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ORIGINAL ARTICLE

The role of OR51E2 in colon cancer and rectal adenocarcinoma and the potential underlying mechanism

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Abstract

Objective Short-chain fatty acids (SCFAs) produced by intestinal microbiota influence the pathogenesis and development of several intestinal diseases. OR51E2 is a newly discovered SCFA receptor. At present, research on the link between OR51E2 and intestinal cancer is limited. This study aimed to analyze the relationship between OR51E2 and colorectal cancer.

Methods Bioinformatic analysis revealed the OR51E2 protein expression pattern in different parts of the intestine, regulation of related proteins, and immune cell infiltration. The expression pattern and prognostic value of OR51E2 in colon and rectal cancer was determined, and the miRNAs targeting OR51E2 were predicted.

Results The expression level of OR51E2 was relatively high in the colon, small intestine, and duodenum. In addition, OR51E2 expression level was significantly reduced in colon and rectal cancer. A positive correlation between OR51E2 and immune cells was observed, which was associated with the survival of patients with colon and rectal cancer (hazard ratio: 1.5). Further, miR-96-5p and miR-1271-5-p were predicted to target OR51E2.

Conclusion OR51E2 plays an important positive role in the survival of patients with colon cancer and rectal adenocarcinoma.

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Key words: OR51E2; colon cancer; rectal cancer; targeted microRNA; protein-protein interaction

Short-chain fatty acids (SCFAs) are the main metabolites produced by specific colonic anaerobic bacteria after fermenting dietary fiber. They mainly include acetic, propionic, butyric, valeric, and caproic acid [1]. SCFAs diffuse through or are actively absorbed by colonic epithelial cells and then enter the blood circulation. On the one hand, most SCFAs can be metabolized to produce energy. On the other hand, they can be used to epigenetically regulate HDACi genes [2]. SCFAs regulate peripheral immunity and the immune response in the central nervous system (CNS). Decreased dietary fiber intake or restriction of the intestinal microbiota is related to the development of inflammatory bowel disease [3–6], emphasizing that SCFAs are important immunomodulatory metabolites. SCFAs function by binding to SCFA receptors. Most of the SCFA receptors discovered to date are G protein-coupled receptors (GPRs), including GPR41, GPR43, and GPR109. GPR41 and GPR43, which are also known as free fatty acid receptors 3 (FFAR3) and 2 (FFAR2), are widely present in various cell types, such as immune and intestinal epithelial endocrine cells. For example, GPR43 can be expressed in large amounts in enteroendocrine cells, immune cells, and adipocytes, while GPR41 is widely expressed in smooth muscle, enteroendocrine, and nerve cells, among others. GPR109A can be expressed in large intestinal epithelial cells, colon cells, and hepatocytes and is abundantly expressed in fat and immune cells.

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GPR43 and GPR41 is activated by acetic, propionic, and butyric acid, and GPR109A is activated by butyric acid. By activating GPRs, SCFAs can activate the signal transduction pathway of the immune response and exert anti-inflammatory effects on the intestinal mucosa. Olfactory receptor 78 (Olfr78), also known as OR51E2, is a newly discovered SCFA receptor that is expressed in a variety of tissues [7]. The *OR51E2* gene is upregulated in prostate cancer [8]. Ligand-induced activation of *OR51E2* also affects the proliferation, differentiation, and melanogenesis of melanocytes [9]. *OR51E2* plays a pathophysiological role in blood pressure regulation, as well as in the development of melanoma and prostate tumors. As an SCFA receptor, *OR51E2* may help maintain the physiological function of the intestine in intestinal cancer; however, current research on the role of *OR51E2* in intestinal cancer remains limited. Therefore, an in-depth analysis of its practical significance in the intestine is needed.

Recent studies have shown the relationship between *OR51E2* and immune and blood pressure regulation [9–10], but there is little data on differential *OR51E2* expression and and its role in the physiology and pathology of intestinal cancer. This study aimed to analyze the pathological and clinical features of the SCFA receptor *OR51E2* in colon and rectal adenocarcinoma. It also aimed to predict *OR51E2*-targeting miRNAs using bioinformatics analysis and provide a scientific basis for the treatment and prevention of colon cancer.

Materials and methods

Differential OR51E2 expression in tissues

The expression levels of the SCFA receptors FFAR2, FFAR3, and *OR51E2* in the mouse intestine (large intestine, small intestine, colon, and duodenum) and heart tissue were obtained from the National Center for Biotechnology Information (NCBI) database (https://pubmed.ncbi.nlm.nih.gov/). The results are presented as heatmaps.

GO functional enrichment and network interaction analysis of OR51E2

UniProt database (https://www.uniprot.org/) was used to functionally reannotate OR51E2, and Gene MANIA software was used to analyze the protein–protein interactions involving OR51E2, coexpression, and the signaling pathway of related proteins.

OR51E2 and its correlation with immune cell infiltration in colon and rectal cancer

The Gene Expression Profiling Interactive Analysis (GEPIA) database (http://gepia.cancer-pku.cn/) was used to analyze OR51E2 gene expression and normal tissues in colon cancer and rectal adenocarcinoma, with statistical significance set at $P \leq 0.05$. The Tumor IMmune Estimation Resource (TIMER) database (http://cistrome.dfci.harvard.edu/TIMER/) was used to explore the correlation between *OR51E2* and different immune cells, namely B cells, CD4-T cells, CD8-T cells, neutrophils, macrophages, and dendritic cells. Pearson's correlation coefficient was used to express the correlation between *OR51E2* and immune cells.

Prognostic analyses of OR51E2 in colon cancer and rectal cancer

The online database Kaplan-Meier Plotter (http://www.kmplot.com/) was used to analyze the prognosis of breast cancer patients through receiver operating characteristic curves (ROC) for hub genes. The follow-up period was six months.

OR51E2 targeted by miRNAs and predicted regulatory mechanism

TargetScan 7.2 (http://www.targetscan.org/) software was used to predict the miRNAs targeting OR51E2 strongly and conservatively in vertebrates (human, rat, mouse, rabbit, and chicken). Using the Kyoto Encyclopedia of Genes and Genomes database (https://www.kegg.jp/), miRNAs were predicted that could target OR51E2 to regulate intestinal and intestine-related acute or chronic diseases.

Results

Differential expression in tissues

Proteins can perform specific biological functions only when they are expressed in tissues. To show that OR51E2 plays an important role in the intestine and heart, we screened the expression level data of the large and small intestine, colon, duodenum, and heart using tissue-specific gene expression data obtained from the NCBI database, in which tissue samples were taken from adult mice. The results are shown in Fig. 1. OR51E2 was expressed at relatively high levels in the heart and colon and at low levels in the large intestine, small intestine, and duodenum. This suggests that OR51E2 plays an important role in the function and stabilization of the gut-mandrel.

GO functional enrichment and protein interaction network analysis of OR51E2

Interactions of two or more proteins can achieve certain biological functions. We used STRING (https://www.string-db.org/) to analyze protein–protein interactions of OR51E2, and the results (Fig. 2a) showed that *OR51E2* interacted with RTP4, CNG1, REEP5, GNB1, RTP3, GNAL, REEP3, RTP2, REEP1, and RTP1. Using Gene MANIA to analyze the relationship between

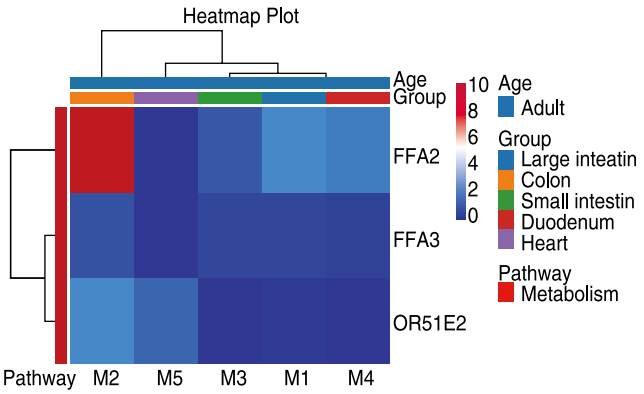

Fig. 1 Expression levels of SCFA receptors in the intestine and heart. Red and blue color scale indicates high and low expression levels, respectively

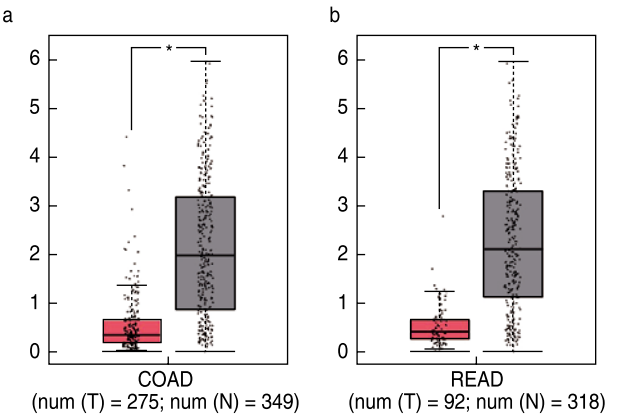

Fig. 3 OR51E2 expression level in intestinal cancer. Panel a shows *OR51E2* expression in 349 healthy people and 275 colon cancer patients. Panel b shows OR51E2 expression in 318 rectal cancer patients 92 healthy people. The red boxes represent cancer patients, and the gray boxes represent normal healthy people

OR51E2 and related proteins (Fig. 2b) and *Homo sapiens* as the data source, the coexpressed proteins were SEMG2, OR51E2, OR51E1, GNGT1, NRL, AKT3, KLK4, NKX3-1, KLK3, TGM4, SLC45A3, KLK2, NPY, PRAC1, ANO7, FAM159A, CPNE4, PAEP. Pathway: OR51E2, OR51E1, ARRB2, GNAL, GNB1, and GNGT1. By analyzing the relationship between OR51E2 and other proteins, it can provide an analytical basis for exploring the pathological mechanism of *OR51E2* in regulating intestinal cells.

Analysis of gene expression and immune cell infiltration in intestinal cancer

To understand whether *OR51E2* plays an important function in intestinal diseases, we analyzed the expression level of *OR51E2* in patients with colon or rectal cancer, including 275 patients with colon cancer with 349 corresponding controls and 318 patients with rectal cancer with 92 corresponding controls. OR51E2 expression level was significantly decreased in colon and rectal cancer ($P < 0.05$). In addition, stromal cell score and immune cell infiltration results can be used to study the immune infiltration between different samples. Through the differential expression of marker genes in different immune cells, the types and distribution of various immune cells in the sample can be analyzed, which can be used to study the differences in immune cell types in different cancers. Using the TIMER database, we analyzed the correlation between OR51E2 and different immune cells (B cells, CD4-T cells, CD8-T cells, dendritic cells, neutrophils, and macrophages). Pearson correlation coefficient was used to calculate the correlation between OR51E2 and different immune cells. Only four types

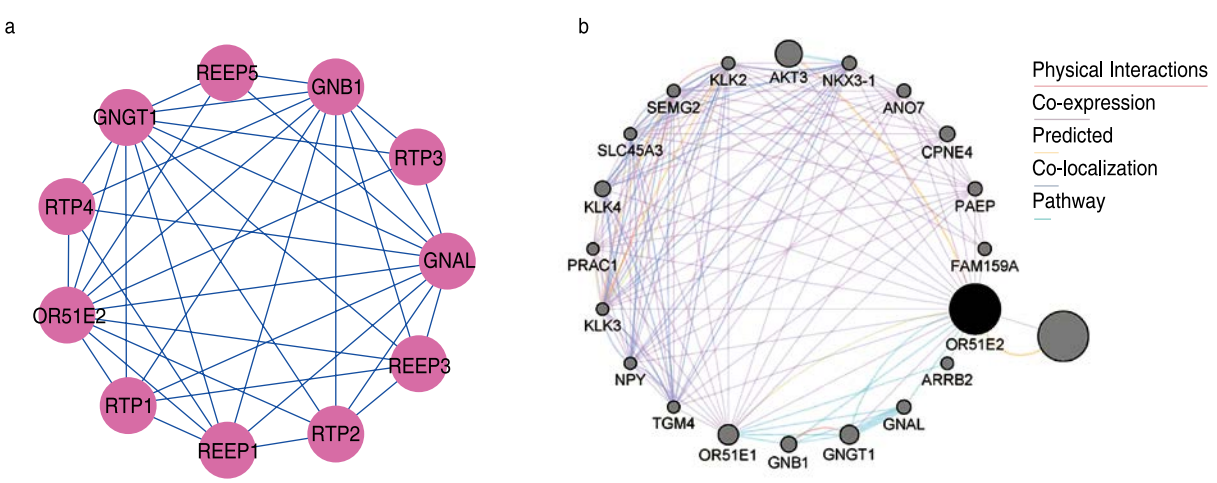

Fig. 2 Interactions between *OR51E2*-related proteins. Panel a shows the proteins interacting with *OR51E2*. Panel b shows the network diagram of *OR51E2*-related proteins mainly involving physical correlation, coexpression, prediction, colocalization, and signaling pathways

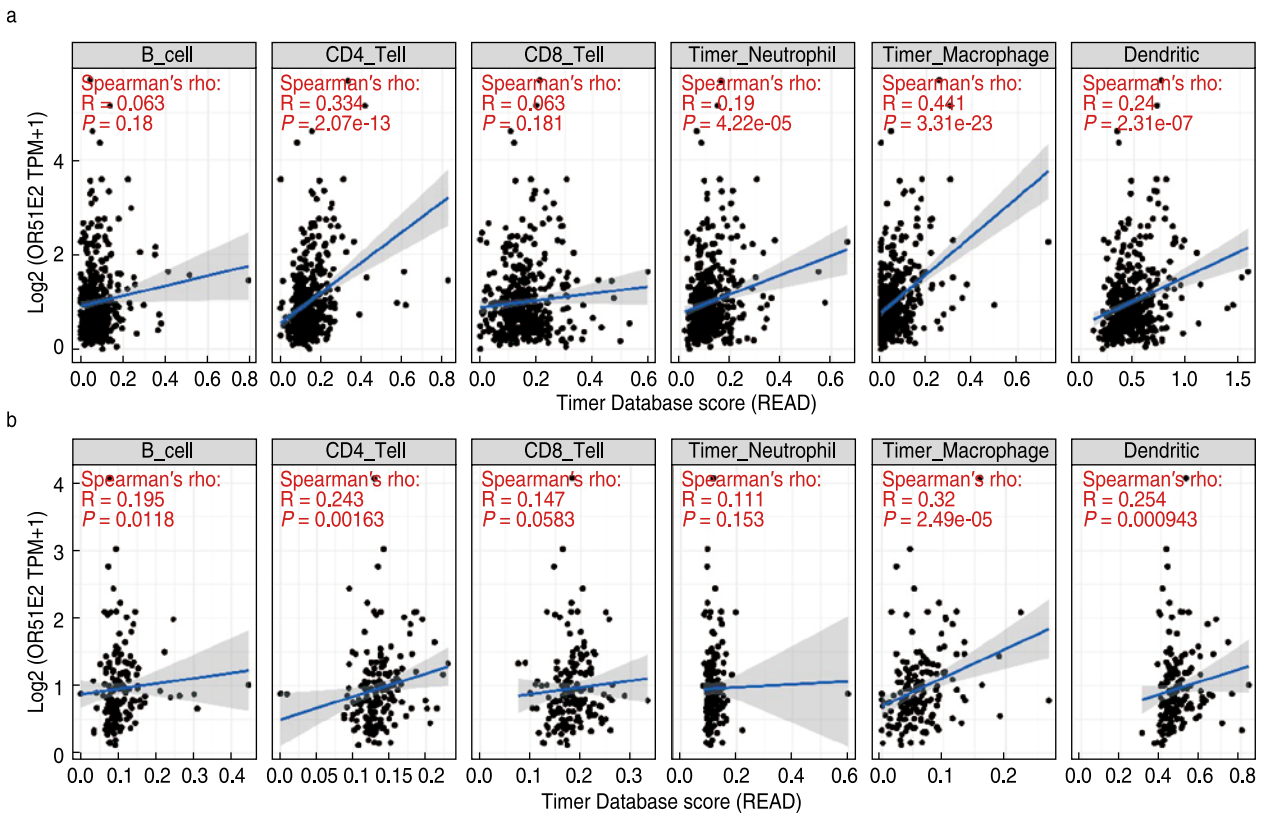

Fig. 4 Analysis of the survival status of *OR51E2* in intestinal cancer. Panel A shows the correlation of *OR51E2* with immune cells of colon cancer. Panel B shows the correlation of *OR51E2* with immune cells of rectal cancer. The R-value indicates the correlation, and statistical significance was set at $P < 0.05$

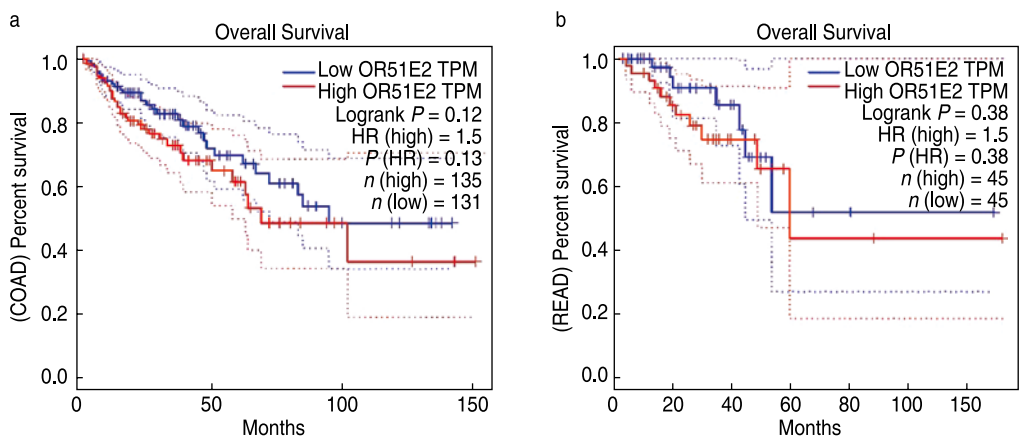

Fig. 5 Correlation between *OR51E2* and related immune cells in intestinal cancer. Panel a shows the survival analysis of *OR51E2* in colon cancer patients, of which 135 had high expression, and 131 had low expression. Panel b shows the survival analysis of *OR51E2* in rectal cancer patients, of which 45 had high expression levels and 45 had low expression levels. Blue represents the low expression group, and red represents the high expression group

of cells were found to be meaningful in colon cancer, namely CD4+ T cells (P = 2.07e-13), neutrophils (P = 4.22e-05), macrophages (P = 2.31e-23), and dendritic cells (P = 2.31e-07). Meanwhile, *OR51E2* gene expression level in rectal cancer was positively correlated with B cells (P = 0.0108), CD4+ T cells (P = 0.00168), macrophages (P = 2.49e-05), and dendritic cells (P = 0.000943).

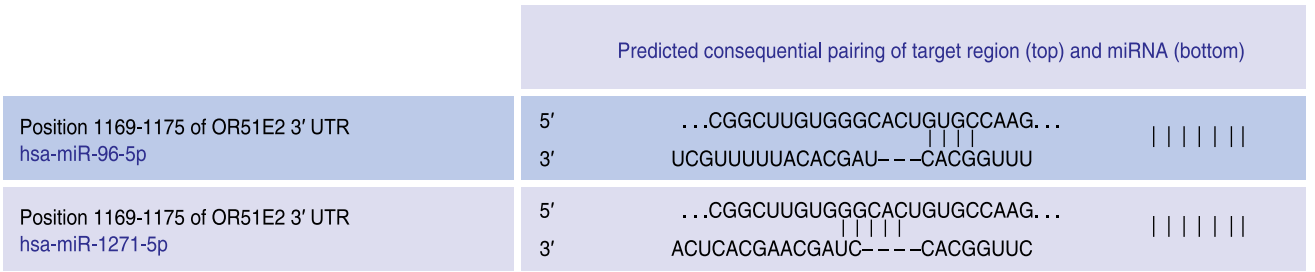

Fig. 6 Prediction of miRNAs targeting *OR51E2*

Survival curve analysis

Prognostic analysis showed that the *OR51E2* gene expression level was related to the survival duration of colon and rectal cancer patients. In Fig. 5a, 266 experimental patients were included, and the hazard ratio (HR) of colon cancer patients was 1.5 (log-rank P = 0.12, P [HR] = 0.13). In Fig. 5b, 90 experimental patients were included, and the HR of rectal cancer patients was 1.5 (log-rank P = 0.38, P [HR] = 0.38).

miRNA prediction

TargetScan 7.2 was used to predict the biological targets of miRNAs by searching for conserved 8mer, 7mer, and 6mer sites matching the seed region of each miRNA, and the reliability of the prediction results had high practicality [11]. TargetScan 7.2 predicted two miRNAs that were conserved in vertebrates, namely miR-96-5P and miR-1271-5-p (Fig. 6).

Discussion

The expression level of OR51E2 is related to the regulation of colitis and related cancer genes [12]; however, the pathological characteristics and role of OR51E2 in colon and rectal cancer remain unclear. This study confirmed that OR51E2 was highly expressed in colon and rectal cancer and was related to the survival time of clinical intestinal cancer patients. Using biological screening methods, we found that miR-96-5p and miR-1271-5-p are key players in the regulation of the pathological processes of the body via the signaling pathway that affects *OR51E2*.

GO functional enrichment analysis showed that *OR51E2* mainly played molecular biological functions in the sense of smell, GPRs, steroid hormone receptors, and signal receptors. Activation of OR51E2 triggers the Ca^{2+} retinal pigment epithelial cell-dependent signaling pathway. The downstream signaling pathway of *OR51E2* involves the activation of adenylate cyclase, ERK1/2, and AKT, and it regulates the migration and proliferation of retinal pigment epithelial cells [13]. In addition, OR51E2 is significantly upregulated in prostate cancer, and coexpression with TMPRSS2 leads to the loss of smell in coronary pneumonia [14]. However, studies on protein-interacting molecules related to OR51E2 are lacking. Multiple protein-phase interaction molecules with OR51E2 screened in this study provide a better understanding of the physiological and pathological mechanisms involved in OR51E2.

As a unique source of various immunoglobulins, B cells are an indispensable part of humoral immunity [15]. The dysregulation of B cells led to changes in space, phenotype, and function, including autoimmune diseases, infections, and cancers [16]. The CD4/CD8 ratio at the tumor-host interface was significantly related to recurrence-free and overall survival and had important implications for clinical prognosis [17]. Neutrophils, macrophages, and dendritic cells played a role in increasing the production and elimination of cytokines and immune cells, and therefore had a profound impact on the morbidity and mortality of cancer patients [18]. Liu *et al.* determined that macrophage migration inhibitor (MIF) promotes the proliferation and invasion of colon cancer cells by promoting the combination of CD74 and STAT1 [19]. Changes in the numbers of immune cells reflect immune disorder. When immune function was inhibited, CD8+ increased while CD4+, the CD4+/CD8+ ratio, and the number of immune cells decreased. The results of this study showed that OR51E2 protein levels were low in patients with colon and rectal cancer, while B cells, CD4+ T cells, macrophages, and dendritic cells were positively correlated with OR51E2 in colon and rectal cancer. This indicates that the expression of OR51E2 in intestinal cancer led to the decline of the body's immune level, which was consistent with our immunoassay results.

SCFA receptor proteins may play important roles not only in the passage of olfactory signals, but also in diseases, such as enteritis [20], prostate cancer [21], and hypertension [22]. Dietary fiber helps maintain the intestinal microbial ecology, host physiology, and health [23]. The SCFAs produced by the intake of dietary fiber are essential for regulating the host's intestinal immune response, which is necessary for the survival of intestinal microbes and metabolites [24]. These findings indicate

that OR51E2 expression is essential for the stability of the intestinal immune system. However, whether this differential expression influences intestinal cancer was unclear. Our study confirmed that OR51E2 expression level was significantly reduced in colon and rectal cancer and this reduced level is a mortality risk factor in patients with colon and rectal cancer.

In summary, after analyzing clinical data, we determined that *OR51E2* may be an effective biomarker for the detection of colon and rectal cancer. OR51E2 protein expression in cancer patients also led to the reduced immune levels, which predicted that *OR51E2* targets and regulates miRNAs. The findings present a target for regulating intestinal cancer, providing a scientific basis for effective detection and treatment of colon and rectal cancer. Despite these findings, our research has some limitations. The role of *OR51E2* was analyzed using bioinformatic tools, but these results should be validated *in vitro* and in clinical samples.

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Conflicts of interest
The authors indicated no potential conflicts of interest.

Author contributions
Not applicable.

Data availability statement
Not applicable.

Ethical approval
Not applicable.

References

1. Wenzel TJ, Gates EJ, Ranger AL, et al. Short-chain fatty acids (SCFAs) alone or in combination regulate select immune functions of microglia-like cells. Mol Cell Neurosci. 2020;105:111-116.
2. Ganapathy V, Thangaraju M, Prasad PD, et al. Transporters and receptors for short-chain fatty acids as the molecular link between colonic bacteria and the host. Curr opin pharmacol. 2013;13:869-874.
3. Katagiri R, Goto A, Sawada N, et al. Dietary fiber intake and total and cause-specific mortality: the Japan Public Health Center-based prospective study. Am J Clin Nutr. 2020;111:1027-1035.
4. Li MM, Zhou Y, Zuo L, et al. Dietary fiber regulates intestinal flora and suppresses liver and systemic inflammation to alleviate liver fibrosis in mice. Nutrition. 2021;81:121-129.
5. Swann OG, Kilpatrick M, Breslin M, et al. Dietary fiber and its associations with depression and inflammation. Nutr Rev. 2020;7: 394-411.
6. Vitale M, Masulli M, Rivellese AA, et al. Pasta consumption and connected dietary habits: associations with glucose control, adiposity measures, and cardiovascular risk factors in people with Type 2 Diabetes-TOSCA. It study. Nutrients. 2019;12:101-110.
7. Miyamoto J, Kasubuchi M, Nakajima A, et al. The role of short-chain fatty acid on blood pressure regulation. Curr Opin Nephrol Hy. 2016; 25:379-383.
8. Xu LL, Stackhouse BG, Florence K, et al. PSGR, a novel prostate-specific gene with homology to a G protein-coupled receptor, is overexpressed in prostate cancer. Cancer Res. 2000;60:6568-6572.
9. Gelis L, Jovancevic N, Veitinger S, et al. Functional characterization of the odorant receptor 51E2 in human melanocytes. J Biol Chem. 2016;291:177-186.
10. Corrêa-Oliveira R, Fachi JL, Vieira A, et al. Regulation of immune cell function by short-chain fatty acids. Clin Transl Immunol. 2016;5:99-107.
11. Agarwal V, Bell G W, Nam J-W, et al. Predicting effective microRNA target sites in mammalian mRNAs. eLife. 2015;4:109-119.
12. Kotlo K, Anbazhagan AN, Priyamvada S, et al. The olfactory G protein-coupled receptor (Olfr-78/OR51E2) modulates the intestinal response to colitis. Am J Physiol Cell Physiol. 2020;318:129-138.
13. Jovancevic N, Khalfaoui S, Weinrich M, et al. Odorant receptor 51E2 agonist β-ionone regulates RPE cell migration and proliferation. Front Physiol. 2017;8:129-139.
14. Kerslake R, Hall M, Randeva HS, et al. Co-expression of peripheral olfactory receptors with SARS-CoV-2 infection mediators: Potential implications beyond loss of smell as a COVID-19 symptom. Int J Mol Med. 2020;46:949-956.
15. McLean KC, Mandal M. It takes three receptors to raise a B cell. Trends Immunol. 2020;41:629-642.
16. Lamaison C, Tarte K. Impact of B cell/lymphoid stromal cell crosstalk in B-cell physiology and malignancy. Immunol lett. 2019;215:12-26.
17. Wang K, Shen T, Siegal GP, et al. The CD4/CD8 ratio of tumor-infiltrating lymphocytes at the tumor-host interface has prognostic value in triple-negative breast cancer. Hum Pathol. 2017;69:111-129.
18. Singbartl K, Formeck CL, Kellum JA. Kidney-Immune System Crosstalk in AKI. Semi Nephrol. 2019;39:96-106.
19. LiuF, Jianxin Z, Jianbin S, et al. Effects of MIF on proliferation, migration, and STAT1 pathway of colon cancer cells. Oncol Transl Med. 2020;6:121-125.
20. Rodriguez M, Siwko S, Liu M. Prostate-specific G-protein coupled receptor, an emerging biomarker regulating inflammation and prostate cancer invasion. Curr Mol Med. 2016;16:526-532.
21. Souza MF, Kuasne H, Barros-Filho MC, et al. Circulating mRNA signature as a marker for high-risk prostate cancer. Carcinogenesis. 2020;41:139-145.
22. RR M, Marques FZ. Diet-related gut microbial metabolites and sensing in hypertension. J Hum Hypertens. 2021;35:162-179.
23. Makki K, Deehan EC, Walter J, et al. The impact of dietary fiber on gut microbiota in host health and disease. Cell Host Microbe. 2018;23:705-715.
24. Danneskiold-Samsøe NB, Dias de Freitas Queiroz Barros H, Santos R, et al. Interplay between food and gut microbiota in health and disease. Food Res Int. 2019;115:132-145.

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