International Journal of
Enteric
Pathogens

Int J Enteric Pathog. 2021 November;9(4):131-137 Original Article

http://enterpathog.abzums.ac.ir

doi 10.34172/ijep.2021.25

Gut Microbiota in Breast Cancer Patients Differs From That of Colon Cancer Patients and Healthy Individuals: A Gut-Tumor Axis

Nafiseh Pakravan[1*], Ardeshir Abbasi[2], Shiva Hatami[3], Nasrin Sehati[1], Ahmad Elahi[4]

[1]Division of Immunology, Medical School, Alborz University of Medical Sciences, Karaj, Iran
[2]Department of Immunology, Medical School, Tarbiat Modares University, Tehran, Iran
[3]Department of Microbiology, Medical School, Alborz University of Medical Sciences, Karaj, Iran
[4]Department of Surgery, Medical School, Alborz University of Medical Sciences, Karaj, Iran

***Corresponding Author:**
Nafiseh Pakravan, Nabowat Blvd, End of West Bou-Ali St, Karaj, Iran. Tel: +98-26-34287315 Ext: 102, Fax: +98-26-32634287425, Email: n.pakravan@abzums.ac.ir

Published Online November 29, 2021

Keywords: Gut-microbiota, Breast-cancer, Colon-cancer, Gut-tumor axis, Bacteroidetes, Firmicutes, Actinobacteria, Proteobacteria, Fusobacteria, Verrucomicrobia

Abstract

Background: The gut microbiota influences human health and disease. Alterations in gut microbiota may have pathological consequences. Scientific knowledge about gut microbiota can facilitate predicting the likelihood of certain intestinal and/or extra-intestinal diseases. There are six main phyla in gut including Bacteroidetes, Firmicutes, Actinobacteria, Proteobacteria, Verrucomicrobia, and Fusobacteria, among which Proteobacteria and Fusobacteria are associated with colon cancer. Association of the gut microbiota pattern with colon cancer is conceivable because of their close proximity. Accordingly, breast tissue microbiota has been associated with breast tumor.

Objective: This study aimed to identify the gut microbiota pattern in breast cancer, therefore, the six phyla in fecal sample from patients with breast cancer were investigated and compared with those from healthy individuals and colon cancer patients.

Methods: Real-time polymerase chain reaction (PCR) was performed on DNA extracted from fecal samples based on variable region of 16S ribosomal DNA gene of the six main phyla in the gut.

Results: Bacteroidetes and Firmicutes levels in breast cancer patients were higher than those in colon cancer patients and healthy individuals. Inversely, Actinobacteria, Verrucomicrobia, Proteobacteria, and Fusobacteria levels in breast cancer were lower than those in colon cancer patients and healthy individuals.

Conclusion: Taking into account the decreased level of oncogenic microbiota in fecal sample from breast cancer patients compared to the level of that from colon cancer or healthy cases as well as the presence of oncogenic microbiota in breast tumor, some bacteria may have translocated from gut to breast tissue in some circumstances which likely contribute to the breast tumorigenesis (gut-tumor axis). Migration of the bacteria from gastrointestinal tract to tumor may have occurred in a similar fashion to that of the bacteria from gastrointestinal to fetus. It is worth mentioning that tumor and fetus are immune privileged sites.

Received November 8, 2021; **Revised** November 15, 2021; **Accepted** November 25, 2021

Background

The ecosystem in gastrointestinal tract, known as the microbiota, typically contains trillions of microbial cells that play an important role in nutrient absorption, digestion, metabolism, as well as host immunity. Since more than five decades ago, scientist have noted that alteration of the microbiome is associated with the development of gut cancer and other inflammatory disease. Specifically, there are researches indicating the association of human gut microbiome with the development of colon cancer. The disease is the third most common type of cancer detected in the world and the second most common cause of the cancer-related mortality worldwide.[1] In addition, breast cancer is the first prevalent cancer and leading cause of death among the world's female population.[2] The disease has been reported to be associated with the pattern of microbiota in the breast tumor.[1,3,4] The association between the pattern of gut microbiota or breast microbiota with colon or breast cancer, respectively, is conceivable due to the close contact of the microbiota with the corresponding tissue. However, the association of gut microbiota with breast cancer is worth considering and deserves to be examined in greater details. Therefore, this study aimed to determine the six major bacterial phyla in patients with colon or breast cancer, and to compare them with those in healthy individuals. This determination was because the microbiota is dominated by five phyla including

Becteroidetes, Firmicutes, Fusobacteria, Actinobacteria, Proteobacteria, and Verrucomicrobia.[5,6]

Materials and Methods

Inclusion and Exclusion Criteria for Selection of Subjects and Sample Collection

Our study subjects included colon or breast cancer patients or healthy control individuals (n = 50), aged between 25–70 years. Breast or colon cancer patients included those diagnosed with breast or colon cancer at early stages. Patients with a history of cancer of any types or with advanced tumor stage with metastasis, those having undergone treatment with antibiotics within a period of three months prior to sampling, and those having received therapy of any types were excluded from the study. Inclusion criteria were patients diagnosed with colon or breast cancer by histopathological examination, and those not having undergone any surgical/physical procedures within 1 week or chemoradiotherapy treatment before sampling. Normal cases were matched to cancer patients by age (±2 years). Healthy volunteers not having undergone any surgical/physical procedures within 1 week or any therapies within a period of three months prior to sampling were considered as the healthy control group. In addition, cases with obesity, intestinal infection, digestive tract symptoms, dementia, chronic diseases (e.g., hypertension, heart disease, and/or diabetes) and low-performance status (e.g., mentally/physically disabled individuals) were excluded from the study.

Fecal Sample Collection and Storage

The subjects were provided with sterile container for stool sample collection along with information about the sample collection procedure including an instruction as to carefully avoid contaminating the samples with urine or sewage. Fresh fecal samples (not less than 6 g) stored in sterile containers were kept at −80°C until DNA was extracted. Stool samples were collected before performing surgery and electronic colonoscopy in patients with colon cancer.

DNA Extraction and Real-Time Polymerase Chain Reaction

DNA was extracted from the stool sample using the QIAamp DNA Stool Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. The DNA concentration of the samples was evaluated using a NanoDrop 2000c (Thermo Fisher Scientific, Waltham, MA, USA) and stored at −20°C for further analysis.

The variable region of 16S ribosomal DNA gene was amplified using ABI Step One (Applied Biosystems, Sequences Detection Systems, Foster City, CA) thermocycler and SYBR® Green PCR master mix (Applied Biosystems, Life Technologies, Paisley, United Kingdom) according to the manufacturer's instructions. Each reaction contained 5 µL master mix, 100 nM primers for Bacteroidetes, Firmicutes, Actinobacteria, Proteobacteria, Verrucomicrobia, and universal bacterial reference gene plus 1 µg DNA. The sequences for primers are presented in Table 1. Thermocycler conditions included an initial step at 95°C for 15 minutes, followed by 40 cycles at 94°C:20 seconds, 58-60°C:40 seconds, and 72°C:30 seconds. The universal bacterial reference gene was chosen as internal control against which DNA level of the target bacteria gene was normalized. The resultant DNA level was presented as $2^{-\Delta\Delta Ct}$, in which ΔCt was the difference between Ct values of target bacteria gene and universal bacterial reference gene.[7]

Statistical Analysis

Data were analyzed using two-tailed Student's *t* test or one-way ANOVA followed by the Tukey's post hoc test whenever applicable. Statistical analysis was performed using GraphPad Prism software (GraphPad Software, San Diego, CA). Statistical significance was set at $P < 0.05$.

Results

The levels of Bacteroidetes and Firmicutes (Figure 1a, b) in breast cancer patients was significantly higher than those in healthy and colon cancer patients ($P < 0.001$). It was also observed that Bacteroidetes level was significantly higher than Firmicutes level in breast cancer and colon cancer patients ($P < 0.0001$). In this regard, as shown in Figure 1c, Bacteroidetes:Firmicutes ratio in breast cancer patients was markedly more than that in colon cancer patients ($P < 0.01$) and healthy individuals ($P < 0.0001$). There was also a significant difference between colon cancer patients and healthy individuals in terms of Bacteroidetes:Firmicutes ratio ($P < 0.0001$).

Table 1. Primer Sequences

Name	Forward	Reverse
Universal	ACTWCTACGYGAGGCAG	GTATTANCGCGYCTGCTG
Bacteroidetes	CATGTGGTYTAATTNGATGAT	AGCTGANGACANCCATGCAG
Firmicutes	TGAAANTYAAAGGAAYTGACG	ACCWTGCANCACCTGTC
Actinobacteria	CGCNGCCTATWAGCTTGTTG	CCGWACTCCCCAGGCGNGG
Fusobacteria	GATNCAGCAATTCTNTGTGC	CYAATTTCACCTNTACACTTWT
Verrucomicrobia	GAATTCTCGGTYTAGCA	GGCATTGTNGTACGTNTGCA
Proteobacteria	CATGNCWTTACCCGYAGNAGAA	CTNTACGAGNCTCAAGCTYG

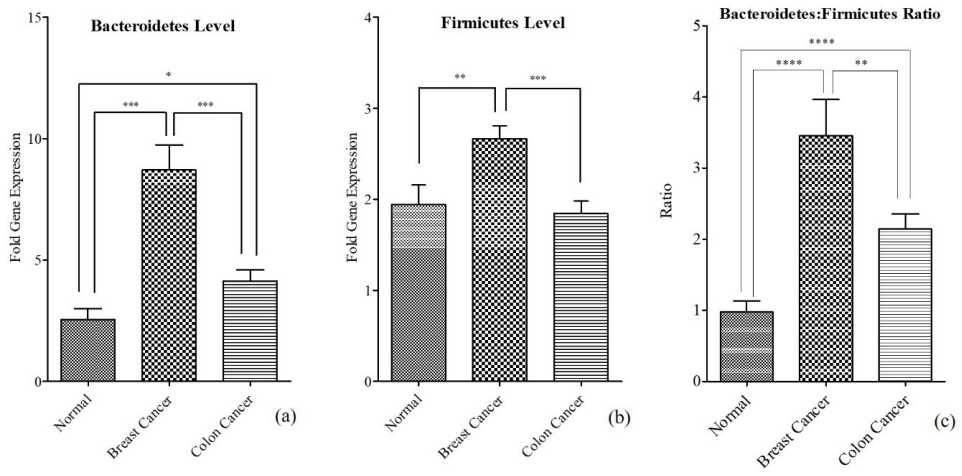

Figure 1. Levels of Bacteroidetes (a), Firmicutes (b), and Bacteroidetes:Firmicutes Ratio (c) in the Fecal Sample of Healthy Subjects, Breast Cancer Patients, and Colon Cancer Patients (n = 50). Real-time PCR using cyber green was performed using phyla-specific 16S ribosomal DNA on the DNA extracted from fecal samples. The quantification of 16S ribosomal DNA of each phylum was normalized against 16S ribosomal DNA-based universal bacterial reference gene. Data are presented as mean ± SEM.

The levels of Actinobacteria and Verrucomicrobia (Figure 2a, b) in healthy individuals were significantly higher than those of breast cancer and colon cancer patients ($P < 0.01$, $P < 0.0001$). In addition, Actinobacteria and Verrucomicrobia levels in colon cancer patients were significantly higher than those of breast cancer patients ($P < 0.05$). Inversely, Proteobacteria and Fusobacteria levels (Figure 2c, d) in colon cancer patients were significantly higher than those of breast cancer patients and healthy individuals ($P < 0.0001$). Nevertheless, Proteobacteria and Fusobacteria levels in healthy subjects were markedly higher than those of breast cancer patients ($P < 0.0001$).

Total level of the six phyla also differed among the three groups where the highest and lowest levels of microbiota were recorded for breast cancer patients and healthy individuals, respectively (Figure 3).

On this basis, the percentages of phyla in each group were in the following order (Figure 3b and Table 2):

- Healthy individual: Fusobacteria < roteobacteria < Verrucomicrobia < Actinobacteria < Firmicutes < Bacteroidetes
- Breast cancer patients: Verrucomicrobia < Fusobacteria < Proteobacteria < Actinobacteria < Firmicutes < Bacteroidetes
- Colon cancer patients: Verrucomicrobia < Fusobacteria < Actinobacteria < Firmicutes < Proteobacteria < Bacteroidetes

Discussion

It has been shown that there is an association between pattern or gut or breast tissue microbiota with colon or breast cancer, respectively, which seems conceivable because of their close proximity. However, the association of gut microbiota with breast cancer has not been investigated yet. In this study, a comparison was made between patients with colon or breast cancer and normal individuals with gut microbiota in terms of six major bacteria phyla in the gut, including Becteroidetes, Firmicutes, Actinobacteria, Verrucomicrobia, Proteobacteria, and Fusobacteria.[8]

Our study results regarding the frequency of six phyla in the normal group were consistent with the findings from previous studies reporting the dominancy of Bacteroidetes over Firmicutes.[8-11] Nevertheless, there are studies reporting different dominancy/frequency for bacterial phyla within the normal human fecal microbiota, and suggesting the dominancy of Firmicutes over Bacteroidetes.[12-19] This may be attributed to genetic differences, age, gender, or life style causing changes in individuals and between individuals.[13,20] However, there is a general consensus that Fusobacteria are less predominant than other phyla present in the gut, and can increase in colon cancer[21,22] along with Bacteroidetes and Proteobacteria whose levels are proportional to tumor mass.[23-28] In contrast, Actinobacteria has been suggested as a family with antitumor potential.[29,30] Our study results regarding Bacteroidetes, Fusobacteria, Actinobacteria, and Proteobacteria levels were consistent with those from previous studies. However, novel results were generated in our study regarding the Firmicutes family in patients with colon cancer with no change in its rate different from others suggesting an increasing or decreasing variation in Firmicutes level.[14,31-34] As for the Verrucomicrobia family, which are able to regenerate the intestinal mucosa by producing proteases[35] and eliminate inflammation,[36] different reports have confirmed their association with colon cancer.[37,38] Our study results were also in line with those highlighting the anti-tumor properties of this family.

It is noteworthy that the levels of six phyla in the fecal

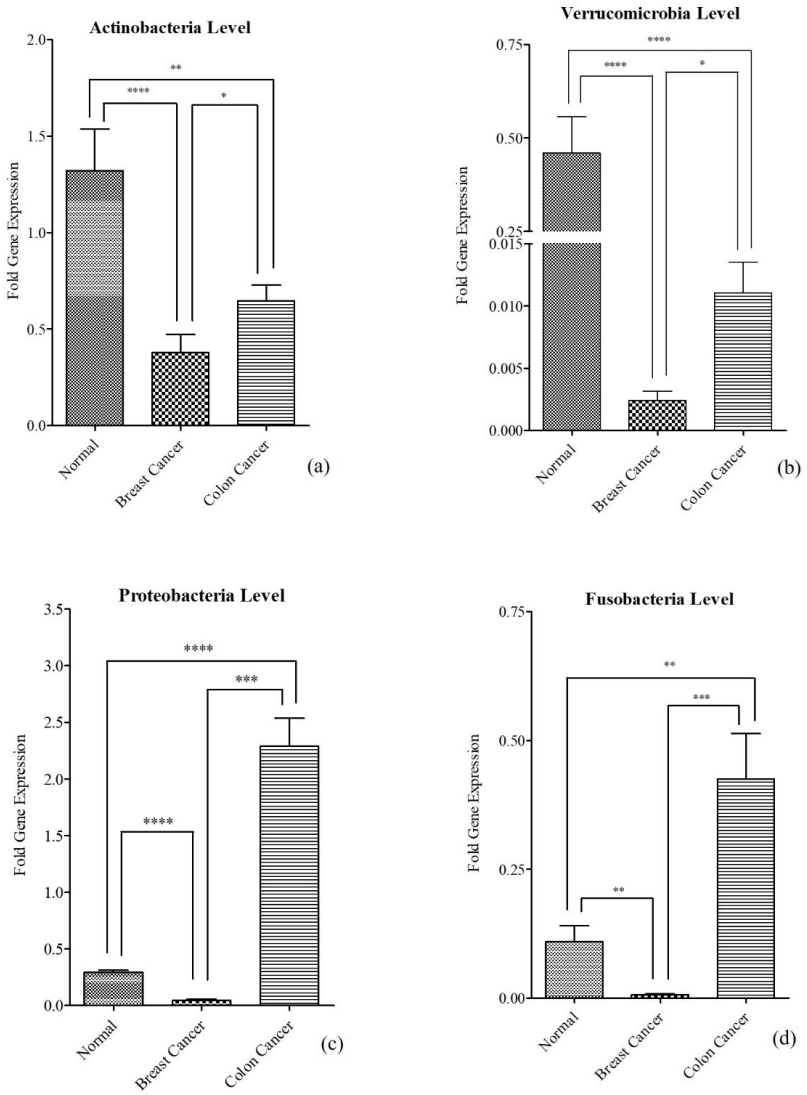

Figure 2. Levels of Actinobacteria (a), Verrucomicrobia (b), Proteobacteria (c), and Fusobacteria (d) in the Fecal Sample of Healthy Subjects, Breast Cancer Patients, and Colon Cancer Patients (n = 50). Real-time PCR was performed, as described in Figure 1 legend. Data are presented as mean ± SEM.

sample from breast cancer patients were completely different than those from colon cancer patients. Fecal sample from breast cancer patients was mainly dominated with Bacteroidetes and Firmicutes, and contained markedly lower levels of Proteobacteria and Fusobacteria. Given the gut microbiome-immune system association with the prevention of tumor development at extra-intestinal tissues, it has been proposed that there is a connection between gut microbiome and microbiome in extra-intestinal tissues.[4,39-45]

The normal breast tissue has been reported to be mostly enriched with Proteobacteria, Firmicutes, and Actinobacteria phyla, among which Proteobacteria is the most abundant phylum represented in breast tissue, while Firmicutes is the second most common phylum.[4] Inversely, previous studies have found significant increase of Proteobacteria and colonization of Fusobacteria in the cancerous breast tissue, accelerating tumor growth and metastatic progression.[46-48] Another study has identified bacteremia with Fusobacterium nucleatum as a risk factor for cancer,[49,50] presumably due to its immunosuppressive activities.[51] However, it has been also argued that Fusobacteria is a passenger for which tumor condition is favorable to multiply, rather than a causal factor in colorectal cancer development.[52,53] Accordingly, the movement of Fusobacterium from primary to distal metastates through blood has been reported.[54-56] This might be explained by the fact that Fusobacteria, specifically home-in to Gal-GalNAc-displaying tumors, binds via fusobacterium adhesin A, fibroblast activation protein 2, and MORN2 (Membrane occupation and recognition nexus repeat containing 2) proteins.[48,52] In addition, the relationship between Fusobacterium species with the emergence and progression of other types of tumors including oral, esophageal, and colon has been investigated.[57] In this regard, the present study

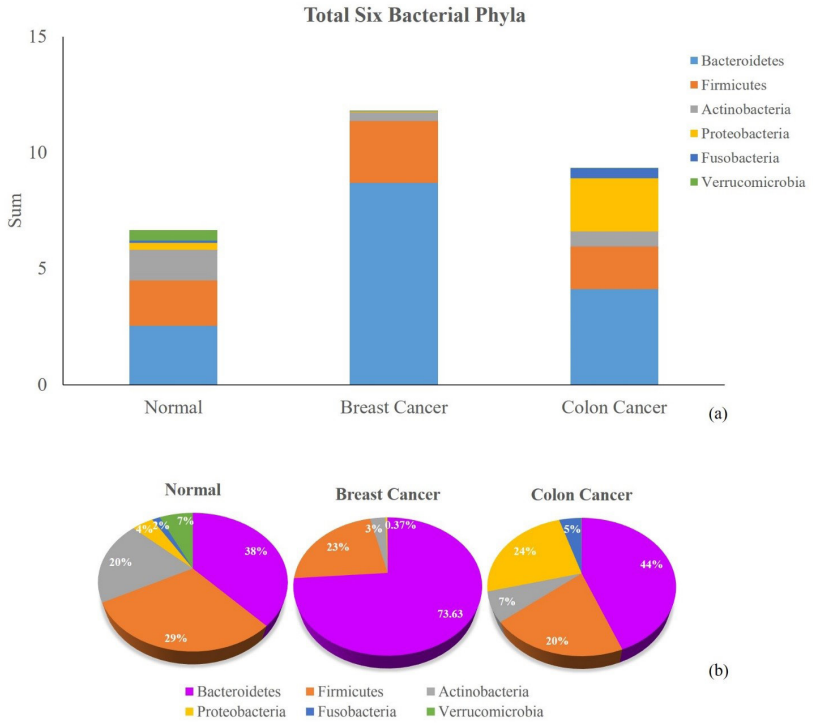

Figure 3. Total Microbiota (a) and Percentage (b) of Each of Six Phyla in Healthy Subjects, Breast Cancer Patients, and Colon Cancer Patients.

Table 2. Percentage of Each Bacterial Phylum in Each Group

	Bacteroidetes	Firmicutes	Actinobacteria	Proteobacteria	Fusobacteria	Verrucomicrobia
Healthy	38.17098139	29.1190724	19.78725058	4.389417923	1.635732595	6.897545125
Breast cancer	73.75536726	22.5884703	3.205231912	0.37571997	0.054749488	0.020461082
Colon cancer	44.16435914	19.7393195	6.926445602	24.49428718	4.557195318	0.118393229

suggested that the decrease in level of Fusobacteria and Proteobacteria in the gut may have been due to their translocation from gut to breast tissue through blood in a similar manner to that seen between mother and fetus.[58,59]

Conclusion

It was concluded that the gut microbiota in breast cancer patients was different from that in colon cancer patients and healthy individuals. It was recommended that the gut microbiota should be closely monitored in order to develop a potential approach for screening extraintestinal and intestinal cancers. Given the alteration of microbiota in the breast and gut of breast cancer patients compared to that in the healthy breast and gut, it was likely that some bacteria, such as Proteobacteria and Fusobacteria, associated with tumorigenesis, may have translocated from gut to breast tissue in some circumstances. This occurrence may have been attributable to the gut-tumor axis presumably participating in breast tumorigenesis. This finding was significant since fetus and tumor are both immune privileged sites, and the migration of microbiota from gastrointestinal tract to tumor may occur in a similar fashion to that of microbiota from mother's gastrointestinal tract to fetus.

Acknowledgements

The authors would like to thank Dr Mojan Asadi from Alborz University of Medical Sciences and Dr Habibollah Mahmoodzadeh from Tehran University of Medical Sciences for providing some samples. Special thanks to Dr Yasliani Fard and Ms Atefe Alirezaee for providing the authors with scientific support and for technical help, respectively.

Authors' Contribution

NP conceived and designed the study. All of the authors were involved in development of methodology. AA, SH, NS, and AE collected the samples and performed the experiments under the supervision of NP. Statistical analysis was performed by NP assisted by other authors. NP supervised overall data generation and analysis, and wrote the manuscript cooperatively with other authors. Authors have no competing interests.

Availability of Data and Materials

The data supporting our study findings may become available by corresponding author Dr Nafiseh Pakravan upon reasonable request.

Conflict of Interest Disclosures

The authors declare that they have no conflict of interests.

Ethical Approval

This study was approved by the Ethical Committee of the Alborz

University of Medical Sciences, Karaj, Iran, under reference No. ABZUMS.REC.1395.117. All collected data were regarded as confidential issues and were only accessible to participants upon request. The subjects participated in the study voluntarily and signed an informed consent form. All participants were provided with information about the nature of research, application of samples, obtained data, importance of the results, and perspective application.

Financial Support

The study was approved initially by Clinical Research Development Unit of Emam Ali Hospital, and then by Deputy of Research at Alborz University of Medical Sciences. The foundation was received from Deputy of Research at Alborz University of Medical Sciences under reference number 3046575.

References

1. Johns MS, Petrelli NJ. Microbiome and colorectal cancer: a review of the past, present, and future. Surg Oncol. 2021;37:101560. doi:10.1016/j.suronc.2021.101560
2. Azamjah N, Soltan-Zadeh Y, Zayeri F. Global trend of breast cancer mortality rate: a 25-year study. Asian Pac J Cancer Prev. 2019;20(7):2015-2020. doi:10.31557/apjcp.2019.20.7.2015
3. Alpuim Costa D, Nobre JG, Batista MV, et al. Human microbiota and breast cancer-is there any relevant link?-A literature review and new horizons toward personalised medicine. Front Microbiol. 2021;12:584332. doi:10.3389/fmicb.2021.584332
4. Toumazi D, El Daccache S, Constantinou C. An unexpected link: the role of mammary and gut microbiota on breast cancer development and management (review). Oncol Rep. 2021;45(5):80. doi:10.3892/or.2021.8031
5. Bull MJ, Plummer NT. Part 1: the human gut microbiome in health and disease. Integr Med (Encinitas). 2014;13(6):17-22.
6. Schloss PD, Handelsman J. Status of the microbial census. Microbiol Mol Biol Rev. 2004;68(4):686-691. doi:10.1128/mmbr.68.4.686-691.2004
7. Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) method. Methods. 2001;25(4):402-408. doi:10.1006/meth.2001.1262
8. Liu W, Zhang R, Shu R, et al. Study of the relationship between microbiome and colorectal cancer susceptibility using 16SrRNA sequencing. Biomed Res Int. 2020;2020:7828392. doi:10.1155/2020/7828392
9. Shin NR, Whon TW, Bae JW. Proteobacteria: microbial signature of dysbiosis in gut microbiota. Trends Biotechnol. 2015;33(9):496-503. doi:10.1016/j.tibtech.2015.06.011
10. Mori G, Rampelli S, Orena BS, et al. Shifts of faecal microbiota during sporadic colorectal carcinogenesis. Sci Rep. 2018;8(1):10329. doi:10.1038/s41598-018-28671-9
11. Gao Z, Guo B, Gao R, Zhu Q, Qin H. Microbiota disbiosis is associated with colorectal cancer. Front Microbiol. 2015;6:20. doi:10.3389/fmicb.2015.00020
12. Sartor RB, Mazmanian SK. Intestinal microbes in inflammatory bowel diseases. Am J Gastroenterol Suppl. 2012;1(1):15-21. doi:10.1038/ajgsup.2012.4
13. Arumugam M, Raes J, Pelletier E, et al. Enterotypes of the human gut microbiome. Nature. 2011;473(7346):174-180. doi:10.1038/nature09944
14. Qin J, Li R, Raes J, et al. A human gut microbial gene catalogue established by metagenomic sequencing. Nature. 2010;464(7285):59-65. doi:10.1038/nature08821
15. Andersson AF, Lindberg M, Jakobsson H, Bäckhed F, Nyrén P, Engstrand L. Comparative analysis of human gut microbiota by barcoded pyrosequencing. PLoS One. 2008;3(7):e2836. doi:10.1371/journal.pone.0002836
16. Eckburg PB, Bik EM, Bernstein CN, et al. Diversity of the human intestinal microbial flora. Science. 2005;308(5728):1635-1638. doi:10.1126/science.1110591
17. Jalanka-Tuovinen J, Salonen A, Nikkilä J, et al. Intestinal microbiota in healthy adults: temporal analysis reveals individual and common core and relation to intestinal symptoms. PLoS One. 2011;6(7):e23035. doi:10.1371/journal.pone.0023035
18. Rajilić-Stojanović M, Heilig HG, Molenaar D, et al. Development and application of the human intestinal tract chip, a phylogenetic microarray: analysis of universally conserved phylotypes in the abundant microbiota of young and elderly adults. Environ Microbiol. 2009;11(7):1736-1751. doi:10.1111/j.1462-2920.2009.01900.x
19. Tap J, Mondot S, Levenez F, et al. Towards the human intestinal microbiota phylogenetic core. Environ Microbiol. 2009;11(10):2574-2584. doi:10.1111/j.1462-2920.2009.01982.x
20. Gerritsen J, Smidt H, Rijkers GT, de Vos WM. Intestinal microbiota in human health and disease: the impact of probiotics. Genes Nutr. 2011;6(3):209-240. doi:10.1007/s12263-011-0229-7
21. Castellarin M, Warren RL, Freeman JD, et al. *Fusobacterium nucleatum* infection is prevalent in human colorectal carcinoma. Genome Res. 2012;22(2):299-306. doi:10.1101/gr.126516.111
22. Kostic AD, Gevers D, Pedamallu CS, et al. Genomic analysis identifies association of *Fusobacterium* with colorectal carcinoma. Genome Res. 2012;22(2):292-298. doi:10.1101/gr.126573.111
23. Bäckhed F, Ley RE, Sonnenburg JL, Peterson DA, Gordon JI. Host-bacterial mutualism in the human intestine. Science. 2005;307(5717):1915-1920. doi:10.1126/science.1104816
24. Pushpanathan P, Mathew GS, Selvarajan S, Seshadri KG, Srikanth P. Gut microbiota and its mysteries. Indian J Med Microbiol. 2019;37(2):268-277. doi:10.4103/ijmm.IJMM_19_373
25. Tremaroli V, Bäckhed F. Functional interactions between the gut microbiota and host metabolism. Nature. 2012;489(7415):242-249. doi:10.1038/nature11552
26. Gagnière J, Raisch J, Veziant J, et al. Gut microbiota imbalance and colorectal cancer. World J Gastroenterol. 2016;22(2):501-518. doi:10.3748/wjg.v22.i2.501
27. Raskov H, Burcharth J, Pommergaard HC. Linking gut microbiota to colorectal cancer. J Cancer. 2017;8(17):3378-3395. doi:10.7150/jca.20497
28. Lee JG, Lee YR, Lee AR, Park CH, Han DS, Eun CS. Role of the global gut microbial community in the development of colitis-associated cancer in a murine model. Biomed Pharmacother. 2021;135:111206. doi:10.1016/j.biopha.2020.111206
29. Silva LJ, Crevelin EJ, Souza DT, et al. Actinobacteria from Antarctica as a source for anticancer discovery. Sci Rep. 2020;10(1):13870. doi:10.1038/s41598-020-69786-2
30. Zhou YJ, Zhao DD, Liu H, et al. Cancer killers in the human gut microbiota: diverse phylogeny and broad spectra. Oncotarget. 2017;8(30):49574-49591. doi:10.18632/oncotarget.17319
31. Fukugaiti MH, Ignacio A, Fernandes MR, Ribeiro Júnior U, Nakano V, Avila-Campos MJ. High occurrence of *Fusobacterium nucleatum* and *Clostridium difficile* in the intestinal microbiota of colorectal carcinoma patients. Braz J Microbiol. 2015;46(4):1135-1140. doi:10.1590/s1517-838246420140665
32. Mima K, Nishihara R, Qian ZR, et al. *Fusobacterium nucleatum* in colorectal carcinoma tissue and patient prognosis. Gut. 2016;65(12):1973-1980. doi:10.1136/gutjnl-2015-310101

33. Peng BJ, Cao CY, Li W, et al. Diagnostic performance of intestinal *Fusobacterium nucleatum* in colorectal cancer: a meta-analysis. Chin Med J (Engl). 2018;131(11):1349-1356. doi:10.4103/0366-6999.232814
34. Tunsjø HS, Gundersen G, Rangnes F, Noone JC, Endres A, Bemanian V. Detection of *Fusobacterium nucleatum* in stool and colonic tissues from Norwegian colorectal cancer patients. Eur J Clin Microbiol Infect Dis. 2019;38(7):1367-1376. doi:10.1007/s10096-019-03562-7
35. Meng X, Wang W, Lan T, et al. A purified aspartic protease from *Akkermansia muciniphila* plays an important role in degrading Muc2. Int J Mol Sci. 2019;21(1):72. doi:10.3390/ijms21010072
36. Plovier H, Everard A, Druart C, et al. A purified membrane protein from *Akkermansia muciniphila* or the pasteurized bacterium improves metabolism in obese and diabetic mice. Nat Med. 2017;23(1):107-113. doi:10.1038/nm.4236
37. Baxter NT, Zackular JP, Chen GY, Schloss PD. Structure of the gut microbiome following colonization with human feces determines colonic tumor burden. Microbiome. 2014;2:20. doi:10.1186/2049-2618-2-20
38. Daisley BA, Chanyi RM, Abdur-Rashid K, et al. Abiraterone acetate preferentially enriches for the gut commensal *Akkermansia muciniphila* in castrate-resistant prostate cancer patients. Nat Commun. 2020;11(1):4822. doi:10.1038/s41467-020-18649-5
39. Xuan C, Shamonki JM, Chung A, et al. Microbial dysbiosis is associated with human breast cancer. PLoS One. 2014;9(1):e83744. doi:10.1371/journal.pone.0083744
40. Dieleman S, Aarnoutse R, Ziemons J, Kooreman L, Boleij A, Smidt M. Exploring the potential of breast microbiota as biomarker for breast cancer and therapeutic response. Am J Pathol. 2021;191(6):968-982. doi:10.1016/j.ajpath.2021.02.020
41. Zhang Z, Tang H, Chen P, Xie H, Tao Y. Demystifying the manipulation of host immunity, metabolism, and extraintestinal tumors by the gut microbiome. Signal Transduct Target Ther. 2019;4:41. doi:10.1038/s41392-019-0074-5
42. Minelli EB, Beghini AM, Vesentini S, et al. Intestinal microflora as an alternative metabolic source of estrogens in women with uterine leiomyoma and breast cancer. Ann N Y Acad Sci. 1990;595(1):473-479. doi:10.1111/j.1749-6632.1990.tb34337.x
43. Erdman SE, Poutahidis T. Gut bacteria and cancer. Biochim Biophys Acta. 2015;1856(1):86-90. doi:10.1016/j.bbcan.2015.05.007
44. Mani S. Microbiota and breast cancer. Prog Mol Biol Transl Sci. 2017;151:217-229. doi:10.1016/bs.pmbts.2017.07.004
45. Alizadehmohajer N, Shojaeifar S, Nedaeinia R, et al. Association between the microbiota and women's cancers - cause or consequences? Biomed Pharmacother. 2020;127:110203. doi:10.1016/j.biopha.2020.110203
46. Lawrance A, Balakrishnan M, Gunasekaran R, et al. Unexplored deep sea habitats in active volcanic Barren Island, Andaman and Nicobar Islands are sources of novel halophilic eubacteria. Infect Genet Evol. 2018;65:1-5. doi:10.1016/j.meegid.2018.07.008
47. Salihoğlu R, Önal-Süzek T. Tissue microbiome associated with human diseases by whole transcriptome sequencing and 16s metagenomics. Front Genet. 2021;12:585556. doi:10.3389/fgene.2021.585556
48. Parhi L, Alon-Maimon T, Sol A, et al. Breast cancer colonization by *Fusobacterium nucleatum* accelerates tumor growth and metastatic progression. Nat Commun. 2020;11(1):3259. doi:10.1038/s41467-020-16967-2
49. Yang Y, Weng W, Peng J, et al. *Fusobacterium nucleatum* increases proliferation of colorectal cancer cells and tumor development in mice by activating toll-like receptor 4 signaling to nuclear factor-κB, and up-regulating expression of microRNA-21. Gastroenterology. 2017;152(4):851-866.e824. doi:10.1053/j.gastro.2016.11.018
50. Yusuf E, Wybo I, Piérard D. Case series of patients with *Fusobacterium nucleatum* bacteremia with emphasis on the presence of cancer. Anaerobe. 2016;39:1-3. doi:10.1016/j.anaerobe.2016.02.001
51. Gholizadeh P, Eslami H, Samadi Kafil H. Carcinogenesis mechanisms of *Fusobacterium nucleatum*. Biomed Pharmacother. 2017;89:918-925. doi:10.1016/j.biopha.2017.02.102
52. Ranjbar M, Salehi R, Haghjooy Javanmard S, et al. The dysbiosis signature of *Fusobacterium nucleatum* in colorectal cancer-cause or consequences? A systematic review. Cancer Cell Int. 2021;21(1):194. doi:10.1186/s12935-021-01886-z
53. Amitay EL, Werner S, Vital M, et al. *Fusobacterium* and colorectal cancer: causal factor or passenger? Results from a large colorectal cancer screening study. Carcinogenesis. 2017;38(8):781-788. doi:10.1093/carcin/bgx053
54. Bullman S, Pedamallu CS, Sicinska E, et al. Analysis of *Fusobacterium* persistence and antibiotic response in colorectal cancer. Science. 2017;358(6369):1443-1448. doi:10.1126/science.aal5240
55. Abed J, Maalouf N, Manson AL, et al. Colon cancer-associated *Fusobacterium nucleatum* may originate from the oral cavity and reach colon tumors via the circulatory system. Front Cell Infect Microbiol. 2020;10:400. doi:10.3389/fcimb.2020.00400
56. Richardson M, Ren J, Rubinstein MR, et al. Analysis of 16S rRNA genes reveals reduced fusobacterial community diversity when translocating from saliva to GI sites. Gut Microbes. 2020;12(1):1-13. doi:10.1080/19490976.2020.1814120
57. Fujiwara N, Kitamura N, Yoshida K, Yamamoto T, Ozaki K, Kudo Y. Involvement of *Fusobacterium* species in oral cancer progression: a literature review including other types of cancer. Int J Mol Sci. 2020;21(17):6207. doi:10.3390/ijms21176207
58. Perez-Muñoz ME, Arrieta MC, Ramer-Tait AE, Walter J. A critical assessment of the "sterile womb" and "in utero colonization" hypotheses: implications for research on the pioneer infant microbiome. Microbiome. 2017;5(1):48. doi:10.1186/s40168-017-0268-4
59. Walker RW, Clemente JC, Peter I, Loos RJF. The prenatal gut microbiome: are we colonized with bacteria in utero? Pediatr Obes. 2017;12(Suppl 1):3-17. doi:10.1111/ijpo.12217