EJMO

DOI: 10.14744/ejmo.2021.65073
EJMO 2021;5(3):260–272

Research Article

Dialister in Microbiome of Cancer Patients: A Systematic Review and Meta-Analysis

Mehmet Demirci

Department of Medical Microbiology, Kirklareli University, Faculty of Medicine, Kirklareli, Turkey

Abstract

Objectives: Dialister is the genus classified within Veillonellaceae family in Firmicutes phylum. Dialister genus has been detected in patients with oral infections and healthy people in their oral cavity, as well as in clinical samples in different parts of the body. Cancers are complex and multifactorial diseases and considered a global problem. End products of Dialister such as acetate, lactate and propionate seem to be important in the mechanism of carcinogenesis. Although it is reported that the composition of Dialister has changed in articles investigating the microbiome relationship with cancer patients, it is seen that it is not taken into consideration. The aim of this review was to investigate cancer studies in humans on the association of microbiome with composition changes in Dialister.

Methods: A systematic literature search was performed using in Pubmed. In vitro and animal studies were excluded. After database search, 510 articles were found. 484 article were excluded based on the exclusion criteria. The remaining 26 articles were identified and analysed for Dialister. Meta-Mar online software was used for metaanalysis results.

Results: The meta-analysis included 26 studies with 1649 control samples and 1961 cancer samples. Compared to healthy controls, Dialister were significantly elevated in samples from cancer patients (Hedges'g=0.907, $p<0.05$, 95%CI [13.19 - 16.746]. Statistical heterogeneity was found hihg (I^2=99.6%).

Conclusion: This review showed that a relationship between different cancer types and Dialister composition of microbiome. however, these data still seem very weak to reveal the Dialister and cancer relationship. Dialister can be an important genus especially in solid tumors but, more comprehensive and wider studies are needed to understand the relationship between Dialister and cancer. In addition, due to rapidly developing new bioinformatics analysis techniques, massive data should be added to public databases by the authors in studies of microbiome or microbiota disease relationship. Thus, it is valuable in terms of detecting different strains such as Dialister, which can be ignored by re-evaluating these data in the future.

Keywords: Cancer, Dialister, microbiome, microbiota, next generation sequencing

Cite This Article: *Demirci M. Dialister in Microbiome of Cancer Patients: A Systematic Review and Meta-Analysis. EJMO 2021;5(3):260–272.*

Cancers are complex and multifactorial diseases and considered a global problem, each year, approximately 9 million people die because of cancer in the world.[1,2] In recent years, although significant advances have been made in prevention and treatment options for some cancer types, the number of cancer patients is still increasing due to the aging global population, as well as risk factors such as smoking, obesity and diet.[3] Incidence of cancer is estimate to increase two times more in 2035. It is expected to affect especially in low-income and middle-income countries [LMICs][2]. Of course not all cancers can be prevented, but prevention is very important and need long-term strategy.[4] Besides surgery and radiotherapy systemic treatment options such as cytotoxic chemotherapy, hormonal

Address for correspondence: Mehmet Demirci, PhD. Kirklareli Universitesi Tip Fakultesi, Tibbi Mikrobiyoloji Anabilim Dali, Kirklareli, Turkey
Phone: +90 288 444 40 39 **E-mail:** demircimehmet@hotmail.com
Submitted Date: March 19, 2021 **Accepted Date:** August 02, 2021 **Available Online Date:** September 24, 2021

therapy, immunotherapy and targeted therapies are used for the treatment of cancer patients.[5] While many types of cancer cannot be cured, when they detect upper stages, early detection of cancerous and precancerous lesions are very important in order to reduce mortality, morbidity, psychological and economical burdens.[6] Despite the complex structure of cancers, technological techniques including medical imaging or minimally invasive biomarkers are used as reliable techniques in the diagnosis, treatment and follow-up of cancers patients. On the other hand, the interpretation of the big data obtained by these techniques is a new challenge.[7]

Many recent studies showed that symbiotic microorganisms that colonize body surfaces in the host are play important role in health or diseases such as cancer and associated with these conditions. The largest symbiotic microorganism concentration is found in the intestine, skin and oral cavity. Our system evolves together with these microorganisms, allowing our immune system to be regulated.[8] In recent years, advances in sequencing technology and bioinformatic techniques have enabled complex symbiotic microorganism communities to be detected in the host. Big data including these techniques such as the Human Microbiome Project, allowed us to understand the metabolic and metagenomic potentials of these symbiotic microorganisms. These data caused a very important change in our perspective. We no longer think of microorganisms as just a cause of disease, but we also believe that microorganisms contribute to the state of health.[9] The term microbiome refers to all habitats containing microorganisms, their genomes and environmental conditions.[10] The main purpose in human microbiome studies is to identify and characterize bacterial taxa and their functions.[11] With the use of culture-independent approaches based methods such as high-throughput sequencing, new culturable or non-culturable bacteria in the microbiome were detected. Thus, it was possible to determine the identity, activities and functional roles of these bacteria in the microbiome. Detection of a conserved fragment of the 16S rRNA gene by the amplification of universal primers using the High-throughput sequencing method is considered the standard method for detecting the complex microbiome profile.[12]

Dialister is the genus classified within Veillonellaceae family in Firmicutes phylum. Although it is in Gram positive phylum, it has Gram negative cell wall. It is nonmotile, nonspore forming, nonfermentative small coccobacilli shaped cells. *Dialister* are obligatory anaerobic or microaerophilic bacteria. *Dialister* pneumosintes, *Dialister* micraerophilus, *Dialister* propionicifaciens, *Dialister* succinatiphilus and *Dialister* invisus species were identified according to their main cellular content and using with 16S rRNA sequencing techniques. *Dialister* genus has been detected in patients with oral infections and healthy people in their oral cavity, as well as in clinical samples in different parts of the body. Acetate, lactate and propionate have been reported as metabolic end products.[13-17] In addition to being such important end products for carcinogenesis, it is seen that although the composition of *Dialister* has changed in the articles investigating the microbiome relationship in cancer patients, it is not taken into consideration.

The present systematic review aims to examine and discuss all available microbiome studies such as case-control, cross-sectional, prospective cohort, observational, interventional, experimental or clinical trials in humans on the association of cancer with changes in Dialister.

Methods

The main questions for this review was; How did changed the amount of *Dialister* spp in microbiome of cancer patients? The PRISMA guidelines was used to design this systemic review.[18]

Searching Strategy

A thorough systematic literature search was performed (March 13, 2020) using the following databases: Pubmed, BioMed Central, Cochrane Library, EBMR, EMBASE, Informa Healthcare. The systematic literature search was structured by means of the PICOs acronym (participants, interventions, comparators, outcome measures, study design). The following query was created by using the Boolean Search Operator: ((dialister[All Fields] AND ("microbiota"[MeSH Terms] OR "microbiota"[All Fields] OR "microbiome"[All Fields])) AND ("microbiota"[MeSH Terms] OR "microbiota"[All Fields])) AND ("neoplasms"[MeSH Terms] OR "neoplasms"[All Fields] OR "cancer"[All Fields])

Eligibility Criteria

Eligibility criteria of this review were I) Articles in english, II) research articles which included studies of case-control, cross-sectional, prospective cohort, observational, interventional, and experimental or clinical trials III) articles focus on patients diagnosed with cancer, IV) microbiome or microbiota studies using with next generation sequencer. V) reported *Dialister* result. In vitro and animal studies were excluded.

Data Extraction

The following information was extracted from each article: author, published time, target cancer type, study population (number of participants) total study population, LDA Score (log10), sample type, sequencer, sequencing protocol, cancer patients age, gender, BMI, country, enrollment

time of study, *Dialister* result from study and *Dialister* status.

Statistical Analysis

Meta-Mar online metaanalysis software was used for the statistical analysis and foresplot figure and p<0.05 value was being considered statistically significant. Effect estimation was performed Hedges' g value (small = 0.2 – 0.49, medium = 0.5 - 0.79 and large ≥ 0.8). Statistical heterogeneity were calculated with I^2 test (0-40% small, 40-70% medium, 70-90% high).

Results

After database search, 510 articles were found. 484 article were excluded based on the exclusion criteria. The remaining 26 studies were identified as using with next generation sequencing to analyse microbiome of cancer patients and mentioned about *Dialister* and then fully reviewed, The process for selecting studies for inclusion in this review is detailed in Figure 1. Main characteristics of studies included in this systematic review showed that in Table 1.

The meta-analysis included 26 studies with 1649 control samples and 1961 cancer samples. Compared to healthy controls, *Dialister* were significantly elevated in samples from cancer patients (Hedges'g=0.907, p<0.05, 95%CI [13.19 - 16.746]. Statistical heterogeneity was found hihg (I^2=99.6%) (Table 2, Table 3, Figure 2).

Wang et al.,[19] (2015), Walther-António et al.,[20] (2016), and Sims et al.[21] (2019) examined the microbiome of cervical cancer patients using with stool, swab & scrape and stool samples respectively. Walther-António et al., (2016), Sims et al. (2019) reported *Dialister* were found to be significantly elevated in cancer patients (p=0.0061; p<0.05 respectively).[20,21] Wang et al.,[19] (2015) reported had increased abundance of Dialister.

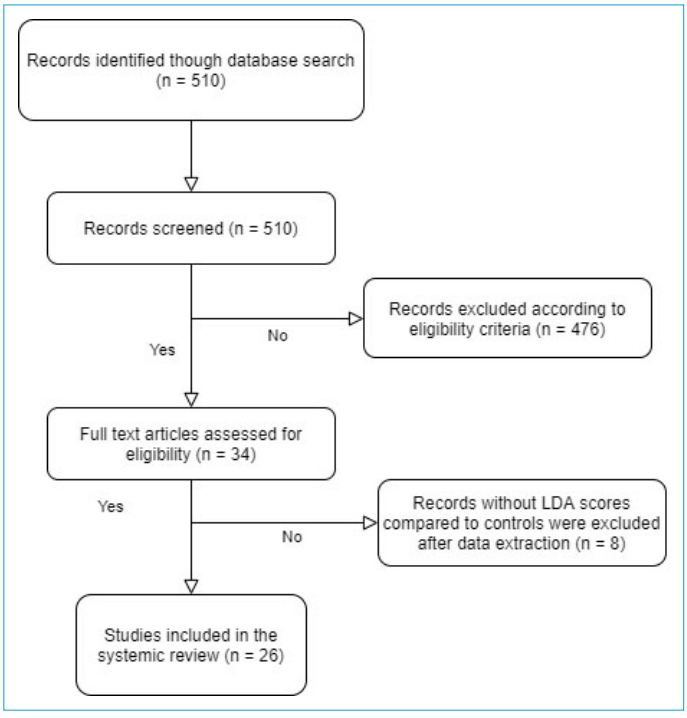


Figure 1. Flow diagram of studies included in these review.

Eight studies were found for association of colorectal cancer with changes in Dialister.[22-27] Different sample types such as stool or tissue samples were used in these studies. Six of these studies reported *Dialister* were found to be significantly elevated in cancer patients, however, two of these studies reported *Dialister* were found decrease in cancer patients.[25,27] Zhang et al. 2018, which also showed increased *Dialister* pneumonsintes.[26] Chen et al., (2012) reported specifically *Dialister* pneumosintes.[22] While the microbiome results of cancer patients generally were compared with the healthy control group, only the Loke et al., (2018) and Chen et al., (2012) studies compared the microbiome results of the cancerous and non-cancerous tissues of cancer patients.[22,27]

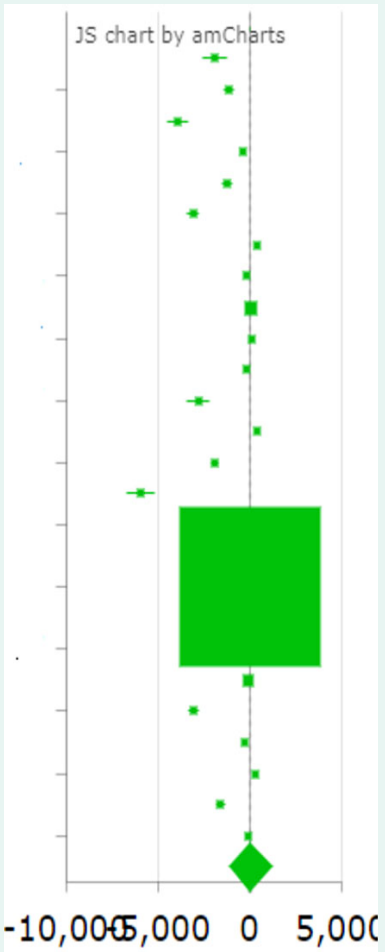
Chen et al., (2015) and Elliot et al., (2017) reported for association of esophageal cancer with changes in Dialister. Saliva and tissue samples were used in these studies respectively.[28,29] Chen et al., (2015) reported a decrease in cancer patients compared to healthy controls,[28] while Elliot et al., (2017) reported an increase in cancer patients compared to healthy controls.[29]

Six studies were found for association of gastric cancer with changes in Dialister.[30-34] In all of these studies except Liang et al.,[31] (2019), *Dialister* were found elevated in microbiome results of cancer patients compared to control. In all of these studies except Liang et al.,[31] (2019), also performed from tissue samples, but Liang et al., (2019) performed their study with stool samples.[31] Interestingly, Liang et al., (2019) reported *Dialister* were found reduced in microbiome results of cancer patients compared to healthy controls. They also found *Dialister* were increased postoperative samples from gastric cancer patients compared to preoperative samples from gastric cancer patients.[31]

Eight studies were found for association of head and neck cancer with changes in Dialister.[35-41] Gong et al. performed two different studies in 2014 and 2017 association of laryngeal carcinoma with *Dialister* and other studies association of head and neck squamous cell carcinoma with Dialister.[35,37] Different sample types such as oral rinse sample, oral swab sample, tissue biopsy sample for buccal mucosa or saliva were used in these studies. All of these studies reported that *Dialister* were increased in the head and neck cancer patient group compared with the control subjects. Yang et al., (2018), reported specifically *Dialister* pneumosintes.[40]

Table 2. Metaanalysis results

Study name	Control		Cancer		Proportion		95%-CI		Weight (%)	
	n	LDA	n	LDA	g	SEg	g_lower	g_upper	Fixed model	Random model
Wang et al., 2015	4	0.001	11	2.001	1882.352941	343.669495	1208.76073	2555.94515	0.000697	0.688215
Walther-António et al., 2016	10	0.001	21	1.201	1168.695652	148.424968	877.782715	1459.60859	0.003736	2.401874
Sims et al., 2019	46	0.001	42	4.001	3965.014577	298.874294	3379.22096	4550.80819	0.000921	0.8753
Chen et al., 2012	56	0.001	46	0.405	400.962406	28.073678	345.937997	455.986815	0.104426	5.343548
Hibberd et al., 2017	21	0.001	15	1.281	1251.555556	147.497607	962.460246	1540.65087	0.003783	2.419079
Xu et al., 2017	61	0.001	99	3.101	3085.26149	172.471437	2747.21747	3423.30551	0.002767	2.001593
Flemer et al., 2018	103	0.341	131	0.001	-338.899676	15.666193	-369.605415	-308.193938	0.335337	5.516151
Zhang et al., 2018	192	0.001	218	0.201	199.632128	6.972156	185.966701	213.297554	1.693068	5.581537
Loke et al., 2018	17	0.03	17	0.001	-28.314961	3.449986	-35.076934	-21.552988	6.91471	5.593903
Chen et al., 2015	85	0.101	87	0.001	-99.558174	5.369964	-110.083303	-89.033044	2.854079	5.588192
Elliot et al., 2017	20	0.001	66	0.201	198.208955	15.115408	168.582756	227.835155	0.360221	5.521723
Castaño-Rodríguez et al., 2017	4	0.001	32	2.881	2816	331.869188	2165.53639	3466.46361	0.000747	0.731518
Liang et al., 2019	22	0.401	20	0.001	-392.45283	42.821187	-476.382357	-308.523303	0.044884	5.039743
Coker et al., 2018	168	0.001	39	1.901	1893.040293	93.038023	1710.68577	2075.39482	0.009508	3.675972
Ling et al., 2019	64	0.001	64	6.001	5964.214712	372.763461	5233.59833	6694.8311	0.000592	0.595969
Liu et al., 2019	230	0.001	476	0.811	809.136767	21.533152	766.93179	851.341745	0.177498	5.445416
Gong et al., 2014	28	0.001	27	0.601	591.469194	56.395002	480.934991	702.003398	0.025878	4.695846
Guerrero-Preston et al., 2016	25	0.001	17	0.01	8.830189	1.011618	6.847418	10.812959	80.422181	5.597579
Gong et al., 2017	32	0.001	31	0.901	888.888889	79.188907	733.678631	1044.09915	0.013124	4.060079
Zhao et al., 2017	40	0.001	40	3.001	2971.061093	234.883107	2510.6902	3431.43198	0.001492	1.292116
Börnigen et al., 2017	242	0.001	121	0.101	99.7921	3.705298	92.529715	107.054485	5.994629	5.593287
Yang et al., 2018	51	0.001	197	3.101	3090.539166	138.769393	2818.55116	3362.52718	0.004274	2.587854
Zhang et al., 2019	50	0.001	50	0.321	317.544757	22.454682	273.53358	361.555934	0.163228	5.432477
Liu et a.l, 2018a	18	0.301	24	0.001	-294.339623	32.116543	-357.288046	-231.391199	0.07979	5.269614
Liu et al., 2018b	44	0.001	40	1.631	1615.045872	124.603682	1370.82265	1859.26909	0.005301	2.888801
Liu et al., 2019	16	0.001	30	0.101	98.285714	10.25151	78.192754	118.378674	0.783128	5.562614
Fixed Effect Model	p<0.05				14.97	0.907	[13.19-16.746]		100%	
Random Effect Model	p<0.05				577.62	30.443	[517.949-637.286]			100%



Test for heterogeneity: I2 = 99.6%, Chi2 = 6938.02, df = 25, Tau2 = 16555.81

Test for overall effect: z = 1.645 (p<0.05)

Table 3. Summary of results - fixed and random effect models

	Hedges'g (SMD)	SEg	95%CI	z score	p	Heterogeneity
Fixed Effect Model	14.97	0.907	[13.19 - 16.746]	16.499	p<0.5	I2=99.6%, Chi2=6938.02, df=25
Random Effect Model	577.62	30.443	[517.949 - 637.286]	18.974	p<0.5	99.6%, Tau2=16555.81

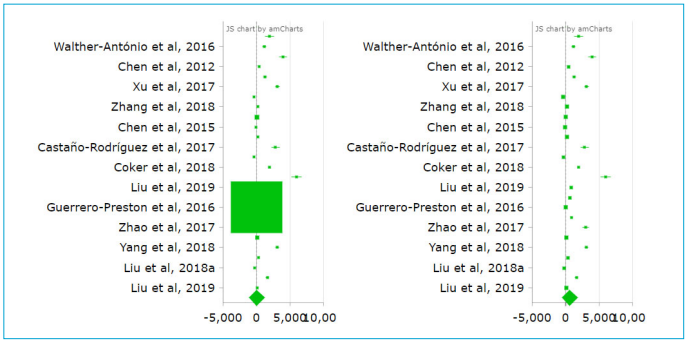


Figure 2. Forestplot - fixed and random effect models.

Three studies were found for association of lung cancer with changes in Dialister.[42-44] In these three lung cancer microbiome studies, they used different types of samples and were protected specimen brushing (PSB) samples, tissue biopsies and stool, respectively. except PSB samples. [42] of other samples.[43,44] reported *Dialister* were elevated in cancer compared the controls. But in PSB samples, Liu et al. (2018a) found *Dialister* was reduced in the microbiome of lung cancer patients.[42] Liu et al., (2018b) reported that patients with lung cancer plus emphysema had the highest *Dialister* amounts compared only emphysema or only lung cancer patients.[43]

Discussion

The interaction between microorganisms, cancer and immune response has not yet been fully discovered. Nevertheless, the evidence on the roles of microbiome studies in carcinogenesis and immunotherapy reveals that the microbiome should be examined.[45] The effect of microbiome changes on the formation of the immune response is indisputable.[46] An important way that the microbiome affects the host is bacterial metabolites. they can reach the target cells by participating in the circulation. these metabolites can affect the host through mitochondrial metabolism and can also regulate important metabolic processes such as lipid metabolism.[47]

Short chain fatty acids (SCFAs) are the ones that are considered to be the most important among the bacterial metabolites that affect the cellular or immunological mechanisms of the host. SCFAs is mainly accepted as butyrate, propionate and acetate[48] and these are essential to maintain intestinal homeostasis, especially in the anaerobic environment of the intestine. SCFAs may have opposite effects that induce or inhibit autophagy and thus inhibit proliferation of cancer cells or induce apoptosis of cancer cells.[49]

Acetate, lactate and propionate have been reported as metabolic end products of Dialister.[13,14] Acetate has been reported as an important energy source for the development of solid tumors.[50] Similar to acetate, lactate has been reported as an important component of primary and metastatic cancer metabolism.[51] Propionate has been reported as an anti-tumor effective prebiotic, unlike acetate and lactate.[52]

Recently, articles also have been published about the relationship between *Dialister* and different diseases other than cancer such as depression,[53] obesity[54] or ankylosing spondylitis.[55] The data in these studies draw attention to the Dialister.

Yost et al., 2018 reported, *Dialister* were more active in the tumour sites.[56] Ling et al., 2019 reported, *Dialister* genus positively correlated with Forkhead box protein P3 (FoxP3)+ T regulatory cells (Tregs).[33] FoxP3+ Tregs cell elevations showed both prognostic effect and a positive correlation with poor clinical outcomes in cancer patients.[57] End products of *Dialister* may also be at play here. Jimma et al., 2010 reported acetate[58] and Angelin et al., 2017 reported lactate[59] induce FoxP3+ Treg cells.

In this review, there are some limitations such as different types of cancer, different sample types in analysis, different gene regions and different number of patients. Despite all these limitations, it is important to reach important conclusions about *Dialister* and cancer relationship.

Conclusion

In a conclusion, although there are interesting results related to *Dialister* in different cancer-microbiome relationship studies, it is not much emphasized. Generally, it is seen that the amount of *Dialister* is elevated in the microbiome of cancer patients. We think that due to the effects of bacterial metabolites on host cells, *Dialister* can be an important genus especially in solid tumors. Nevertheless, more comprehensive and wider studies are needed to understand this relationship between *Dialister* and cancer. In addition, although high-throughput data are obtained with constantly developing new molecular sequencing techniques, some genus with low levels such as *Dialister* can be overlooked among these data. For this reason, these raw data

must be uploaded to public databases by the authors in microbiome or microbiota - disease relationship studies. Thus, raw data will have the chance to be re-evaluated with continuously developing bioinformatics techniques.

Disclosures

Ethics Committee Approval: Not required.

Peer-review: Externally peer-reviewed.

Conflict of Interest: None declared.

References

1. Lochhead P, Chan AT, Nishihara R, Fuchs CS, Beck AH, Giovannucci E, et al. Etiologic field effect: reappraisal of the field effect concept in cancer predisposition and progression. Mod Pathol 2015;28:14–29. [CrossRef]
2. Prager GW, Braga S, Bystricky B, Qvortrup C, Criscitiello C, Esin E, et al. Global cancer control: responding to the growing burden, rising costs and inequalities in access. ESMO Open 2018;3:e000285. [CrossRef]
3. Global Burden of Disease Cancer Collaboration, Fitzmaurice C, Dicker D, Pain A, Hamavid H, Moradi-Lakeh M, et al. The Global Burden of Cancer 2013. JAMA Oncol 2015;1:505–27.
4. Allemani C, Matsuda T, Di Carlo V, Harewood R, Matz M, Nikšić M, et al; CONCORD Working Group. Global surveillance of trends in cancer survival 2000-14 (CONCORD-3): analysis of individual records for 37 513 025 patients diagnosed with one of 18 cancers from 322 population-based registries in 71 countries. Lancet 2018;391:1023–75. [CrossRef]
5. Block KI, Gyllenhaal C, Lowe L, Amedei A, Amin ARMR, Amin A, et al. Designing a broad-spectrum integrative approach for cancer prevention and treatment. Semin Cancer Biol 2015;35 Suppl:S276–S304.
6. Miller JW, Hanson V, Johnson GD, Royalty JE, Richardson LC. From cancer screening to treatment: service delivery and referral in the National Breast and Cervical Cancer Early Detection Program. Cancer 2014;120 Suppl 16:2549–56. [CrossRef]
7. Bi WL, Hosny A, Schabath MB, Giger ML, Birkbak NJ, Mehrtash A, et al. Artificial intelligence in cancer imaging: Clinical challenges and applications. CA Cancer J Clin 2019;69:127–57.
8. Vivarelli S, Salemi R, Candido S, Falzone L, Santagati M, Stefani S, et al. Gut microbiota and cancer: from pathogenesis to therapy. Cancers (Basel) 2019;11:38. [CrossRef]
9. Bastiaanssen TFS, Cowan CSM, Claesson MJ, Dinan TG, Cryan JF. Making sense of … the microbiome in psychiatry. Int J Neuropsychopharmacol 2019;22:37–52. [CrossRef]
10. Marchesi JR, Ravel J. The vocabulary of microbiome research: a proposal. Microbiome 2015;3:31. [CrossRef]
11. Shetty SA, Hugenholtz F, Lahti L, Smidt H, de Vos WM. Intestinal microbiome landscaping: insight in community assemblage and implications for microbial modulation strategies. FEMS Microbiol Rev 2017;41:182–99. [CrossRef]
12. Milani C, Duranti S, Bottacini F, Casey E, Turroni F, Mahony J, et al. The first microbial colonizers of the human gut: composition, activities, and health implications of the infant gut microbiota. Microbiol Mol Biol Rev 2017;81:e00036–17.
13. Wade WG. Dialister. In: Whitman WB, Rainey F, Kämpfer P, Trujillo M, Chun J, DeVos P, editors. Bergey's Manual of Systematics of Archaea and Bacteria. Wiley Online Library; 2015.
14. Downes J, Munson M, Wade WG. Dialister invisus sp. nov., isolated from the human oral cavity. Int J Syst Evol Microbiol 2003;53:1937–40. [CrossRef]
15. Morio F, Jean-Pierre H, Dubreuil L, Jumas-Bilak E, Calvet L, Mercier G, et al. Antimicrobial susceptibilities and clinical sources of Dialister species. Antimicrob Agents Chemother 2007;51:4498–501. [CrossRef]
16. Jumas-Bilak E, Jean-Pierre H, Carlier JP, Teyssier C, Bernard K, Gay B, et al. Dialister micraerophilus sp. nov. and Dialister propionicifaciens sp. nov., isolated from human clinical samples. Int J Syst Evol Microbiol 2005;55:2471–8. [CrossRef]
17. Morotomi M, Nagai F, Sakon H, Tanaka R. Dialister succinatiphilus sp. nov. and Barnesiella intestinihominis sp. nov., isolated from human faeces. Int J Syst Evol Microbiol 2008;58:2716–20.
18. Hutton B, Salanti G, Caldwell DM, Chaimani A, Schmid CH, Cameron C, et al. The PRISMA extension statement for reporting of systematic reviews incorporating network meta-analyses of health care interventions: checklist and explanations. Ann Intern Med 2015;162:777–84. [CrossRef]
19. Wang A, Ling Z, Yang Z, Kiela PR, Wang T, Wang C, et al. Gut microbial dysbiosis may predict diarrhea and fatigue in patients undergoing pelvic cancer radiotherapy: a pilot study. PLoS One 2015;10:e0126312. [CrossRef]
20. Walther-António MR, Chen J, Multinu F, Hokenstad A, Distad TJ, Cheek EH, et al. Potential contribution of the uterine microbiome in the development of endometrial cancer. Genome Med 2016;8:122. [CrossRef]
21. Sims TT, Colbert LE, Zheng J, Delgado Medrano AY, Hoffman KL, Ramondetta L, et al. Gut microbial diversity and genus-level differences identified in cervical cancer patients versus healthy controls. Gynecol Oncol 2019;155:237–44. [CrossRef]
22. Chen W, Liu F, Ling Z, Tong X, Xiang C. Human intestinal lumen and mucosa-associated microbiota in patients with colorectal cancer. PLoS One 2012;7:e39743. [CrossRef]
23. Hibberd AA, Lyra A, Ouwehand AC, Rolny P, Lindegren H, Cedgård L, et al. Intestinal microbiota is altered in patients with colon cancer and modified by probiotic intervention. BMJ Open Gastroenterol 2017;4:e000145. [CrossRef]
24. Xu K, Jiang B. Analysis of mucosa-associated microbiota in colorectal cancer. Med Sci Monit 2017;23:4422–30. [CrossRef]
25. Flemer B, Warren RD, Barrett MP, Cisek K, Das A, Jeffery IB, et al. The oral microbiota in colorectal cancer is distinctive and predictive. Gut 2018;67:1454–63. [CrossRef]
26. Zhang Y, Yu X, Yu E, Wang N, Cai Q, Shuai Q, et al. Changes in

gut microbiota and plasma inflammatory factors across the stages of colorectal tumorigenesis: a case-control study. BMC Microbiol 2018;18:92. [CrossRef]
27. Loke MF, Chua EG, Gan HM, Thulasi K, Wanyiri JW, Thevambiga I, et al. Metabolomics and 16S rRNA sequencing of human colorectal cancers and adjacent mucosa. PLoS One 2018;13:e0208584. [CrossRef]
28. Chen X, Winckler B, Lu M, Cheng H, Yuan Z, Yang Y, et al. Oral microbiota and risk for esophageal squamous cell carcinoma in a high-risk area of China. PLoS One 2015;10:e0143603.
29. Elliott DRF, Walker AW, O'Donovan M, Parkhill J, Fitzgerald RC. A non-endoscopic device to sample the oesophageal microbiota: a case-control study. Lancet Gastroenterol Hepatol 2017;2:32–42. [CrossRef]
30. Castaño-Rodríguez N, Goh KL, Fock KM, Mitchell HM, Kaakoush NO. Dysbiosis of the microbiome in gastric carcinogenesis. Sci Rep 2017;7:15957. [CrossRef]
31. Liang W, Yang Y, Wang H, Wang H, Yu X, Lu Y, et al. Gut microbiota shifts in patients with gastric cancer in perioperative period. Medicine (Baltimore). 2019;98:e16626. [CrossRef]
32. Coker OO, Dai Z, Nie Y, Zhao G, Cao L, Nakatsu G, et al. Mucosal microbiome dysbiosis in gastric carcinogenesis. Gut 2018;67:1024–32. [CrossRef]
33. Ling Z, Shao L, Liu X, Cheng Y, Yan C, Mei Y, et al. Regulatory T cells and plasmacytoid dendritic cells within the tumor microenvironment in gastric cancer are correlated with gastric microbiota dysbiosis: a preliminary study. Front Immunol 2019;10:533. [CrossRef]
34. Liu X, Shao L, Liu X, Ji F, Mei Y, Cheng Y, et al. Alterations of gastric mucosal microbiota across different stomach microhabitats in a cohort of 276 patients with gastric cancer. EBioMedicine 2019;40:336–48. [CrossRef]
35. Gong H, Shi Y, Zhou X, Wu C, Cao P, Xu C, et al. Microbiota in the throat and risk factors for laryngeal carcinoma. Appl Environ Microbiol 2014;80:7356–63. [CrossRef]
36. Guerrero-Preston R, Godoy-Vitorino F, Jedlicka A, Rodríguez-Hilario A, González H, Bondy J, et al. 16S rRNA amplicon sequencing identifies microbiota associated with oral cancer, human papilloma virus infection and surgical treatment. Oncotarget 2016;7:51320–34. [CrossRef]
37. Gong H, Shi Y, Xiao X, Cao P, Wu C, Tao L, et al. Alterations of microbiota structure in the larynx relevant to laryngeal carcinoma. Sci Rep 2017;7:5507. [CrossRef]
38. Zhao H, Chu M, Huang Z, Yang X, Ran S, Hu B, et al. Variations in oral microbiota associated with oral cancer. Sci Rep 2017;7:11773. [CrossRef]
39. Börnigen D, Ren B, Pickard R, Li J, Ozer E, Hartmann EM, et al. Alterations in oral bacterial communities are associated with risk factors for oral and oropharyngeal cancer. Sci Rep 2017;7:17686. [CrossRef]
40. Yang CY, Yeh YM, Yu HY, Chin CY, Hsu CW, Liu H, et al. Oral microbiota community dynamics associated with oral squamous cell carcinoma staging. Front Microbiol 2018;9:862.
41. Zhang L, Liu Y, Zheng HJ, Zhang CP. The oral microbiota may have influence on oral cancer. Front Cell Infect Microbiol 2020;9:476. [CrossRef]
42. Liu HX, Tao LL, Zhang J, Zhu YG, Zheng Y, Liu D, et al. Difference of lower airway microbiome in bilateral protected specimen brush between lung cancer patients with unilateral lobar masses and control subjects. Int J Cancer 2018;142:769–78.
43. Liu Y, O'Brien JL, Ajami NJ, Scheurer ME, Amirian ES, Armstrong G, et al. Lung tissue microbial profile in lung cancer is distinct from emphysema. Am J Cancer Res 2018;8:1775–87.
44. Liu F, Li J, Guan Y, Lou Y, Chen H, Xu M, et al. Dysbiosis of the gut microbiome is associated with tumor biomarkers in lung cancer. Int J Biol Sci 2019;15:2381–92. [CrossRef]
45. Thomas S, Izard J, Walsh E, Batich K, Chongsathidkiet P, Clarke G, et al. The Host microbiome regulates and maintains human health: a primer and perspective for non-microbiologists. Cancer Res 2017;77:1783–812. [CrossRef]
46. Borges-Canha M, Portela-Cidade JP, Dinis-Ribeiro M, Leite-Moreira AF, Pimentel-Nunes P. Role of colonic microbiota in colorectal carcinogenesis: a systematic review. Rev Esp Enferm Dig 2015;107:659–71. [CrossRef]
47. Mikó E, Kovács T, Sebő É, Tóth J, Csonka T, Ujlaki G, et al. Microbiome-microbial metabolome-cancer cell interactions in breast cancer-familiar, but unexplored. Cells 2019;8:293.
48. Ratajczak W, Rył A, Mizerski A, Walczakiewicz K, Sipak O, Laszczyńska M. Immunomodulatory potential of gut microbiome-derived short-chain fatty acids (SCFAs). Acta Biochim Pol 2019;66:1–12. [CrossRef]
49. Casanova MR, Azevedo-Silva J, Rodrigues LR, Preto A. Colorectal cancer cells increase the production of short chain fatty acids by Propionibacterium freudenreichii impacting on cancer cells survival. Front Nutr 2018;5:44. [CrossRef]
50. Comerford SA, Huang Z, Du X, Wang Y, Cai L, Witkiewicz AK, et al. Acetate dependence of tumors. Cell 2014;159:1591–602.
51. Goodwin ML, Pennington Z, Westbroek EM, Cottrill E, Ahmed AK, Sciubba DM. Lactate and cancer: a "lactatic" perspective on spinal tumor metabolism (part 1). Ann Transl Med 2019;7:220. [CrossRef]
52. Bindels LB, Porporato P, Dewulf EM, Verrax J, Neyrinck AM, Martin JC, et al. Gut microbiota-derived propionate reduces cancer cell proliferation in the liver. Br J Cancer 2012;107:1337–44.
53. Valles-Colomer M, Falony G, Darzi Y, Tigchelaar EF, Wang J, Tito RY, et al. The neuroactive potential of the human gut microbiota in quality of life and depression. Nat Microbiol 2019;4:623–32. [CrossRef]
54. Naderpoor N, Mousa A, Gomez-Arango LF, Barrett HL, Dekker Nitert M, de Courten B. Faecal microbiota are related to ınsulin sensitivity and secretion in overweight or obese adults. J Clin Med 2019;8:452. [CrossRef]

55. Chen Z, Qi J, Wei Q, Zheng X, Wu X, Li X, Liao Z, Lin Z, Gu J. Variations in gut microbial profiles in ankylosing spondylitis: disease phenotype-related dysbiosis. Ann Transl Med 2019;7:571. [CrossRef]
56. Yost S, Stashenko P, Choi Y, Kukuruzinska M, Genco CA, Salama A, et al. Increased virulence of the oral microbiome in oral squamous cell carcinoma revealed by metatranscriptome analyses. Int J Oral Sci 2018;10:32. [CrossRef]
57. Saleh R, Elkord E. FoxP3+ T regulatory cells in cancer: Prognostic biomarkers and therapeutic targets. Cancer Lett 2020;490:174–85.
58. Jimma F, Takeda Y, Kaneda K, Wakabayashi I. Induction of Foxp3 expression in T cells by cellulose acetate beads in vitro. J Clin Apher 2010;25:216–22. [CrossRef]
59. Angelin A, Gil-de-Gómez L, Dahiya S, Jiao J, Guo L, Levine MH, et al. Foxp3 reprograms T cell metabolism to function in low-glucose, high-lactate environments. Cell Metab 2017;25:1282–93.e7. [CrossRef]

Table 1. Main characteristics of studies included in the systematic review

Author	Published time	Target cancer type	Study population	Total population	LDA scores (log10)	Sample type	Sequencer	Sequencing protocol	Cancer patients mean age	Cancer patients gender (F/M)	Cancer patients BMI	Country	Enrolment time	Dialister status in cancer patients
Wang et al.[19]	2015	Cervical cancer	Before and after pelvic radiotherapy for Pelvic Cancer patients (n=11), and healthy control (n=4)	15	2	Stool	Roche/454, GS-FLX	V3 region of 16s rRNA	51	9/2	21.5	China	N/A	Elevated
Walther-António et al.[20]	2016	Cervical cancer	Benign gynecologic condition (control cohort) (n=10), endometrial hyperplasia (n=4), and endometrial cancer (n=17) patients	31	1.2	Vaginal and cervical swab and scrape samples	Illumina MiSeq	V3-V5 region of 16s rRNA	64	17/0	32.1	USA	N/A	Elevated
Sims et al.[21]	2019	Cervical cancer	Cervical cancer patients (n=42) and healthy controls (n=46)	88	4	Stool	Illumina MiSeq	V4 region of the 16s rRNA	48.9±10.4	42/0	29.0±6.6	USA	between 2015 to 2017	Elevated
Chen et al.[22]	2012	Colorectal cancer (CRC)	Colorectal cancer patients (CRC, n=46) and healthy controls (n=56)	102	0.404	Swab, stool and tissue samples	Roche/454, GS-FLX	V1-V3 region of 16s rRNA	65	N/A	N/A	China	N/A	Elevated
Hibberd et al.[23]	2017	Colorectal cancer (CRC)	Colon cancer patients (n=15), and non-cancer healthy controls (n=21)	36	1.28	Tissue and stool samples	Illumina MiSeq	V4 region of the 16s rRNA	77	9/6	24.1	Sweden	between 2010 to 2016	Elevated
Xu et al.[24]	2017	Colorectal cancer (CRC)	Colorectal adenomas (n=47), invasive adenocarcinomas (n=52), and healthy control (n=61)	160	3.1	Tissue biopsies	Roche/454, GS-FLX	V1-V4 region of 16s rRNA	67.85±13.18	N/A	N/A	China	N/A	Elevated
Flemer et al.[25]	2018	Colorectal cancer (CRC)	Colorectal cancer (CRC n=99), colorectal polyps (n=32) and healthy controls (n=103)	234	-0.34	Oral swabs, colonic mucosae and stool	Illumina MiSeq	V3-V4 region of 16s rRNA	65	N/A	N/A	Ireland	N/A	Reduced
Zhang et al.[26]	2018	Colorectal cancer (CRC)	Initially diagnosed CRC patients (n=130), advanced	410	0.2	Stool	Illumina MiSeq	V3-V4 region of 16s rRNA	60.5	65/65	N/A	China	Between 2014 to 2015	Elevated

Table 1. CONT.

Author	Published time	Target cancer type	Study population	Total population	LDA scores (log10)	Sample type	Sequencer	Sequencing protocol	Cancer patients mean age	Cancer patients gender (F/M)	Cancer patients BMI	Country	Enrolment time	Dialister status in cancer patients
			colorectal adenoma patients (A-CRA, n=88), patients with benign intestinal polyps (n=62), and healthy controls (n=130)											
Loke et al.[27]	2018	Colorectal cancer (CRC)	Tumor and umor-free tissues from Colorectal cancer patients (CRC, n=17)	17	-0.029	Tissue biopsies	Illumina	V3-V4 region of 16s rRNA	N/A	10/7	N/A	Malaysia	Between 2013 to 2014	Reduced
Chen et al.[28]	2015	Esophageal cancer	dysplasia (n=63), esophageal squamous cell carcinoma (ESCC n=87), and healthy control (n=85)	235	-0.1	Saliva	Roche/454, GS-FLX	V3-V4 region of 16s rRNA	64.8±8.0	28/59	N/A	China	between 2010 to 2012	Reduced
Elliot et al.[29]	2017	Esophageal cancer	Normal squamous controls (n=20), non-dysplastic (n=24), dysplastic Barrett's oesophagus (n=23), and oesophageal adenocarcinoma (n=19) patients	86	0.2	Tissue biopsies	Illumina MiSeq	V1-V2 region of 16s rRNA	70	4/15	N/A	UK	N/A	Elevated
Castaño-Rodríguez et al.[30]	2017	Gastric cancer (GC)	gastric cancer (n=12) and controls (functional dyspepsia (FD), (n=20), and gastric ulcers (n=4)	36	2.88	Tissue biopsies	Illumina MiSeq	N/A	N/A	N/A	N/A	Australia	N/A	Elevated
Liang et al.[31]	2019	Gastric cancer (GC)	Gastric cancer patients (n=20) and healthy controls (n=22) & microbiota shifts of the patients with	6	-0.4	Stool	Illumina MiSeq	16s rRNA	61.3±5.8	2/4	20.8±1.81	China	between 2017 to 2018	Reduced

Table 1. CONT.

Author	Published time	Target cancer type	Study population	Total population	LDA scores (log10)	Sample type	Sequencer	Sequencing protocol	Cancer patients mean age	Cancer patients gender (F/M)	Cancer patients BMI	Country	Enrolment time	Dialister status in cancer patients
Coker et al.[32]	2018	Gastric cancer (GC)	GC (n=6) before and after the radical distal gastrectomy (RDG) Superficial gastritis (SG) (n=77), atrophic gastritis (AG) (n=74), intestinal metaplasia (IM) (n=17) and gastric cancer (GC) (n=39) patients	207	1.9	Tissue biopsies	Illumina MiSeq	V4 region of the 16s rRNA	N/A	N/A	N/A	China	N/A	Elevated
Ling et al.[33]	2019	Gastric cancer (GC)	Tumor and tumor-free tissues from Gastric cancer patients (n=64)	64	6	Tissue biopsies	Illumina MiSeq	V3 region of 16s rRNA	60.30±12.75	24/40	22.37±3.25	China	between 2014 to 2017	Elevated
Liu[1] et al.[34]	2019	Gastric cancer (GC)	primary gastric cancer tumoral tissues (n=229), peritumoral tissues (n=247), and normal tissues (n=230)	276	0.81	Tissue biopsies	Illumina MiSeq	V3 region of 16s rRNA	61.11±11.82	81/195	22.46±3.32	China	Between 2009 to 2013	Elevated
Gong et al.[35]	2014	Head and neck cancer	laryngeal carcinoma patients (n=27) and subjects with vocal cord polyps (n=28)	55	0.6	Swab and tissue samples	Roche/454, GS-FLX	V1-V3 region of 16s rRNA	N/A	2/25	N/A	China	Between 2011 to 2012	Elevated
Guerrero-Preston et al.[36]	2016	Head and neck cancer	Normal Mucosa (Control) HPV Negative (n=25), HNSCC patients (n=17), [Oropharynx Squamous cell carcinoma (OPSCC) HPV Negative (n=4), Oropharynx Squamous cell carcinoma (OPSCC) HPV Positive (n=7), and Oral Cavity Squamous cell	42	0.009	Saliva	Roche/454, GS Junior	V3-V5 region of 16s rRNA	66	7/10	N/A	USA	between 2000 to 2011	Elevated

Table 1. CONT.

Author	Published time	Target cancer type	Study population	Total population	LDA scores (log10)	Sample type	Sequencer	Sequencing protocol	Cancer patients mean age	Cancer patients gender (F/M)	Cancer patients BMI	Country	Enrolment time	Dialister status in cancer patients
			carcinoma (OSCC) HPV Negative (n=6)]											
Gong et al.[37]	2017	Head and neck cancer	Tumor and tumor-free tissues from laryngeal carcinoma patients (n=31) and subjects with vocal cord polyps (n=32)	63	0.9	Tissue biopsies	Roche/454, GS-FLX	V3 region of 16s rRNA	56.4	N/A	N/A	China	Between 2011 to 2012	Elevated
Zhao et al.[38]	2017	Head and neck cancer	Oral squamous cell carcinoma (OSCC) patients (n=40)	40	3	Swabs of oral lesions and anatomically matched normal sites	Illumina MiSeq	V4-V5 region of 16s rRNA	62	16/24	N/A	China	N/A	Elevated
Börnigen et al.[39]	2017	Head and neck cancer	oral cancer patients (n=121) (oral cavity (n=43), oropharynx (n=64), or unknown primary (n=5) squamous cell carcinoma) and healthy controls (n=242)	363	0.1	Oral rinse samples	Illumina MiSeq	V4 region of 16s rRNA	58	27/94	N/A	USA	Between 2011 to 2013	Elevated
Yang et al.[40]	2018	Head and neck cancer	Oral squamous cell carcinoma (OSCC, n=197) [OSCC stage 1 (n=41), OSCC stages 2 and 3 (n=66), and OSCC stage 4 (n=90)], healthy controls (n=51)	248	3.1	Oral rinse samples	Illumina MiSeq	V3-V4 region of 16s rRNA	53	20/177	N/A	Taiwan	N/A	Elevated
Zhang et al.[41]	2019	Head and neck cancer	Tumor and tumor-free tissues from Oral squamous cell carcinoma patients (OSCC, n=50)	50	0.32	Buccal mucosa	Illumina MiSeq	V3-V4 region of 16s rRNA	60.7	18/32	N/A	China	Between Jan to July 2018	Elevated
Liu et al.[42]	2018	Lung Cancer (LC)	Lung cancer patients (n=24)	42	-0.3	Protected specimen brushing (PSB)	Illumina MiSeq	V3-V4 region of 16s rRNA	60.58±1.275	8/16	N/A	China	N/A	Reduced

Table 1. CONT.

Author	Published time	Target cancer type	Study population	Total population	LDA scores (log10)	Sample type	Sequencer	Sequencing protocol	Cancer patients mean age	Cancer patients gender (F/M)	Cancer patients BMI	Country	Enrolment time	Dialister status in cancer patients
			and healthy controls (n=18)			samples								
Liu et al.[43]	2018	Lung Cancer (LC)	Lunc Cancer (n=40) (Emphysema-only (n=10), LC-only (n=11), LC with emphysema (n=19), and heavy smoker (n=44)	84	1.63	Tissue biopsies	Illumina MiSeq	V4 region of 16s rRNA	65	4/36	N/A	USA	N/A	Elevated
Liu et al.[44]	2019	Lung Cancer (LC)	newly diagnosed lung cancer patients (n=30), and healthy control (n=16)	46	0.1	Stool	Illumina Hiseq	V4 region of 16s rRNA	60	9/21	N/A	China	N/A	Elevated

N/A: Not avaliable.