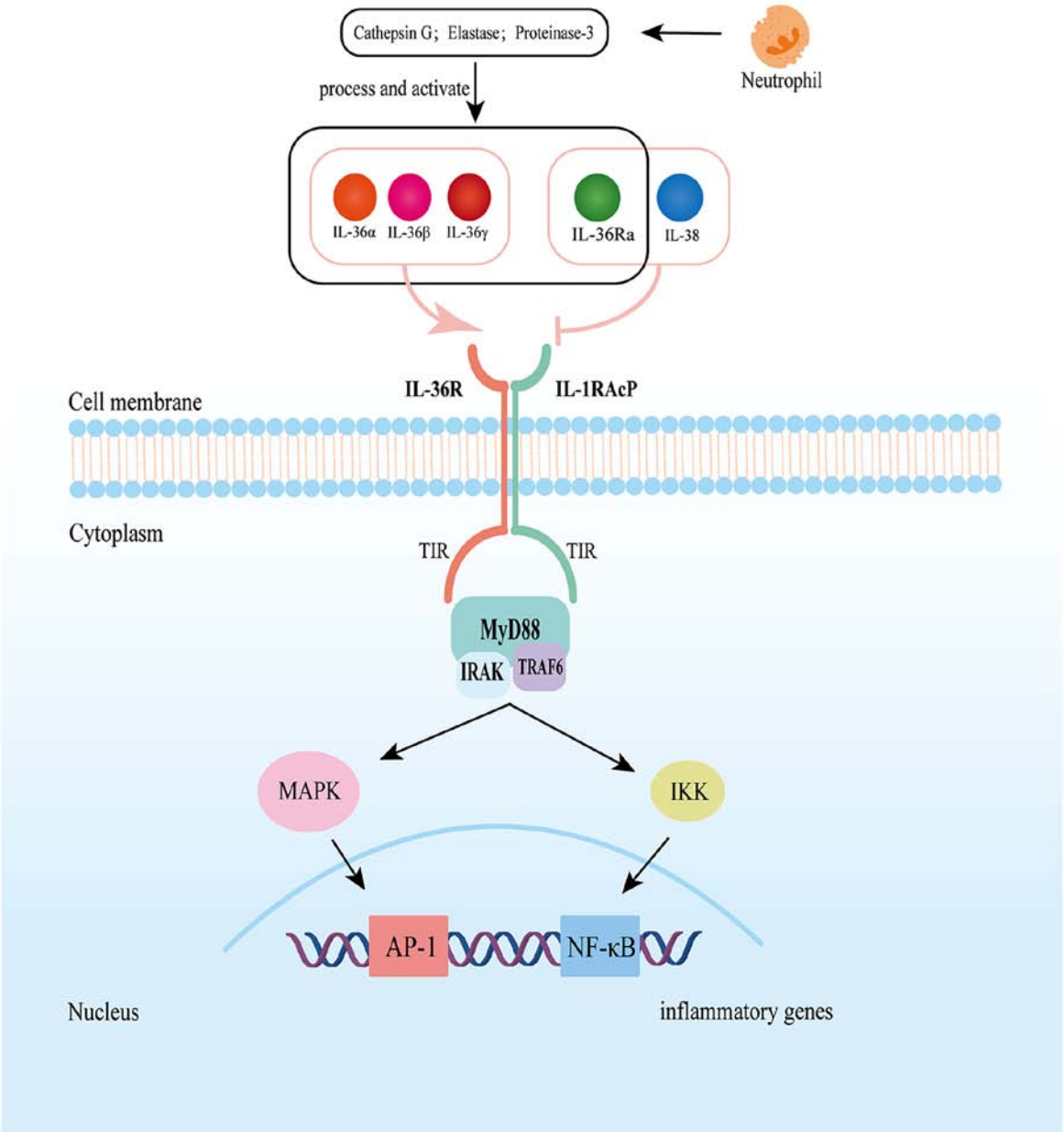

Figure 1. IL-36 signaling pathway. Neutrophil-derived proteases process and activate IL-36 cytokines. Activated IL-36 agonists bind IL-36R and recruit IL-1RAcP, which then relies on MyD88 to trigger downstream inflammatory signaling. This process is inhibited by IL-36Ra and IL-38. IL, interleukin; IL-36Ra, IL-36 receptor antagonist; MAPK, mitogen-activated protein kinase; TIR, MyD88; TRAF6, IRAK, IKK, AP-1.

with the degree of inflammation (14,47). The expression of IL-36β has been observed to be higher in the plasma membrane, muscle and submucosa of active CD (48). Since IL-36α and IL-36γ have a more comparable gene sequence compared with IL-36β, they are also more functionally similar and hence more consistent in intestinal inflammation (3,14). Mucosal tissue biopsies from patients with IBD have demonstrated that both non-immune and immune cells, such as intestinal epithelial cells, macrophages, monocytes and T cells, can produce IL-36α, IL-36β, IL-36γ and IL-36Ra (48). According to Scheibe *et al*, IL-36α is mostly expressed in CD14 inflammatory macrophages, whereas intestinal epithelial cells primarily express IL-36γ (49). Overall, IL-36 is broadly expressed in inflammatory tissues of the intestine, and its degree of expression corresponds with tissue inflammation.

The complex role of IL-36 in IBD has been further demonstrated by several studies in experimental models of colitis (15,47,50). It has been revealed that dextran sodium sulfate (DSS)-induced colitis is ameliorated in IL-36R-/- mice (IL-36R knockout), accompanied by a decreased infiltration of innate inflammatory cells in the colonic lamina propria (50). This has also been observed in the *Citrobacter rodentium*-infected IL36R-/- mouse model. Unlike the DSS model, which often induces only an innate immune response, the *Citrobacter rodentium*-infected colitis model also induces adaptive immunity, resulting in an altered T helper cell response, manifested by an enhanced Th17 response and a diminished Th1 response (50). These findings suggest that IL-36R signaling can modulate both innate and T-cell immunity in the intestinal mucosa. Thus, in an oxazolone-induced T cell-dependent colitis model, IL-36 or IL-36R

absence reduces colonic inflammation (51). This is associated with IL-36γ inhibiting regulatory T cell (Treg) differentiation through MyD88 and NFκBp50-dependent signaling and promoting IL-9-producing pathogenic CD4 helper T cell (TH9) polarization via an IL-2-signal transducer and activator of transcription 5 (STAT5) and IL-4-STAT6-dependent manner. Therefore, mice blocking IL-36γ/IL-36R signaling are protected from effector T cell-driven intestinal inflammation and colonic immune cells exhibit elevated Treg cells and decreased Th9 cells (51). Kanda *et al* have also demonstrated that IL-36α and IL-36γ promotes intestinal inflammation through the induction of pro-inflammatory mediators, such as IL-6 and chemokine C-X-C ligand (CXCL)1, CXCL2 and CXCL8, by colonic subepithelial myofibroblasts (52). This process can be synergized by the pro-inflammatory cytokines IL-17A and TNF-α (52). Another report also revealed the pathogenic role of IL-36β. The research revealed an exacerbation of colitis compared with the control group by intraperitoneal injection of IL-36β in a DSS mouse model (53). It was hypothesized that this result is associated with enhanced Th2 response and inhibition of Treg response by IL-36β (53). In conclusion, all three IL-36 agonists demonstrate pro-inflammatory effects in the experimental model. Hence, in both DSS and 2,4,6-trinitro benzene sulfonic acid-induced colitis mice models, inhibiting IL-36R activation through gene deletion or neutralizing antibodies decreases colonic mucosal inflammation and histopathology, the degree of colonic mucosal inflammation and histopathology (15). Moreover, IL-38, another natural antagonist of IL-36, is highly expressed in IBD-inflamed tissues, and blocking IL-36 signaling with IL-38 improves DSS-induced colitis and inhibits the expression of macrophage-associated pro-inflammatory molecules, such as IL-1β and TNF-α (54). All of these findings indicate that IL-36 has a pro-inflammatory pathogenic property in chronic intestinal inflammation.

However, the detrimental role of IL-36 in the gut is somewhat controversial. Numerous studies have discovered that IL-36 may act as a protective factor in intestinal mucosal repair, particularly in acute inflammation, which appears to be associated with the role of IL-22 in stimulating intestinal epithelial cell proliferation, promoting mucosal healing and maintaining intestinal epithelial barrier integrity (16,49,55). IL-22 is currently found to be mainly derived from group 3 innate lymphocytes, which are key sentinels of the intestinal innate immune response, after sensing cytokine signals such as IL-1 and IL-23 from mononuclear phagocytes activate rapidly and secrete IL-22 effector molecules to participate in mucosal immunity (56). In the acute DSS-induced colitis model, mice with IL-36R deficiency are unable to recover from acute colitis and have increased disease activity and decreased survival, accompanied by a significant decrease in IL-22 (16,49,57). Meanwhile, the impairment of intestinal mucosal repair capacity caused by IL-36R deficiency is restored by exogenous administration of IL-23 (16). In addition, IL-36γ can stimulate the increased expression of IL-22 and IL-23 in an *in vitro* experiment with colonic explants, suggesting a role for the cytokine network between IL-36 and IL-23/IL-22 in intestinal mucosal barrier repair and innate immune response (16).

In addition, IL-36 potentially affects intestinal mucosal healing by influencing the proliferation and activation of intestinal epithelial cells and fibroblasts. Analysis of intestinal epithelial cell proliferation marker Ki-67 and antimicrobial protein lipocalin-2 (LCN2) expression by immunofluorescence and quantitative PCR after injection of IL-36R ligand into the peritoneal cavity of mice has revealed that Ki-67 and LCN2 expression levels are reduced in IL-36R-/- knockout mice compared with wild-type mice (49). The IL-36R agonists IL-36α and IL-36γ also promote the expression of colonic fibroblasts and promote mucosal repair by inducing chemokines, granulocyte-macrophage colony stimulating factor and IL-6 to recruit leukocytes (49,58). Although inflammatory factors adversely affect the intestinal mucosal barrier, they also have a host protective role during the acute inflammatory phase (59). The release of large amounts of inflammatory mediators by cells into the tissue microenvironment also participates in mucosal immune defense, recruiting immune cells to remove pathogens and necrotic material and stimulating epithelial repair responses and the synthesis of mediators that contribute to the restoration of mucosal homeostasis (59). The intestinal epithelium, the interface between the luminal microenvironmental and the mucosal immune system, is exposed to additional challenges and undertakes more complex and demanding tasks, the integrity of which is fundamental to the maintenance of intestinal homeostasis (60). Therefore, the impact of IL-36, which is widely present in a variety of cells and tissues, in intestinal inflammation and mucosal repair is not destined to be singular, and its main role as pathogenic or protective needs to be seen in context (Fig. 2) (17,43).

4. Function of IL-36 in intestinal fibrosis

The essence of fibrosis is the healing and repair response to tissue injury. The recruitment of immune cells and the activation of the inflammatory response are the first steps in this process. Following the activation of immune cells such as macrophages, eosinophils, basophils, innate lymphocytes and T cells, a series of soluble pro-fibrotic cytokines are produced to stimulate myofibroblast proliferation and activation and to generate extracellular matrix (ECM) (61). Transforming growth factor-β is considered to be the most effective fiber-activating cytokine, which is not only derived from fibroblasts, but also expressed by macrophages and eosinophils (62). Additionally, peripheral non-immune cells such epithelial, endothelial and smooth muscle cells can also transform to myofibroblast-like phenotypes during fibrosis, generating soluble cytokines that hasten the progression of fibrosis. Therefore, the most notable feature of fibrosis is the excessive synthesis and aberrant deposition of collagen-rich ECM, which is mainly derived from mesenchymal cells including fibroblasts and myofibroblasts (61,63). Owing to its ability to stimulate fibroblasts and promote the secretion of pro-fibrotic soluble mediators, IL-36 is implied to play a role in fibrotic diseases (64). Several studies have revealed the function of IL-36 in fibrotic diseases of the kidney, lung, intestine and pancreas, and it has been hypothesized that IL-36 may mediate the fibrotic process by regulating immune cells, such as macrophages, fibrotic cytokines such as IL-17 and collagen remodeling (65-67). Intestinal fibrosis is a serious complication of IBD that is frequently encountered in patients with CD of the small intestine (68). Under the long-term stimulation of chronic inflammatory agents, intestinal fibrosis remodeling in patients with IBD proceeds

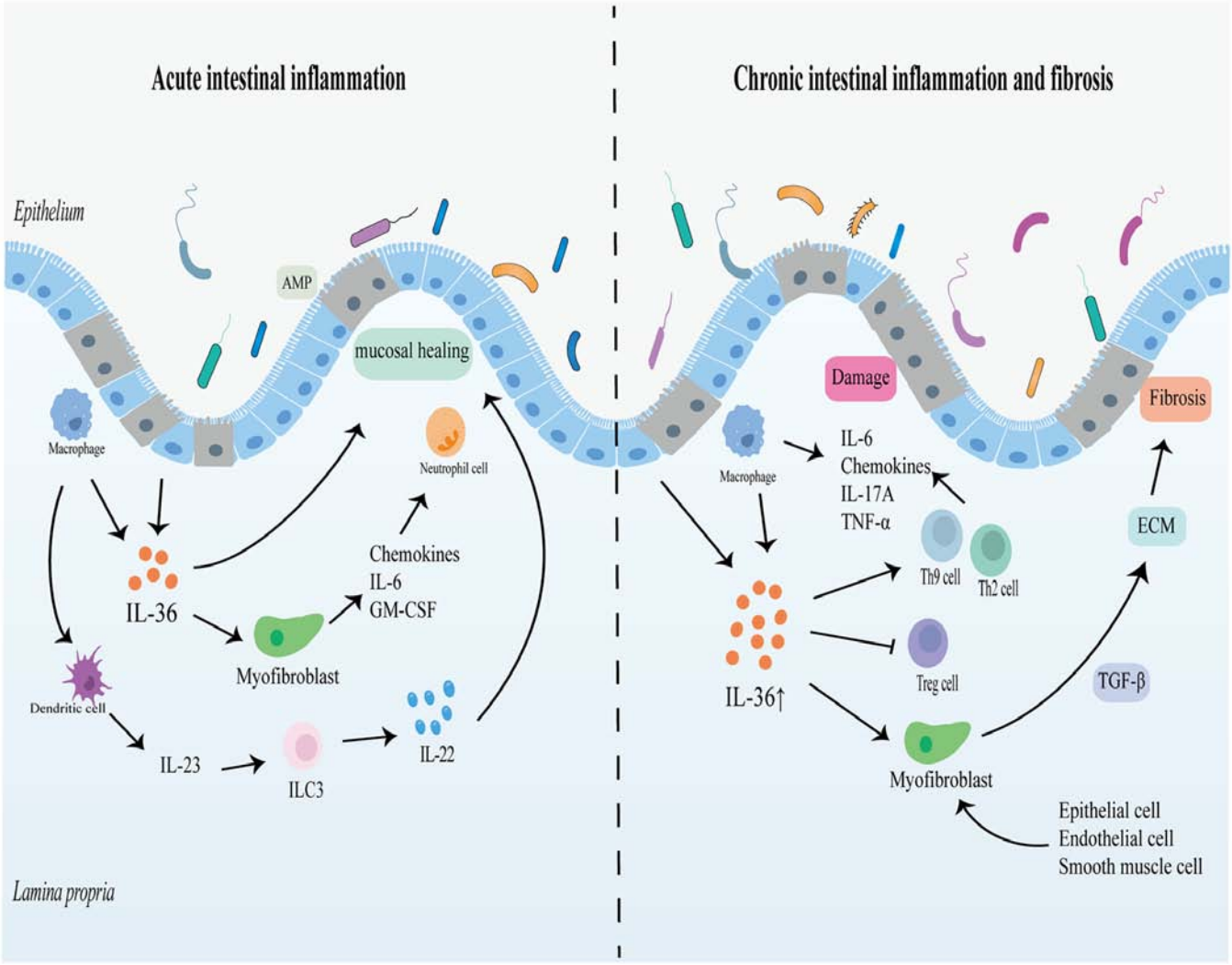

Figure 2. Role of IL-36 in intestinal inflammation. In acute intestinal inflammation, IL-36 drives healing of epithelial injury by promoting the proliferation of intestinal epithelial cells and fibroblasts. While in chronic inflammatory conditions, IL-36 regulates the activation of T cells and fibroblasts exacerbating the progression of intestinal inflammation and fibrosis. IL-, interleukin; TH, T helper cell; Treg, regulatory T cell; ECM, extracellular matrix.

constantly, resulting in a thickening of the intestinal wall and structural alterations of the intestinal canal, eventually leading to intestinal stenosis and even serious consequences such as intestinal obstruction requiring surgical treatment (69).

Scheibe *et al* revealed elevated IL-36α expression in the tissues of patients with fibrous stenosis CD, and macrophages were its main source (15). Subsequently, it was revealed in a mouse model that activation of IL-36R consistently induces fibroblast activation and type VI collagen production, while the intestinal fibrosis process is significantly reduced in IL-36R-/- mice or in mice with antibodies that inhibit IL-36R activity (15). These results supported the hypothesis that the IL-36R signaling pathway is associated with intestinal fibrosis. Previous studies have reported that IL-36 can be derived from a variety of cells, such as fibroblasts, epithelial cells and macrophages, and that IL-36R-expressing epithelial cells and fibroblasts are also regulated by IL-36 cytokines (49,52,66). Furthermore, human colonic myofibroblasts can produce large amounts of IL-36γ when stimulated with IL-1β or IL-1β plus TNF-α *in vitro* (70). RNA-sequencing and gene enrichment analyses of human and mice colonic fibroblasts have indicated that IL-36R signaling upregulates the inflammatory factor IL-6 as well as fibrosis-related genes, such as CXCL1, CXCL5, matrix metalloproteinase (MMP)1, MMP3, MMP10 and MMP13 (15). Therefore, it is postulated that the IL-36 in intestinal fibrosis may predominantly be IL-36α, which boosts fibroblast activation and collagen generation by modulating cytokines involved in fibrosis and tissue remodeling (15). This procedure is dependent on MyD88 signaling, which is consistent with earlier research (15,46). In addition, the IL-36R antibody has also showed positive effects on reversing chronic intestinal fibrosis in established mouse models, as evidenced by a decrease in tissue fibrosis scores, α-smooth muscle fibroblasts and type VI collagen (15). Notably, this research demonstrates the potential value of IL-36R inhibition in halting and reversing the fibrosis process, as well as the potential of IL-36 for the treatment of intestinal fibrosis and stricture in IBD.

In summary, IL-36 cytokine regulates the intestinal immune response and promotes intestinal inflammation and fibrosis, therefore targeting IL-36 may be one of the strategies to treat IBD and inhibit the progression of IBD fibrosis.

Table I. Role of IL-36 in colorectal cancer.

Cytokine	Function	Mechanism	(Refs.)
IL-36α	Predict prognosis	-	(19,74)
	Suppress tumor	Enhance the function of CD8 T lymphocytes	(78)
IL-36β	Promote tumor	Regulate p42/44 MAPK and PI3K/AKT pathway	(88)
	Suppress tumor	Enhance CD8 T cells proliferation and activation	(75)
IL-36γ	Predict prognosis	-	(19)
	Promote tumor	Regulate p42/44 MAPK and PI3K/AKT pathway	(88)
	Promote tumor	Induce cell-matrix adhesion molecules expression and Wnt signaling	(87)
	Promote tumor	Synergize with IL-17/IL-23 axis	(32)
	Suppress tumor	Recruit immune cell infiltration and promote the formation and maintenance of TLS	(81,84)
	Suppress tumor	Combination with OX40L and IL-23 increases tumor sensitivity to immune checkpoint blockade therapy	(92)
IL-36Ra	Suppress tumor	-	(87,88)

IL-36, interleukin 36; IL-36Ra, IL-36 receptor antagonist; MAPK, mitogen-activated protein kinase; PI3K/Akt, phosphatidylinositol 3 kinase/protein kinase B; TLS, tertiary lymphoid structures.

However, its practical application to human intestinal fibrosis requires further investigation in more animal models and clinical trials.

5. Role of IL-36 in colorectal cancer

To the best of our knowledge, although evidence on IL-36 in cancer are few, it is reasonable to infer that IL-36 has a role in cancer response since it is an important modulator of intestinal inflammation, and inflammation has been linked to cancer. Several experimental investigations have demonstrated that IL-36 has an anticancer impact in a variety of cancers, including ovarian cancer, breast cancer, hepatocellular carcinoma and melanoma (17,21). CRC, as the most dominant malignant tumor of the intestine, has become the third most prevalent cancer in humans and the second leading cause of cancer-related deaths worldwide based on cancer statistics analysis in 2020 (71). Evidence has indicated that it can develop from IBD through the inflammatory pathway such as NF-κB, IL-6/STAT3 and IL-23/IL-17 pathways (72,73). Therefore, the search for sensitive early screening and diagnostic markers remains one of the aims of CRC research. Current research evidence of IL-36 in CRC seems to indicate its diagnostic and therapeutic value (Table I). Wang *et al* in 2014 detected a negative correlation between high IL-36α expression in tumor tissues and corresponding clinicopathological parameters such as tumor size and TNM stage (74). Kaplan-Meier analysis and Cox regression analysis have demonstrated that low IL-36α expression levels predict poorer prognosis and decreased survival in CRC. However, this finding needs to be further validated because it was not compared with non-cancerous tissues (74). Following that, Chen *et al* revealed that colonic IL-36α, IL-36β and IL-36γ are significantly lower in CRC (19). Moreover, high IL-36α expression and low IL-36γ expression are associated with higher survival in patients with CRC through immunohistochemical and histopathological analysis of 185 tissue arrays from patients undergoing colorectal cancer surgery and non-cancerous tissues. This indicates that IL-36α and IL-36γ levels in CRC tissues might be used as biomarkers to predict CRC prognosis. This also implies that IL-36α and IL-36γ play distinct roles in tumor growth, despite both having pro-inflammatory effects in reports of chronic intestinal inflammation (19). Although there was no statistically significant difference in survival, IL-36β has been demonstrated to be reduced by 80% in CRC colon tissues, suggesting that IL-36β may contribute to colorectal cancer development (19). An experiment has shown that IL-36β can boost $CD8^+$ T cell activation and then enhance anti-tumor immune responses by activating phosphatidylinositol 3 kinase (PI3K)/protein kinase B (Akt), IκB kinase and MyD88 dependent mammalian target of rapamycin complex1 pathways (75).

Considering IL-36α drives antitumor immune responses in hepatocellular carcinoma cells by recruiting CD3 and CD8 T lymphocytes to activate adaptive immune cells, it hypothesized that the anticancer impact of IL-36α in CRC may potentially be connected with T lymphocyte activation (76). This speculation was confirmed in a study by Wei *et al* (77). This revealed that IL-36α inhibits the growth and metastasis of colorectal cancer by constructing CT26-IL-36α and HT29-IL-36α cell lines overexpressing IL-36α, which enhances the infiltration and activity of CD8 T lymphocytes by upregulating the expression of the chemokines CXCL10 and CXCL11 (77). By contrast, the role of IL-36γ in CRC is more complicated. *In vitro* experiments, IL-36γ stimulates the proliferation and secretion of IFN-γ by type 1 lymphocytes, including $CD8^+$ T cells, natural killer cells and γδ T cells, which are significant antitumor immune effector cells (78-80). Wang *et al* then demonstrated in melanoma and breast cancer models that IL-36γ/IL-36R signaling promotes the differentiation of type 1 lymphocytes in the tumor microenvironment and inhibits the tumor growth, implying that IL-36 has an important tumor immunomodulatory role

by regulating the tumor microenvironment and tumor infiltrating cells (78). Furthermore, IL-36γ expression has been indicated to be adversely linked with the advancement of human melanoma and lung cancer, supporting the anticancer potential of IL-36γ (78). In the MC38 mice model of CRC, IL-36γ delivered by dendritic cells (DC) has previously been shown to slow tumor development with recruitment of T cells into the tumor microenvironment and the formation of tumor-associated tertiary lymphoid structures (TLS) (81). T lymphocytes are the most common lymphocytes seen in tumors and are linked to tumor immune response and prognosis (82). The presence of TLS has also been linked to an improved prognosis in a variety of malignancies including CRC, breast cancer, lung cancer and head neck squamous cell carcinoma (83).Some studies reported that IL-36γ can be generated by M1 macrophages, vascular endothelial cells and smooth muscle cells in the CRC tumor microenvironment and contributes to the activation of anti-tumor response by upregulating vascular cell adhesion molecule, intercellular adhesion molecule-1 and the chemokines CCL2 and CCL20 to recruit immune cell populations and promote CD4 central memory T cell infiltration, as well as the maintenance and formation of TLS (78,84-86).

However, several reports have proposed the opposite view that IL-36γ promotes the development of CRC (32,87,88). In the intestinal cancer model, injection of anti-IL-36γ significantly inhibits the tumorigenesis and tumor development in the colon and the small intestine along with the expression of cell-matrix adhesion molecules and Wnt downstream genes in the colon tumors (87). Therefore, IL-36γ may contribute to tumorigenesis by indirectly regulating Wnt signaling through inducing the expression of cell-matrix adhesion molecules (87). Another study has reported that IL-36R agonists induce pro-tumor gene expression and promote tumor cell proliferation, migration and invasion *in vitro* colon cancer cell line. Among them, IL-36β and IL-36γ were more prominent in tumor invasion compared with IL-36α, which were possibly mediated through the p42/44 MAPK and PI3K/AKT pathways (75). Subsequently, inhibition of the IL-36R signaling pathway has been shown to reduce tumor cell proliferation and tumor burden in a CT26 mouse colon cancer model by administration of IL-36Ra or knockdown of IL-36R gene (88). The reduced expression of IL-36R in tumors has also been shown to be associated with an improved prognosis for patients (32). IL-36Ra administration more strongly inhibits tumor growth in mice, which is consistent with previous findings of increased incidence of colon tumorigenesis in the absence of IL-36Ra, both supporting IL-36Ra as a potentially potent target for the treatment of CRC and the anticancer potential of inhibiting the IL-36R (87,88). IL-36 also synergizes with inflammatory factors to promote tumors. Differential gene expression analysis of patient samples and cell lines has revealed the role of the IL-36/IL-17/IL-23 axis in colon tumorigenesis, showing that IL-36γ synergistically induces various genes involved in the IL-17/IL-23 axis in CRC cells, thereby inducing cancer cell proliferation (32).

The discovery of immune checkpoints such as programmed cell death (PD)-1/PD ligand 1 and cytotoxic T-lymphocyte antigen-4 has revolutionized the course of tumor immunotherapy (89,90). However, the responsiveness to immune checkpoint inhibitors varies by cancer type and by mutation type in the same cancer. Therefore, new research approaches are exploring how to improve the therapeutic sensitivity of immune checkpoint inhibitors, such as combination with co-stimulatory signals and pro-inflammatory factors to modulate the recruitment and activation of tumor-infiltrating lymphocytes, thus enhancing the anti-tumor immune response (91). As IL-36γ is involved in reducing the expression of immune checkpoint molecules and coordinately activating DCs and T cells in the TME in support of strong anti-tumor $CD8^+$ T cell responses, it has been explored as a direction to modulate antitumor immunity and immune checkpoint inhibitor therapy (78). Treatments with IL-23/IL-36γ/OX40L triplet mRNA mixture facilitates infiltration of immune cells, including cross-presenting DCs and cytotoxic $CD8^+$ T cells, into tumor tissue and effectively destroys tumor cells in HT22 hepatoma, MC38 colon carcinoma and B16 melanoma (92). Notably, the combination therapy of IL-36γ with OX40L and IL-23 significantly increases tumor susceptibility to immune checkpoint blockade therapy in a murine colon cancer model (92). Collectively, these findings reveal the complexity of the IL-36 family function in colorectal cancer, but also provide lateral evidence of the potential value of the IL-36 family in the treatment of CRC.

6. Clinical trials of IL-36 antibody in IBD

Targeting the IL-36 signaling pathway in inflammatory diseases is currently dominated by targeting IL-36R. Several clinical trials are currently investigating the neutralization of antibodies targeting IL-36R in GPP, palmoplantar pustulosis (PPP), atopic dermatitis (AD) and IBD to evaluate the safety and efficacy of clinical treatment. Spesolimab (BI 65513), a novel humanized IL-36R monoclonal immunoglobulin G1 antibody developed by Boehringer Ingelheim, has demonstrated a good safety profile and significant efficacy in phase I and II clinical trials for the treatment of GPP and was approved by the FDA as a treatment option for GPP flares in adults on September of this year (12,93). In the three clinical trials of patients with moderate to severe active UC, although the efficacy endpoints were not reached, the spesolimab shown to be generally well tolerated in patients with UC with no unexpected safety concerns or clinically relevant hypersensitivity or opportunistic infections (NCT03482635; NCT03123120; NCT03100864) (94). Another trial on patients who have moderate to severely active UC and have completed a previous treatment trial is evaluating the long-term safety of spesolimab (NCT03648541). Moreover, IL-36R targeted antibody are also undergoing phase II clinical trial in patients with CD specifically targeting structuring and fistulizing CD. The causes of fistula development in CD and the effect of spesolimab on the treatment of patients with fistulizing CD have been studied in a phase II trial (NCT03752970), while another study on whether spesolimab administration improves intestinal stricture caused by CD was terminated in 2022 (NCT05013385). In addition, the long-term safety and efficacy of spesolimab is currently under evaluation in patients with perianal fistulizing CD who had completed

previous treatments (NCT04362254). In conclusion, the safety of IL-36 in patients with IBD has been demonstrated, but clinical efficacy remains to be determined by additional clinical trials and data.

7. Conclusion

Inflammatory bowel disease and colorectal cancer, two of the most predominant heterogeneous diseases of the intestine, are now becoming increasingly common worldwide. The search for markers and targets for the diagnosis and treatment of both diseases still remains an important research direction. The IL-36 cytokine, an important member of the IL-1 superfamily, has been identified to have role in regulating innate and adaptive immunity in a variety of tissues, including the intestine, lung, joints and kidney, and from experimental models and a growing body of evidence from clinical samples supports the function of the IL-36 signaling pathway in promoting inflammation and fibrosis in the context of chronic intestinal inflammation. Some of the agents that target IL-36 signaling have been or are being evaluated in phase II clinical trials in patients with CD and UC and have shown a favorable safety profile. Although investigations of IL-36 in cancer are still scarce, the existing data suggests that IL-36 has an anticancer effect in the tumor microenvironment by regulating tumor-infiltrating cells and tertiary lymphoid bodies, although this conclusion is being challenged by numerous studies. In conclusion, all of these findings revealed that the IL-36 signaling pathway has therapeutic potential in IBD and CRC and more exploration is required in the future to investigate more precise mechanisms of IL-36 function.

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Authors' contributions

JZ and ML conceived and designed the article. ML, WJ, ZW, YL and JZ were involved in drafting of the manuscript and in revising it critically for important intellectual content. All authors have read and approved the final manuscript. Data authentication is not applicable.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

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Competing interests

The authors declare that they have no competing interests.

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