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Identifying and validating immunological biomarkers in obstructive sleep apnea through bioinformatics analysis

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Obstructive sleep apnea (OSA) is a prevalent sleep disorder characterized by disrupted breathing patterns and dysfunctions in multiple organ systems. Although studies support a close correlation between OSA and immune function, the broader implications and specific manifestations remain unclear. Therefore, it is pressingly needed to identify potential immune-related markers and elucidate underlying immunological mechanisms of OSA. OSA-related datasets (GSE38792) and immune-related genes were downloaded from the GEO and ImmPort databases and intersected to obtain differentially expressed immune-related genes (DEIRGs). GO, KEGG, and GSEA were employed to explore the biological functions of DEIRGs. Immune cells and immune regulation were analyzed by CIBERSORT. The ROC curve was constructed to assess the accuracy of each DEIRG. The co-regulatory networks of transcription factors, microRNAs, and drugs were built using the NetworkAnalyst database and visualized by Cytoscape. The levels of DEIRGs in clinical samples were validated by RT-qPCR. GO, KEGG, and GSEA revealed that DEGs were mainly enriched in negative regulation of immune response and antigen processing and presentation in OSA. IL33, IL10RB, ANGPTL1, EIF2AK2, SEM1, IFNA16, SLC40A1, FCER1G, IL1R1, TNFRSF17, and ERAP2 were identified as DEIRGs among 175 differentially expressed genes in OSA. Memory B cells, mast cells resting, and dendritic cells resting were the predominant immune cells related to DEIRGs. The co-regulatory network contained 128 miRNAs, 40 transcription factors, and 172 drugs/compounds. Finally, IL33, EIF2AK2, IL10RB, and ANGPTL1 were also upregulated in clinical OSA samples. The present study identified potential immune-related biomarkers and systematically elucidated underlying immunological mechanisms of OSA. These findings provide novel insights into the diagnosis, mechanism research, and management strategies for future studies.

Keywords Obstructive sleep apnea, Immune-related genes, Immune function, Biomarkers, Bioinformatics analysis

Abbreviations

AHI	Apnea-hypopnea index
CI	Confidence interval
DC	Dendritic cells
DEG	Differentially expressed genes
DEIRG	Differentially expressed-related and immune-related gene
GEO	Gene expression omnibus
GSEA	Gene Set Enrichment Analysis
GO	Gene oncology
HLA	Human leukocyte antigen
IFN	Type I interferon

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IRG	Immune-related gene
KEGG	Kyoto Encyclopedia of Genes and Genomes
MC	Mast cells
miRNA	Micro-RNA
NOSA	Non-obstructive sleep apnea
OSA	Obstructive sleep apnea
ROC	Receiver operating characteristic
RT-qPCR	Reverse transcriptionquantitative PCR
TF	Transcription factor

Obstructive sleep apnea (OSA), a prevalent yet frequently underestimated sleep-related breathing disorder, has a prevalence of up to 10% in the general population across all age groups[1,2]. OSA is characterized by recurrent upper airway obstruction, intermittent hypoxemia, and sleep fragmentation, leading to secondary sympathetic activation, oxidative stress, and systemic inflammation[3]. Its physiological functions mainly rely on the balance between pro- and anti-inflammatory factors. The progression of OSA often results in a shift towards pro-inflammatory cytokines[4], contributing to immune disorder, cellular dysfunction, tissue failure, and developmental retardation, regardless of age[5,6]. These outcomes are strongly associated with the development of cardiovascular diseases, metabolic disorders, and cognitive dysfunction[7–10].

Intermittent hypoxia (IH) during OSA and its complications can release the overexpression of hypoxia-inducible factor 1-α (HIF-1α), which exerts systemic effects on the immune surveillance system through the regulation of immune cells, immune checkpoint axis, and various immune components, ultimately disrupting the physiological microenvironment across multiple organ systems[11–13]. IH also releases TGFβto establish immunosuppression in the monocytes and natural killer cells of OSA patients[14]. An immune checkpoint PSGL-1 might cause T-cell dysfunction in OSA patients[13]. Additionally, in the tumor microenvironment, monocytes of OSA impair cytotoxicity by secreting more HIF-1α. Moreover, both IH and sleep fragmentation induce M2-polarized macrophage infiltration and drive tumor cell proliferation, migration, and invasion[15,16]. However, although studies support the close correlation between OSA and immune function, the macro cognition and micro manifestation remain unclear. Exploring OSA pathogenesis from an immunological perspective is essential to further understand OSA and provide references for early detection and non-invasive treatment.

Bioinformatics analysis has made great strides and has become an important tool for identifying pathogenic genes[17]. This study first combined differentially expressed genes (DEGs) of OSA from the gene expression omnibus (GEO) database with immune-related genes (IRGs), immunologic functions, and regulatory networks from public databases. Furthermore, in-depth bioinformatics analysis was employed to uncover OSA-related IRGs and molecular mechanisms, which were then validated in clinical samples. This comprehensive approach sought to preliminarily elucidate the immune immunological mechanisms and clinical implications of OSA, thereby offering valuable insights into OSA management.

Materials and methods

Data source

The GSE38792 dataset of gene expression in OSA was downloaded from the GEO database (https://www.ncbi.nlm.nih.gov/geo/) using the R package GEOquery. The GSE38792 dataset is a training dataset with 8 normal samples and 10 OSA visceral fat tissue samples. GPL6244 (Affymetrix Human Gene 1.0 ST Array) is the platform for the GSE38792 dataset. Gene probes were annotated as gene symbols. Probes that did not match gene symbols or matched many symbols were removed. Gene expression of duplicate symbols was calculated at the maximum. Through R packages clusterProfiler, org.Hs.eg.db, enrichplot, and ggplot2, the biological functions of DEGs were determined by Gene Oncology (GO), Kyoto Encyclopedia of Genes and Genomes (KEGG), and Gene Set Enrichment Analysis (GSEA)[18,19].

ImmPort is a vast open database of subject-level human immunological data that is created to facilitate efficient data sharing among the basic, clinical, and translational research communities. In this paper, information on IRGs was obtained from the ImmPort database (https://www.immport.org/shared/). The workflow is displayed in Fig. 1.

Alteration analysis of IRGs between OSA and control samples

The limma R package was utilized to discern DEGs between OSA and control samples in the GSE38792 dataset, with p value < 0.05 and log2 |fold change (FC)| > 0.5 as the threshold. A Venn diagram was adopted to filter differentially expressed IRGs (DEIRGs) via the intersection of DEGs and IRGs. Through clusterProfiler, org.Hs.eg.db, enrichplot, and ggplot2 packages, the biological functions of DEIRGs were determined through GO and Friends analysis.

Analysis of immune cells and immunity regulation

The CIBERSORT algorithm was utilized for immune infiltration analysis. The corrplot in R was employed for Pearson correlation analysis of infiltrating immune cells and diagnostic genes, and the correlations were visualized with lollipop.

Construction of potential gene regulatory networks

NetworkAnalyst (https://www.networkanalyst.ca/) is a comprehensive website for gene expression analysis for diverse species and meta-analysis. This tool was applied for data collection from the RegNetwork repository to build the networks of transcription factor (TF), microRNA (miRNA), and drug to unveil the regulatory

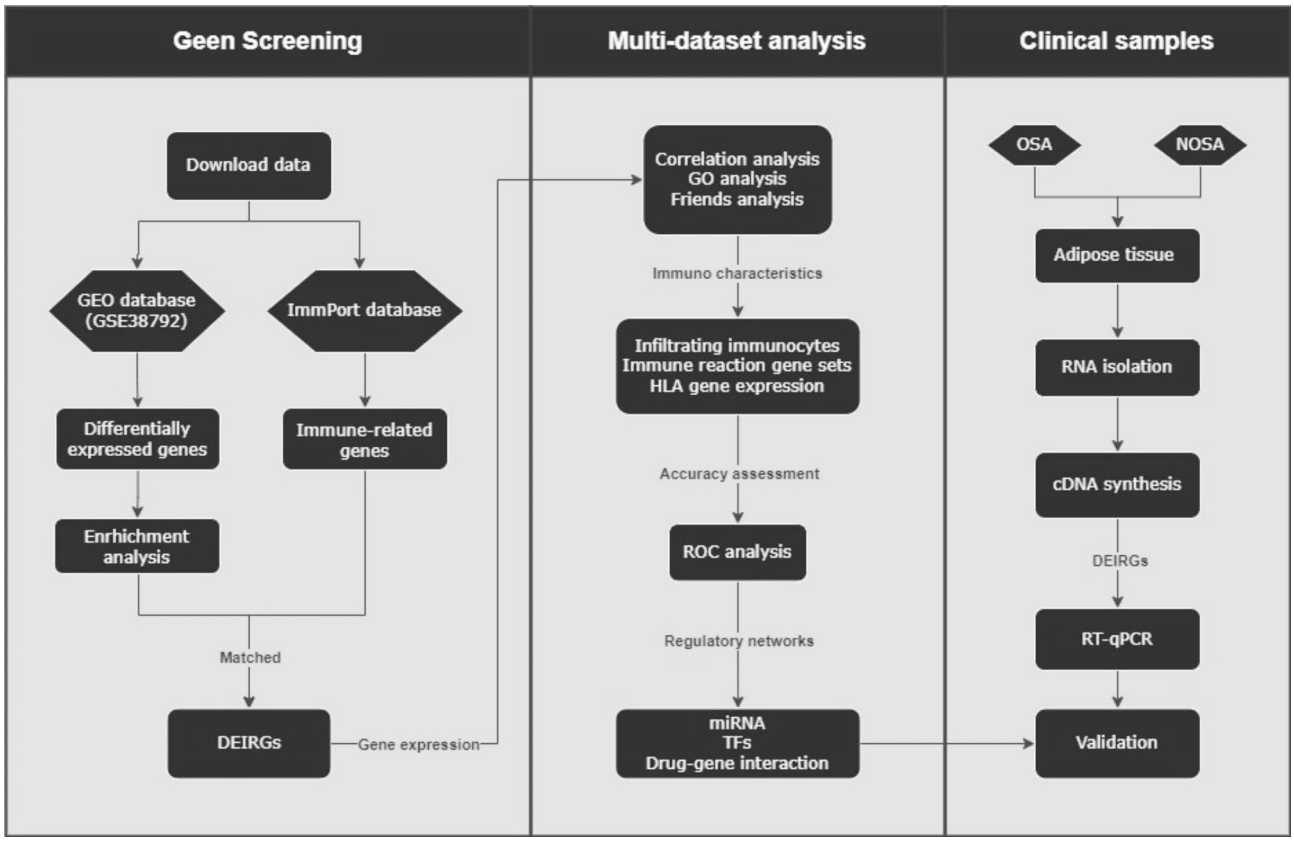

Fig. 1. The flowchart of the study.

mechanism of DEIRGs at the transcriptional and post-transcriptional level. Moreover, the interaction network was visualized with Cytoscape.

The ROC curve analysis and expression analysis

In the GSE38792 dataset, receiver operating characteristic (ROC) curve analysis was done on each screened hub gene to verify its accuracy using the pROC package. Hub genes with AUC > 0.7 were regarded as having high diagnostic accuracy. The levels of hub genes in OSA and control samples were presented in the boxplots with the ggplot2 package. The functional similarity of genes was analyzed using the GOSemSim package and their correlations were assessed using the corrplot package.

Sample sources for validation

Eight subjects (four OSA patients and four controls) were enrolled at the otolaryngology head and neck surgery department of the Shanghai Sixth People's Hospital between December 2023 and January 2024. Subjects underwent standard overnight polysomnography before treatment. Four OSA patients were aged over 18 years old, with total sleep duration of > 240 min and an apnea-hypopnea index (AHI) of ≥ 5 events per hour. The other four patients with benign neck mass were selected as controls with AHI < 5 events/h. Both OSA and control groups were matched by sex, age, body mass index, absence of other comorbid sleep disorders (such as insomnia, upper airway resistance syndrome, narcolepsy, or restless legs syndrome), and psychotropic medication. Adipose tissue is a key player in OSA[20]. Tissue samples in the GSE38792 dataset were obtained from visceral adipose tissue[21]. To ensure tissue homogeneity and the representativeness of OSA, specimens for validation in this study were specifically collected from soft palate adipose tissue during uvulopalatopharyngoplasty procedures in OSA patients. Additionally, control specimens were obtained from paratracheal adipose tissue during surgical procedures in patients with benign cervical masses. Details of patient data are displayed in Table 1.

RNA isolation and cDNA synthesis

Total RNA was isolated with a Tissue RNA Purification Kit (for adipose tissue) (EZBioscience, Cat.No.: EZB-RN001A) based on the manufacturer's instructions. Optical densities were tested using a NanoDrop™ One spectrophotometer (Thermo Scientific) to determine the concentration of obtained RNAs. The obtained RNA samples were synthesized into cDNA using a Color Reverse Transcription Kit (EZBioscience, Cat.No.: A0010CGQ) under the following thermal conditions for enzyme inactivation: 42 °C for 15 min and 95 °C for 5 min. The cDNAs obtained were stored at − 20 °C and the remaining RNAs were stored at − 80 °C.

	OSA ($n=4$)	Control ($n=4$)	*P* value
Male, n(%)	2 (50)	2 (50)	1.000
Age, years	33.00 ± 8.10	25.74 ± 4.18	0.437
BMI, kg/m^2	25.74 ± 4.18	23.87 ± 1.65	0.292
AHI, events/h	36.03 ± 13.45	2.18 ± 1.10	0.015
$LSaO_2$, %	79.50 ± 4.65	93.75 ± 1.26	0.001
Comorbidities			
Hypertension, n(%)	1 (25)	1 (25)	1.000
Diabetes, n(%)	0 (0)	0 (0)	/
Heart disease, n(%)	0 (0)	0 (0)	/
Smoking, n(%)	1 (25)	2 (50)	0.465

Table 1. Subject characteristics at baseline. Continuous variables are are shown as mean ± SD. OSA, obstructive sleep apnea; BMI, body mass index; AHI, apnea hyponea index; LSaO2, lowest oxygen saturation.

Human	Forward Primer (5'-3')	Reverse Primer (5'-3')	Fragment length (bp)
IL10RB	ATGAGCATTCAGACTGGGTAAAC	TTTTAGGGGCTAAGAAACGCAT	123
SLC40A1	CTACTTGGGGAGATCGGATGT	CTGGGCCACTTTAAGTCTAGC	176
FCER1G	AGCAGTGGTCTTGCTCTTACT	TGCCTTTCGCACTTGGATCTT	151
IFNA16	GATATGATTTCGGATTCCCCCAG	TAGGAGGGTCTCATCCCAAGC	149
SEM1	GAAAAAGCAGCCGGTAGACTT	ATCCCAATTATCCTCCCAGACA	124
EIF2AK2	TGGAAAGCGAACAAGGAGTAAG	CCAAAGCGTAGAGGTCCACTT	98
IL33	GTGACGGTGTTGATGGTAAGAT	AGCTCCACAGAGTGTTCCTTG	94
ANGPTL1	AGAAAGGAAAGCCGTAACATGAA	TCCCTGTATCTTGTTGCCATCT	164
TNFRSF17	GAACGAATGCGATTCTCTGGA	TCATCACCAGTCCTGCTCTTT	184
PPIA	ATCCTAGAGGTGGCGGATTT	CACTCAGGTCTGAGCCACAA	184

Table 2. The primer sets for RT-qPCR.

Reverse transcriptionquantitative PCR (RTqPCR)

According to the significant expression of DEIRGs in the GSE38792 dataset, RT-qPCR was performed to verify their expression in OSA and control adipose tissues using LightCycler™ 480 (Roche Applied Science) with the SYBR Green qPCR Mater Mix (ROX2 plus) (EZBioscience, Cat.No.: A0012-R2). RT-qPCR was carried out at 95 °C for 5 min, followed by 40 cycles of 95 °C for 10s and 60 °C for 30s. Primers from the primer bank are listed in Table 2. Expression levels were normalized to PPIA[22,23] and computed using the comparative threshold cycle method ($2^{-\Delta\Delta CT}$).

Statistical analysis

All data were calculated and analyzed using R programming (https://www.r-project.org/, version 4.3.1). Wilcoxon test and Kruskal–Wallis test were adopted to compare subgroup differences. Pearson correlation coefficient was utilized for correlation analysis. GraphPad Prism 9 (La Jolla, CA, USA) and SPSS 26.0 were utilized for statistical analyses. The Shapiro-Wilk normality test and the variance homogeneity test were first performed. Normally distributed continuous variables were compared using an unpaired t-test and non-normally distributed continuous variables were compared using the Mann-Whitney U test. The level of confidence was set at 95%, and $P<0.05$ implied statistical significance (ns: no significance, *: $p<0.05$, **: $p<0.01$, ***: $p<0.001$).

Results

Differential gene expression analysis

175 DEGs were obtained from the GSE38792 dataset (Fig. 2A; Supplementary Table 1). To identify DEIRGs, all 1793 IRGs were downloaded from the ImmPort database. Then, IRGs and DEGs were matched and 11 DEIRGs were obtained (Fig. 2B; Supplementary Table 2). In the OSA group, 7 DEIRGs were significantly upregulated and 2 DEIRGs were significantly downregulated compared to the normal group (Fig. 2C, D). In 11 DEIRGs, Pearson correlation analysis showed strong positive correlations between IL33, IL10RB, ANGPTL1, and EIF2AK2 expression (Fig. 2E). Friends analysis revealed that ANGPTL1 had the strongest correlation with other DEIRGs, followed by TNFRSF17, IL10RB, IL1R1, and other hub genes (Fig. 2F). GSEA results revealed that Alzheimer's disease, antigen processing and presentation, autophagy—animal, cell cycle, and mitophagy–animal and protein processing in endoplasmic reticulum were enriched in the OSA group, while drug metabolism—cytochrome P450 and metabolism of xenobiotics by cytochrome P450 were enriched in the control group (Fig. 2G). GO analysis revealed that DEGs were mainly enriched in endoplasmic reticulum lumen, secretory granule membrane, extracellular matrix, negative regulation of immune response, and immune response—regulating

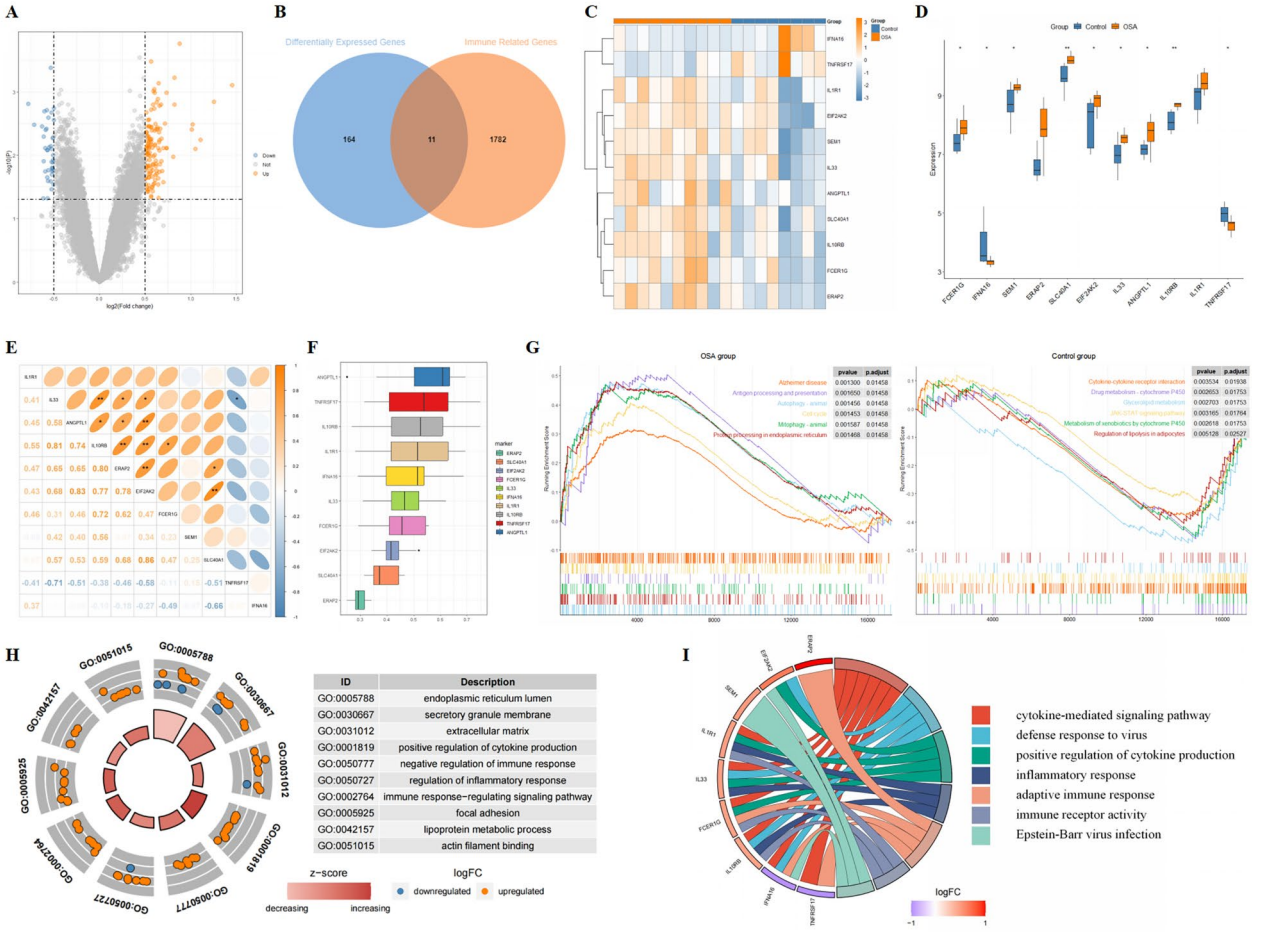

Fig. 2. Screening DEGs and DEIRGs in OSA. **(A)** Volcano plot showing DEGs in GSE38792. **(B)** Venn diagram showed DEIRGs. **(C)** and **(D)** Heatmap and differentially expression analysis of 11 DEIRGs in GSE38792. **(E)** Correlation analysis of DEIRGs. **(F)** GSEA result of DEIRGs. The enrichment score of curve above 0 points indicates that the gene sets up-regulated, and the curve below 0 points indicates that the gene sets down-regulated. **(G)** and **(H)** GO analysis of DEIRGs. **(I)** Friends analysis of DEIRGs. DEGs, differentially expressed genes; DEIRGs, differentially expressed immune-related genes; OSA, obstructive sleep apnea; GSEA, Gene Set Enrichment Analysis; GO, Gene Oncology; p.adjust, adjusted p-value.

signaling pathway (Fig. 2H). Furthermore, DEIRGs were related to cytokine-mediated signaling pathways, defense response to virus, positive regulation of cytokine production, inflammatory response, adaptive immune response, immune receptor activity, and Epstein-Barr virus infection (Fig. 2I).

DEIRGs are associated with immune characteristics of OSA

To uncover the biological behaviors of DEIRGs in the immune microenvironment, the correlation of DEIRGs with infiltrating immune cells, immune checkpoints, and human leukocyte antigen (HLA) was analyzed. DEIRGs were closely linked to many immune cells. FCER1G had the most positive correlation with macrophages M2 ($p < 0.001$), and IL10RB had the most positive correlation with dendritic cells (DCs) resting ($p = 0.007$); IL33 had the most passive correlation with memory B cell ($p = 0.005$), and IFNA16 had the most passive correlation with macrophages M2 ($p = 0.022$) (Fig. 3A). The cell composition of monocytes was greatly lower in the OSA group, profoundly affected by FCER1G and IL10RB expression, and strongly correlated with the function of T cell follicular helper; DCs resting abundance was notably higher in the OSA group, positively correlated with FCER1G, IL33, IL10RB, and strongly associated with the function of memory B cells; mast cells (MCs) resting abundance was markedly higher in the OSA group, positively correlated with ERAP2, EIF2AK2, ANGPTL1, and strongly correlated with the function of eosinophils; macrophages M1 abundance was negatively correlated with IL10RB, IL1R1, EIF2AK2 and SEM1; macrophages M2 abundance was positively linked to FCER1G and negatively linked to IFNA16; both macrophages M1 and M2 were closely related to DCs resting (Fig. 3B, C). Analysis of immune checkpoints showed that LAG3 and PDCD1 were negatively correlated with most DEIRGs, while CD274 was positively correlated with most DEIRGs (Fig. 3D). IFNA16 and TNFRSF17 were negatively correlated with most HLA-related genes. Except for HLA-DOA, EIF2AK2, SLC40A1, IL33, and IL10RB had strongly positive correlations with other HLA-related genes (Fig. 3E). In addition, ERAP2, EIF2AK2, IL33,

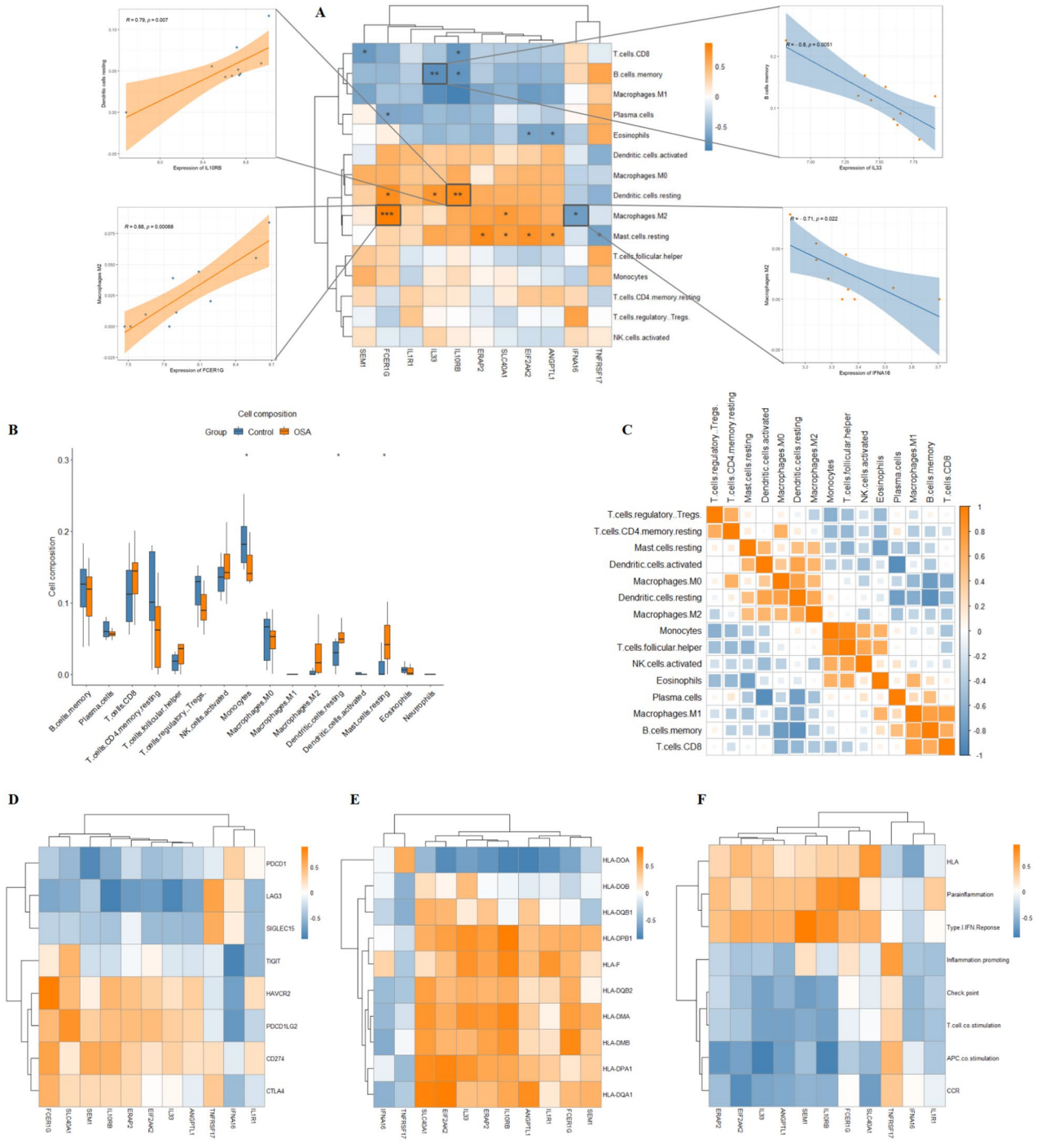

Fig. 3. Immune microenvironment of DEIRGs in OSA. **(A)** Immunocytes related to DEIRGs. **(B)** Differentially expression of immunocytes in GSE38792 dataset. **(C)** Correlation analysis between the various immunocyte types in DEIRGs. **(D)** Correlation between immune checkpoint data and DEIRGs. **(E)** Correlation between HLA-related genes and DEIRGs. **(F)** Correlation between immune reaction gene-sets and DEIRGs. DEIRGs, differentially expressed immune-related genes; OSA, obstructive sleep apnea; HLA, human leukocyte antigen.

ANGPTL1, SEM1, IL10RB, FCER1G, and SLC40A1 expression was positively correlated with HLA, para-inflammation, type I interferon (IFN) response, and negatively correlated with the inflammation-promoting, checkpoint, T cell co-stimulation, APC co-stimulation, and CCR (Fig. 3F).

ROC analysis of DEIRGs

The diagnostic value of 11 DEIRGs was validated via the ROC curve (Fig. 4). The AUC of IL10RB in the GSE38792 was 0.9 (95% CI, 0.7255–1.000), SLC40A1 was 0.9 (95% CI, 0.7584–1.000), IL33 was 0.8375 (95% CI, 0.6224–1.000), EIF2AK2 was 0.825 (95% CI, 0.6306–1.000), TNFRSF17 was 0.825 (95% CI, 0.613–1.000), FCER1G was 0.825 (95% CI, 0.6066–1.000), ANGPTL1 was 0.8125 (95% CI, 0.5794–1.000), SEM1 was 0.7875 (95% CI, 0.5381–1.000), IFNA16 was 0.7875 (95% CI, 0.5713–1.000), IL1R1 was 0.775 (95% CI, 0.5526–1.000), and ERAP2 was 0.775 (95% CI, 0.5255–1.000).

Construction of the related regulatory network

The miRNA, TFs, and drug co-regulatory networks on 11 DEIRGs were identified using the NetworkAnalyst database. Cross-linked miRNAs were selected to ensure the accuracy and stability of the results. The regulatory network contained 128 miRNAs and 10 DEIRGs (Fig. 5A). The drug–gene interaction network contained 172 potential target drugs/compounds and 11 DEIRGs (Fig. 5B). For the TF-DEIRG regulatory network, 40 TFs and 11 DEIRGs were obtained (Fig. 5C).

Validation of significant DEIRG expression in clinical samples

No significant differences in sex (2 females and 2 males in each group), age (33.00 ± 8.10 vs. 41.75 ± 12.81, $P = 0.437$), and body mass index (25.74 ± 4.18 vs. 23.87 ± 1.65, $P = 0.292$) were found between OSA and control clinical samples. To verify the results of bioinformatics analysis, we performed RT-qPCR in clinical adipose tissue samples. Consistently, IL33, EIF2AK2, IL10RB, and ANGPTL1 were significantly upregulated in the OSA group, while no differences were found in the expression of SEM1, FCER1G, IFNA16, SLC40A1, and TNFRSF17 between the OSA and NOSA groups (Fig. 6).

Discussion

OSA is an intricate systemic disorder characterized by recurrent episodes of total or partial upper airway obstruction during sleep, leading to hypoxemia, hypercapnia, or sleep fragmentation[1]. It demonstrates strong associations with excessive daytime sleepiness, cardiovascular disease, metabolic disorder, and cognitive

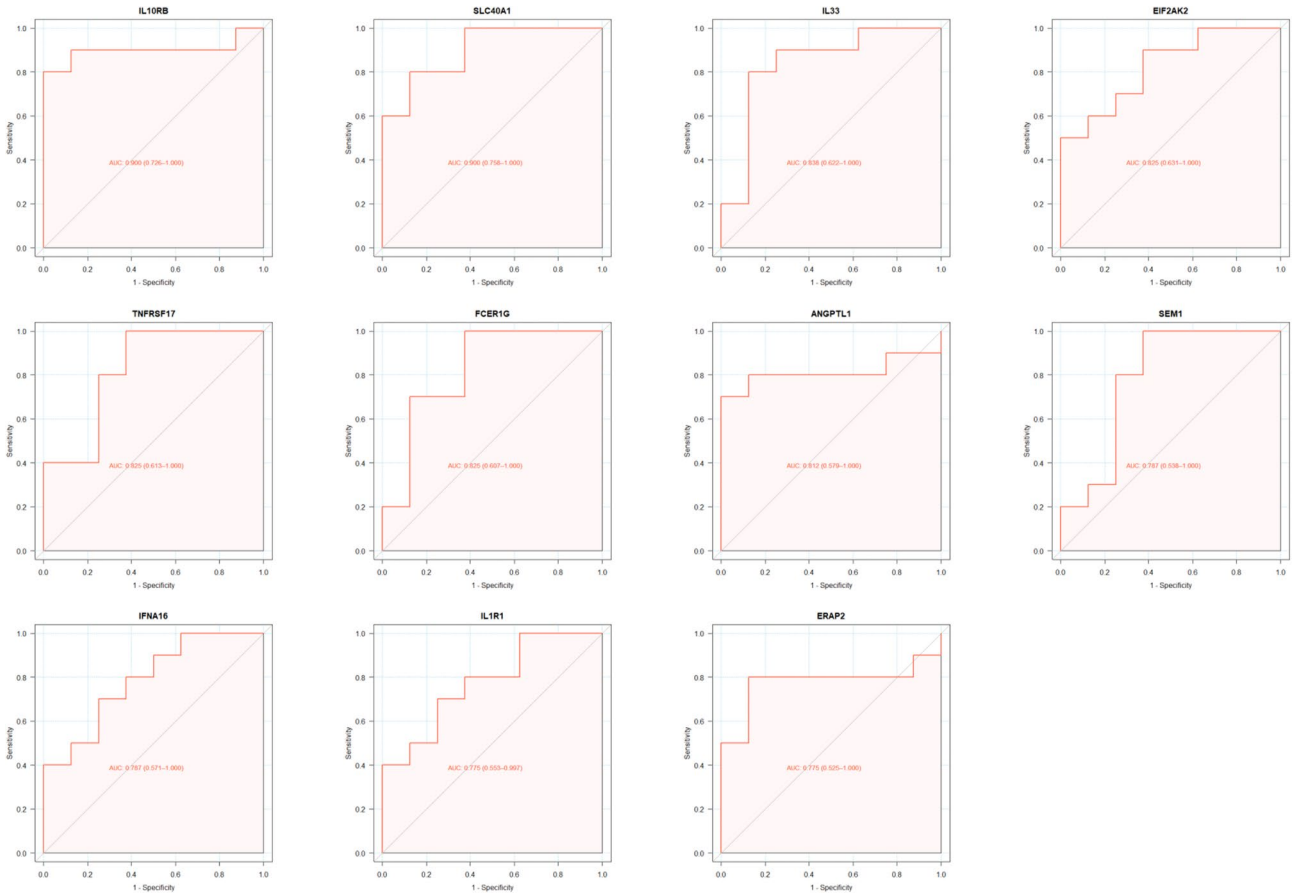

Fig. 4. ROC curve analysis of DEIRGs in OSA.

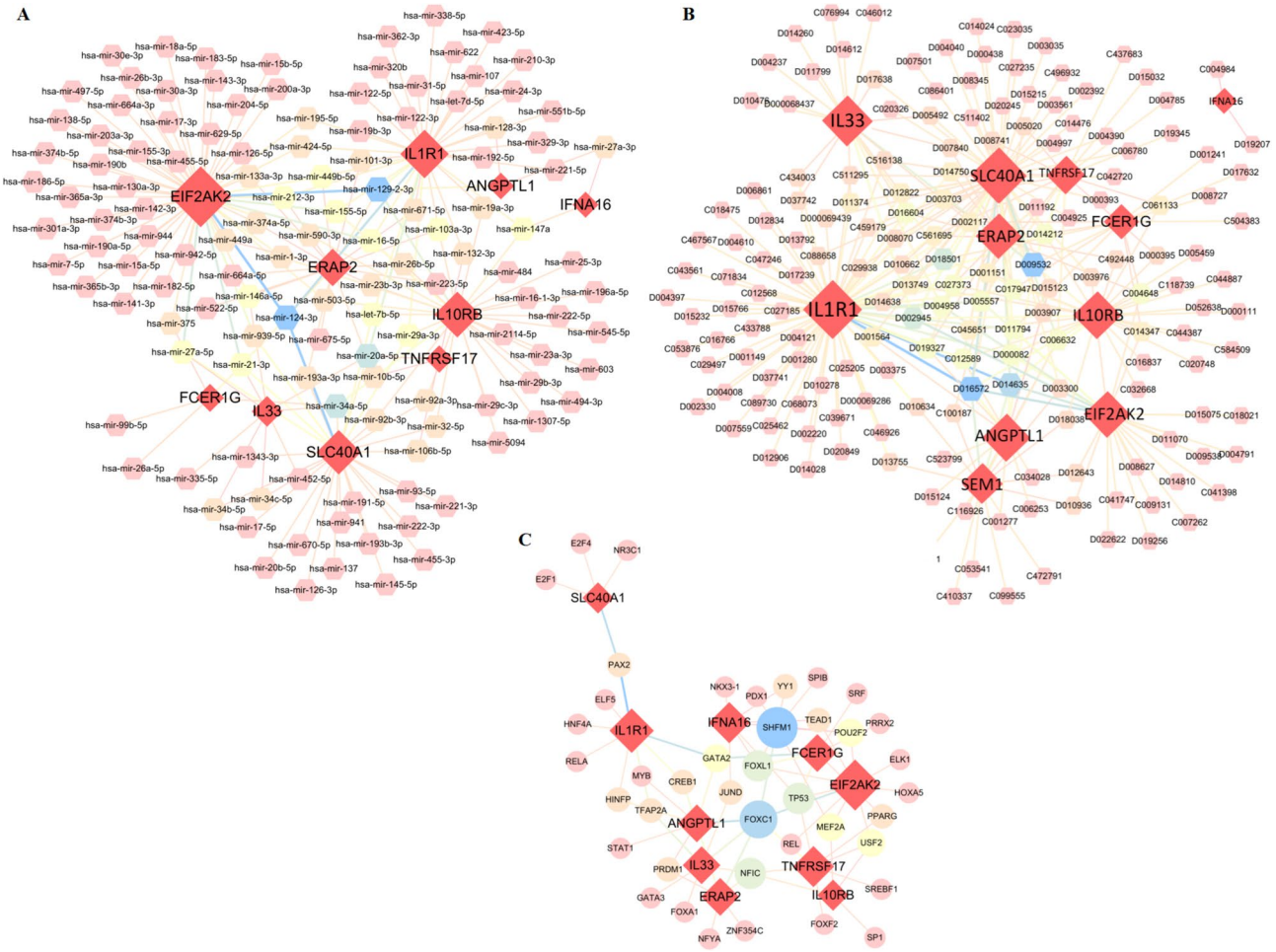

Fig. 5. The regulatory networks of DEIRGs in OSA. **(A)** The regulatory network of 128 miRNAs and 10 DEIRGs. **(B)** The regulatory network of 40 TFs and 11 DEIRGs. **(C)** The drug–gene interaction network of 172 potential target drugs/compounds (represented by ID cord in Comparative Toxicogenomics Database) and 11 DEIRGs. DEIRGs, differentially expressed immune-related genes; OSA, obstructive sleep apnea; miRNA, micro-RNA; TF, transcription factor.

dysfunction[7–9]. Due to its increasing incidence and significant limitations in early detection and clinical treatment, OSA has recently garnered considerable attention[9]. Nevertheless, its pathogenesis has not been fully elucidated.

In the present study, we uncovered 11 DEIRGs using differential expression analysis by intersecting transcriptome data from GEO (GSE38792 dataset) and IRGs from ImmPort. Furthermore, 4 common DEIRGs (IL33, EIF2AK2, IL10RB, and ANGPTL1) were verified in clinical samples.

Interleukin 33 (IL33) is an alarmin cytokine of the interleukin family and is mainly expressed in myofibroblasts, fibroblasts-like cells, epithelial cells, and endothelial cells, with close relations to respiratory diseases[24,25]. IL33 is produced by adipocytes[26] and is substantially higher in adipose tissue of OSA patients, possibly due to IH, leading to systemic inflammation, vascular remodeling, and cognitive dysfunction through the IL-33/ST2, HIF-1α/VEGFA, and NF-κB pathways[24,25,27,28]. Interleukin 10 (IL10), a potent regulatory cytokine, is essential for maintaining self-tolerance and limiting tissue damage during inflammation[29]. Deficiencies in IL10 or its receptor correlate with poor immune surveillance[30]. The single nucleotide polymorphisms of IL10 receptor beta (IL10RB), which is strongly correlated with IL33 in the present study, are associated with ischemic stroke and hypertension[31]. Additional investigation is necessary for the interaction between IL10RB and IL33.

Eukaryotic translation initiation factor 2 alpha kinase 2 (EIF2AK2) is highly expressed in brain tissue and is a critical protein in fatty acid-induced endoplasmic reticulum stress, which bridges the signals between lipid metabolism and inflammation and modulates multiple signal transduction pathways[32–34]. Angiopoietin-like protein 1 (ANGPTL1) is a member of the ANGPTL family that encompasses a coiled-coil domain and a fibrinogen-like domain and can regulate angiogenesis[35]. Recent research has highlighted that ANGPTLs are therapeutic targets for cardiovascular, metabolic, and obesity-related diseases[36,37]. A strong correlation between EIF2AK2 and ANGPTL1 was found in our present study, indicating their potential interactions in OSA. The above 4 DEIRGs simultaneously regulate metabolic homeostasis, vascular stability, and tumorigenesis, which

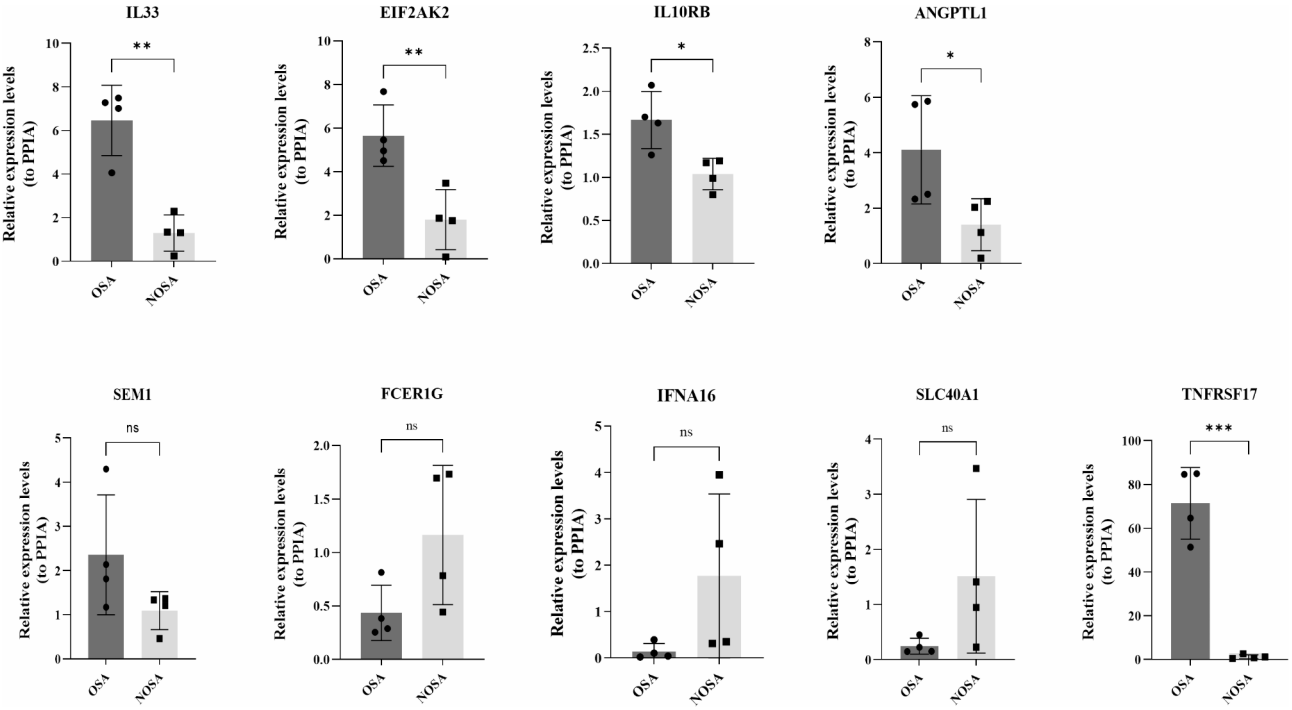

Fig. 6. Validation of significant DEIRGs by RT-qPCR. * indicates p value < 0.05, ** indicates p value < 0.01, *** indicates p value < 0.001. OSA, obstructive sleep apnea; NOSA, non-obstructive sleep apnea.

are obvious physiological changes under hypoxia. The pathophysiological mechanisms are worth further investigation.

Our immune filtration analysis investigated OSA-related immune cells and their interactions and unraveled higher DCs/MCs resting abundance and lower monocyte abundance in OSA patients. Systemic hypoxia could maintain inflammation by reducing IFN signaling, thereby suppressing monocyte egress, while overcoming the direct effects of hypoxia on monocyte dynamics could improve outcomes in patients with hypoxic respiratory and cardiovascular injuries[38,39]. DCs are distributed in lymphoid and extra-lymphoid tissues and sense their surroundings via the membrane and cytoplasmic receptors. Hypoxia inhibits monocyte-derived DC maturation and upregulates chemokine receptor expression[40,41]. Recent research has elucidated that MCs adapt to long-term hypoxia by stabilizing HIF-1α, increasing MC extracellular traps, and decreasing phagocytic active cells[42]. However, higher CD8 + T cells and macrophages M2, and lower memory B cells and plasma cells were observed in OSA patients. Consistently, previous findings stated that a hypoxic burden on such IRGs might lead to alterations in metabolic substrates, local inflammatory molecules, hormones, mechanical stretch, and hypoxia-induced pathways, potentially contributing to metabolic and vascular damage on the target organ[43–45]. Although the therapeutic medicines for OSA remain unclear, drug targets based on DEIRGs may theoretically prevent OSA complications. HLA, positively related to most DEIRGs, is the expression product of the human major histocompatibility complex (MHC). MHC genes exhibit complex polygenic structures, high polymorphisms, and sensitivity to oxygen content[46]. Adequate evidence reveals the correlation between hypoxia and MHC, especially in tumor progression[47,48]. Additionally, IH in OSA upregulates the programed cell death 1 (PDCD1) ligand/receptor crosstalk and reduces CD8 + T-cell activation and cytotoxicity, thus increasing tumor incidence and aggressiveness and the risk of infections in these patients[49].

Apart from IRGs, we also explored miRNAs and TFs on DEIRGs. miRNAs are short non-coding RNAs that control gene expression post-transcriptionally, while TFs represent the central hub of multiple pathways in eukaryotic cells, both of which are key elements in response to hypoxia[50,51]. Numerous studies have noted that miRNAs and TFs are implicated in pathophysiological processes, like mitochondrial function, endoplasmic reticulum stress, cellular proliferation, and DNA repair, which may ultimately lead to tumor progression, cardiovascular sequelae, and abnormal lipid metabolism[51–54]. Our study constructed a network of miRNAs and TFs based on target genes, which contained 128 miRNAs and 40 TFs, providing a basis for further research on the immunological mechanisms of OSA.

Continuous positive airway pressure is the main highly effective treatment regimen for OSA, but its clinical effectiveness is restricted by poor tolerance and poor adherence in many patients. The effectiveness of alternative therapies like upper airway surgeries, oral appliances, and hypoglossal nerve stimulation depends on patients' specific conditions and is difficult to predict[55,56]. Although pharmacologic therapies for OSA cover drugs that enhance airway muscle tone, ventilatory drive, and arousal threshold, these therapies are still under investigation[56,57]. The present study predicted 172 potential target drugs/compounds associated with IRGs in OSA and its complications. Better matching of drugs to OSA patients according to dominant mechanisms may yield better results.

The present investigation identified the most promising DEIRGs and elucidated underlying immune-related pathophysiological performance in OSA. Two major limitations in this study warrant attention in future research. Firstly, the sample size for clinical validation was relatively small, which may have constrained the generalizability of the findings. Secondly, variations in different tissues could influence gene expression profiles. However, the results, in conjunction with bioinformatic analysis and validation after controlling for confounding factors, might be critical for screening DIERGs in OSA. Future large-scale longitudinal cohort studies should include diverse tissue samples and conduct in vitro validation to investigate the triggering signals and underlying pathophysiological mechanisms and provide direct evidence of complications.

Conclusions

This study systematically evaluated the immune-related patterns in OSA, investigated the immunologic functions of immunocytes, and constructed a drug network using bioinformatics approaches. Additionally, we identified potential immune-related biomarkers and offered novel insight into diagnosis, mechanism research, and management strategies for future studies on OSA.

Data availability

The original contributions presented in the study are included in the article/Supplementary Materials. Further inquiries can be directed to the corresponding author.

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Author contributions

The corresponding authors are responsible for the authenticity of the data. Conception and study design: J.Y.Z., H.L.Y. and W.J.H. Manuscript drafting and critical revision: E.H.Z., T.J.Z., S.K.Y., H.L.Y. Data collection and analysis: E.H.Z., X.T.W., T.J.Z. and J.G. Obtained funding: H.L.Y, S.K.Y., and W.J.H. All authors approved the final version of the manuscript to be published; they agreed on submission to this journal. All authors have agreed to be accountable for all aspects of the work.

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Competing interests

The authors declare no competing interests.

Ethics approval and consent to participate

This study was approved by the Ethics Committee of Shanghai Sixth People's Hospital Affiliated to Shanghai Jiao Tong University School of Medicine (Approval No: 2019-KY-050[K]) and registered at the Chinese

Clinical Trial Registry (No. ChiCTR1900025714; URL: http://www.chictr.org.cn/showproj.aspx?proj=43057). The research was conducted in accordance with the Declaration of Helsinki.

Consent for publication

All patients provided written informed consent.

Additional information

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