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Clostridium difficile Toxin B: Insights into Its Target Genes

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Abstract

Clostridium difficile is a grossly Gram-positive anaerobic bacterium that has been a key factor in inducing imbalances in the gut microbiota in recent years, leading to intestinal-associated inflammation. The main pathogenic toxins of *Clostridium difficile* are toxin A (TcdA) and toxin B (TcdB). TcdB is the main pathogenic factor of *Clostridium difficile* infection. This review revealed the pathogenic mechanism of *Clostridium difficile* toxin B, expounded the impact of *Clostridium difficile* on the intestinal system, and predicted the genes on which TcdB may act, thereby providing a new therapeutic target for *Clostridium difficile* infection, offering theoretical basis and new strategies for clinical prevention and control.

Keywords

Clostridium difficile Infection, TcdB, Gene Regulation

1. Introduction

In recent years, the misuse of drugs such as broad-spectrum antibiotics or immunosuppressants [1] has led to the suppression of normal intestinal flora. As a conditional pathogen, *Clostridium difficile* (*CD*) is highly resistant to therapeutic drugs, proliferates excessively in the intestines and releases toxins, causing serious clinical infectious diseases such as diarrhea, pseudomembranous enteritis and toxic megacolon [2], threatening human health. Oysters grown in contaminated water have even been found to bioaccumulate toxigenic *CD* and pose a health risk by acting as a vector for the transmission of the pathogen to humans [3]. Traditional cooking methods may not adequately eliminate *CD* spores from meat, which leads to more serious foodborne transmission.

In 2013, the US Centers for Disease Control and Prevention (CDC) listed *CD* as the main pathogens of hospital-acquired infections [4]. Toxin is the main pathogenic factor of *CD*. *CD* can produce 6 kinds of toxins, namely toxin A (TcdA), toxin B (TcdB), toxin C (TcdC), toxin D (TcdD), toxin E (TcdE) and clostridium airficilebinary toxin (CDT), of which TcdA and TcdB cause infection symptoms, which are the main virulence factors [5]. Toxin A, known as enterotoxin, acts on the intestinal mucosa epithelium, causing increased secretion and even bleeding of the intestinal mucosa [6]. Toxin B, also known as a cytotoxin, destroys intestinal cells and even causes degeneration, apoptosis, necrosis and shedding of intestinal cells, worsening the inflammatory response [7]. The human colon was 10 times more sensitive to damage caused by TcdB than TcdA in the study [8]. TcdB poses a more serious risk to humans. Several studies also showed that TcdB is a condition for the pathogenesis of CDI, causing more mucosal oedema in the tissue as well as manifestations of cellular infiltration, and is 100 - 10,000 times more virulent than TcdA [9] [10]. There is no doubt that TcdB is the relatively more important *CD* toxin in humans. For CDI, the therapeutic drugs metronidazole and vancomycin are commonly used clinically [11], but gradually cases of resistance to metronidazole and vancomycin have emerged [12] [13] [14], and the relapse rate of CDI treated with these two drugs remains high [15], placing a great burden on social and medical resources. Both patients and clinicians need a more effective treatment measure. It is worth noting that TcdB is associated with a variety of genes when it works. The relationship between changes in the expression of these genes and the toxin response suggested that gene targeting may be used to prevent, treat or reduce the recurrence of CDI.

The aim of this paper is to provide an overview of gene targeting therapy and further mechanisms of action by describing the effects of toxins secreted by *CD* on the intestinal system as recently discovered by researchers, screening for genes that may play a role in the TcdB response.

2. Pathogenic Mechanism of TcdB

A common feature of most CDI patients is the long-term use of antibacterial drugs. This results in a gradual weakening of the colonization resistance of the normal intestinal flora against pathogen adhesion, and the normal composition of the intestinal flora is disrupted by the adhering pathogens, which take the opportunity to disrupt. This is how *Clostridium difficile* sticks to the intestines to release toxins [16].

In general, the effect of toxins is initially caused by the binding of TcdA to the toxin receptors on colon cells, which damages colon villi and brush borders, destroys the cytoskeleton and tight junctions, and activates the mucosal epithelial cell cAMP system to secrete more water and salt, corrodes the mucous membrane, and chemoattracts white blood cells, causing inflammation, diarrhea and even bleeding in the intestine [17] [18]. TcdB enters the tissues after TcdA damages the intestinal mucosa, inactivates the guanosine triphosphatase (GTPase) protein of the Rho family after glycosylation, inhibits its effect, and blocks cell

signals that depend on Rho, thus exerting cytotoxic effects [19]. Afterwards, the toxin stimulates monocytes, macrophages and neutrophils to release inflammatory factors to destroy intestinal wall cells, increase the permeability of intestinal epithelial cells, cause inflammation, secrete more intestinal cell mucus and electrolytes, and ultimately lead to cell apoptosis, degeneration, necrosis, shedding, and even the formation of pseudomembranes causing pseudomembranous colitis [7]. However, a growing body of research suggested that there is not necessarily a sequential relationship between the actions of TcdA and TcdB, and that TcdB may not need to enter cells to produce effects after TcdA has destroyed the intestinal mucosa [20]. Pathogenic *CD* strains that are TcdB+ but TcdA− were clinically isolated and were more likely to cause severe systemic disease [21]. TcdB is a critical presence that requires greater attention.

In addition to the general mechanisms described above, TcdB has been found to accomplish damage to cellular tissues by activating multiple pathways. One is that TcdB activates NF-κB signaling via the JAK1/2, ERK1/2 and p38/MAPK pathways, inducing the release of inflammatory factors such as IL-1β, IL-6, IL-8 and TNF-α from epithelial cells, initiating downstream tissue edema, cell chemotaxis and apoptosis responses and exacerbating inflammatory effects [22] [23]. Secondly, TcdB induces the production of VEGF-A in human colon through HIFα, p38/MAPK and MEK1/2 signaling pathways, increasing pro-inflammatory cytokines and making the intestinal vascular barrier more permeable [24]. Thirdly, colonic stem cells are the main targets of TcdB. Chondroitin sulfate proteoglycan 4 (CSPG4), poliovirus receptor-like 3 (PVRL3) and frizzled proteins (FZDs) have recently been identified as TcdB receptors [25] [26] [27] [28] [29], of which FZDs are thought to be the major receptors in the colonic epithelium [25] [30]. When TcdB binds to FZDs, FZD1, 2 and 7 mediate the entry of toxins into cells, inhibit Wnt signaling and interfere with the self-renewal and differentiation of colonic stem cells [25] [31] [32]. Fourth, TcdB can also induce caspase-dependent and caspase-independent apoptotic pathways through mitochondrial ATP-dependent potassium channels, causing apoptosis and damage to tissues [33]. Fifth, in recent studies, it has been proposed that toxin self-processing mediated by the cysteine protease domain (CPD) can positively regulate cytotoxicity and negatively regulate pro-inflammatory activity [34] [35] [36] [37]. Sixth, TcdB activates the release of IL-1β from Inflammasome containing the ASC gene, a pathway that also plays an important role in the pathogenesis of CDI [38]. We can therefore conclude that TcdB initially disrupts the intestinal barrier by altering tight junctions and intracellular signaling between intestinal epithelial cells. After the intestinal epithelial barrier has been breached, the toxin interacts with immune cells in the body, causing a strong inflammatory response that leads to subsequent tissue damage.

CDI has become one of the important factors threatening human health. After the author explained the possible pathogenic mechanism, the importance of TcdB is self-evident. Although many studies have revealed the role of *CD* toxin

B, there are still many unsolved problems in the prevention and treatment of CDI.

3. Genes That TcdB May Act on

The author searched the GEO database in NCBI for datasets related to *CD* (keyword *Clostridium difficile*) and found dataset GSE63880. 6 of these colon stem cell samples treated with TcdB for 24 h and 2 controls were analyzed. (Because the effect of the toxin on each person infected with *CD* is different and there were three different levels in this dataset, the levels treated were not differentiated here, only time was chosen as a fixed factor, just enough to better simulate the real situation, while 24 hours was chosen as the principle of the longest duration of action.) The data were then analyzed for variance using the limma package in R (3.6.1), and the results of the variance analysis were obtained and visualized in R. The thresholds were chosen as $p < 0.05$ and $|\log FC| > 1$. A total of 273 differential genes were obtained, of which 126 were up-regulated and 147 were down-regulated (**Figure 1**). The resulting differential genes were enriched for analysis and visualised using the online tool gprofiler (http://biit.cs.ut.ee/gprofiler/gost). The main entries for MF enrichment in GO (Gene Ontology) were found to be alcohol dehydrogenase (NAD+) activity, RNA-DNA hybrid ribonuclease activity, alcohol dehydrogenase [NAD(P)+] activity, catalytic activity acting on DNA, and transcription factor binding. The main entries in BP enrichment were response to stress, response to oxygen-containing compound, response to hormone, response to lipid and nuclear DNA replication. The main entries for CC enrichment included membrane-bounded

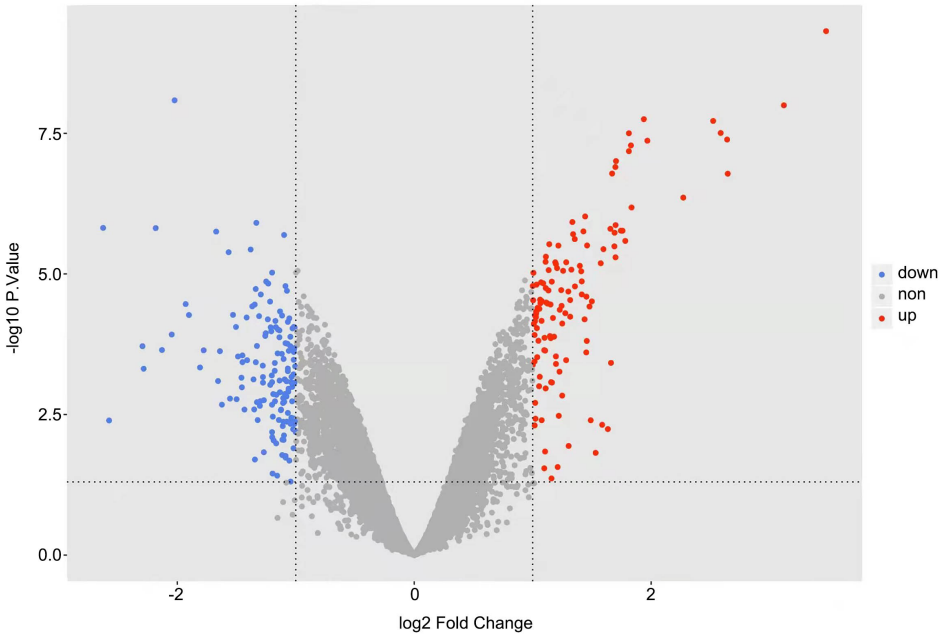
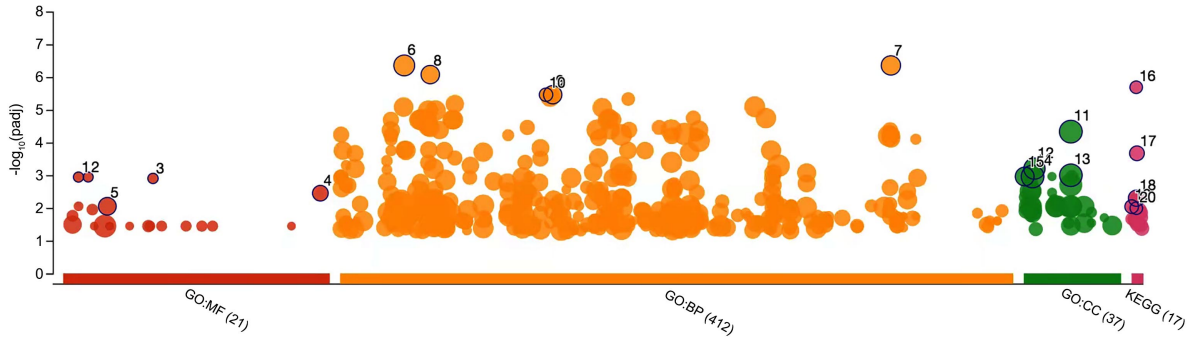


Figure 1. Volcano plot of gene distribution (horizontal coordinates are Fold Change taken as log2 values, vertical coordinates are p values taken as log negative values; where red dots represent 126 genes up-regulated and blue dots represent 147 genes down-regulated).

organelle, cytosol, intercellular membrane-bounded organelle, cytoplasm, and nuclear chromosome. In addition, the main pathways enriched in KEGG (Kyoto Encyclopedia of Genes and Genomes) were DNA replication, Cell cycle, MAPK signalling pathway, Glycolysis/Gluconeogenesis and Base excision repair (**Figure 2**). These enriched entries revealed the impact of the toxin produced by *CD* on intestinal cells and the microenvironment. It also suggested that the differential genes analyzed may indeed be associated with the action of the TcdB secreted by *CD*. Finally, 273 genes with differential expression were subjected to PPI network construction in string (https://string-db.org/) and network visualization designed using cytoscape (3.7.1) to find genes that play a key role in response to the action of TcdB (**Figure 3**). The expression of the top 20 genes by degree number was obtained in **Figure 4**, and a table of genes with degree numbers greater than or equal to 20 was also produced (**Table 1**).

The author used the above steps to make a preliminary prediction of the genes in which TcdB may act, and presented a short review of some of these genes to describe their possible role in CDI, in the hope that these genes may serve as targets for future prevention, treatment or recurrence of CDI.



ID	Source	Term ID	Term Name	Padj (query_1)
1	GO:MF	GO:0004022	alcohol dehydrogenase (NAD+) activity	1.132×10^{-3}
2	GO:MF	GO:0004523	RNA-DNA hybrid ribonuclease activity	1.132×10^{-3}
3	GO:MF	GO:0018455	alcohol dehydrogenase [NAD(P)+] activity	1.245×10^{-3}
4	GO:MF	GO:0140097	catalytic activity, acting on DNA	3.524×10^{-3}
5	GO:MF	GO:0008134	transcription factor binding	8.848×10^{-3}
6	GO:BP	GO:0006950	response to stress	4.385×10^{-7}
7	GO:BP	GO:1901700	response to oxygen-containing compound	4.385×10^{-7}
8	GO:BP	GO:0009725	response to hormone	8.346×10^{-7}
9	GO:BP	GO:0033993	response to lipid	3.445×10^{-6}
10	GO:BP	GO:0033260	nuclear DNA replication	3.445×10^{-6}
11	GO:CC	GO:0043227	membrane-bounded organelle	4.627×10^{-5}
12	GO:CC	GO:0005829	cytosol	6.257×10^{-4}
13	GO:CC	GO:0043231	intracellular membrane-bounded organelle	9.978×10^{-4}
14	GO:CC	GO:0005737	cytoplasm	1.089×10^{-3}
15	GO:CC	GO:0000228	nuclear chromosome	1.089×10^{-3}
16	KEGG	KEGG:03030	DNA replication	2.038×10^{-6}
17	KEGG	KEGG:04110	Cell cycle	2.127×10^{-4}
18	KEGG	KEGG:04010	MAPK signaling pathway	5.006×10^{-3}
19	KEGG	KEGG:00010	Glycolysis / Gluconeogenesis	9.086×10^{-3}
20	KEGG	KEGG:03410	Base excision repair	1.014×10^{-2}

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organism	hsapiens

g:Profiler

Figure 2. GO and KEGG enrichment (the horizontal coordinates are the corresponding entries, the vertical coordinates are the negative logarithm of Padj, the size of the bubbles represents the number of genes enriched, and the table below shows the top 5 entries sorted by Padj).

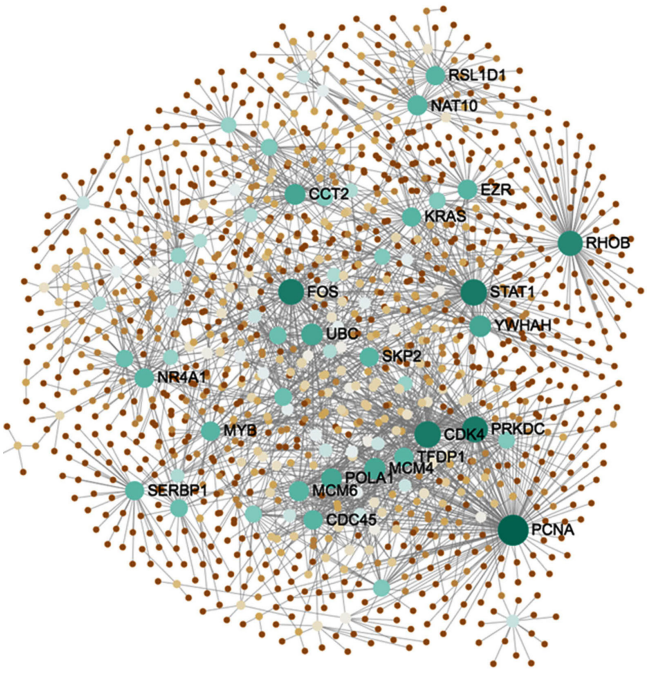

Figure 3. Topological network of 273 differential genes (larger and bluer nodes in the diagram representing more connections to other genes).

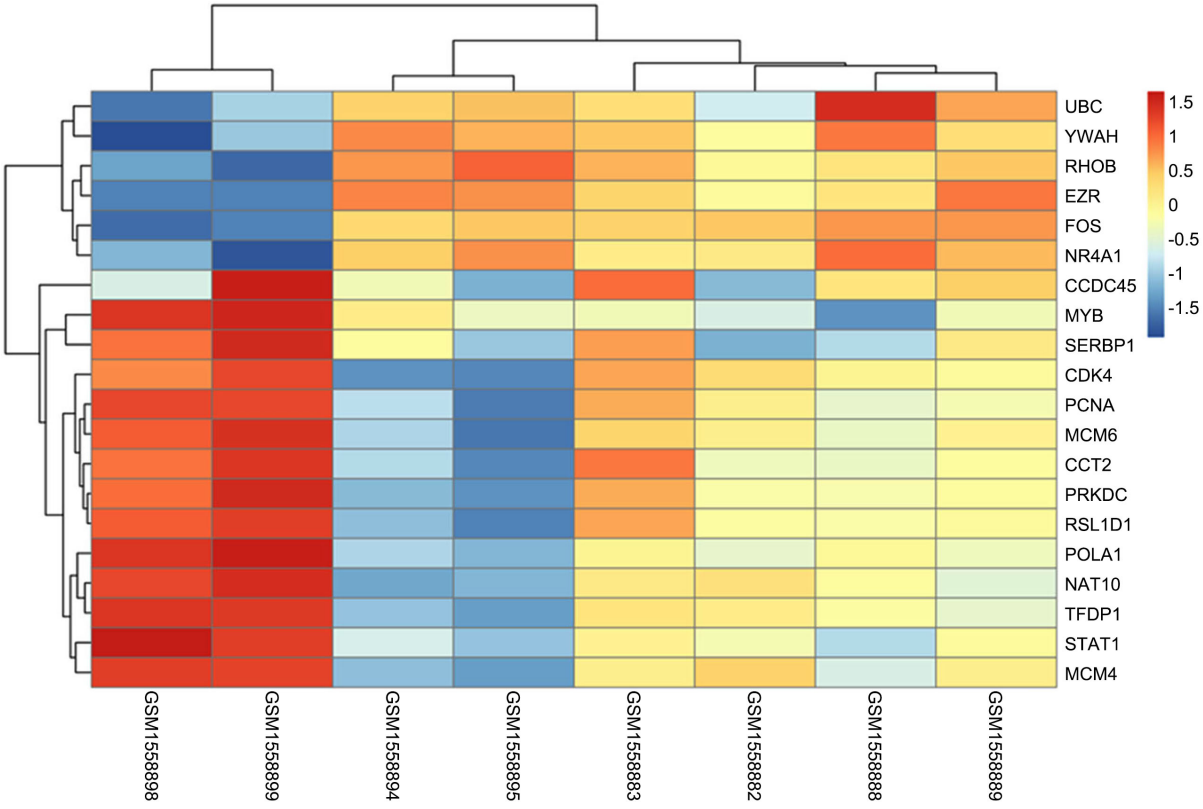

Figure 4. Expression of the top 20 genes ranked by degree (horizontal coordinates are the corresponding samples, GSM1558898 and GSM1558899 are controlled, the rest are toxin treatment groups. The vertical coordinates are the genes, the redder the colour the higher the expression, the bluer the lower the expression).

Table 1. Genes with a degree greater than or equal to 20.

Gene	Degree	Betweenness
PCNA	134	117,321.47
CDK4	85	74,659.75
STAT1	84	99,114.06
PRKDC	80	100,947.23
FOS	79	95,936.42
RHOB	73	81,295.11
UBC	49	320,040.16
YWHAH	48	41,039.68
MCM4	46	13,871.56
CCT2	44	38,120.64
POLA1	42	14,123.16
NAT10	40	64,192.27
MCM6	40	12,287.29
EZR	38	39,279.2
NR4A1	37	26,347.93
MYB	36	30,916.56
RSL1D1	36	20,755
TFDP1	36	14,878.26
CDC45	36	12,022.94
SERBP1	35	36,532.52
KRAS	35	35,376.37
SKP2	35	23,152.04
SUPT16H	29	27,611.45
HSPB1	29	25,015.58
JUNB	28	16,343.29
NR4A2	28	6692.65
APEX1	27	17,424.94
FEN1	27	10,234.49
CXCR4	26	42,835.52
TUBA1B	26	17,552.58
NBN	26	11,375.66
TUBB2A	25	11,582.12
MEF2D	23	10,644.4
BRCC3	22	18,984.65
EFNA1	22	17,594.26
NME1	20	29,894.24

3.1. Increased PCNA Suggests Proliferation of Intestinal Epithelial Cells to Repair Damaged Intestine

Proliferating Cell Nuclear Antigen (PCNA), also known as Cyclin, is a marker protein specific to proliferating cells [39]. Because PCNA is an auxiliary protein of DNA polymerase δ, it is closely related to DNA replication and synthesis and plays an important role in the initiation of cell proliferation, so it can be used as a good indicator of the proliferative state of cells and the development of aggressive lesions [40] [41] [42]. In diseases such as gastric cancer, non-small cell lung cancer and liver cancer, increased expression of PCNA indicates an increased degree of malignant tissue [43] [44] [45]. In the differential gene table produced by the author (**Table 1**), it can be found that PCNA with a degree as high as 134 was highly correlated with TcdB action and was most likely the key gene for TcdB action. In previous studies, few or no people mentioned the relationship between *CD* toxin and PCNA. However, it can be found from PCNA-related studies that when small intestinal epithelial cells were overactivated, that is, when the small intestinal epithelium was in a hyperproliferative state, PCNA expressed strongly positive correspondingly [46]. The effect of *CD* toxin starts in the intestinal epithelial cells, destroying permeability and intestinal mucosa, intestinal cell apoptosis, necrosis, shedding while the body proliferates a large number of cells to repair the place damaged by the toxin. A large number of proliferating cells make PCNA expression elevated. It is therefore worth trying to assist in the diagnosis of CDI by detecting PCNA expression and predicting the grading of the degree of CDI. PCNA can be used as a therapeutic target for CDI to enable proper proliferation of intestinal cells, thereby repairing the damaged intestine and creating a good environment for intestinal bacteria to survive and alleviate flora disorders.

3.2. STAT1 May Exacerbate Inflammation Caused by *Clostridium difficile* Toxin

Signal transducer and activator of transcription (STAT) is a family of transcription factors involved in cellular signal transduction, which when activated can be involved in regulating the expression of genes for cell growth, differentiation, proliferation and apoptosis through a variety of signaling pathways. STAT1 is the first and extremely important member of the STAT family to be discovered. It acts as a transcriptional activator in the nucleus [47]. It is related to inflammation caused by immune system disorders and induces the expression of pro-inflammatory factors such as TNF-α and TGF-β [48]. Janus kinases (JAK) and mitogen-activated protein kinases (MAPK) can activate STAT1, phosphorylate the conserved tyrosine and serine residues in its C-terminal activation region, form a dimer and then translocate into the nucleus to regulate target genes [49] [50]. These two pathways are closely related to intestinal immune function. TcdB can activate STAT1 via JAK and p38 MAPK [51]. STAT1 activation promotes TNF-α-induced apoptosis and exacerbates *CD* toxin-induced inflammation. In addition, STAT1 can also induce the synthesis of caspase precursors

[52], paving the way for the strong activation of the caspase pathway that depends on the glycosylation modification of Rho protein after TcdB enters macrophages. It is currently believed that the STAT pathway is highly conserved in vertebrates, so this gene target is undoubtedly an excellent choice for the treatment of *CD*.

3.3. Increased c-Fos Expression Is Associated with CDI

The immediate early gene c-fos is rapidly inducible, and its expression increases after being activated, encoding the Fos protein. People believe that because the expression of c-fos in neuronal cells is proportional to the intensity of the stimulus it bears, c-fos is often used as a sign of neuron activation by noxious stimuli. Further, c-fos also regulate cell proliferation, differentiation and apoptosis and other physiological functions. In the experiment of culturing the human colon in vitro, it was found that the toxin B of *CD* can cause an inflammatory response in the human colon in vitro, releasing IL-1β and other cytokines. The expression of c-fos is induced in human submucosal neuronal populations through four pathways: IL-1β-mediated, specific neurochemically encoded, smooth muscle or vascular, and is increased in a dose-dependent manner [53]. Phosphorylation of the p38 MAPK pathway has also been found to upregulate c-fos expression in animal experiments, leading to inflammation [54] [55]. Accordingly, when severe inflammation is caused by the effect of *CD* toxins, it may be useful to try to alleviate the inflammatory response by inhibiting c-fos genes, using c-fos as an observational indicator, which can also be used as an auxiliary diagnostic criterion and provide new strategies for the clinical treatment of *CD* infections.

3.4. DUSP1 Blocks Pathways That TcdB May Affect

Dual specificity phosphatase (DUSP) is also known as mitogen-activated protein kinases phosphatase (MKPs). DUSP1 or MKP-1 is the first member of the MKP family to be discovered. It is also an essential part of the body [56]. Studies have found that DUSPs can inhibit the activity of MAPKs in mammalian cells [57], especially DUSP1 can cause all three MAPKs pathways: p38 mitogen-activated protein kinase (p38 MAPK), c-Jun N-terminal kinase (JNK) and extracellular regulated protein kinase (ERK1/2) and NF-κB signaling pathway are inactivated. It is precisely the release of the pro-inflammatory factors TNF-α and IL-1β following the action of TcdB that activates the three pathways of MAPK cascade signaling and the NF-κB signaling pathway [58] [59] [60]. These four pathways in turn continue to produce TNF-α and IL-1β, creating a vicious cycle in which intestinal inflammation becomes more severe and worsens the condition. It was also found that under the influence of TcdB, DUSP1 is the most suppressed by the toxin in the DUSPs protein, that is, DUSP1 is the lowest expressed in the family in CDI [58]. Then in the future, therapeutic drugs such as DUSP1 activator can be used in CDI patients, so that inflammatory factors such as TNF-α and

IL-1β induced by TcdB are inhibited by DUSP1, thereby reducing the inflammatory response caused by CDI. Therefore, DUSP1 can be used as a regulator of inflammatory cytokine production and a new potential target for inhibiting the production of pro-inflammatory factors by TcdB.

3.5. Five Predictive Genes

Thymidylate synthase (TS) encoded by TYMS (thymidylate synthase) gene is a key enzyme for pyrimidine nucleotide synthesis, and its abnormal expression is often closely related to malignant tumors [61] [62], so it is currently mostly used as 5-fluorouracil (5-fluorouracil, 5-FU) and other anti-tumor chemotherapy drugs. Among a variety of malignant tumors closely related to the role of TYMS, the expression of TYMS in patients with colorectal cancer can determine whether the patient is resistant to chemotherapeutic drugs such as 5-FU and predict the efficacy and prognosis of the patient [63] [64] [65].

The mechanistic target of rapamycin kinase (mTOR) is a highly conserved atypical serine/threonine kinase in mammals, existing in both complex 1 (mTOR complex 1, mTORC1) and mTORC2 forms [66] [67]. Among them, mTORC1 is more extensively studied and can be considered as a central regulator of cellular activity, involved in the regulation of cell proliferation, differentiation and apoptosis *in vivo*, influenced by growth factors as well as environmental factors. Inactivation of mTORC1 has been found to lead to proliferation and regeneration of colonic crypts, inducing IL-6-related reparative inflammation and damaging colonic tissue [68]. CD36, an important pattern recognition receptor, plays a key role in the immune response [69], for example by activating JNK1/2 kinase to release inflammatory factors such as IL-8 [70]. mTORC1 has been found to form the CD36/mTORC1 signaling pathway with CD36, which is involved in the regulation of inflammatory immune response [71] [72] [73]. Several studies also mentioned that mTORC1 affects the STAT family, and affects the differentiation and function of CD4+T cells and CD8+T cells [74] [75]. These studies have shown that mTORC1 is closely related to inflammation, and the inactivation of mTORC1 may cause colitis, thereby inducing the occurrence and development of colorectal cancer.

Cyclin-dependent kinase 4 (CDK4) is an important member of the cyclin-dependent kinase family, whose main role is to regulate the cell proliferation cycle G1~S. The overexpression of CDK4 can disrupt the cell cycle, lead to uncontrolled cell proliferation, and promote the development of malignant tumors. More and more studies have shown that CDK4 is negatively correlated with the degree of differentiation of cancer tissues [76]. For example, in colorectal cancer, CDK4 is mostly overexpressed and may be an oncogene for colorectal cancer [76] [77].

Flap Endonuclease 1 (FEN1) is a 5' nuclease that forms a "sliding clip" with PCNA and plays a role in DNA replication and repair [78]. It has been found that FEN1 is highly expressed in a variety of tumors, such as breast, kidney, and

ovarian cancer [79]. Knockout of FEN1 gene also inhibits the growth of human colorectal cancer cells with RAD54B mutation [80]. The high correlation between FEN1 and tumors makes it one of the possible targets for tumor therapy.

DNA-dependent protein kinase gene (PRKDC) is a serine/threonine protein kinase and an important part of the DNA repair mechanism [81]. PRKDC has been proven to be an important factor in tumor development and metastasis, and its high expression often appears in cancer patients with low survival rates [82]. The expression of DNA-dependent protein kinase catalytic subunits (DNA-PKcs) encoded by PRKDC gene in colorectal cancer tissues is significantly higher than that in normal tissues adjacent to cancer [83]. In the study of Davidson *et al.*, small molecule inhibitors of DNA-PKcs can effectively enhance the efficacy of SN38 in colorectal cancer [84]. It can be seen that the potential connection between PRKDC and colorectal cancer may be a future treatment target for colorectal cancer.

The above-mentioned TYMS, mTORC1, CDK4, FEN1 and PRKDC have not been found to have a clear relationship with the toxin of *CD*, but the author found that these five genes are more or less related to malignant tumors, especially in colorectal cancer. With the increasing research on colorectal cancer, many researchers have pointed out in recent years that the occurrence and development of colorectal cancer are related to the imbalance of the intestinal microbial environment [85], and the infection of *CD* is positively correlated with the incidence of colorectal cancer [86] [87]. Although there has not been a clear study suggesting that there is a causal relationship between CDI and colorectal cancer, there is no doubt that there is a strong correlation between them. Even though the current research cannot explain that these five genes are related to the role of TcdB, it cannot be denied that they may be one of the targets of TcdB. And among the genes predicted by the author to be associated with TcdB, they are clearly listed. These five genes, including the other genes in the heat map and the table (**Figure 4** and **Table 1**), may have little effect on the early infection of *CD*, but they may play a key role when the disease worsens and causes cancer. They may become one of the directions of future research on the mechanism of action of *CD* toxin, and the future target of gene-targeted therapy to avoid the further development of tumors.

4. Discussion

The infection of *CD* is more serious worldwide, and its drug resistance is rising rapidly and the situation is serious. The high recurrence of *CD* also makes clinical treatment difficult. The efficacy of traditional antibiotic treatments has stagnated or even receded. Therefore, there is an urgent clinical need for alternative non-antibiotic therapies for targeted treatment of CDI. In this review, the author took the effect of the toxin secreted by *CD* on the intestinal system as the main level, deeply analyzed the possible mechanism of the pathogenicity of TcdB, and screened for genes that may play a role in the body's response to toxins. The

importance of TcdB makes its genes have broad prospects. For the predicted genes provided by the author, some may not have been mentioned in *CD*-related studies, but this does not prevent them from being one of the possible important targets for the treatment of CDI in the future. Although little research has been done on TcdB-acting genes, it would be useful if more researchers were invested in validating against possible genes to explore whether these genes are indeed critical mechanisms for CDI and whether they could be the subject of follow-up studies for the treatment of CDI, so that genes that are truly useful can be applied to the treatment of the disease and provide a scientific basis for the clinic. The author believes that the influence of TcdB on colon tissue or inflammation will cause changes in host genes. The changes in genes can indicate the key sites of targeted therapy for CDI and open up a new path for the study of *CD*.

Availability of Data and Materials

All data generated or analyzed during this study are included in this published article.

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Author Contributions

Hu wrote the original draft, including substantive translation. Yang reviewed and edited this article. Shan collected the important information and confirmed the authenticity of all the raw data. Yang supported the financial and approved the final version to be published. All authors read and approved the final manuscript.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

References

[1] Velarde Ruiz-Velasco, J.A., Aldana-Ledesma, J.M., Ibarra-Estrada, M.A., Aguirre Díaz, S.A., Fernández-Ramírez, J.A., Cárdenas-Lara, F., *et al.* (2017) Clinical and Endoscopic Features in Patients with Hospital-Acquired Diarrhea Associated with *Clostridium difficile* Infection. *Revista de Gastroenterología de México* (*English Edition*), **82**, 301-308. https://doi.org/10.1016/j.rgmxen.2017.08.002

[2] Bartlett, J.G. (1994) *Clostridium difficile*: History of Its Role as an Enteric Pathogen and the Current State of Knowledge about the Organism. *Clinical Infectious Diseases*, **18**, S265-S272. https://doi.org/10.1093/clinids/18.Supplement_4.S265

[3] Montazeri, N., Liu, D. and Janes, M. (2015) Occurrence of Toxigenic *Clostridium difficile* in Louisiana Oysters (*Crassostrea virginica*) and Environmental Waters. *Food and Nutrition Sciences*, **6**, 1065-1070. https://doi.org/10.4236/fns.2015.611110

[4] Peterson, L.R., Mehta, M.S., Patel, P.A., Hacek, D.M., Harazin, M., Nagwekar, P.P., *et al.* (2011) Laboratory Testing for *Clostridium difficile* Infection: Light at the End of the Tunnel. *American Journal of Clinical Pathology*, **136**, 372-380. https://doi.org/10.1309/AJCPTP5XKRSNXVIL

[5] Voth, D.E. and Ballard, J.D. (2005) *Clostridium difficile* Toxins: Mechanism of Action and Role in Disease. *Clinical Microbiology Reviews*, **18**, 247-263. https://doi.org/10.1128/CMR.18.2.247-263.2005

[6] Pruitt, R.N. and Lacy, D.B. (2012) Toward a Structural Understanding of *Clostridium difficile* Toxins A and B. *Frontiers in Cellular and Infection Microbiology*, **2**, Article No. 28. https://doi.org/10.3389/fcimb.2012.00028

[7] Zhang, L.-H., Dong, D.-F., Jiang, C. and Peng, Y.-B. (2012) Toxin Detection and Ribotyping of Nosocomial *Clostridium difficile* Strains. *Journal of Shanghai Jiaotong University* (*Medical Science*), **32**, 1436-1439.

[8] Riegler, M., Sedivy, R., Pothoulakis, C., Hamilton, G., Zacherl, J., Bischof, G., *et al.* (1995) *Clostridium difficile* Toxin B Is More Potent than Toxin A in Damaging Human Colonic Epithelium *in Vitro*. *Journal of Clinical Investigation*, **95**, 2004-2011. https://doi.org/10.1172/JCI117885

[9] Tam, J., Beilhartz, G.L., Auger, A., Gupta, P., Therien, A.G. and Melnyk, R.A. (2015) Small Molecule Inhibitors of *Clostridium difficile* Toxin B-Induced Cellular Damage. *Chemistry & Biology*, **22**, 175-185. https://doi.org/10.1016/j.chembiol.2014.12.010

[10] Di Bella, S., Ascenzi, P., Siarakas, S., Petrosillo, N. and Di Masi, A. (2016) *Clostridium difficile* Toxins A and B: Insights into Pathogenic Properties and Extraintestinal Effects. *Toxins*, **8**, Article No. 134. https://doi.org/10.3390/toxins8050134

[11] Cohen, S.H., Gerding, D.N., Johnson, S., Kelly, C.P., Loo, V.G., McDonald, L.C., *et al.* (2010) Clinical Practice Guidelines for *Clostridium difficile* Infection in Adults: 2010 Update by the Society for Healthcare Epidemiology of America (SHEA) and the Infectious Diseases Society of America (IDSA). *Infection Control & Hospital Epidemiology*, **31**, 431-455. https://doi.org/10.1086/651706

[12] Peláez, T., Alcalá, L., Alonso, R., Rodríguez-Créixems, M., García-Lechuz, J.M. and Bouza, E. (2002) Reassessment of *Clostridium difficile* Susceptibility to Metronidazole and Vancomycin. *Antimicrobial Agents and Chemotherapy*, **46**, 1647-1650. https://doi.org/10.1128/AAC.46.6.1647-1650.2002

[13] Peláez, T., Cercenado, E., Alcalá, L., Marín, M., Martín-López, A., Martínez-Alarcón, J., *et al.* (2008) Metronidazole Resistance in *Clostridium difficile* Is Heterogeneous. *Journal of Clinical Microbiology*, **46**, 3028-3032. https://doi.org/10.1128/JCM.00524-08

[14] Johnson, S., Sanchez, J.L. and Gerding, D.N. (2000) Metronidazole Resistance in *Clostridium difficile*. *Clinical Infectious Diseases*, **31**, 625-626. https://doi.org/10.1086/313955

[15] Louie, T.J., Miller, M.A., Mullane, K.M., Weiss, K., Lentnek, A., Golan, Y., *et al.* (2011) Fidaxomicin versus Vancomycin for *Clostridium difficile* Infection. *New England Journal of Medicine*, **364**, 422-431. https://doi.org/10.1056/NEJMoa0910812

[16] Theriot, C.M., Koenigsknecht, M.J., Carlson Jr., P.E., Hatton, G.E., Nelson, A.M., Li, B., *et al.* (2014) Antibiotic-Induced Shifts in the Mouse Gut Microbiome and Metabolome Increase Susceptibility to *Clostridium difficile* Infection. *Nature Commu-*

nications, **5**, Article No. 3114. https://doi.org/10.1038/ncomms4114

[17] Vedantam, G., Clark, A., Chu, M., McQuade, R., Mallozzi, M. and Viswanathan, V.K. (2012) *Clostridium difficile* Infection: Toxins and Non-Toxin Virulence Factors, and Their Contributions to Disease Establishment and Host Response. *Gut Microbes*, **3**, 121-134. https://doi.org/10.4161/gmic.19399

[18] Keel, M.K. and Songer, J.G. (2007) The Distribution and Density of *Clostridium difficile* Toxin Receptors on the Intestinal Mucosa of Neonatal Pigs. *Veterinary Pathology*, **44**, 814-822. https://doi.org/10.1354/vp.44-6-814

[19] Carter, G.P., Lyras, D., Allen, D.L., Mackin, K.E., Howarth, P.M., O'Connor, J.R., *et al.* (2007) Binary Toxin Production in *Clostridium difficile* Is Regulated by CdtR, A LytTR Family Response Regulator. *Journal of Bacteriology*, **189**, 7290-7301. https://doi.org/10.1128/JB.00731-07

[20] Zheng, Y.-J. and Zhang, G.-C. (2011) *Clostridium difficile* Infection in Children. *Journal of Clinical Pediatrics*, **29**, 501-505.

[21] Carter, G.P., Chakravorty, A., Pham Nguyen, T.A., Mileto, S., Schreiber, F., Li, L., *et al.* (2015) Defining the Roles of TcdA and TcdB in Localized Gastrointestinal Disease, Systemic Organ Damage, and the Host Response during *Clostridium difficile* Infections. *MBio*, **6**, Article No. e00551. https://doi.org/10.1128/mBio.00551-15

[22] Bobo, L.D., El Feghaly, R.E., Chen, Y.S., Dubberke, E.R., Han, Z., Baker, A.H., *et al.* (2013) MAPK-Activated Protein Kinase 2 Contributes to *Clostridium difficile*-Associated Inflammation. *Infection and Immunity*, **81**, 713-722. https://doi.org/10.1128/IAI.00186-12

[23] Kelly, C.P., Becker, S., Linevsky, J.K., Joshi, M.A., O'Keane, J.C., Dickey, B.F., *et al.* (1994) Neutrophil Recruitment in *Clostridium difficile* Toxin A Enteritis in the Rabbit. *Journal of Clinical Investigation*, **93**, 1257-1265. https://doi.org/10.1172/JCI117080

[24] Huang, J., Kelly, C.P., Bakirtzi, K., Villafuerte Gálvez, J.A., Lyras, D., Mileto, S.J., Larcombe, S., *et al.* (2019) *Clostridium difficile* Toxins Induce VEGF-A and Vascular Permeability to Promote Disease Pathogenesis. *Nature Microbiology*, **4**, 269-279. https://doi.org/10.1038/s41564-018-0300-x

[25] Chen, P., Tao, L., Wang, T., Zhang, J., He, A., Lam, K.H., Liu, Z., He, X., Perry, K., Dong, M. and Jin, R. (2018) Structural Basis for Recognition of Frizzled Proteins by *Clostridium difficile* Toxin B. *Science*, **360**, 664-669. https://doi.org/10.1126/science.aar1999

[26] Tao, L., Zhang, J., Meraner, P., Tovaglieri, A., Wu, X., *et al.* (2016) Frizzled Proteins Are Colonic Epithelial Receptors for C. Difficile Toxin B. Nature, **538**, 350-355. https://doi.org/10.1038/nature19799

[27] Yuan, P., Zhang, H., Cai, C., Zhu, S., Zhou, Y., *et al.* (2015) Chondroitin Sulfate Proteoglycan 4 Functions as the Cellular Receptor for *Clostridium difficile* Toxin B. *Cell Research*, **25**, 157-168. https://doi.org/10.1038/cr.2014.169

[28] LaFrance, M.E., Farrow, M.A., Chandrasekaran, R., Sheng, J., Rubin, D.H. and Lacy, D.B. (2015) Identification of an Epithelial Cell Receptor Responsible for *Clostridium difficile* TcdB-Induced Cytotoxicity. *Proceedings of the National Academy of Sciences of the United States of America*, **112**, 7073-7078. https://doi.org/10.1073/pnas.1500791112

[29] Gupta, P., Zhang, Z., Sugiman-Marangos, S.N., Tam, J., Raman, S., *et al.* (2017) Functional Defects in *Clostridium difficile* TcdB Toxin Uptake Identify CSPG4 Receptor-Binding Determinants. *Journal of Biological Chemistry*, **292**, 17290-17301. https://doi.org/10.1074/jbc.M117.806687

[30] Terada, N., Ohno, N., Murata, S., Katoh, R., Stallcup, W.B. and Ohno, S. (2006) Immunohistochemical Study of NG2 Chondroitin Sulfate Proteoglycan Expression in the Small and Large Intestines. *Histochemistry and Cell Biology*, **126**, 483-490. https://doi.org/10.1007/s00418-006-0184-3

[31] Gregorieff, A. and Clevers, H. (2005) Wnt Signaling in the Intestinal Epithelium: From Endoderm to Cancer. *Genes & Development*, **19**, 877-890. https://doi.org/10.1101/gad.1295405

[32] Nusse, R. and Clevers, H. (2017) Wnt/β-Catenin Signaling, Disease, and Emerging Therapeutic Modalities. Cell, **169**, 985-999. https://doi.org/10.1016/j.cell.2017.05.016

[33] Qa'Dan, M., Ramsey, M., Daniel, J., Spyres, L.M., Safiejko-Mroczka, B., Ortiz-Leduc, W., *et al.* (2002) *Clostridium difficile* Toxin B Activates Dual Caspase-Dependent and Caspase-Independent Apoptosis in Intoxicated Cells. *Cellular Microbiology*, **4**, 425-434. https://doi.org/10.1046/j.1462-5822.2002.00201.x

[34] Li, S., Shi, L., Yang, Z. and Feng, H. (2013) Cytotoxicity of *Clostridium difficile* Toxin B Does Not Require Cysteine Protease-Mediated Autocleavage and Release of the Glucosyltransferase Domain into the Host Cell Cytosol. *Pathogens and Disease*, **67**, 11-18. https://doi.org/10.1111/2049-632X.12016

[35] Kreimeyer, I., Euler, F., Marckscheffel, A., Tatge, H., Pich, A., *et al.* (2011) Auto-proteolytic Cleavage Mediates Cytotoxicity of *Clostridium difficile* Toxin A. *Naunyn-Schmiedeberg's Archives of Pharmacology*, **383**, 253-262. https://doi.org/10.1007/s00210-010-0574-x

[36] Lanis, J.M., Hightower, L.D., Shen, A. and Ballard, J.D. (2012) TcdB from Hypervirulent *Clostridium difficile* Exhibits Increased Efficiency of Autoprocessing. *Molecular Microbiology*, **84**, 66-76. https://doi.org/10.1111/j.1365-2958.2012.08009.x

[37] Chumbler, N.M., Farrow, M.A., Lapierre, L.A., Franklin, J.L., Haslam, D.B., Goldenring, J.R. and Lacy, D.B. (2012) *Clostridium difficile* Toxin B Causes Epithelial Cell Necrosis through an Autoprocessing-Independent Mechanism. *PLoS Pathogens*, **8**, Article ID: e1003072. https://doi.org/10.1371/journal.ppat.1003072

[38] Ng, J., Hirota, S.A., Gross, O., Li, Y., Ulke-Lemee, A., *et al.* (2010) *Clostridium difficile* Toxin-Induced Inflammation and Intestinal Injury Are Mediated by the Inflammasome. *Gastroenterology*, **139**, 542-552.e3. https://doi.org/10.1053/j.gastro.2010.04.005

[39] Bravo, R., Frank, R., Blundell, P.A. and Macdonald-Bravo, H. (1987) Cyclin/PCNA Is the Auxiliary Protein of DNA Polymerase-Delta. *Nature*, **326**, 515-517. https://doi.org/10.1038/326515a0

[40] Kannouche, P.L., Wing, J. and Lehmann, A.R. (2004) Interaction of Human DNA Polymerase Eta with Monoubiquitinated PCNA: A Possible Mechanism for the Polymerase Switch in Response to DNA Damage. *Molecular Cell*, **14**, 491-500. https://doi.org/10.1016/S1097-2765(04)00259-X

[41] Bravo, R. and Macdonald-Bravo, H. (1987) Existence of Two Populations of Cyclin/proliferating Cell Nuclear Antigen during the Cell Cycle: Association with DNA Replication Sites. *Journal of Cell Biology*, **105**, 1549-1554. https://doi.org/10.1083/jcb.105.4.1549

[42] Danova, M., Riccardi, A., Giordano, M., Girino, M., Mazzini, G., Dezza, L., *et al.* (1988) Cell Cycle-Related Proteins: A Flow Cytofluorometric Study in Human Tumors. *Biology of the Cell*, **64**, 23-28. https://doi.org/10.1016/0248-4900(88)90089-5

[43] Jin, E.H., Lee, D.H., Jung, S.A., Shim, K.N., Seo, J.Y., Kim, N., *et al.* (2015) Clinicopathologic Factors and Molecular Markers Related to Lymph Node Metastasis in

Early Gastric Cancer. *World Journal of Gastroenterology*, **21**, 571-577. https://doi.org/10.3748/wjg.v21.i2.571

[44] Wang, L., Kong, W., Liu, B. and Zhang, X. (2018) Proliferating Cell Nuclear Antigen Promotes Cell Proliferation and Tumorigenesis by Up-Regulating STAT3 in Non-Small Cell Lung Cancer. *Biomedicine & Pharmacotherapy*, **104**, 595-602. https://doi.org/10.1016/j.biopha.2018.05.071

[45] Ma, S., Yang, J., Li, J. and Song, J. (2016) The Clinical Utility of the Proliferating Cell Nuclear Antigen Expression in Patients with Hepatocellular Carcinoma. *Tumor Biology*, **37**, 7405-7412. https://doi.org/10.1007/s13277-015-4582-9

[46] Sheen-Chen, S.M., Ho, H.T., Chen, W.J. and Eng, H.L. (2003) Obstructive Jaundice Alters Proliferating Cell Nuclear Antigen Expression in Rat Small Intestine. *World Journal of Surgery*, **27**, 1161-1164. https://doi.org/10.1007/s00268-003-6992-x

[47] Ohmori, Y., Schreiber, R.D. and Hamilton, T.A. (1997) Synergy Between Interferon-Gamma and Tumor Necrosis Factor-Alpha in Transcriptional Activation Is Mediated by Cooperation Between Signal Transducer and Activator of Transcription 1 and Nuclear Factor κB. *Journal of Biological Chemistry*, **272**, 14899-14907. https://doi.org/10.1074/jbc.272.23.14899

[48] De Nardo, D.G. and Coussens, L.M. (2007) Inflammation and Breast Cancer. Balancing Immune Response: Crosstalk between Adaptive and Innate Immune Cells during Breast Cancer Progression. *Breast Cancer Research*, **9**, Article No. 212. https://doi.org/10.1186/bcr1746

[49] Vinkemeier, U. (2004) Getting the Message Across, STAT! Design Principles of a Molecular Signaling Circuit. *Journal of Cell Biology*, **167**, 197-201. https://doi.org/10.1083/jcb.200407163

[50] Chen, X., Vinkemeier, U., Zhao, Y., Jeruzalmi, D., Darnell Jr., J.E. and Kuriyan, J. (1998) Crystal Structure of a Tyrosine Phosphorylated STAT-1 Dimer Bound to DNA. *Cell*, **93**, 827-839. https://doi.org/10.1016/S0092-8674(00)81443-9

[51] Darnell Jr., J.E., Kerr, I.M. and Stark, G.R. (1994) Jak-STAT Pathways and Transcriptional Activation in Response to IFNs and Other Extracellular Signaling Proteins. *Science*, **264**, 1415-1421. https://doi.org/10.1126/science.8197455

[52] Lee, C.K., Smith, E., Gimeno, R., Gertner, R. and Levy, D.E. (2000) STAT1 Affects Lymphocyte Survival and Proliferation Partially Independent of Its Role Downstream of IFN-Gamma. *The Journal of Immunology*, **164**, 1286-1292. https://doi.org/10.4049/jimmunol.164.3.1286

[53] Neunlist, M., Barouk, J., Michel, K., Just, I., Oreshkova, T., Schemann, M., *et al.* (2003) Toxin B of *Clostridium difficile* Activates Human VIP Submucosal Neurons, in Part via an IL-1β-Dependent Pathway. *American Journal of Physiology: Gastrointestinal and Liver Physiology*, **285**, G1049-G1055. https://doi.org/10.1152/ajpgi.00487.2002

[54] Han, S.Y. and Kim, Y.K. (2019) Berberine Suppresses RANKL-Induced Osteoclast Differentiation by Inhibiting c-Fos and NFATc1 Expression. *The American Journal of Chinese Medicine*, **47**, 439-455. https://doi.org/10.1142/S0192415X19500228

[55] Tzeng, H.E., Tsai, C.H., Ho, T.Y., Hsieh, C.T., Chou, S.C., Lee, Y.J., *et al.* (2018) Radix Paeoniae Rubra Stimulates Osteoclast Differentiation by Activation of the NF-κB and Mitogen-Activated Protein Kinase Pathways. *BMC Complementary Medicine and Therapies*, **18**, Article No. 132. https://doi.org/10.1186/s12906-018-2196-7

[56] Hunter, R.H. (1993) Sperm: Egg Ratios and Putative Molecular Signals to Modulate Gamete Interactions in Polytocous Mammals. *Molecular Reproduction and Development*, **35**, 324-327. https://doi.org/10.1002/mrd.1080350315

[57] Keyse, S.M. (2008) Dual-Specificity MAP Kinase Phosphatases (MKPs) and Cancer. *Cancer and Metastasis Reviews*, **27**, 253-261. https://doi.org/10.1007/s10555-008-9123-1

[58] Li, Y., Xu, S., Xu, Q. and Chen, Y. (2020) *Clostridium difficile* Toxin B Induces Colonic Inflammation through the TRIM46/DUSP1/MAPKs and NF-κB Signalling Pathway. *Artificial Cells, Nanomedicine, and Biotechnology*, **48**, 452-462. https://doi.org/10.1080/21691401.2019.1709856

[59] Hammer, M., Echtenachter, B., Weighardt, H., Jozefowski, K., Rose-John, S., Männel, D.N., *et al.* (2010) Increased Inflammation and Lethality of Dusp1$^{-/-}$ Mice in Polymicrobial Peritonitis Models. *Immunology*, **131**, 395-404. https://doi.org/10.1111/j.1365-2567.2010.03313.x

[60] Gil-Araujo, B., Toledo Lobo, M.V., Gutiérrez-Salmerón, M., Gutiérrez-Pitalúa, J., Ropero, S., Angulo, J.C., *et al.* (2014) Dual Specificity Phosphatase 1 Expression Inversely Correlates with NF-κB Activity and Expression in Prostate Cancer and Promotes Apoptosis through A P38 MAPK Dependent Mechanism. Molecular Oncology, **8**, 27-38. https://doi.org/10.1016/j.molonc.2013.08.012

[61] Li, X.Y., Liang, J.W., Mohamed, O.K., Zhang, T.J., Lu, G.Q. and Meng, F.H. (2018) Design, Synthesis and Biological Evaluation of N-Phenyl-(2,4-Dihydroxypyrimidine-5-Sulfonamido) Benzoyl Hydrazide Derivatives as Thymidylate Synthase (TS) Inhibitors and as Potential Antitumor Drugs. *European Journal of Medicinal Chemistry*, **154**, 267-279. https://doi.org/10.1016/j.ejmech.2018.05.020

[62] Lu, G.Q., Li, X.Y., Mohamed, O.K., Wang, D. and Meng, F.H. (2019) Design, Synthesis and Biological Evaluation of Novel Uracil Derivatives Bearing 1, 2, 3-Triazole Moiety as Thymidylate Synthase (TS) Inhibitors and as Potential Antitumor Drugs. *European Journal of Medicinal Chemistry*, **171**, 282-296. https://doi.org/10.1016/j.ejmech.2019.03.047

[63] Bai, W., Wu, Y., Zhang, P. and Xi, Y. (2015) Correlations Between Expression Levels of Thymidylate Synthase, Thymidine Phosphorylase and Dihydropyrimidine Dehydrogenase, and Efficacy of 5-Fluorouracil-Based Chemotherapy for Advanced Colorectal Cancer. *International Journal of Clinical and Experimental Pathology*, **8**, 12333-12345.

[64] Jiang, H., Li, B., Wang, F., Ma, C. and Hao, T. (2019) Expression of ERCC1 and TYMS in Colorectal Cancer Patients and the Predictive Value of Chemotherapy Efficacy. *Oncology Letters*, **18**, 1157-1162. https://doi.org/10.3892/ol.2019.10395

[65] Torén, W., Ansari, D., Andersson, B., Spelt, L. and Andersson, R. (2018) Thymidylate Synthase: A Predictive Biomarker in Resected Colorectal Liver Metastases Receiving 5-FU Treatment. *Future Oncology*, **14**, 343-351. https://doi.org/10.2217/fon-2017-0431

[66] Nagalakshmi, M.L., Rascle, A., Zurawski, S., Menon, S. and De Waal Malefyt, R. (2004) Interleukin-22 Activates STAT3 and Induces IL-10 by Colon Epithelial Cells. *International Immunopharmacology*, **4**, 679-691. https://doi.org/10.1016/j.intimp.2004.01.008

[67] Dudakov, J.A., Hanash, A.M. and Van Den Brink, M.R. (2015) Interleukin-22: Immunobiology and Pathology. *Annual Review of Immunology*, **33**, 747-785. https://doi.org/10.1146/annurev-immunol-032414-112123

[68] Brandt, M., Grazioso, T.P., Fawal, M.A., Tummala, K.S., Torres-Ruiz, R., Rodriguez-Perales, S., *et al.* (2018) MTORC1 Inactivation Promotes Colitis-Induced Colorectal Cancer But Protects from APC Loss-Dependent Tumorigenesis. *Cell Metabolism*, **27**, 118-135.e8. https://doi.org/10.1016/j.cmet.2017.11.006

[69] McGilvray, I.D., Serghides, L., Kapus, A., Rotstein, O.D. and Kain, K.C. (2000) Non-opsonic Monocyte/Macrophage Phagocytosis of Plasmodium Falciparum-Parasitized Erythrocytes: A Role for CD36 in Malarial Clearance. *Blood*, **96**, 3231-3240. https://doi.org/10.1182/blood.V96.9.3231

[70] Baranova IN, Kurlander R, Bocharov AV, Vishnyakova TG, Chen Z, Remaley AT, *et al.* (2008) Role of Human CD36 in Bacterial Recognition, Phagocytosis, and Pathogen-Induced JNK-Mediated Signaling. *The Journal of Immunology*, **181**, 7147-7156. https://doi.org/10.4049/jimmunol.181.10.7147

[71] Wullschleger, S., Loewith, R. and Hall, M.N. (2006) TOR Signaling in Growth and Metabolism. *Cell*, **124**, 471-484. https://doi.org/10.1016/j.cell.2006.01.016

[72] Düvel, K., Yecies, J.L., Menon, S., Raman, P., Lipovsky, A.I., Souza, A.L., *et al.* (2010) Activation of A Metabolic Gene Regulatory Network Downstream of MTOR Complex 1. *Molecular Cell*, **39**, 171-183. https://doi.org/10.1016/j.molcel.2010.06.022

[73] Bononi, A., Agnoletto, C., De Marchi, E., Marchi, S., Patergnani, S., Bonora, M., *et al.* (2011) Protein Kinases and Phosphatases in the Control of Cell Fate. *Enzyme Research*, **2011**, Article ID: 329098. https://doi.org/10.4061/2011/329098

[74] Yang, K., Shrestha, S., Zeng, H., Karmaus, P.W., Neale, G., Vogel, P., *et al.* (2013) T Cell Exit from Quiescence and Differentiation into Th2 Cells Depend on Raptor-MTORC1-Mediated Metabolic Reprogramming. *Immunity*, **39**, 1043-1056. https://doi.org/10.1016/j.immuni.2013.09.015

[75] Dan, H.C., Cooper, M.J., Cogswell, P.C., Duncan, J.A., Ting, J.P. and Baldwin, A.S. (2008) Akt-Dependent Regulation of NF-κB Is Controlled by MTOR and Raptor in Association with IKK. *Genes & Development*, **22**, 1490-1500. https://doi.org/10.1101/gad.1662308

[76] Wang, C., Gao, C. and Li, C. (2020) Expression and Correlation of CDK4 and PD-L1 in Colorectal Cancer Tissues. *Journal of Medical Research*, **49**, 158-162.

[77] Dong, X., Wang, J. and Yu, B. (2018) Relationship between the Expression of Geminin, CDK4 and Biological Behaviors of Colorectal Carcinoma. *Chinese Journal of Operative Procedures of General Surgery* (*Electronic Version*), **12**, 346-348. http://doi.org/10.3877/cma.j.issn.1674-3946.2018.04.024

[78] Chapados, B.R., Hosfield, D.J., Han, S., Qiu, J., Yelent, B., Shen, B., *et al.* (2004) Structural Basis for FEN-1 Substrate Specificity and PCNA-Mediated Activation in DNA Replication and Repair. *Cell*, **116**, 39-50. https://doi.org/10.1016/S0092-8674(03)01036-5

[79] Singh, P., Yang, M., Dai, H., Yu, D., Huang, Q., Tan, W., *et al.* (2008) Overexpression and Hypomethylation of *Flap Endonuclease* 1 Gene in Breast and Other Cancers. *Molecular Cancer Research*, **6**, 1710-1717. https://doi.org/10.1073/pnas.0813414106

[80] McManus, K.J., Barrett, I.J., Nouhi, Y. and Hieter, P. (2009) Specific Synthetic Lethal Killing of RAD54B-Deficient Human Colorectal Cancer Cells by FEN1 Silencing. *Proceedings of the National Academy of Sciences of the United States of America*, **106**, 3276-3281. https://doi.org/10.1016/j.jaci.2015.01.040

[81] Mathieu, A.L., Verronese, E., Rice, G.I., Fouyssac, F., Bertrand, Y., Picard, C., *et al.* (2015) PRKDC Mutations Associated with Immunodeficiency, Granuloma, and Autoimmune Regulator-Dependent Autoimmunity. *Journal of Allergy and Clinical Immunology*, **135**, 1578-1588.e5.

[82] Sun, S., Cheng, S., Zhu, Y., Zhang, P., Liu, N., Xu, T., *et al.* (2016) Identification of PRKDC (Protein Kinase, DNA-Activated, Catalytic Polypeptide) as an Essential

Gene for Colorectal Cancer (CRCs) Cells. *Gene*, **584**, 90-96. https://doi.org/10.1016/j.gene.2016.03.020

[83] Lu, Y., Zhang, H.L., Li, Y.Z. and Zhao, P. (2008) Clinicopathological Significance of Expressions of DNA Dependent Protein Kinase Catalytic Subunit and P16 in Colorectal Carcinoma. *National Medical Journal of China*, **88**, 2025-2029.

[84] Davidson, D., Coulombe, Y., Martinez-Marignac, V.L., Amrein, L., Grenier, J., Hodkinson, K., *et al.* (2012) Irinotecan and DNA-PKcs Inhibitors Synergize in Killing of Colon Cancer Cells. *Investigational New Drugs*, **30**, 1248-1256. https://doi.org/10.1007/s10637-010-9626-9

[85] Wu, N., Yang, X., Zhang, R., Li, J., Xiao, X., Hu, Y., *et al.* (2013) Dysbiosis Signature of Fecal Microbiota in Colorectal Cancer Patients. *Microbial Ecology*, **66**, 462-4670. https://doi.org/10.1007/s00248-013-0245-9

[86] Kostic, A.D., Gevers, D., Pedamallu, C.S., Michaud, M., Duke, F., Earl, A.M., *et al.* (2012) Genomic Analysis Identifies Association of Fusobacterium with Colorectal Carcinoma. *Genome Research*, **22**, 292-298. https://doi.org/10.1101/gr.126573.111

[87] Li, S., Liu, H., Xin, Y., Wang, L., Fu, S., Ma, L., *et al.* (2018) Correlation between *Clostridium difficile* Infection and Colorectal Cancer and Colorectal Adenoma. *Chinese Journal of Microecology*, **30**, 300-303.