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Original Article

Differential expression of intestinal microbiota in colorectal cancer compared with healthy controls: a systematic review and meta-analysis

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Abstract: The purpose of this study was to review the differential expression of intestinal microbiota in colorectal cancer (CRC) with quantitative real-time PCR (q-PCR). An online search within PubMed, Web of Science and Wanfang Database up to June 6, 2015 was conducted. We used an original assessment tool to assess the quality of included articles. A systematic review and meta-analysis described the altered intestinal microbiota in colorectal cancer versus healthy control (HC). Six articles, involving 192 CRC patients and 264 healthy controls were included. Scores of quality assessment of these articles ranged from 7 to 16. Significant differences of microbial expression in CRC compared with HC were found in Bifidobacterium (SMD=-3.303, P=0.048), Faecalibacterium prausnitzii (SMD=-0.33, P=0.018) and Enterobacteriaceae (SMD=2.69, P=0.009), while total bacteria, Lactobacillus, Bacteroides-Prevotella group and Escherichia coli failed to display significant difference. We can conclude that there is down regulation of some colonization bacteria such as Bifidobacterium and Faecalibacterium prausnitzii, and up regulation of Enterobacteriaceae in CRC. Microbiota changes also participate in the pathogenesis of CRC.

Keywords: Intestinal microbiota, microbial expression, colorectal cancer, systematic review, meta-analysis

Introduction

Colorectal cancer (CRC), also named large bowel cancer, has the third highest cancer morbidity in the world [1]. The risk factors of colorectal cancer include poor eating habits, genetic susceptibility, colorectal adenoma evolution, and chronic inflammatory stimulation, etc [2]. In addition, intestinal microbiota are considered to have an impact on the pathogenesis of CRC as well. Many studies have shown direct contact between microbiota and intestinal mucosal cells, wherein some bacteria are closely relate to the formation and development of CRC, while the metabolites of other bacteria provide a protective effect to mucosal cells of the large bowel [3].

Intestinal microbiota contain up to 1014 bacteria [4]. In recent decades, scientists have invented various methods to investigate the microbial community structure. Besides traditional culture-based methods, new molecular techniques, such as fluorescence in situ hybridization (FISH), DNA microarrays, denaturing gradient gel electrophoresis (DGGE), real-time quantitative PCR (q-PCR), and pyrosequencing method, have been applied by scores of studies [5]. Real-time quantitative PCR is a quantitative experiment technique with much higher accuracy relative to previous qualitative or semi-quantitative methods. Compared with pyrosequencing, the new generation of sequencing technique, q-PCR is much cheaper and becomes a validation tool after pyrosequencing screening.

After reviewing several studies investigating the abundant variations in bacterial flora, we found many differences and even contradictions among the studies. Based on the advantages of q-PCR, we retrieved studies using q-PCR and performed a systematic review and meta-analysis in order to confirm the differential expres-

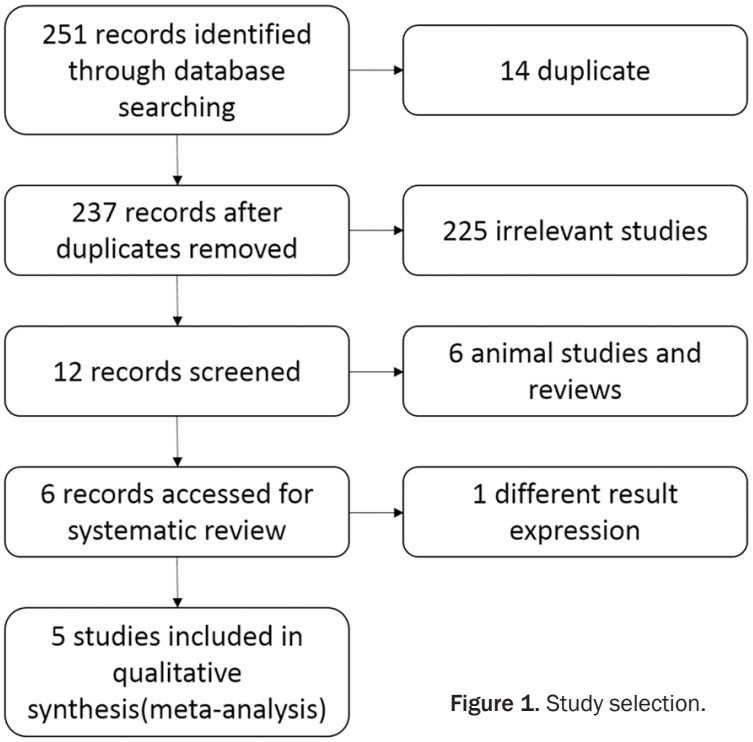

Figure 1. Study selection.

sion of intestinal microbiota in colorectal cancer (CRC).

Materials and methods

Search strategy

We retrieved articles from PubMed using keywords "("Colorectal Neoplasms" [Mesh] OR "colorectal cancer" OR "colorectal carcinoma") AND (qPCR OR q-PCR OR real-time) AND (microbiome OR microbiota OR flora)", and retrieved articles from Web of Science and Wanfang Database using keywords "("Colorectal Neoplasms" OR "colorectal cancer" OR "colorectal carcinoma") AND (qPCR OR q-PCR OR real-time) AND (microbiome OR microbiota OR flora))" with an end date of June 6, 2015. All references of the studies included were also carefully scrutinized.

Inclusion criteria and exclusion criteria

For a study to be included in this systematic review, several criteria had to be met: (1) studies had to be microbiota studies in CRC versus healthy controls; (2) samples had to use feces; (3) the methods implemented had to be quantitative real-time PCR techniques. Exclusion criteria were: (1) studies lacked an abundance of data concerning microbiota; (2) studies had already been reported by identical authorities; (3) intervention studies, animal studies, letters and reviews.

Quality assessment

We formulated an original assessment tool as there were no appropriate quality assessment tools for these laboratory studies. We used the following twelve criteria based on diagnostic tests as elaborated by Bossuyt et al [6]: (1) Describe the study population: the inclusion and exclusion criteria, setting and locations where data were collected. (2) Describe participant sampling: was the study population a consecutive or random series of participants? (3) Describe the diagnostic standard. (4) Describe the primers and their references. (5) Describe technical specifications of methods involved including how and when measurements were taken, and/or cite references for index tests and reference standard. (6) Describe methods for calculating test reproducibility, if done. (7) Report when the study was done, including beginning and ending dates of recruitment. (8) Report clinical and demographic characteristics of the study population (e.g., age, sex, comorbidity, recruitment centers). (9) Report the number of participants satisfying the criteria for inclusion that did or did not undergo the index tests and/or the reference standard; describe why participants failed to receive either test. (10) Report the distribution of the test results by the results of the reference standard for continuous results. (11) Report every sample's expression data or scatter chart. (12) Report how indeterminate results, missing responses, and outliers of the index tests were handled. Studies were assigned scores to each criterion by: (1) completely satisfactory; (2) partially satisfactory; (3) unsatisfactory or unclear.

Data extraction

Two reviewers independently extracted the following data from all eligible studies: (1) basic

Table 1. Characteristics of the 6 studies included in the systematic review

Study	Year	Region	Case/Control			Experiment methods		Reference
			Number	Mean age	Male ratio (%)	Fluorescent dye	q-PCR instrument	
Mira-Pascual L et al	2015	Spain	7/10	71.1/52.6	100/60	SYBR Green I	Light Cyder	[9]
Miao HF et al	2014	China	19/19	58.0/54.0	47/58	SYBR Green I	ABI 7300	[10]
Dong Y et al	2014	China	30/30	49/46	50/50	SYBR Green I	ABI 7500	[11]
Wang T et al	2012	China	46/56	60/49[a]	52/48	SYBR Green I	/	[12]
Sobhani I et al	2011	France	60/119	67.1/55.8	52/46	/	ABI 7000	[13]
Guo SK et al	2010	China	30/30	65.4/60.5	53/57	SYBR Green I	Light Cyder	[14]

[a]Median age.

Table 2. Scores of the 6 studies included using above assessment tool

Author	Year	1	2	3	4	5	6	7	8	9	10	11	12	Score
Mira-Pascual L et al	2015	2	2	2	2	2	2	0	2	0	2	0	0	16
Miao HF et al	2014	1	0	0	2	1	0	0	1	0	2	0	0	7
Dong Y et al	2014	1	0	0	1	1	2	2	1	0	2	0	0	10
Wang T et al	2012	1	0	2	2	2	2	0	2	0	2	0	0	13
Sobhani I et al	2011	1	0	0	2	2	0	2	1	0	2	0	1	11
Guo SK et al	2010	1	0	2	2	1	0	2	1	0	2	0	0	11

No/unclear =0 points; partial =1 point; complete =2 points.

characteristics of studies, including name of the first author, year of publication, country of origin, sample size, mean age, male to female ratio, diagnostic criteria; (2) experimental methods: fluorescent dye, q-PCR instrument; (3) effect size of microbiota: mean (or median), standard deviation (or quartile) and *P* value. Means and standard deviations were estimated with the method proposed by Stela PH et al [7], if the expressions were shown by medians and quartiles.

Statistical analysis

Meta-analysis methods were used to assess the standardized mean difference (SMD) of bacterial expressions. The random-effect model was applied. The degree of heterogeneity was quantified by I^2 test. Level of significance was set at $P<0.05$. The effect of publication bias was tested by Egger bias [8]. All analysis used Stata 12.0 software.

Results

Study selection and characteristics

251 records were identified from the databases, of which 18 were from PubMed, 31 were from Web of Science and 204 were from Wanfang. After removing 14 duplicate records, 237 were reserved. Finally, 6 articles met our inclusion criteria after eliminating animal studies and reviews (**Figure 1**). A total of 192 CRC patients and 264 healthy controls were included.

Population characteristics and experiment methods were listed in **Table 1**. Four studies were from Asia, while two studies were from Europe. Most of studies used SYBR Green I as fluorescent dye, which had a low specificity than TaqMan Probe.

Methodological quality assessment

We assessed the selected studies with the aforementioned twelve criterion assessment tool. Scores of each study were listed in **Table 2**. Scores of quality assessment of these articles ranged from 7 to 16.

Microbiota expression

6 kinds of bacteria and total bacteria in 5 articles were included in the meta-analysis. The results were expressed as the logarithmic number of bacteria per gram stool. Sample sizes, means, standard deviations and *P* values of Lactobacillus, Bifidobacterium, Bacteroides-Prevotella group, Faecalibacterium prausnitzii, Escherichia coli, Enterobacteriaceae and total bacteria are shown in **Table 3**.

Total bacteria, Escherichia coli and Enterobacteriaceae in CRC patients were up-regulated, while only Enterobacteriaceae (SMD=2.69, P=0.009, 95% CI=0.66 to 4.72) had the significantly changed expression (**Figure 2**). Lactoba-

Table 3. Effect size and *P* value of various bacteria

Bacteria	Author	Year	CRC			Healthy Control			*P* value
			N	Mean	Std	N	Mean	Std	
Total bacteria	Mira-Pascual L et al	2015	7	11.2	0.20	9	10.88	0.20	/
	Miao HF et al	2014	19	11.4	0.80	19	11.5	0.50	0.954
	Sobhani I et al	2011	60	11.8	0.56	119	11.88	0.35	0.21
*Lactobacil*lus	Mira-Pascual L et al	2015	7	6.59	1.16	9	5.93	0.24	/
	Dong Y et al	2014	30	6.54	0.43	30	9.53	0.74	0.076
	Sobhani I et al	2011	60	9.56	0.85	119	9.56	0.95	0.27
	Guo SK et al	2010	30	4.52	0.49	30	9.25	0.83	0.00
*Bifidobacteri*um	Mira-Pascual L et al	2015	7	8.6	0.34	9	9.09	0.32	/
	Dong Y et al	2014	30	6.54	0.43	30	9.53	0.74	0.082
	Sobhani I et al	2011	60	9.89	1.06	119	9.85	1.22	0.9
	Guo SK et al	2010	30	4.52	0.49	30	9.25	0.83	0.02
*Bacteroides-Prevotella g*roup	Mira-Pascual L et al	2015	7	10.36	0.34	9	10.4	0.26	/
	Dong Y et al	2014	30	9.83	0.53	30	11.14	0.57	0.009
	Sobhani I et al	2011	60	10.76	0.55	119	10.48	0.83	0.009
*Faecalibacterium prausnitz*ii	Mira-Pascual L et al	2015	7	8.19	0.24	9	8.15	0.25	/
	Miao HF et al	2014	19	8.9	1.70	19	9.6	0.90	0.35
	Sobhani I et al	2011	60	10.75	1.02	119	11.04	0.80	0.72
*Escherichia c*oli	Sobhani I et al	2011	60	8.14	1.34	119	8.14	1.28	0.25
	Guo SK et al	2010	30	5.82	0.47	30	4.68	0.32	0.01
*Enterobacteriacea*e	Mira-Pascual L et al	2015	7	8.25	0.48	9	7.41	0.55	/
	Dong Y et al	2014	30	10.59	0.63	30	8.51	0.49	0.047
*Enterococcus faecali*s	Guo SK et al	2010	30	10.6	0.3	30	4.95	0.24	0.00
Enterococcus faecium	Guo SK et al	2010	30	5.74	0.16	30	5.03	0.43	0.00
Fusobacterium	Miao HF et al	2014	19	9.1	1.40	19	7.2	1.50	0.002
Eubacterium rectale	Miao HF et al	2014	19	9.2	1.20	19	9.2	1.30	0.840

cillus, Bifidobacterium, Bacteroides-Prevotella group and Faecalibacterium prausnitzii in CRC patients were down-regulated, while Bifidobacterium (SMD=-3.303, P=0.048, 95% CI=-6.57 to -0.03) and Faecalibacterium prausnitzii (SMD=-0.33, P=0.018, 95% CI=-0.60 to -0.05) had the significantly different expression (**Figure 3**).

In addition, Enterococcus faecalis, Enterococcus faecium, Fusobacterium and Eubacterium rectale were reported to be differently expressed in CRC patients from that in the healthy controls in one single article.

Publication bias

Egger bias test did not show evident publication bias with the *P* value of 0.179 (**Figure 4**).

Discussion

Recently, abundant scientific and clinical studies have identified that intestinal microbiota play an essential role in the mechanisms of obesity, diabetes mellitus, irritable bowel syndrome (IBS), inflammatory bowel disease (IBD) and colorectal cancer [15]. Some evidence suggested that the differential expression of bacteria and tumorigenesis of the large bowel is a relationship of reciprocal causation. A certain combination of life styles, microbiota and their metabolites leads to intestinal inflammation. The study by Junhai Ou et al linked different CRC incidence and microbiota to distinct dietary habits of rural Africans and African Americans [16]. On the other hand, CRC pertained as a systemic disease releases cytokines into circulation and influences the microenvironment of intestines, leading to changes in the microbial community structure.

In this study, we observed a significant decreased expression in Bifidobacterium and Faecalibacterium prausnitzii, as well as a significant increased expression in Enterobacteriaceae. According to the results, we specu-

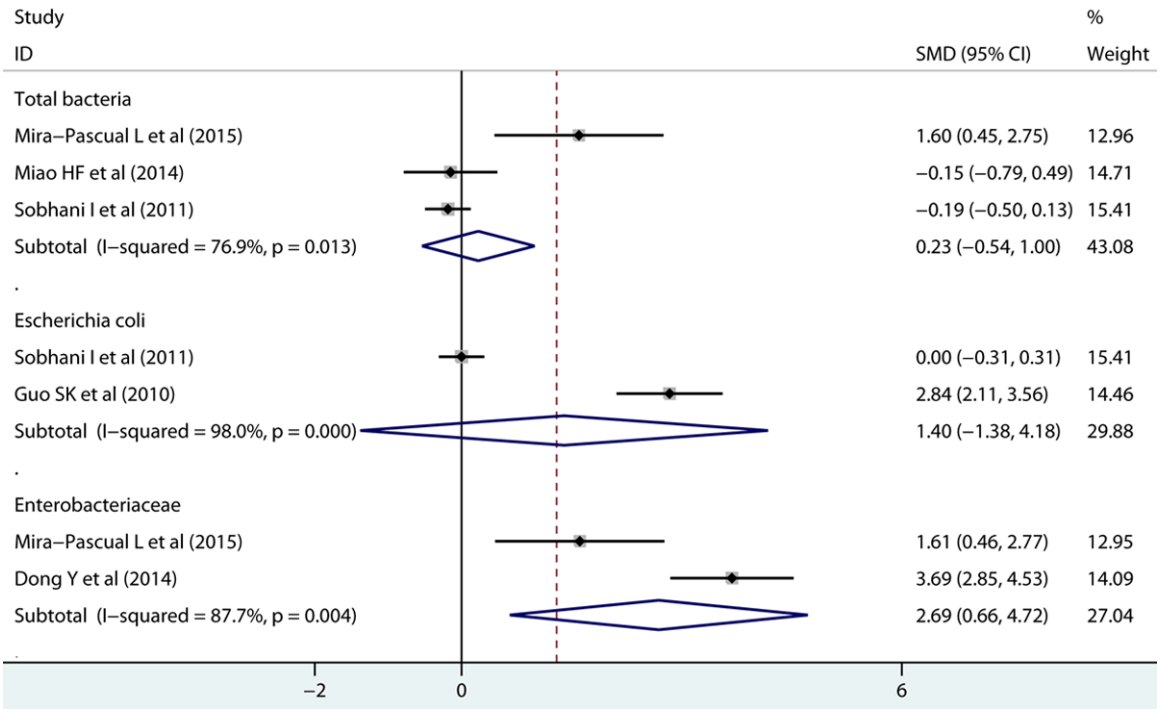

Figure 2. Forest plot of up-regulated bacteria including total bacteria, Escherichia coli and Enterobacteriaceae.

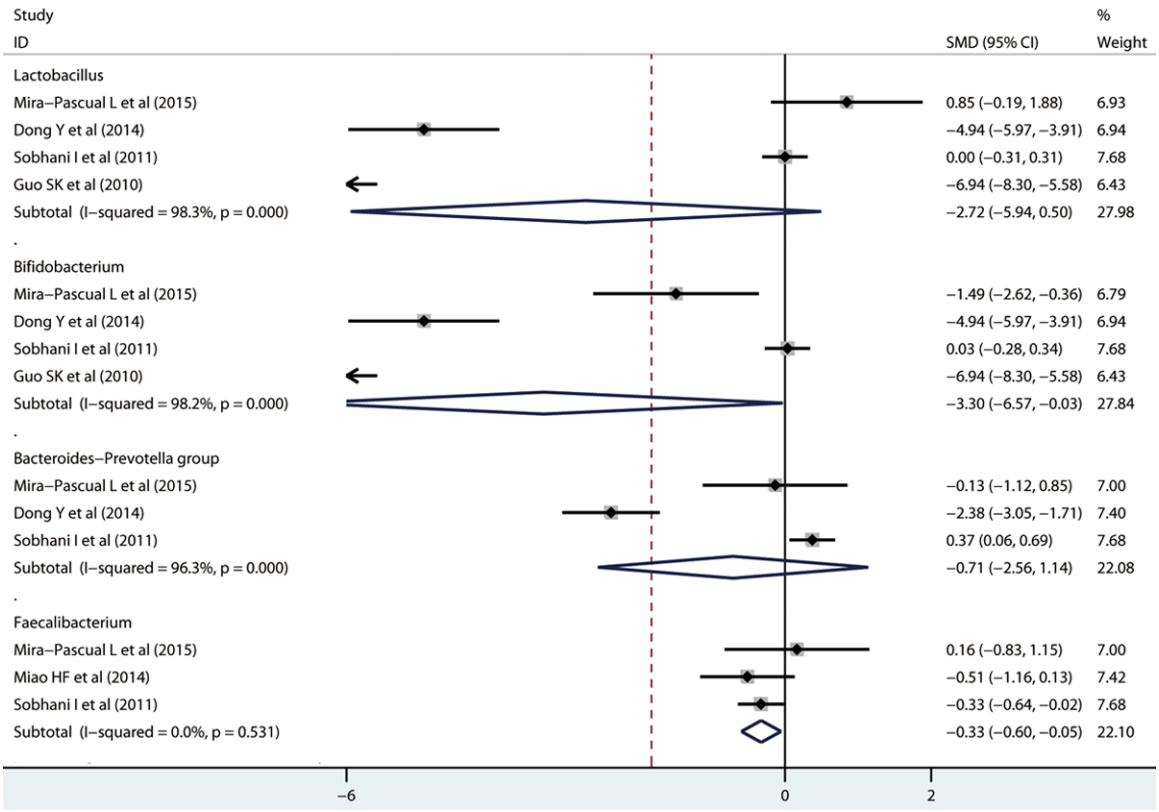

Figure 3. Forest plot of down-regulated bacteria including *Lactobacillus*, *Bifidobacterium*, *Bacteroides-Prevotella* group and *Faecalibacterium prausnitzii*.

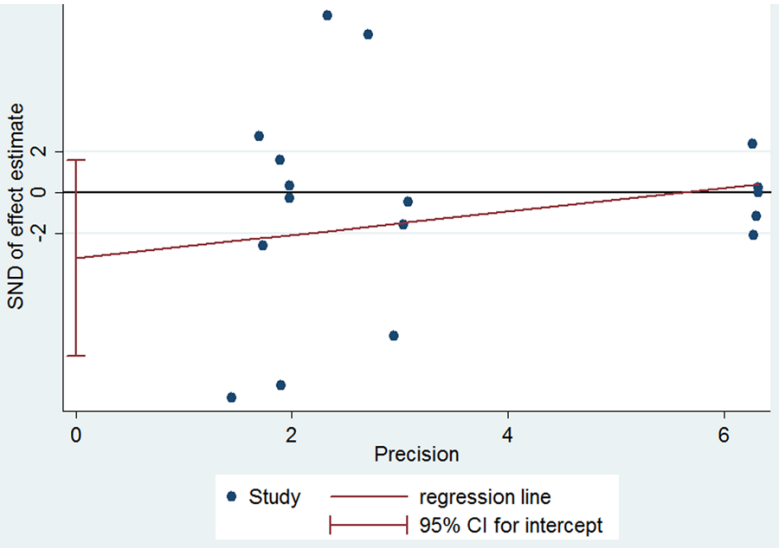

Figure 4. Egger's funnel plot of various bacteria.

late that Lactobacillus failed to reveal a significant difference due to inadequate sample size; however, total bacteria, Bacteroides-Prevotella and Escherichia Coli did not display a fundamental association with CRC. Otherwise, numerous bacteria such as Enterococcus faecalis, Enterococcus faecium and Fusobacterium were observed to have significant increases, but were not included in the meta-analysis due to the limited number of studies available. In short, it can be determined that there is down-regulation of normal bacterial flora and up-regulation of pathogenic bacteria in those with CRC.

The high heterogeneity of meta-analysis cannot be ignored; therefore we should be conscientious in accepting the synthetic result. A subgroup analysis, sensitivity analysis or meta-regression was not appropriate because the number of included studies was less than 10. For our part, the heterogeneity was derived from experimental methodology. Although experimenters used the same q-PCR technique, primers, and Fluorescent dye, the storage of fecal samples and the quantity of DNA standard samples might account for errors. The storage condition of fecal samples prior to storage at -80°C was uncontrollable. Under prolonged exposure at room temperature, the reproduction of aerobic bacteria may have caused damage to DNA of anaerobic bacteria, which are the main flora in the large bowel. The quantification methods of DNA standard samples were different in included studies. DNA quantification using ultraviolet spectrophotometer in early studies [14] contributed the largest errors in this study.

We tried to discuss the consequences of altered microbiota from the perspective of mechanisms. A decrease in Bifidobacterium, one of the most crucial colonization bacteria, leads to immunodeficiency of hosts, which allows tumor cells to escape from immune surveillance [17]. Faecalibacterium prausnitzii, a member of Clostridium cluster IV, is one of the main butyrate producers. Butyrate can be absorbed and utilized by intestinal mucosal cells, and then inhibit pro-inflammatory mediators such as tumor necrosis factor (TNF) and IL-6 [3]. In addition, Faecalibacterium prausnitzii is an anti-inflammatory bacterium with secreted metabolites which can block nuclear factor κB activation and IL-8 production [18]. Enterobacteriaceae, including Enterobacter, Escherichia, Klebsiella, Salmonella, Shigella and Yersinia, are pathogenic or conditional pathogenic species which would reproduce at greater rates when colonization bacterial flora is decreased. Some genera of Enterobacteriaceae can produce polyamines to enhance their invasiveness. Oxidative stress as the result of high levels of polyamines is associated with CRC [19, 20]. Enterococcus faecalis can induce oxidative DNA damage and mitochondrial dysfunction through reactive oxygen species (ROS) in epithelial cells [21]. Fusobacterium nucleatum can stimulate the proliferation of CD11b+ myeloid cells and overexpression of pro-inflammatory factors like Ptgs2, Scyb1, Tnf and Mmp. These pro-inflammatory factors belong to NF-κB signaling pathway, which leads to the tumorigenesis of epithelial cells [22, 23].

In summary, we had identified the association between the differential expression of intestinal microbiota and colorectal cancer through a meta-analysis of 5 case-control studies, and had discussed its corresponding mechanisms.

Further studies with larger samples and more species/clusters of bacteria or fungi are needed to identify the association and mechanisms are needed to be elaborated.

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Disclosure of conflict of interest

None.

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