The Role of Cyclophilins in Inflammatory Bowel Disease and Colorectal Cancer

Lifang Liang¹, Rongxiao Lin², Ying Xie², Fangyuan Shao³, Wen Rui²,4,5,6, Hongyuan Chen¹,4,5,6

¹Department of Pathogenic Biology and Immunology, School of Life Sciences and Biopharmaceuticals, Guangdong Pharmaceutical University, Guangzhou 510006, Guangdong Province, PR China.

²Centre for Novel Drug Research and Development, Guangdong Pharmaceutical University, Guangzhou 510006, Guangdong Province, PR China.

³Cancer Center, Faculty of Health Sciences, University of Macau, Macau SAR, China.

⁴Guangdong Engineering & Technology Research Center of Topical Precise Drug Delivery System, Guangdong Pharmaceutical University, Guangzhou 510006, Guangdong Province, PR China.

⁵Key Laboratory of Digital Quality Evaluation of Chinese Materia Medica of State Administration of TCM, Guangzhou 510006, Guangdong Province, PR China.

⁶Guangdong Cosmetics Engineering & Technology Research Center, Guangzhou 510006, Guangdong Province, PR China.

Corresponding authors: Hong-Yuan Chen, E-mail: hychen@gdpu.edu.cn, Tel & Fax: +86-20-3935-2186. Wen Rui, E-mail: gyrw@gdpu.edu.cn, Tel & Fax: +86-20-3935-2527.
Abstract

Cyclphilins (Cyps) are a kind of ubiquitous protein family in organisms. They have biological functions such as promoting intracellular protein folding and participating in the pathological processes of inflammation and tumor. Inflammatory bowel disease (IBD) and colorectal cancer (CRC) are two common intestinal diseases, but the etiology and pathogenesis of these two diseases are still unclear. IBD and CRC are closely associated. IBD has always been considered as one of the main risks of CRC. However, the role of Cyps in these two related intestinal diseases is rarely studied and reported. In this review, the expression of several Cyps family members in IBD, especially ulcerative colitis (UC), and CRC, the relationship between those members and the development of the two intestinal diseases, as well as the possible pathogenesis, were summarized in order to provide an modest reference for clinical researches and treatments.

Keywords: inflammatory bowel disease; colorectal cancer; cyclophilins; CypA; CypB; CypD

Introduction

Cyps are a family of highly conserved proteins, which are found in all cells of all organisms studied, in both prokaryotes and eukaryotes [1]. Member of this family posses peptidyl prolyl isomerase (PPIase) activity, which catalyzes the cis–trans isomerization of peptide bonds at proline residues. Indeed, due to the PPIase activity, Cyps have been demonstrated to play a role in protein folding [2, 3], as well as protein trafficking and chaperone activity [3, 4]. Through this characteristic, on the one hand, Cyps exert immunosuppressive effects. Cyps inhibit the activity of calcineurin through the interaction with cyclosporin A (CsA), an immunosuppressive drugs, via theirs PPIase active site, blocking the translocation of the nuclear factor of activated T cells (NF-AT) from the cytosol to the nucleus, and thus preventing the activation of T cells [5]. On the other hand, Cyps inhibit the proliferation and differentiation of cells, promote apoptosis etc. [6, 7]. In addition, Cyps are known to have relationship with the pathological processes of many diseases, such as viral infection [8, 9],
cardiovascular diseases [10], inflammatory responses [11, 12] and cancers [13].

Up till now, there are at least 16 known human Cyps, which are structurally distinct [14], including Cyclophilin A (CypA), Cyclophilin B (CypB), Cyclophilin C (CypC), Cyclophilin D (CypD), Cyclophilin 40 (Cyp40), Cyclophilin NK (CypNK) etc. [15]. Among them, most of the studies mainly focused on CypA, CypB and CypD. These members are found ubiquitously in different subcellular compartments. They have their own unique biological functions in cells consequently.

CypA, expressed in the cytosol, is the most abundantly expressed and first identified cyclophilin [16]. Substantial evidence showed that intracellular CypA (iCypA) is secreted by several cell types, including vascular smooth muscle cells (VSMC), macrophages, and endothelial cells (EC), in response to inflammatory stimuli [17-24]. Apart from the ordinary functions of Cyps, secreted CypA or named extracellular CypA (eCypA) participates in both inflammatory response and signal transduction [12, 23, 25]. Additionally, the eCypA, through autocrine and paracrine, could mediate intercellular communications, serving as a chemokine that recruits inflammatory cells, as well as aggravate oxidative stress and inflammation [26, 27]. Furthermore, studies suggested that high CypA expression correlates with poor outcome in patients with inflammatory diseases [20]. Meanwhile, various reports have shown that CypA is upregulated in cancer [28-33] and is involved in diverse pathological processes of cancer development, such as synthesis of tumor-associated proteins, signal transmission of tumor cell growth, regulation of transcription factors, apoptosis, metastasis, and drug resistance [13, 16, 34-36]. However, it is worth mentioning that a number of mechanistic details about CypA in IBD and CRC are still unknown and await further studies.

CypB is mainly located in the endoplasmic reticulum (ER), where it attenuates ER stress-induced cell injury by interacting with the ER-related chaperones [37]. Cystolic CypB can also be stimulated by inflammation to form extracellular CypB (eCypB) [38-40]. eCypB has multiple functions, including chemotaxis and signaling transduction [41-47]. In addition, CypB is closely associated with the replication of hepatitis virus [48-52] and human immunodeficiency virus (HIV) [53], and found
high expression in breast cancer, pancreatic cancer, glioblastoma, liver cancer and gastric cancer [54-59].

CypD, a component of the mitochondrial permeability transition pore (mPTP), is uniquely located in the mitochondrial matrix. It is responsible for regulating the opening of the mPTP [60]. mPTP is a mitochondrial channel complex, primarily composed by several proteins, including voltage-dependent anion channel (VDAC), adenine nucleotide translocator-1 (ANT-1), and CypD [61], whose main function is to maintain the balance of mitochondrial respiratory chain [62]. Under resting conditions, CypD shuts down the channel complex [62-64]. When facing stimuli of hypoxia, calcium overload, and oxidative stress, CypD travels to the inner membrane and binds to ANT-1, which leads to mPTP sustained opening [63, 65-68], followed by mitochondrial membrane depolarization, mitochondria swelling, Ca^{2+} release, and eventually, cell death [62, 69-71]. CypD is the basic component of mitochondrial function, and may contribute to regulating the opening state of mPTP to regulate inflammation [72] and cancer [73].

IBD has emerged as global diseases [74-80]. New data suggest that the incidence and prevalence of IBD affecting five million patients worldwide, and approximately 0.3% of the European and North American population suffers from IBD at the present time [79, 81, 82]. IBD is a group of chronic. IBD characterized by macrophages and neutrophils infiltration. Primarily, there are two clinical types of IBD: ulcerative colitis (UC) and Crohn's disease (CD) [83-88].

UC, the most common type of IBD, occurs mostly in the colon, affecting the entire intestinal tract in a discontinuous manner [89, 90]. CD, on the other hand, mainly occurs in the rectum and can affect part or all of the colon in a continuous manner. [91, 92]. According to statistics, in the countries with the highest incidence of IBD, the annual incidence of UC and CD was 24.3 and 12.7 per 100,000 person-years in Europe, 6.3 and 5 per 100,000 person-years in Asia and the Middle East, and 19.2 and 20.2 per 100,000 person-years in North America [81, 93, 94]. The overall incidence is coalescing around a range between 15 and 5 per 100,000 person years for both UC and CD [94]. It can be seen that as two of most common types of IBD
diseases, the prevalence and incidence of UC and CD are rapidly increasing in the world. Although the research on IBD has been growing and deepening in recent years, the exact etiology and pathogenesis remain unclear, which brings certain difficulties for clinical researches and disease treatments.

Clinical studies have shown that both UC and CD patients are at an increased risk for developing CRC compared with the general population [79, 95, 96]. Furthermore IBD can induce oncogene instability, tumor suppressor gene activation and regulation of cell proliferation through intestinal adenomas, eventually leading to intestinal malignancy [97-100]. CRC is a common malignant tumor of the digestive tract. Its incidence is increasing every year, with affecting approximately 1.23 million patients worldwide each year and accounting for almost 10% of all cancers [101-103]. According to statistics, from 2015 to 2020, CRC has become one of the leading causes of cancer deaths in China, ranking firmly in the top five cancer-related deaths [104]. Its occurrence and development are affected by many factors, among which is closely related to inflammation and damage. Surgery is still the most effective treatment of CRC. Although great progress has been made in the prevention and treatment of tumors, its morbidity and mortality are still high [105]. The main reason is that the disease has tumor features, such as invasion, metastasis, resistance and recurrence and other characteristics[106].

IBD and CRC are currently two of the common diseases in the intestinal tract, they are related but different. In the pathological development of two diseases, IBD can be regarded as one of the main causes of CRC, but they are different in disease characteristics, so this article will discuss the two diseases separately. In a number of studies, it has been found that CRC [107-109] and IBD [110-112] patients generally have high expression of Cyps. The previous research of our group has shown that the lack of Cyclophilin J (CypJ) caused the loss of its protective effect in mouse colitis induced by dextran-sulfate-sodium (DSS), and this is related to the ability of CypJ blocking the binding of ubiquitin chains, thereby negatively regulating nuclear factor kappa B (NF-κB) signaling [113]. More relevant experiments are still needed to confirm the role of Cyps in enteritis and bowel cancer.
This article mainly reviews the expression of Cyps in IBD and CRC as well as the possible mechanisms related to the occurrence and development of these two diseases, aiming to provide clues for finding an accurate and detectable biomarker for the diagnosis of the diseases.

The relationship between Cyps and inflammatory bowel disease

CypA

It was found that the expression of CypA was significantly increased in the crypt tissue [114], serum [110] and lymphocytes [111] of UC patients. Compared with the concentration of 2 ng/ml in the serum of health subjects, the CypA level in the serum of UC patients can reach 6 ng/ml [110]. Furthermore, CypA also showed characteristics related to UC disease progression. Expression of CypA in active UC patients was higher than that in remission UC patients [111]. However, studies have further shown that CypA is not significantly elevated in colon tissue of UC patients, nor in serum of CD patients [110]. This indicates that CypA plays an important role in IBD, especially in UC, but it is worth mentioning that the expression level of CypA may be different at different detection levels in UC patients. Simultaneously, in addition to the increase of CypA, the serum anti-CypA antibody in UC patients also increased, and the expression level increased with the course of disease [111, 112], illustrating that the expression level of anti-CypA antibody may be positively correlated with the increase of CypA level, which suggests that anti-CypA also has a certain preoperative diagnostic value in inflammatory enteritis.

Early studies have found that eCypA can be produced after lipopolysaccharide (LPS) stimulation of macrophages [18] (Figure 1), and it is found to be one of the stable reference genes for evaluating LPS-stimulated macrophages [14]. Additionally, eCypA can also upregulate and bind to macrophage surface differentiation cluster 147 (CD147) [115]. In addition, eCypA induces the expression of inflammatory factors such as MMP-9, MMP2, TIMP1 [115, 116] or IL-1β, IL-6, IL-17 [117, 118] through phosphorylation of ERK1/2/JNK/P38MAPK and NF-κB [117, 118], or induces autophagy [119, 120], apoptosis [120], M1 polarization [118], infiltration [12],
chemotaxis and adhesion [121, 122] of monocytes/macrophages through these signals, which play a role in various inflammatory diseases (Figure 1). Others speculated that eCypA-induced autophagy in macrophages may be related to PI3K/Akt/mTOR signaling pathway, but no experimental study has been confirmed [95] (Figure 1). However, it is noteworthy that some research results have proved that iCypA can promote the migration of dendritic cells [123] and the proliferation of macrophages [124] by inducing ERK1/2/MAPK and NF-κB phosphorylation. This opposite effect of eCypA and iCypA on macrophages indicates that different forms of CypA may have opposite biological significance to the same cell by activating the same signal.

Not only macrophages, some researchers speculated that CypA is related to the obvious activation of lymphocytes in patients with UC, and the increase of CypA after lymphocytes activation may participate in the apoptosis of UC [111]. Clinical studies showed that the levels of MMP-9 and TNF-α in UC patients can be significantly increased with the increase of serum CypA, and the levels of TIMP-1/MMP-9 complex in UC and CD patients are also significantly increased [110], suggesting serum CypA may influence MMPs and TIMPs in IBD patients. This result is consistent with the previous discoveries [115, 121, 122], speculating that the high expression of serum CypA in IBD may regulate the expression of TIMP-1/MMP-9 by activating ERK1/2, which promotes the pathogenesis and development of IBD, especially UC (Figure 1). Further research is needed to confirm this hypothesis. In short, the difference between eCypA and iCypA lies in that the former may need to combine with the receptor CD147 and act on it first. Both receptor-mediated eCypA and iCypA, seems to activate MAPK, NF-κB and other signals to promote the proliferation or apoptosis, migration of a variety of immune cells and the expression of TIMP1, MMP9, MMP2, which may regulate IBD and other inflammatory diseases, but the specific mechanism is still unclear.

CD147, also known as extracellular matrix metalloproteinase inducer (EMMPRIN) or Basigin, is a transmembrane glycoprotein that can induce extracellular matrix metalloproteinases (MMPs) [125, 126]. As matrix metalloproteinases, MMPs have been widely studied in the migration of inflammatory
cells, cancer invasion and metastasis due to their universal function of degrading extracellular matrix components [121, 127, 128]. In addition, CD147 is the cell surface receptor of eCypA and eCypB [121, 125, 129, 130]. Heparin may be involved in the signal transduction induced by the binding of these two types of cyclophilins with CD147. Heparin firstly binds to eCypB and eCypA through sulfated glycosaminoglycans (GAG) and heparan sulphates (a subtype of GAG) molecular binding sites, and then presents them to CD147. The interaction of eCypA-CD147/eCypB-CD147 and the transfer of eCypA/eCypB into the cells were promoted by the transduction activity of proline 180 (P180) and glycine 181 (G181) in the extracellular region of CD147, thereby activating the ERK signaling cascade [121, 125, 129, 130] (Figure 1).

Up till now, the importance of CD147 has been generally recognized by researchers [121, 125, 129, 130]. In inflammatory, CD147 can mediate the migration of monocytes/macrophages after binding to eCypA [121, 122] (Figure 1). In cancer, CD147 interacts with a variety of proteins, induces the secretion of MMPs, and promotes tumor invasion and metastasis [129, 131-134]. Recent studies have shown that CD147 significantly increases in intestinal mucosa of IBD patients and aggravates IBD inflammatory response by activating NF-κB [135]. This indicates the important significance of CD147 in inflammatory diseases, and further confirms the results of previous studies [116-124] that eCypA firstly binds to CD147 on cell surface, activates multiple signal pathways to regulate inflammatory cells and promotes the expression of MMPs and other factors that can promote the occurrence and development of inflammation such as IBD, especially UC.

Since the binding of CsA with CypA can inhibit its PPIase activity and exert immunosuppressive effect, it may have adverse effects on the normal immune function or disease treatment of the body [136-139]. Therefore, a variety of CsA analogues binding to Cyps without causing immunosuppression have been developed clinically [140-144]. In recent years, the researches on CypA have focused on the application of antibodies to diseases. Recombinant purified CypA proteins from different sources [117, 145] were used as immunogens to prepare polyclonal
antibodies for the treatment of inflammatory diseases such as acute pneumonia [117] and sepsis [145]. However, the application of anti-CypA antibodies in the treatment of IBD has not been found so far, which may indicate a new direction for the treatment of IBD and other inflammations in the future.

**CypD**

The regulation of CypD on IBD is major related to mitochondrial permeability transition (mPT). In vivo and in vitro studies have shown that inhibition or knockout of CypD can attenuate mitochondrial necrosis induced by inflammatory stimuli such as non-steroidal anti-inflammatory drugs (NSAID), LPS and Ca^{2+} or oxidative stress in intestinal epithelial cells [146], macrophages [147] and eosinophils [148], respectively, and further regulate enteritis, which is related to the continuous opening of mPTP after CypD deletion (Figure 2). Interestingly, contrary to the results that CypD knockout or inhibition in macrophages and intestinal epithelial cells can reduce inflammation, CypD knockout in eosinophils aggravates colon inflammation in mice. However, this may be related to the different regulatory roles of different target cells in IBD (Figure 2). In summary, the absence of CypD in intestinal epithelial cells, macrophages and eosinophils can protect cells from a series of mitochondrial reactions caused by the continuous opening of mPTP, such as mitochondrial membrane depolarization, increased reactive oxygen species and oxidative stress [66-68], thereby reducing cell death caused by mitochondrial necrosis (Figure 2). However, the decrease in the death of intestinal macrophages and endothelial cells plays a positive role in inflammation, while the decrease in the necrosis of eosinophils aggravates intestinal inflammation. Therefore, the difference in immune cells makes the lack of CypD also two sides for IBD.

**Other Cyps**

In addition to CypA and CypD, other Cyps have also been found to play an important role in the development of IBD. CypJ, also known as PPIL3, is a newly discovered member of the cyclophilin family in recent years. It mainly exists in the cytoplasm and nucleus, and it also has PPIase activity [113]. Previous studies have found that CypJ can interact with the NZF domain of TGF-β-activated kinase 1.
binding protein 2/3 and the components of linear ubiquitin chain assembly complexes, blocking the ubiquitin chain binding, negatively regulating NF-κB signaling, thereby inhibiting DSS-induced colitis[113].

The relationship between Cyps and colorectal cancer

Cyps are high expression in colorectal cancer

In many studies, the expression of Cyps was confirmed to be significantly different between CRC and normal tissues. Over the years, many researchers detected the tissue samples of patients with CRC, and found that CypA [21, 32, 107, 149], CypB [108, 150, 151], CypE [152] and CypJ [153] in CRC tissue samples were highly expressed compared with those in normal tissues or adjacent tissues. Even CypB in the serum of patients [107, 151] and CypA in different colorectal cancer cell lines in vitro [107, 151], were found to be overexpressed. Moreover, the high expression of CypB has been confirmed to be related to the poor prognosis and low survival rate of patients [108, 150, 151].. Some studies also found that the high expression of CypA in colon cancer was accompanied by the up-regulation of CD147 expression, and the expression changes of the two were consistent. It is suggested that CD147 may be positively correlated with the high expression of CypA in colon cancer tissues, which provides clues for further exploring the mechanism of CypA in CRC.

In addition to the high expression of Cyps in colorectal cancer, the expression of Cyps is closely related to the progression of colorectal cancer. The expression intensity of Cyps increased with the decrease of CRC differentiation, the occurrence of lymph node metastasis [21, 154], TNM (tumor, node, metastases) stage and tumor invasion depth [109, 153]. In accordance with these experimental results, in addition to directly studying the pathological tissues of patients with CRC, we also established animal models of early submucosal non-invasive CRC and submucosal invasive CRC in rats. Proteomic analysis showed that compared to non-invasive CRC and normal control group, the expression of CypA and other proteins in invasive CRC was significantly higher [155]. Interestingly, Yeonghwan Kim's team has also studied the relationship between CypB expression and tumor progression, but has not found any
relevance between CypB overexpression and the grading or development of colon cancer [150], which seems to contradict the results of previous researchers' finding that the expression of CypB is significantly increased in CRC with lymph node metastasis [154]. Yeonghwan Kim et al. suggested that CypB was mainly distributed in ER, and its expression might be mainly affected by ER oxidative stress rather than tumor invasion and metastasis [37]. The expression level of CypB in the progression of CRC needs more experiments to explain, but these results clearly suggest that the general high expression of CypB may be closely related to the occurrence, development and metastasis of CRC.

**The possible mechanisms of Cyps in CRC**

In addition to its high expression in CRC, different members of Cyps can participate in the development of CRC in different ways. The mechanism of Cyps in CRC will be briefly reviewed in the form of family members.

**CypA**

The role of CypA in CRC mainly focuses on the proliferation, invasion and metastasis of cancer cells. The effect of eCypA on CRC is also closely related to the signal regulation of CD147 receptor. The combination of ecypa and CD147 on cell surface can activate downstream pathways through ERK1/2 [122, 156], and MAPK activation can promote tumor metastasis [34] (Figure 3). It is speculated that eCypA may play a role in the proliferation and metastasis of CRC by binding to CD147 and regulating the downstream MAPK signaling pathway. However, a number of experiments have shown that the regulation of MMPs in tumors is controlled by the activation of the p38MAPK signal [157-160], and plays an important role in promoting the invasion and metastasis of cancer cells [35, 157-160]. Combined with recent studies, it was found that the activity of MMP-9 promoter was significantly enhanced in CRC [161] (Figure 3). It can be speculated that CypA may regulate the expression of MMPs through the MAPK signaling pathway after binding to CD147, which has a certain effect on the invasion and metastasis of CRC and other cancer cells (Figure 3).
Apart from eCypA, the inhibition or knockout of iCypA also affects the proliferation, migration and invasion of CRC. In the early years, it has been found that sanglifehrin A (SFA, one kind of immunosuppressor) can inhibit the proliferation of macrophages through iCypA [124]. Similarly, SFA combined with iCypA can promote the transcription of tumor suppressor gene p53 in human colon cancer cell line HCT116 through the NF-κB signal, stimulate the expression of p21 and inhibit the activity of cell cycle kinase activity cyclinE-Cdk2, thus inhibiting the proliferation of colon cancer cells [162] (Figure 3). Unlike the inhibition of ERK1/2 activity in macrophages, SFA promoted NF-κB signaling in colon cancer cells [162] (Figure 3). However, the inhibition of iCypA can not only negatively regulate the migration and proliferation of immune cells [124], but is also related to the inhibition of CRC cell proliferation [162]. However, the difference may be that iCypA has different effects on immune cells such as macrophages and colon cancer cells (Figure 1 and 3).

However, in a recent study to explore the effect of CypA on the progression of colon cancer [163], the invasion and metastasis of SW480 cell line were significantly inhibited, but the cell proliferation was not affected (Figure 3). The up-regulation of E-cadherin (regulating the epithelial properties of cells) and the down-regulation of N-cadherin (regulating the mesenchymal properties of cells) were also noted [163], which indicated that iCypA could promote the invasion and migration of colon cancer cells by regulating epithelial mesenchymal transition (EMT). It is worth noting that, similar to the phosphorylation of NF-κB signal caused by the inhibition of iCypA, the knockout of iCypA also causes the activation of p38MAPK (Figure 3). The p38 inhibitors can increase the invasion number of CypA knockout colon cancer cells [163], which once again proves our previous conjecture that the inhibition or knockout of iCypA in CRC cells can activate NF-κB and MAPK signals. However, it may have the opposite effect in immune cells. Interestingly, however, the expression and release of major MMPs involved in cell invasion were also detected. The expression levels of these MMPs did not change after CypA knockout, and were even lower than the detection limit [163] (Figure 3). This seems to be in contradiction with many experimental results indicating that the activation of MAPK promotes the
expression of MMPs [34, 121, 132-134], but this suggests that extracellular and intracellular CypA may have different effects on MAPK in different cancer cells. At the same time, the expression of MMPs can not exclude the possibility of other signal pathway regulation, which needs further study. But it is certain that CypA does play an important role in the invasion and metastasis of CRC.

**CypB**

In cancer biology, CypB is related to the malignant progression and regulation of a variety of tumors [164-169], but research on it in CRC is rarely reported.

In the recent decade, Sung Soo Kim et al. mainly devoted to the study of CypB [108, 150, 170]. In 2011, the team first studied the induction of CypB under hypoxia and its function in tumor cells in vivo and in vitro [150]. These results suggest that CypB may be a new candidate target for the development of anticancer drugs for hepatocellular carcinoma and colon cancer, which also lays a foundation for further study on the correlation between CypB and chemotherapy resistance in patients with CRC. However, in this study, the relevant mechanism of hypoxia-induced up-regulation of CypB expression has not been deeply explored. In 2015, they demonstrated for the first time that hypoxia up-regulates the transcription of CypB by activating activation of transcription factor 6 (ATF-6) [170], thus elucidating the mechanism relatively completely. In recent years, they began to study the mechanism of CypB regulating chemotherapy resistance in CRC [108]. The results showed that p53 wild-type (p53WT) tumor suppressor gene can up-regulate CypB mRNA and protein levels, and overexpressed CypB can interact with ubiquitin E3 ligase (MDM2), so as to make p53WT ubiquitination degradation and shorten its half-life, thus inhibiting oxaliplatin-induced apoptosis of CRC cells (Figure 4). On the contrary, CypB knockout can prolong the half-life of p53WT and stimulate apoptosis of cancer cells [108]. These results suggest that CypB is an effective target molecule for drug development to improve chemotherapy resistance in patients with CRC. In conclusion, these results suggest that CypB is regulated by oncogene transcription factors such as hypoxia inducible factor-1α (HIF-1α), ATF-6 and p53WT, which may be related to ER stress and extensive signaling pathways, and determine the role of CypB in
In addition to participating in the regulation of chemotherapy resistance of CRC, CypB has been found to play a critical role in the invasion and metastasis of CRC [151, 154]. CypB was knocked out by RNA interference plasmid in CRC cell line. The results showed that the migration and invasion ability of cancer cells were significantly reduced after CypB knockout [154] (Figure 4). However, the specific mechanism of CypB in promoting the migration and invasion of cancer cells was not revealed in this study. A recent study [151] showed that CypB silencing can reduce the proliferation, invasion and migration of colon cancer in vivo and in vitro by blocking the phosphorylation and nuclear translocation of signal transducer and activator of transcription-3 (STAT-3) induced by IL-6 (Figure 4). In addition, CypB silencing can also block the hydroxylation of type I collagen and the formation of strip, thereby inhibiting the metastasis of cancer cells [151] (Figure 4). In conclusion, STAT3/CypB/collagen regulatory axis may play a key role in the development of CRC, and CypB may be an effective target to prevent the proliferation, invasion and migration of CRC.

**CypD**

CypD is a component of mitochondrial permeability transition pore (mPTP). The role of CypD in mPTP has been widely used in experiments in recent years. It seems that the effect of CypD on CRC also revolves around cell death induced by mPTP [171-174]. Some chemotherapeutic drugs have been found to promote the combination of mitochondrial ANT-1 and CypD, reduce the mitochondrial membrane potential, promote the opening of mPTP, and exert a toxic effect on CRC cells through programmed mitochondrial necrosis, and induce the necrosis of cancer cells, but not apoptosis (Figure 2). Consistent with this, when CypD inhibitors or corresponding siRNA were used, the toxicity was significantly reduced [171, 172]. In the mouse model, the inhibition of chemotherapy drugs on the growth of HCT-116 tumor was significantly reduced after CsA treatment [172]. Correspondingly, the overexpression of CypD significantly enhanced the sensitivity and cytotoxicity of CRC cells to chemotherapy drugs [172]. This shows that CypD plays an important role in the toxic
effects of chemotherapeutic drugs-induced CRC cells, and this effect is closely related to the pathway of programmed mitochondrial necrosis. In the study of Chunxian Zhou, it was also found that the chemotherapeutic drug icariin (ICT) that promoted the opening of mPTP may be induced by the JNK activation pathway [171]. It is suggested that the JNK signal pathway may be one of the regulatory mechanisms that CypD participates in chemotherapy drugs-induced necrosis of CRC cells (Figure 2).

However, it is interesting that in addition to the pathway of mitochondrial programmed necrosis, some researchers discovered that a new anti-cancer drug candidate can also induce apoptosis of CRC cells by destroying mPTP [173]. The drug is a VDAC-1 binding small molecule (Erastin), which can promote the binding of VDAC-1 with CypD, release of Cytochrome C, regulate the opening of mPTP, and then induce mitochondrial oxidative stress and caspase-9-dependent cell apoptosis on the cytotoxic effects of various CRC cell lines (Figure 2). After the corresponding inhibitors, the cytotoxicity and apoptosis induced by the drug were significantly reduced [173]. This not only indicates the emergence of a potential new type of drug against CRC, but also suggests that CypD may also be involved in the drug-induced apoptosis of CRC cells by regulating mPTP. To sum up, CypD can participate in the cytotoxic effect of drugs on CRC not only through the necrosis pathway, but also through the apoptosis pathway. The difference of these pathways may be related to the types of drugs, the molecules that bind to CypD, and the way to induce the opening of mPTP.

In a recent study, it was found that Ganoderma Acid D (GAD) could induce the deacetylation of CypD by up-regulating the level of mitochondrial deacetylase Sirtuin 3 (SIRT3) in a dose-dependent manner, thus changing the open state of mPTP, thereby inhibiting the Warburg effect of colon cancer cells and causing cell death [174]. This suggests a possible new way and mechanism for CypD to participate in drugs to inhibit CRC. But different routes have the same goal. The inhibitory effect of different drugs on CRC cells is always related to the involvement of CypD in the regulation of the open state of mPTP.
Summary

Cyps is generally highly expressed in inflammatory bowel disease and CRC, and correlated with the course of disease and prognosis, which suggests that Cyps may be an indicator for diagnosis and prognosis. In CRC, Cyps is mainly involved in the invasion, metastasis, apoptosis, necrosis and drug resistance of cancer cells. In IBD, Cyps plays a role in inflammation through apoptosis of immune cells, autophagy, necrosis and secretion of inflammatory factors, and its mechanism research focuses on the common signal molecules MAPK, NF-κB and mitochondrial programmed death. The specific mechanism of action still needs to be further exploration. However it is certain that Cyps will play a pivotal role in future research on IBD and CRC, and can provide effective clues for disease treatment and/or new drug development.

Abbreviations

Cyps: Cyclophilins; IBD: Inflammatory bowel disease; CRC: CRC; PPIase: Peptidyl prolyl isomerase; CsA: Cyclosporin A; NF-AT: Nuclear factor of activated T cells; CypA: Cyclophilin A; CypB: Cyclophilin B; CypC: Cyclophilin C; CypD: Cyclophilin D; Cyp40: Cyclophilin 40; CypNK: Cyclophilin NK; VSMC: Vascular smooth muscle cells; EC: Endothelial cells; iCypA: Intracellular CypA; eCypA: Extracellular CypA; ER: Endoplasmic reticulum; eCypB: Extracellular CypB; HIV: Human immunodeficiency virus; mPTP: Mitochondrial permeability transition pore; VDAC: Voltage-dependent anion channel; ANT-1: Adenine nucleotide translocator-1; UC: Ulcerative colitis; CD: Crohn's disease; CypJ: Cyclophilin J; DSS: Dextran sulftesodium; NF-κB: Nuclear factor kappa B; LPS: Lipopolysaccharide; CD147: Differentiation cluster 147; MMPs: Matrix metalloproteinases; TIMP-1: Tissue inhibitor of matrix metalloproteinases-1; EMMPRIN: Extracellular matrix metalloproteinase inducer; GAG: Sulfated glycosaminoglycans; MPT: Mitochondrial permeability transition; NSAID: Non-steroidal anti-inflammatory drugs; TNM: tumor, node, metastases; SFA: Sanglifehrin A; EMT: Epithelial mesenchymal transition; ATF-6: Activation of transcription factor-6; p53WT: p53 wild-type; MDM2: Ubiquitin E3 ligase; HIF-1α: Hypoxia inducible factor-1α; STAT-3: Signal transducer
and activator of transcription-3; ICT: Icarin; GAD: Ganoderma acid D; SIRT3: Sirtuin 3.

Competing Interests

The authors have declared that no competing interest exists.

References


120. Xue Z, Yuan W, Li J, Zhou H, Xu L, Weng J, et al. Cyclophilin A mediates the ox-LDL-induced
136. Matsuda S, Moriguchi T, Koyasu S, Nishida E. T lymphocyte activation signals for interleukin-2 production involve activation of MKK6-p38 and MKK7-SAPK/JNK signaling pathways sensitive to
152. Laidlaw AM, Copeland B, Ross CM, Hardingham JE. Extent of over-expression of hepatocyte growth factor receptor in colorectal tumours is dependent on the choice of normaliser. Biochemical and


Figure 1. A summary diagram of the possible mechanism of CypA in inflammatory bowel disease. iCypA is secreted by vascular smooth muscle cells, macrophages and endothelial cells to form eCypA (dotted arrow). iCyps combined with CsA, inhibited the activity of calcineurin, thus blocking the NF-AT and inhibiting the activation of T cells (orange arrow). SFA inhibits macrophage proliferation by blocking ERK1/2 phosphorylation through binding to CypA (purple arrow). eCypA induces the up-regulation of CD147 on macrophage surface and together with it, induce the expression of inflammatory factors through phosphorylation of ERK and NF-κB, or induce macrophage autophagy, apoptosis, M1 polarization and infiltration (black arrow). CypA induces macrophage autophagy through the PI3K/Akt/mTOR signaling pathway. CypA may also activate ERK1/2 via CD147 to regulate the expression of TIMP1/MMP9, thus promoting the occurrence and development of IBD (black arrow).
Figure 2. CypD is involved in the pathogenesis of enteritis and CRC. The lack of CypD in macrophages, intestinal epithelial cells and eosinophils can protect cells from a series of mitochondrial responses caused by the continuous opening of mPTP induced by LPS, non-steroidal anti-inflammatory drugs, Ca^{2+} overload and oxidative stress. In turn, it inhibits the necrosis or apoptosis of immune cells, but the reduction of eosinophil lysis and necrosis promotes the development of enteritis (solid line). GAD induces the deacetylation of CypD by up-regulating the level of SIRT3 in a dose-dependent manner, changes the open state of mPTP, inhibits the Warburg effect of colon cancer cells, and makes cancer cells apoptosis. Chemotherapy drugs (PF-543, ICT) can promote the combination of mitochondrial ANT-1 and CypD, keep mPTP open, exert toxic effects on CRC cells through programmed mitochondrial necrosis, and induce necrosis of cells. Erastin can induce mitochondrial oxidative stress and caspase-9-dependent cell apoptosis by inducing VDAC-1 binding with CypD and regulating MPTP opening (dotted line).
Figure 3. The mechanism of CypA in CRC. SFA combined with CypA can promote the transcription of tumor suppressor gene p53 in human colon cancer cells by activating NF-κB signal, and then inhibit the proliferation of cancer cells (black arrow). Knockout of CypA gene can activate p38MAPK signal, change EMT, reduce the expression of MMPs and other factors, and then inhibit the invasion and metastasis of CRC cells (green arrow). The expression of MMPs may be the up-regulated by activation of MAPK, NF-κB and other signaling pathways after the secreted CypA binds to CD147, which promotes the invasion and metastasis of CRC cells (blue arrow).
**Figure 4.** Related mechanisms of CypB involved in CRC. Through HIF-1α, hypoxia and cisplatin can induce VEGF expression and CypB transcription, promote angiogenesis and protect tumor cells from stress-induced apoptosis. Overexpressed of CypB binds to STAT3 and positively promotes HIF-1α transcription (Black arrow). CypB can induce STAT3 phosphorylation, nuclear translocation and collagen hydroxylation under the action of IL-6, and promote the proliferation and metastasis of cancer cells (black and orange arrow). The siRNA plasmid targeting CypB can inhibit the metastasis and invasion of cancer cells (purple arrow). p53 upregulates the expression of CypB, the interaction between CypB and N-terminal of MDM2 can degrade p53 protein and inhibit oxaliplatin induced apoptosis of cancer cells (blue arrow).