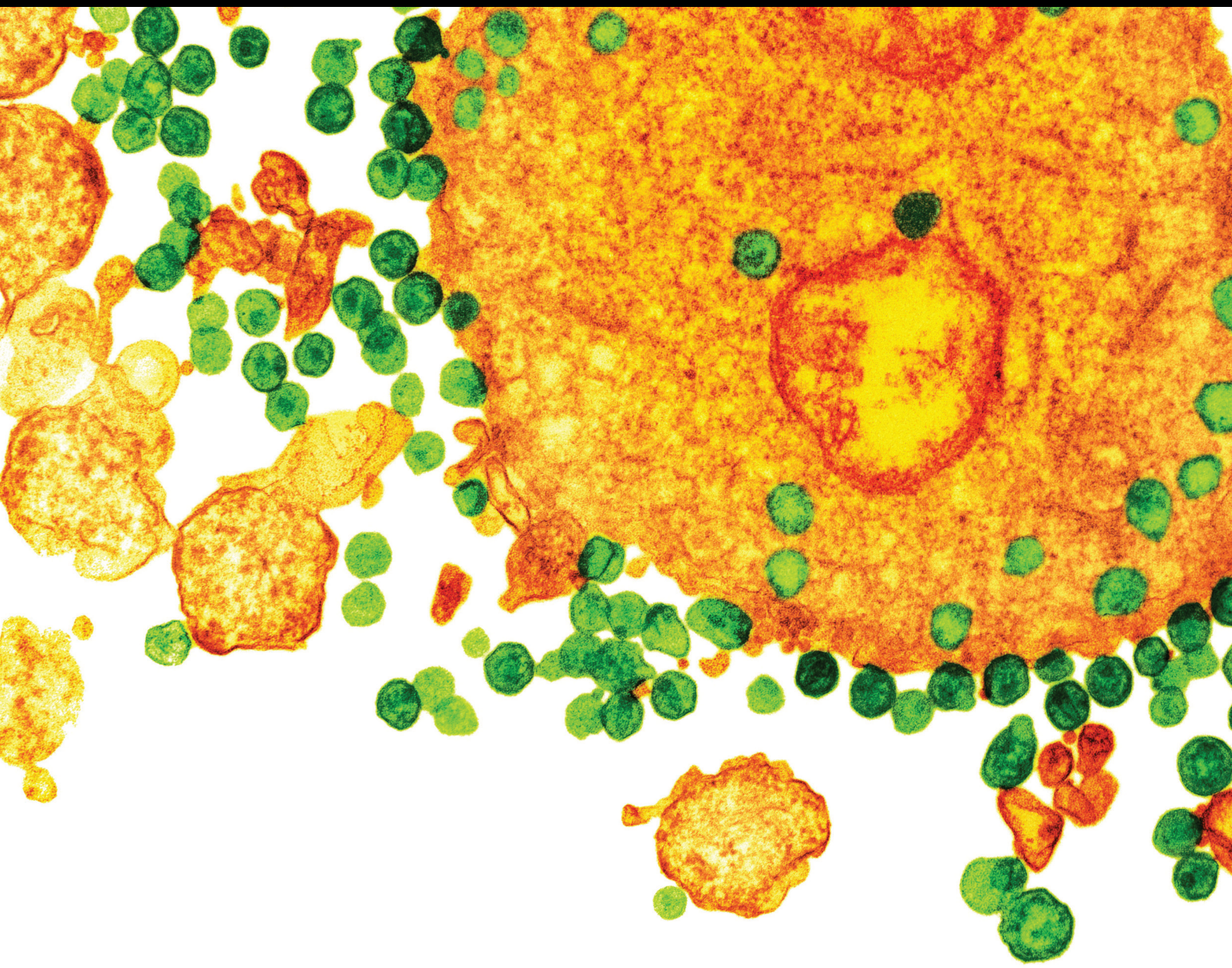
Canadian Journal of Infectious Diseases and Medical Microbiology

The Role of Gut Microbiota in Health and Disease 2021

Lead Guest Editor: Tingtao Chen
Guest Editors: Menghao Huang, Hua Zhang, Jie Luo, and Xiaorong Deng



The Role of Gut Microbiota in Health and Disease 2021

Canadian Journal of Infectious Diseases and Medical Microbiology

The Role of Gut Microbiota in Health and Disease 2021

Lead Guest Editor: Tingtao Chen
Guest Editors: Menghao Huang, Hua Zhang, Jie Luo, and Xiaorong Deng

Contents

Hindawi
Canadian Journal of Infectious Diseases and Medical Microbiology
Volume 2022, Article ID 4899824, 10 pages
https://doi.org/10.1155/2022/4899824

Research Article

Dataset for Genome Sequencing and De Novo Assembly of the Candidate Phyla Radiation in Supragingival Plaque

Song Jiang,[1] Jie Nie,[1] Yuxing Chen,[1] Shiying Zhang,[2] Xiaoyan Wang ,[1] and Feng Chen [2]

[1]*Department of Endodontics, Peking University School and Hospital of Stomatology, Beijing 100081, China*
[2]*Central Laboratory, Peking University School and Hospital of Stomatology, Beijing 100081, China*

Correspondence should be addressed to Xiaoyan Wang; wangxiaoyan@pkuss.bjmu.edu.cn and Feng Chen; chenfeng2011@bjmu.edu.cn

Received 6 November 2021; Accepted 26 January 2022; Published 19 March 2022

Academic Editor: Tingtao Chen

The Candidate Phyla Radiation (CPR), as a newly discovered and difficult-to-culture bacterium, accounts for the majority of the bacterial domain, which may be related to various oral diseases, including dental caries. Restricted by laboratory culture conditions, there is limited knowledge about oral CPR. Advances in metagenomics provide a new way to study CPR through molecular biology. Here, we used metagenomic assembly and binning to reconstruct more and higher quality metagenome-assembled genomes (MAGs) of CPR from oral dental plaque. These MAGs represent novel CPR species, which differed from all known CPR organisms. Relative abundance of different CPR MAGs in the caries and caries-free group was estimated by mapping metagenomic reads to newly constructed MAGs. The relative abundance of two CPR MAGs was significantly increased in the caries group, indicating that there might be a relationship with caries activity. The detection of a large number of unclassified CPR MAGs in the dataset implies that the phylogenetic diversity of CPR is enormous. The results provide a reference value for exploring the ecological distribution and function of uncultured or difficult-to-culture microorganisms.

1. Introduction

The human oral cavity, as one of five major microecological systems in the human body, has been used as the main model system for microbial community research to understand microbial ecology and function [1]. The data from the Human Microbiome Project [2] show that the oral cavity can contain up to 700 types of microorganisms, 30% of which are uncultured. Oral microorganisms include bacteria, fungi, viruses, and bacteriophages. Oral bacteria, which are relatively abundant and easy to be detected and cultured, have always been a hot topic in the study of oral microorganisms. In recent years, although we have made great progress in understanding the complex microbial communities in the oral cavity, the research on some microorganisms is still not deep enough, for example, a new bacterial group called "Candidate Phyla Radiation" (CPR) has not been fully studied. With regard to such microorganisms, studies have shown that they may be associated with a variety of oral diseases. For example, the Saccharibacteria bacterium (once known as TM7), belonging to CPR organisms, is widespread in the human oral microbiota, and there is increasing evidence that they are associated with a variety of mucosal diseases, including periodontitis, halitosis, and inflammatory bowel disease [3–6]. TM7 may have a certain effect on the microecology of oral flora, playing a vital but little-known role in the occurrence and development of oral diseases.

Caries is the most common and frequently occurring chronic oral infectious disease, which is characterized by progressive destruction of hard tissues of teeth. The aetiological mechanism of caries has always been a hot research topic and the basis for caries treatment and prevention. The four-factor theory of caries considers that cariogenic bacteria, susceptible host, appropriate substrate, and time are necessary conditions for caries [7], and cariogenic bacteria is the most important link. Now, most scholars tend to agree with the "ecological plaque hypothesis" [8], believing that

the bacteria with the so-called "cariogenic" are by no means limited to one or some bacteria, and the bacterial community is considered as a whole. These bacteria are originally the resident flora existing in the oral cavity, and numerous microorganisms maintain close contact with each other, thus maintaining the demineralization and remineralization on the tooth surface as a whole through complex interaction. When the microenvironment changes, one or more microflora in dental plaque obtains a competitive advantage and breaks the ecological balance between microorganisms, between dental plaque and tooth surface, and between the oral environment, thus leading to the occurrence of dental caries [9]. As a kind of difficult-to-cultivate and poorly understood bacteria, whether the CPR organisms acting as the aetiological factor of caries, or the type of the CPR organisms changed from health to disease states is still unknown.

Unlike fungi and viruses, CPR is a recently discovered species of bacterial organisms that has greatly influenced our perception of the diversity of life on Earth [10]. This previously unknown bacterium may contain a total of more than 70 phyla, accounting for more than 25% of the bacterial domain [11]. CPR organism is found in a variety of niches and has similar characteristics, such as containing self-splicing introns of 16S subunit ribosomal RNA (16S rRNA) genes and archaeal-specific RuBisCO genes [12], lacking genes that can be used to encode the CRISPR/Cas phage defence system [13], etc. Due to the reduction of the genome, their biosynthetic and metabolic capacities are limited, and they have no electron transfer chains, tricarboxylic acid cycles, amino acid and membrane biosynthetic pathway, and various ribosome subunits [14–19]. High-resolution frozen transmission electron microscopy shows that the CPR cell size is $0.009 \pm 0.002\,\mathrm{mm}^3$, which is consistent with the size of their small genome [18]. To sum up, these shared properties indicate that members of the CPR may show a symbiotic lifestyle and rely on essential metabolites of partner cells, while potentially providing unstable fermentation (e.g., acetate) in return [15, 20–22]. This codependent lifestyle may explain their resistance to culture in vitro [23]. However, it is unclear whether environmental conditions change from health status to caries will affect the composition of CPR organisms.

The challenges of cultivating CPR limit research on their phylogeny and functional diversity. With the development of bioinformatics analyses and high-throughput sequencing, more and more metagenome sequencing data have been obtained from diverse ecological niches and multiple parts of the human body including the oral cavity. Metagenomics is a DNA sequencing methodology based on shotgun sequencing, which sequences the DNA directly separated from the environment and then, assigns the reconstructed genome segments to the genome sketches [24]. This polymerase chain reaction (PCR)-independent genomic (rather than gene)-based approach is valuable for researchers to overcome the obstacles described above and provide information about the metabolic potential of uncultured organisms. In addition, there are various phylogenetically informative sequences that can be used to classify CPR organisms.

Here, we used metagenome shotgun sequencing data obtained from the study [25] for metagenomic analysis. Limited to the memory of the server, only 25% raw data were assembled and analysed downstream in the original study. However, we used all the raw data to characterize the distribution, abundance, and functional differences of CPR organisms in supragingival biofilm swabs of twin pairs, including caries and healthy children using shotgun metagenomic sequencing, de novo assembly, and binning techniques. Previous metagenomic data analysis was incomplete, and data mining was insufficient. Our work utilizes resources of public databases to conduct in-depth metagenomic analysis to obtain more valuable microbial information is significant for metagenomic research.

2. Materials and Methods

2.1. Metagenomic Datasets. The individual reads of 88 subjects were downloaded from the NCBI SRA database, with accession numbers SRR6865436 to SRR6865523. In the original study [25], International Caries Detection and Assessment System (ICDAS II) criteria were used for caries assessment. Subjects with either enamel caries or dentinal caries are referred to as diseased, while subjects without enamel or dentinal caries are referred to as healthy, unless otherwise stated. Dental plaque samples were collected, and DNA was extracted according to a previously published method [26]. The metagenomic libraries were constructed using the NEBNext Illumina DNA library preparation kit (New England Biolabs, Ipswich, MA) and then were sequenced for 300 cycles using the Illumina NextSeq 500 High-Output kit according to standard manufacturer's specifications (Illumina Inc., La Jolla, CA).

2.2. Data Quality Control. Sequence data quality was assessed using FastQC v0.11.7 (https://www.bioinformatics.babraham.ac.uk/projects/fastqc/). The raw reads from each sample were subjected to adapter trimming and low-quality filtering using Trimmomatic v0.36 [27] with parameters of "LEADING : 3 TRAILING : 3 SLIDINGWINDOW : 4 : 15 MINLEN : 36." Each sample's reads were then aligned to human genome build hg38 using Bowtie2 (v2.3.5.1) [28] to filter any genes that were of host origin and avoid host contamination. FastQC was performed again to evaluate the quality of the remaining reads.

2.3. De Novo Assembly and Binning. Total cleaned reads were first assembled on a per sample basis using the metaSPAdes v3.14.0 [29] with option--meta and default parameters [30]. The required coverage depth for the binning was inferred by mapping the raw cleaned reads back to their assemblies using Bowtie2 (v2.3.5.1) and then, calculating the corresponding read depths of each individual contig using SAMtools v1.9 [31] ("samtools view-Sbu" followed by "samtools sort") together with the jgi_summarize_bam_contig_depths function from MetaBAT 2 (v2.12.1) [32]. The MetaBAT 2 program was then called using a minimum contig length threshold of 1,500 bp (option--

minContig 1500) and default parameters (minCV 1.0, minCVSum 1.0, maxP 95%, minS 60, and maxEdges 200), leading to the generation of 424 bins covering 3,408,272,494 bases.

2.4. Taxonomic Analyses and Phylogenetic Analysis. We used Kraken2 v2.0.8 [33] to classify the contigs with default parameters according to the Kraken2 database, and each genome bin was assigned the lowest taxonomic label that was assigned to at least 70% of the contigs in the genome bin. Twenty-nine CPR genome bins were recovered and the completeness and contamination of each bin were estimated with CheckM v1.1.2 [34] using the lineage_wf workflow. Thirteen low-quality (<50% completeness) genome bins were removed, and the remaining sixteen CPR genome bins were used for downstream analysis as metagenome-assembled genomes (MAGs). 16S SSU rRNA genes reads were extracted from bins using the command–ssu_finder of CheckM v1.1.2 with default parameters. 16S SSU rRNA genes reads of 22 representative CPR genomes (including sixteen TM7; three Gracilibacteria; and three Absconditabacteria) were downloaded from the expanded Human Oral Microbiome Database (eHOMD) (https://www.homd.org). The alignment of total 16S SSU rRNA was conducted using the ClustalW method. The misaligned ends and regions with >95% gaps were trimmed, and the final alignment was used to generate a 16S rRNA phylogenetic tree by using the maximum-likelihood method through the MEGA7 software [35], with a bootstrap test of 1000 replicates. Firstly, a JTT model was used to estimate a matrix of pairwise distances, then to which applied Neighbor-Join and BioNJ algorithms, obtaining the initial tree for the heuristic search automatically. Secondly, a tree with the highest log-likelihood was constructed by choosing the topology with the highest log-likelihood value. At last, the branch lengths were adjusted with the number of substitutions per site.

2.5. Comparative Analysis of MAGs. Five MAGs for CPR were downloaded from the original literature, including three TM7 MAGs and two GN02 MAGs. Quast v5.0.2 [36] was used to determine the quality of the sixteen newly assembled genomes and five original literature's genomes for further comparison. Then, pairwise average nucleotide identity (ANI) values of newly assembled genomes and the original literature's genomes were calculated by using JSpecies (https://imedea.uib-csic.es/jspecies/) [37]. Whole-genome comparisons were conducted by aligning sequenced genomes with Progressive MAUVE v2.4.0 [38], using the MAUVE Multiple Genome Alignment software (https://asap.ahabs.wisc.edu/mauve/).

As reference genome, each individual assembled MAG was indexed using Bowtie2 v2.3.5.1 with the command "bowtie2-build." Clean reads of the caries group and the caries-free group were aligned to the reference genomes, respectively, with Bowtie2 v2.3.5.1. The resulting count tables were converted to include length, coverage, and relative abundance in the measurements by adjusting the transcript per million (TPM) [39] calculation for the contigs, a fundamental standardization of metagenomic assembly, as the contigs length has an inherently wide distribution. The relative abundance for each CPR MAG in the caries and caries-free group was calculated, and the differences in relative abundance were analysed using Fisher's exact test with Benjamini–Hochberg False Discovery Rate (FDR) correction using STAMP v2.1.3 [40]. A p value <0.01 was taken to denote statistical significance.

2.6. Metagenomic Annotation and Functional Characterization. The significantly increased MAGs in the caries group were selected. The genes from these MAGs were predicted using Prodigal v2.6.3 [41]. Predicted protein sequences were annotated against KEGG with GhostKOALA (genus_prokaryotes + family_eukaryotes) (https://www.kegg.jp/ghostkoala/) [42]. Marker genes for central metabolic pathways and key environmental element transformations were identified based on K number assignments. The metabolic capacity of MAG is determined by the similarity of gene content with the KEGG genome with known functions and the presence of key genes and pathways in MAG. KEGG ids extracted from the MGENE group associated with each KEGG genome (a total of 5647 comparisons) were added to the matrix of all MAG KEGG ids, respectively. The functional ability of MAG was evaluated by using the functional ability of up to four nearest KEGG genomes.

3. Results and Discussion

3.1. Data Overview. In the original literature, the shotgun sequencing of metagenome was conducted on 88 dental plaque samples from 44 twin pairs including 50 from caries patients, and 38 from healthy persons (the information for the samples is shown in Table S1). We downloaded the SRA files with serial accession numbers from SRR6865436 to SRR6865523 (BioProject accession number PRJNA383868) and converted them to FASTQ files using SRA Toolkit 2.9.6 (https://www.ncbi.nlm.nih.gov/sra). A total of 96 Gb of paired-end sequence data were generated, and each sample had an average of 5.52 million reads (1.1 Gb). After filtering low-quality and human reads, about 47.8% of the sequence reads remained. The remaining, quality-filtered reads were assembled with metaSPAdes produced contigs that could undergo genomic binning by MetaBAT 2, generating a total of 424 bins covering 3,408,272,494 bases.

3.2. More Discoveries of Uncultured CPR Organisms. Having identified 424 bins in the dental plaque, we sought to determine their taxonomic classification and evaluated their quality. By complementing the phylogenetic inference method of CheckM with exact alignment based on k-mers using Kraken2 against the Kraken2 database, we attempted to assign the most likely taxonomic lineage to each bin. We screened 29 CPR bins, but 13 of them were of poor quality (<50% completeness) and were removed. The remaining 16 CPR genome bins were used for downstream analysis as MAGs including thirteen genomes for the TM7 lineage and three genomes for the Gracilibacteria (GN02) lineage (see Table 1). Of these MAGs, eight were high-quality genomes

Table 1: Basic information about CPR bins.

Identifier	Family	Genus	Species	Completeness	Contamination	No. of contigs
bin.138 (MAGI.A)	Unclassified Saccharibacteria	*Candidatus saccharimonas*	*Candidatus saccharibacteria* oral taxon TM7	91.67	132.01	135
bin.366 (MAGI.J)				91.67	50.00	28
bin.158 (MAGI.C)				91.67	88.02	44
bin.339 (MAGI.H)				91.67	66.67	58
bin.404 (MAGI.M)				91.67	66.67	32
bin.335 (MAGI.G)				91.67	72.19	19
bin.344 (MAGI.I)				91.67	0.85	28
bin.149 (MAGI.B)				89.47	61.46	162
bin.374 (MAGI.L)				81.03	76.88	75
bin.321 (MAGI.F)				66.67	30.95	104
bin.229 (MAGI.D)				65.95	59.65	144
bin.247 (MAGI.E)				65.53	43.90	13
bin.367 (MAGI.K)				62.92	22.81	29
bin.376 (MAGII.C)	Unclassified Gracilibacteria	Unclassified *Gracilibacteria*	*Candidatus gracilibacteria* bacterium	94.64	7.27	15
bin.181 (MAGII.A)				93.10	80.22	963
bin.354 (MAGII.B)				62.07	6.90	34

(>90% completeness), and eight were medium-quality genomes (<90% completeness and >50% completeness) as estimated by CheckM.

We used 16S SSU rRNA gene reads extracted from the sixteen CPR MAGs and twenty-two representative CPR genomes to generate a 16S rRNA phylogenetic tree by using the maximum-likelihood method to further verify the classification of MAGs (see Figure 1). Analysis of the phylogenetic tree showed MAG I.A I.C I.J and MAG II.C have a distant lineage with all other species. It may mean they correspond to entirely "novel" genomes that we found. There are also some species with particularly similar lineage, such as MAG I.B and TM7 G-1 HMT348, MAG I.I and TM7 G-1 HMT347, and MAG I.F and TM7 G-1 HMT346 suggesting that they may be the same flora. Most MAGs have a pedigree relationship with the reference genomes at a medium distance.

The abovementioned results indicate that all 16 new MAGs are derived from the CPR organisms, including thirteen TM7 and three GN02. There are many research studies on TM7 but still few studies on GN02. TM7 is a kind of highly diversified bacteria in the world, which was first identified in peat bog in Germany [43]. TM7 can be found in a variety of globally distributed environments, including fresh water, seawater, hot spring, and soils [44]. It has also been detected in many parts of human bodies, including skin [45], the distal esophagus [46] and intestinal tract [4], which is especially common in the oral cavity [47]. It is considered that TM7 is a common and possibly permanent component of the oral flora, which has the ability to maintain growth under both healthy and severe disease state conditions [48].

3.3. The Quality of MAGs Was Higher Than the Results in the Original Literature. Quast was used to determine the quality of the sixteen MAGs and five original literature's MAGs including three TM7 MAGs and two GN02 MAGs for further comparison. The contig N50 in this study was 28,065 significantly larger than the N50 of 5,586 in the original literature. All our MAGs contain a total of 215 contigs greater than 50,000 bp in length. However, the number of contigs larger than 50,000 bp in the original literature is zero (see Table 2). Compared with the original literature, we have obtained more MAGs and higher quality. Then, we compared MAG pairwise using MAUVE and JSpecies to calculate ANI. At least 95% (ANI) is considered the identification standard of the same species. Species identical to those in the original literature were not found but there are some similar species (see Tables 3 and 4). MAGs that are not matched to the original literature are newly excavated CPR genomic information.

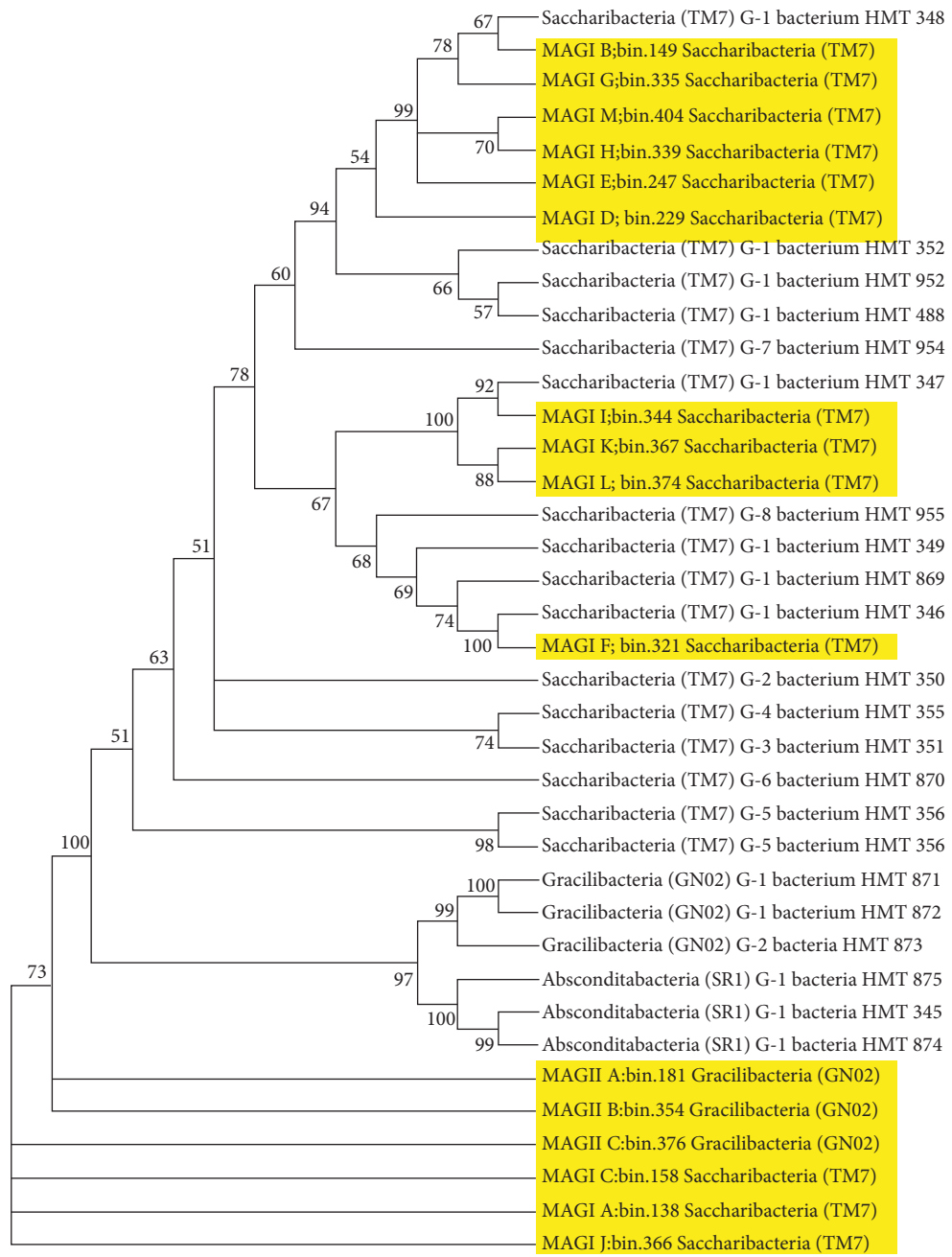


Figure 1: Phylogenetic relationships within the CPR of assembled in the study and derived from public ribosomal databases and available genomes using maximum-likelihood 16S rRNA. Highlights show CPR assembled for this study.

Table 2: Comparison of CPR bins' features in the original literature and in this study.

	CPR bins in the original literature	CPR bins in this study
No. of bins	5	16
No. of contigs (≥50000 bp)	0	215
N50	5586	28065
N75	3037	7917

3.4. The Difference of CPR in Caries and Caries-Free Groups. The results of this study showed that in the caries-free group, the TM7 and the GN02 were second in abundance to the major phyla, indicating that CPR is an important component of the oral flora. The results of this study also showed that the CPR at the species level were mainly *Candidatus Saccharibacteria* oral taxon TM7 of phylum TM7 and *Candidatus Gracilibacteria* bacterium of phylum

Table 3: ANI values for assembled CPR bins and original CPR bins (TM7).

Identifier	RBJO01	RBJP01	RBJQ01
bin.138	73.56	67.09	67.10
bin.366	70.32	67.07	66.33
bin.158	76.34	67.12	68.63
bin.229	69.27	91.12	66.05
bin.339	70.10	66.77	94.28
bin.344	69.18	64.87	94.53
bin.404	81.95	67.02	69.55
bin.149	81.41	67.09	69.27
bin.321	82.86	66.02	69.61
bin.247	79.23	66.84	68.85
bin.367	72.85	65.77	87.21
bin.335	71.05	67.00	66.41
bin.374	68.31	66.29	94.06

Table 4: ANI values for assembled CPR bins and original CPR bins (GN02).

Identifier	RBJV01	RBJW01
bin.181	92.03	63.46
bin.376	94.65	64.71
bin.354	67.10	85.06

GN02, both of which had higher abundance at the oral flora species level, which further proved the importance of CPR in the oral microbiota. TM7 and GN02 were common to both caries and caries-free groups and were detected in all samples, indicating that CPR are members of the oral "core microbiome," and they may play an important role in the stability and function of the oral microecological environment. Based on the annotation level of the existing database, LefSe difference analysis showed that there was no significant difference between the bacteria of phylum TM7 and phylum GN02 in the caries and healthy groups in general. In this study, we followed up with an in-depth analysis of CPR obtained by genome assembly and binning, and the results showed that there were differences in CPR at the level of unknown strains in the caries and caries-free groups.

We used STAMP to calculate the relative abundance for each CPR MAG in the caries group and caries-free group (see Figure 2). The relative abundance for MAG I.I and MAG II.C are significantly higher in caries than in caries-free groups. We selected the two MAGs with the greatest difference to study the functional predictions of the encoded proteins in their genomes (see Figures 3 and 4). Most of the functional genes within the two genomes encoded proteins are related to genetic information processing, in addition to signaling and cellular processes. Genetic information processing and cellular processes are of great significance to all microorganisms. The two of them together comprised 38.1% and 38.8% of the genes in the MAG I.I and MAG II.C, respectively, suggesting the importance of cell motility, cell envelope biogenesis, and signal transduction for these organisms. These results are consistent with the phenotypic characteristics of TM7 isolates. A recent study showed that TM7 species in the human oral cavity had undergone morphological changes, which showed that they changed from ultramicrococcus to slender cells in response to environmental cues including oxygen levels and nutritional status [49]. These cues could also be involved in cell signal transduction and activation of the cell movement pathway.

Compared with other bacterial genomes [50], genes involved in metabolism only account for a small proportion in the newly constructed CPR genomes, with 25.2% and 27.2% in the MAG I.I and MAG II.C. These results are consistent with the hypothesis that the biological metabolic capacity of CPR lineage is limited. Some previous studies have shown that the genomes of CPR organisms lack genes for the biosynthesis of most nucleotides, amino acids, lipids, and vitamins. In addition, the genomes lack most of the genes for the tricarboxylic acid cycle and electron transport chain components. In view of the fact that the metabolic capacities of some CPR organisms have recently been extended to members of the Parcubacteria, it is based on new genomes encoding putative components of the dissimilatory nitrate reduction to ammonia pathway [51, 52], and those genomes involved in hydroxylamine oxidation [51]. Metaproteomic analyses also showed that fermented CPR may play an important role in the hydrogen and carbon cycle of underground ecosystems [15, 53]. In this study, we identified genes encoding various transporters and enzymes in these two newly constructed genomes with significant differences, including genes encoding glycolysis-specific proteins of triosephosphate isomerase, glyceraldehyde-3-phosphate dehydrogenase, 6-phosphofructokinase, and enolase. We did not find a complete tricarboxylic acid cycle pathway in either of these MAGs. These results are consistent with previous hypotheses, that is, cardiopulmonary resuscitation organisms may show a symbiotic lifestyle and only have a part of the metabolic pathway. However, because the genome is incomplete, they may lack a complete metabolic pathway.

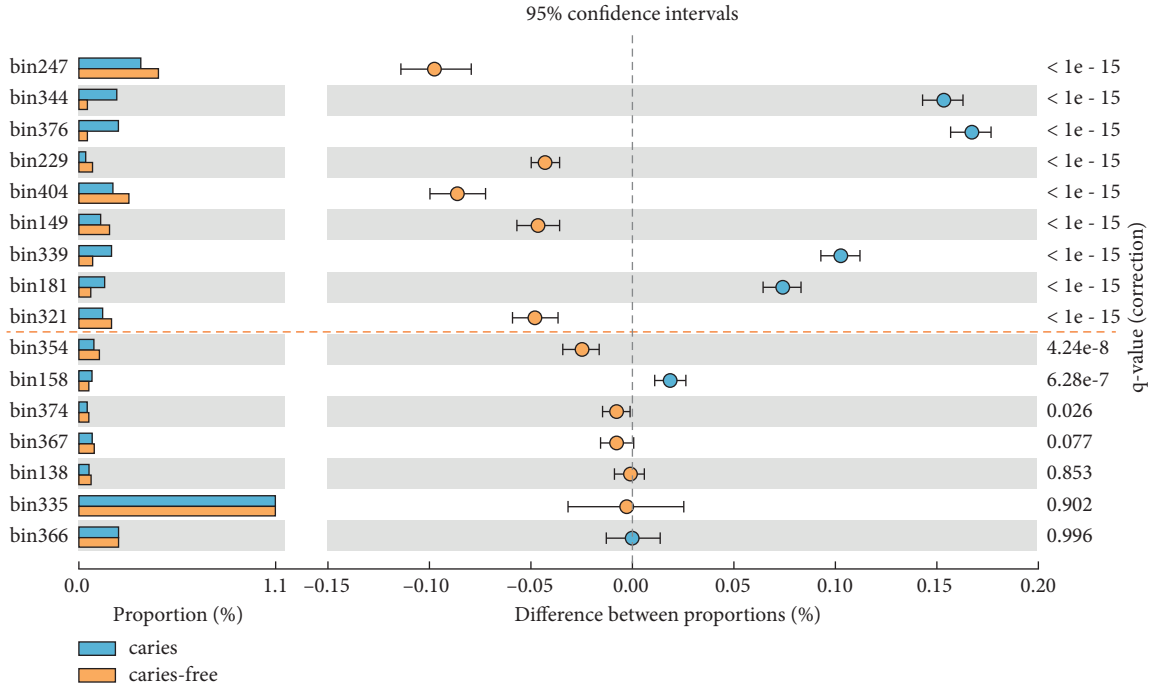


Figure 2: Comparison of relative abundance of each CPR MAGs between caries and caries-free groups. CPR MAGs above the dotted line are considered to be significantly different.

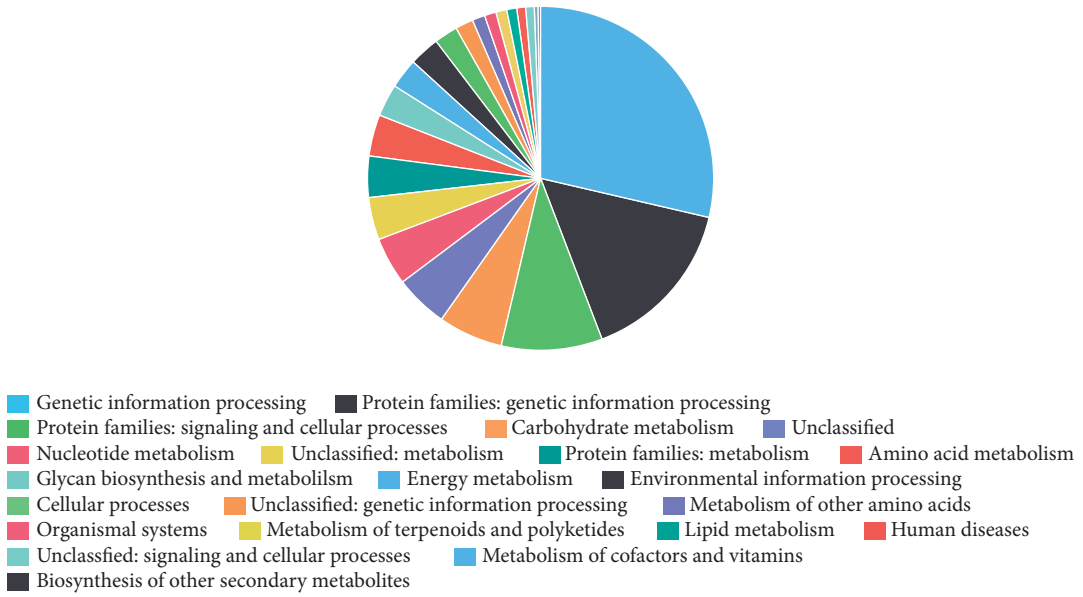


Figure 3: The functional predictions of proteins encoded in the genomes of MAG I.I.

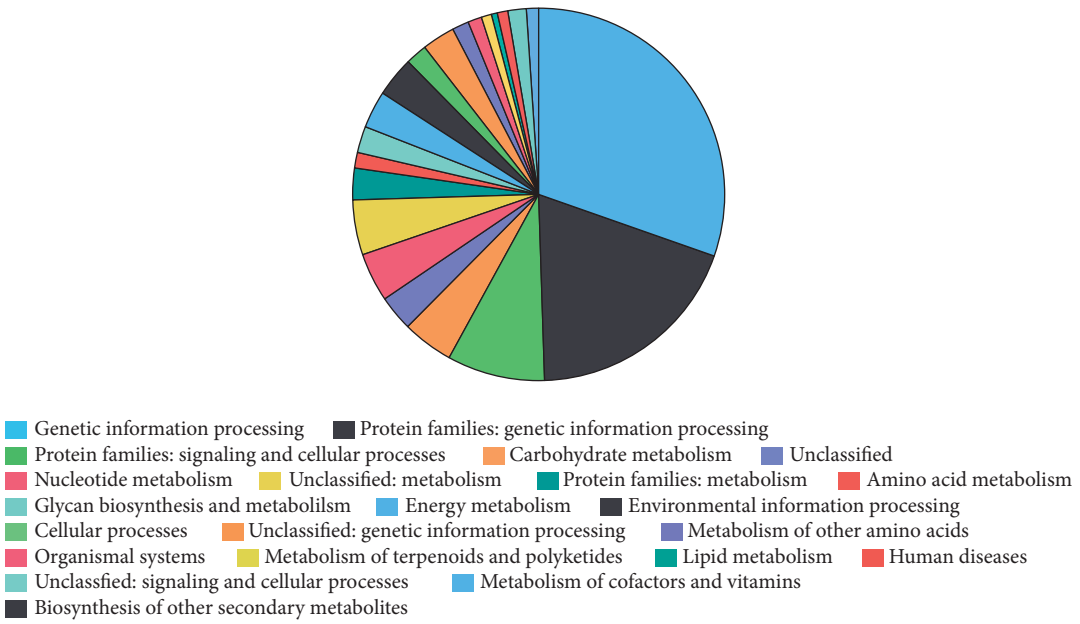


Figure 4: The functional predictions of proteins encoded in the genomes of MAG II.C.

4. Conclusions

In conclusion, we identified and characterized CPR organisms that were difficult to culture using metagenomic sequencing approaches via a metagenomic approach. Using this approach, we reconstructed more and higher quality CPR MAGs from human oral supragingival plaque with or without dental caries using metagenomic data and confirmed the affiliation of genomes. By comparing the abundance and function differences of each MAG between caries and healthy subjects, two MAGs are suspected to be related to caries activity. In order to better understand the characteristics of the CPR organisms, more efforts should be conducted to develop a method to cultivate them in the future.

Data Availability

The data used to support the findings of the study are available from the corresponding author upon request. The raw data are available through accession numbers SRR6865436 to SRR6865523, and the genome bines are available through biosample accession numbers SAMN17152884 to SAMN17152899.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Authors' Contributions

Song Jiang developed the experimental strategy, analysed the data, and wrote this manuscript. Yuxing Chen and Shiying Zhang assist in analyzing the data and writing this manuscript. Jie Nie, Xiaoyan Wang, and Feng Chen refined the strategy and mentored the work. All authors approved the final manuscript.

Acknowledgments

This study was supported by grants-in-aid from the National Science Foundation China (grant no. 81991501).

Supplementary Materials

Human subject metadata: metadata for human subjects used in this analysis. (*Supplementary Materials*)

References

[1] P. E. Kolenbrander, R. J. Palmer, S. Periasamy, and N. S. Jakubovics, "Oral multispecies biofilm development and the key role of cell-cell distance," *Nature Reviews Microbiology*, vol. 8, no. 7, pp. 471–480, 2010.

[2] Human Microbiome Project Consortium, "Structure, function and diversity of the healthy human microbiome," *Nature*, vol. 486, no. 7402, pp. 207–214, 2012.

[3] N. Kianoush, C. J. Adler, K.-A. T. Nguyen, G. V. Browne, M. Simonian, and N. Hunter, "Bacterial profile of dentine caries and the impact of pH on bacterial population diversity," *PLoS One*, vol. 9, no. 3, Article ID e92940, 2014.

[4] T. Kuehbacher, A. Rehman, P. Lepage et al., "Intestinal TM7 bacterial phylogenies in active inflammatory bowel disease," *Journal of Medical Microbiology*, vol. 57, no. 12, pp. 1569–1576, 2008.

[5] B. J. Paster, S. K. Boches, J. L. Galvin et al., "Bacterial diversity in human subgingival plaque," *Journal of Bacteriology*, vol. 183, no. 12, pp. 3770–3783, 2001.

[6] V. Soro, L. C. Dutton, S. V. Sprague et al., "Axenic culture of a candidate division TM7 bacterium from the human oral cavity and biofilm interactions with other oral bacteria," *Applied and Environmental Microbiology*, vol. 80, no. 20, pp. 6480–6489, 2014.

[7] P. D. Marsh, "Microbial ecology of dental plaque and its significance in health and disease," *Advances in Dental Research*, vol. 8, no. 2, pp. 263–271, 1994.

[8] P. D. Marsh, "Are dental diseases examples of ecological catastrophes?" *Microbiology*, vol. 149, no. 2, pp. 279–294, 2003.

[9] N. Takahashi and B. Nyvad, "The role of bacteria in the caries process: ecological perspectives," *Journal of Dental Research*, vol. 90, no. 3, pp. 294–303, 2011.

[10] L. A. Hug, B. J. Baker, K. Anantharaman et al., "A new view of the tree of life," *Nature Microbiology*, vol. 1, no. 5, Article ID 16048, 2016.

[11] C. J. Castelle and J. F. Banfield, "Major new microbial groups expand diversity and alter our understanding of the tree of life," *Cell*, vol. 172, no. 6, pp. 1181–1197, 2018.

[12] K. C. Wrighton, C. J. Castelle, V. A. Varaljay et al., "RubisCO of a nucleoside pathway known from Archaea is found in diverse uncultivated phyla in bacteria," *The ISME Journal*, vol. 10, no. 11, pp. 2702–2714, 2016.

[13] D. Burstein, C. L. Sun, C. T. Brown et al., "Major bacterial lineages are essentially devoid of CRISPR-Cas viral defence systems," *Nature Communications*, vol. 7, Article ID 10613, 2016.

[14] X. He, J. S. McLean, A. Edlund et al., "Cultivation of a human-associated TM7 phylotype reveals a reduced genome and epibiotic parasitic lifestyle," *Proceedings of the National Academy of Sciences*, vol. 112, no. 1, pp. 244–249, 2015.

[15] C. T. Brown, L. A. Hug, B. C. Thomas et al., "Unusual biology across a group comprising more than 15% of domain bacteria," *Nature*, vol. 523, no. 7559, pp. 208–211, 2015.

[16] J. H. Campbell, P. O'Donoghue, A. G. Campbell et al., "UGA is an additional glycine codon in uncultured SR1 bacteria from the human microbiota," *Proceedings of the National Academy of Sciences*, vol. 110, no. 14, pp. 5540–5545, 2013.

[17] C. Rinke, P. Schwientek, A. Sczyrba et al., "Insights into the phylogeny and coding potential of microbial dark matter," *Nature*, vol. 499, no. 7459, pp. 431–437, 2013.

[18] B. Luef, K. R. Frischkorn, K. C. Wrighton et al., "Diverse uncultivated ultra-small bacterial cells in groundwater," *Nature Communications*, vol. 6, Article ID 6372, 2015.

[19] L. Solden, K. Lloyd, and K. Wrighton, "The bright side of microbial dark matter: lessons learned from the uncultivated majority," *Current Opinion in Microbiology*, vol. 31, pp. 217–226, 2016.

[20] J. Gong, Y. Qing, X. Guo, and A. Warren, ""*Candidatus* Sonnebornia yantaiensis," a member of candidate division OD1, as intracellular bacteria of the ciliated protist *Paramecium bursaria* (Ciliophora, Oligohymenophorea)," *Systematic & Applied Microbiology*, vol. 37, no. 1, pp. 35–41, 2014.

[21] R. S. Kantor, K. C. Wrighton, K. M. Handley et al., "Small genomes and sparse metabolisms of sediment-associated bacteria from four candidate phyla," *mBio*, vol. 4, no. 5, pp. e00708–e00713, 2013.

[22] W. C. Nelson and J. C. Stegen, "The reduced genomes of Parcubacteria (OD1) contain signatures of a symbiotic lifestyle," *Frontiers in Microbiology*, vol. 6, p. 713, 2015.

[23] R. E. Danczak, M. D. Johnston, C. Kenah, M. Slattery, K. C. Wrighton, and M. J. Wilkins, "Members of the candidate phyla radiation are functionally differentiated by carbon- and nitrogen-cycling capabilities," *Microbiome*, vol. 5, no. 1, p. 112, 2017.

[24] G. J. Dick, A. F. Andersson, B. J. Baker et al., "Community-wide analysis of microbial genome sequence signatures," *Genome Biology*, vol. 10, no. 8, p. R85, 2009.

[25] J. L. Espinoza, D. M. Harkins, M. Torralba et al., "Supragingival plaque microbiome ecology and functional potential in the context of health and disease," *mBio*, vol. 9, no. 6, 2018.

[26] A. Gomez, J. L. Espinoza, D. M. Harkins et al., "Host genetic control of the oral microbiome in health and disease," *Cell Host & Microbe*, vol. 22, no. 3, pp. 269–278, 2017.

[27] A. M. Bolger, M. Lohse, and B. Usadel, "Trimmomatic: a flexible trimmer for Illumina sequence data," *Bioinformatics*, vol. 30, no. 15, pp. 2114–2120, 2014.

[28] B. Langmead and S. L. Salzberg, "Fast gapped-read alignment with Bowtie 2," *Nature Methods*, vol. 9, no. 4, pp. 357–359, 2012.

[29] A. Bankevich, S. Nurk, D. Antipov et al., "SPAdes: a new genome assembly algorithm and its applications to single-cell sequencing," *Journal of Computational Biology*, vol. 19, no. 5, pp. 455–477, 2012.

[30] S. Nurk, D. Meleshko, A. Korobeynikov, and P. A. Pevzner, "metaSPAdes: a new versatile metagenomic assembler," *Genome Research*, vol. 27, no. 5, pp. 824–834, 2017.

[31] L. Heng, B. Handsaker, A. Wysoker et al., "The sequence alignment/map format and SAMtools," *Bioinformatics*, vol. 25, no. 16, pp. 2078-2079, 2009.

[32] D. D. Kang, J. Froula, R. Egan, and Z. Wang, "MetaBAT, an efficient tool for accurately reconstructing single genomes from complex microbial communities," *PeerJ*, vol. 3, no. 8, Article ID e1165, 2015.

[33] D. E. Wood, J. Lu, and B. Langmead, "Improved metagenomic analysis with Kraken 2," *Genome Biology*, vol. 20, no. 1, p. 257, 2019.

[34] D. H. Parks, M. Imelfort, C. T. Skennerton, P. Hugenholtz, and G. W. Tyson, "CheckM: assessing the quality of microbial genomes recovered from isolates, single cells, and metagenomes," *Genome Research*, vol. 25, no. 7, pp. 1043–1055, 2015.

[35] S. Kumar, G. Stecher, and K. Tamura, "MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets," *Molecular Biology and Evolution*, vol. 33, no. 7, pp. 1870–1874, 2016.

[36] M. Alla, A. Prjibelski, V. Saveliev, D. Antipov, and A. Gurevich, "Versatile genome assembly evaluation with QUAST-LG," *Bioinformatics*, vol. 34, no. 13, pp. i142–i150, 2018.

[37] M. Richter and R. Rosselló-Móra, "Shifting the genomic gold standard for the prokaryotic species definition," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 106, no. 45, pp. 19126–19131, 2009.

[38] A. E. Darling, B. Mau, N. T. Perna, A. E. Darling, B. Mau, and N. T. Perna, "progressiveMauve: multiple genome alignment with gene gain, loss and rearrangement," *PLoS One*, vol. 5, no. 6, Article ID e11147, 2010.

[39] A. Conesa, P. Madrigal, S. Tarazona et al., "A survey of best practices for RNA-seq data analysis," *Genome Biology*, vol. 17, no. 1, p. 13, 2016.

[40] D. H. Parks, G. W. Tyson, P. Hugenholtz, and R. G. Beiko, "STAMP: statistical analysis of taxonomic and functional profiles," *Bioinformatics*, vol. 30, no. 21, pp. 3123-3124, 2014.

[41] D. Hyatt, G.-L. Chen, P. F. LoCascio, M. L. Land, F. W. Larimer, and L. J. Hauser, "Prodigal: prokaryotic gene recognition and translation initiation site identification," *BMC Bioinformatics*, vol. 11, no. 1, p. 119, 2010.

[42] M. Kanehisa, Y. Sato, and K. Morishima, "BlastKOALA and GhostKOALA: KEGG tools for functional characterization of genome and metagenome sequences," *Journal of Molecular Biology*, vol. 428, no. 4, 2015.

[43] H. Rheims, C. Spröer, F. A. Rainey, and E. Stackebrandt, "Molecular biological evidence for the occurrence of uncultured members of the actinomycete line of descent in different

environments and geographical locations," *Microbiology*, vol. 142, no. 10, Article ID 2863, 1996.

[44] P. Hugenholtz, G. W. Tyson, R. I. Webb, A. M. Wagner, and L. L. Blackall, "Investigation of candidate division TM7, a recently recognized major lineage of the domain bacteria with no known pure-culture representatives," *Applied and Environmental Microbiology*, vol. 67, no. 1, pp. 411–419, 2001.

[45] J. M. Dinis, D. E. Barton, J. Ghadiri et al., "In search of an uncultured human-associated TM7 bacterium in the environment," *PLoS One*, vol. 6, no. 6, Article ID e21280, 2011.

[46] Z. Pei, E. J. Bini, L. Yang, M. Zhou, F. Francois, and M. J. Blaser, "Bacterial biota in the human distal esophagus," *Proceedings of the National Academy of Sciences*, vol. 101, no. 12, pp. 4250–4255, 2004.

[47] L. S. Weyrich, S. Duchene, J. Soubrier et al., "Neanderthal behaviour, diet, and disease inferred from ancient DNA in dental calculus," *Nature*, vol. 544, no. 7650, pp. 357–361, 2017.

[48] M. M. Brinig, P. W. Lepp, C. C. Ouverney, G. C. Armitage, and D. A. Relman, "Prevalence of bacteria of division TM7 in human subgingival plaque and their association with disease," *Applied and Environmental Microbiology*, vol. 69, no. 3, pp. 1687–1694, 2003.

[49] B. Bor, N. Poweleit, J. S. Bois et al., "Phenotypic and physiological characterization of the epibiotic interaction between TM7x and its basibiont actinomyces," *Microbial Ecology*, vol. 71, no. 1, pp. 243–255, 2016.

[50] P. Meng, C. Lu, Q. Zhang, J. Lin, and F. Chen, "Exploring the genomic diversity and cariogenic differences of Streptococcus mutans strains through pan-genome and comparative genome analysis," *Current Microbiology*, vol. 74, no. 10, pp. 1200–1209, 2017.

[51] C. J. Castelle, C. T. Brown, B. C. Thomas, K. H. Williams, and J. F. Banfield, "Unusual respiratory capacity and nitrogen metabolism in a parcubacterium (OD1) of the candidate phyla radiation," *Scientific Reports*, vol. 7, Article ID 40101, 2017.

[52] R. Leon-Zayas, L. Peoples, J. F. Biddle et al., "The metabolic potential of the single cell genomes obtained from the Challenger Deep, Mariana Trench within the candidate superphylum Parcubacteria (OD1)," *Environmental Microbiology*, vol. 19, no. 7, pp. 2769–2784, 2017.

[53] K. C. Wrighton, C. J. Castelle, M. J. Wilkins et al., "Metabolic interdependencies between phylogenetically novel fermenters and respiratory organisms in an unconfined aquifer," *The ISME Journal*, vol. 8, no. 7, pp. 1452–1463, 2014.

Hindawi
Canadian Journal of Infectious Diseases and Medical Microbiology
Volume 2021, Article ID 4668565, 18 pages
https://doi.org/10.1155/2021/4668565

Research Article

KDM1A Identified as a Potential Oncogenic Driver and Prognostic Biomarker via Multi-Omics Analysis

Lingyue Li,[1] Yiyu Wang,[1] Yuan Mou,[1] Hao Wu,[2] and Ye Qin[1]

[1]*Hubei Key Laboratory of Tumor Microenvironment and Immunotherapy, China Three Gorges University, Yichang 443002, Hubei Province, China*
[2]*Department of Oncology, The First Affiliated Hospital of Nanjing Medical University, Nanjing 210029, Jiangsu Province, China*

Correspondence should be addressed to Hao Wu; whdactor@163.com and Ye Qin; ycqinye@163.com

Received 3 October 2021; Accepted 12 November 2021; Published 9 December 2021

Academic Editor: Meng-Hao Huang

Background. Lysine-specific demethylase 1A (KDM1A) is a histone demethylation enzyme and a crucial epigenetic factor for multiple pathological pathways that mediate carcinogenesis and immunogenicity. Although increasing evidence supposes the association between KDM1A and cancers, no systematic multi-omics analysis of KDM1A is available. *Methods*. We systematically evaluated the KDM1A expression of various cancer and normal tissues and the unique relationship between KDM1A expression and prognosis of cancer cases based on The Cancer Genome Atlas (TCGA), Genotype Tissue Expression (GTEx), and Clinical Proteomic Tumor Analysis Consortium (CPTAC) database. The genetic variations, phosphorylation, and DNA methylation of KDM1A were analyzed via various tools. We further analyzed the correlation of KDM1A expression and fibroblasts and immune cell infiltration score of TCGA samples via TIMER2.0. *Results*. *KDM1A* was highly expressed in 17 types of total 33 cancers, while it expressed low levels in only 4 cancers. High KDM1A expression was associated with worse survival status in various cancers. KDM1A expression was positively correlated with the cancer-associated fibroblasts and myeloid-derived suppressor cells infiltration levels in most cancer types. Additionally, KDM1A in most cancer types was negatively correlated with Th1 cell infiltration and positively correlated with Th2 cells. Moreover, spliceosome, cell cycle, and RNA transport pathways were involved in the functional mechanisms of KDM1A via enrichment analysis. *Conclusions*. Our study describes the epigenetic factor KDM1A as an oncogene and prognostic biomarker. Our findings provide valuable guidance for further analysis of KDM1A function in pathogenesis and potential clinical treatment.

1. Introduction

Epigenetics has been proved as one of the fundamental mechanisms leading towards carcinogenesis [1]. The irregularities of the epigenome associated with cancer are regulated via histone modifications, DNA methylation, chromatin remodeling, and stability of RNA transcripts. The advancement in genomic technologies over the last two decades provided us with a bird's eye view of the epigenetic factors in oncogenesis, including oncogenic and tumor-suppressor networks. Moreover, the epigenetic changes in cancer cells exposed a key role in the effects of tumor-host interactions, especially with immune cells and stromal cells [2]. With improved understanding, epigenetic modifications in cancer are possibly reversible, indicating that epigenetic regulation is a promising therapeutic target to explore.

The lysine-specific demethylase 1A (KDM1A), also known as LSD1or AOF2, was the first histone demethylation enzyme identified by Shi et al. [3]. It revealed the dynamic regulation of histone methylation by both histone methylases and demethylases. KDM1A has been shown to demethylate histone H3 on lysine 4 (H3K4) and lysine 9 (H3K9), which functions in the regulation of gene expression as a transcriptional repressor or activator [3, 4]. Furthermore, a neuron-specific isoform of LSD1 (KDM1A), LSD1n, was described to acquire a new substrate specificity targeting H4K20me2 methylation for transcription activation of neuronal-regulated genes [5]. The expression of KDM1A has

been found upregulated and correlated with poor prognosis in various cancer types [6–9]. KDM1A plays a pivotal role in various cancer-related physiological processes, such as maintenance of stemness, regulation of hypoxia, epithelial-to-mesenchymal transition (EMT), and escape of immune surveillance [7, 10–13]. Our group and Shi's group have proved inhibition of KDM1A can convert tumors from "cold" to "hot" via regulating the tumor immunogenicity [7, 13] and suppose KDM1A as a target to enhance the efficacy of immunotherapy on poor immunogenic cancers. However, the role of KDM1A in other cancers remains unknown. To date, there is no comprehensive study on the prognostic significance of KDM1A in pan-cancer.

In this study, we performed pan-cancer analysis by using the TCGA project and GTEx databases to systematically characterize the role of KDM1A across various cancer types. We conducted analyses of a set of elements, such as RNA level, protein level, survival curve, DNA methylation, genetic alteration, post-translation modification, microenvironment score, and relevant cellular pathway, to explore the potential mechanism of KDM1A in the pathogenesis or clinical prognosis of different cancers [14]. The current evidence suggested that KDM1A plays different roles in diverse cancers, and the underlying molecular mechanisms that occur in several cancers merit further investigation.

2. Materials and Methods

2.1. Gene Expression Analysis. The TIMER2.0 database was used to detect the expression difference of *KDM1A* using TCGA pan-cancer data [15]. GEPIA2 was used to draw the expression level of *KDM1A* in tumors and compare with related normal tissue from Genotype Tissue expression (GTEx) database, setting as $|\log2FC| = 1$, p value = 0.05, and "Match TCGA normal and GTEx data" [16]. Additionally, GEPIA2 was used to obtain violin plots of the KDM1A expression according to the tumor pathological stages.

To evaluate differences in KDM1A expression at the protein level, Clinical Proteomic Tumor Analysis Consortium (CPTAC) was analyzed using the UALCAN portal [17]. The expression levels of the total protein and phosphorylated protein of KDM1A (NP_001350583.1, NP_055828.2) were analyzed by comparison of the primary tumor and normal tissues.

The Oncomine database (https://www.oncomine.org/resource/main.html) was also applied to obtain the different expressional levels of KDM1A between cancer and normal tissues by entering the word "KDM1A" and setting the threshold of p value = 0.05, fold change = 2, and gene rank in top 10%.

2.2. Survival Analysis. We used the "Survival Analysis-Survival Map" module of GEPIA2 to obtain the effect of KDM1A expression on overall survival (OS) and disease-free survival (DFS) of various cancers based on TCGA. The high- and low-expression cohorts were cut with the ratio of 50 : 50. The hypothesis test used a log-rank test. The "Survival Analysis" module was used to analyze the survival curve of each cancer type. The hazards ratio (HR) based on Cox PH model was calculated, and the 95% confidence interval (CI) as the dotted line is added in the figures.

2.3. Genetic Alteration Analysis. The cBioPortal (http://cbioportal.org) website was used to rank the genetic variation of *KDM1A* via the "Cancer Types Summary" module, including the gene alteration frequency, mutation type, and copy number alteration (CNA) [18]. The mutated site of KDM1A was shown in the schematic diagram of the protein structure via the "Mutations" module. PyMol software was used to label mutation sites of KDM1A. The "Comparison" module was used to obtain the Kaplan–Meier curves of the OS, DFS, progression-free survival (PFS), and disease-specific survival (DSS) for various cancer types according to the *KDM1A* genetic alteration. The log-rank p value was shown. The mutation of *KDM1A* in the different subtypes of breast cancer was analyzed with the Breast Invasive Carcinoma data set (TCGA, Pan-Cancer Atlas) through cBioPortal.

2.4. DNA Methylation Analysis. MethSurv is an interactive and user-friendly web portal providing univariable and multivariable survival analysis based on DNA methylation biomarkers using TCGA (The Cancer Genome Atlas) data [19]. We evaluated survival data of all cancer types using DNA methylation of KDM1A as conditions, selecting the curves with p value < 0.05 to exhibit. Moreover, MEXPRESS was applied to visualize DNA methylation, expression, and clinical data [20].

2.5. Immune Infiltration Analysis. The TIMER2.0 database was used to analyze associations between KDM1A and tumor stromal cells, tumor-infiltrating immune cells, including cancer-associated fibroblasts, $CD8^+$ T cells, $CD4^+$ T cells, Tregs, B cells, macrophages, myeloid-derived suppressor cells (MDSCs), neutrophils, and dendritic cells. The EPIC, MCPCOUNTER, TIDE, TIMER, CIBERSORT, CIBERSORT-ABS, QUANTISEQ, and XCELL algorithms were applied for estimations. The purity-adjusted Spearman's rank correlation test was used to obtain the p values and partial correlation (cor) values, and then heatmaps and corresponding scatter plots were generated.

2.6. KDM1A-Related Gene Enrichment Analysis. The STRING database was used to acquire *KDM1A*-binding proteins [21]. We searched "KDM1A" in "*Homo sapiens*" and set main parameters, including Network type as "full STRING", the meaning of network edges as "evidence", active interaction sources as "experiments", the minimum required interaction score as "low confidence (0.150)", and the max number of interactors to show as "custom value; max interactors (100)" in the 1st shell. Finally, the available experiment-determined *KDM1A*-binding proteins were obtained as Set 1.

GEPIA2 was used to obtain 100 top KDM1A-correlated genes based on TCGA and GTEx databases as Set 2 via the "Similar Gene Detection" module. The "Correlation

Analysis" module was used to execute a pairwise gene Pearson correlation analysis based on expression data. The dot plots showed $\log_2$ (TPM) with p values and the correlation coefficient (R). TIMER2.0 was applied to generate the heatmap to demonstrate the relationship between KDM1A and selected genes via the "Gene_Corr" module in the "Exploration" part.

Venny2.1.0 (https://bioinfogp.cnb.csic.es/tools/venny/index.html) was applied to conduct an intersection analysis of Set 1 and Set 2 for the common genes. Moreover, we combined Set 1 and Set 2 to perform KEGG (Kyoto Encyclopedia of Genes and Genomes) pathway analysis and GO (Gene Ontology) enrichment analysis. We used the "clusterProfiler" R package to conduct KEGG enrichment analysis and GO enrichment analysis [22]. The enriched pathways were visualized with the bubble plots. GO enrichment analyses were visualized as bubble plots and cnetplots. The *R* language software [*R*-3.6.3, 64-bit] (https://www.r-project.org/) was used in this analysis. Two-tailed $p < 0.05$ was considered statistically significant.

3. Results

3.1. KDM1A Gene Differentially Expressed between Normal and Tumor Tissues. TIMER2.0 was used to detect the differential expression of KDM1A between tumor and corresponding normal tissues from TCGA. The results showed that KDM1A was highly expressed in 15 cancer types compared with normal samples, including bladder urothelial carcinoma (BLCA), breast invasive carcinoma (BRCA), cholangiocarcinoma (CHOL), colon adenocarcinoma (COAD), esophageal carcinoma (ESCA), head and neck squamous cell carcinoma (HNSC), liver hepatocellular carcinoma (LIHC), lung adenocarcinoma (LUAD), lung squamous cell carcinoma (LUSC), prostate adenocarcinoma (PRAD), rectum adenocarcinoma (READ), stomach adenocarcinoma (STAD), uterine corpus endometrial carcinoma (UCEC), cervical squamous cell carcinoma and endocervical adenocarcinoma (CESC), and glioblastoma multiforme (GBM), and was lowly expressed only in kidney chromophobe (KICH), kidney renal clear cell carcinoma (KIRC), and kidney renal papillary cell carcinoma (KIRP) (Figure 1(a)).

As the corresponding normal tissues of 10 cancer types are unavailable in the TCGA database, we used the expression data of normal tissues from the GTEx database to compare with TCGA data (Figures 1(b) and S1(a)). It was shown that the *KDM1A* gene was highly expressed in tumor samples of lymphoid neoplasm diffuse large B-cell lymphoma (DLBC) and thymoma (THYM) and was lower in acute myeloid leukemia (LAML) compared with normal tissues ($p < 0.05$). Moreover, 7 cancers showed no significant difference in the expression of *KDM1A* compared with normal tissues (Figure S1(a)).

We further explored the transcription levels of *KDM1A* in cancer using the Oncomine database (Figure 1(c)). Relative to normal tissues, KDM1A in bladder cancer, colorectal cancer, kidney cancer, leukemia, and lung cancer was overexpressed, while it was downregulated in brain and CNS cancer and breast cancer, which made the potential function as either oncogenic or antitumor activities based on the cancer types. Part of Oncomine data was inconsistent with the analysis of TCGA data, perhaps caused by different sample sources and different tumor classifications. Hence, detailed analyses of KDM1A are considered for further analysis.

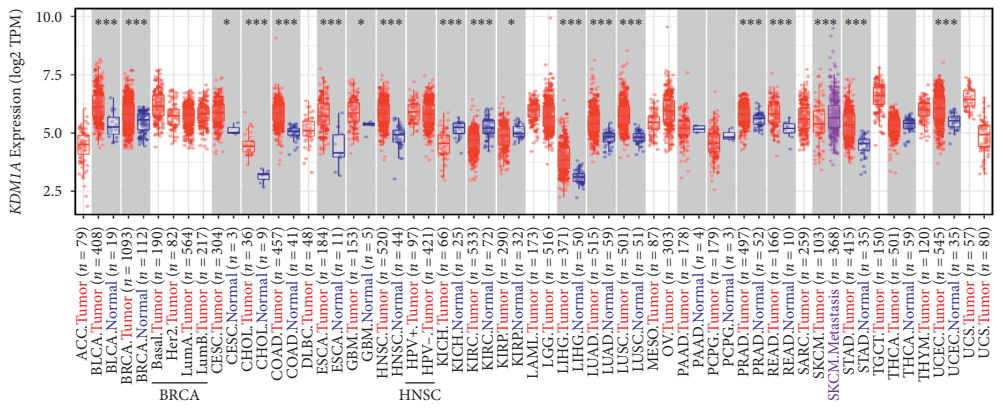
To evaluate the protein level of KDM1A, CPTAC was utilized to analyze the TCGA data. As shown in Figure 1(d), the total protein level of KDM1A was higher in breast cancer, uterine corpus endometrial carcinoma (UCEC), colon cancer, ovarian cancer, lung adenocarcinoma ($p < 0.001$), and clear cell RCC ($p < 0.05$) compared with normal tissues.

Moreover, we applied GEPIA2 to investigate the correlation of KDM1A with the pathological stages. KDM1A expression was a positive correlation with pathological stages in 4 cancers, including LIHC, HNSC, SKCM, and OV, but not others (Figures 1(e) and Figure S1(b)).

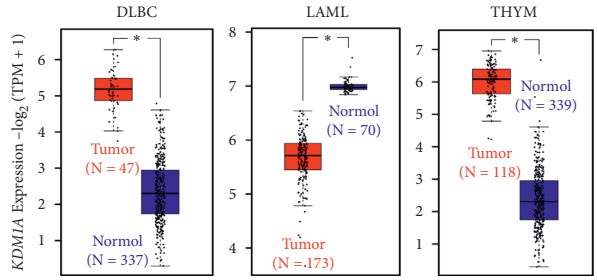
3.2. Survival Analysis of KDM1A. To investigate the association of KDM1A expression with prognosis, survival association analysis was performed via GEPIA2 based on the expression level of KDM1A. The cancer cases were dichotomized into high and low groups according to KDM1A expression. As shown in Figures 1(f) and S2, the high-expression group was linked to poor OS (overall survival) for cases of ACC ($p = 0.0014$), LIHC ($p = 0.0053$), and SARC ($p = 0.011$), and the contrary result was shown for cases of COAD ($p = 0.023$) and KIRC ($p = 0.025$). Additionally, DFS (disease-free survival) was analyzed and showed that 4 cancer types with high KDM1A were positively related to poor prognosis, including ACC ($p = 4.2e-05$), LIHC ($p = 0.021$), KICH ($p = 0.026$), and LGG ($p = 0.017$), and low KDM1A was associated with poor DFS for KIRC ($p = 0.015$).

The Kaplan–Meier plotter tool was also utilized to analyze the expression of the KDM1A gene concerning clinical prognosis. The result presented that the high expression of KDM1A was associated with better OS ($p = 0.0068$) but the reverse effect to RFS ($p = 0.001$) in patients with breast cancer (Figure S3(a)). In ovarian cancer, the high KDM1A group was related to poor OS ($p = 0.043$) and PFS ($p = 0.02$) (Figure S3(b)). The low expression of KDM1A in gastric cancer was associated with poor PPS ($p = 0.0013$) (Figure S3(c)). The upregulation of KDM1A was correlated with poor OS ($p = 0.0031$) in LUAD (Figure S3(d)). The downregulation of KDM1A was linked to poor PPS ($p = 0.072$) in LUSC (Figure S3(e)). Moreover, highly expressed KDM1A was coupled with poor OS, RFS, PFS, and DSS (all $p < 0.001$) for the cases of liver cancer (Figure S3(f)). The summary of the differential association between KDM1A expression and the prognosis of different cancers is shown in Table 1, according to both methods of GEPIA2 and Kaplan–Meier plotter.

3.3. Genetic Alterations of KDM1A. We applied cBioPortal to observe the chromosomal abnormalities and mutation status of *KDM1A* in various cancers using the TCGA data.

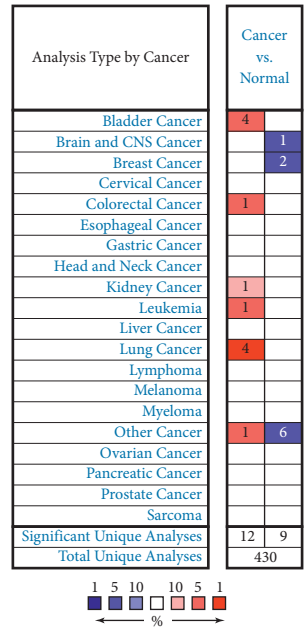


(a)



(b)

Disease Summary for *KDM1A*



(c)

Figure 1: Continued.

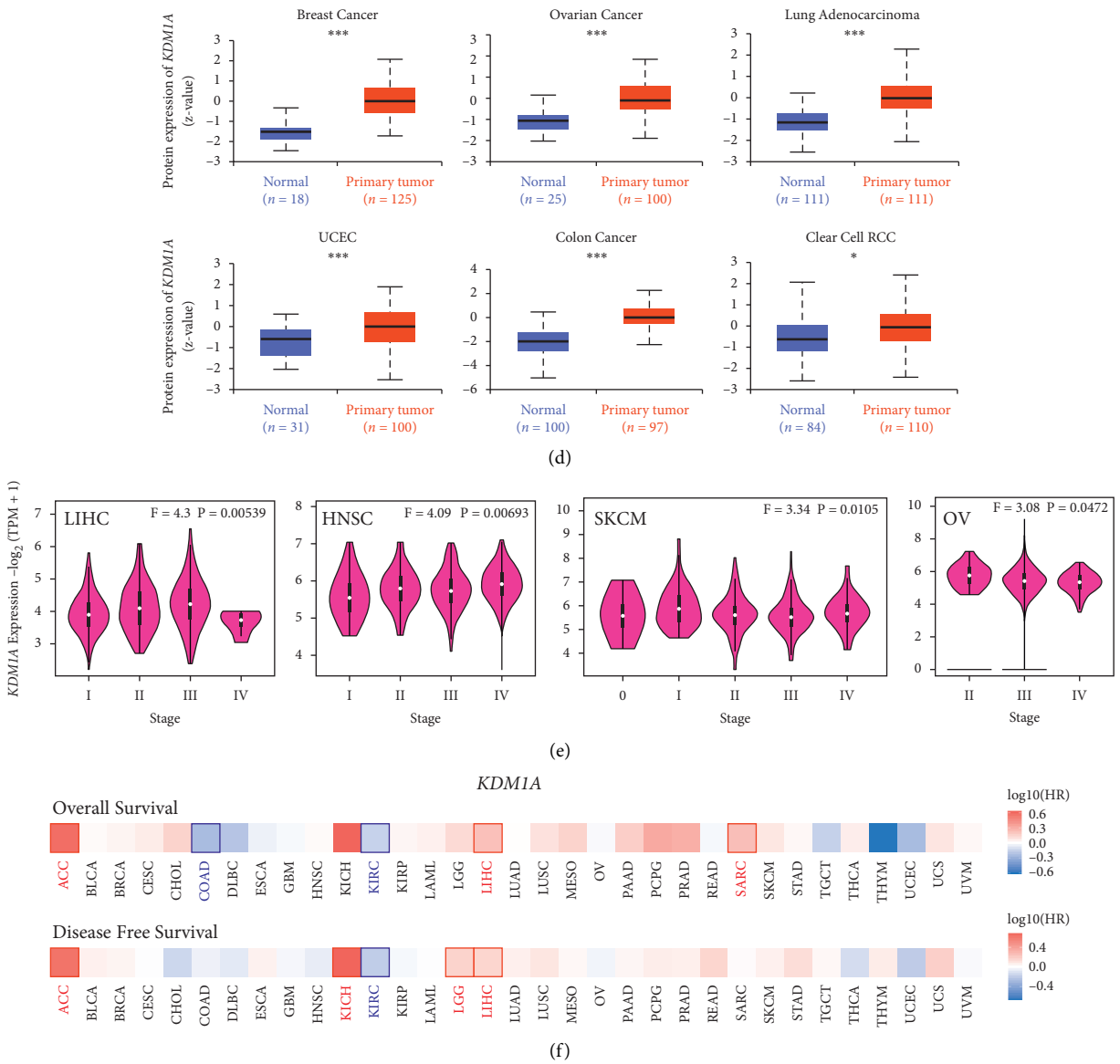


Figure 1: Analysis of the expressional level of KDM1A gene and survival prognosis of cancers. (a) TIMER2.0 was used to analyze the expressional level of the KDM1A in different cancers. (b) The box plot data were supplied for the type of DLBC, LAML, and THYM in the TCGA project, and the corresponding normal tissues of the GTEx database were included as controls. (c) Expressional levels of KDM1A in different types of tumors according to the Oncomine database. The plot indicated the numbers of datasets with statistically significant ($p < 0.05$) mRNA overexpression (red) or downexpression (blue) of KDM1A (different types of cancer vs. corresponding normal tissue). (d) The protein expressional levels of KDM1A were analyzed according to the CPTAC dataset. (e) The main pathological stages of KDM1A expression levels in LIHC, HNSC, SKCM, and OV based on the TCGA data. (f) Survival prognosis of cancers including overall survival and disease-free survival. $^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$.

As illustrated in Figure 2(a), uterine cancer owned the highest alteration frequency of *KDM1A* (>4%) with mutation frequency as the main proportion. It is worth mentioning that deep deletion of *KDM1A* accounted for all cases of genetic alteration in CHOL, pheochromocytoma and paraganglioma (PCPG), DLBC, mesothelioma (MESO), THYM, TGCT, and KIRC. Meanwhile, all cases of *KDM1A* alteration were the amplification of copy number in UCS and SARC. We further present the sites and types of *KDM1A* mutation and related case numbers in Figure 2(b). The missense mutation was the highest among genomic alterations, which include the alterations of R321C/H, E477K, and R591 ∗ /L in the amino oxidase domain, including 3 cases each and involving SKCM, UCEC, BLCA, LUSC, and CESC (Figure 2(b)). As shown in the 3D structure of KDM1A protein, R321 and R591 located at the region of the KDM1A catalytic pocket, while E477 stood at the binding region of KDM1A with the nucleosome and coeffector (Figure 2(c)). Moreover, we present the alteration sites of all TCGA cancer types in Table S1. Furthermore, we investigated the association between the clinical survival of cases and *KDM1A* mutations with various cancers. As

Table 1: The summary of analysis on KDM1A expression and prognosis in different tumors of TCGA

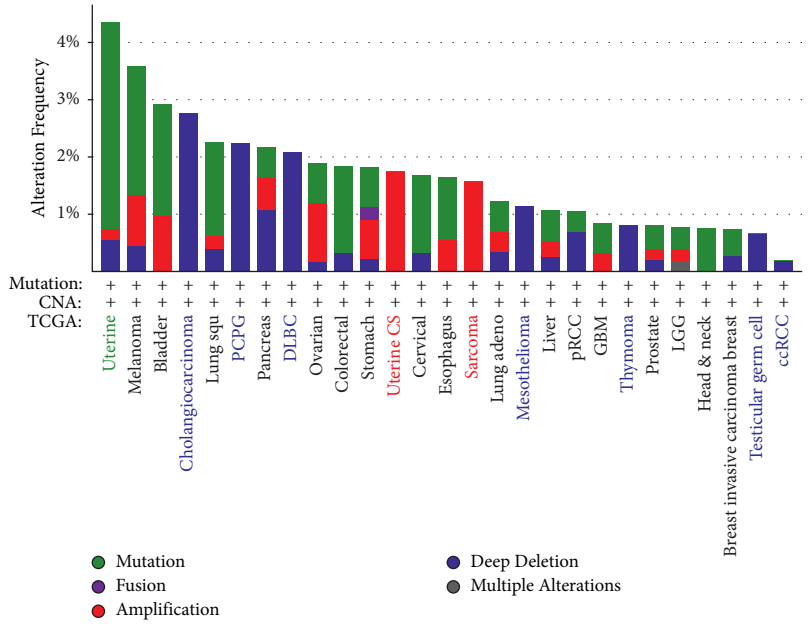
	Tumor type	mRNA expression	Protein expression	Stage level	Poor prognosis of OS	Poor prognosis of DFS
ACC	Adrenocortical carcinoma	ns	NA	ns	**Positive****	**Positive*****
BLCA	Bladder urothelial carcinoma	**High*****	NA	ns	ns	ns
BRCA	Breast invasive carcinoma	**High*****	**High*****	ns	**Negative****	ns
CESC	Cervical squamous cell carcinoma and endocervical adenocarcinoma	**High***	NA	ns	ns	ns
CHOL	Cholangiocarcinoma	**High*****	NA	ns	ns	ns
COAD	Colon adenocarcinoma	**High*****	**High*****	ns	**Negative***	ns
DLBC	Lymphoid neoplasm diffuse large B-cell lymphoma	**High****	NA	ns	ns	ns
ESCA	Esophageal carcinoma	**High*****	NA	ns	ns	ns
GBM	Glioblastoma multiforme	**High***	NA	NA	ns	ns
HNSC	Head and neck squamous cell carcinoma	**High*****	NA	**F = 4.09****	ns	ns
KICH	Kidney chromophobe	**Low*****	NA	ns	ns	**Positive***
KIRC	Kidney renal clear cell carcinoma	**Low*****	**High***	ns	**Negative***	**Negative***
KIRP	Kidney renal papillary cell carcinoma	**Low***	NA	ns	ns	ns
LAML	Acute myeloid leukemia	**Low****	NA	NA	ns	ns
LGG	Brain lower grade glioma	ns	NA	NA	ns	**Positive***
LIHC	Liver hepatocellular carcinoma	**High*****	NA	**F = 4.3****	**Positive****	**Positive***
LUAD	Lung adenocarcinoma	**High*****	**High*****	ns	**Positive****	ns
LUSC	Lung squamous cell carcinoma	**High*****	NA	ns	ns	ns
MESO	Mesothelioma	NA	NA	NA	ns	ns
OV	Ovarian serous cystadenocarcinoma	ns	**High*****	**F = 3.08***	**Positive***	ns
PAAD	Pancreatic adenocarcinoma	ns	NA	ns	ns	ns
PCPG	Pheochromocytoma and paraganglioma	ns	NA	NA	ns	ns
PRAD	Prostate adenocarcinoma	**High*****	NA	NA	ns	ns
READ	Rectum adenocarcinoma	**High*****	NA	ns	ns	ns
SARC	Sarcoma	ns	NA	NA	**Positive***	ns
SKCM	Skin cutaneous melanoma	ns	NA	**F = 3.34***	ns	ns
STAD	Stomach adenocarcinoma	**High*****	NA	ns	ns	ns
TGCT	Testicular germ cell tumors	ns	NA	ns	ns	ns
THCA	Thyroid carcinoma	ns	NA	ns	ns	ns
THYM	Thymoma	**High****	NA	NA	ns	ns
UCEC	Uterine corpus endometrial carcinoma	**High*****	**High*****	ns	ns	ns
UCS	Uterine carcinosarcoma	ns	NA	ns	ns	ns
UVM	Uveal melanoma	NA	NA	NA	ns	ns

OS, overall survival; DFS, disease-free survival; NA, not available; ns, no significance; $*p < 0.05$; $**p < 0.01$; $***p < 0.001$.

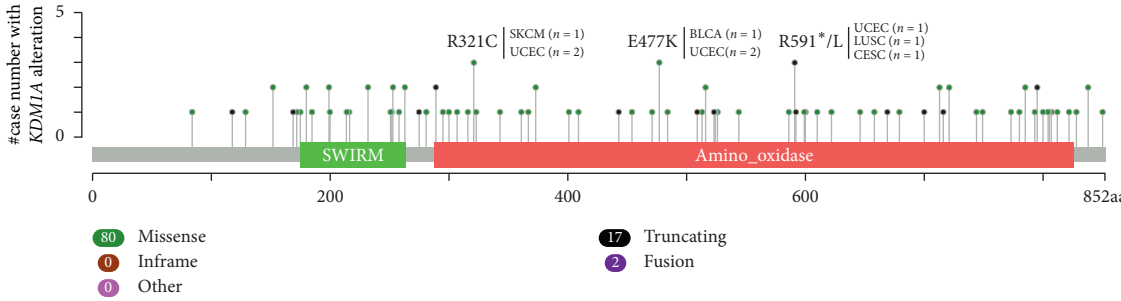
shown in Figure 2(d), breast invasive carcinoma cases with *KDM1A* alteration indicated poor OS ($p = 0.0391$), DSS ($p = 2.493e-03$), PFS ($p = 0.0284$) survival, but not DFS ($p = 0.230$), compared with cases without KDM1A mutation. Subsequently, we surveyed the association of breast cancer subtype and the *KDM1A* alteration and found 5 of 7 cases with *KDM1A* alteration were luminal A type of breast cancer (Figure 2(e)).

3.4. DNA Methylation Analysis of KDM1A. To investigate the DNA methylation of *KDM1A*, we explore the data of *KDM1A* DNA methylation of different cancer types in the TCGA project. As displayed in Table 2, the methylation level of the *KDM1A* promoter region was negatively correlated with gene expression in BRCA, KIRC, MESO, READ, SKCM, and UCEC and positively correlated in HNSC and LUSC. In LGG, the methylation level at cg22683154 was negatively correlated with gene expression, whereas methylation at cg06958034 was a positive correlation with gene expression. Moreover, the level of methylation was a negative correlation with gene expression based on multiple probes of the nonpromoter region ($p < 0.05$). We further analyzed the potential correlation of *KDM1A* DNA methylation with the prognosis of different cancers via MethSurv and MEXPRESS approach, and the results showed that hypermethylation of *KDM1A* is positively correlated with good prognosis in most tumors (Figures 3 and S4).

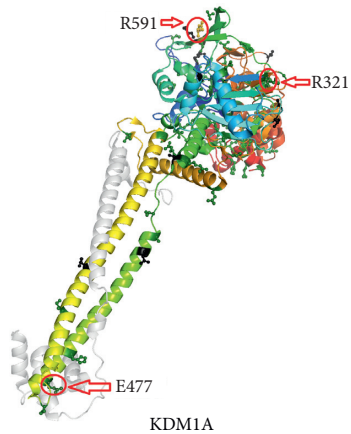
3.5. Phosphorylation Levels of KDM1A Protein. To compare phosphorylation levels of KDM1A between normal tissues and primary tumor tissues, six cancer types (breast cancer, ovarian cancer, clear cell RCC, LUAD, UCEC, and COAD) were analyzed via the CPTAC dataset. The phosphorylation levels of KDM1A protein in different tumors are framed in Table S2. As shown in Figure 4(a), the phosphorylation sites of KDM1A with significant differences ($p < 0.05$) were summarized, and the most frequent phosphorylation sites were located at the N-terminal. Compared with normal tissues, the phosphorylation levels of different sites were upregulated in breast cancer, colon cancer, UCEC, and LUAD and downregulated in clear cell RCC, ovarian cancer, and colon cancer. Interestingly, different phosphorylation



(a)



(b)



KDM1A

RefSeq: NM_015013
Ensembl: ENST00000356634
CCDS: CCDS30627
UniProt: KDM1A_HUMAN

(c)

Figure 2: Continued.

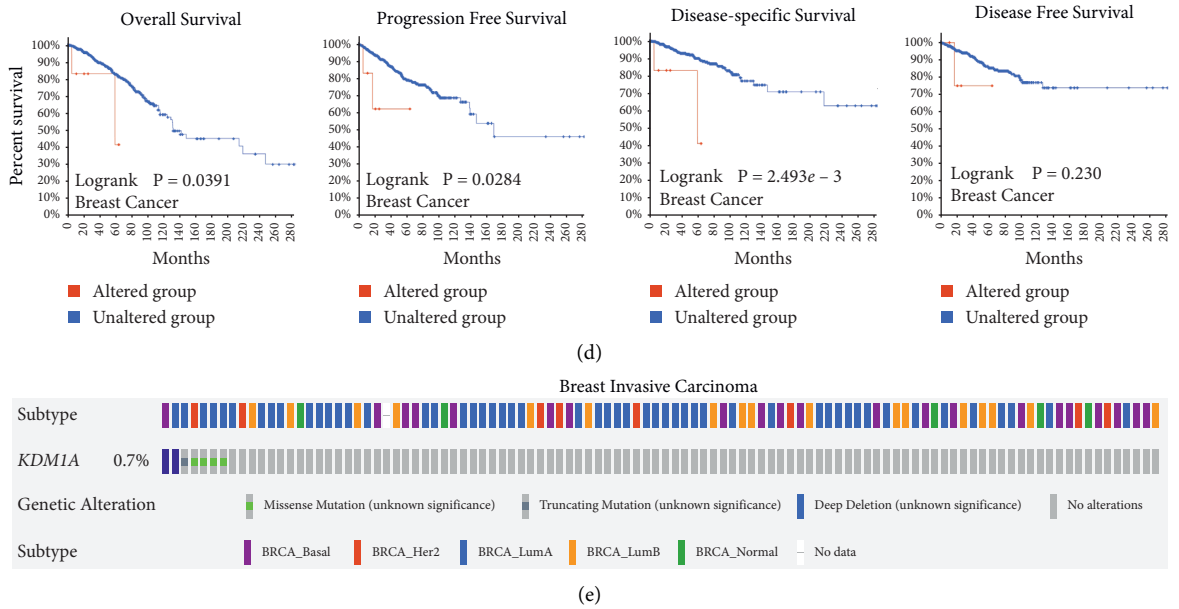


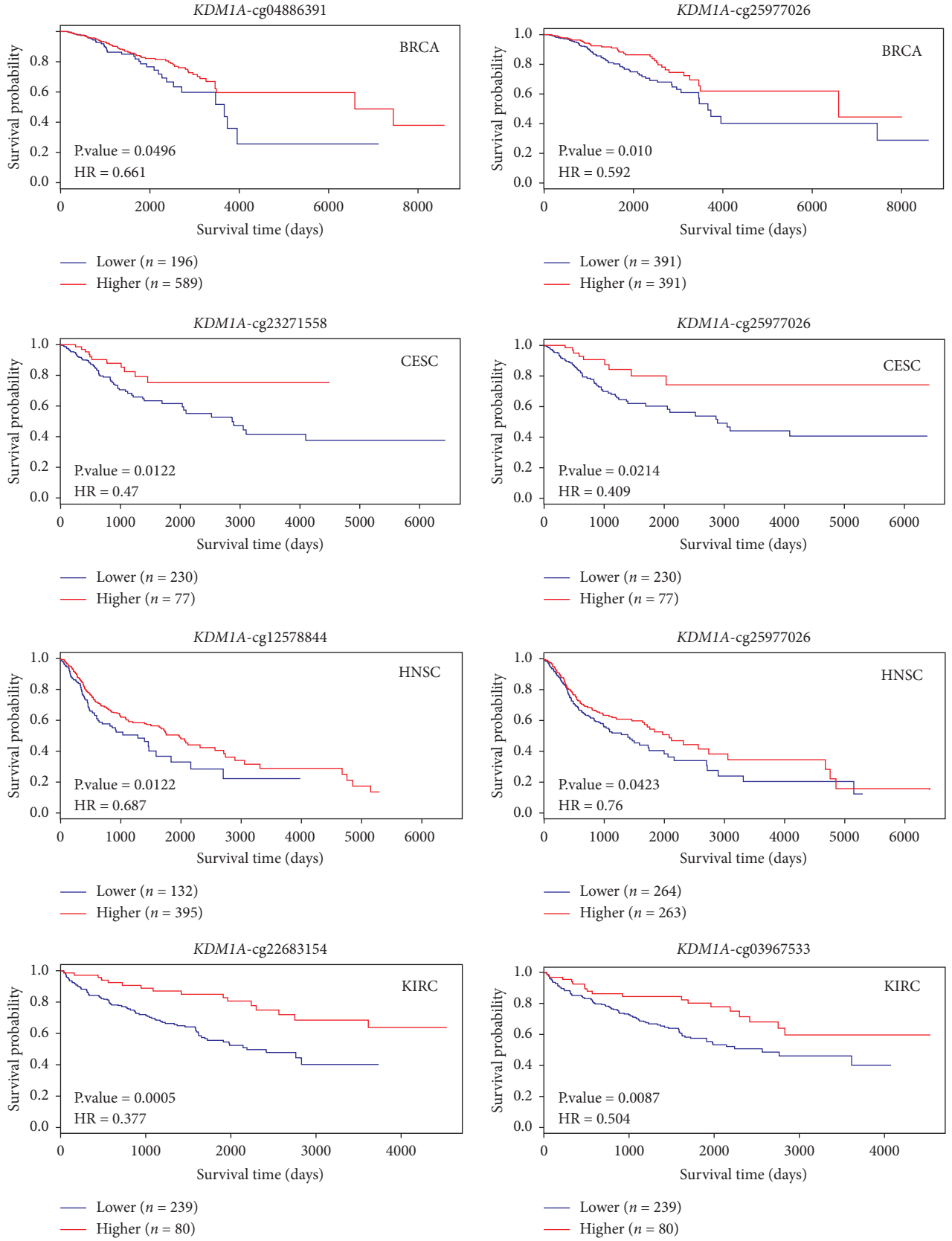
Figure 2: KDM1A mutations in different tumors according to the TCGA data. (a) The alteration frequency of KDM1A with mutation type using the cBioPortal tool. (b) KDM1A mutation site and corresponding diseases of the highest number of cases are displayed. (c) The top three mutation sites including R321C/H, E477K, and R591 ∗ /L showed in the 3D structure of KDM1A. (d) Mutation status of KDM1A was relevant to the OS, PFS, DSS, and DFS of breast cancer analyzed by the cBioPortal tool. (e) Breast cancer samples with KDM1A mutation were identified from the TCGA Invasive Breast Carcinoma data set.

Table 2: Relationship between *KDM1A* DNA methylation and gene expression.

Cancer	Name	pearson_r	*p* value	The promoter probe
BRCA	cg04886391	−0.0870	0.010281191	Yes
	cg25977026	−0.5540	5.95276*E*−40	No
CESC	cg25977026	−0.4934	2.31587*E*−18	No
	cg23271558	−0.1294	0.022886159	No
HNSC	cg12578844	0.1234	0.004768733	Yes
	cg25977026	−0.4335	1.0084*E*−21	No
KIRC	cg22683154	−0.2429	5.65216*E*−06	Yes
	cg03967533	−0.1554	0.003970689	No
LGG	cg22683154	−0.0875	0.045136678	Yes
	cg06958034	0.2343	6.17708*E*−08	Yes
LIHC	cg25977026	−0.5302	3.75586*E*−25	No
LUAD	cg25977026	−0.5043	1.48899*E*−25	No
LUSC	cg26662347	0.1615	0.001653858	Yes
MESO	cg07118078	−0.3149	0.003163089	Yes
READ	cg22683154	−0.2815	0.004356805	Yes
SARC	cg25977026	−0.4172	4.05178*E*−12	No
SKCM	cg22683154	−0.1241	0.007028282	Yes
STAD	cg23271558	−0.2353	1.25499*E*−05	No
UCEC	cg04886391	−0.1419	0.002202533	Yes

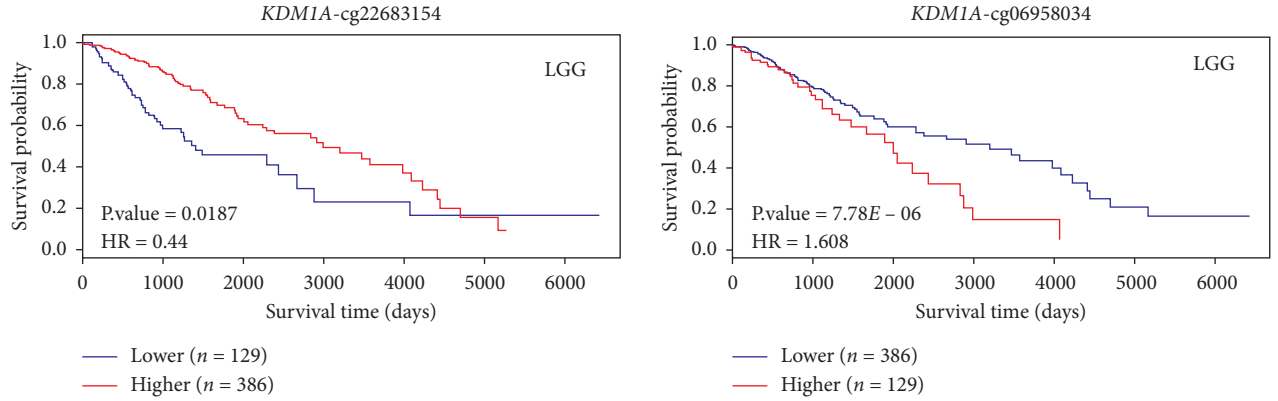
sites showed converse regulation in colon cancer. The phosphorylation levels of S69 and S131 were upregulated and the level of S166 was downregulated in colon cancer. We further found that the S131 locus exhibits a higher phosphorylation level in breast cancer, colon cancer, UCEC, and LUAD compared with normal tissues but lower in renal clear cell carcinoma and the S131 locus can undergo double phosphorylation in conjunction with other phosphorylation sites (Figures 4(b), 4(c), and S5). Furthermore, we also utilized PhosphoNET to analyze the phosphorylation of KDM1A in the CPTAC database (Table S3). One publication experimentally revealed the biological significance of phosphorylation of LSD1 at S131 and S137 mediated by CK2, which benefited cell proliferation and survival after DNA damage [23]. This discovery indicates the significance of further experimental exploration for the role of KDM1A phosphorylation in tumorigenesis.

3.6. Relationship between KDM1A Expression and Tumor Microenvironment. Various algorithms in TIMER2.0 were applied to measure the potential correlation between KDM1A and cancer-associated fibroblast (CAF) and immune cells in diverse cancer types. Through multiple analyses, we observed a statistically positive correlation between KDM1A expression and CAF in most cancer types, but a negative correlation in THYM (Figure 5(a)). As for myeloid-derived suppressor cells (MDSCs), it can be learned from the TIDE algorithm that MDSCs were positively correlated with KDM1A expression (Figure 5(c)). In addition, we noticed a negative correlation of *KDM1A* expression with the infiltration of CD^{8+} T cells in TGCT, LGG, KIRP, KIRC, and HNSC-HPV+ based on most algorithms (Figure S6). The scatter plots are shown in Figures 5(b) and S6(b). For instance, the KDM1A level in CESC was positively associated with CAF (Figure 5, cor = 0.362, $p = 5.20e{-}10$) depending on the EPIC algorithm. The correlation between the other tumor-infiltrating immune cells and KDM1A expression is shown in Figures S7 and S8. Interestingly, in most cancer types, KDM1A was negatively correlated with CD^{4+} Th1 cells



(a)

Figure 3: Continued.



(b)

Figure 3: Correlation between DNA methylation of *KDM1A* and survival prognosis in TCGA tumors using MethSurv. The p value (<0.05) and the hazard ratio (HR) are displayed.

and positively correlated with CD^{4+} Th2 cells (Figure S7(a)). In addition, there was a positive correlation between Tregs and KDM1A expression in LIHC and LGG, but a negative correlation in TGCT (Figure S7(b)). B-cell infiltration was negatively correlated with KDM1A expression in STAD, READ, and HNSC (Figure S7(c)). Moreover, neutrophil infiltration was positively correlated with KDM1A expression in multiple tumors from various algorithms (Figure S8(c)), whereas other myeloid cells, such as macrophages and dendritic cells, showed no obvious correlations with KDM1A in cancer types via different algorithms (Figures S8(a) and S8(b)).

3.7. Enrichment Analysis of KDM1A-Related Genes. To study the molecular significance of KDM1A in tumorigenesis and development, we screened out the KDM1A-binding proteins and expression-correlated genes for downstream analyses. We generated Set 1 including 100 KDM1A-binding proteins stood by experimental evidence via the STRING database. The protein-protein interaction networks of these proteins excluding histone-associated proteins are shown in Figure 6(a). GEPIA2 was applied to analyze all expression data of TCGA and yield Set 2 including the top 100 genes correlating with KDM1A expression. The expression of top 6 genes in Set 2 were shown to maintain positive correlation with KDM1A (Figure 6(b)), including DHX9 (DExH-box helicase 9) (R = 0.58), SNRNP40 (small nuclear ribonucleoprotein U5 subunit 40) (R = 0.59), HNRNPR (heterogeneous nuclear ribonucleoprotein R) (R = 0.63), PPM1G (protein phosphatase, Mg^{2+}/Mn^{2+}-dependent 1G) (R = 0.51), HDAC2 (histone deacetylase 2) (R = 0.54), and SMARCA4 (SWI/SNF related, matrix associated, actin-dependent regulator of chromatin, subfamily a, member 4) (R = 0.48) (all $p < 0.001$). The positive correlations between KDM1A and the above six genes in different cancer types were displayed via a heatmap (Figure 6(c)). A Venn analysis of Set 1 and Set 2 generated two common genes, HDAC2 and SMARCA4 (Figure 6(d)).

Furthermore, we merged Set 1 and Set 2 to execute pathway and GO enrichment analyses. The KEGG-based pathway enrichment indicated that "spliceosome", "cell cycle", and "RNA transport" pathways were involved in the effect of KDM1A (Figure 6(e)). GO enrichment analysis indicated that KDM1A-related genes were enriched to the terms related to DNA and RNA, such as nucleosome binding, repressing transcription factor binding, chromatin DNA binding, RNA polymerase II transcription factor binding, RNA splicing, RNA localization, and others (Figures 7(a)–7(c)).

4. Discussion

Histone lysine methylation is an important covalent post-translational modification (PTM) of chromatin. To date, two different families of histone demethylases (KDMs) have been identified as the flavin-dependent amine oxidase-containing and the Jumonji C (JmjC)-domain-containing enzymes that both use oxidative mechanisms to catalyze N-methyl-lysine demethylation [24]. The first KDM (LSD1 or KDM1A) was identified by Shi's group in 2004 as a member of the FAD amine oxidase family [3]. KDM1A can demethylate H3K4me1/2 and H3K9me1/2 based on its interacting partners [3, 25]. KDM1A demethylates H3K4me1/2 and renders genes transcriptional repression via binding with CoREST (REST (RE1-silencing transcription factor) corepressor), CtBP (C-terminal-binding protein 1), and NuRD (nucleosome remodeling and deacetylase) complexes [26–29]. In addition, KDM1A interacting with androgen receptor (AR) or estrogen receptor (ER) induces transcriptional activation by demethylating H3K9me1/2 [4, 30, 31]. Furthermore, LSD1n, a neuron-specific isoform of LSD1 (KDM1A), was verified to specially target H4K20me2 for transcription activation of neuronal-regulated genes [5]. KDM1B/AOF1, as another member of the KDM1s family, is a histone H3K4 demethylase [32]. KDM1B plays different roles in the regulation of proliferation, apoptosis, and stemness in several cancers, such as breast cancer, ovarian cancer, and pancreatic cancer [24, 33–35]. In this study, we used pan-cancer analysis to systematically characterize the roles of KDM1A.

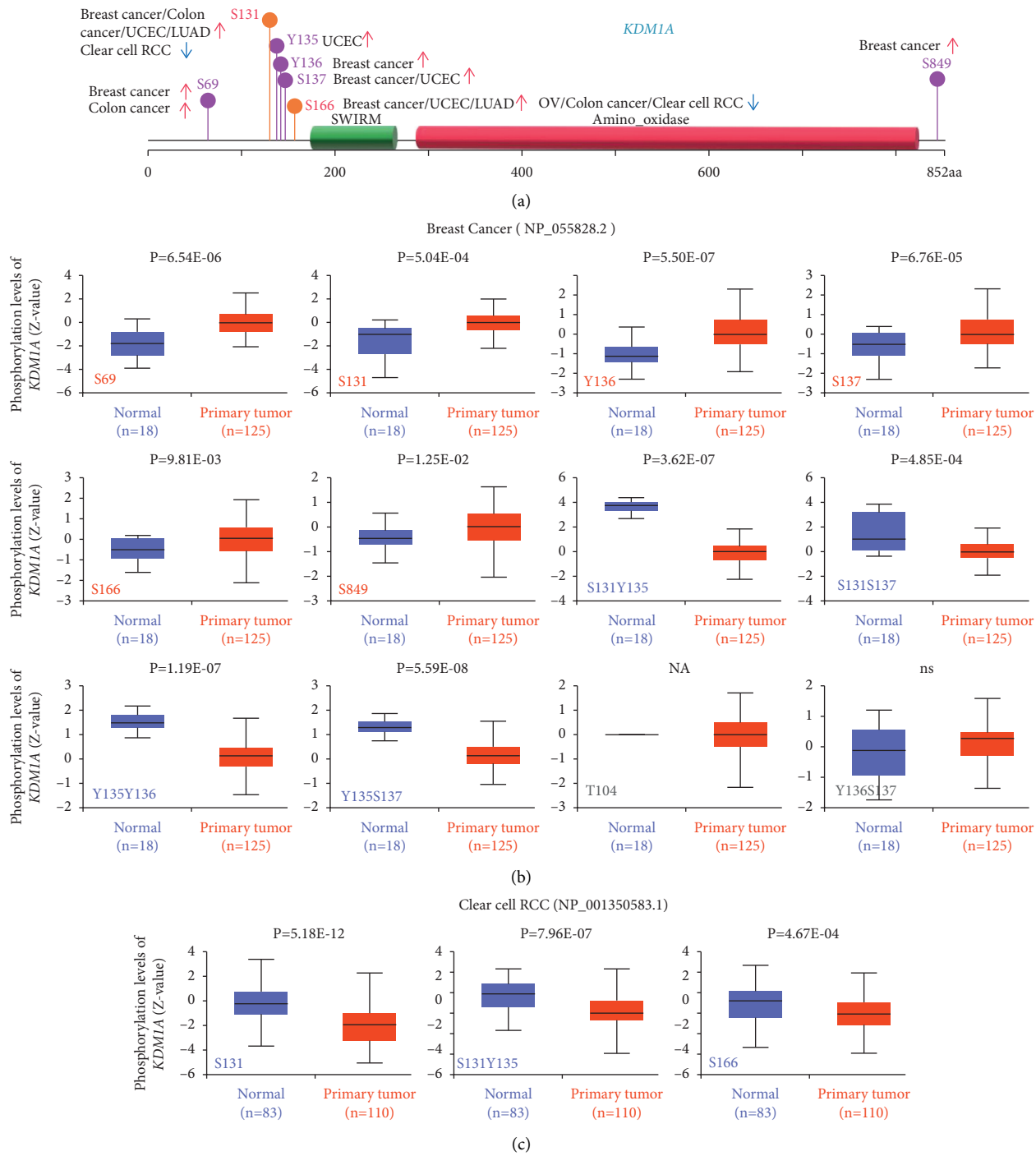


FIGURE 4: Analysis of phosphorylation levels of KDM1A in different cancers based on the CPTAC data set via the UALCAN. (a) Schematic diagram showed the phosphoprotein sites of KDM1A (NP_001350583.1) that were expressed at different levels in tumors compared with normal tissues. (b) and (c) Phosphorylation analysis of KDM1A protein in breast cancer and clear cell RCC, respectively.

Multiple studies showed that KDM1A expression is high in various cancers and plays an important role in different cancer-related processes. Considerable studies have highlighted the pivotal role of KDM1A in several cellular processes of normal and cancer cells such as stemness maintaining, differentiation [36, 37], cell migration, epithelial-to-mesenchymal transition [12], autophagy [38], senescence [39], neurodegenerative diseases [40], and metabolism [41]. However, a pan-cancer analysis of KDM1A was still urgently needed to reveal its relationship with cancer from the overall perspective. Thus, we comprehensively investigated the expression and efficacy of KDM1A on a total of 33 different cancer types in TCGA, GTEx, and CPTAC databases from the following aspects including gene expression, mutations, protein phosphorylation, DNA methylation, and tumor-infiltrating immune.

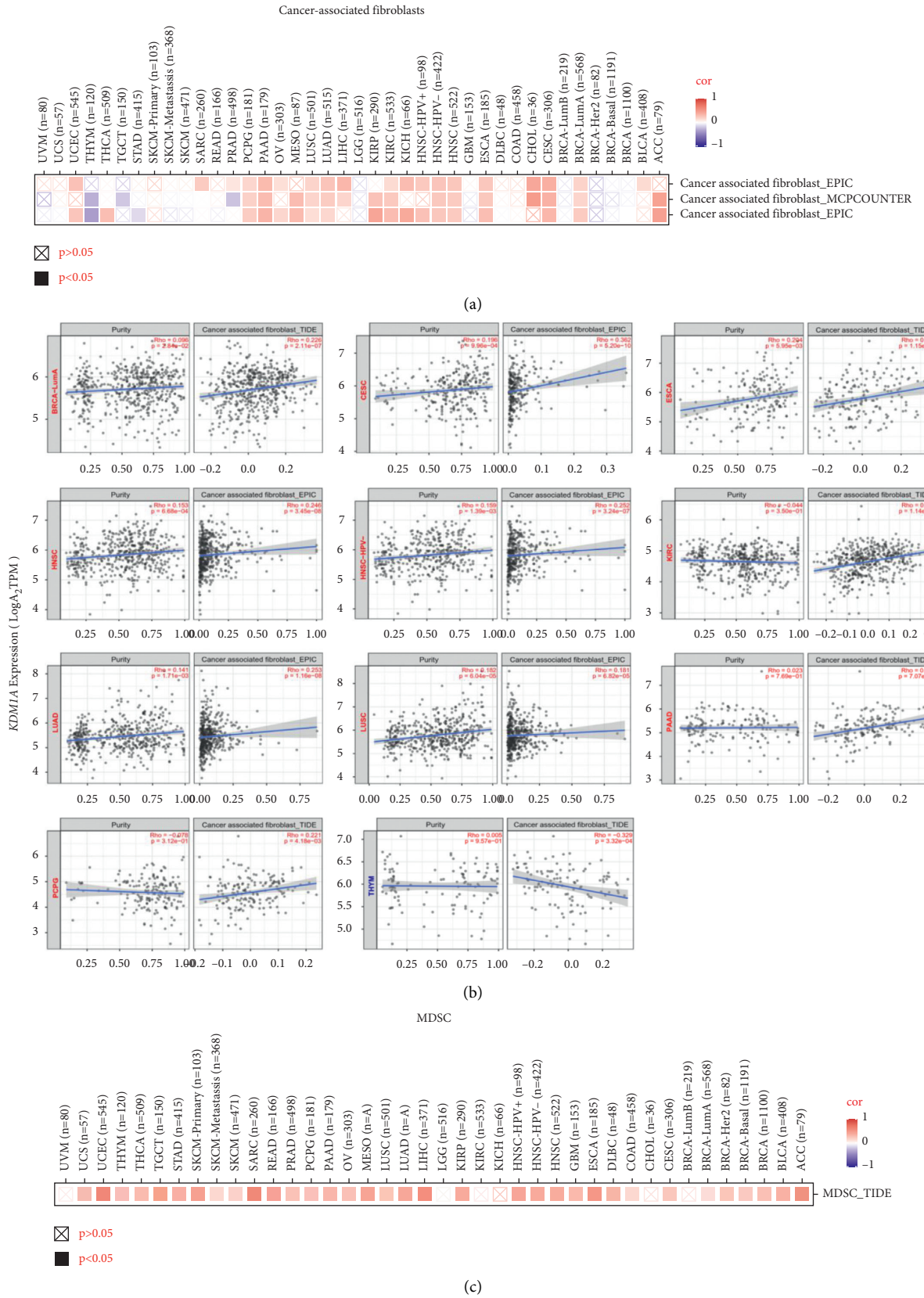


Figure 5: Relationship of KDM1A with cancer-associated fibroblasts (CAFs) and myeloid-derived suppressor cells (MDSCs) in the tumor microenvironment. (a) The scores of CAF were associated with the expression of *KDM1A* gene via EPIC, MCPCOUNTER, and TIDE algorithms. (b) Correlation between KDM1A expression and infiltration level of CAFs. (c) TIDE algorithm showed MDSCs were positively correlated with KDM1A in most cancer types.

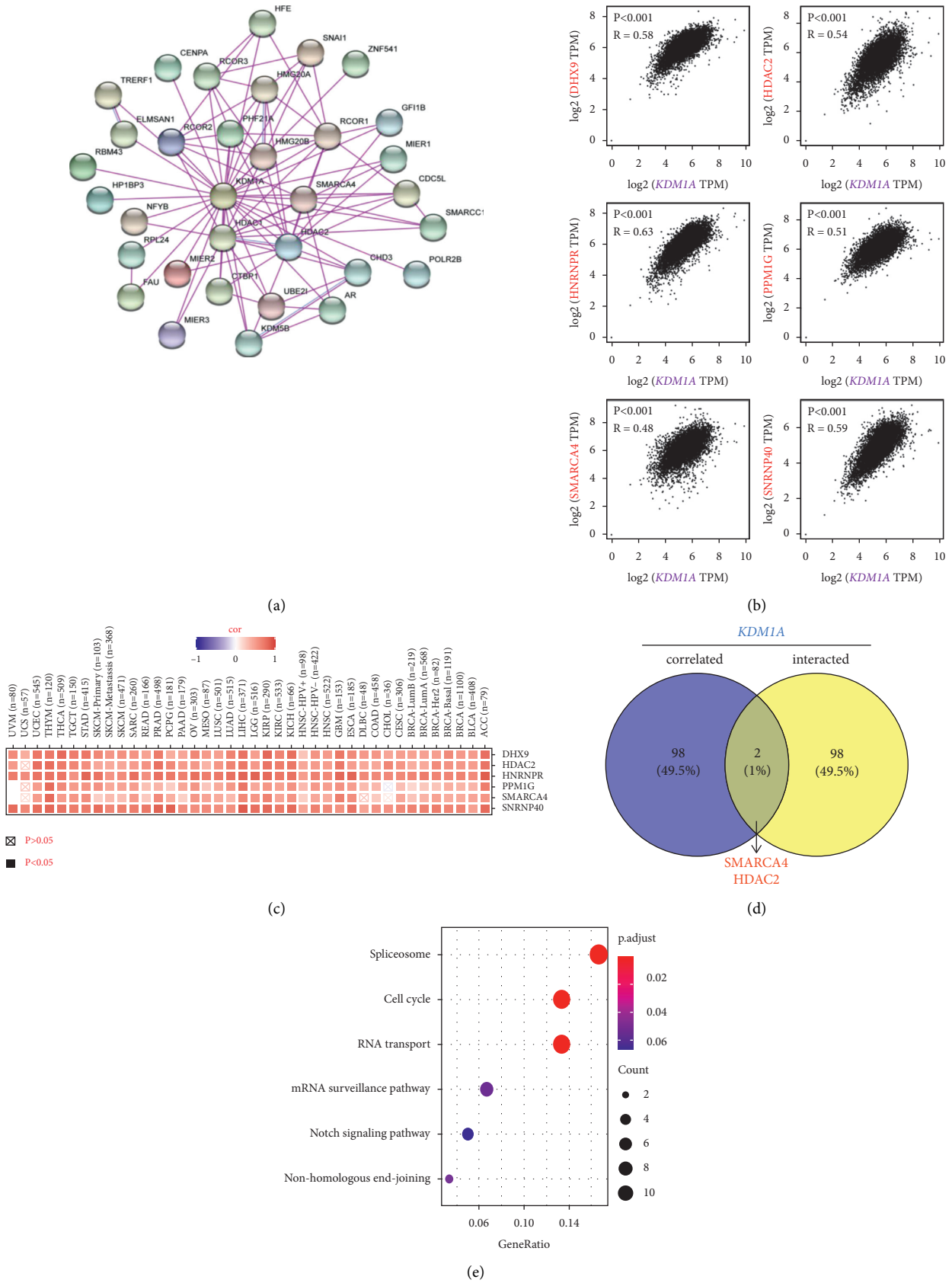


Figure 6: KDM1A-related gene enrichment analysis. (a) KDM1A-binding proteins were determined using the STRING tool. (b) The correlation of KDM1A and 6 top targeting genes was analyzed by GEPIA2. (c) The heatmap showed a corresponding relationship in the detailed cancer types. (d) An intersection analysis was conducted with the KDM1A-binding and correlated genes. (e) The bubble plot displayed KEGG pathway analysis based on the KDM1A-binding and interacted genes.

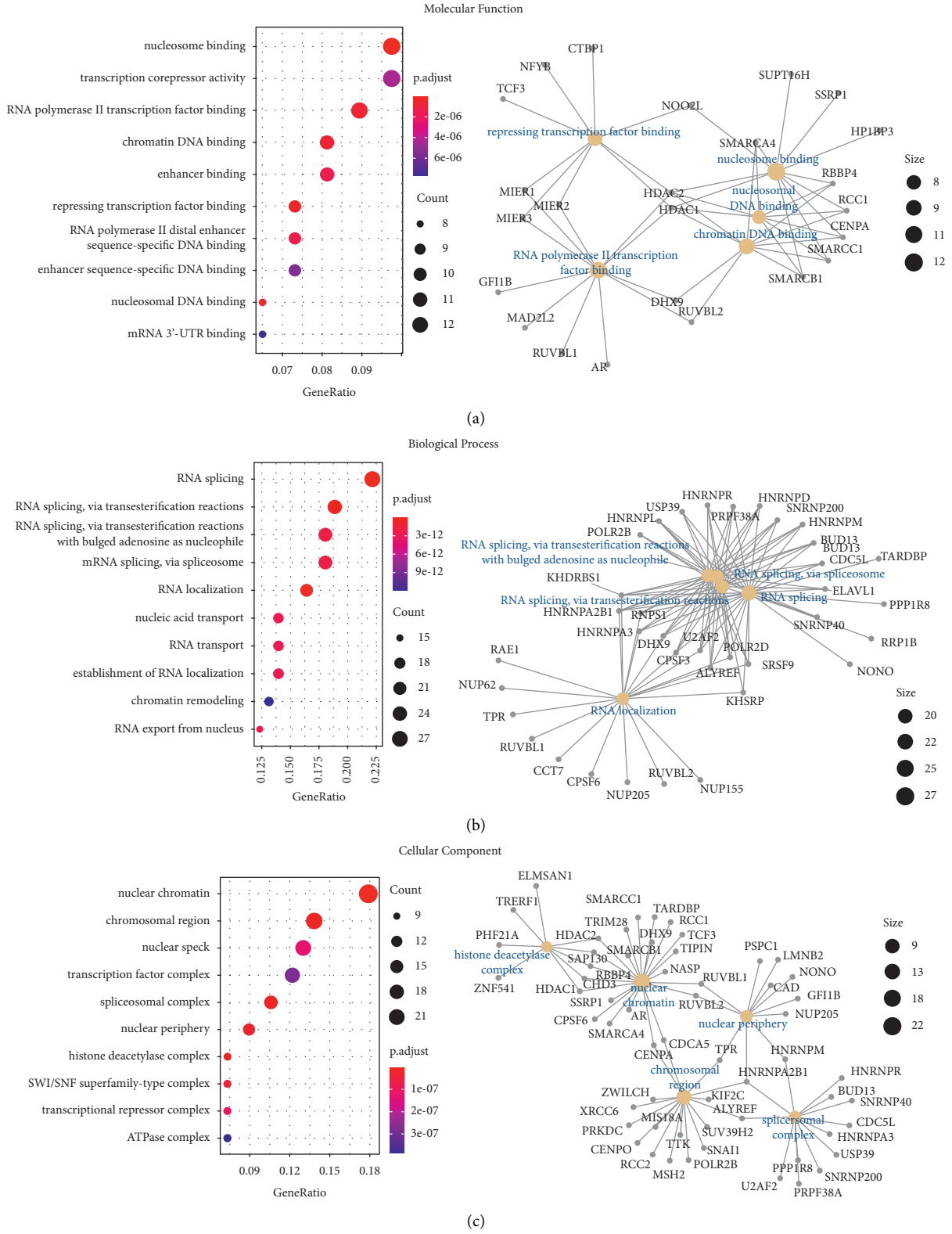


Figure 7: GO enrichment analysis of two gene sets referring to genes of KDM1A-binding and KDM1A-correlated genes. And the cnetplot for GO analysis of the first five was also shown: (a) molecular function analysis, (b) biological process analysis, and (c) cellular component analysis.

In the present study, we compared the expression of KDM1A in 33 tumors and their corresponding normal tissues and found that KDM1A was differentially highly expressed in up to 21 tumors, and 17 types in them were highly expressed in tumors compared with normal tissues. Meanwhile, we explored whether KDM1A expression is related to survival prognosis. We found that in most tumors, the high expression of KDM1A was a risk factor and associated with poor OS and DFS. Furthermore, the survival analysis revealed that KDM1A in LIHC and LUAD was the high expression and associated with poor survival prognosis (Table 1). In addition, the mutation of KDM1A in BRCA exhibited poor survival, yet the high DNA methylation of KDM1A foreboded a better survival prognosis of breast cancer via decreasing KDM1A expression. Moreover, the phosphorylation levels of KDM1A were upregulated in breast cancer, UCEC, and LUAD, and the phosphorylation of KDM1A at S131 and S137 was experimentally supposed to play a role in regulating RNF168-dependent 53BP1 recruitment in response to DNA damage and resisting DNA damaging agents [23, 42]. Meanwhile, Liu et al. showed that the overexpression of KDM1A is a potential prognostic factor in patients with liver cancer and KDM1A promotes tumorigenesis and malignancy in vitro [43]. Interestingly, high KDM1A expression in KICH was linked to poor prognosis, although it was the low expression in KICH compared with normal tissue from the TCGA database. Meanwhile, it has been reported that KDM1A can regulate kidney cancer cell growth via epigenetic control of AR transcription factors and that KDM1A inhibitors may be good candidate drugs for treating kidney cancer [44]. For UCEC cases, KDM1A is highly expressed, and the proportion of mutations is highest in all 33 tumors. Chen et al. demonstrated that silencing of KDM1A can abolish estrogen-driven endometrial cancer cell (ECC) proliferation and induce G1 cell arrest and apoptosis via PI3K/AKT/cyclinD1 signal [45]. These indicated that KDM1A is a potential prognostic biomarker in several cancers. Numerous KDM1A inhibitors had been discovered, and 8 of them had been used in clinical trials for multiple solid tumors and hematologic malignancy. Our result implied KDM1A inhibitors could have a potential effect on a wider spectrum of tumors, which can be further proved via experimental evidence.

Tumor microenvironment, including the immune and stromal microenvironment, constitutes a vital element of tumor tissue, which was closely related to oncogenesis and metastasis. Cancer-associated fibroblasts (CAFs) in the stroma participate in modulating the infiltration and function of various immune cells [46, 47]. Our analysis observed a statistically positive correlation between KDM1A expression and cancer-associated fibroblasts in most cancer types via multiple algorithms. Moreover, Liu and colleagues reported that upregulated KDM1A expression in CAFs is a driver of Notch3-mediated cancer stem-like cells self-renewal in hepatocellular carcinoma [43]. In addition, we illustrated that the positive correlation between KDM1A expression and MDSC infiltration happened in most cancers. MDSCs, as a heterogeneous group of myeloid cells, own potent immunosuppressive activity via interacting with innate and adaptive immune cells and perform a significant role in modulating antitumor immunity [48]. For adaptive immune cells, a statistically negative correlation was shown between KDM1A expression and CD^{8+} T cell infiltration in TGCT, LGG, KIRP, KIRC, and HNSC-HPV^{+}. KDM1A was negatively correlated with Th1 cells and B memory cells but positively correlated with Th2 cells in most cancer types. This implied KDM1A potentially related to immunosurveillance escape. Our previous study reported that KDM1A ablation stimulated tumor immunogenicity and increased T cell infiltration in breast cancer [7]. Sheng et al. also verified that LSD1 inhibition in tumor cells stimulated antitumor T cell immunity and overcame resistance to checkpoint blockade therapy [13]. These studies demonstrated that inhibition of KDM1A could increase the infiltration of CD^{8+} T cells from different perspectives, which promoted the efficacy of immunotherapy. We suggested that KDM1A could become a new prognostic biomarker for antitumor immunotherapy, and the combination of KDM1A inhibitors and immunotherapy could exert a potent efficacy of tumor suppression.

In this study, we combined the KDM1A-binding components and KDM1A expression-related genes for downstream analyses and evaluated the potential roles of KDM1A on "cell cycle pathway," "RNA transport pathway," "DNA binding," and "RNA splicing." The intersection of KDM1A-binding components and KDM1A-related genes included HDAC2 and SMARCA4, which indicated the efficacy of KDM1A on cancer mainly through cooperating with other epigenetic regulatory factors to finely regulate downstream genes. It implied the combination therapy of multiple epigenetic inhibitors could increase synergy effect and safety.

Gut microbiota have been found to link with both local gastrointestinal cancers and other distal tumors [49]. Microbial metabolites were proved to regulate the development of cancer via epigenetic regulators, such as propionic and butyric acids [49, 50]. Wang et al. demonstrated that the expression of KDM1A is upregulated by microbial metabolite butyrate in adipocytes [51]. It suggested that microbial metabolites may impact the KDM1A level in cancer cells to regulate tumor progression, which needs to be proved via experimental evidence.

Carcinogenic infections with certain viruses, bacteria, and parasites are strong risk factors for specific cancers [52]. KDM1A can impact viral and parasitic infections via the epigenetic regulation of viral genes and immune response. KDM1A activates replication of herpes simplex virus and varicella-zoster virus from latency via demethylating H3K9 at the viral immediate-early (IE) gene promoters [53]. KDM1A mediates the activation of the hepatitis B virus via demethylating H3K9 and synergizing with Set1A methylating H3K4 [54]. On the other hand, Douce et al. reported that LSD1 cooperating with CTIP2 silences HIV-1 transcription and viral expression [55]. Furthermore, KDM1A downregulates PD-1 expression of CD8 T cells via histone H3K4 modification following acute viral infection [56]. KDM1A is also important for goblet cell maturation and effector responses of gut immunity to bacterial and helminth

infections [57]. Meanwhile, KDM1A protects from endotoxin-induced death via regulating hematopoietic stem cells homeostasis [58]. The above studies show that KDM1A may have various effects on different types of infections.

5. Conclusion

Our comprehensive pan-cancer analysis illustrates the role of KDM1A as an oncogene and predictor of worse survival in most tumor types. KDM1A correlated with immunosuppressive tumor microenvironment via various approaches based on pan-cancer analysis. These findings highlight the role of KDM1A in tumorigenesis and development and potentially enable more precise and personalized immunotherapy in the future.

Abbreviations

ACC: Adrenocortical carcinoma
BLCA: Bladder urothelial carcinoma
BRCA: Breast invasive carcinoma
CESC: Cervical squamous cell carcinoma and endocervical adenocarcinoma
CHOL: Cholangiocarcinoma
COAD: Colon adenocarcinoma
DLBC: Lymphoid neoplasm diffuse large B-cell lymphoma
ESCA: Esophageal carcinoma
GBM: Glioblastoma multiforme
HNSC: Head and neck squamous cell carcinoma
KICH: Kidney chromophobe
KIRC: Kidney renal clear cell carcinoma
KIRP: Kidney renal papillary cell carcinoma
LAML: Acute myeloid leukemia
LGG: Brain lower grade glioma
LIHC: Liver hepatocellular carcinoma
LUAD: Lung adenocarcinoma
LUSC: Lung squamous cell carcinoma
MESO: Mesothelioma
OV: Ovarian serous cystadenocarcinoma
PAAD: Pancreatic adenocarcinoma
PCPG: Pheochromocytoma and paraganglioma
PRAD: Prostate adenocarcinoma
READ: Rectum adenocarcinoma
SARC: Sarcoma
SKCM: Skin cutaneous melanoma
STAD: Stomach adenocarcinoma
TGCT: Testicular germ cell tumors
THCA: Thyroid carcinoma
THYM: Thymoma
UCEC: Uterine corpus endometrial carcinoma
UCS: Uterine carcinosarcoma
UVM: Uveal melanoma
OS: Overall survival
DMFS: Distant metastasis-free survival
RFS: Relapse-free survival
PPS: Post-progression survival
FP: First progression
DSS: Disease-specific survival
PFS: Progression-free survival.

Data Availability

The data used to support the findings of this study are available from the corresponding authors upon request.

Conflicts of Interest

The authors declare that no competing interest exists.

Authors' Contributions

Y. Q. and H. W. conceived and supervised the study; L. L. and Y. W. performed data analyses; Y. M. helped in the analysis; all the authors contributed to drafting the manuscript; and Y. Q., L. L., and H. W. produced the final version of the manuscript.

Acknowledgments

This work was supported by grants from the Scientific Research Program of Hubei Education Department (D20201202) and Three Gorges University Master's Dissertation Training Fund (2021SSPY099).

Supplementary Materials

Survival analysis of Kaplan–Meier plotter. The Kaplan–Meier plotter (http://kmplot.com/analysis/) is a web-based tool of which aim is meta-analysis-based discovery and validation of survival biomarkers. The Kaplan–Meier plotter was used to analyze the correlations between KDM1A expression and patient survival of OS, DMFS (distant metastasis-free survival), RFS (relapse-free survival), PPS (post-progression survival), FP (first progression), DSS (disease-specific survival), and PFS (progress-free survival) in breast, ovarian, lung (LUAD and LUSC), gastric, and liver cancers. The data of breast, ovarian, lung (LUAD and LUSC), and gastric cancer came from gene chip (Affy ID: 212348_s_at (KDM1)), while one of liver cancer came from RNAseq (ID: 23028 (KDM1A)). The cases of these cancers were split into two groups by setting "autoselect best cutoff". The hazard ratio (HR), 95% confidence intervals, and log-rank p-value were computed, and the Kaplan–Meier survival plots were generated. *Phosphorylation feature prediction.* The open-access PhosphoNET database ((http://www.phosphonet.ca/) was used to obtain the predicted phosphorylation features of the S69, S131, Y135, Y136, S137, S166, and S849 sites by searching the protein name "KDM1A". Figure S1: *KDM1A* expression in various cancers and pathological stages. (a) The expression levels of *KDM1A* gene in different cancers from TCGA were compared with the corresponding normal tissues based on GTEx databases. (b) KDM1A expression in different pathological stages in selected cancer types. Figure S2: Survival prognosis of cancers was related to the expression of KDM1A analyzed by the GEPIA2 tool. Figure S3: Correlation between KDM1A gene expression and prognosis of cancers. The Kaplan–Meier plot showed the survival curve by comparison of the cases with high and low expression of KDM1A in breast cancer (a), ovarian cancer (b), gastric cancer (c), LUAD (d), LUSC (e), and liver cancer (f)

and the curves were plotted from the Kaplan–Meier plotter database. OS, overall survival; DMFS, distant metastasis-free survival; RFS, relapse-free survival; PPS, post-progression survival; FP, first progression; DSS, disease-specific survival; PFS, progress-free survival. The data of breast, ovarian, lung (LUAD and LUSC), and gastric cancer came from gene chip (Affy ID: 212348_s_at (KDM1)), while one of liver cancer came from RNAseq (ID: 23028 (KDM1A)). Figure S4: Correlation between DNA methylation of *KDM1A* and survival prognosis in TCGA tumors using MethSurv. We used the MethSurv website to perform multivariable survival analysis using DNA methylation data. The p value (<0.05) and the hazard ratio (HR) are displayed. Figure S5: Phosphorylation level of KDM1A protein (NP_0055828.2) in different tumors based on the CPTAC data set, including ovarian cancer (a), UCEC (b), LUAD (c), and colon cancer (d). Figure S6: Correlation between KDM1A expression and CD^{8+} T cell infiltration across all types of cancer in TCGA based on different algorithms. Figure S7: Correlation between KDM1A expression and the infiltration of CD^{4+} T cells, Tregs, and B cells across all types of cancer in TCGA based on different algorithms. (a) CD^{4+} T cells, (b) Tregs, and (c) B cells. Figure S8: Correlation between *KDM1A* expression and the infiltration of myeloid-derived cells across all types of cancer in TCGA based on different algorithms. (a) Macrophage, (b) myeloid dendritic cell, and (c) neutrophil. Table S1: Alteration sites of KDM1A in tumors. Table S2: Phosphorylation level of KDM1A protein in different tumors. Table S3: Analysis of CPTAC-identified phosphorylation sites of KDM1A via the PhosphoNET database. (*Supplementary Materials*)

References

[1] S. Biswas and C. M. Rao, "Epigenetics in cancer: fundamentals and beyond," *Pharmacology & Therapeutics*, vol. 173, pp. 118–134, 2017.

[2] S. J. Hogg, P. A. Beavis, M. A. Dawson, and R. W. Johnstone, "Targeting the epigenetic regulation of antitumour immunity," *Nature Reviews Drug Discovery*, vol. 19, no. 11, pp. 776–800, 2020.

[3] Y. Shi, F. Lan, C. Matson et al., "Histone demethylation mediated by the nuclear amine oxidase homolog LSD1," *Cell*, vol. 119, no. 7, pp. 941–953, 2004.

[4] E. Metzger, "LSD1 demethylates repressive histone marks to promote androgen-receptor-dependent transcription," *Nature*, vol. 437, pp. 436–439, 2005.

[5] J. Wang, "LSD1n is an H4K20 demethylase regulating memory formation via transcriptional elongation control," *Nature Neuroscience*, vol. 18, pp. 1256–1264, 2015.

[6] D. Kim, K. I. Kim, and S. H. Baek, "Roles of lysine-specific demethylase 1 (LSD1) in homeostasis and diseases," *Journal of Biomedical Science*, vol. 28, p. 41, 2021.

[7] Y. Qin, "Inhibition of histone lysine-specific demethylase 1 elicits breast tumor immunity and enhances antitumor efficacy of immune checkpoint blockade," *Oncogene*, vol. 38, pp. 390–405, 2019.

[8] Q. Xie, "LSD1 promotes bladder cancer progression by upregulating LEF1 and enhancing EMT," *Front Oncol*, vol. 10, p. 1234, 2020.

[9] S. Gao, "Chromatin binding of FOXA1 is promoted by LSD1-mediated demethylation in prostate cancer," *Nature Genetics*, vol. 52, pp. 1011–1017, 2020.

[10] J. Wang, "Novel histone demethylase LSD1 inhibitors selectively target cancer cells with pluripotent stem cell properties," *Cancer Research*, vol. 71, pp. 7238–7249, 2011.

[11] J. Y. Lee, "LSD1 demethylates HIF1α to inhibit hydroxylation and ubiquitin-mediated degradation in tumor angiogenesis," *Oncogene*, vol. 36, pp. 5512–5521, 2017.

[12] T. Lin, A. Ponn, X. Hu, B. K. Law, and J. Lu, "Requirement of the histone demethylase LSD1 in Snai1-mediated transcriptional repression during epithelial-mesenchymal transition," *Oncogene*, vol. 29, pp. 4896–4904, 2010.

[13] W. Sheng, "LSD1 ablation stimulates anti-tumor immunity and enables checkpoint blockade," *Cell*, vol. 174, pp. 549–563, 2018.

[14] X. Cui, "A pan-cancer analysis of the oncogenic role of staphylococcal nuclease domain-containing protein 1 (SND1) in human tumors," *Genomics*, vol. 112, pp. 3958–3967, 2020.

[15] T. Li, J. Fu, Z. Zeng et al., "TIMER2.0 for analysis of tumor-infiltrating immune cells," *Nucleic Acids Research*, vol. 48, pp. W509–w514, 2020.

[16] Z. Tang, B. Kang, C. Li, T. Chen, and Z. Zhang, "GEPIA2: an enhanced web server for large-scale expression profiling and interactive analysis," *Nucleic Acids Research*, vol. 47, pp. W556–w560, 2019.

[17] D. S. Chandrashekar, B. Bashel, S. A. H. Balasubramanya et al., "UALCAN: a portal for facilitating tumor subgroup gene expression and survival analyses," *Neoplasia*, vol. 19, pp. 649–658, 2017.

[18] E. Cerami, "The cBio cancer genomics portal: an open platform for exploring multidimensional cancer genomics data," *Cancer Discovery*, vol. 2, pp. 401–404, 2012.

[19] V. Modhukur, T. Iljasenko, T. Metsalu, K. Lokk, T. Laisk-Podar, and J. Vilo, "MethSurv: a web tool to perform multivariable survival analysis using DNA methylation data," *Epigenomics*, vol. 10, pp. 277–288, 2018.

[20] A. Koch, J. Jeschke, W. Van Criekinge, M. van Engeland, and T. De Meyer, "MEXPRESS update 2019," *Nucleic Acids Research*, vol. 47, pp. W561–W565, 2019.

[21] D. Szklarczyk, "STRING v11: protein-protein association networks with increased coverage, supporting functional discovery in genome-wide experimental datasets," *Nucleic Acids Research*, vol. 47, pp. D607–d613, 2019.

[22] G. Yu, L. G. Wang, Y. Han, and Q. Y. He, "clusterProfiler: an R package for comparing biological themes among gene clusters," *Omics*, vol. 16, pp. 284–287, 2012.

[23] B. Peng, "Modulation of LSD1 phosphorylation by CK2/WIP1 regulates RNF168-dependent 53BP1 recruitment in response to DNA damage," *Nucleic Acids Research*, vol. 43, pp. 5936–5947, 2015.

[24] L. Chen, "Functional characterization of lysine-specific demethylase 2 (LSD2/KDM1B) in breast cancer progression," *Oncotarget*, vol. 8, pp. 81737–81753, 2017.

[25] B. Majello, F. Gorini, C. D. Saccà, and S. Amente, "Expanding the role of the histone lysine-specific demethylase LSD1 in cancer," *Cancers (Basel)*, vol. 11, 2019.

[26] F. Gu, Y. Lin, Z. Wang et al., "Biological roles of LSD1 beyond its demethylase activity," *Cellular and Molecular Life Sciences*, vol. 77, pp. 3341–3350, 2020.

[27] M. G. Lee, C. Wynder, N. Cooch, and R. Shiekhattar, "An essential role for CoREST in nucleosomal histone 3 lysine 4 demethylation," *Nature*, vol. 437, pp. 432–435, 2005.

[28] Y. Shi, "Coordinated histone modifications mediated by a CtBP co-repressor complex," *Nature*, vol. 422, pp. 735–738, 2003.

[29] Y. Wang, "LSD1 is a subunit of the NuRD complex and targets the metastasis programs in breast cancer," *Cell*, vol. 138, pp. 660–672, 2009.
[30] B. Laurent, "A specific LSD1/KDM1A isoform regulates neuronal differentiation through H3K9 demethylation," *Molecular Cell*, vol. 57, pp. 957–970, 2015.
[31] J. Wang, "Opposing LSD1 complexes function in developmental gene activation and repression programmes," *Nature*, vol. 446, pp. 882–887, 2007.
[32] D. N. Ciccone, "KDM1B is a histone H3K4 demethylase required to establish maternal genomic imprints," *Nature*, vol. 461, pp. 415–418, 2009.
[33] T. A. Katz, S. N. Vasilatos, E. Harrington, S. Oesterreich, N. E. Davidson, and Y. Huang, "Inhibition of histone demethylase, LSD2 (KDM1B), attenuates DNA methylation and increases sensitivity to DNMT inhibitor-induced apoptosis in breast cancer cells," *Breast Cancer Research and Treatment*, vol. 146, pp. 99–108, 2014.
[34] Y. K. Lee, J. Lim, S. Y. Yoon, J. C. Joo, S. J. Park, and Y. J. Park, "Promotion of cell death in cisplatin-resistant ovarian cancer cells through KDM1B-DCLRE1B modulation," *International Journal of Molecular Sciences*, vol. 20, 2019.
[35] Y. Wang, L. Sun, Y. Luo, and S. He, "Knockdown of KDM1B inhibits cell proliferation and induces apoptosis of pancreatic cancer cells," *Pathology, Research & Practice*, vol. 215, pp. 1054–1060, 2019.
[36] A. Maiques-Diaz, "Enhancer activation by pharmacologic displacement of LSD1 from GFI1 induces differentiation in acute myeloid leukemia," *Cell Reports*, vol. 22, pp. 3641–3659, 2018.
[37] W. A. Whyte, S. Bilodeau, D. A. Orlando et al., "Enhancer decommissioning by LSD1 during embryonic stem cell differentiation," *Nature*, vol. 482, pp. 221–225, 2012.
[38] S. Ambrosio, A. Ballabio, and B. Majello, "Histone methyltransferases and demethylases in the autophagy regulatory network: the emerging role of KDM1A/LSD1 demethylase," *Autophagy*, vol. 15, pp. 187–196, 2019.
[39] J. Castex, "Inactivation of Lsd1 triggers senescence in trophoblast stem cells by induction of Sirt4," *Cell Death & Disease*, vol. 8, Article ID e2631, 2017.
[40] M. A. Christopher, "LSD1 protects against hippocampal and cortical neurodegeneration," *Nature Communications*, vol. 8, p. 805, 2017.
[41] A. Sakamoto, "Lysine demethylase LSD1 coordinates glycolytic and mitochondrial metabolism in hepatocellular carcinoma cells," *Cancer Research*, vol. 75, pp. 1445–1456, 2015.
[42] R. Costa, G. Arrigoni, G. Cozza et al., "The lysine-specific demethylase 1 is a novel substrate of protein kinase CK2," *Biochimica et Biophysica Acta*, vol. 1844, pp. 722–729, 2014.
[43] C. Liu, "LSD1 stimulates cancer-associated fibroblasts to drive notch3-dependent self-renewal of liver cancer stem-like cells," *Cancer Research*, vol. 78, pp. 938–949, 2018.
[44] K. H. Lee, B. C. Kim, S. H. Jeong et al., "Histone demethylase LSD1 regulates kidney cancer progression by modulating androgen receptor activity," *International Journal of Molecular Sciences*, vol. 21, 2020.
[45] C. Chen, "LSD1 sustains estrogen-driven endometrial carcinoma cell proliferation through the PI3K/AKT pathway via di-demethylating H3K9 of cyclin D1," *International Journal of Oncology*, vol. 50, pp. 942–952, 2017.
[46] M. Paauwe, "Endoglin expression on cancer-associated fibroblasts regulates invasion and stimulates colorectal cancer metastasis," *Clinical Cancer Research*, vol. 24, pp. 6331–6344, 2018.
[47] X. Chen and E. Song, "Turning foes to friends: targeting cancer-associated fibroblasts," *Nature Reviews Drug Discovery*, vol. 18, pp. 99–115, 2019.
[48] C. Zalfa and S. Paust, "Natural killer cell interactions with myeloid derived suppressor cells in the tumor microenvironment and implications for cancer immunotherapy," *Frontiers in Immunology*, vol. 12, p. 633205, 2021.
[49] S. Vivarelli, "Gut microbiota and cancer: from pathogenesis to therapy," *Cancers (Basel)*, vol. 11, 2019.
[50] W. S. Garrett, "Cancer and the microbiota," *Science*, vol. 348, pp. 80–86, 2015.
[51] D. Wang, "LSD1 mediates microbial metabolite butyrate-induced thermogenesis in brown and white adipose tissue," *Metabolism*, vol. 102, p. 154011, 2020.
[52] M. Plummer, C. de Martel, J. Vignat, J. Ferlay, F. Bray, and S. Franceschi, "Global burden of cancers attributable to infections in 2012: a synthetic analysis," *Lancet Glob Health*, vol. 4, pp. e609–16, 2016.
[53] Y. Liang, D. Quenelle, J. L. Vogel, C. Mascaro, A. Ortega, and T. M. Kristie, "A novel selective LSD1/KDM1A inhibitor epigenetically blocks herpes simplex virus lytic replication and reactivation from latency," *mBio*, vol. 4, pp. e00558–12, 2013.
[54] V. Alarcon, "The enzymes LSD1 and Set1A cooperate with the viral protein HBx to establish an active hepatitis B viral chromatin state," *Scientific Reports*, vol. 6, Article ID 25901, 2016.
[55] V. Le Douce, "LSD1 cooperates with CTIP2 to promote HIV-1 transcriptional silencing," *Nucleic Acids Research*, vol. 40, pp. 1904–1915, 2012.
[56] A. P. R. Bally, D. K. Neeld, P. Lu et al., "PD-1 expression during acute infection is repressed through an LSD1-blimp-1 Axis," *The Journal of Immunology*, vol. 204, pp. 449–458, 2020.
[57] N. Parmar, "Intestinal-epithelial LSD1 controls goblet cell maturation and effector responses required for gut immunity to bacterial and helminth infection," *PLoS Pathogens*, vol. 17, Article ID e1009476, 2021.
[58] J. Wang, K. Saijo, D. Skola et al., "Histone demethylase LSD1 regulates hematopoietic stem cells homeostasis and protects from death by endotoxic shock," *Proceedings of the National Academy of Sciences of the USA*, vol. 115, pp. E244–e252, 2018.

Hindawi
Canadian Journal of Infectious Diseases and Medical Microbiology
Volume 2021, Article ID 9278441, 8 pages
https://doi.org/10.1155/2021/9278441

Review Article

Gut-Lung Microbiota in Chronic Pulmonary Diseases: Evolution, Pathogenesis, and Therapeutics

Chang Yi Shi,[1] Chen Huan Yu,[2,3] Wen Ying Yu,[1] and Hua Zhong Ying[1]

[1]*Zhejiang Provincial Laboratory of Experimental Animal's & Nonclinical Laboratory Studies, Hangzhou Medical College, Hangzhou, China*
[2]*Institute of Cancer and Basic Medicine, Chinese Academy of Sciences, Hangzhou, China*
[3]*Cancer Hospital of the University of Chinese Academy of Sciences (Zhejiang Cancer Hospital), Hangzhou, China*

Correspondence should be addressed to Hua Zhong Ying; hzying0101@163.com

Received 19 September 2021; Accepted 20 November 2021; Published 3 December 2021

Academic Editor: Hua Zhang

The microbiota colonized in the human body has a symbiotic relationship with human body and forms a different micro-ecosystem, which affects human immunity, metabolism, endocrine, and other physiological processes. The imbalance of microbiota is usually linked to the aberrant immune responses and inflammation, which eventually promotes the occurrence and development of respiratory diseases. Patients with chronic respiratory diseases, including asthma, COPD, bronchiectasis, and idiopathic pulmonary fibrosis, often have alteration of the composition and function of intestinal and lung microbiota. Gut microbiota affects respiratory immunity and barrier function through the lung-gut microbiota, resulting in altered prognosis of chronic respiratory diseases. In turn, lung dysbiosis promotes aggravation of lung diseases and causes intestinal dysfunction through persistent activation of lymphoid cells in the body. Recent advances in next-generation sequencing technology have disclosed the pivotal roles of lung-gut microbiota in the pathogenesis of chronic respiratory diseases. This review focuses on the association between the gut-lung dysbiosis and respiratory diseases pathogenesis. In addition, potential therapeutic modalities, such as probiotics and fecal microbiota transplantation, are also evaluated for the prevention of chronic respiratory diseases.

1. Introduction

With the development of high-throughput second-generation sequencing technology and through the analysis and sequencing of the whole gene spectrum of microbiota, a certain correlation between the respiratory tract and the intestine has been gradually found [1, 2], and certain microbiota disorders or microbial pathogens in the lungs and intestines have been discovered to be capable of affecting the occurrence, development, and prognosis of diseases through different means, such as inflammation, metabolism, and cell signaling [3, 4]. Clinically, lung diseases, such as asthma, chronic obstructive pulmonary disease (COPD), and even lung cancer, are often associated with digestive tract diseases, resulting in prolonged disease courses, aggravated diseases, and increased mortality [5–8].

In these circumstances, the concept of the lung-gut axis was put forward in modern medicine. This theory uses the immune system and microbial flora, which colonize in the lung and gut, as a link hub to form a two-way axis that connects the lungs and intestines; in other words, intestinal flora influences the development of lung diseases, and in turn, lung diseases, especially infectious diseases caused by various bacteria, can also affect the digestive tract through immunoregulation. The lung-gut link proposed by the lung-gut axis provides a new insight for clinical diagnosis and treatment of the lung diseases through modulating the intestine system and vice versa. This link further explains the scientific nature of the concept of the "exterior-interior relationship between the lung and the large intestine" in Chinese medicine. In this study, the progress of research on the lung-gut axis and the effects of lung and intestinal microecology on lung diseases are reviewed and surveyed.

2. Interaction between Lung and Intestinal Microbiota

A large and varied number of microorganisms live in the human body and are mainly distributed on mucosal surfaces, such as the oral cavity, intestinal tract, respiratory tract, skin, and vagina, forming a highly complex microecosystem [9–11]. Moreover, the numerous and various microorganisms in different parts of the body not only help the human body to maintain normal physiological functions but also play an important role in the occurrence and development of disease.

2.1. Gut Microbiota and Respiratory Diseases. At present, more than 1000 kinds of intestinal flora are known. They mainly include *Bacteroides*, *Firmicutes*, *Actinomycetes*, and *Verrucomicrobia* [12, 13]. Gut flora consists of approximately 38 trillion bacteria, which can encode approximately 3.3 million specific genes [14, 15]. Each microbiome is distributed in different parts of the gastrointestinal tract in accordance with pH gradient and oxygen content.

Intestinal flora is not only involved in the immune development of the intestinal mucosa but also known as an important innate immune system regulator. Research has found that the development of the immune system is greatly affected by gut microbes [16–18]. In the early stage of life, the incidence of immune system diseases, asthma, and other allergic diseases is significantly increased due to the lack of the irritation of gut microbes [19–22]. This incidence shows the trend of being higher in developed countries than in developing countries and in cities than in rural areas. This trend is related to the reduction in intestinal flora diversity due to the improved hygiene and good medical conditions in developed countries and cities. Epidemiological studies have also confirmed that the use of broad-spectrum antibiotics in infants and young children reduces the variety of gut microbes [19, 23–25]. This effect is a contributing factor to allergic asthma in adulthood [26–28]. Therefore, intestinal flora, especially those in early life, have an important effect on the development of immune system diseases and respiratory diseases.

2.2. Lung Microbiota and Respiratory Diseases. Up to now, less is known about lung microecology than about intestinal microecology. In a healthy state, *Prevotella*, *Streptococcus*, *Veronococcus*, *Fusobacterium*, and *Haemophilus* are the dominant bacteria in the human respiratory tract and lungs [29], but their relative abundances are remarkably less than those in the intestine (Figure 1). It has been proven that the lung-based microorganisms play the biologic roles primarily through regulation of the immune system [10, 30, 31]. In the early stage of life, lung microorganisms migrate into the lungs from pharyngeal secretions or gastric juice mainly through microaspiration and finally are removed through phagocytosis by alveolar macrophages and transported by mucociliary cilia, thereby promoting the maturation of the immune system to achieve the balance and stability of lung microecology. However, in the state of disease, microbial homeostasis in the lungs is disturbed due to the following: (1) changes in the respiratory tract environment caused by chronic inflammation are conducive to the growth and reproduction of certain flora (Figure 2). It is now clear that the number of *Pseudomonas aeruginosa*, *Staphylococcus*, and *Burkholderia* is significantly increased in the respiratory tracts of patients with cystic fibrosis. In patients with COPD, the number of *Moraxella* and *Haemophilus* bacteria in the lungs is increased [32, 33]. The bacteria from the genera *Fusobacterium*, *Lachnospira*, *Veillonella*, and *Rothia* are more common in asthmatic patients than in healthy [29]. The supplementation of these genera in nude mice can reduce the number of pulmonary eosinophils, reduce the immune response of Th1/Th2 or Th17 [34–36], and alleviate the symptoms of those abovementioned respiratory diseases [37–39]. Notably, *Haemophilus influenzae*, *Moraxella catarrhalis*, *Streptococcus pneumoniae*, and *Klebsiella pneumoniae* were found to be the most common bacterial species in patients with severe respiratory diseases, which were also considered to be the potential pathogenic factors [40–42]. (2) The pulmonary epithelial barrier dysfunction impacts the removal mechanism for lung microorganisms (e.g., damaged mucosa cilia) or promotes the migration of microorganisms to the lungs (e.g., secondary infections). Although the mechanism of action through which lung flora influence the development of disease is not clear, it can be used as a potential target for the diagnosis and treatment of diseases and provide a new basis for reasonable disease classification and thus has a good clinical application value.

2.3. Mucosal Immunity Bridges the Lung-Gut Axis. From the perspective of embryonic development, the lungs, trachea, and large intestines are homologous, in which the alveolar, glandular, and mucosal epithelia all develop from the endoderm of the archenteron. The mucosal structure of the respiratory tract and gastrointestinal tract is not only an important site for the survival of microflora but also protects the body from pathogen invasion through the mucosal immune system [43, 44]. The physiological conditions on the surface of the mucosa, such as temperature, humidity, and pH, as well as secretions, can affect the growth and migration of microorganisms. In addition, immunoglobulin sIgA, which is secreted by the mucosa, has a selective effect on microorganisms on the surfaces of the mucosa. For example, some pathogens are removed by binding to sIgA, whereas some nonpathogenic and beneficial bacteria can be retained on the mucosal surface by binding to sIgA. Moreover, the body's own congenital immunity and adaptive immunity also play a regulatory role in microecology. The immune system can use inherent immune cells or epithelial cells to identify the presence of microbes and release antimicrobial peptides (such as α-defensins) and inflammatory factors to further activate lymphocytes to produce an immune response. In addition to endogenous factors, such as mucosal properties and the immune system, exogenous factors, such as diet structure, glucocorticoids, antibiotics, lifestyle, and environment, can affect the composition and function of bacteria in the lung and gut microbiota [45–47].

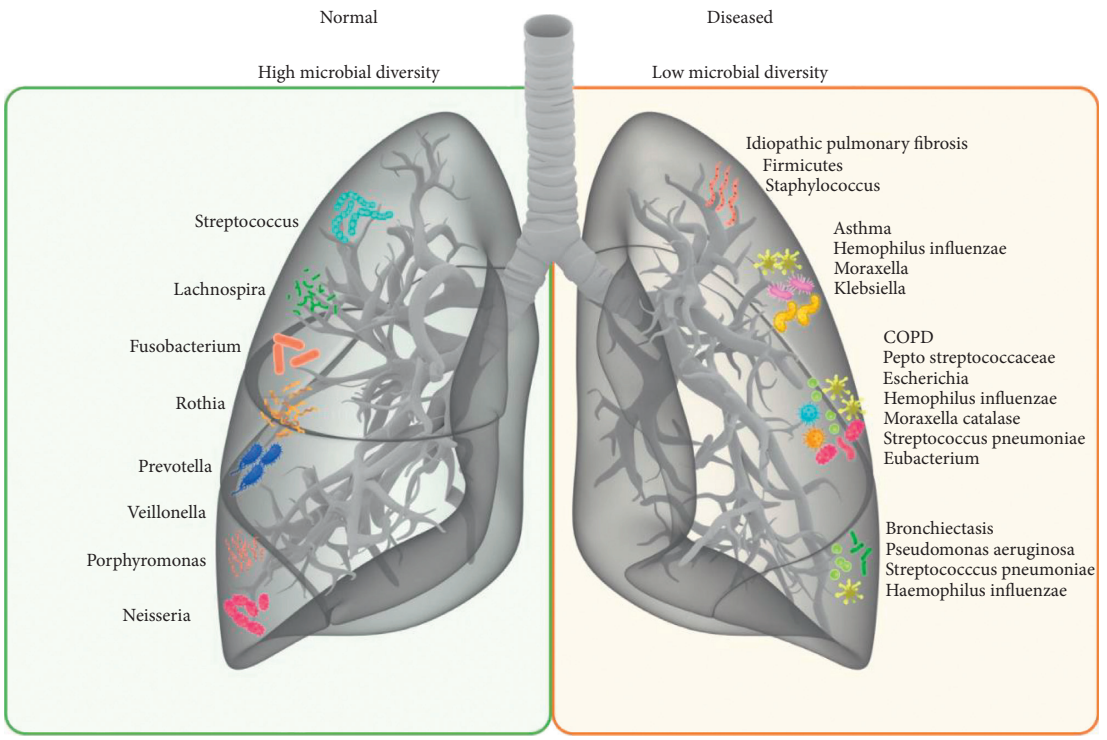


Figure 1: The profiles of lung microbiota in the lung tissues of healthy people and the patients with various chronic pulmonary diseases.

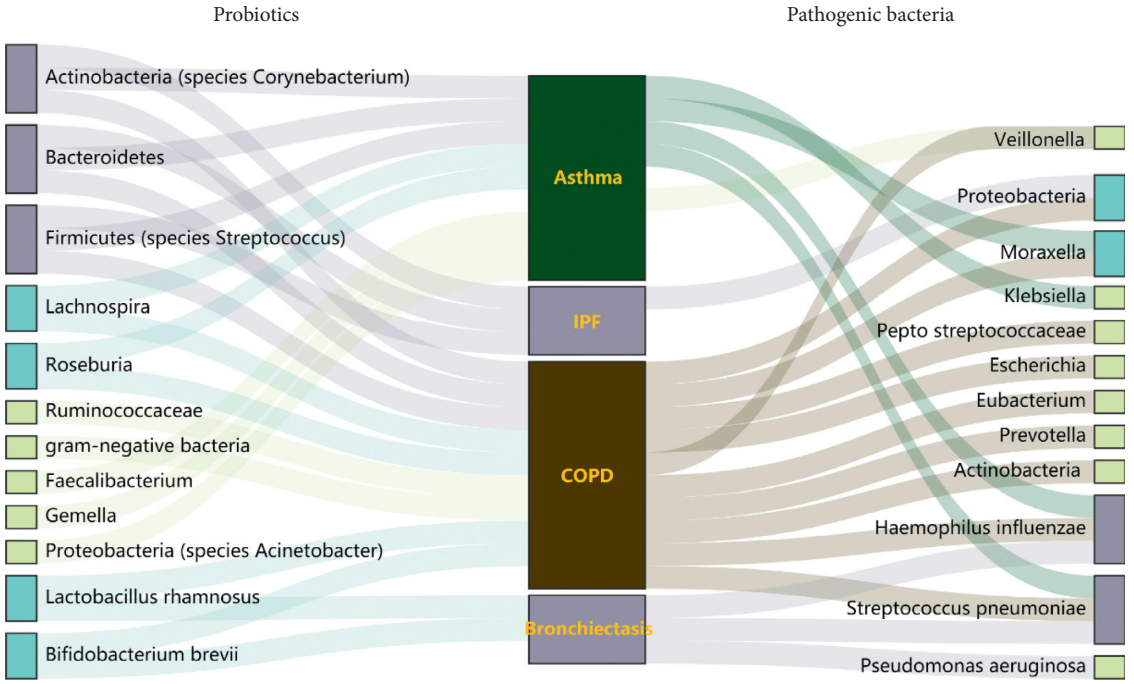


Figure 2: Sankey diagram of gut-lung microbiota composition at genus or species level during the development of various respiratory diseases.

3. The Lung-Gut Microbiome Crosstalk

The intestines and lungs interact with and restrict each other through microorganisms, immune functions, and metabolites, thus achieving two-way regulation (Figure 3).

3.1. Direct Interaction between Lung and Gut Microbiome. The microorganisms that have colonized the mucosa of the respiratory and digestive tracts can have a regulatory effect on tissues and are the material basis for lung-gut connections. For example, gavage with a suspension of feces from

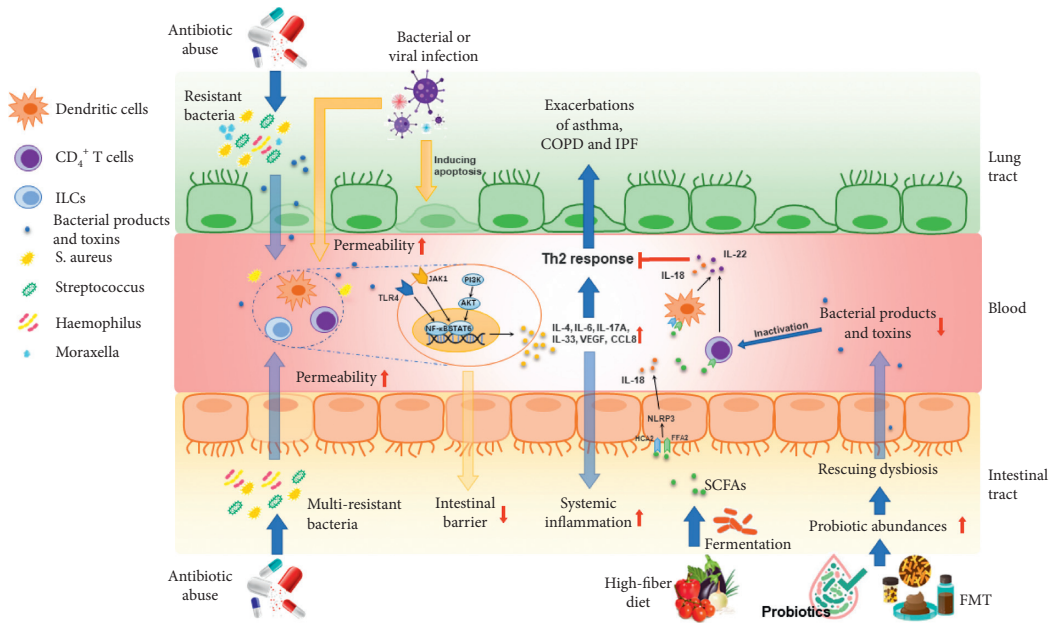


Figure 3: The role of lung and gut microbiota in the pathology of respiratory diseases.

healthy mice can alleviate the symptoms of pneumonia in mice infected with *Streptococcus pneumoniae* under antibiotic treatment [48, 49]. In children, oral administration of *Lactobacillus* and *Bifidobacterium* can help relieve asthma symptoms and reduce the frequency of seizures [50]. These results have shown that changes in gut microbes can cause changes in lung immunity and lung diseases. Conversely, *S. pneumoniae* and *Haemophilus* flu in the lungs activate the MAPK pathways of intestinal tissue cells and enhance the inflammatory response [51–53]. In addition, gut microbes can be transferred to the lungs [54]. For example, the deterioration of sepsis and acute respiratory distress syndrome has been clinically found to be promoted when the integrity of the intestinal mucosa is destroyed, causing the intestinal flora to transfer into blood and even the lungs [55, 56].

3.2. Immunomodulation of Lung and Gut Microbiome. Studies have shown that certain lung and intestinal flora can affect the body's immune system. For example, segmented filamentous bacteria in the gut can stimulate the body to produce Th17 immune cells, thus reducing the infection rate and mortality rate of *S. pneumoniae* [57, 58]. In mice, gut inoculation with *Lactobacillus johnsonii* can significantly reduce the inflammatory response of Th2 in the lungs [59]. In addition, when intestinal or lung flora disorders occur in the body, immune cells, such as ILC2s, can migrate through blood in the lungs and intestines, releasing excessive inflammatory media and thus affecting the microecological environment of the lungs and the type and intensity of the immune response.

3.3. Gut Microbiota Metabolites and Respiratory Diseases. Certain components or metabolites of gut microbiota, such as short chain fatty acids (SCFAs) [60, 61], lipopolysaccharide (LPS), and peptide peptidoglycans, also play an important role in the body when it is in a diseased or healthy state [17, 62, 63]. Studies on SCFA functions are the most detailed. SCFAs in the intestinal lumen provide energy to colon cells and regulate immune response in the intestine to maintain the stability of the intestinal microecology. In addition, SCFAs can activate downstream effect molecules (e.g., MAPK, PI3K, and NLRP3) by binding to G protein-coupled receptors (e.g., GRP43, FFA2, and HCA2) on cell membranes, thus changing dendritic cells (DCs) and auxiliary T cells [64], which can also enter the cell via the transporters SLC5A8 or SLC16A1 [65, 66], inhibit the activity of histone deacetylase, and increase the number of Ly6c− monocytes in the bone marrow and lungs, thereby reducing the production of neutrophils and improving allergic inflammation in the lungs. In addition to SCFAs, metabolites produced by intestinal flora, such as desaminotyrosine, indole derivatives, niacin, polyamine, urolithin A, pyruvate, and lactic acid, have anti-inflammatory and antiinfection activities. For example, indoles and indoles' derivatives can inhibit central nervous system inflammation by activating aryl hydrocarbon receptor signaling in astrocytes and regulate intestinal ecosystem function, thus playing anti-inflammatory and antioxidant roles [67, 68].

4. Probiotics and Fecal Microbiota Transplantation for Treatment of Respiratory Diseases

Given that intestinal flora play an important role in the human body, attempts have been made to treat diseases with complementary probiotics (mainly composed of *Bifidobacteria* and *Lactobacilli*) [23–25] or fecal microbiota transplantation [48, 49].

It was reported previously that 6 h after FMT, the pulmonary bacterial counts as well as TNF-α and IL-10 levels

were remarkably normalized in microbiota-depleted mice, indicating the protection of gut microbiota against pneumococcal pneumonia [18]. Similarly, FMT downregulated the activity of the TLR4/NF-kB signaling pathway and relieved oxidative stress in animals with acute lung injury by restoring the gut microecology [69]. They can not only treat all kinds of intestinal diseases caused by intestinal flora disorders but also have a positive effect on the prevention and treatment of infectious diseases. In particular, the clinical treatment guidelines made by the United States, China, and other countries for the prevention of COVID-19 pneumonia have clearly proposed that intestinal microecological regulators can be used to maintain intestinal microbiota hemostasis and prevent secondary lung infection [70, 71]. However, a certain risk for pathogenic bacterial contamination, which can increase the occurrence of immune-related adverse events, may exist regardless of the use of flora regulation agents or flora transplantation. Therefore, in clinical practice, we should pay attention to the safety and quality control of microflora regulation agents or flora transplantation and prevent and reduce the occurrence of adverse events as much as possible while enhancing efficacy.

5. The Immunomodulation of Traditional Chinese Medicine on Lung Dysbiosis

The theory of the exterior-interior relationship between the lung and large intestine is an important part of the Tibetan elephant theory in traditional Chinese medicine. As early as 3000 years ago, the classic Huangdi Neijing of traditional Chinese medicine recorded the physiological and pathological relationship between the lungs and the large intestine in detail. Xuanbai Chengqi decoction, Gegen Qinlian decoction, and other tonic Chinese medicines, such as Ginseng Radix et Rhizoma, Gardeniae Fructus Praeparatus, Angelicae Sinensis Radix, and Astragali Radix, can improve LPS-induced acute lung tissue damage and pathological colon tissue damage by adjusting the lung-gut mucosal immune function and are thus candidate drugs in innovative drug development based on the concept of treating the lung and intestine together [72–74].

However, the current research on the mechanism of traditional Chinese medicine has mainly focused on the changes in the expression levels of secreted IgA and cytokines and the number of immune cells, such as T lymphocytes. In-depth studies on airway/intestinal mucus secretion, changes in immune cell function in mucosal systems, and changes in the local microecological components of the lung-gut axis are lacking.

6. Future Challenges and Prospects

With the further development of microbiome research, people have increasingly realized the important role of lung and gut microecology in the body, and the mechanism behind the lung-gut axis has been gradually uncovered in many clinical phenomena and experimental data. However, due to the differences in the sources of clinical trial samples, the consistency and repeatability of the results are poor. Given the lack of longitudinal or intrusive research on the microbiome, the study of the specific mechanisms and pathways of the gut-lung axis remains difficult, and oral probiotic administration, flora transplantation, or antibiotic prevention and treatment still need further verification. In the future, with the updating of sample-handling methods, advances in biotechnology, and increased interpretation of sequencing results, this area could lead to revolutionary advances in the prevention and treatment of lung diseases and provide new ideas and therapeutic targets for the clinical treatment of related diseases.

Data Availability

No data were used to support this study.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Acknowledgments

This work was supported by the National Natural Science Foundation of China (81603368 and 81673583), Natural Science Foundation of Zhejiang Province (LY19H280012), and Zhejiang Medical Science and Technology Project (2020KY527).

References

[1] D. H. Parks, C. Rinke, M. Chuvochina et al., "Recovery of nearly 8,000 metagenome-assembled genomes substantially expands the tree of life," *Nature Microbiology*, vol. 2, no. 11, pp. 1533–1542, 2017.

[2] G. B. Huffnagle, R. P. Dickson, and N. W. Lukacs, "The respiratory tract microbiome and lung inflammation: a two-way street % J Mucosal Immunology," *The official publication of the Society for Mucosal Immunology*, vol. 10, no. 2, 2017.

[3] R. P. Dickson, J. R. Erb-Downward, N. R. Falkowski, E. M. Hunter, S. L. Ashley, and G. B. Huffnagle, "The lung microbiota of healthy mice are highly variable, cluster by environment, and reflect variation in baseline lung innate immunity," *American Journal of Respiratory and Critical Care Medicine*, vol. 198, no. 4, pp. 497–508, 2018.

[4] L. N. Segal, J. C. Clemente, J.-C. J. Tsay et al., "Enrichment of the lung microbiome with oral taxa is associated with lung inflammation of a Th17 phenotype," *Nature microbiology*, vol. 1, no. 5, Article ID 16031, 2016.

[5] D. N. O'Dwyer, X. Zhou, C. A. Wilke et al., "Lung dysbiosis, inflammation, and injury in hematopoietic cell transplantation," *American Journal of Respiratory and Critical Care Medicine*, vol. 198, no. 10, pp. 1312–1321, 2018.

[6] J. Wang, M. Lesko, M. H. Badri et al., "Lung microbiome and host immune tone in subjects with idiopathic pulmonary fibrosis treated with inhaled interferon-γ," *ERJ open research*, vol. 3, no. 3, 2017.

[7] B. J. McVerry and A. Morris, "The lung microbiome in hematopoietic stem cell transplant. Where the money lies," *American Journal of Respiratory and Critical Care Medicine*, vol. 198, no. 10, pp. 1249–1251, 2018.

[8] D. N. O'Dwyer, S. L. Ashley, S. J. Gurczynski et al., "Lung microbiota contribute to pulmonary inflammation and

disease progression in pulmonary fibrosis," *American Journal of Respiratory and Critical Care Medicine*, vol. 199, no. 9, pp. 1127–1138, 2019.

[9] A. Morris, J. M. Beck, P. D. Schloss et al., "Comparison of the respiratory microbiome in healthy nonsmokers and smokers," *American Journal of Respiratory and Critical Care Medicine*, vol. 187, no. 10, pp. 1067–1075, 2013.

[10] C. M. Bassis, J. R. Erb-Downward, R. P. Dickson et al., "Analysis of the upper respiratory tract microbiotas as the source of the lung and gastric microbiotas in healthy individuals," *mBio*, vol. 6, no. 2, Article ID e00037, 2015.

[11] R. P. Dickson, J. R. Erb-Downward, F. J. Martinez, and G. B. Huffnagle, "The microbiome and the respiratory tract," *Annual Review of Physiology*, vol. 78, no. 1, pp. 481–504, 2016.

[12] P. B. Eckburg, E. M. Bik, C. N. Bernstein et al., "Diversity of the human intestinal microbial flora," *Science*, vol. 308, no. 5728, pp. 1635–1638, 2005.

[13] J. Qin, R. Li, R. Li et al., "A human gut microbial gene catalogue established by metagenomic sequencing," *Nature*, vol. 464, no. 7285, pp. 59–65, 2010.

[14] A. Zhernakova, A. Kurilshikov, M. J. Bonder et al., "Population-based metagenomics analysis reveals markers for gut microbiome composition and diversity," *Science*, vol. 352, no. 6285, pp. 565–569, 2016.

[15] T. T. Rasmussen, L. P. Kirkeby, K. Poulsen, J. Reinholdt, and M. Kilian, "Resident aerobic microbiota of the adult human nasal cavityNote," *Apmis*, vol. 108, no. 10, pp. 663–675, 2000.

[16] T. Ichinohe, I. K. Pang, Y. Kumamoto et al., "Microbiota regulates immune defense against respiratory tract influenza A virus infection," *Proceedings of the National Academy of Sciences*, vol. 108, no. 13, pp. 5354–5359, 2011.

[17] L. W. Chen, P. H. Chen, and C. M. Hsu, "Commensal microflora contribute to host defense against *Escherichia coli* pneumonia through Toll-like receptors," *Shock*, vol. 36, no. 1, pp. 67–75, 2011.

[18] T. J. Schuijt, J. M. Lankelma, B. P. Scicluna et al., "The gut microbiota plays a protective role in the host defence against pneumococcal pneumonia," *Gut*, vol. 65, no. 4, pp. 575–583, 2016.

[19] M. C. Noverr, N. R. Falkowski, R. A. McDonald, A. N. McKenzie, and G. B. Huffnagle, "Development of allergic airway disease in mice following antibiotic therapy and fungal microbiota increase: role of host genetics, antigen, and interleukin-13," *Infection and Immunity*, vol. 73, no. 1, pp. 30–38, 2005.

[20] T. R. Abrahamsson, H. E. Jakobsson, A. F. Andersson, B. Björkstén, L. Engstrand, and M. C. Jenmalm, "Low gut microbiota diversity in early infancy precedes asthma at school age," *Clinical and Experimental Allergy*, vol. 44, no. 6, pp. 842–850, 2014.

[21] E. J. Feuille and A. Nowak-Węgrzyn, "Reduced diversity of the intestinal microbiota during infancy is associated with increased risk of allergic disease at school age," *The Journal of Pediatrics*, vol. 130, no. S1, 2012.

[22] S. L. Russell, M. J. Gold, B. P. Willing, L. Thorson, K. M. McNagny, and B. B. Finlay, "Perinatal antibiotic treatment affects murine microbiota, immune responses and allergic asthma," *Gut Microbes*, vol. 4, no. 2, pp. 158–164, 2013.

[23] M. Reale, P. Boscolo, V. Bellante et al., "Daily intake of Lactobacillus casei Shirota increases natural killer cell activity in smokers," *British Journal of Nutrition*, vol. 108, no. 2, pp. 308–314, 2012.

[24] V. Kim, J. v. Bergenhenegouwen, J. Garssen, G. F. G. Bezemer, A. D. Kraneveld, and F. Gert, "Treatment with specific prebiotics or probiotics prevents the development of lung emphysema in a mouse model of COPD," *J European Journal of Pharmacology*, vol. 668, 2011.

[25] E. Mortaz, I. M. Adcock, F. L. M. Ricciardolo et al., "Anti-inflammatory effects of Lactobacillus rahmnosus and bifidobacterium breve on cigarette smoke activated human macrophages," *PLoS One*, vol. 10, no. 8, Article ID e0136455, 2015.

[26] S. L. Russell, M. J. Gold, M. Hartmann et al., "Early life antibiotic-driven changes in microbiota enhance susceptibility to allergic asthma," *EMBO Reports*, vol. 13, no. 5, pp. 440–447, 2012.

[27] S. L. Russell, M. J. Gold, L. A. Reynolds et al., "Perinatal antibiotic-induced shifts in gut microbiota have differential effects on inflammatory lung diseases," *The Journal of Allergy and Clinical Immunology*, vol. 135, no. 1, pp. 100–109, 2015.

[28] M. C. Arrieta, L. T. Stiemsma, P. A. Dimitriu et al., "Early infancy microbial and metabolic alterations affect risk of childhood asthma," *Science Translational Medicine*, vol. 7, no. 307, p. 307ra152, 2015.

[29] M. Hilty, C. Burke, H. Pedro et al., "Disordered microbial communities in asthmatic airways," *PLoS One*, vol. 5, no. 1, Article ID e8578, 2010.

[30] L. N. Segal, A. V. Alekseyenko, J. C. Clemente et al., "Enrichment of lung microbiome with supraglottic taxa is associated with increased pulmonary inflammation," *Microbiome*, vol. 1, no. 1, p. 19, 2013.

[31] A. Venkataraman, C. M. Bassis, J. M. Beck et al., "Application of a neutral community model to assess structuring of the human lung microbiome," *mBio*, vol. 6, no. 1, 2015.

[32] Z. Wang, B. Maschera, S. Lea et al., "Airway host-microbiome interactions in chronic obstructive pulmonary disease," *Respiratory Research*, vol. 20, no. 1, Article ID 113, 2019.

[33] A. A. Pragman, K. H. Bum, C. S. Reilly, W. Christine, and R. E. Isaacson, "The lung microbiome in moderate and severe chronic obstructive," *Pulmonary Disease*, vol. 7, no. 10, pp. S77–S78, 2012.

[34] K. Tomoda, K. Kubo, K. Dairiki et al., "Whey peptide-based enteral diet attenuated elastase-induced emphysema with increase in short chain fatty acids in mice," *BMC Pulmonary Medicine*, vol. 15, no. 1, p. 64, 2015.

[35] M. Vasakova, I. Striz, A. Slavcev, S. Jandova, L. Kolesar, and J. Sulc, "Th1/Th2 cytokine gene polymorphisms in patients with idiopathic pulmonary fibrosis," *Tissue Antigens*, vol. 67, no. 3, pp. 229–232, 2010.

[36] T. A. Wynn, "Integrating mechanisms of pulmonary fibrosis," *Journal of Experimental Medicine*, vol. 208, no. 7, pp. 1339–1350, 2011.

[37] A. T. Essilfie, J. L. Simpson, M. L. Dunkley et al., "CombinedHaemophilus influenzaerespiratory infection and allergic airways disease drives chronic infection and features of neutrophilic asthma," *Thorax*, vol. 67, no. 7, pp. 588–599, 2012.

[38] A.-T. Essilfie, J. C. Horvat, R. Y. Kim et al., "Macrolide therapy suppresses key features of experimental steroid-sensitive and steroid-insensitive asthma," *Thorax*, vol. 70, no. 5, pp. 458–467, 2015.

[39] R. Y. Kim, J. W. Pinkerton, A. T. Essilfie et al., "Role for NLRP3 inflammasome-mediated, IL-1β-dependent responses in severe, steroid-resistant asthma," *American Journal of Respiratory and Critical Care Medicine*, vol. 196, no. 3, pp. 283–297, 2017.

[40] L. G. Wood, J. L. Simpson, P. M. Hansbro, and P. G. Gibson, "Potentially pathogenic bacteria cultured from the sputum of

stable asthmatics are associated with increased 8-isoprostane and airway neutrophilia," *Free Radical Research*, vol. 44, no. 2, pp. 146–154, 2010.

[41] K. M. Kloepfer, W. M. Lee, T. E. Pappas et al., "Detection of pathogenic bacteria during rhinovirus infection is associated with increased respiratory symptoms and asthma exacerbations," *The Journal of Allergy and Clinical Immunology*, vol. 133, no. 5, pp. 1301–1307, 2014.

[42] D. Agarwal, D. Dhotre, R. Patil, Y. Shouche, S. Juvekar, and S. Salvi, "Potential of health and demographic surveillance system in asthma and chronic obstructive pulmonary disease microbiome research," *Frontiers in public health*, vol. 5, Article ID 196, 2017.

[43] S. Verschuere, K. R. Bracke, T. Demoor et al., "Cigarette smoking alters epithelial apoptosis and immune composition in murine GALT," *Laboratory Investigation*, vol. 91, no. 7, pp. 1056–1067, 2011.

[44] L. Allais, F.-M. Kerckhof, S. Verschuere et al., "Chronic cigarette smoke exposure induces microbial and inflammatory shifts and mucin changes in the murine gut," *Environmental Microbiology*, vol. 18, no. 5, pp. 1352–1363, 2016.

[45] S. Quercia, M. Candela, C. Giuliani et al., "From lifetime to evolution: timescales of human gut microbiota adaptation," *Frontiers in Microbiology*, vol. 5, Article ID 587, 2014.

[46] S. H. Chotirmall, S. L. Gellatly, K. F. Budden et al., "Microbiomes in respiratory health and disease: an Asia-Pacific perspective," *Respirology*, vol. 22, no. 2, pp. 240–250, 2017.

[47] J. C. Young, C. Chehoud, K. Bittinger et al., "Viral metagenomics reveal blooms of anelloviruses in the respiratory tract of lung transplant recipients," *American Journal of Transplantation*, vol. 15, no. 1, pp. 200–209, 2015.

[48] Y. Umesaki, Y. Okada, S. Matsumoto, A. Imaoka, and H. Setoyama, "Segmented filamentous bacteria are indigenous intestinal bacteria that activate intraepithelial lymphocytes and induce MHC class II molecules and fucosyl asialo GM1 glycolipids on the small intestinal epithelial cells in the ex-germ-free mouse," *Microbiology and Immunology*, vol. 39, no. 8, pp. 555–562, 1995.

[49] I. Ekmekciu, E. von Klitzing, U. Fiebiger et al., "Immune responses to broad-spectrum antibiotic treatment and fecal microbiota transplantation in mice," *Frontiers in Immunology*, vol. 8, Article ID 397, 2017.

[50] J. Durack, N. E. Kimes, D. L. Lin et al., "Delayed gut microbiota development in high-risk for asthma infants is temporarily modifiable by Lactobacillus supplementation," *Nature Communications*, vol. 9, no. 1, Article ID 707, 2018.

[51] L. Wang, X. Zhang, G. Wu et al., "Streptococcus pneumoniae aminopeptidase N contributes to bacterial virulence and elicits a strong innate immune response through MAPK and PI3K/AKT signaling," *Journal of Microbiology*, vol. 58, no. 4, pp. 330–339, 2020.

[52] S. Yang, Y. Yin, W. Xu et al., "Type I interferon induced by DNA of nontypeable Haemophilus influenza modulates inflammatory cytokine profile to promote susceptibility to this bacterium," *International Immunopharmacology*, vol. 74, Article ID 105710, 2019.

[53] H. Shen, H. Yoshida, F. Yan et al., "Synergistic induction of MUC5AC mucin by nontypeable Haemophilus influenzae and Streptococcus pneumoniae," *Biochemical and Biophysical Research Communications*, vol. 365, no. 4, pp. 795–800, 2008.

[54] A. L. Raftery, E. Tsantikos, N. L. Harris, and M. L. Hibbs, "Links between inflammatory bowel disease and chronic obstructive pulmonary disease," *Frontiers in Immunology*, vol. 11, Article ID 2144, 2020.

[55] W. H. W. Tang, D. Y. Li, and S. L. Hazen, "Dietary metabolism, the gut microbiome, and heart failure," *Nature Reviews Cardiology*, vol. 16, no. 3, pp. 137–154, 2019.

[56] T. Peschel, M. Schönauer, H. Thiele, S. Anker, G. Schuler, and J. Niebauer, "Invasive assessment of bacterial endotoxin and inflammatory cytokines in patients with acute heart failure," *European Journal of Heart Failure*, vol. 5, no. 5, pp. 609–614, 2003.

[57] I. I. Ivanov, K. Atarashi, N. Manel et al., "Induction of intestinal Th17 cells by segmented filamentous bacteria," *Cell*, vol. 139, no. 3, pp. 485–498, 2009.

[58] S. Gauguet, S. D'Ortona, K. Ahnger-Pier et al., "Intestinal microbiota of mice influences resistance to *Staphylococcus aureus* pneumonia," *Infection and Immunity*, vol. 83, no. 10, pp. 4003–4014, 2015.

[59] K. E. Fujimura, T. Demoor, M. Rauch et al., "House dust exposure mediates gut microbiome Lactobacillus enrichment and airway immune defense against allergens and virus infection," *Proceedings of the National Academy of Sciences*, vol. 111, no. 2, pp. 805–810, 2014.

[60] A. Trompette, E. S. Gollwitzer, K. Yadava et al., "Gut microbiota metabolism of dietary fiber influences allergic airway disease and hematopoiesis," *Nature Medicine*, vol. 20, no. 2, pp. 159–166, 2014.

[61] A. N. Thorburn, C. I. McKenzie, S. Shen et al., "Evidence that asthma is a developmental origin disease influenced by maternal diet and bacterial metabolites," *Nature Communications*, vol. 6, no. 1, Article ID 7320, 2015.

[62] C. T. Fagundes, F. A. Amaral, A. T. Vieira et al., "Transient TLR activation restores inflammatory response and ability to control pulmonary bacterial infection in germfree mice," *Journal of immunology (Baltimore)*, vol. 188, no. 3, pp. 1411–1420, 2012.

[63] T. B. Clarke, "Early innate immunity to bacterial infection in the lung is regulated systemically by the commensal microbiota via nod-like receptor ligands," *Infection and Immunity*, vol. 82, no. 11, pp. 4596–4606, 2014.

[64] K. Hussain, D. P. Letley, A. B. Greenaway et al., "Helicobacter pylori-mediated protection from allergy is associated with IL-10-secreting peripheral blood regulatory T cells," *Frontiers in Immunology*, vol. 7, p. 71, 2016.

[65] M. A. Felmlee, B. L. Morse, and M. E. Morris, "γ-Hydroxybutyric acid: pharmacokinetics, pharmacodynamics, and toxicology," *The AAPS Journal*, vol. 23, no. 1, p. 22, 2021.

[66] C. Kent-Dennis and G. B. Penner, "Effects of a proinflammatory response on metabolic function of cultured, primary ruminal epithelial cells," *Journal of Dairy Science*, vol. 104, no. 1, pp. 1002–1017, 2021.

[67] V. Rothhammer, I. D. Mascanfroni, L. Bunse et al., "Type I interferons and microbial metabolites of tryptophan modulate astrocyte activity and central nervous system inflammation via the aryl hydrocarbon receptor," *Nature Medicine*, vol. 22, no. 6, pp. 586–597, 2016.

[68] P. Vernocchi, T. Gili, F. Conte et al., "Network analysis of gut microbiome and metabolome to discover microbiota-linked biomarkers in patients affected by non-small cell lung cancer," *International Journal of Molecular Sciences*, vol. 21, no. 22, 2020.

[69] J. Tang, L. Xu, Y. Zeng, and F. Gong, "Effect of gut microbiota on LPS-induced acute lung injury by regulating the TLR4/NF-kB signaling pathway," *International Immunopharmacology*, vol. 91, Article ID 107272, 2021.

[70] D. Dhar and A. Mohanty, "Gut microbiota and Covid-19-possible link and implications," *Virus Research*, vol. 285, Article ID 198018, 2020.

[71] T. Zuo, F. Zhang, G. C. Y. Lui et al., "Alterations in gut microbiota of patients with COVID-19 during time of hospitalization," *Gastroenterology*, vol. 159, no. 3, pp. 944–955, 2020, e8.

[72] S. Adesso, R. Russo, A. Quaroni, G. Autore, and S. Marzocco, "Astragalus membranaceus extract attenuates inflammation and oxidative stress in intestinal epithelial cells via NF-κB activation and Nrf2 response," *International Journal of Molecular Sciences*, vol. 19, no. 3, 2018.

[73] Y. Cui, Q. Wang, R. Sun et al., "Astragalus membranaceus (Fisch.) Bunge repairs intestinal mucosal injury induced by LPS in mice," *BMC Complementary and Alternative Medicine*, vol. 18, no. 1, Article ID 230, 2018.

[74] K. L. Su and X. J. Xiong, "Treatment strategy and thought on classical herbal formulae for coronavirus disease 2019," *China journal of Chinese materia medica*, vol. 46, no. 2, pp. 494–503, 2021.

Hindawi
Canadian Journal of Infectious Diseases and Medical Microbiology
Volume 2021, Article ID 8046368, 6 pages
https://doi.org/10.1155/2021/8046368

Research Article

The Role of Exhaled Hydrogen Sulfide in the Diagnosis of Colorectal Adenoma

Nian Liu,[1] **Yujen Tseng**,[2] **Huilu Zhang**,[2] **and Jian Chen**[2]

[1]*Sunvou Medical Electronics Company, Wuxi, China*
[2]*Department of Gastroenterology, Huashan Hospital of Fudan University, Shanghai, China*

Correspondence should be addressed to Jian Chen; chen5120@126.com

Received 10 September 2021; Revised 27 October 2021; Accepted 11 November 2021; Published 30 November 2021

Academic Editor: Meng-Hao Huang

Purpose. Exhaled determination can detect metabolite hydrogen sulfide in the intestine. We aim to analyze the predictive value of hydrogen sulfide in the diagnosis of colorectal adenoma. *Methods*. We recruited seventy patients diagnosed with colorectal adenoma as the observation group and sixty-six healthy subjects as the control group. The colorectal adenoma was diagnosed by colonoscopy at the Endoscopy Center of Huashan Hospital affiliated to Fudan University from June 2018 to November 2019. Exhaled gas was collected through the nose and mouth, respectively, and hydrogen sulfide in exhaled gas was determined according to the manufacturer's instructions. *Results*. Receiver operating characteristic (ROC) curve was analyzed based on the exhaled data of the observation group and the control group. The ROC curve showed an area under ROC curve (AUC) 0.724 for nasal exhaled H_2S, which had a diagnostic value. When nasal exhaled H_2S was >13.3 part per billion (ppb), the sensitivity and the specificity of predicting colorectal adenoma were 57% and 78%, respectively. The exhaled H_2S of the observation group was significantly different from that of the control group. The AUC value was 0.716 as a prognostic factor of colorectal adenoma. As exhaled H_2S was >28.8 ppb, the sensitivity and the specificity of predicting colorectal adenoma were 63% and 77%, respectively. *Conclusion*. Exhaled and nasal H_2S determination has a predictive value for colorectal adenoma as a novel and noninvasive method. Therefore, it is worth conducting more research to analyze exhaled and nasal H_2S.

1. Introduction

Colorectal cancer (CRC) incidence ranks the third in the world and ranks the fourth in China among malignant diseases [1, 2]. The high incidence of CRC imposes a considerable economic and humanistic burden on the society and patients. 70%–90% of CRC is developed from colorectal adenoma (CRA) [3]. The synthesis and metabolism of signaling molecule-H_2S is one of the clinical manifestations that provide a theoretical basis for CRA/CRC screening and detection in the progression from CRA to CRC. There are two main sources of H_2S in the body. H_2S in the tissue and cells is synthesized by endogenous enzymes: cystathionineγ-lyase (CSE), cystathionine-β-synthase (CBS), and 3-mercaptopyruvate sulfurtransferase (3-MST). In addition, H_2S is produced from cystine by the intestinal bacteria [4, 5]. Yamagishi et al. demonstrated that the amount of H_2S and its derivative methyl mercaptan in CRC patients is significantly higher than that of normal people in the study of digestive tract gas analysis [6]. As a gas molecule, H_2S can diffuse into the bloodstream and can be exhaled along with expiratory movements. Therefore, H_2S has the potential to become a biomarker for screening out colon tumors as a simple and noninvasive method [7].

How to screen out adenoma early and interdict it efficiently is an effective way of reducing incidence and mortality of colorectal cancer [8]. Traditional detecting methods including endoscopy, blood test, and stool DNA testing are invasive, expensive, and complicated to implement. As a result, there is an urgent need to look for a more convenient, accurate, and easy-to-carryout method for clinical practice. Gastrointestinal gas analysis is one of the optimistic methods to develop, while the gas-collecting technique is not easy in the antecedent research (anal exhaust and fecal

fermentation), and the technique of gas analysis is complex (near edge X-ray absorption fine structure (NEXAFS) spectra and spectrophotometric determination of gas after solution preparation) [6, 9]. Thus, a better gas-determination method is required. For the above reasons, our study determines concentration of nasal and exhaled H_2S by electrochemical sensors to predict the occurrence of CRA. We hope that exhaled H_2S determination could provide scientists and researchers some new insight into early screening and warning of CRC.

2. Study Design and Methodology

2.1. Study Design. 70 patients diagnosed with CRA were enrolled as the observation group and 66 healthy subjects without organic lesions were selected as the control group. The diagnosis of CRA was made via colonoscopy screening at the Endoscopy Center of Huashan Hospital Affiliated to Fudan University from June 2018 to November 2019. Inclusion criteria: (1) age 18–80 y old, normal cognitive function, and able to complete exhaled determination; (2) not take any antibiotics or probiotics within 2 weeks before enrollment. Exclusion criteria: (1) take gastrointestinal motility drugs, acid suppressants, psychotropic drugs, immunosuppressants, intestinal microecological agents, laxatives, or antidiarrheal drugs for more than 3 days within 2 weeks of enrollment; (2) have serious systemic diseases (such as abnormal liver and kidney function and abnormal heart and lung function); (3) have a history of gastrointestinal or abdominal surgery.

This study was approved by the Ethics Committee of Huashan Hospital, ethics number: (2019), Linshen No. (471). All subjects signed an informed consent form.

2.2. Exhaled H_2S Determination. The breath test instrument is the Nanocoulomb breath analyzer DA6000 (Wuxi Sunvou Medical Electronics Co., Ltd., Wuxi, China). The measured concentration of hydrogen sulfide is one part per billion (part per billion, ppb). The instrument uses the electrochemical H_2S gas sensor. The lower limit of H_2S concentration detection is 3 ppb, detection error is ±3 ppb or 10%, and detection range is 0–3000 ppb. The instrument is designed to operate in accordance with the sampling techniques in 2005 ATS/ERS [10] and the 2019 Rome Consensus [11] as well as 2017 ERS technical standards for exhaled biomarkers [12]. In order to ensure the reliability and repeatability of breath sampling, the flow rate, expiratory pressure, and duration of exhalation are set at 50 ml/s, 10 cm H_2O, and 10 s, respectively. In order to eliminate the influence of H_2S in the environment, the subject first inhales through the H_2S filter and then exhales according to the set flow rate, expiratory pressure, and duration of exhalation. Standard gas of 50 and 200 ppb H_2S/N_2 provided by the manufacturer is used for calibration before the test every day in order to ensure the accuracy of the detection.

Taking into account the impact of oral H_2S on the detection of H_2S in the digestive tract, nasal exhalation is adopted to determine H_2S concentration besides oral exhalation. Results of nasal and exhaled determination are then compared and analyzed.

The detailed operation process is as follows. (1) Subject preparation: all subjects only eat rice and meat the day before the test, must fast 12 h before the test, and avoid workout and smoking on the day of the test. (2) Operation process: gargle before the test; during exhaled determination, the subject uses a disposable filter to wrap the lips tightly. After inhaling through the filter, hold the breath for 15 s and then exhale with some force. Coordinate the exhalation rhythm through animation software. The analyzer will automatically collect the end-expiratory air. During the nasal exhalation measurement, the subject uses the disposable nasal filter to align with the single test nostril and then holds breath after 15 s natural inhalation. As you block opposite nostril with your hand, exhale with a certain strength and coordinate the exhalation rhythm through the animation software. The analyzer will automatically collect the end-expiratory gas. After the above collection process is completed, the analyzer will automatically analyze the exhaled gas and displays the result immediately.

2.3. Statistical Method. SPSS 15.0 statistical software was used for data analysis. Normally, distributed data in the measurement data are represented by the mean ± standard deviation, and nonnormally distributed data are represented by the median (interquartile range). $P < 0.05$ is considered statistically different. $P < 0.01$ is considered significantly different.

3. Result

3.1. Rank-Sum Test Result. The ratio of male and female is 39 : 31, and the average age is 61 ± 13 in the observation group. The ratio of male and female is 34 : 32, and the average age is 56 ± 14 in the control group. Two groups' baseline values are comparable, and there is no significant difference ($P > 0.05$).

The nasal exhalation indicator H_2S in 70 cases of colorectal adenoma and 66 cases of the control group were analyzed by the rank-sum test. The results showed that (1) the nasal exhaled H_2S in the observation group was significantly different from that in the control group ($P < 0.05$) (Table 1, Figure 1).

The exhaled indicator (H_2S) in 70 cases of colorectal adenoma and 66 cases of the control group was analyzed by the rank-sum test. The results showed that there was a significant difference in exhaled H_2S between the CRA group and Huashan control group ($P \leq 0.001$) (Table 1 and Figure 2).

3.2. AUC Curve Analysis. Diagnostic predictive value of exhaled H_2S in colorectal adenoma: ROC curve analysis of the nasal exhaled H_2S of the two groups showed that the AUC was 0.724, which had a diagnostic value. When the nasal exhaled H_2S >13.3 ppb, the sensitivity and specificity of predicting CRA were 57% and 78%, respectively (Figure 3). ROC curve analysis of exhaled H_2S of the two groups showed

Table 1: Results of exhaled determination in the observation group and control group.

	Observation group	Control group
Number of people (male/female)	70 (39/31)	66 (34/32)
Age	61 ± 13	56 ± 14
Nasal exhaled H_2S (ppb)	19.32 ± 15.71**	10.59 ± 4.53
Exhaled H_2S (ppb)	47.47 ± 44.95**	25.00 ± 17.94

**Statistically significant difference between groups, $P < 0.01$.

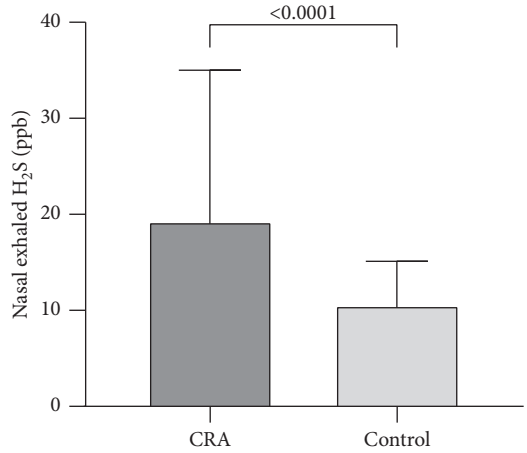


Figure 1: Nasal exhaled H_2S comparison between the observation group and control group.

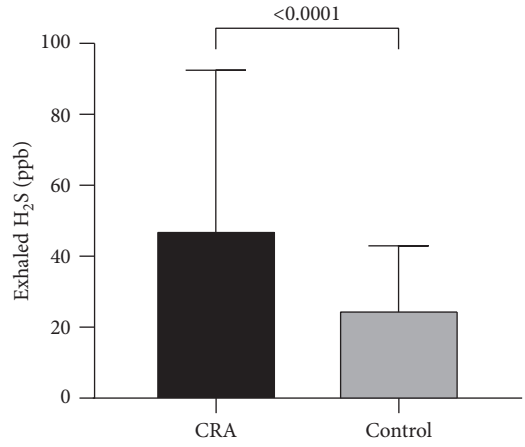


Figure 2: Exhaled H_2S comparison between two groups.

that the AUC was 0.716, which had a certain diagnostic value (Figure 3). When exhaled H_2S >28.8 ppb, the sensitivity for predicting CRA was 63% and the specificity was 77%. Above analysis suggested that exhaled determination was better than nasal exhaled determination.

4. Discussion

CRA is recognized as precancerous lesion of CRC, and CRA has common pathophysiological basis as CRC. The increase of hydrogen sulfide production is one of those typical pathophysiological characters. Although, H_2S in the intestine may play a two-way role in the occurrence and development process of CRA/CRC [13], in which the overall trend of H_2S production is rising amongst people with CRA based on current research. On the one hand, the synthesis of endogenous H_2S goes up in tumor cells. On the other hand, gut microbiota metabolism shifts to prone to H_2S production during tumor genesis.

Currently, cystathionine beta-synthase CBS, cystathionine beta-synthase CSE, and 3-MST are the three main enzymes that endogenously synthesize H_2S. These enzymes are all related to the occurrence and development of malignant tumors [13]. Some studies have found that CBS increases significantly in CRA/CRC [14–16]. According to Phillips et al. [16], the expression of CBS in intestinal adenoma epithelium was upregulated during the development of CRA, leading to increased H_2S. CBS expression was

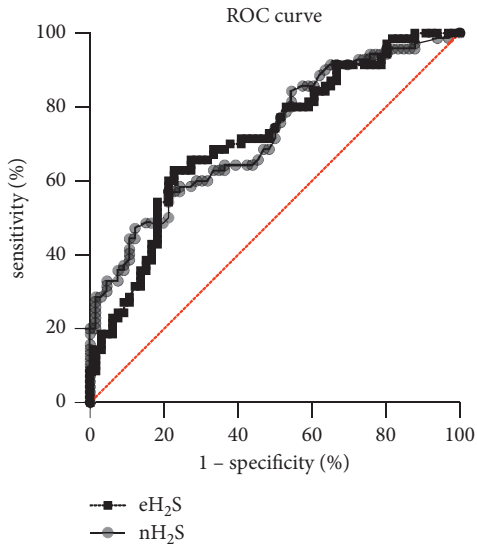


Figure 3: ROC curve of nasal exhaled H_2S (nH_2S) and oral exhaled H_2S (eH_2S).

related to the upregulation of NF-κB, K-RAS, and $p53$ signaling pathways as well. H_2S further promoted glycolysis of adenoma epithelium and production of ATP, thereby boosting the abnormal division and proliferation of adenoma epithelium and accelerating CRA development.

Besides endogenous synthesis, H_2S can derive from gut microbial metabolism [17]. Flannigan et al. [18] demonstrated that more than half H_2S was generated by intestinal microbiome. Shen et al. [19] also illustrated that the proportion of H_2S produced by intestinal microbiota accounted for the majority of H_2S produced by human body according to an experimental study on sterile mice and bacterial colonized mice. The H_2S-producing intestinal microbiome is divided into two main types. The first type of bacteria generates H_2S by metabolizing sulfur-containing amino acids (similar to the CBS pathway of tissue cells), including *Fusobacterium*, *Clostridium*, *Escherichia coli*, *Salmonella*, *Klebsiella*, *Streptococcus*, *Desulfovibrio*, and some *Enterobacter*. Another type of bacteria produces H_2S through the sulfate metabolism, mainly *Desulfovibrio* [7]. Vacante [7] discovered that Biliophilus and *Desulfovibrio* increased significantly (control = 547) in the flora of tumor tissue and peripheral intestinal epithelium among CRA patients ($n = 233$). Moreover, the increase of Biliophilus and *Desulfovibrio* were obviously related to the increase of metabolite-H_2S, which had the potential value for diagnosing CRA as a characteristic change. In the follow-up studies, the author found that the H_2S ion current test for colon cancer epithelium, peripheral epithelium, and distal epithelium showed a high-to-low change, which was statistically significant among CRC patients ($n = 106$). Meanwhile, some intestinal bacteria associated with H_2S change became different, such as *Clostridium sclerotium*, *Clostridium perfringens*, nematodes gingivalis, and *Bacteroides fragilis* [20]. These findings clearly indicated that H_2S strains derived from CRA were closely related to the progression of CRC.

After clarifying the potential value of H_2S in the diagnosis of CRA, many scholars explored the specific methods of H_2S application in CRA/CRC as well. These studies focused primarily on the detection of H_2S in human peripheral blood and feces, using methods such as methylene blue, monobromodimarane, S2-electrode ion detection, and mass spectrometry. However, methylene blue, monobromodimarane, and S2-electrode ion detection methods are susceptible to pH changes, so measured results may have large fluctuations. In addition, H_2S is relatively unstable and can be converted to methyl mercaptan and dimethyl disulphide in the body, which makes it difficult to draw high repeatable conclusions. Mass spectrometry is complicated and expensive and is rarely used. Therefore, a high-precision electrochemical sensor was adopted in this study to determine exhaled H_2S content among CRA patient population by the point-of-care test. The advantages of exhaled determination are as follows: (1) the pH and temperature in the circulating blood are relatively stable, so H_2S is less affected, and the concentration of free H_2S is relatively stable; (2) the POCT avoids H_2S being oxidized or converted to other forms; (3) holding the breath for 15 s ensures the full exchange of circulated gas molecules in the alveoli, which can truly reflect H_2S concentration; (4) the accuracy of the measurement is ppb level (ambient gas detection is usually ppm level), which is more sensitive to indicate the degree of H_2S change.

In this study, exhaled H_2S of the CRA group was significantly higher than that of the control group, which was statistically different. Both two sampling methods of oral exhalation (AUC 0.716) and nasal exhalation (AUC 0.724) obtained from the above results had good diagnostic value based on ROC analysis, confirming the accuracy of exhaled H_2S determination for CRA screening. Hampton [21] reported that the oral flora was associated with colonic bacterial colonization. For example, *Fusobacterium nucleatum* was one of the common facultative anaerobes in the oral cavity, but it was rare to be seen in the healthy people's intestine. The association between oral and colonic flora may be the key to maintaining the same trend for oral exhalation and nasal exhalation. This conclusion was in line with conclusions of previous studies [4, 6, 9], which reflected that the overall H_2S was at a high level within the CRA population. From the realm of the physiological mechanism, exhaled H_2S is derived from the total H_2S excreted from the body through the alveolar gas exchange. A portion of total H_2S is synthesized by the CRA tissue epithelium and the rest H_2S is produced by intestinal flora. Besides, change tendency of H_2S from the two sources is determined, which is conducive to the consistent judgment of the results.

In the process of exhalation, the influence of H_2S from other sites on the results mainly included the sources of upper airway (nasal cavity) [22], lower airway (lung tissue and trachea) [23], and oral cavity [24]. How to eliminate the effect is one of the key considerations. In terms of H_2S source of upper airway (nasal cavity), the median of H_2S measured by nasal ventilation was 2 ppb. Nasal H_2S further reduced in patients with seasonal allergic rhinitis compared with the nonallergic rhinitis group according to the study of Li et al.

[22]. For the H_2S source of lower airway, Zhang et al. [23, 25] demonstrated that exhaled H_2S concentration in the lower airway among some patients with either asthma or COPD increased compared with the control group. However, increase was inversely proportional to the number of eosinophils. From the perspective of inflammatory cell types and exhaled H_2S concentration, oligogranulocytic > neutrophilic > eosinophilic granulocytosis [25]. Even the more obvious inflammatory cell infiltration in the airway (regardless of nasal or tracheal origin) was, the less H_2S was produced. In other words, there was a negative correlation between the granulocytic infiltration and H_2S production. Therefore, based on the above research results, it was concluded that airway inflammation had little impact on the total exhaled H_2S volume. Oral cavity is the third part of H_2S source. Due to the presence of oral bacteria, Pysanenko et al. [24] found that H_2S measured orally was significantly higher than nasally, while Dryahina suggested that using nasal exhaled H_2S determination could better reflect the H_2S from the intestinal tract. Therefore, we added nasal exhaled H_2S determination on the basis of oral exhaled H_2S determination, which meant that the exhalation site was replaced with the nose. Nevertheless, the breath-holding time, flow rate, and end-expiratory sampling method remained unchanged in order to eliminate the influence of the oral cavity, which further verified the predictive value of exhaled H_2S on CRA. Results revealed that the nasal exhaled H_2S (mean: 19.32 ± 15.71 ppb) in the CRA group was significantly higher than that in the control group (mean: 25.00 ± 17.94 ppb). In the ideal model, the nasally exhaled sampling method that excludes the influence of the oral cavity should have a better diagnostic value. Although, in this study, the ROC of the nasal exhaled sampling was slightly higher than that of the oral exhaled sampling (nasal exhaled AUC 0.724 vs. oral exhaled AUC 0.716), there is no significance between two AUCs ($P = 0.86$).

However, study design had some disadvantages. For example, fasting exhaled H_2S was a reflection of the total H_2S in the human body. Some factors may limit the diagnostic value of fasting H_2S on CRA, such as whether H_2S derived from adenoma tissue or other human tissues was affected by other factors and whether intestinal flora metabolism was affected by food, drugs, and H_2S peaks. Banik et al. [26] used fasting H_2S as a baseline value in an IBS study. The exhaled H_2S determination was performed 45 min after oral administration of the substrate lactulose. Subtract the baseline value from the test value as $\triangle H_2S$ to determine whether IBS merges SIBO. This research method was to optimize the exhalation method under the condition of limiting the microbiota metabolism. How to choose a better preparation and sampling method for detecting CRA patients remained to be further explored in order to achieve an optimal diagnostic value.

In conclusion, CRA screening is essential for detecting early cancer of colon tumors. The study used exhaled H_2S determination that was a noninvasive, accurate, and innovative method. Exhaled H_2S determination provides a potential CRA inspection method and compensates shortcomings of traditional endoscopy and plasma laboratory testing, so it is worthy of further research and generalization.

Data Availability

The data used to support the findings of this study are currently under embargo while the research findings are commercialized. Requests for data, 12 months after publication of this article, will be considered by the corresponding author.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Authors' Contributions

Jian Chen conceptualized and designed the study. Nian Liu, Yuzhen Tseng, and Huilu Zhang involved in administrative support and provision of study materials or patients. Yuzhen Tseng and Huilu Zhang collected data. Nian Liu and Jian Chen analyzed and interpreted data. All authors reviewed and approved the final article.

References

[1] F. Bray, J. Ferlay, I. Soerjomataram, R. L. Siegel, L. A. Torre, and A. Jemal, "Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries," *CA: A Cancer Journal for Clinicians*, vol. 68, no. 6, pp. 394–424, 2018.

[2] R. S. Zheng, K. X. Sun, S. W. Zhang et al., "Report of cancer epidemiology in China, 2015," *Chinese Journal of Oncology*, vol. 41, no. 1, pp. 19–28, 2019.

[3] D. R. Rudy and M. J. Zdon, "Update on colorectal cancer," *American Family Physician*, vol. 61, no. 6, pp. 1759–1770, 2000.

[4] F. F. Guo, T. C. Yu, J. Hong, and J. Y. Fang, "Emerging roles of hydrogen sulfide in inflammatory and neoplastic colonic diseases," *Frontiers in Physiology*, vol. 7, p. 156, 2016.

[5] J. Chen, Z. B. Qiu, Z. G. Luo, H. L. Zhang, and J. Liu, "Small intestinal bacterial overgrowth and low-grade systemic inflammation in 50 patients with irritable bowel syndrome," *Chinese Journal of Digestion*, vol. 38, no. 11, pp. 769–773, 2018.

[6] K. Yamagishi, "Generation of gaseous sulfur-containing compounds in tumour tissue and suppression of gas diffusion as an antitumour treatment," *Gut*, vol. 61, no. 4, pp. 554–561, 2012.

[7] M. Vacante, R. Ciuni, F. Basile, and A. Biondi, "Gut microbiota and colorectal cancer development: a closer look to the adenoma-carcinoma sequence," *Biomedicines*, vol. 8, no. 11, 2020.

[8] F. Amersi, M. Agustin, and C. Y. Ko, "Colorectal cancer: epidemiology, risk factors, and health services," *Clinics in Colon and Rectal Surgery*, vol. 18, no. 3, pp. 133–140, 2005.

[9] J. Jorgensen and P. B. Mortensen, "Hydrogen sulfide and colonic epithelial metabolism: implications for ulcerative colitis," *Digestive Diseases and Sciences*, vol. 46, no. 8, pp. 1722–1732, 2001.

[10] American Thoracic Society and European Respiratory Society, "ATS/ERS recommendations for standardized procedures for the online and offline measurement of exhaled lower respiratory nitric oxide and nasal nitric oxide," *American Journal of*

Respiratory and Critical Care Medicine, vol. 171, no. 8, pp. 912–930, 2005.

[11] A. Gasbarrini, G. R. Corazza, G. Gasbarrini et al., "Methodology and indications of H_2-breath testing in gastrointestinal diseases: the Rome consensus conference," *Alimentary Pharmacology & Therapeutics*, vol. 29, no. Suppl 1, pp. 1–49, 2009.

[12] I. Horvath, P. J. Barnes, S. Loukides et al., "A European respiratory society technical standard: exhaled biomarkers in lung disease," *European Respiratory Journal*, vol. 49, no. 4, 2017.

[13] X. Cao, L. Ding, Z. Z. Xie, Y. Whiteman, M. Moore, and J. S. Bian, "A review of hydrogen sulfide synthesis, metabolism, and measurement: is modulation of hydrogen sulfide a novel therapeutic for cancer?" *Antioxidants and Redox Signaling*, vol. 31, no. 1, pp. 1–38, 2019.

[14] C. Szabo, C. Coletta, C. Chao et al., "Tumor-derived hydrogen sulfide, produced by cystathionine-β-synthase, stimulates bioenergetics, cell proliferation, and angiogenesis in colon cancer," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 110, no. 30, pp. 12474–12479, 2013.

[15] G. Olah, K. Modis, G. Toro, M. R. Hellmich, C. Szczesny, and BandSzabo, Role of endogenous and exogenous nitric oxide, carbon monoxide and hydrogen sulfide in HCT116 colon cancer cell proliferation," *Biochemical Pharmacology*, vol. 149, pp. 186–204, 2018.

[16] C. M. Phillips, J. R. Zatarain, M. E. Nicholls, M. E. Porter et al., Upregulation of cystathionine-β-synthase in colonic epithelia reprograms metabolism and promotes carcinogenesis," *Cancer Research*, vol. 77, no. 21, pp. 5741–5754, 2017.

[17] L. Tomasova, P. Konopelski, and M. Ufnal, "Gut bacteria and hydrogen sulfide: the new old players in circulatory system homeostasis," *Molecules*, vol. 21, no. 11, 2016.

[18] K. L. Flannigan, T. A. Agbor, J. P. Motta et al., "Proresolution effects of hydrogen sulfide during colitis are mediated through hypoxia-inducible factor-1α," *The FASEB Journal*, vol. 29, no. 4, pp. 1591–1602, 2015.

[19] X. Shen, M. Carlstrom, S. Borniquel, C. Jädert, C. Kevil, and J. O. GandLundberg, "Microbial regulation of host hydrogen sulfide bioavailability and metabolism," *Free Radical Biology & Medicine*, vol. 60, pp. 195–200, 2013.

[20] V. L. Hale, P. Jeraldo, M. Mundy et al., "Synthesis of multi-omic data and community metabolic models reveals insights into the role of hydrogen sulfide in colon cancer," *Methods*, vol. 149, pp. 59–68, 2018.

[21] T. Hampton, "How oral microbes exacerbate colorectal cancer," *Journal of the American Medical Association*, vol. 316, no. 13, p. 1349, 2016.

[22] L. Li, Y. Liu, and Q. Wang, "Levels of nasal exhaled hydrogen sulfide in the general population and allergic rhinitis patients," *Journal of Clinical Laboratory Analysis*, vol. 35, no. 3, Article ID e23678, 2021.

[23] J. Zhang, X. Wang, Y. Chen, and W. Yao, "Exhaled hydrogen sulfide predicts airway inflammation phenotype in COPD," *Respiratory Care*, vol. 60, no. 2, pp. 251–258, 2015.

[24] A. Pysanenko and P. Spanel, D. Smith, A study of sulfur-containing compounds in mouth-and nose-exhaled breath and in the oral cavity using selected ion flow tube mass spectrometry," *Journal of Breath Research*, vol. 2, no. 4, p. 46004, 2008.

[25] J. Zhang, X. Wang, Y. Chen, and W. Yao, "Correlation between levels of exhaled hydrogen sulfide and airway inflammatory phenotype in patients with chronic persistent asthma," *Respirology*, vol. 19, no. 8, pp. 1165–1169, 2014.

[26] G. D. Banik, D. Anulekha, and S. Som, "Hydrogen sulphide in exhaled breath: a potential biomarker for small intestinal bacterial overgrowth in IBS," *Journal of Breath Research*, vol. 10, no. 2, p. 26010, 2016.

Hindawi
Canadian Journal of Infectious Diseases and Medical Microbiology
Volume 2021, Article ID 3044534, 12 pages
https://doi.org/10.1155/2021/3044534

Review Article

The Intestinal Dysbiosis of Mothers with Gestational Diabetes Mellitus (GDM) and Its Impact on the Gut Microbiota of Their Newborns

Xinke Li,[1] Da Yu,[2] Yushuang Wang,[1] Huimin Yuan,[3] Xixi Ning,[1] Binqi Rui,[1] Zengjie Lei,[1] Jieli Yuan,[1] Jingyu Yan,[4] and Ming Li[1]

[1]*Dalian Medical University, Dalian, Liaoning 116044, China*
[2]*Department of Obstetrics, Dalian Women and Children's Medical Group, Dalian, China*
[3]*Department of Obstetrics and Gynecology, Suihua First Hospital, Suihua, China*
[4]*Dalian Institute of Chemical Physics, Chinese Academy of Sciences, Key Laboratory of Separation Science for Analytical Chemistry, Dalian, China*

Correspondence should be addressed to Ming Li; vivianmarat@163.com

Received 23 July 2021; Accepted 11 September 2021; Published 22 September 2021

Academic Editor: Tingtao Chen

Gestational diabetes mellitus (GDM) is defined as "diagnosed as impaired glucose tolerance for the first time during pregnancy," which can lead to adverse pregnancy outcomes and produces divergent effects on mothers and newborns. In recent years, with the continuous expansion of obese people, GDM shows an upward trend. The abundant and diverse members of the human gut microbiota exert critical roles in the maintenance of human health. Studies have shown that GDM may be associated with disordered gut microbiota in both mothers and newborns. Taking into account the potential effects on maternal and consequently neonatal health, in this review, we analyzed the available data and discussed the current knowledge about the potential relationship between GDM and intestinal dysbiosis in mothers and newborns. In addition, we also discussed the influencing factors derived from GDM mothers on the gut microbiome of their newborns, including the vertical transmission of microbiota from mothers, the alteration of milk components of GDM mothers, and using of probiotics. Hoping that new insights into the role of the gut microbiota in GDM could lead to the development of integrated strategies to prevent and treat these metabolic disorders.

1. Introduction

Gestational diabetes mellitus (GDM) is an increasing public health concern that affects approximately 5~20% of pregnancies [1]. The prevalence of GDM has continued to increase during the past few decades and is likely to see a further rise in the future. GDM affects both mother and child with short-term complications such as preeclampsia, cesarean delivery, neonatal hypoglycemia, and congenital malformation, while long-term complications included maternal T2DM and cardiovascular diseases, as well as obesity, and other metabolic diseases in the offspring [2]. Metabolic disturbance usually occurs in GDM women, including decreased insulin secretion and increased insulin resistance, which are typically related to obesity/overweight [3].

There are rich and diverse microbiota in the gut of humans, which play an important role in maintaining human health. A substantial body of evidence supports that gut microbiota plays a pivotal role in the regulation of metabolic, endocrine, and immune functions. The gut microbiota can use polysaccharides in food, and they produce short-chain fatty acids (SFCAs) by fermenting and absorbing polysaccharides. Studies in mice have shown that SFCA supplementation improves insulin sensitivity and dyslipidemia, prevents weight gain, and increases energy expenditure in

diet-induced obese mice [4]. Depletion of SCFA-producing bacterial species might therefore contribute to the increased inflammatory tone often found in patients with obesity and diabetes. Changing the quantity and quality of the gut microbiota destroys the homeostasis of the gut environment and leads to the occurrence or development of many human diseases. In recent years, people have become increasingly aware of the importance of the microbiota during pregnancy and early life, as they are closely related to reproductive health. The early colonization of the microbiota may affect the development of newborns and may cause long-term adverse consequences in the future [5]. At present, most studies have analyzed the effects and related mechanisms of GDM on mothers, but few studies on infants (especially the effect on the gut microbiota of infants). The present review analyzes the correlation between changes of the gut microbiota in mothers with GDM and their infants. In particular, we focus on the possible influencing aspects of GDM mothers on the gut microbiota in infants, including the vertical transmission of maternal microbiota, breastfeeding, and the use of probiotics. We aim to prompt the development of innovative therapeutic targets for the slowing of adverse effects of GDM by highlighting the role of the gut microbiota in GDM infants.

2. The Influencing Factors and Adverse Pregnancy Outcome of GDM

The well-documented risk factors for GDM include prepregnancy body mass index (BMI) within the range of overweight or obesity, advanced maternal age, family history of diabetes, or any form of diabetes and cigarette smoking [6]. Genetic factors are also one of the risk factors of GDM. At present, we have found some genes related to GDM, but they are very limited [7]. We are aware of only one published genome-wide association study (GWAS) of GDM to date. This was conducted among Korean women and demonstrated a potentially shared genetic basis between GDM and type 2 diabetes [8].

GDM is related to diverse adverse pregnancy outcomes for both the mother and their kids. For the mother, gestational diabetes increases the risk of obstetrical complications such as preterm delivery and dystocia. For infants, fetal macrosomia is a common adverse infant outcome in GDM, which is more likely to be large and macrosomic, and infants more easily suffer from shoulder dystocia, clavicle fracture, and brachial plexus injury at birth [9]. After birth, infants from GDM mothers are likely to develop childhood obesity, metabolic syndrome, T2DM, and impaired insulin secretion [9]. Emerging, yet suggestive data indicate that these children may be at high risk for atopic dermatitis and allergen sensitization. The clinical study found that GDM infants are more sensitive to allergens and their sensitization risk increases more than 5-fold. It is also more likely to suffer from atopic dermatitis, which increases its risk by more than 7-fold [10].

3. The Gut Microbiota of Pregnant Women with GDM

3.1. Changes of Gut Microbiota in Normal Pregnant Women. During pregnancy, the body of pregnant women undergoes weight and metabolism changes, which is accompanied by changes in the gut microbiota. Weight gain during pregnancy was positively correlated with the relative abundance of *Bacteroides*, *Escherichia coli*, and *Enterobacteriaceae* [11] and negatively correlated with the abundance of *Bifidobacterium* and *Akkermansia muciniphila* [12]. The gut microbes in early pregnancy are similar to those in nonpregnant women; however, O. Koren et al. found that intestinal microbiota changed in the early and third trimesters of pregnancy, characterized by increased diversity (β diversity) and decreased richness (α-diversity) in pregnant women [13]. Some obesity-related bacteria such as Actinobacteria and Proteobacteria Phyla were found to increase significantly in the third trimester of pregnancy. Notably, the researchers also reported a decrease in butyric acid-producing bacteria *Faecalibacterium* that have an anti-inflammatory activity in pregnant women [14]. All of these changes seem to lead to weight gain (high obesity) and insulin resistance (IR) in pregnant women, which mainly occur in the third trimester of pregnancy.

Confirming the influence of microbiota on metabolic function, Koren et al. transplanted fecal samples from early and late pregnant women into germ-free mice and found that mice with late gestational fecal samples were more likely to be obese and more likely to induce inflammation [13]. At present, the relationship between different metabolic variables of pregnancy and some specific bacteria has been found; for example, there is a negative correlation between insulin values and *Blautia*; arterial blood pressure and *Odoribacter*; and ghrelin insulin and *Prevotellaceae*. One study conducted a prospective observational and exploratory study of 41 patients with GDM and found that there was a correlation between C-reactive protein and *Sutterella*; circulating levels of insulin and *Collinsella*; and ghrelin and *Bacteroidaceae* [15]. Therefore, gut microbiota may affect the changes of some metabolic indexes during pregnancy in different ways, but the internal mechanism is still unclear and needs further study.

3.2. Changes of Gut Microbiota in Pregnant Women with GDM. Some metabolic changes in pregnancy promote the accumulation of adipose tissue in the early stage. With the advancing of gestational age, the ability to decompose fat in the body increases. In the third trimester of pregnancy, the ability of insulin to prevent fat decomposition is inhibited, which is further aggravated in women with GDM, resulting in an increase in free fatty acids in the body of pregnant women, accelerating the production of hepatic glucose and severe insulin resistance (IR). This severe IR has been found to be associated with a decline in the numbers of *Roseburia* and *Faecalibacterium prausnitzii* in the third trimester of

pregnancy in women with GDM, which are butyric acid-producing bacteria with anti-inflammatory properties [16]. In addition, studies have reported that chronic low-grade inflammation in women with gestational diabetes mediates an imbalance in tryptophan metabolism. The study found that the maternal tryptophan–kynurenine pathway was upregulated in women diagnosed with GDM compared with the control group [17]. Some specific bacteria have the ability to produce tryptophan, such as *Escherichia coli*. In the intestinal tract, it has also been clearly proved that major tryptophan metabolism pathways such as 5-hydroxytryptamine and kynurenine are directly or indirectly regulated by microbiota [18]. It is suggested that the imbalance of the array of metabolites in GDM is closely related to the microbiota.

The changes of the gut microbiota in women with GDM compared with those without GDM have been reported. Table 1 shows the specific changes in intestinal microbes in GDM mothers shown in these studies. Compared with healthy pregnant women, the gut microbial community diversity of women with GDM changed, including the decrease of α-diversity and the increase of β-diversity. In addition, GDM also showed various types of abnormal bacterial composition, including changes in phylum, genus, and species levels. At the phylum level, an increase in Firmicutes/Bacteroidetes (F/B) ratio in late pregnancy was exhibited in the GDM group when compared with non-GDM [19]. As reported, a higher F/B ratio is more likely to cause obesity and aggravate inflammation. At the genus level, some intestinal bacteria such as *Parabacteroides*, *Prevotella, Haemophilus,* and *Desulfovibrio* are more abundant in women with GDM when compared with those of healthy women in both second and third trimesters of pregnancy [16, 19–22]. Most of these are Gram-negative bacteria. Lipopolysaccharide (LPS) is a unique structure exposed to the outer membrane of the cell wall of Gram-negative bacteria and is an important endotoxin of most intestinal pathogens. It is highly immunogenic and stimulates B lymphocytes to produce specific antibodies, resulting in low-grade inflammation and IR. LPS has strong immunogenicity and can stimulate B lymphocytes to produce specific antibodies, which can contribute to low-grade inflammation and insulin resistance [23]. At the individual level, the biosynthesis and transport system of LPS have always been positively correlated with the blood glucose level of the oral glucose tolerance test (OGTT) [21]. Meanwhile, the relative abundance of SCFA-producing genera such as *Faecalibacterium*, *Ruminococcus*, *Roseburia*, *Coprococcus*, *Akkermansia*, *Phascolarctobacterium*, and *Eubacterium* in the gut of GDM women was significantly lower than that of the healthy women [2, 16, 19–21, 24, 25]. These changes are reported to be associated with elevated blood glucose levels in individuals [16, 19–21].

Therefore, changes in the intestinal microbiota during the first trimester of pregnancy may be considered as a potential diagnostic tool for GDM or may be one of the causes of GDM. However, previous studies have shown that the gut microbiota composition of women diagnosed with GDM at early pregnancy is similar when compared with those of women without GDM at the same gestational stage [26], suggesting that the imbalance of the gut microbiota may be a result of GDM. Therefore, the debate about whether intestinal microbiota is the cause or consequence of gestational diabetes is still unclear, and further research on their relationship is needed.

4. The Gut Microbiota of GDM Infants

4.1. Early Colonization of Gut Microbiota in Healthy Infants. The early colonization of intestinal bacteria in infants usually occurs at birth. In the first few days, only a few groups of alien microbes, unrelated to the source of nutrition, settled in the intestines and became more stable in the first week of life. At that time, facultative anaerobes belonging to *Enterobacteriaceae*, *Streptococcus*, *Staphylococcus*, and *Enterococcus* already existed, mainly due to the initial supply of oxygen in the gut of newborns [27]. *Escherichia coli*, *Enterococcus faecium,* and *Enterococcus faecalis* are the most represented species in the first batch of colonizers.

With the gradual increase of oxygen consumption of facultative anaerobes, there is an anoxic environment in the gut, which leads to an increase in some obligate anaerobes such as *Bifidobacterium*, *Bacteroides,* and *Clostridium* [27]. With the introduction of solid food, the colonization and diversity of bacteria in the gut have undergone continuous changes, and one of the most prominent features is the increase in the number of *Bacteroides*.

Among the gut bacteria in the early stage of healthy infants, *Bifidobacterium* is the dominant bacteria in the colonization microbiome. *Bifidobacterium* appeared on the 3rd-4th day after birth, then increased gradually, and peaked in the first year. With the increase of age, the number of *Bifidobacterium* began to decrease in the second year, other intestinal microbiota species began to expand, and the intestinal microbial community of infants became more diversified [28].

Bifidobacteria and *Lactobacilli* contribute to both natural and acquired immune responses in healthy neonates. According to the research report, there is an association between a low level of fecal *Bifidobacteria* in the early stage and a high risk of noncommunicable diseases (such as atopic diseases and obesity) in the later stage [29]. The presence of *Bifidobacteria* in the human adult intestinal microbiota is minor, indicating that *Bifidobacteria* is specific for early life [30].

4.2. Changes of Gut Microbiota in GDM Infants. A large number of convincing experimental data show that maternal metabolic disorders are closely related to the development of related metabolic diseases such as obesity in offspring [31]. Some metabolic diseases that mothers often suffer from, such as gestational diabetes, overweight, or obesity, increase the offspring's risk of metabolic disorders associated with inflammation and weight gain. The establishment of intestinal barrier function and the maturation of the immune system depend on early bacterial colonization [32]. Early colonization is the decisive factor of mucosal dynamic

Table 1: Comparison of changes in the gut microbiota between GDM women and non-GDM women.

Surveyed country	No.		GW (weeks)		Features of gut microbial community		Reference
	G+	G−	G+	G−	Increase	Decrease	
China	11	11	31.2 ± 0.5	32.7 ± 0.3	Verrucomicrobia (P) *Akkermansia* (G)	*Faecalibacterium* (G)	[20]
China	43	81	26.2 ± 1.2	25.9 ± 1.9	*Parabacteroides* (G) *Megamonas* (G) *Phascolarctobacterium* (G) *Streptococcus agalactiae* (S) *Lachnospiraceae bacterium* (S)	*Ruminiclostridium* (G) *Roseburia* (G) *Fusobacterium* (G) *Haemophilus* (G) *Clostridium* (G) *Bifidobacterium* (S) *Eubacterium siraeum* (S) *Alistipes shahii* (S)	[21]
Denmark	50	157	28.7 ± 1.4	28.4 ± 1.1	Actinobacteria (P) *Collinsella* (G) *Desulfovibrio* (G) *Blautia* (G) *Ruminococcus* (G)	*Bacteroides* (G) *Faecalibacterium* (G) *Ruminococcus* (G) *Isobaculum* (G)	[16]
Brazil	26	42	32.45 ± 7.04	28.23 ± 5.68	Firmicutes (P) *Ruminococcus* (G) *Collinsella* (G) *Lachnospiraceae* (G) *Dorea* (G)	Bacteroides (P) *Eubacterium rectale* (G)	[19]
China	74	73			*Fusobacterium* (G) *Prevotella* (G)	*Faecalibacterium* (G)	[5]
China	23	26	38.6–39.7	39.0–40.6	*Bacteroides dorei* (S)	*Alistipes putredinis* (S) *Lactobacillus casei* (S)	[25]
China	30	31	38.3 ± 0.7	38.5 ± 0.8	*Haemophilus* (G)	*Alistipes* (G) *Rikenellaceae* (G)	[22]
China	36	16	25.6 ± 1.0	25.9 ± 1.1	*Blautia* (G)	*Faecalibacterium* (G) *Phascolarctobacterium Roseburia* (G)	[2]
China	45	45	25.55 ± 1.17	25.68 ± 1.26	*Blautia* (G) *Faecalibacterium* (G)	Bacteroides (P) *Akkermansia* (G) *Odoribacter* (G) *Butyricimonas* (G)	[26]
China	31	103	24.5 ± 0.5	24.5 ± 0.5	*Holdemania* (G) *Megasphaera* (G) *Eggerthella* (G)	*Streptococcus* (G)	[24]

No., number; G+, GDM; G−, non-GDM; GW, gestational weeks; P, phylum; G, genus; S, species. The increased/decreased microbiota in GDM women when compared with non-GDM.

balance. So it is particularly important to observe the effect of GDM on gut microbiota in infants.

Some previous studies have reported significant changes in intestinal microorganisms in the offspring of mothers with GDM, including a decrease in α-diversity and changes in the relative abundance of some specific bacteria. Table 2 shows the specific changes in intestinal microbes in the offspring of mothers with GDM shown in these studies.

Ponzo et al. [33] have found that GDM infants showed a higher relative abundance of proinflammatory bacterial taxa and a lower α-diversity than infants from healthy women. Hu et al. [34] collected the first intestinal discharge from 23 newborns stratified by maternal diabetes status and found that the maternal diabetes status was significantly associated with the relative abundance of *Bacteroidetes*. Wang et al. [5] found an increase in the number of lactic acid bacteria in the meconium of newborns of mothers with GDM, indicating that some specific colonizing bacteria in the gut of infants may be affected by maternal GDM status. Su et al. [35] have found that there are differences in gut microbiota between the newborns from GDM mothers and the control group. The gut microbiota of the GDM infants showed lower α-diversity than that of the control group. At the phyla level, the abundance of Proteobacteria and Actinobacteria increased and that of Bacteroidetes decreased in the GDM group. Besides, a few unique gut microbiota belonging to the phylum of Proteobacteria, Firmicutes, and Actinobacteria were found in the neonatal fecal samples of healthy infants and were absent in GDM ones. At the genus level, the number of *Prevotella* and *Lactobacillus* decreased in newborns from GDM mothers.

Correlation analysis showed that maternal fasting blood glucose levels had a positive correlation with the relative abundance of phylum Actinobacteria and genus *Acinetobacter* but a negative correlation with the relative abundance of *Bacteroidetes* and genus *Prevotella*. Finally, the study also

Table 2: Changes of gut microbiota in the newborns of GDM mothers compared with the newborns of mothers without GDM.

Surveyed country	No.		Features of gut microbial community		Ref.
	G+	G−	Increase	Decrease	
China	24	24	*Lactobacillus iners* (S)		[5]
Italy	29	19	Actinobacteria (p) Bacteroidetes (p) *Escherichia* (G) *Parabacteroides* (G)	*Staphylococcus* (G) *Ralstonia* (G) *Lactobacillus* (G) *Enterobacteriaceae* (G)	[33]
America	5	13	Bacteroidetes (p)		[34]
China	20	14	Actinobacteria (p) Proteobacteria (p)	Bacteroidetes (p) *Prevotella* (G) *Lactobacillus* (G)	[35]

No., number; G+, GDM; G−, non-GDM; P, phylum; G, genus; S, species. The increased/decreased microbiota in the newborns of GDM mothers when compared to the newborns of mothers without GDM.

found that the total amount of bacteria in newborns differs significantly from the severity of diabetes in mothers [35]. Intestinal dysregulation not only leads to various gastrointestinal diseases, such as acute diarrhea and chronic enteritis, but also leads to the occurrence of several metabolic syndromes and neurogenic diseases, including obesity, hyperglycemia, and autism [36]. Previous studies have reported changes in the gut microbiota in children with diabetes and found a significant decrease in the number of *Lactobacillus* and *Prevotella* [37]. In addition, another study reported that gastrointestinal diseases caused by autism may be related to the absence of *Prevotella* [38]. These results are consistent with the changes in the gut microbiota in infants with GDM. It is speculated that the variation of these intestinal bacterial genera may be related to diabetes and gastrointestinal diseases, which may lead to a higher risk of these diseases in neonates with GDM than in control newborns. The results of these studies are of great significance for understanding the internal relationship of GDM with neonatal gut microbiota and thus on their future healthy development, which is worthy of in-depth study.

5. The Maternal Factors That Affect Gut Microbiota of GDM Infants

It is well known that the maternal internal environment affects the health of offspring. The intestinal microbiota of newborns is strongly affected by maternal health and pregnancy status and participates in the developmental programming of the newborns. Overweight, obesity, and allergies in children are related to maternal/newborn dysbiosis. Many prenatal and postnatal factors have been shown to affect the colonization of early intestinal microbiota in infants, such as mode of delivery and breastfeeding. GDM is the most common complication of pregnancy, which increases the risk of metabolic disorders such as obesity and diabetes in offspring. At present, there is little data on the relationship between maternal characteristics of GDM and neonatal microbiota. Next, we will break down the following points to introduce the effect of GDM mothers on infants' gut microbiota in different ways, as shown in Figure 1.

5.1. Vertical Transmission of Maternal Microbiota. The early colonized microbiota is important for the establishment and maturation of metabolic pathways. Evidence from analysis of experimental data supports that the vertical transmission of microbiota from mother to offspring is an important source of early colonization of infant gut microbiota [36]. In this context, Azad et al. collected fecal samples from 24 Canadian healthy infants at 4 months of age and found that some microbes including *Bifidobacteria, Clostridium,* and viral organisms have a different genetic diversity between mothers and infants of different individuals but have the same genetic characteristics in mothers and their babies [39]. Some animal experiments found that, compared with the control group, lower levels of *Lactobacillus* appear in the vagina of maternal mice which are exposed to stress, and the abundance of *Lactobacillus* in the gut of the mother was positively correlated with the abundance of *Lactobacillus* in the gut of offspring [33].

In another mouse study [40], the researchers fed pregnant mice normal milk or milk containing genetically tagged bacteria, then obtained the fetuses by aseptic cesarean section, and analyzed their fecal samples. The results showed that fecal samples from mothers fed milk containing genetically tagged bacteria were found to contain the same genetically tagged bacteria, which was not detected in the control mothers or children. However, despite the consensus view of vertical transmission from mother to infant, knowing the exact source of early colonizers and the modes of transmission is still a challenge. Given the potential relationship between the vertical transmission of the maternal microbiome and the gut microbiota of infants, more research on the mechanism is needed.

The gut microbiota, which is the most abundant of microbial flora in the body, may be a potential source of the transmission of mother-to-infant bacteria [41]. The gut has barrier properties, which can prevent harmful substances passing through the intestinal epithelium. During pregnancy, the intestinal permeability of mothers increases, which leads to an increase in the ability of intestinal contents to cross the intestinal epithelial barrier. The placental endothelial integrity also changes during pregnancy, which may allow the bacteria from the gut to cross the barrier into

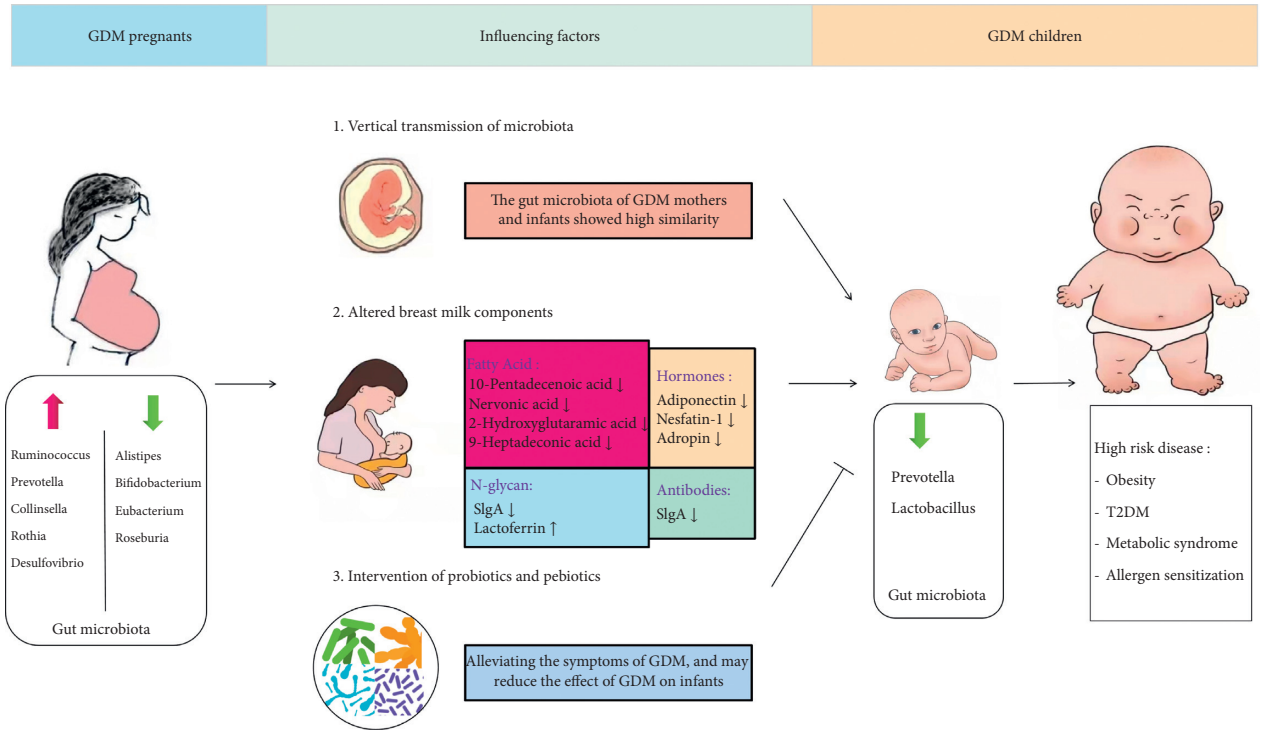


Figure 1: GDM mothers influence the gut microbiota of infants in different ways.

umbilical cord blood and amniotic fluid [42]. Therefore, it was speculated that maternal bacteria may participate in vertical transmission by crossing the placenta [43]. It has been found that antibiotic resistance genes in maternal intestinal bacteria can be detected in fecal samples of newborns. Through the study of mother-infant pairs, Ferretti et al. longitudinally sampled the microbiome of 25 mother-infant pairs across multiple body sites from birth up to 4 months postpartum in the Italy cohort and found that on the day of delivery, the proportion of the gut microbial species of the infant that were transmitted from the mother reached up to 50.7%, and this fraction was relatively stable over the next 4 months [44]. The most contribution was from the mothers' gut, accounting for 22.1% [44]. Roswall et al. studied with a larger population size of 98 mother-infant pairs and reported that 72% of the colonized microbial species in the vaginally delivered infant gut within 2–5 days after birth were shared species, such as *Bifidobacterium longum*, *Bacteroides fragilis*, and *Enterococcus faecalis* [45]. The number of other species that are not transmitted from mother to baby is very low and drops to an undetectable level after four months [45]. In addition, six species consisting of three *Bacteroides species (B. uniformis, B. vulgatus, and B. dorei*), two *Bifidobacterium species (B. adolescentis* and *B. longum*), and *E. coli* were found in pairs of mothers and infants in the Finnish cohort [46]. Microbiota from the maternal gut are more persistent over time compared to other maternal sources [45]. Although unrelated individuals often shared the same species, the number of species shared by infants and their mothers in the first three days was significantly higher than that shared with other mothers [44].

Vertical transmission of maternal microbes has been confirmed to be widespread, so it is worth exploring whether vertical transmission of maternal microbes will have an impact on newborns of GDM mothers. The clinical study of the intestinal microbiota of mothers and offspring of GDM recently revealed that there were two shared genera including *Bacteroides* and *Brucellosis* colonized in GDM mothers and their babies, suggesting that GDM offspring have maternal microbial imprints. The study also showed that abundant proinflammatory microbial groups appear in the gut of infants with GDM, such as *Escherichia coli* and *Parabacteroides,* compared with the infants of the healthy mother [33]. Another study [5] investigated the possibility of maternal and neonatal microbiota disorders associated with GDM by collecting and analyzing samples from 581 pregnant women (oral, intestinal, and vaginal) and 248 newborns (oral, pharynx, meconium, and amniotic fluid) and estimated the potential risk of microbial transfer to newborns. The study revealed that there were a large number of high abundant OTUs that vary with the same trend by counting maternal and neonatal microbiota, in which *Prevotella*, *Streptococcus*, and *Bacteroides* are the most common genus in the tested samples, reflecting the consistency of microbiological variation between mother and infant. In addition, the study calculated correlations between bacterial genera in samples from mothers and neonates with and without GDM. Notably, the proportion of the microbiota which had the same cooccurrence trend between generations reached up to 88.8%, in which 69.1% were only detected in GDM+ but not in GDM−. Despite body part-specific variations, the effects of GDM on maternal and neonatal microbiota may be similar. Animal experiments also confirm the above point of

view. Yao et al. established a GDM mouse model and explored the effect of GDM on the gut microbiota of maternal mice [47]. It was found that the *Bacteroides* and *Clostridiales_vadinBB60* were more abundant, while *Prevotella* was much lower in GDM mice than in control mice. However, most of these bacteria were found to have a common trend in GDM offspring; it was found that *Bacteroides* were more abundant, while *Lactobacillus* and *Prevotella* were less abundant in the gut of offspring fed by GDM mothers [48]. GDM can change the microbiota of pregnant women and newborns, revealing another mode of heredity. However, there are few studies on the vertical transmission of GDM maternal microbiome to newborns, and more data are needed to be analyzed.

5.2. Effect of Breast Milk of GDM Mothers on the Gut Microbiota of Their Offspring. Breast milk plays an important role in the growth and development of infants. In addition to providing the nutrients that babies need, breast milk also provides complex carbohydrates and proteins, which have a wide range of biological activities and can promote the development and maturity of the infant immune system, as well as early healthy intestinal colonization [49].

Breastfeeding may affect the composition of gut microbiota. One study found that *Bifidobacteria* and *Clostridium difficile* are more abundant in breastfed newborns, whereas *Bacteroides* and *Clostridium perfringens* prevail in formula-fed infants [50]. The difference of intestinal microbiota in infants with healthy mothers caused by different feeding methods also existed in infants with GDM. One study compared the gut microbiota of newborns of 29 GDM puerpera (10 were breastfed and 19 were formula-fed) and found that there were differences in gut microbiota between breastfed babies and formula-fed babies.

At the phylum level, breastfed infants showed more Actinobacteria and Proteobacteria, while in formula-fed infants, we observed a higher proportion of Firmicutes phyla. At the genus level, breastfed infants showed more *Escherichia* and *Bifidobacterium*, while formula-fed infants had different microbiota composed mainly of *Bacteroides*, *Clostridium*, *Enterococcaceae*, *Escherichia*, *Streptococcus*, *Staphylococcus*, and *Streptococcus*. In the multiple regression analysis, breastfeeding was significantly associated with the relative abundance of *Bifidobacterium* in the intestinal microbiota of the 11 infants ($P = 0.0017$). Remarkably, the breastfed infants had a higher number of *Bifidobacterium* compared with the formula-fed infants, which was considered to have a positive effect on babies. However, in comparison with the infants of healthy women, a higher relative abundance of proinflammatory taxa was shown in infants with GDM, such as *Escherichia* and *Parabacteroides* [33]. This may be related to the change in the composition of breast milk. Next, we will analyze it from this perspective.

5.2.1. Breast Milk Oligosaccharides and Glycans. Human Milk Oligosaccharides (HMOs) are free oligosaccharides with multiple biological functions, which are the third largest component of human milk. It is completely indigestible to newborns but can be used by some intestinal bacteria. In addition to HMOs, the glycoprotein is another large source of breast milk glycobiome. Glycoprotein is a kind of protein in which one or more sugars are connected to the peptide chain by a covalent bond. According to the connection mode, the glycans on glycoproteins are divided into N-polysaccharides and O-polysaccharides. It was found that more than 70% of human milk proteins are glycosylated, and human lactose proteins play a defensive role against infectious diseases by producing antibacterial and immunomodulatory activities of passive immunity to breastfed infants [48]. Breast milk glycobiome has been shown to selectively enrich the infant gut microbiome with beneficial bacteria [51]. These beneficial bacteria are able to quickly consume HMOs as the sole carbon source and successfully become the dominant bacteria in the gut [52]. However, some intestinal bacteria consume HMOs poorly or not at all, such as *Clostridium perfringens*, *E. faecalis,* and *Veillonella parvula* [52]. In this way, breast milk oligosaccharides help babies establish a healthy gut environment.

Due to the different abilities of intestinal microbiomes to use different types of glycans as carbon sources for growth and metabolism [48], differences in breast milk glycans may also affect the composition of intestinal microbial communities in offspring. In this context, some research groups have studied the glycobiome patterns in the breast milk of mothers with GDM, it was found that [53], compared to healthy women, the content of free oligosaccharides in the breast milk of GDM women was not different, but the total protein concentration and glycosylation level of sIgA in GDM breast milk were reduced; in contrast, the glycosylation of lactoferrin in the milk of GDM mothers was increased compared with the breast milk of healthy control mothers. They found that the content of total N-glycan of sIgA was 32–43% lower than that of normal pregnant women ($P < 0.0001$), and the content of total N-glycan of lactoferrin was 45% higher than that of normal pregnant women. These results suggested that maternal glucose regulation disorder has been happening in GDM women during pregnancy. Because breast milk glycobiome is closely related to the gut microbiota of infants, differences in milk glycans may also affect the composition of the gut microbiome in the offspring. However, there are few studies at present.

Previously, our team established a GDM mouse model and collected milk and fecal samples of GDM maternal and offspring mice to observe the changes of oligosaccharides and protein N-glycans in the milk of GDM mice and their possible effects on the gut microbiota of offspring [48]. Different from the main proportion of fucosylated milk oligosaccharides in human milk, mouse milk mostly contains sialylated milk oligosaccharides. We found that there are no significant differences in the abundance of milk oligosaccharides between the CON and GDM mice, which is consistent with the findings in human milk. However, through further analysis, we found the levels of fucosylation and sialylation of N-glycan in the milk of GDM mice were significantly higher than those of CON mice. On this basis,

we analyzed the gut microbiota of offspring mice. Our results showed that the abundance of *Bacteroides spp*. was significantly increased in the gut of offspring mice fed by GDM mothers when compared with those fed by healthy control mothers. *Bifidobacteria* and *Lactobacillus* are the major microbial genera in the gut of healthy breastfed infants. They promote the healthy growth and development of babies, and the decrease of these bacteria may indicate the poor state of newborns. On the contrary, some *Bacteroides* spp. have the strong ability to use complex polysaccharides, which promotes their growth in the gastrointestinal tract. So large amounts of fucosylated and sialylated N-glycans may provide a major carbon source for *Bacteroides,* resulting in their dominance in the intestines of newborns fed GDM. In particular, through further experiments in vitro, we found that the metabolites of *Bacteroides* could stimulate the lymphocyte, whereas they inhibit the production of Treg cells. Treg cells can inhibit the immune response of other cells and maintain the immune balance of the body [54]. Our results suggest an immune imbalance in the offspring with GDM, which may be a predisposing factor for this type of disease. However, the mechanism of action is still not clear, which is worthy of our more in-depth study.

5.2.2. Antibodies in Breast Milk. Newborns are exposed to an environment that contains a large number of viruses and bacteria when they are born. Lacking a mature immune system, newborns initially rely on antibodies transferred by their mothers. These antibodies are transmitted through the placenta and breast milk. In the placenta, the mother mainly transmits IgG, which helps to prevent neonatal infection [55, 56]. In addition, other studies have also shown that the mother transfers IgE to the fetus through the placenta, which is closely related to neonatal allergies [57]. After birth, the baby continues to gain maternal immunity through breast milk. Unlike placentally transferred IgG, BM mainly contains SIgA, which plays a leading role in neonatal mucosal immunity. The antibodies in breast milk populate the intestinal mucosal surface of newborns, providing the first line of defense for the healthy development of the intestinal system in the early stages of infants.

The antibody concentration in breast milk changes dynamically throughout the lactation period according to the needs of the baby. In colostrum, the antibody content is high, while in mature milk, the antibody concentration of breast milk decreases, replaced by an increase in carbohydrates and fat. Antibodies in breast milk are mainly synthesized by plasma cells in the breast. Recently, increasing evidence shows that a large part of antibodies in breast milk are related to antigen specificity of intestinal origin [58]. The mother selectively transfers mucosal immunity-related antibodies to the baby through breast milk, which provide a barrier against the same antigens found in the mother's environment, which newborns are most likely to encounter.

Increasing evidence shows that immunoglobulin plays a key role in the establishment and maintenance of early healthy microbiota in infants. Maternal immunoglobulin selectively wraps microorganisms in the small intestine, promotes the colonization of symbiotic bacteria, and delivers antigens to antigen-presenting cells, thus inhibiting the proliferation of pathogens. Most of the SIgA in the mucosa is considered to be nonspecific, highly cross-reactive, and widely reactive with the microbiota. Through a process called immune exclusion, SIgA captures microbes and enables the immune system to selectively sample complex bacteria to produce immunity by limiting the translocation of bacteria between mucosal epithelial cells [59]. In addition, SIgA can cause immune rejection to viruses and bacteria by promoting pathogens to gather or neutralize pathogens in the intestinal lumen [60]. Unlike IgA, IgG promotes tolerization by forming IgG-allergen complexes promoting the uptake of allergens by epithelial cells and assisting in the immune presentation of allergen [61].

In the individuals with IgA deficiency, *Enterobacter* accounted for a higher proportion of the microbiota, which is the dominant bacteria in the infant's gut [62]. Interestingly, an increase in the incidence of allergies and autoimmune diseases has been observed in patients with IgA deficiency, which may be the result of this change in microbiota [63]. Similar results have occurred in the infant from GDM women, which may be closely related to the change of antibody concentration in the breast milk of GDM mothers. It was found that the level of SIgA in the breast milk of GDM patients was significantly lower than that of healthy controls. Therefore, antibodies in breast milk are essential to promote the development and maintenance of healthy intestinal microbiota in infants [53].

5.2.3. Free Fatty Acids in Breast Milk. Free fatty acids are the main nutrients in breast milk, which are very important for the growth and development of newborns. Some studies have shown that free fatty acids in breast milk may affect early intestinal microbiota colonization in infants [64]. In one study, Heerup et al. examined the effect of selected nonesterified fatty acids, monoacylglycerols, and sphingosine on the composition of fecal microbial communities derived from infants aged 2–5 months during a 24 h anaerobic in vitro fermentation.

The results showed that the number of acid-producing bacteria such as *Lactobacillus* and *Bifidobacterium* increased significantly in the presence of a high concentration of medium-chain nonesterified fatty acids. In the mixture containing long-chain nonesterified fatty acids and sphingosine, *Bifidobacterium* was also found to increase significantly. However, the relative abundance of *Enterobacteriaceae* decreased significantly in the presence of the mixture of two lipids. It is also worth noting that oleic acid (18 : 1), the most common fatty acid in human milk, has been found to stimulate the growth of several types of *Lactobacillus* [65]. These findings suggest that the high concentration of nonesterified fatty acids in breast milk might have functional effects on the establishment of the gut microbiota in early life. In the early stages of life, the establishment of the immune system is very important for growth and development. It may be very beneficial to

promote the growth of lactic acid-producing bacteria such as *Bifidobacterium* and *Lactobacillus* and reduce the number of *Proteobacteria* in the intestinal microbiota. One study [66] compared the metabolites of colostrum, transitional milk, and mature milk between normal pregnant women ($n = 94$) and GDM women ($n = 90$). The results showed that quite a lot of free fatty acids in breast milk significantly declined in the GDM group compared to the control group. It is suggested that there is a disorder of fatty acids in the breast milk of mothers with GDM. However, the effect of disturbed fatty acids in GDM breast milk on gut microbiota in infants has not been reported and needs to be further explored.

5.2.4. Hormones in Breast Milk. Hormones in breast milk were suggested to protect infants from the short-term acceleration of adipose deposits and long-term obesity and diabetes. Some studies have assessed hormone levels in breast milk in women with GDM. Adiponectin and ghrelin concentrations were found to decrease in the breast milk of pregnant women with GDM [67]. And adiponectin was inversely associated with early infant growth in both women with GDM and healthy babies who grow up with low levels of adiponectin in the breast milk of GDM women and are more likely to be obese than healthy babies. However, with favorable controlled blood glucose, breastfeeding can help babies of women with GDM regain a healthy growth trajectory [68]. Aydin [69] evaluated the concentration of Nesfatin-1 in the breast milk of GDM rats, which is a peptide that derives from the precursor peptide nucleobindin 2. It has been found that Nesfatin-1 has an anorexia effect on rats and can make rats lose weight [70].

The authors found that the concentration of Nestitin-1 in the colostrum of rats with GDM was significantly lower than that of non-GDM rats, while the concentration of Nestitin-1 in the mature breast milk of GDM rats was lower, but the difference was not statistically significant, which might be due to the normalization of their blood glucose over time [69]. Thus, in the first week of life, offspring fed with breast milk with lower levels of Nesfatin-1 may be more likely to be hungry, so they drink more breast milk than those who are fed normal breast milk. Previous studies have shown that the concentration of plasma Nesfatin-1 in newborns is negatively correlated with the degree of hunger (calorie intake). Obese patients tend to have lower circulating Nesfatin-1 levels and higher calorie intake [71]. The same group of investigators evaluated adropin concentrations in the breast milk of GDM mothers. Adropin is a peptide hormone that is involved in the regulation of metabolic homeostasis [72].

Aydin et al. [73] found that the adrenaline concentration in the colostrum of GDM women was lower than that in non-GDM women, but the adropin level in immature milk during the transitional period (7 days after delivery) was not different between the two groups. Adrenaline deficiency has been shown to be associated with increased fat content in mice, suggesting that exposure to lower levels of adrenaline in GDM breast milk may also lead to the increased fat content in children [72]. These suggest that the level of breast milk hormone in parturient women with GDM is a disorder, which leads to an increase in the probability of obesity in infants. It is generally believed that obesity can cause disorders of the gut microbiota in infants; therefore, we speculate that the disorder of hormone levels in breast milk may affect the gut microbiota of infants. Luoto et al. reported differences in adiponectin concentrations in the maternal colostrum and in fecal *Bifidobacteria* counts at age 3 months between normal children ($n = 15$) and overweight children ($n = 15$) [74]. The authors found that higher *Bifidobacteria* was detected in normal children at the age of 3 months compared with overweight children, and the level of adiponectin in breast milk was significantly higher in mothers with normal children than in those with overweight children. These results suggest that hormones in breast milk have a more complex effect on the gut microbiota of an infant than previously anticipated. However, there is little research in this area, and more data is needed to support it.

5.3. Intervention of Probiotic and Prebiotics. At present, the microbial intervention has received widespread attention. Probiotics usually contain live, freeze-dried bacterial microbes, mainly from intestinal beneficial bacteria such as *Lactobacillus and Bifidobacterium*. When given sufficient amounts of probiotics, probiotics regulate and promote the intestinal health of the host. During pregnancy, most pregnant women use probiotics orally, and few use vaginal administration. Probiotic interventions are not live bacteria but are made up of indigestible food substances that can be broken down into HMOs and used by beneficial bacteria in the intestines, thereby promoting the expansion of these beneficial bacteria. Synbiotics combine probiotics and probiotics intervention. Synbiotics promote the survival of living microorganisms in the intestinal tract by stimulating the growth and/or metabolic activity of one or more probiotics, thus producing beneficial effects. Probiotics intervention measures were used during pregnancy, and probiotics were used to a lesser extent to improve maternal and infant outcomes.

Recently, using probiotics to prevent or treat GDM has become a hot research direction. Dolatkhah et al. enrolled 64 pregnant women with GDM into the clinical trial and randomly divided them into three groups, which were treated with probiotics capsule or placebo capsule and dietary advice for 8 weeks. They found that fasting blood glucose and insulin resistance index decreased significantly in patients treated with probiotic capsules or placebo capsules ($P < 0.05$) [75]. Luoto et al. also found that probiotic intervention reduced the risk of GDM [76]. Specific probiotic therapy may change the composition and activity of intestinal microbiota, to improve the intestinal microecological environment, repair the intestinal barrier, and enhance the intestinal ability to regulate inflammation. Recently, the gut microbiota are considered one of the keys to participate in the dynamic balance of host energy, affecting the acquisition of energy from the outside and storage in the body. In addition, it also regulates plasma endotoxin concentration and insulin sensitivity to prevent the occurrence of metabolic syndrome. Considering that the

maternal microbiota is the first inoculum to the development of the child's microbiota, GDM mothers receiving probiotic intervention during pregnancy may promote the establishment of early healthy intestinal microbiota in infants. A systematic review and meta-analysis looking at the effect of treatment of GDM on pregnancy outcomes showed that treatment significantly reduced the risks of fetal macrosomia, large-for-gestational-age births, shoulder dystocia, and gestational hypertension, as well as a tendency to reduction of perinatal/neonatal mortality and birth trauma [77]. A review of probiotics for the prevention of GDM included one study that reported lower rates of women diagnosed with GDM and lower birth weight with probiotics [78]. These results suggest that probiotics taken by GDM mothers during pregnancy can reduce the adverse pregnancy outcome and promote the healthy growth of the baby. Taking probiotics may be a good way to prevent or treat GDM, and more research on the mechanism is needed.

6. Summary

Altered gut microbial structures of the GDM mother and their offspring have been proved by many studies, and some of the changed bacteria have the same trend in the intestines of the mother and their offspring, which suggests that the mother's microbiome may be transmitted to the child, reflecting the influence of the GDM mother's gut microbiota on the colonization process of the child's gut microbiota. This provides a new direction for early prevention and treatment of GDM to reduce the incidence of adverse pregnancy outcomes in GDM. At the same time, we found that changes in the composition of GDM breast milk have a potential impact on the healthy development of babies, which may provide a theoretical basis for future studies aimed at developing specific nutritional care for children of mothers with gestational diabetes.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Authors' Contributions

Xinke Li and Da Yu contributed equally to this work.

Acknowledgments

This work was supported by the National Natural Science Foundation of China (Nos. 31900920 and 21934005) and the Nutrition and Care of Maternal & Child Research Fund Project of Guangzhou Biostime Institute of Nutrition & Care (2019BINCMCF02).

References

[1] B. E. Metzger, "International association of diabetes and pregnancy study groups recommendations on the diagnosis and classification of hyperglycemia in pregnancy," *Diabetes Care*, vol. 33, no. 3, pp. 676–682, 2010.

[2] G. Ye, L. Zhang, M. Wang et al., "The gut microbiota in women suffering from gestational diabetes mellitus with the failure of glycemic control by lifestyle modification," *Journal of Diabetes Research*, vol. 2019, Article ID 6081248, 12 pages, 2019.

[3] S. Radzicka, M. Pietryga, R. Iciek, and J. Brązert, "The role of visfatin in pathogenesis of gestational diabetes (GDM)," *Ginekologia Polska*, vol. 89, no. 9, pp. 518–521, 2018.

[4] Y. Lu, C. Fan, P. Li, Y. Lu, X. Chang, and K. Qi, "Short chain fatty acids prevent high-fat-diet-induced obesity in mice by regulating g protein-coupled receptors and gut microbiota," *Scientific Reports*, vol. 6, Article ID 37589, 2016.

[5] J. Wang, J. Zheng, W. Shi et al., "Dysbiosis of maternal and neonatal microbiota associated with gestational diabetes mellitus," *Gut*, vol. 67, no. 9, pp. 1614–1625, 2018.

[6] T. Filardi, F. Tavaglione, M. Di Stasio, V. Fazio, A. Lenzi, and S. Morano, "Impact of risk factors for gestational diabetes (GDM) on pregnancy outcomes in women with GDM," *Journal of Endocrinological Investigation*, vol. 41, no. 6, pp. 671–676, 2018.

[7] C. Zhang, S. Rawal, and Y. S. Chong, "Risk factors for gestational diabetes: is prevention possible?" *Diabetologia*, vol. 59, no. 7, pp. 1385–1390, 2016.

[8] S. H. Kwak, S.-H. Kim, Y. M. Cho et al., "A genome-wide association study of gestational diabetes mellitus in Korean women," *Diabetes*, vol. 61, no. 2, pp. 531–541, 2012.

[9] K. C. Kamana, S. Shakya, and H. Zhang, "Gestational diabetes mellitus and macrosomia: a literature review," *Annals of Nutrition and Metabolism*, vol. 66, no. 2, pp. 14–20, 2015.

[10] D. A. Ravcheev and I. Thiele, "Comparative genomic analysis of the human gut microbiome reveals a broad distribution of metabolic pathways for the degradation of host-synthetized mucin glycans and utilization of mucin-derived monosaccharides," *Frontiers in Genetics*, vol. 8, pp. 1–22, 2017.

[11] M. A. Stanislawski, D. Dabelea, B. D. Wagner, M. K. Sontag, C. A. Lozupone, and M. Eggesbø, "Pre-pregnancy weight, gestational weight gain, and the gut microbiota of mothers and their infants," *Microbiome*, vol. 5, p. 113, 2017.

[12] T. Anjos, R. M. Lopez, J. Florido, C. Campoy, Y. Sanz, and M. Martı, "Gut microbiota composition is associated with body weight, weight gain and biochemical parameters in pregnant women," *British Journal of Nutrition*, vol. 2, pp. 83–92, 2010.

[13] O. Koren, J. K. Goodrich, T. C. Cullender et al., "Host remodeling of the gut microbiome and metabolic changes during pregnancy," *Cell*, vol. 3, pp. 470–480, 2013.

[14] C. Haro, S. Garcia-Carpintero, J. F. Alcala-Diaz et al., "The gut microbial community in metabolic syndrome patients is modified by diet," *The Journal of Nutritional Biochemistry*, vol. 27, pp. 27–31, 2016.

[15] I. Ferrocino, V. Ponzo, R. Gambino et al., "Changes in the gut microbiota composition during pregnancy in patients with gestational diabetes mellitus (GDM)," *Scientific Reports*, vol. 8, Article ID 12216, 2018.

[16] M. K. W. Crusell, T. H. Hansen, T. Nielsen et al., "Gestational diabetes is associated with change in the gut microbiota composition in third trimester of pregnancy and postpartum," *Microbiome*, vol. 6, no. 1, p. 89, 2018.

[17] K. P. Law, T.-L. Han, X. Mao, and H. Zhang, "Tryptophan and purine metabolites are consistently upregulated in the urinary metabolome of patients diagnosed with gestational diabetes mellitus throughout pregnancy: a longitudinal metabolomics study of Chinese pregnant women part 2," *Clinica Chimica Acta*, vol. 468, pp. 126–139, 2017.

[18] A. Agus, J. Planchais, and H. Sokol, "Gut microbiota regulation of tryptophan metabolism in health and disease," *Cell Host & Microbe*, vol. 23, no. 6, pp. 716–724, 2018.

[19] R. V. Cortez, C. R. Taddei, L. G. Sparvoli et al., "Microbiome and its relation to gestational diabetes," *Endocrine*, vol. 64, no. 2, pp. 254–264, 2019.

[20] H. Liu, L. L. Pan, S. Lv et al., "Alterations of gut microbiota and blood lipidome in gestational diabetes mellitus with hyperlipidemia," *Frontiers in Physiology*, vol. 10, pp. 1–10, 2019.

[21] Y. S. Kuang, J. H. Lu, S. H. Li et al., "Connections between the human gut microbiome and gestational diabetes mellitus," *GigaScience*, vol. 6, pp. 1–12, 2017.

[22] X. Yajuan, Z. Miao, Z. Jingzhe et al., "Differential intestinal and oral microbiota features associated with gestational diabetes and maternal inflammation," *American Journal of Physiology. Endocrinology and Metabolism*, vol. 319, pp. E247–E253, 2020.

[23] C. V. Rosadini and J. C. Kagan, "Early innate immune responses to bacterial LPS," *Current Opinion in Immunology*, vol. 44, pp. 14–19, 2017.

[24] W. Zheng, Q. Xu, W. Huang et al., "Gestational diabetes mellitus is associated with reduced dynamics of gut microbiota during the first half of pregnancy," *mSystems*, vol. 5, pp. 1–13, 2020.

[25] Y. Wu, P. W. Bible, S. Long et al., "Metagenomic analysis reveals gestational diabetes mellitus-related microbial regulators of glucose tolerance," *Acta Diabetologica*, vol. 57, no. 5, pp. 569–581, 2020.

[26] Y. Liu, S. Qin, Y. Feng et al., "Perturbations of gut microbiota in gestational diabetes mellitus patients induce hyperglycemia in germ-free mice," *Journal of Developmental Origins of Health and Disease*, vol. 11, no. 6, pp. 580–588, 2020.

[27] A. R. Pacheco, D. Barile, M. A. Underwood, and D. A. Mills, "The impact of the milk glycobiome on the neonate gut microbiota," *Annual Review of Animal Biosciences*, vol. 3, no. 1, pp. 419–445, 2015.

[28] S. Subramanian, S. Huq, T. Yatsunenko et al., "Persistent gut microbiota immaturity in malnourished Bangladeshi children," *Nature*, vol. 510, no. 7505, pp. 417–421, 2014.

[29] M. Kalliomäki, M. C. Collado, S. Salminen, and E. Isolauri, "Early differences in fecal microbiota composition in children may predict overweight," *American Journal of Clinical Nutrition*, vol. 87, pp. 534–538, 2008.

[30] F. Turroni, A. Ribbera, E. Foroni, D. van Sinderen, and M. Ventura, "Human gut microbiota and bifidobacteria: from composition to functionality," *Antonie van Leeuwenhoek*, vol. 94, no. 1, pp. 35–50, 2008.

[31] J. E. Friedman, "Developmental programming of obesity and diabetes in mouse, monkey, and man in 2018: where are we headed?" *Diabetes*, vol. 67, no. 11, pp. 2137–2151, 2018.

[32] P. A. M. J. Scholtens, R. Oozeer, R. Martin, K. B. Amor, and J. Knol, "The early settlers: intestinal microbiology in early life," *Annual Review of Food Science and Technology*, vol. 3, no. 1, pp. 425–447, 2012.

[33] V. Ponzo, I. F. Id, A. Zarovska et al., "The microbiota composition of the offspring of patients with gestational diabetes mellitus (GDM)," *PLoS One*, vol. 14, no. 12, Article ID e0226545, 2019.

[34] J. Hu, Y. Nomura, A. Bashir et al., "Diversified microbiota of meconium is affected by maternal diabetes status," *PLoS One*, vol. 8, no. 11, Article ID e78257, 2013.

[35] M. Su, Y. Nie, R. Shao, S. Duan, and Y. Jiang, "Diversified gut microbiota in newborns of mothers with gestational diabetes mellitus," *PLoS One*, vol. 13, no. 10, Article ID e0205695, 2018.

[36] S. M. Profiling, A. Pedrotti, and A. Tett, "Studying vertical microbiome transmission from mothers to infants by strain-level metagenomic profilin," *mSystems*, vol. 2, pp. 1–13, 2017.

[37] M. Murri, I. Leiva, J. M. Gomez-zumaquero et al., "Gut microbiota in children with type 1 diabetes differs from that in healthy children: a case-control study," *BMC Medicine*, vol. 11, no. 1, p. 46, 2013.

[38] D. Kang, J. G. Park, Z. E. Ilhan et al., "Reduced incidence of prevotella and other fermenters in intestinal microflora of autistic children," *PLoS One*, vol. 8, no. 7, Article ID e68322, 2013.

[39] M. B. Azad, T. Konya, H. Maughan et al., "Gut microbiota of healthy Canadian infants: profiles by mode of delivery and infant diet at 4 months," *Canadian Medical Association Journal*, vol. 185, no. 5, pp. 385–394, 2013.

[40] M. T. Bailey, G. R. Lubach, and C. L. Coe, "Prenatal stress alters bacterial colonization of the gut in infant monkeys," *Journal of Pediatric Gastroenterology and Nutrition*, vol. 38, no. 4, pp. 414–421, 2004.

[41] N. Jain and W. A. Walker, "Diet and host-microbial crosstalk in postnatal intestinal immune homeostasis," *Nature Reviews Gastroenterology & Hepatology*, vol. 12, pp. 14–25, 2014.

[42] K. Aagaard, J. Ma, K. M. Antony, R. Ganu, J. Petrosino, and J. Versalovic, "The placenta harbors a unique microbiome," *Science Translational Medicine*, vol. 6, Article ID 237ra65, 2014.

[43] S. Rautava, R. Luoto, S. Salminen, and E. Isolauri, "Microbial contact during pregnancy, intestinal colonization and human disease," *Nature Reviews Gastroenterology & Hepatology*, vol. 9, no. 10, pp. 565–576, 2012.

[44] P. Ferretti, E. Pasolli, A. Tett et al., "Mother-to-Infant microbial transmission from different body sites shapes the developing infant gut microbiome," *Cell Host & Microbe*, vol. 24, no. 1, pp. 133–145, 2018.

[45] J. Roswall, Y. Peng, Q. Feng, H. Jia, and P. Kovatcheva-datchary, "Dynamics and stabilization of the human gut microbiome during the first year of life," *Cell Host & Microbe*, vol. 17, pp. 690–703, 2015.

[46] M. Yassour, E. Jason, L. J. Hogstrom et al., "Strain-level analysis of mother-to-child bacterial transmission during the first few months of life," *Cell Host & Microbe*, vol. 24, no. 1, pp. 146–154, 2018.

[47] Z. Yao, Y. Long, J. Ye, P. Li, Y. Jiang, and Y. Chen, "16S rRNA gene-based analysis reveals the effects of gestational diabetes on the gut microbiota of mice during pregnancy," *Indian Journal of Microbiology*, vol. 60, pp. 239–245, 2020.

[48] J. Zhou, Y. Wang, Q. Fan, M. Li, W. Dong, and W. Li, "High levels of fucosylation and sialylation of milk N-glycans from mothers with gestational diabetes mellitus alter the offspring gut microbiome and immune balance in mice," *FASEB Journal*, vol. 34, pp. 3715–3731, 2020.

[49] P. Brandtzaeg, "The mucosal immune system and its integration with the mammary glands," *Journal of Pediatrics*, vol. 156, pp. S8–S15, 2010.

[50] I. Huërou-Luron, S. Blat, and G. Boudry, "Breast- v. formula-feeding: impacts on the digestive tract and immediate and long-term health effects," *Nutrition Research Reviews*, vol. 23, pp. 23–36, 2010.

[51] Z.-T. Yu, C. Chen, D. E. Kling et al., "The principal fucosylated oligosaccharides of human milk exhibit prebiotic properties

on cultured infant microbiota," *Glycobiology*, vol. 23, no. 2, pp. 169–177, 2013.

[52] A. Marcobal, M. Barboza, J. W. Froehlich et al., "Consumption of human milk oligosaccharides by gut-related microbes," *Journal of Agricultural and Food Chemistry*, vol. 58, no. 9, pp. 5334–5340, 2010.

[53] J. T. Smilowitz, S. M. Totten, J. Huang et al., "Human milk secretory immunoglobulin A and lactoferrin N-glycans are altered in women with gestational diabetes mellitus," *Journal of Nutrition*, vol. 143, pp. 1906–1912, 2013.

[54] J. K. Kolls and A. Lindén, "Interleukin-17 family members and inflammation," *Immunity*, vol. 21, no. 4, pp. 467–476, 2004.

[55] P. Palmeira, C. Quinello, A. L. Silveira-Lessa, C. A. Zago, and M. Carneiro-Sampaio, "IgG placental transfer in healthy and pathological pregnancies," *Clinical and Developmental Immunology*, vol. 2012, Article ID 985646, 13 pages, 2012.

[56] N. Simister, "Placental transport of immunoglobulin G," *Vaccine*, vol. 21, no. 24, pp. 3365–3369, 2003.

[57] R. Msallam, J. Balla, A. P. S. Rathore et al., "Fetal mast cells mediate postnatal allergic responses dependent on maternal IgE," *Science*, vol. 370, no. 6519, pp. 941–950, 2020.

[58] K. P. Gopalakrishna, B. R. Macadangdang, M. B. Rogers et al., "Maternal IgA protects against the development of necrotizing enterocolitis in preterm infants," *Nature Medicine*, vol. 25, no. 7, pp. 1110–1115, 2019.

[59] E. W. Rogier, A. L. Frantz, M. E. C. Bruno et al., "Secretory antibodies in breast milk promote long-term intestinal homeostasis by regulating the gut microbiota and host gene expression," *Proceedings of the National Academy of Sciences*, vol. 111, no. 8, pp. 3074–3079, 2014.

[60] K. Moor, M. Diard, M. E. Sellin et al., "High-avidity IgA protects the intestine by enchaining growing bacteria," *Nature*, vol. 544, no. 7651, pp. 498–502, 2017.

[61] E. Mosconi, A. Rekima, B. Seitz-Polski et al., "Breast milk immune complexes are potent inducers of oral tolerance in neonates and prevent asthma development," *Mucosal Immunology*, vol. 3, no. 5, pp. 461–474, 2010.

[62] J. R. Catanzaro, J. D. Strauss, A. Bielecka et al., "IgA-deficient humans exhibit gut microbiota dysbiosis despite secretion of compensatory IgM," *Scientific Reports*, vol. 9, pp. 13574–10, 2019.

[63] R. Yazdani, G. Azizi, H. Abolhassani, and A. Aghamohammadi, "Selective IgA deficiency: epidemiology, pathogenesis, clinical phenotype, diagnosis, prognosis and management," *Scandinavian Journal of Immunology*, vol. 85, no. 1, pp. 3–12, 2017.

[64] R. G. Nejrup, M. I. Bahl, L. K. Vigsnæs, C. Heerup, T. R. Licht, and L. I. Hellgren, "Lipid hydrolysis products affect the composition of infant gut microbial communities in vitro," *British Journal of Nutrition*, vol. 114, no. 1, pp. 63–74, 2015.

[65] J. A. Muller, R. P. Ross, W. F. H. Sybesma, G. F. Fitzgerald, and C. Stanton, "Modification of the technical properties of *Lactobacillus johnsonii* NCC 533 by supplementing the growth medium with unsaturated fatty acids," *Applied and Environmental Microbiology*, vol. 77, no. 19, pp. 6889–6898, 2011.

[66] L. Wen, Y. Wu, Y. Yang et al., "Gestational diabetes mellitus changes the metabolomes of human colostrum, transition milk and mature milk," *Medical Science Monitor*, vol. 25, pp. 6128–6152, 2019.

[67] X. Yu, S. S. Rong, X. Sun et al., "Associations of breast milk adiponectin, leptin, insulin and ghrelin with maternal characteristics and early infant growth: a longitudinal study," *British Journal of Nutrition*, vol. 120, pp. 1380–1387, 2018.

[68] T. J. Whitmore, N. J. Trengove, D. F. Graham, and P. E. Hartmann, "Analysis of insulin in human breast milk in mothers with type 1 and type 2 diabetes mellitus," *International Journal of Endocrinology*, vol. 2012, Article ID 296368, 9 pages, 2012.

[69] S. Aydin, "The presence of the peptides apelin, ghrelin and nesfatin-1 in the human breast milk, and the lowering of their levels in patients with gestational diabetes mellitus," *Peptides*, vol. 31, no. 12, pp. 2236–2240, 2010.

[70] A. Stengel, M. Goebel, and Y. Taché, "Nesfatin-1: a novel inhibitory regulator of food intake and body weight," *Obesity Reviews*, vol. 12, pp. 261–271, 2011.

[71] K. Mirzaei, A. Hossein-nezhad, S. Ali et al., "Association of nesfatin-1 level with body composition, dietary intake and resting metabolic rate in obese and morbid obese subjects," *Diabetes & Metabolic Syndrome*, vol. 9, pp. 292–298, 2014.

[72] K. G. Kumar, J. Zhang, S. Gao et al., "Adropin deficiency is associated with increased adiposity and insulin resistance," *Obesity (Silver Spring)*, vol. 20, pp. 1394–1402, 2012.

[73] S. Aydin, T. Kuloglu, and S. Aydin, "Copeptin, adropin and irisin concentrations in breast milk and plasma of healthy women and those with gestational diabetes mellitus," *Peptides*, vol. 47, pp. 66–70, 2013.

[74] R. Luoto, M. Kalliomäki, K. Laitinen et al., "Initial dietary and microbiological environments deviate in normal-weight compared to overweight children at 10 years of age," *Journal of Pediatric Gastroenterology and Nutrition*, vol. 52, no. 1, pp. 90–95, 2011.

[75] N. Dolatkhah, M. Hajifaraji, F. Abbasalizadeh, N. Aghamohammadzadeh, Y. Mehrabi, and M. M. Abbasi, "Is there a value for probiotic supplements in gestational diabetes mellitus? A randomized clinical trial," *Journal of Health, Population and Nutrition*, vol. 33, 2015.

[76] R. Luoto, K. Laitinen, M. Nermes, and E. Isolauri, "Impact of maternal probiotic-supplemented dietary counselling on pregnancy outcome and prenatal and postnatal growth: a double-blind, placebo-controlled study," *British Journal of Nutrition*, vol. 103, no. 12, pp. 1792–1799, 2010.

[77] N. Poolsup, N. Suksomboon, and M. Amin, "Effect of treatment of gestational diabetes mellitus: a systematic review and meta-analysis," *PLoS One*, vol. 9, no. 3, Article ID e92485, 2014.

[78] A. L. Brantsæter, R. Myhre, M. Haugen et al., "Intake of probiotic food and risk of preeclampsia in primiparous women the Norwegian mother and child cohort study," *American Journal of Epidemiology*, vol. 174, pp. 807–815, 2011.