

## Research Article

# To Determine Pivotal Genes Driven by Methylated DNA in Obstructive Sleep Apnea Hypopnea Syndrome

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Obstructive sleep apnea syndrome (OSAHS) is a widespread respiratory dysfunction that has attracted more and more attention in recent years. Recently, a large number of studies have shown that abnormal DNA methylation epigenetically silences genes necessary for the pathogenesis of human diseases. However, the exact mechanism of abnormal DNA methylation in OSAHS is still elusive. In this study, we downloaded the OSAHS data from the GEO database. Our data for the first time revealed 520 hypermethylated genes and 889 hypomethylated genes in OSAHS. Bioinformatics analysis revealed that these abnormal methylated genes exhibited an association with the regulation of angiogenesis, apoptosis, Wnt, and ERBB2 signaling pathways. PPI network analysis displayed the interactions among these genes and validated several hub genes, such as GPM2, CCR8, TAS2R20, TAS2R4, and TAS2R5, which were related to regulating liganded Gi-activating GPCR and the transition of mitotic metaphase/anaphase. In conclusion, our study offers a new hint of understanding the molecular mechanisms in OSAHS progression and will provide OSAHS with newly generated innovative biomarkers.

## 1. Introduction

Obstructive sleep apnea hypopnea syndrome (OSAHS), a widely occurred breathing dysfunction, in recent years, has attracted a growing amount of attention [1]. OSAHS patients present constant respiratory failure when sleeping [2]. OSAHS can exacerbate the occurrence of cardiac arrhythmias, pulmonary and systemic hypertension, myocardial infarction, type 2 diabetes mellitus, cerebrovascular accidents, impaired cognition, and traffic accidents [3]. Previous reports have revealed that the five-year mortality ratio of patients in the absence of treatment approximately reaches 11-13% worldwide [4]. In the past decades, several genes had been revealed to be related to OSAHS. For example, HIF-1 $\alpha$  mRNA was significantly upregulated in the plasma of OSAHS patients, suggesting that hypoxia signaling may have a crucial role in this disease. miR-130 was related to regulating apoptosis in the pathogenesis of OSAHS by targeting the GAX gene. Nevertheless, the exact etiology of OSAHS is still elusive [5].

Several pieces of research have illustrated that epigenetic mechanisms, comprising DNA methylation, demethylation, and chromatin remodeling, exhibit association with human disease development [6-8]. DNA methylation is governed by a series of DNA methyltransferases and demethylases [9]. Numerous studies have displayed that abnormal DNA methylation epigenetically silences genes essential for human disease pathogenesis, and some markers for DNA methylation perhaps become human disease prognosis and treatment biomarkers [10]. DNA methylation profile is extremely useful for exploring the molecular mechanisms towards diseases [11]. In the past few decades, a large amount of DNA methylation data have recently been obtained from high-throughput DNA methylation platforms [12]. Some research groups use this method to identify abnormal methylation of genes associated with the disease. For instance, Xu et al. reported that MAOB and RTP4 were the methylated hub genes in prostate cancer and new biomarkers for the diagnosis and treatment of PCa [13]. As Liang et al. reported, 5 increased and 81 decreased methylated genes were identified

in colon cancer [14]. Nevertheless, the details of specified gene methylation in OSAHS are still unknown.

In our study, the *in silico* data and clinical data of OSAHS were downloaded from the Gene Expression Omnibus (GEO) database [15]. The molecular functions and mechanisms towards modulating OSAHS development were evaluated by bioinformatics analysis. The identification of prognosis-related MeDEGs was determined by protein-protein interaction (PPI) networks. Collectively, our data would offer newly produced innovational biomarkers for OSAHS.

## 2. Methods

**2.1. Data Source.** In our research, the expression profile of DNA methylation of specified genes and clinical details of OSAHS were obtained from the GSE61463 database (<https://tcga-data.nci.nih.gov/tcga/>). The microarray datasets of gene methylation consisted of 16 peripheral blood mononuclear cell (PBMC) samples from OSAHS patients and 7 PBMC samples from normal subjects.

**2.2. To Single Out Gene Driven by DNA Methylation.** R 3.4.4 software (<https://www.r-project.org/>) was applied to analyze gene expression and methylation. The R package MethylMix was executed to analyze gene expression and methylation data in view of GSE61463 data [16]. MethylMix was applied to validate the probable association existing in gene expression and DNA methylation. As previously shown, three parts of the methyl mixture analysis were used totally. In the light of GEO data, the R package limma was used to identify the DMEs. At the same time, the VennDiagram package in R software was executed to screen genes driven by DNA methylation.

**2.3. Gene Ontology Analysis.** The annotation of differentially methylated genes under the control of DNA methylation was analyzed by Gene Ontology (GO) analysis (<http://david.abcc.ncifcrf.gov/>). The Database for Annotation, Visualization, and Integrated Discovery (DAVID) (<http://david.abcc.ncifcrf.gov/>) tool was applied to acquire selected GO terms of differentially methylated genes driven by DNA methylation in view of hypergeometric distribution, followed by calculating the values as previously shown.  $FDR < 0.05$  represented the threshold.

**2.4. PPI Network Construction.** To validate PPI information, we conducted the search Tool for the Retrieval of Interacting Genes (STRING) database (version 10.5) in this part. In order to explore the interplay amid these genes, we blasted all differentially methylated genes driven by DNA methylation with the STRING database. Cytoscape software (version 3.6.1) was applied to establish PPI networks. The Molecular Complex Detection (MCODE) was executed to select modules of the PPI network with degree cutoff = 2, node score cutoff = 0.2,  $k$ -core = 2, and max. depth = 100. DAVID was used to analyze the function and pathway in all modules.

**2.5. Term and Pathway Enrichment Analyses.** The Cytoscape plug-in ClueGO was used for enriched item analysis and clas-

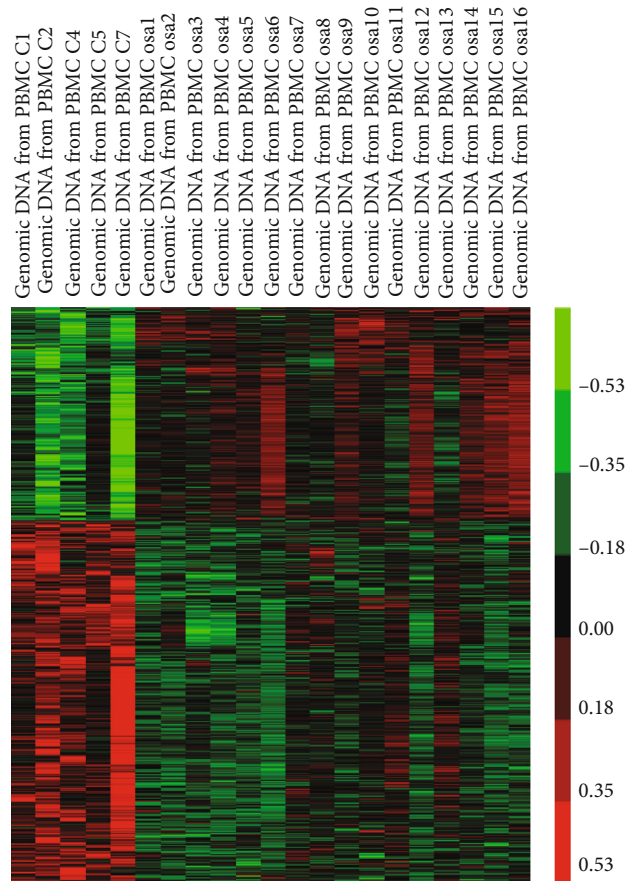


FIGURE 1: The heatmap of the DNA methylation profile in OSAHS based on GSE61463. The differences in differential methylation between tumor and normal samples were shown using a heatmap. Red indicates high methylation, and blue indicates low DNA methylation.

sification. The details in the Kyoto Encyclopedia of Genes and Genomes (<http://www.genome.jp/kegg/pathway.html>) database were combined. According to the ClueGO results, a  $\kappa$  coefficient reflecting the association between two pathways or functional terms was calculated, and its threshold was 0.4. Similarly, the same color represented analogous functional terms. The Pathview package (version 1.4.2) of R software, displaying enriched pathways, revealed the details of DEGs in a certain pathway.  $FDR < 0.05$  was thought to be significant among all selected and compared pathways.

**2.6. Establishment of Functional Annotation Maps.** For further evaluating the functions of the proteins in PPI networks in the light of their expression including upregulation, downregulation, and total DEGs, we conducted ClueGO plug-in v2.5.0 to determine the biological process (BP) terminology of protein members (14). ClueGO integrated GO terms into a PPI network and then generated a functional annotation map, representing the links between terms. The  $\kappa$  score with 0.4 showed that the GO items of related genes were similar. Additionally, KEGG pathways were applied to explore the probable pathways corresponding to related genes.  $FDR < 0.05$  showed a significant difference.

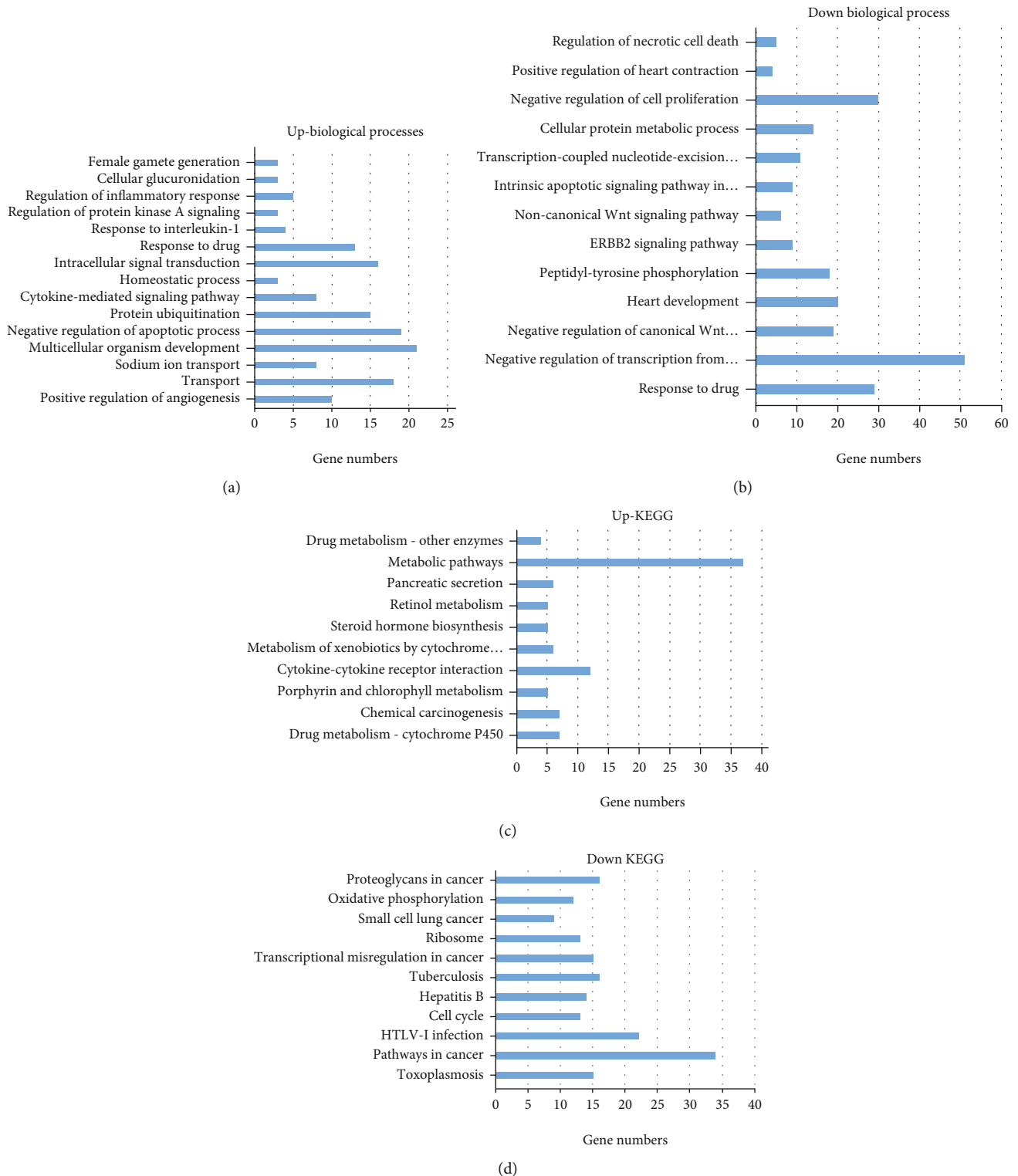


FIGURE 2: GO terms and KEGG pathway analysis of genes driven by DNA methylation. (a, b) The enriched GO terms of hypermethylated (a) and hypomethylated (b) genes. (c, d) The enriched KEGG pathways of hypermethylated (c) and hypomethylated (d) genes. The x-axis means the number of genes that fall inside the indicated GO term or KEGG pathway. The y-axis means the enriched GO terms or KEGG pathways.

### 3. Results

3.1. To Identify Genes Driven by DNA Methylation. Totally, 1409 genes were differentially methylated in 16 PBMC sam-

ples from OSAHS patients when compared to those in normal ones referring to the MethylMix standard. Figure 1 illustrates the difference in differential methylation between tumor and normal samples. Among these genes, 520 genes

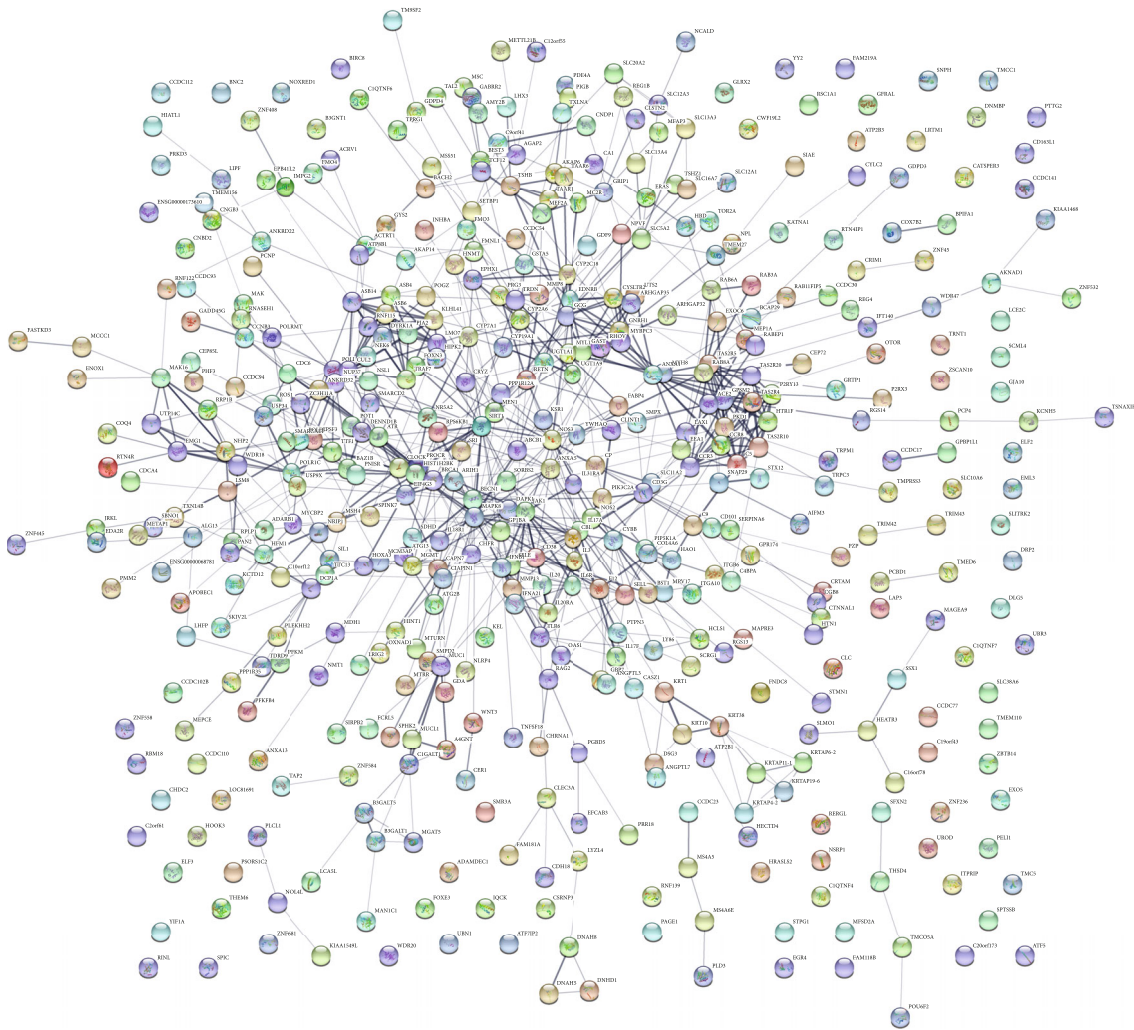


FIGURE 3: PPI network of hypermethylated genes. Each node presents a differently methylated gene.

(36.91%) were hypermethylated, while the remaining 889 genes (63.09%) were hypomethylated.

**3.2. To Analyze Gene Ontology Terms of Genes Driven by DNA Methylation.** Classification of GO terms might alter with GO enrichment. Hypermethylated genes exhibited links with positively modulated angiogenesis, transport, sodium ion transport, multicellular organism development, negatively regulated apoptotic process, protein ubiquitination, cytokine-mediated signaling pathway, homeostatic process, intracellular signal transduction, response to drug or interleukin-1, protein kinase A signaling regulation, inflammatory response regulation, cellular glucuronidation, and female gamete generation (Figure 2(a)). Meanwhile, hypomethylated genes displayed an association with drug response, negative regulation of RNA polymerase II promoter transcription or canonical Wnt signaling pathway, heart development, peptidyl-tyrosine phosphorylation, Erb-B2 Receptor Tyrosine Kinase 2 (ERBB2) and non-canonical Wnt, as well as intrinsic apoptotic signaling pathways, transcription-coupled nucleotide-excision repair, cellular protein metabolic process, negatively regulated cell

proliferation, and positively regulated heart contraction (Figure 2(b)).

**3.3. KEGG Pathway Analysis of Gene Expression Driven by DNA Methylation in OSAHS.** The data revealed that hypermethylated genes had an association with drug metabolism-cytochrome P450, chemical carcinogenesis, porphyrin and chlorophyll metabolism, the interaction between cytokine-cytokine receptor, cytochrome P450-induced xenobiotics metabolism, steroid hormone biosynthesis, retinol metabolism, pancreatic secretion, metabolic pathways, and drug metabolism-other enzymes (Figure 2(c)). Hypomethylated genes had an association with toxoplasmosis, pathways in cancer, HTLV-I infection, cell cycle, hepatitis B, tuberculosis, misregulation of transcription in cancer, ribosome, and oxidative phosphorylation (Figure 2(d)).

**3.4. PPI Network Analysis of Gene Expression Driven by DNA Methylation.** The PPI network of hypermethylated genes comprising 395 nodes and 820 edges (Figure 3) and hypomethylated genes containing 756 nodes and 3780 edges (Figure 4) were established by Cytoscape software in the

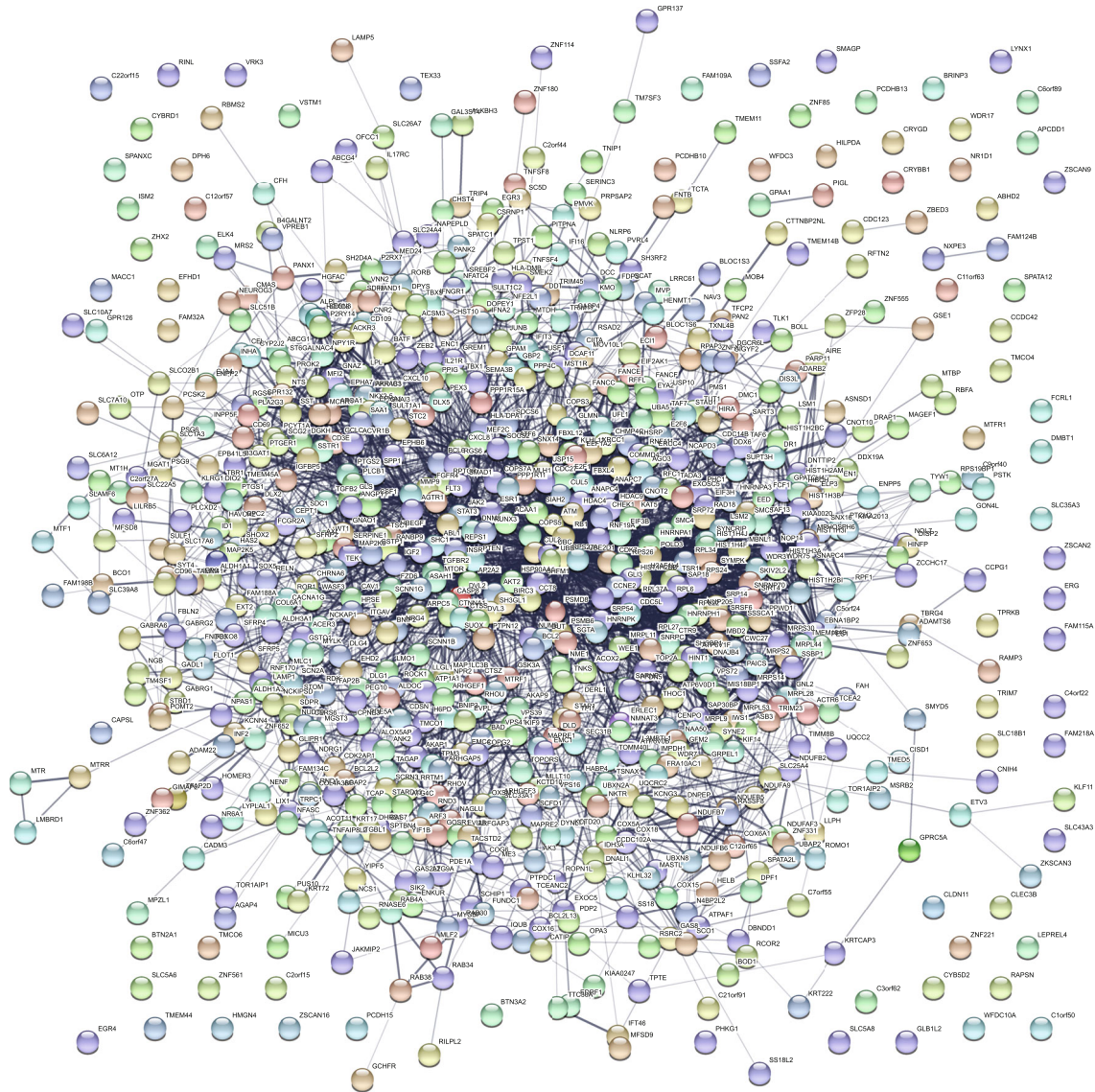


FIGURE 4: PPI network of hypomethylated genes. Each node presents a differently methylated gene.

STRING database. CytoHubba was applied to screen the top hub genes under the control of DNA methylation utilizing Cytoscape software. Then, a module possessing an MCODE score  $\geq 10$  was chosen. It contained 11 hypermethylated genes and 111 edges and many genes, such as GPM2, CCR8, CCR3, HTR1F, TAS2R20, TAS2R4, TAS2R5, P2RY13, TAS2R10, ANXA1, and C5 (Figure 5(a)). Hypomethylated hub networks included UFL1, FBXL4, RPS27A, SIAH2, CUL3, UBC, KLHL11, UBB, FBXL12, RNF19A, RNF111, GLMN, CUL5, UBE2D1, ANAPC7, UBA5, CDC27, and ANAPC4 (Figure 6(a)).

3.5. *Functional Annotation of Hub PPI Networks in OSAHS.* ClueGO offered a functional annotation map for PPI subnetworks. The hub hypermethylated hub network was involved in regulating the ligand:GPCR:Gi complex dissociates, and liganded Gi-activating GPCR acted as a GEF for Gi (Figure 5(b)). And the hub hypomethylated hub network was involved in modulating ubiquitinated proteins in the cat-

abolic process and the transition of mitotic metaphase/anaphase (Figure 6(b)).

#### 4. Discussion

OSAHS is a common breathing disorder, and its mechanisms in modulating this disease were still unclear [17]. Over the past decades, a large number of studies have shown that abnormal DNA methylation epigenetically silences genes necessary for the pathogenesis of human diseases. Previous researches have shown that methylation alternation of specified genes was linked to human disease progression, including OSAHS [18]. For example, Huang et al. showed that TLR6 and TLR2 were aberrantly methylated in OSAHS tissues [19]. Chen and Kong found that hypomethylation of IL1R2, hypermethylation of AR, hypomethylation of NPR2, and hypermethylation of SP140 might display as probable OSAHS biomarkers [20]. Additionally, DNA methylation of FOXP3 is reported to be a potential biomarker in children with

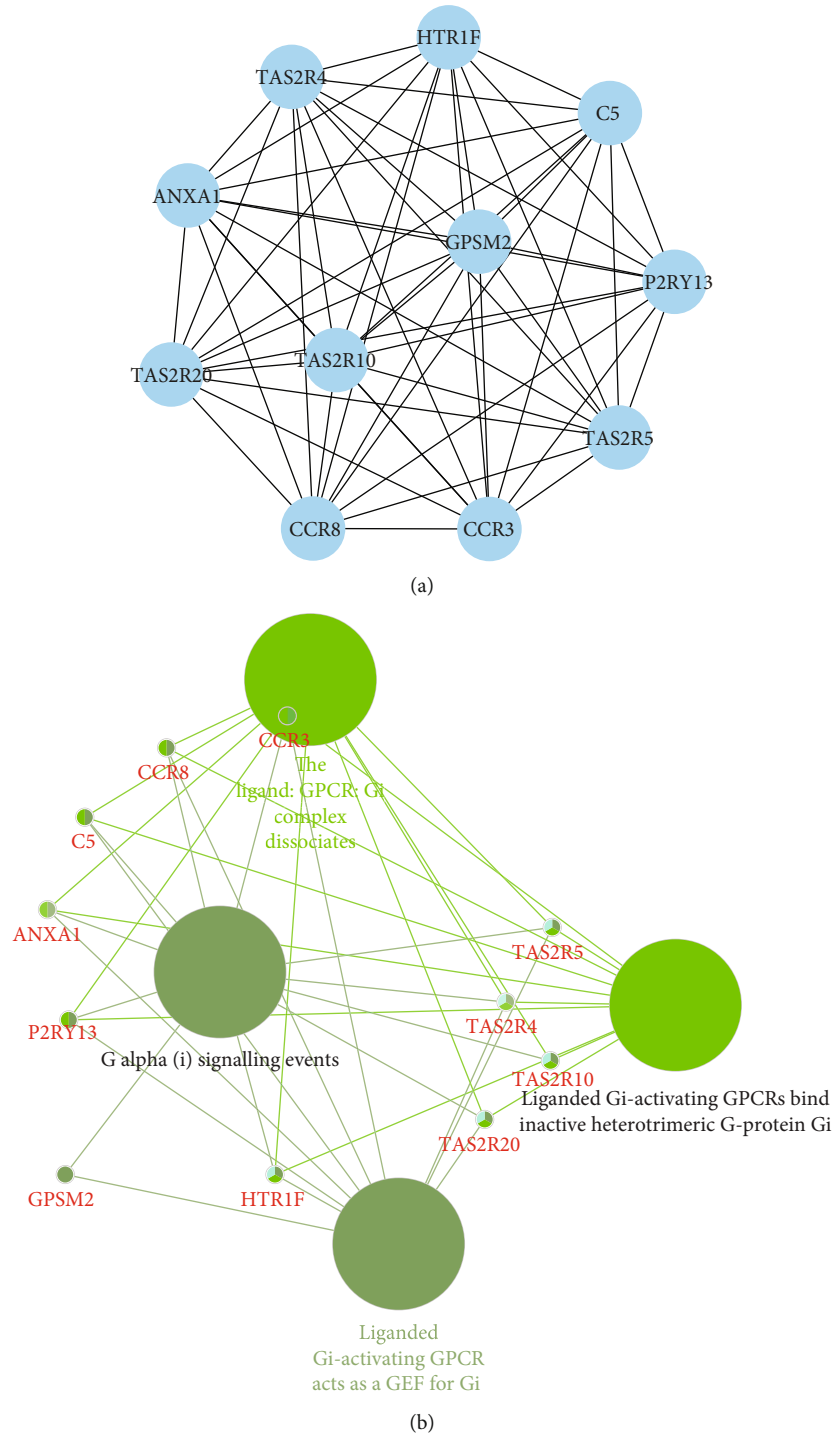


FIGURE 5: Module analysis of hub hypermethylated genes. (a) The PPI subnetwork of hub hypermethylated genes with an MCODE score  $\geq 10$ . Each blue node presents a differently methylated gene. (b) Functional annotation map for hub hypermethylated genes. Brown or green node presents a pathway.

obstructive sleep apnea [21]. However, there was still a lack of comprehensive analysis of differently methylated genes in the progression of OSAHS. In this study, we downloaded the OSAHS data from the GEO database. Our data for the first time revealed screened 1409 abnormal methylated genes in OSAHS, including 520 hypermethylated genes and 889 hypomethylated genes. Bioinformatics analysis revealed that these

abnormally methylated genes exhibited an association with the regulation of angiogenesis, apoptosis, Wnt, and ERBB2 signaling pathways. PPI network analysis displayed the interactions among these genes and validated several hub genes, such as GPSM2, CCR8, TAS2R20, TAS2R4, and TAS2R5.

Bioinformatics analysis suggested that hypermethylated genes were linked to the regulation of angiogenesis, sodium

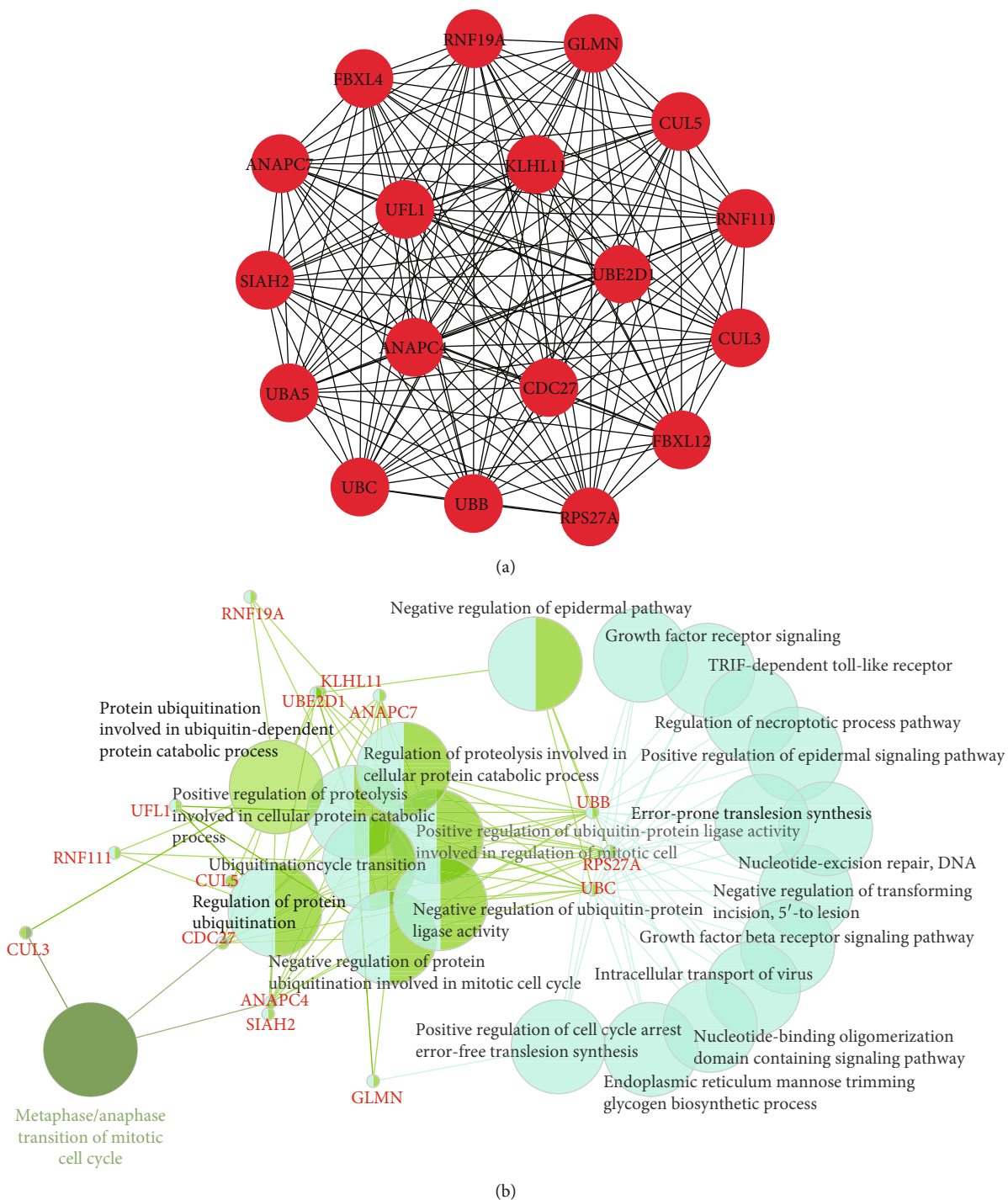


FIGURE 6: Module analysis of hub hypomethylated genes. (a) The PPI subnetwork of hub hypomethylated genes with an MCODE score  $\geq 10$ . (b) Functional annotation map for hub hypomethylated genes.

ion transport, apoptotic process, and cytokine-induced signaling pathway. Nevertheless, the hypomethylated genes had a relation with Wnt, ERBB2, and intrinsic apoptotic signaling pathways. The abovementioned pathways were reported to play as primary modulators of human diseases. For instance, angiogenesis is a new blood vessel formed by the preexisting vascular system [22]. Previous researches have shown that angiogenesis displays importance in chronic

obstructive pulmonary disease (COPD) development [23]. VEGF members are the primary angiogenesis mediator, and targeting VEGF was a feasible strategy to treat COPD. The activated Wnt signaling pathway is completed after Wnt ligand binding to the seven-way transmembrane receptor frizzled motivating Wnt/ $\beta$ -catenin or other signaling pathways, thus important for developmental and physiological processes. Overexpression or activation of erbB-2 causes

the activation of multiple signaling pathways mediated by MAP, PI3 kinase, and the STAT family. A previous study demonstrates that phosphorylation of erbB2 receptor is activated by intermittent hypoxia, which is involved in promoting the proliferation of vascular smooth muscle cells in the OSAHS model [24].

What was more, we constructed a PPI network of hypermethylated genes and hypomethylated genes. Hub PPI networks revealed a series of key methylated genes in OSAHS, including GPSM2, CCR8, CCR3, HTR1F, TAS2R20, TAS2R4, TAS2R5, P2RY13, TAS2R10, ANXA1, C5, UFL1, FBXL4, RPS27A, SIAH2, CUL3, UBC, KLHL11, UBB, FBXL12, RNF19A, RNF111, GLMN, CUL5, UBE2D1, ANAPC7, UBA5, CDC27, and ANAPC4. GPSM2 belongs to the protein family, which can modulate the activation of G protein and transduce the extracellular signal when cell surface receptors receive an integrated cellular response. Depleted GPSM2-induced random distribution of mitotic spindle and aberrant location of apical NPCs in the cortex did not affect cell proliferation and neurogenic divisions. CCR8 was reported to cause skin IL-10 producer T cells homing to the inflammatory tissue. Yabe et al. reported that CCR8 modulated DC migration from skin to the draining lymph nodes in contact allergy (Type IV allergy) associated inflammation. Type 2 taste receptors (T2Rs, TAS2Rs), a type of G protein-coupled receptors (GPCRs), participated in the transduction of cell membranes signal, particularly in response to bitter substances. Generally, expressed T2R could distinguish beneficial or harmful exogenous and endogenous molecules and then motivate the following processes including utilizing or eliminating these stimuli, thus modulating consequent metabolism and disease development. More and more evidence revealed that T2Rs exhibited importantly in the etiology of diseases, of which genetic variation was considered modifiers. In addition, ClueGO analysis showed that the hub hypermethylated hub network was involved in regulating Gi-activating GPCR signaling and the hub hypomethylated hub network was involved in modulating the transition of mitotic metaphase/anaphase. GPCRs are overexpressed in the carotid body (CB), which played a crucial role in monitoring the blood supplying the brain. GPCRs have a very significant part in evoking CB hyperactivity, hypertension, and cardiac arrhythmia associated with key conditions such as OSA and HF. GPCRs could be activated by multiple ligands, including Gs, Gi, Gq, and G12/13. This study for the first time revealed potential regulators related to the Gi-GPCR complex, including CCR3 and CCR8.

## 5. Conclusion

Collectively, our data for the first time revealed differently methylated genes in OSAHS. Further bioinformatics analysis revealed that these abnormally methylated genes had a crucial role in OSAHS by modulating angiogenesis, apoptosis, Wnt, and ERBB2 signaling pathways. Although more confirmation is still needed, our study offers a new hint of understanding the molecular mechanisms in OSAHS progression.

## Data Availability

All the data and materials are available with the authors' permission.

## Conflicts of Interest

All authors declared no competing interests.

## Authors' Contributions

Writing, review, and revision of the manuscript were handled by Yan Li. Conception and design were taken care of by Yajuan Zhang. All authors have read and approved the content and agreed to submit it for consideration for publication in the journal.

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