

Gastrointestinal disasters of cetuximab in the treatment of metastatic colorectal cancer: Mechanism and its effect on prognosis

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Abstract

Colorectal cancer (CRC) is among the top three cancers worldwide in terms of incidence and associated mortality. Colorectal cancer (CRC) is responsible for more than 880,000 deaths annually. The number of CRC cases worldwide continues to increase, posing a serious threat to human health. Surgery and chemotherapy are the first treatments for CRC patients. The majority of CRC patients are diagnosed at an advanced stage, as symptoms are usually not apparent and difficult to diagnose in the early stage. The prognosis of metastatic CRC (mCRC) has long been unsatisfactory. Targeted drugs therapy, which targeting at specific genes and proteins, is a new treatment approach to CRC. Cetuximab is one of the most widely studied targeted drugs. By competitively binding to the epithelial growth factor receptor (EGFR), cetuximab inhibits the EGF and binding of the EGF ligand to the EGFR, thereby inhibiting tumor cell growth, invasion, and metastasis and inducing tumor cell apoptosis. The curative effect of cetuximab as a treatment for many kinds of tumors, especially mCRC, has been confirmed. Cetuximab combined with chemotherapy or monotherapy is used as first-line treatment in patients with RAS(rat sarcoma,Ras) wild-type mCRC. However, adverse drug reactions (ADRs) associated with the clinical application of cetuximab are attracting increasing attention, with numerous studies reporting adverse effects of cetuximab on the gastrointestinal system, with these effects having adverse consequences for the prognosis of CRC. In this review, we focus mainly on gastrointestinal disasters on cetuximab treatment for mCRC from three areas: the intestinal mucosal barrier (IMB), gut microbiota (GM)-host immune balance, and bacterial metabolites short chain fatty acids (SCFAs), and the impact of these effects on the prognosis of mCRC. We also make suggestions aimed at aiding oncological understanding of cetuximab as a treatment for mCRC.

Keywords: Bacterial metabolites, cetuximab, colorectal cancer, gut microbiota-host immune balance, gastrointestinal disasters

Introduction

Cetuximab is an immunoglobulin G1(IgG1)human/mouse chimeric monoclonal antibody targeting the extracellular region of the EGFR [1]. It can specifically bind to EGFR-related domains on the surface of a variety of cancer cells, competitively block the corresponding ligands, and inhibit the activation of RAS-RAF. RAS-RAF can induce the

phosphorylation and activation of receptor-related kinases (MAPK, MEK, and ERK) and regulate the expression of transcription factors [2]. Cetuximab can also activate PI3K and affect the SH3 domain of AKT, thereby regulating cell growth and apoptosis. Moreover, by inhibiting the activation of PLC- γ 1 through the EGFR, EGFR inhibitors affect cell movement, growth, and differentiation. This can lead to membrane wrinkling, which is critical for the proliferation and apoptosis of cancer cells [3]. In addition to inhibiting EGFRs, cetuximab exerts antitumor effects in many other ways, such as inhibiting the production of vascular endothelial growth factor (VEGF), inducing natural killer cells (NK cells) to kill tumor cells through antibody-dependent cell mediated cytotoxicity, regulating hypoxia factor 1-a and Bcl-2 proto oncogenes, activating the autophagy genes BECLIN1 and HVPS34, and inducing the autophagy of tumor cells [4]. Thus, cetuximab plays an antitumor role at multiple levels and paths.

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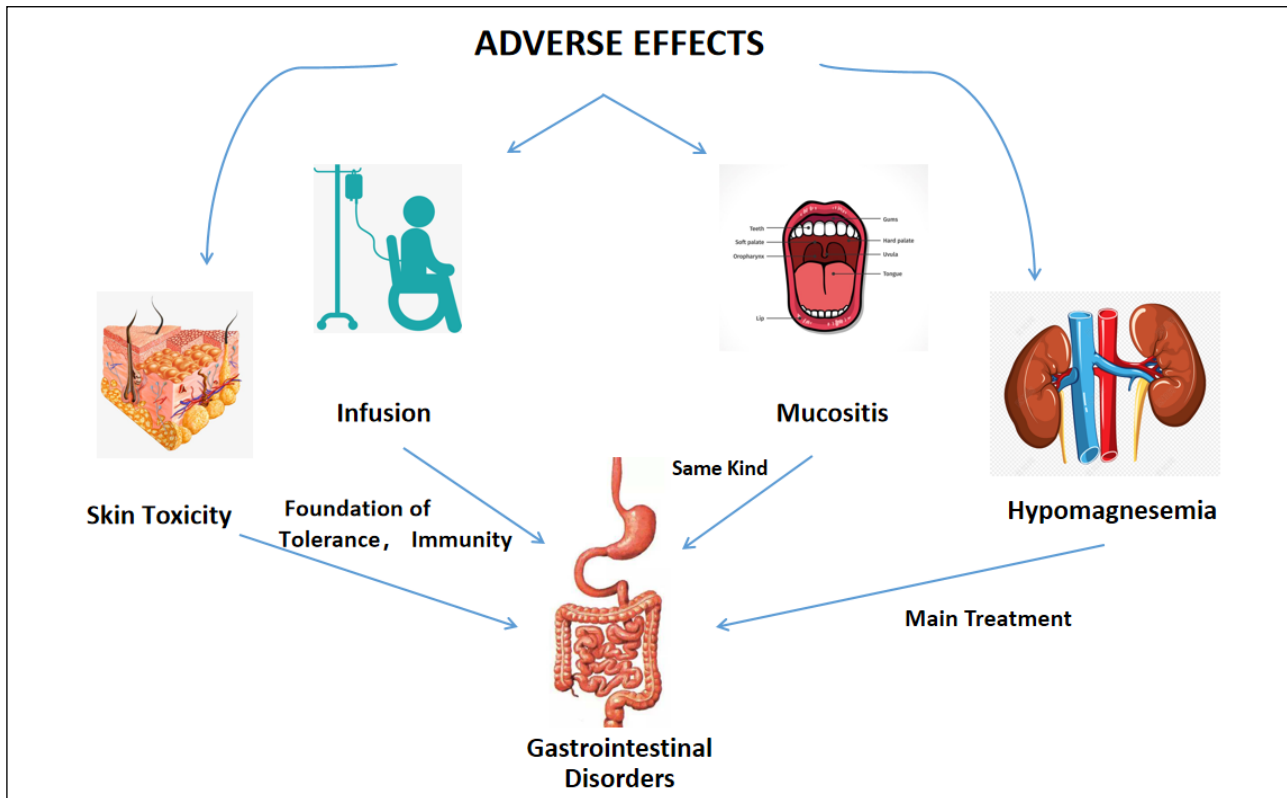


Figure 1. The role of gastrointestinal disorders in major adverse drug reactions.

Phase III clinical trials showed that combination treatment with cetuximab and irinotecan [5], FOLFOX [6], or multitarget drugs [7-9] was more effective in mCRC treatment than cetuximab treatment alone. The National Comprehensive Cancer Network and Food and Drug Administration have recommended that cetuximab combined with classical chemotherapy can be used as a first-line treatment in patients with RAS wild-type mCRC [10]. Notably, not all RAS wild-type CRC patients are sensitive to cetuximab, with studies showing that mutations of the RAS gene (NRAS and KRAS) [11] and V600E BRAF [12] caused drug resistance. In previous research, a PI3K mutation and PTEN loss affected the efficacy of cetuximab combined with chemotherapy in mCRC treatment [13].

In terms of adverse drug reactions (ADRs), skin reactions, hypomagnesemia, mucositis, and infusion-related reactions have attracted much attention [14]. Gastrointestinal disorders (GDs) refer to the expression of mucositis in the intestinal mucosa [15]. We find GDs play significant roles in all ADRs (Figure 1) and relate to the occurrence, treatment, and tolerance. In addition, GDs affect the efficacy of cetuximab in many ways and have a profound impact on the prognosis of CRC.

Previous reviews focused on the mechanism of action and resistance of cetuximab [16,17]. We review mainly adverse drug reactions (ADRs) of cetuximab when administered as monotherapy or in combination with classical chemotherapy, focusing on the mechanism underlying its effects on the gastrointestinal system and its influence on the prognosis of mCRC. We review clinical studies of ce-

tuximab in combination with chemotherapy, radiotherapy, and multitarget drugs combinations.

ADRs of Cetuximab

Cetuximab is generally well tolerated. However, with the clinical application of cetuximab, its adverse drug reactions (ADRs) are attracting increasing attention. The main ADRs include skin reactions, hypomagnesemia, mucositis, and transfusion-related reactions [18]. It should be noted that GDs jump to the most important ADRs after the application of cetuximab combined with chemotherapy [19]. According to the Food and Drug Administration Adverse Event Reporting System and the EudraVigilance database, the majority of ADRs of cetuximab affect the gastrointestinal system (Figure 2). However, in most cases, these skin reactions are mild or moderate rashes [20]. Based on our review of clinical studies, the incidence of severe rashes associated with cetuximab is low [21]. Infusion-related reactions may recur, they can be effectively controlled by conventional antihistamines and corticosteroids [22]. Although the digestive tract is not directly related to skin reactions and infusion reactions, its condition is closely related to the nutrition and immune status of the patient, which is the basis of ADR tolerance [23]. The EGF regulates the activity and distribution of TRPM6 and mutations in the EGFR gene. Cetuximab acts on EGFRs expressing a lot in the kidney, resulting in hypomagnesemia. Controlling GDs, especially diarrhea, is the first step

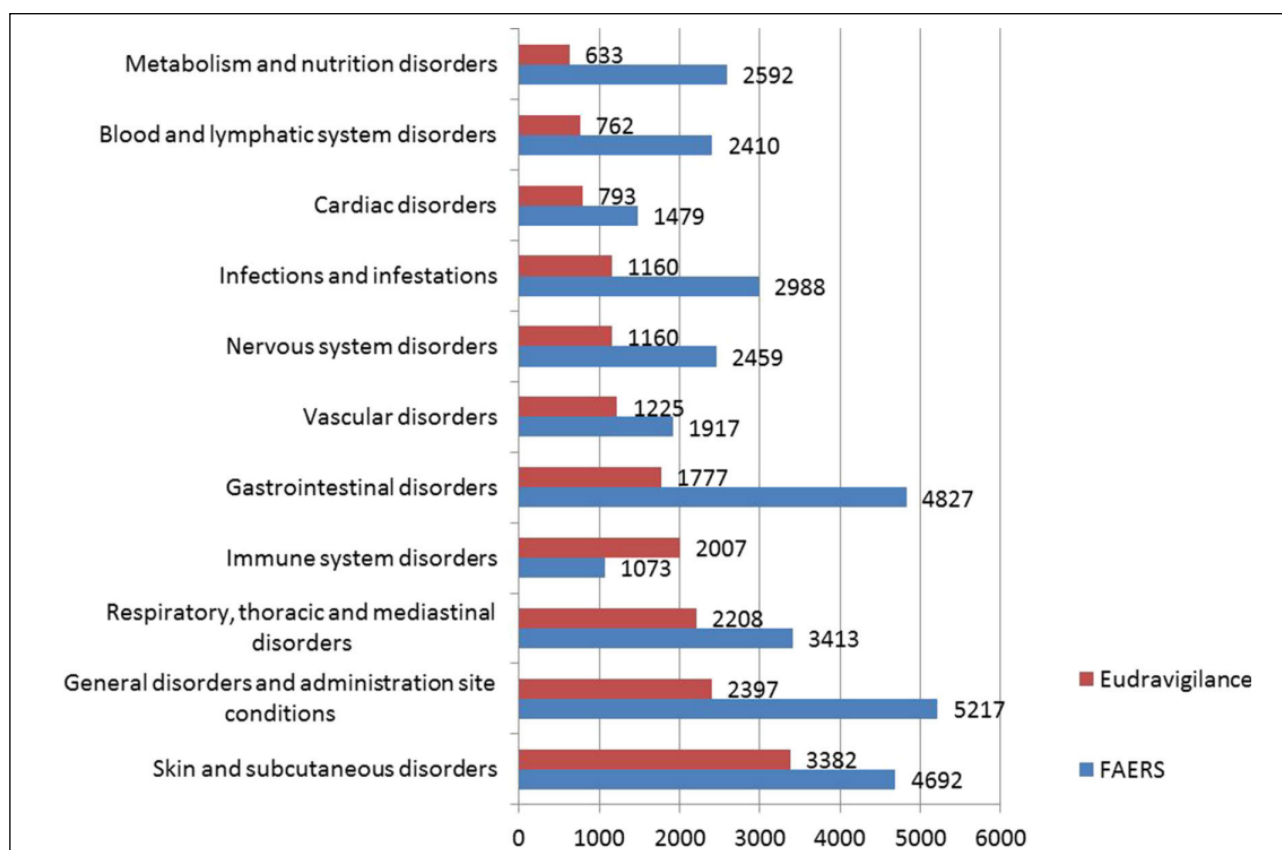


Figure 2. Numbers of cases of ADRs reported in EudraVigilance and FEARS (cited from Fornasier, G) [18].

in alleviating hypomagnesemia [24]. In terms of mucositis, most reports have focused on oral mucosal lesions [25]. However, mucosa are widely distributed in the digestive tract, where EGFRs are widely expressed. Thus, GDs are symptoms of mucositis in the digestive tract. Several studies have confirmed a direct causal relation between gastrointestinal disorders (GDs) and intestinal mucositis [26,27]. Based on the discussion above, it can be concluded that ADRs of cetuximab include GDs.

To sum up, Many studies have found that ADRs of anti-tumor drugs, including cetuximab, occur through multiple channels and that these ADRs, affect the anti-tumor effect of the drugs, tumoral progression, and the prognosis of CRC [28]. In this review, we focus on the impacts of ADRs of cetuximab on the intestinal mucosal barrier (IMB), gut microbiota (GM)-host immune balance, and microbial metabolites short chain fatty acids (SCFAs) (Figure 3).

IMB induces GDs and Contributes to the Tumor Growth Microenvironment

The destruction of the IMB plays a vital role in inducing mucositis [29]. Mucositis in the gastrointestinal tract is a major ADR of cetuximab and the most common ADR of cetuximab when it is combined with irinotecan and FOLFOX [6,30]. The main mechanism underlying

mucositis induced by cetuximab of EGFR inhibition as epidermal growth factor receptors (EGFRs) are widely distributed in the digestive tract, and 25–77% of CRC cases overexpress EGFRs [31]. According to our review, EGFR inhibitors affect almost all the major components of the IMB. The IMB can broadly be divided into a physical barrier and chemical barrier [32]. The physical barrier consists of four types of intestinal epithelial cells: absorptive intestinal cells, goblet cells producing mucin, Paneth cells producing antimicrobial peptides (AMPs), and endocrine cells produced by hormones. The chemical barrier mainly consists of a mucus layer containing mucin and AMPs and secretory immunoglobulin A (sIgA) [33]. Mucin is the skeleton of the mucus layer and works as isolation. IgA and AMPs can kill GM directly and work with mucin to isolate bacteria from intestinal epithelial cells, including symbionts [34]. Yasuda-Onozawa et al. found that the EGFR/Akt serine/threonine kinase 1 pathway induced the expression of mucin 2 and oligomeric mucus/gel forming mRNA and promoted the production of mucin in goblet cells [35]. EGFR inhibitors reduce the production of mucin, and the integrity of the IMB is difficult to maintain when mucin production is reduced. Gut microbiota (GM) and other components in the gut directly break through the gap of the IMB and come into contact with intestinal epithelial cells, resulting in mucosal inflammation [36]. This may be the initial mechanism of EGFR inhibition, leading to GDs and directly or indirectly inducing

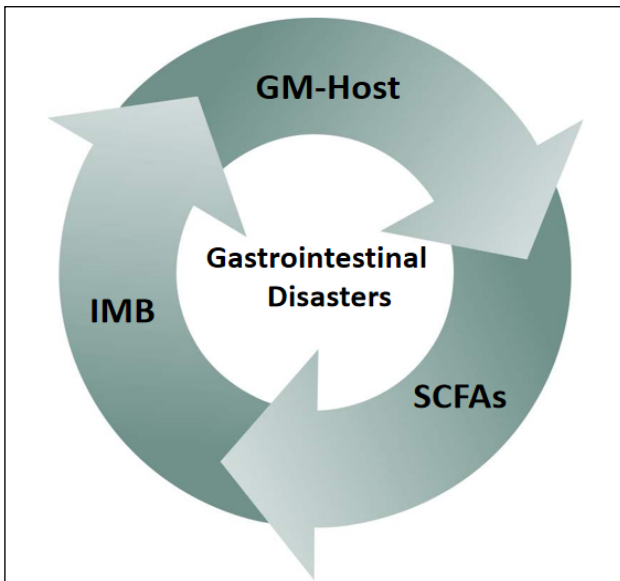


Figure 3. Interactions between the intestinal mucosal barrier (IMB), gut microbiota (GM)-host immune balance, and short chain fatty acids (SCFAs) leading to GDs.

multiple domino effects. Previous research showed that butyrate, a bacterial metabolite, increased mucin secretion [37]. If GMs are disordered by drugs and the inflammatory environment, butyrate will decrease significantly, and then, mucin production decrease with it. ErbB 3 is a member of the EGFR family, which inhibits Atoh 1 levels, mediated by PI3K, to limit the number of Paneth cells and AMPs will reduce after that [38]. Meanwhile, dendritic cells (DCs) are activated in the inflammatory state and induce B cells to produce IgA [39]. Although AMPs and IgA increase in response to EGFR inhibitors and kill invasive GM, they do not act as a physical barrier. In addition, a suitable reaction place for AMPs and IgA decrease due to mucin deficiency [40]. Thus, increasing the concentration of AMP and IgA cannot completely prevent bacteria from contacting with the IMB intestinal epithelial cells. On the contrary, Extensive GM mortality results in a dramatic reduction in butyrate production and an associated reduction in mucin. In addition, previous research showed that inflammation led to high permeability in and between epithelial cells and diarrhea exacerbates the loss of active ingredients of the IMB [41,42].

In mCRC, the status of the IMB is closely related to intestinal inflammation which is associated with the tumor growth environment and prognosis of CRC patients [43]. Since 1863, inflammation has been recognized as a high risk factor for cancer with less than 10% of cancers caused by gene mutations, and more than 20% related to microbial infections [44]. Chronic inflammation is a recognized risk factor for CRC, and most patients with mCRC have chronic inflammation [45]. Intestinal flora disturbance caused by drugs and tumor rejection aggravate the original intestinal inflammation [46]. Following the destruction of the IMB, the immune response and inflammatory response induce the proliferation and differentiation of a variety of

immune cells, which produce a large number of cytokines, forming a microenvironment for tumor growth that facilitates the occurrence, maintenance, and development of tumors [47]. Therefore, the destruction of the IMB caused by EGFR inhibitors initiates mucositis in the gut and not only contributes to ADRs but also antagonizes the curative effect of antitumor drugs, thereby having a profound impact on the prognosis.

The Destruction of the GM-Host Immune Balance Affects the Prognosis of mCRC

The immune balance between GM and the host is the result of coevolution [48]. The host provides a stable environment for GM, which have a wide range of functions, affecting the occurrence and development of various diseases, such as inflammatory bowel disease (IBD) and CRC [49]. The gut immune system must maintain a delicate balance between tolerance and immunity. It is found that this effect is realized by butyrate. Butyrate, a short chain fatty acid (SCFA), is the main metabolite of intestinal bacteria [50]. Butyrate can inhibit the differentiation of bone marrow stem cells into DCs, thereby maintaining host immunoreactivity at a low level [51]. Drugs, especially antibiotics and antitumor drugs, can lead to the destruction of the GM-host immune balance, with greater effects than either diet or inflammation [52]. A previous study showed that cetuximab combined with XELOX did not significantly improve overall survival and progression-free survival (PFS) of patients with mCRC as compared with XELOX alone [53]. These suggest that the intestinal environment influence the drug efficacy. In the following, we will illustrate this effect from two aspects: host and GMs.

In terms of the host, a variety of host immune cells are activated by inflammation and the immune response, among which the proliferation and differentiation of DCs upregulate a proportion of B cells and T (Th1, Th2, Th17, and Treg) cells [54], leading to chemotherapy-induced enteritis and other side effects. Various growth factors, reactive oxygen species, and nitrogen produced by inflammatory cells persisting damage DNAs under and even after the inflammatory state. As a result, DNAs damaging give rise to gene mutations and the potential development of cancers [55].

Under pathological conditions, the proliferation and differentiation of DCs induced by intestinal bacteria due to translocation of bacteria across the IMB, allowing the bacteria to come into contact with intestinal epithelial cells [56]. In contrast, under normal conditions, Metabolites, such as butyrate, can pass the IMB and contact with intestinal epithelial cells easily [57]. These metabolites will inhibit DCs proliferation and differentiation, but bacteria face many difficulties in front of IMB (Figure 4). Panbianco [58] confirmed that the immune and inflammation effect causing by bacteria was not realized by its translocation but metabolite. The latter explains why the host immune system can be activated, as well as inhibited, by GM.

Specifically, GM include bacteria, fungi, archaea, viruses

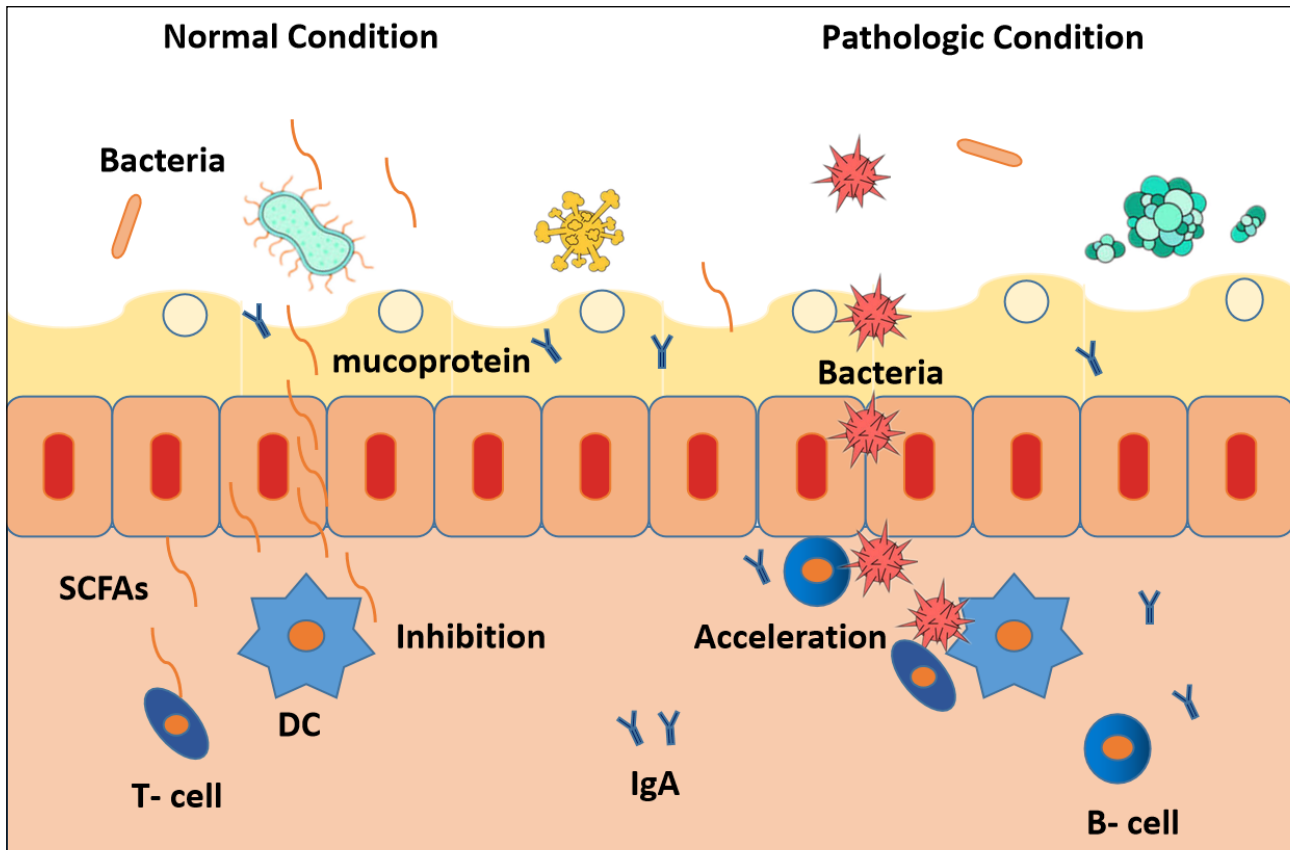


Figure 4. The action mode of IBM, GT-Host , SCFAs working under normal condition versus pathologic condition.

and parasites [59]. We're concerned about the effects of bacteria on the host and disease because bacteria are dominant in GM. GM disorders caused by drugs are mainly manifested by a decrease in the quantity and type [60]. In patients treated with irinotecan and FOLFOX, the number and species of intestinal bacteria all decrease shown by 16S rRNA gene detection [61]. In an epidemiological investigation, a decrease in GM was associated with an increase in CRC morbidity [62]. Another study found that bacteria favored mucin as a carbon source in the absence of dietary fiber, which further aggravated IMB damage, increased bacterial contact with intestinal epithelial cells, and promoted the formation of a tumor microenvironment in the host gut [63]. However, whether this phenomenon exists in CRC patients treated with antibiotics and chemotherapy drugs has not been studied. Therefore, it can be concluded the destruction of the IMB by EGFR inhibitors leads to the destruction of the GM-host immune balance. The latter is a vital mechanism underlying gastrointestinal disorders, drug efficacy, and prognosis of mCRC.

SCFAs Have Anti-Inflammatory and Antitumor Effects

It can be seen from the above discussion that the integrity of the IMB and the immune balance between GM and the host are interdependent and interacted. SCFAs are running

with them working as important media and signal [64]. SCFAs, metabolites of bacteria degraded from dietary fiber, include acetate, propionate, and butyrate. GMs exert an antitumor effect mainly through their metabolites [65]. Histone deacetylase inhibitors are widely used in cancer treatment [66]. Both propionate and butyrate inhibit histone deacetylase activity [67]. Propionate and butyrate decrease because of bacteria are damaged by antitumor drugs. As a result, the antitumor power of the host itself is clipped. According to some studies, the SCFA acetate plays a protective signal molecule acting on G protein-coupled receptor 109A(GPR109A) [68] and GPR43/41 [69] of host cells to regulate their energy metabolism. In CRC, tumor location is an important factor in determining the reactivity of cetuximab [70]. Colorectal cancer (CRC) is usually classified as left or right sided, depending on the location of the tumor or tumors. In left-sided CRC, the tumors originate in the flexure of the spleen, descending colon, and sigmoid colon, whereas in right-sided CRC, the tumors originate in the cecum, ascending colon, flexure of the liver, and transverse colon [71]. As shown by multiple studies, EGFR inhibitors (such as cetuximab) are superior to left colon cancer with RAS wild-type comparing with the right, and it is recommended that patients with left RAS wild-type colon cancer should be given EGFR inhibitors [72,73]. Interestingly, SCFAs show a remarkable distribution and absorption gradient [74]. SCFAs are well absorbed in the distal colon but not fully

absorbed in the proximal colon. According to Cummings et al., the transport effect of the sodium-coupled monocarboxylate transporter SLC5A8 and H-coupled low-affinity monocarboxylate transporter SLC16A makes the absorption efficiency of SCFAs greater in the distal colon than that in the proximal [75]. SCFAs play a biological role on the host only after they are absorbed [76]. As SCFAs have been shown to be effective as intestinal mucosal protective agents and to have antitumor functions, the absorption efficiency of SCFAs potentially explain the efficacy of cetuximab in CRC patients. However, the reason for the difference in the positional sensitivity of cetuximab in the colon is not clear, and research is lacking on the specific mechanism of SCFAs related to the positional sensitivity of cetuximab. However, we can conclude that there is a correlation between the efficacy of cetuximab and the function of SCFAs.

Conclusion

Most ADRs (skin reactions, hypomagnesemia, mucositis, and transfusion-related reactions) of cetuximab are related to the inhibition of EGFRs and the mode of administration. Diarrhea and nausea are also common ADRs of cetuximab. ADRs of the digestive tract are common in patients treated with cetuximab combined with chemotherapy. Gastrointestinal disorders (GDs) are a manifestation of mucositis in the digestive tract. The health of the digestive tract is closely related to the nutritional status and immune status of the human body, which is the basis of tolerance to ADRs, such as skin reactions and infusion reactions. Moreover, the management of the digestive tract to treat diarrhea is a necessary measure in the treatment of hypomagnesemia. The integrity of the IMB, GM-host immune balance, and biological effects of bacterial metabolites (SCFAs) all play a role in intestinal mucositis in CRC patients treated with cetuximab. Gastrointestinal disorders (GDs) are closely related to ADRs in the digestive tract, drug efficacy and the health of the gastrointestinal system affect drug reactivity and efficacy and ultimately the progression of mCRC and prognosis of colorectal patients. Recently, increasing numbers of studies have found that probiotics and prebiotics can restore the balance between GM and the host immune system, reduce ADRs, and improve the antitumor effect [77,78]. Probiotics have been proven to be a safe and beneficial choice for IBD and CRC patients [79], with randomized clinical placebo-controlled trials reporting that probiotics did not increase the risk of ADRs, unless the patients with a poor immune system or severely damaged IMB [80]. Therefore, we suggest taking measures to maintain the IMB, regulate the GM-host immune balance, and control intestinal bacterial metabolism in CRC patients treated with cetuximab monotherapy or cetuximab combined with chemotherapy. Probiotics and prebiotics represent promising measures to alleviate ADRs associated with cetuximab, enhance the efficacy of cetuximab, and improve the prognosis of mCRC.

Declarations

Conflict of interest: The authors declare no conflict of interest.

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