Summary of the alteration of gut microbiota in patients with CRC in from 2014 to 2019

Object	Year	Alteration of gut microbiota	Sequencing methods	References	Jounal
human	2015	A number of Bacteroides and Parabacteroides species, along with Alistipes putredinis, Bilophila wadsworthia, Lachnospiraceae bacterium and Escherichia coli were enriched in carcinoma samples compared with both healthy and advanced adenoma samples. Gut commensals such as Bifidobactium animalis and Streptococcus thermophilus, on the other hand, decreased in faeces from adenoma or carcinoma patients, consistent with deviation from a healthy microbiome. Ruminococcus, Bifidobacterium and Streptococcus were significantly overrepresented in the controls, while Bacteroides, Alistipes, Escherichia, Parvimonas, Bilophila and Fusobacterium were overrepresented in the carcinoma patients .	metagenome-wide association study	Gut microbiome development along the colorectal adenoma-carcinoma sequence	Nat Commun
human	2014	Relative to healthy subjects, subjects with adenomas had higher relative abundances of OTUs affiliated with the Ruminococcaceae (OTU 21), Clostridium (OTU 60), Pseudomonas (OTU 3322), and Porphyromonadaceae (OTUs 1901 and 1903); they had lower relative abundances of OTUs affiliated with the Bacteroides (OTUs 1889 and 1913), Lachnospiraceae (OTU 36), Clostridiales (OTU 38), and Clostridium (OTUs 20, 97, 99; Supplementary Fig. S1) We observed that relative to healthy subjects, subjects with carcinomas had higher abundances of OTUs associated with Fusobacterium (OTU 2458), Porphyromonas (OTU 1905), Lachnospiraceae (OTUs 31, 59, 32, 116, 85), and Enterobacteriaceae (OTU 2479); they had lower relative abundances of OTUs affiliated with the Bacteroides (OTU 1889), Lachnospiraceae (OTUs 23, 30, 253, 136), and Clostridiales (OTU 42; Supplementary Fig. S2). T	16S rRNA gene sequencing	The human gut microbiome as a screening tool for colorectal cancer	Cancer Prev Res (Phila)
human	2017	Significant stepwise increase of C. symbiosum abundance was found in CRA, early CRC and advanced CRC ($P<0.01$).	qTPCR	Fecal Clostridium symbiosum for Noninvasive Detection of Early and Advanced Colorectal Cancer: Test and Validation Studies.	EBioMedicine

human	2019	First, the relative abundance of Fusobacterium nucleatum spp. was significantly (P < 0.005) elevated continuously from intramucosal carcinoma to more advanced stages. Second, Atopobium parvulum and Actinomyces odontolyticus, which co-occurred in intramucosal carcinomas, were significantly (P < 0.005) increased only in multiple polypoid adenomas and/or intramucosal carcinomas. Compared to the healthy controls, we found microbiome shifts in MP and S0, in addition to SI/II and SIII/IV, which were highly distinct across stages. A number of species in the phyla Firmicutes, Fusobacteria and Bacteroidetes was predominantly elevated in samples from S0, SI/II and SIII/IV, increasing with the degree of malignancy. In addition, we identified species newly associated with CRC, of which Colinsella aerofaciens (P=0.000840, q=0.0544), Dorea longicatena (P=0.000925, q=0.0557), Porphyromonas uenonis (P=0.000439, q=0.0475), Selenomonas sputigena (P=0.00369, q=0.101) and Streptococcus anginosus (P=0.00177, q=0.0788) were significantly elevated in SIII/IV with all four analytic pipelines used (see Methods). In line with a previous study5 , two butyrate producers, Lachnospira multipara (S0, P=0.000596, q=0.585; SI/II, P=0.000801, q=0.725; SIII/IV, P=0.000116, q=0.877) and Eubacterium eligens (S0, P=0.00147, q=0.698), were significantly depleted in CRC stages. Sulfide-producing bacteria, including Desulfovibrio vietnamensis (SIII/IV, P=0.00109, q=0.0565), D. longreachensis (S0, P=0.00164, q=0.188) and Bilophila wadsworthia (SIII/IV, P=0.00408, q=0.101), were elevated.	fecal metagenomic and metabolomic studies	Metagenomic and metabolomic analyses reveal distinct stage-specific phenotypes of the gut microbiota in colorectal cancer	Nat Med
human	2016	stool of CA cases was depleted in a network of Clostridia operational taxonomic units from families Ruminococcaceae, Clostridiaceae, and Lachnospiraceae, and enriched in the classes Bacilli and Gammaproteobacteria, order Enterobacteriales, and genera Actinomyces and Streptococcus (all q < 0.10). class Erysipelotrichi was depleted in SSA cases.	16S rRNA gene sequencing	The gut microbiota in conventional and serrated precursors of colorectal cancer	Microbiome
human	2017	Eubacterium ventriosum was consistently enriched in control microbiomes across all three methods (IMG: q=0.002; mOTU: q=0.0049; MLG: q=3.33×10−4). On the other hand, Parvimonas micra (q<7.73×10−6), Solobacterium moorei (q<0.011) and F. nucleatum (q<0.00279) were consistently enriched in CRC patient microbiomes across all three methods (figure 1A and online supplementary figure S5), while Peptostreptococcus stomatis (q<7.73×10−6) was enriched according to two methods. PERMANOVA analysis showed that only CRC status (p≤0.013 from all three methods) and colonoscopy (p=0.079 from two methods) explained the quantitative variation in the three CRC-enriched species	metagenome-wide association studies	Metagenomic analysis of faecal microbiome as a tool towards targeted non-invasive biomarkers for colorectal cancer	Gut
human	2017	We found that individual markers for clbA+ bacteria and F. nucleatum were more abundant in stool of patients with CRC	16S rRNA gene sequencing	Cancer-associated fecal microbial markers in colorectal cancer detection.	Int J Cancer
human	2017	Significantly elevated abundances of Fn, Ch, and m7 and decreased abundances of Bc and Ri in CRC patients compared to healthy controls	metagenome sequencing analysis	Fecal Bacteria Act as Novel Biomarkers for Noninvasive Diagnosis of Colorectal Cancer.	Clin Cancer Res

human	2017	The relative abundance in patientswith CRC compared with controls was 132-fold, 37-fold and41-fold for the markers Fn, Pa and Pm, respectively. As for patients with advanced adenoma, their relative abundance of marker Fn was significantly higher than the control group (3.8-fold, p=0.022, online supplementary figure S3A). Nevertheless, there was no significant difference in abundance between advanced adenoma and control groups for markers Pa (p=0.545) and Pm (p=0.232) (see online supplementary figure S3B, C).	quantitative PCR	Quantitation of faecal Fusobacterium improves faecal immunochemical test in detecting advanced colorectal neoplasia	Gut

Summary of the alteration of gut microbiota in patients with T2DM and the common alteration with both T2DM and CRC from 2012 to 2019

Object	Year	Alteration of gut microbiota	Sequencing methods	References	Jounal
human	2018		sequencing of the 16S rRNA	Characteristics of gut microbiota in adult patients with type 1 and type 2 diabetes based on next-generation sequencing of the 16S rRNA gene fragment	Pol Arch Intern Med
human	2012		metagenome-wide association study	A metagenome-wide association study of gut microbiota in type 2 diabetes.	Nature
human	2019	The sequence analysis revealed that bacteria from Firmicutes were predominant along with those from Clostridia and Negativicutes, whereas bacteria from Verrucomicrobia, Bacteroidetes, Proteobacteria, and Elusimicrobia were less abundant among the obese T2DM patients	16S rRNA sequencing targeting V3–V4	Analysis of gut microbiota of obese individuals with type 2 diabetes and healthy individuals.	PLoS One

human	2019	Participants with T2DM presented a reduction in the amounts of A. muciniphila and F. prausnitzii compared with those without diabetes (P≤0.036). Furthermore, A muciniphila is	quantitative PCR (qPCR)	Participants with T2DM presented a reduction in the amounts of A. muciniphila and F. prausnitzii compared with those without diabetes (P≤0.036). Furthermore, A muciniphila is	Biosci Rep
human	2019	The T2DM patients consumed more carbohydrates, and had lower fecal propionate and butyrate concentrations, larger fecal populations of Bifidobacterium spp. and bacteria of the order Lactobacillales, and smaller fecal Bacteroides spp. populations than the control individuals.		Gut microbiota disorders cause type 2 diabetes mellitus and homeostatic disturbances in gut-related metabolism in Japanese subjects	J Clin Biochem Nutr.
human	2017	While, the level of Lactobacillus was significantly higher in the patients with T2DM (P value < 0.001), Bifidobacterium was significantly more frequent in the healthy people (P value < 0.001)	quantitative real-time polymerase chain reaction (qPCR) method using bacterial 16S rRNA gene.	Comparison of gut microbiota in adult patients with type 2 diabetes and healthy individuals	Microb Pathog

Summary of alteration of gut microbiota by metformin in patients with T2DM that may affect CRC from 2014 to 2020

Object	Year	Alteration of gut microbiota	Sequencing methods	References	Jounal
patients with T2DM	2020	At the phylum level, subtle increases of Bacteroidetes and Actinobacteria, and a decrease of Firmicutes, were observed, though these were not statistically significant (Figure 3A). In addition, the ratio of Firmicutes to Bacteroidetes decreased after four weeks of taking metformin, although this was not statistically significant	16S rDNA sequences	The Effects of Metformin on the Gut Microbiota of Patients with Type 2 Diabetes: A Two-Center, Quasi-Experimental Study.	

patients with T2DM	2015	n, we next compared T2D metformin-treated (n=93) and T2D metformin-untreated (n=106) samples to characterize the treatment effect in more detail. Multivariate contrasts of T2D metformin-treated with T2D metformin-untreated samples appeared weaker than those between T2D metformin-untreated and ND control samples, the former only significant at the bacterial family level (PERMANOVA FDR<0.1), suggesting that the effects of metformin treatment on gut microbial composition are poorly captured by multivariate analysis. Univariate tests of the effects of metformin treatment showed a significant increase of Escherichia spp. and a reduced abundance of Intestinibacter spp.,	16S rDNA sequences	Disentangling type 2 diabetes and metformin treatment signatures in the human gut microbiota.	Nature
patients with T2DM	2018	In the metformin treatment group, four CAGs were increased by metformin and three were significantly decreased (Fig. 3b; see Table S3 in the supplemental material). Among the four CAGs enriched by metformin, CAG21 showed significant inverse correlations with HbA1c, and CAG25 with FBG, 2-h PBG, and LDL-c (Fig. 4a). CAG21 consisted of 10 OTUs: two OTUs belong to Clostridium XIVa and one to each of the following genera: Erysipelotrichaceae incertae sedis, Escherichia/Shigella, Fusobacterium, Flavonifractor, Lachnospiraceae, Lachnospiracea incertae sedis, and Clostridium XVIII and IV. CAG25 contained four OTUs from Blautia and one from Anaerostipes. Among the three CAGs inhibited by metformin, CAG7 and CAG8 were significantly correlated with the alleviation of hyperglycemia (Fig. 4a). CAG7 and -8 showed significantly negative correlations with HOMA-β. CAG7 contained three OTUs from Bacteroides and one from Parabacteroides; CAG8 consisted of three OTUs from Bacteroides, two OTUs from Alistipes, and one OTU each from Oscillibacter and un-Ruminococcaceae, respectively. To sum up, metformin significantly enriched CAG21 and CAG25 and inhibited CAG7 and CAG8, which were significantly correlated with the amelioration of hyperglycemia and hyperlipidemia.	V3 and V4 regions of the 16S rRNA gene by Illumina sequencing and multivariate statistical methods.	Structural Alteration of Gut Microbiota during the Amelioration of Human Type 2 Diabetes with Hyperlipidemia by Metformin and a Traditional Chinese Herbal Formula: a Multicenter, Randomized, Open Label Clinical Trial	mBio.
patients with T2DM	2018	metformin treatment, disturbances of the intestinal microbes lead to increased abundance ofEscherichia spp., Akkermansia muciniphila, Subdoligranulum variabile and decreased abundance of Intestinibacter bartlettii. T	16S rDNA sequences	Understanding the Representative Gut Microbiota Dysbiosis in Metformin-Treated Type 2 Diabetes Patients Using Genome-Scale Metabolic Modeling.	
patients with T2DM	2020	At the genus level, SB treatment increased the levels of Megamonas, Mobilitalea, Acetivibrio_g1, AB606281_g, and AB606237_g, while it decreased Clostridium_g23, Oscilibacter, and Alloprevotella. Additionally, eight species were increased and seven species were decreased in response to SB treatment (Figure 3A). Relative abundance in genus level including lactic acid bacteria and Akkermansia was compared by t-test analysis and found to differ between treatment groups. The relative abundance of Bifidobacterium was significantly lower in the SB group than the placebo group, while those of Lactobacillus and Akkermansia were significantly higher in the SB group than the placebo group (Figure 3B). The relative abundance of Weissella did not differ between treatment groups.	MiSeq system based on 16S rRNA gene	Scutellaria baicalensisCombined effects of with metformin on glucose tolerance of patients with type 2 diabetes via gut microbiota modulation.	

FIGURE 1 Relative percentage distribution of bacteria at the phylum level (L2) in the study groups

	Control, %	T1DM, %	T2DM, %
Verrucomicrobia	5.843	6.450	10.961
Tenericutes	0.072	0.028	0.034
Proteobacteria	0.260	0.579	0.468
Fusobacteria	0.002	0.004	0.001
Firmicutes	81.512	79.297	77.894
Bacteroidetes	6.426	8.222	3.630
Actinobacteria	5.771	5.204	6.542
Euryarchaeota	0.114	0.216	0.469

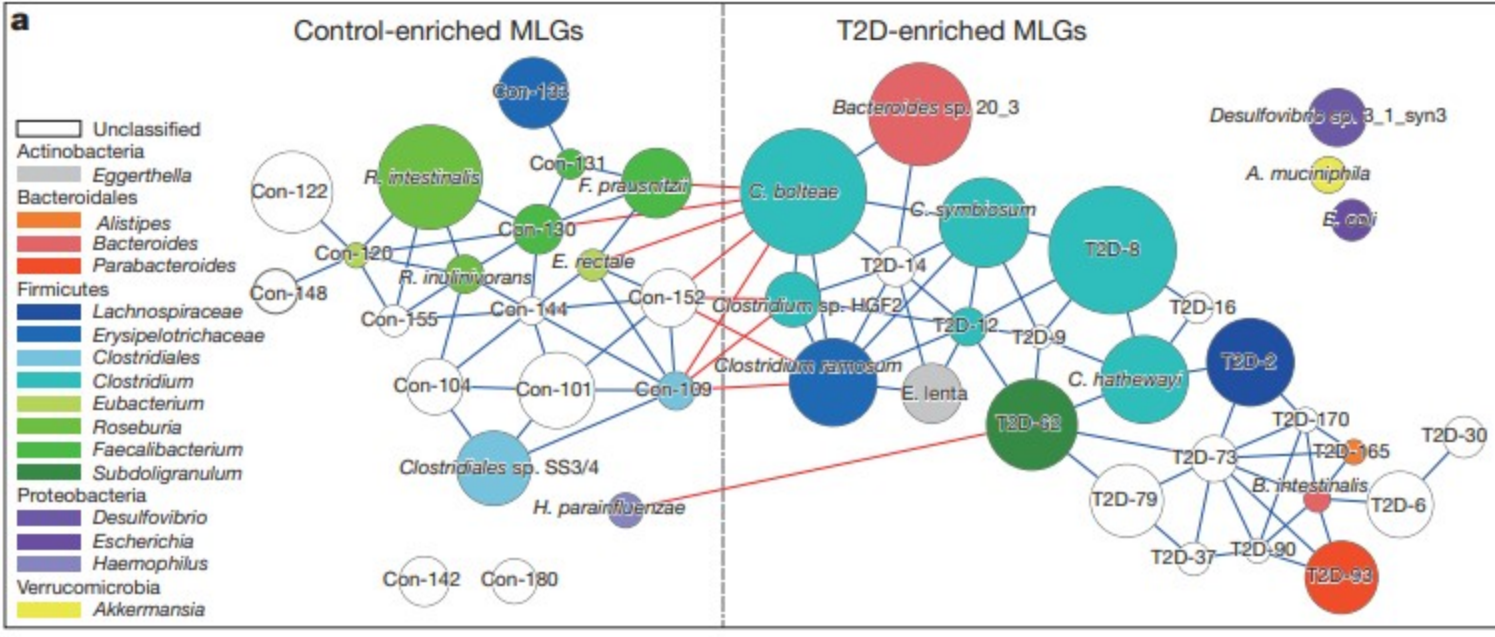
TABLE 2 Differences in the relative percentages of the microbial types between the study groups at the genus level (L6)

Genus (L6)	Controls (n = 23)	T1DM group (n = 22)	T2DM group (n = 23)	*P* value
g__*Bacteroides*	5.22%	5.96%	2.74%	0.02[a,c]
f__Clostridiaceae; Other	2.29%	2.53%	0.77%	0.03[a] 0.006[b] 0.01[c]
f__Clostridiaceae; g__	2.09%	1.96%	0.88%	0.02[a] 0.04[b]
f__Lachnospiraceae; Other	3.23%	1.63%	1.74%	0.02[a,c]
g__*Ruminococcus*	5.30%	6.66%	10.69%	0.02[a] 0.04[b,c]
g__*Anaerostipes*	0.34%	0.28%	0.21%	0.049[a] 0.04[c]
g__*Roseburia*	0.45%	0.29%	0.13%	0.005[a] 0.003[b]
f__Peptostreptococcaceae;g__	0.12%	0.12%	0.03%	0.001[a] 0.003[b] 0.02[c]
f__Enterobacteriaceae;g__	0.05%	0.53%	0.42%	0.001[a,c]
f__Flavobacteriaceae;g__	0.06%	0.06%	0.02%	0.007[a,c]

A *P* value of less than 0.05 is considered significant.

Clostridiales (6.43%, 5.98%, and 7.07%, respectively); *Akkermansia* (5.84%, 6.45%, and 10.96%, respectively); *Ruminococcus* (5.3%, 6.66%, and 10.69%, respectively); *Bacteroides* (5.22%, 5.96%, and 2.74%, respectively); *Blautia* (4.86%, 7.45%, and 5.61%, respectively); an isolated, but not yet identified, genus belonging to the family Lachnospiraceae (3.23%, 1.63%, and 1.74%, respectively); *Faecalibacterium* (3.14%, 2.71%, and 1.62%, respectively); *Bifidobacterium* (2.89%, 2.68%, and 2.02%, respectively); *Coprococcus* (2.76%, 2.42%, and 3.49%, respectively); an unnamed genus in the family Clostridiaceae (2.09%, 1.96%, and 0.88%, respectively); an isolated, but not yet identified, genus belonging to the family Clostridiaceae (2.29%, 2.53%, and 0.77%, respectively); *Collinsella* (1.75%, 1.37%, and 3.13%, respectively); *Dorea* (1.24%, 1.01%, and 1.58%, respectively); and a genus with the suggested name of *Ruminococcus*, belonging to the family Lachnospiraceae (1.05%, 3.89%, and 1.78%, respectively). The remaining 108 OTUs (corresponding to the genus) constituted a fraction of the gut microbiota composition in the samples examined. A comparison of the relative percentages of the microbial types in the study groups showed significant differences for 10 types (TABLE 2).

An α-diversity analysis showed a slightly low-


a
Control-enriched MLGs
T2D-enriched MLGs
Unclassified
Actinobacteria
Eggerthella
Bacteroidales
Alistipes
Bacteroides
Parabacteroides
Firmicutes
Lachnospiraceae
Erysipelotrichaceae
Clostridiales
Clostridium
Eubacterium
Roseburia
Faecalibacterium
Subdoligranulum
Proteobacteria
Desulfovibrio
Escherichia
Haemophilus
Verrucomicrobia
Akkermansia
Con-133
Con-131
Con-122
R. intestinalis
F. prausnitzii
Con-130
Con-120
R. inulinivorans
E. rectale
Con-148
Con-155
Con-144
Con-152
Con-104
Con-101
Con-109
Clostridiales sp. SS3/4
H. parainfluenzae
Con-142
Con-180
Bacteroides sp. 20_3
C. bolteae
C. symbiosum
T2D-8
T2D-14
Clostridium sp. HGF2
T2D-12
T2D-9
T2D-16
Clostridium ramosum
E. lenta
C. hathewayi
T2D-2
T2D-62
T2D-170
T2D-73
T2D-165
T2D-30
B. intestinalis
T2D-79
T2D-6
T2D-37
T2D-90
T2D-93
Desulfovibrio sp. 3_1_syn3
A. muciniphila
E. coli