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MKI67 an potential oncogene of oral squamous cell carcinoma via the high throughput technology

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Abstract

Oral squamous cell carcinoma is a malignant tumor that occurs in the oral cavity, with poor prognosis and easy recurrence. However, the relationship between MKI67 and oral squamous cell carcinoma remains unclear. The oral squamous cell carcinoma datasets GSE138206, GSE146483 and GSE184616 were downloaded from the gene expression omnibus database, and the differentially expressed genes (DEGs) were screened. The protein-protein interaction network was constructed and analyzed by search tool for the retrieval of interacting genes database and Cytoscape software. Gene ontology (GO) and Kyoto encyclopedia of genes and genomes (KEGG) were used for functional enrichment analysis. GO and KEGG analyses were performed on the whole genome, as formulated by gene set enrichment analysis. comparative toxicogenomics database was used to identify the diseases most associated with the core genes. TargetScan was used to screen miRNA regulating central DEGs. A total of 1472 DEGs were identified. GO analysis showed that the differentially expressed genes were mainly enriched in the tissues of extracellular matrix, type i interferon signaling pathway, human papillomavirus infection, adhesion spot, hepatitis C and ECM-receptor interaction. Enrichment items were similar to GO and KEGG enrichment items of differentially expressed genes. 10 core genes were obtained, and their expression was different between oral squamous cell carcinoma and normal tissue samples. MKI67 is highly expressed in oral squamous cell carcinoma and may be an oncogene in oral squamous cell carcinoma.

Abbreviations: CTD = comparative toxicogenomics database, DEGs = differentially expressed genes, FDR = false discovery rate, GO = gene ontology, GSEA = gene set enrichment analysis, KEGG = Kyoto encyclopedia of genes and genomes, PPI = protein-protein interaction, STRING = search tool for the retrieval of interacting genes, TOM = topological overlap matrix, WGCNA = weighted gene co-expression network analysis.

Keywords: bioinformatics technology, gene targets, MKI67, oral squamous cell carcinoma

1. Introduction

Oral squamous cell carcinoma is the most common malignant tumor in the oral and maxillofacial region, which generally causes squamous cell carcinoma in the tongue, gingiva and mouth floor.[1] Oral squamous cell carcinoma has a poor prognosis and is usually prone to recurrence.[2] In recent years, with the increase of environmental pollution and people's living pressure, the number of patients with oral squamous cell carcinoma has increased year by year, and is on the rise.[3] Oral squamous cell carcinoma is characterized by mucosal leukoplakia and rough surface in the early stage, then develops into papillary or ulcerative type, and mucosal hyperplasia ulcer, pain, limited mouth opening, dysphagia and so on in the late stage. Oral squamous cell carcinoma will metastasiize, first to the cervical regional lymph nodes, and metastasis to the cervical lymph nodes will lead to swelling, and the enlarged mass is cancer. If the cervical nerves and blood vessels are invaded, it will cause serious consequences. If the important organs and tissues are invaded, it will cause death.[4] Oral squamous cell carcinoma is usually treated with radical resection, combined with chemotherapy and radiotherapy.[5] However, the pathogenesis of oral squamous cell carcinoma is still unclear, and the disease may be related to genetic factors, chromosome abnormalities, gene fusion and other factors. Therefore, it is particularly important to study the molecular mechanism of oral squamous cell carcinoma.

Bioinformatics technology is an interdisciplinary subject of biology and computer, and its research focus is mainly reflected in Genomics and Proteomics.[6] Bioinformatics is an emerging interdisciplinary subject in biological science, which starts from nucleic acid and protein sequences to analyze the biological information of the structure and function expressed

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The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

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in the sequence. With the development of bioinformatics technology, it is not only a simple analysis of genome and proteome data, but also a comprehensive analysis of known or new gene products.[7]

Marker of Proliferation MKI67 (MKI67) is a protein-coding gene that encodes a nuclear protein that is associated with and may be essential for cell Proliferation.[8] Mki67-related diseases include intracranial meningiomas and cervical intraepithelial neoplasia, and its related pathways include primary focal segmental glomerulosclerosis and DNA damage. Gene ontology (GO) annotations associated with this gene include RNA binding and protein C-terminal binding. MKI67 is usually used in pathological immunohistochemical reports, and its expression level often indicates the degree of cell proliferation activity.[9] In the pathological reports of patients, the higher the expression level of MKI67, the more active the cell proliferation, the higher the malignant degree, and the more likely to relapse and metastasis.[10] However, the relationship between MKI67 and oral squamous cell carcinoma remains unclear.

Therefore, this paper intends to use bioinformatics technology to explore the core genes between oral squamous cell carcinoma and normal tissues, and perform enrichment analysis and pathway analysis. Public datasets were used to validate the significant role of MKI67 in oral squamous cell carcinoma.

2. Methods

2.1. Oral squamous cell carcinoma dataset

In this study, the oral squamous cell carcinoma datasets GSE138206, GSE146483, and GSE184616 profiles were downloaded from the gene expression omnibus database(http://www.ncbi.nlm.nih.gov/geo/) generated by GPL570, GPL17077, and GPL24676. GSE138206 included 6 oral squamous cell carcinoma and 6 normal tissue samples, and GSE146483 included 8 oral squamous cell carcinoma and 3 normal tissue samples. GSE184616 included 15 oral squamous cell carcinoma and 15 normal tissue samples. Differentially expressed genes (DEGs) were used to identify oral squamous cell carcinoma.

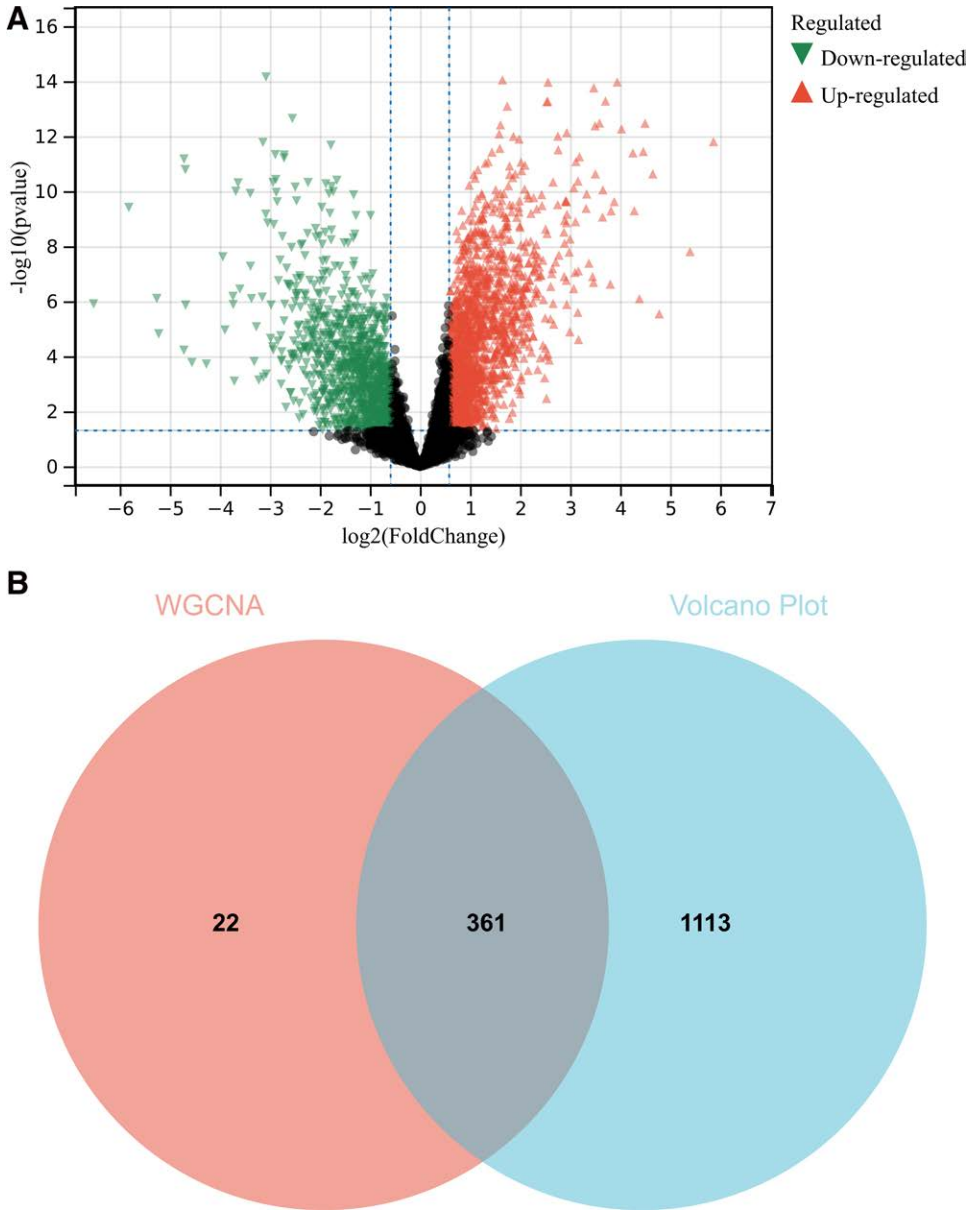

Figure 1. Functional enrichment analysis of DEGs. (A) 1474 DEGs were identified (B) Venn diagram was drawn for differential genes and intersection was taken. DEGs = differentially expressed genes.

2.2. To batch processing

For the merging and debatching of multiple datasets, we first combined the datasets GSE138206, GSE146483 and GSE184616 using the R software package. For the combination of multiple data sets, we first merged the data sets using the R package inSilicoMerging [DOD:10.1186/1471-2105-13-335] to obtain the merge matrix. Furthermore, we used the remove Batch Effect function of the R package limma (version 3.42.2) to remove the batch effect, and finally obtained the matrix after removing the batch effect and applied it to the subsequent analysis.

2.3. Screening of DEGs

The R package "limma" was used for probe summary and background correction of the merging matrices for GSE138206,

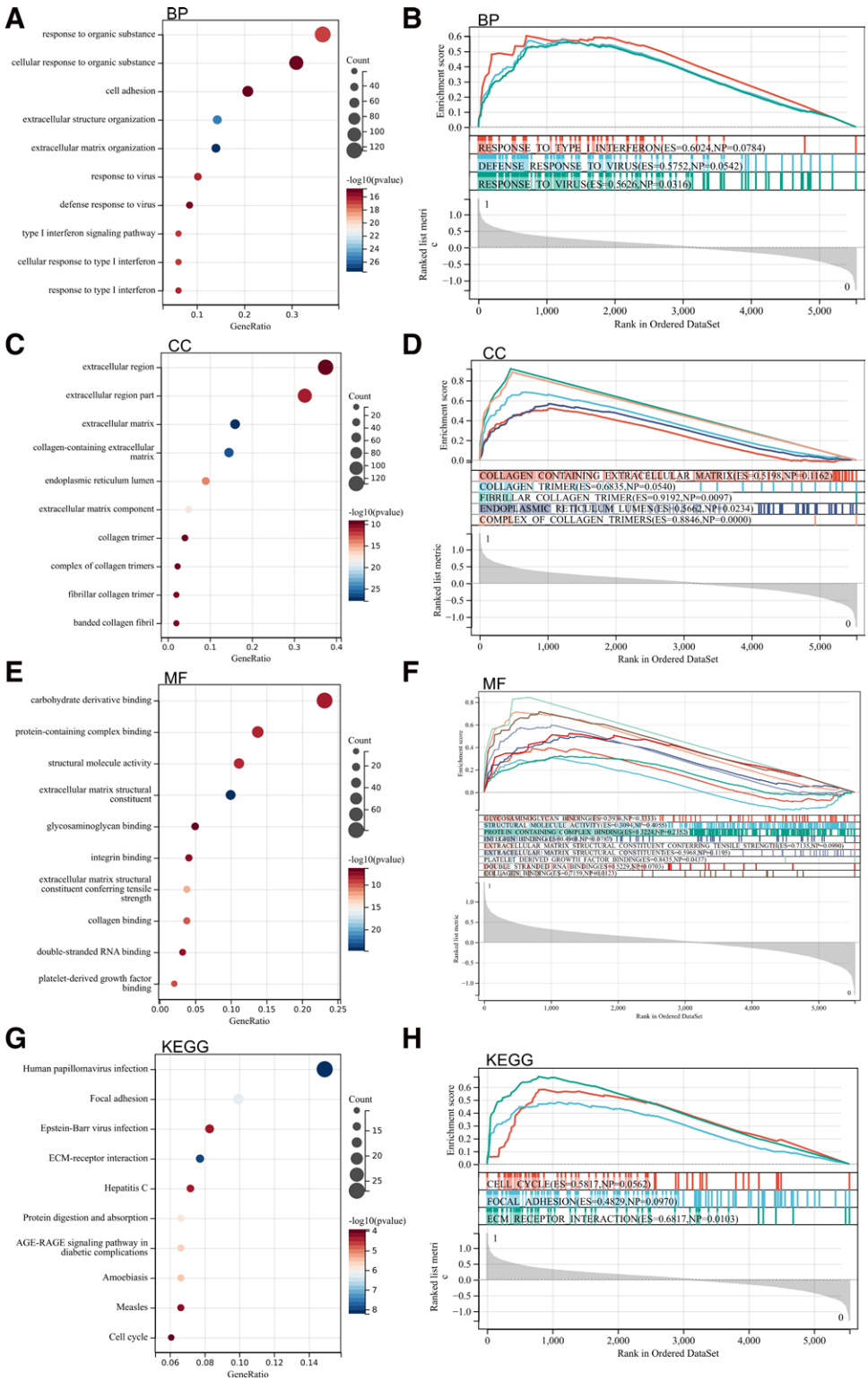

Figure 2. Functional enrichment analysis of DEGs and GSEA enrichment analysis. DEGs = differentially expressed genes, GSEA = gene set enrichment analysis.

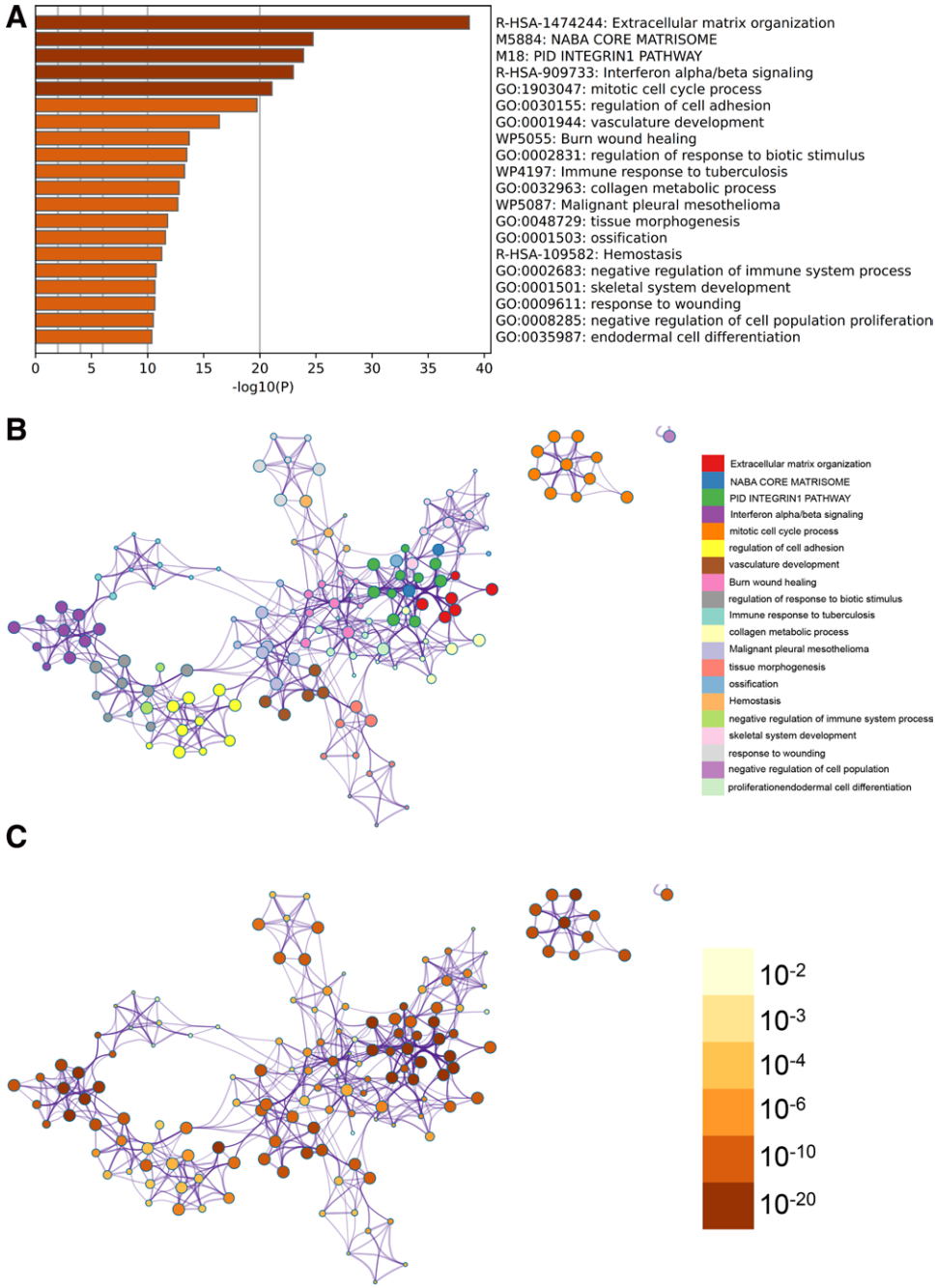

Figure 3. Enrichment analysis by Metascape. (A) GO enrichment term. (B) enriched networks colored by enriched terms (C) enriched networks colored by *P* values. GO = gene ontology.

GSE146483, and GSE184616. Benjamini-Hochberg method was used to adjust raw *P* values. Fold change was calculated using the false discovery rate (FDR). The cutoff criterion for DEG was FDR < 0.05. The volcano diagram was made, and the intersection DEGs were taken by Venn diagram.

2.4. Weighted gene co-expression network analysis (WGCNA)

First, we calculated the median absolute deviation of each gene separately using the post-batch merging matrices of GSE138206, GSE146483 and GSE184616, and excluded the top 50% of genes with the smallest median absolute deviation. The outlier genes and samples were removed by the good Samples Genes method of R software (https://www.r-project.org/) package weighted gene co-expression network analysis (WGCNA), and WGCNA was further used to construct a scale-free co-expression network. Specifically, first, Pearson correlation matrix and average linkage method were performed for all paired genes. Then, using the power function A_mn = | C_mn | ^ β to construct weighted adjacency matrix (Pearson correlation between C_mn = Gene_m and Gene_n; A_mn = adjacency between Gene m and Gene n). β is a soft threshold parameter that can emphasize strong correlations between genes and attenuate the effects of weak and negative correlations. After selecting a power of 10, adjacency is transformed into a topological overlap matrix (TOM), which measures the network connectivity of a gene, defined as the sum of its adjacencies with all other genes, for the network

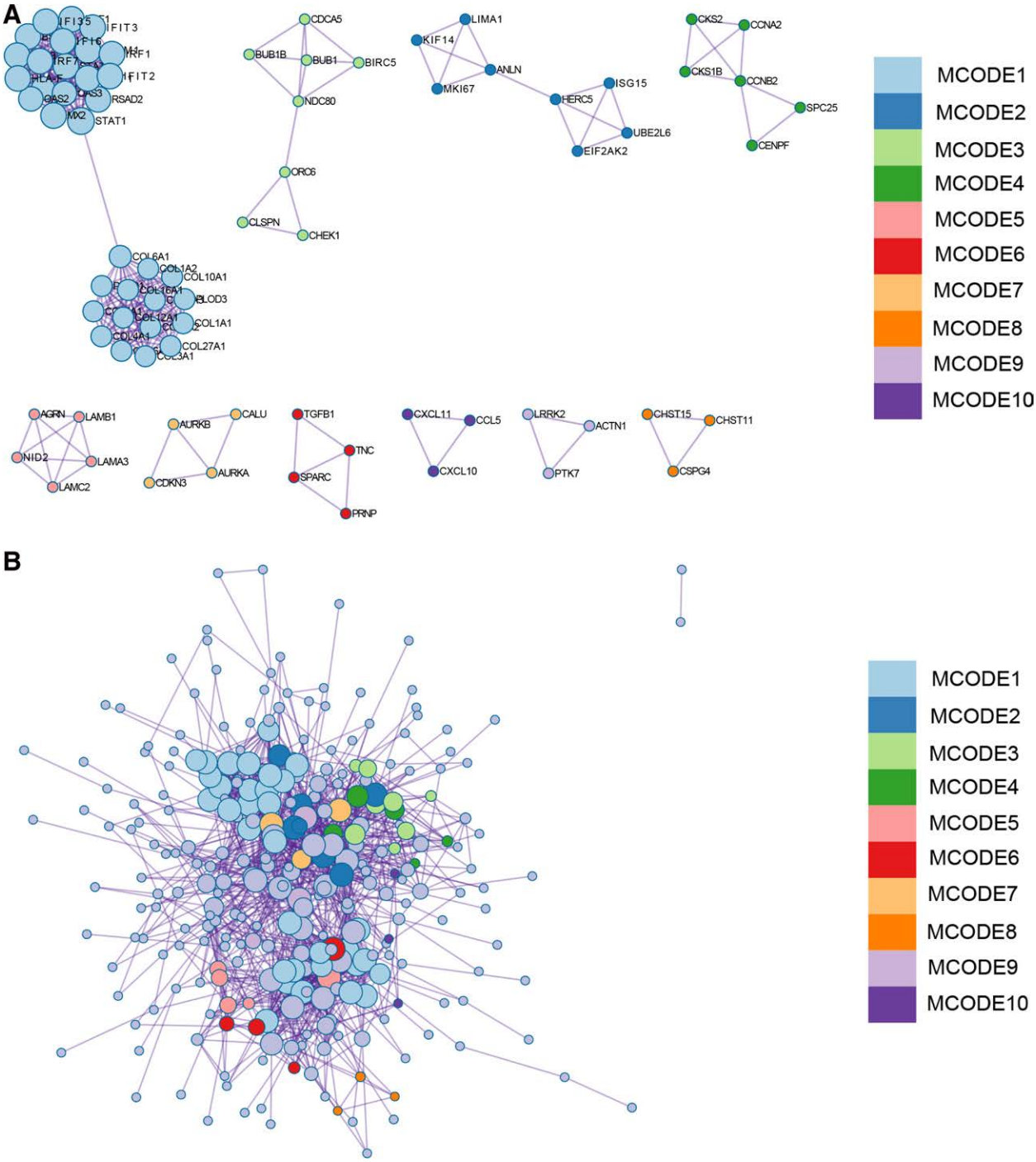

Figure 4. Enrichment analysis by Metascape.

gene ratio, and the corresponding degree of dissimilarity (1-TOM) is calculated. To classify genes with similar expression profiles into gene modules, average linkage hierarchical clustering was performed according to TOM-based dissimilarity measures, with a minimum size (genome) of 30 for the gene dendrogram. The sensitivity was set to: 3. To further analyze modules, we calculated the dissimilarity of module characteristic genes, selected a cut line for the module dendrogram, and merged some modules. In addition, modules with distance < 0.25 were merged, and finally 12 co-expression modules were obtained. It is worth to note that the gray module is considered to be a gene set that cannot be assigned to any module.

2.5. Construction and analysis of protein-protein interaction (PPI) network

Search tool for the retrieval of interacting genes (STRING) database (http://string-db.org/) designed to collect, scoring and integrate all publicly available protein sources - protein interaction, and predicted by calculation to complement these sources. In this study, the list of differential genes was input into the STRING database to construct a protein-protein interaction (PPI) network for predicting core genes (confidence > 0.4). Cytoscape software can provide biologists with biological network analysis and 2-dimensional (2D) visualization. In this study, Cytoscape software was used to visualize the PPI network formed by STRING

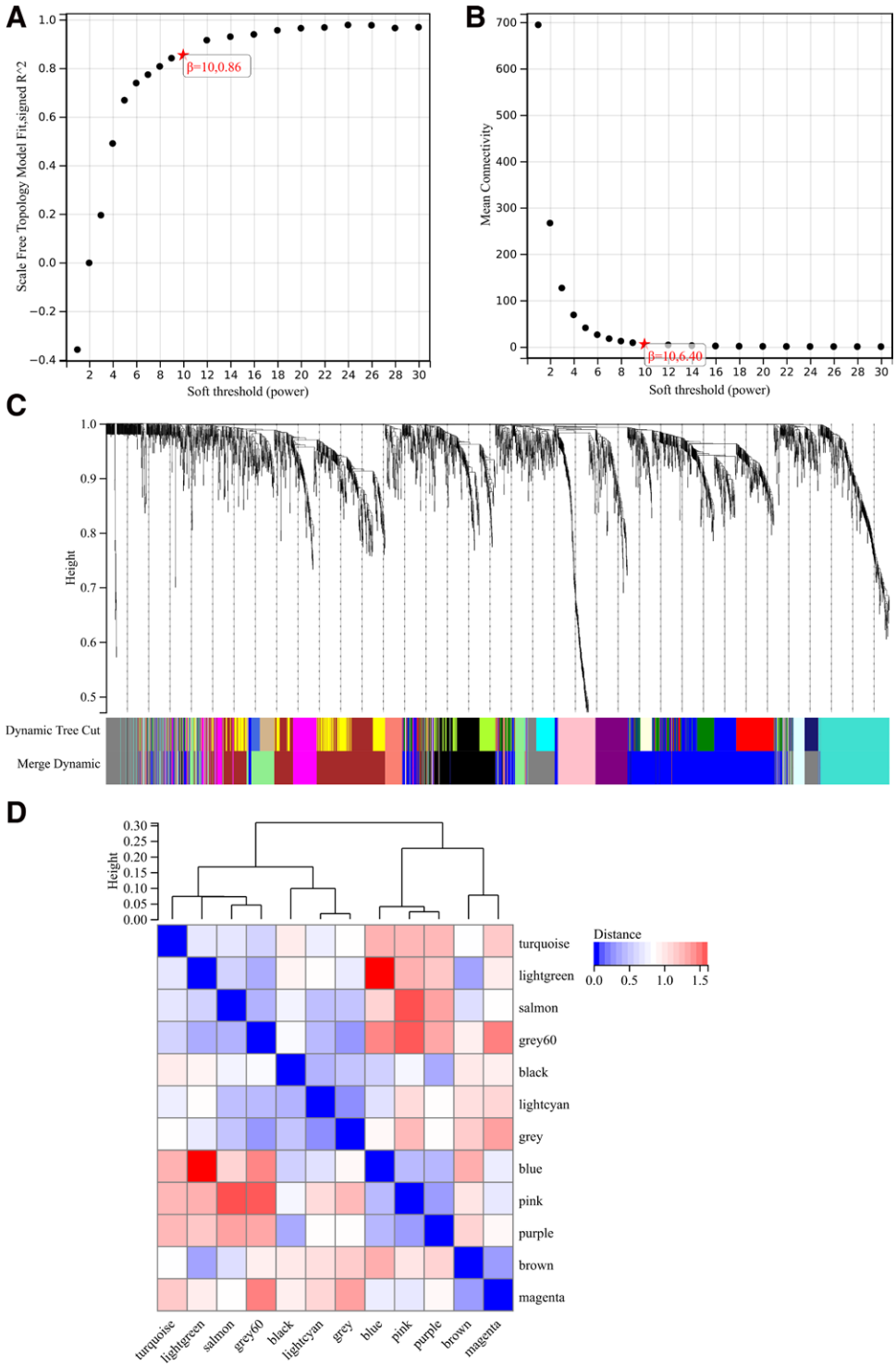

Figure 5. WGCNA analysis. (A) β = 10,0.86 (B) β = 10,6.40 (C) Hierarchical clustering trees of all genes were constructed and 22 important modules were generated. (D) Interaction between modules.

database and predict core genes. First, we imported the PPI network into Cytoscape software, found the module with the best correlation by MCODE, calculated the ten genes with the best correlation and took the intersection by MCC algorithm, and exported the list of core genes after visualization.

2.6. Functional enrichment analysis

GO and Kyoto encyclopedia of genes and genomes (KEGG) analysis are computational methods to evaluate gene functions and biological pathways. This study will Wayne figure out the difference of gene list input KEGG rest API (https://www.kegg.jp/kegg/rest/keggapi.html) to obtain the latest KEGG Pathway gene annotation, as the background, to map genes to background in the collection. The R package cluster Profiler (version 3.14.3) was used for enrichment analysis to obtain the results of gene set enrichment. The GO annotation of genes in the R package org.Hs. for example,.db (version 3.1.0) was also used as the background, and the genes were mapped to the background set, with the minimum gene set of 5 and the maximum gene set of

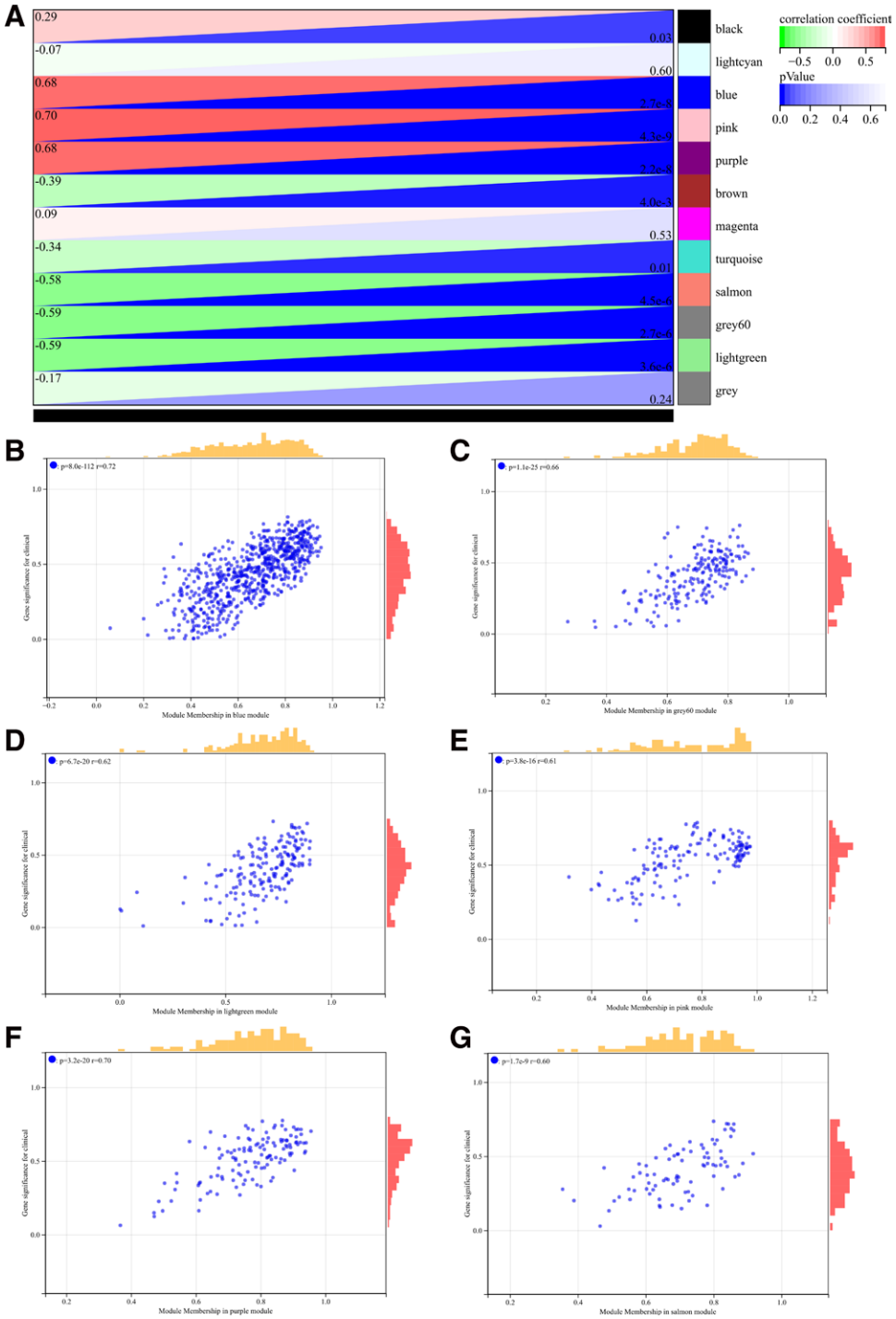

Figure 6. WGCNA analysis. (A) Heat map of module and phenotype correlation. (B-G) Scatter plot of GS and MM correlation of related hub genes.

5000. A *P* value of < .05 and a FDR of < 0.25 were considered to be measures of statistical significance

In addition, the Metascape database can provide comprehensive gene list annotation and analysis resources with visual export. We use the Metascape database (http://metascape.org/gp/index.html), for the above differences in gene enrichment of function analysis and export list.

2.7. GSEA analysis

For Gene Set enrichment analysis (GSEA), we derived from GSEA (DOI: 10.1073/ pnas. 0506580102, http://software.broadinstitute.org/gsea/index.jsp) web site for the GSEA software (version 3.0). Samples were divided into 2 groups based on oral squamous cell carcinoma and normal tissues, and obtained from Molecular Signatures Database (DOI: 10.1093/ bioinformatics/btr260, http://www.gsea-msigdb.org/gsea/downloads.jsp) to download the c2.cp.kegg.v7.4.symbols.gmt to evaluate relevant pathways and molecular mechanisms. Based on gene expression profile and phenotype grouping, the minimum gene set was 5, the maximum gene set was 5000, 1000 resampling times, *P* value < .05 and a FDR of < 0.25 were considered statistically significant. GO and KEGG analyses were performed on the whole genome. Developed by GSEA.

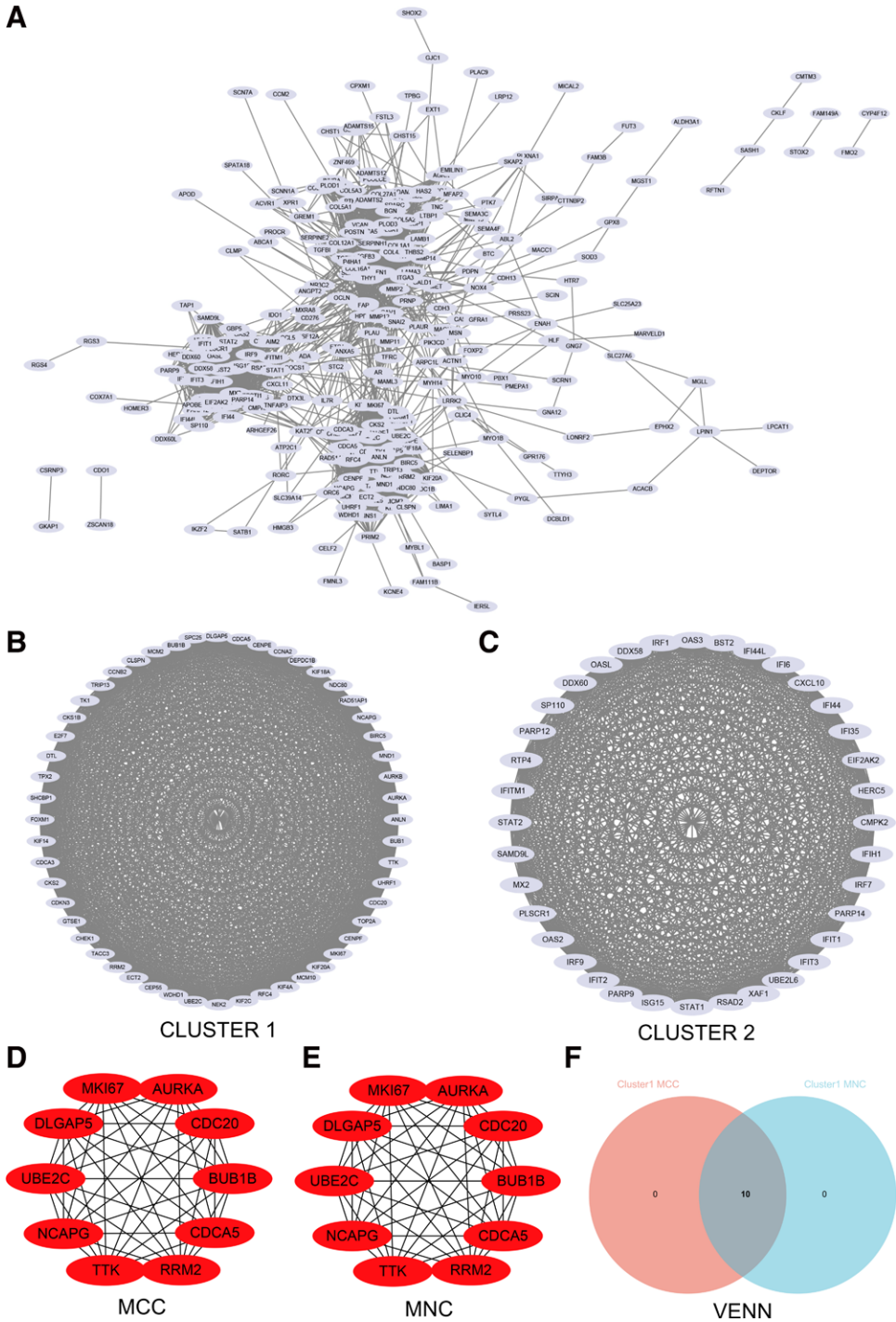

Figure 7. Construction and analysis of protein-protein interaction (PPI) network. (A) PPI network of DEGs (B) Core gene clusters: CLUSTER 1 (C) Core gene clusters: CLUSTER 2 (D) The algorithm of MCC identified the central genes (E) the MNC algorithm identified the central genes (F) Venn diagram and took the intersection to obtain 10 core genes (CDCA5, MKI67, CDC20, AURKA, BUB1B, TTK, DLGAP5, UBE2C, NCAPG, RRM2). DEGs = differentially expressed genes, PPI = protein-protein interaction.

2.8. Heat map of gene expression

The R package heatmap was used to make a heatmap of the expression levels of the core genes found by the 2 algorithms in the PPI network in GSE138206, GSE146483 and GSE184616 to visualize the expression differences of core genes between oral squamous cell carcinoma and normal tissue samples.

2.9. CTD analysis

Comparative toxicogenomics database (CTD) integrates a large number of interaction data between chemicals, genes, functional phenotypes and diseases, which provides great convenience for the study of disease-related environmental exposure factors and potential drug mechanisms. We entered the core genes into the CTD website, found the most relevant diseases to the core genes, and used Excel to draw the differential expression radar map of each gene.

2.10. miRNA

TargetScan (www.targetscan.org) is an online database, is used to forecast analysis of micrornas and target genes. TargetScan was used to screen miRNA regulating central DEGs.

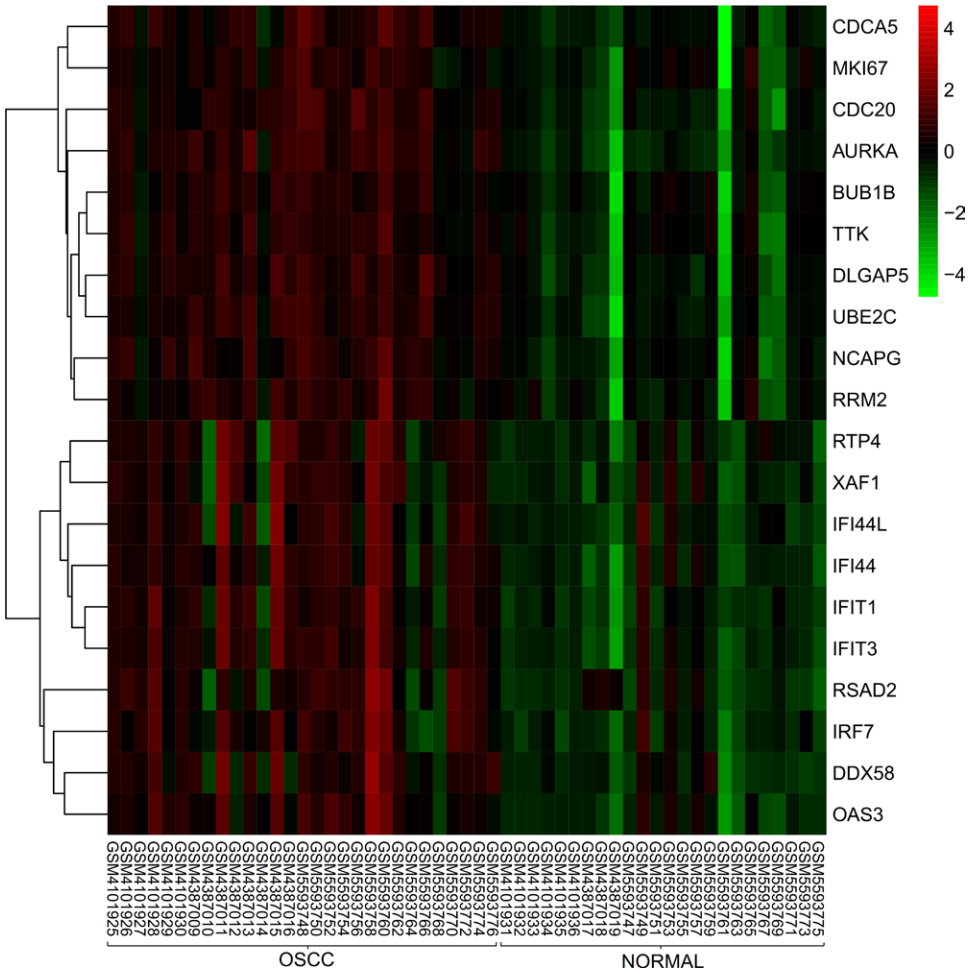

Figure 8. Heat map of gene expression. Core genes were differentially expressed between oral squamous cell carcinoma and normal tissue samples.

3. Results

3.1. Functional enrichment analysis

1.3.1. Functional enrichment analysis of DEGs. In this study, a total of 1474 DEGs were identified based on DEGs identified in the debatching merger matrix of GSE138206, GSE146483, and GSE184616 (Fig. 1A).

We then performed GO and KEGG analysis of these differentially expressed genes. According to GO analysis, they were mainly enriched in the organization of extracellular matrix, type i interferon signaling pathway, human papillomavirus infection, focal adhesion, hepatitis C, and ECM-receptor interaction. (Fig. 2A, C, E, G).

2.3.1. GSEA analysis. In addition, we performed GSEA enrichment analysis on the whole genome to find possible enrichment terms in non-differentially expressed genes. The results are shown in the figure, and the enrichment terms are similar to GO and KEGG enrichment terms for differentially expressed genes. (Fig. 2B, D, F, H)

3.3.1. Enrichment analysis by metascape. The content enriched by Metascape includes GO enriched terms (Fig. 3A) and has an enriched network colored by enriched terms and *P* value (Fig. 3B, C and Fig. 4).

4.3.1. WGCNA analysis. The selection of soft threshold power is an important step in WGCNA analysis. Network topology analysis was performed to determine the soft threshold power. The soft threshold power in the WGCNA analysis was set to 9, which is the lowest power for a scale-free topological fit index of 0.9 (Fig. 5A, B). Hierarchical clustering trees were constructed for all genes and 22 significant modules were generated (Fig. 5C). The interaction between these modules was then analyzed (Fig. 5D). Heatmaps of module and phenotype correlations (Fig. 6A) and scatter plots of GS and MM correlations of related hub genes (Fig. 6B–G) were also generated.

We calculated the module characteristic vector correlation with the expression of genes for MM, according to the cutting standard (|MM| > 0.8), and in the clinical significant module 12 high connectivity gene was identified as hub. We also plotted the Venn diagram and took the intersection of the differential genes screened by WGCNA with DEGs (Fig. 1B).

5.3.1. Construction and analysis of protein-protein interaction (PPI) network. The PPI network of DEGs was constructed from the STRING online database and analyzed by Cytoscape software (Fig. 7A), and 2 core gene clusters (Fig. 7B, C) were obtained. Two different algorithms were used to identify the central genes (Fig. 7D, E), Venn diagram was drawn and intersection was taken (Fig. 7F). 10 core genes (CDCA5, MKI67, CDC20, AURKA, BUB1B, TTK, DLGAP5, UBE2C, NCAPG, RRM2) were obtained.

6.3.1. Heat map of gene expression. The difference in expression of core genes between oral squamous cell carcinoma and normal tissue samples is shown in the heat map (Fig. 8).

7.3.1. Survival analysis. Figure 9 shows box plot information related to oral squamous cell carcinoma.

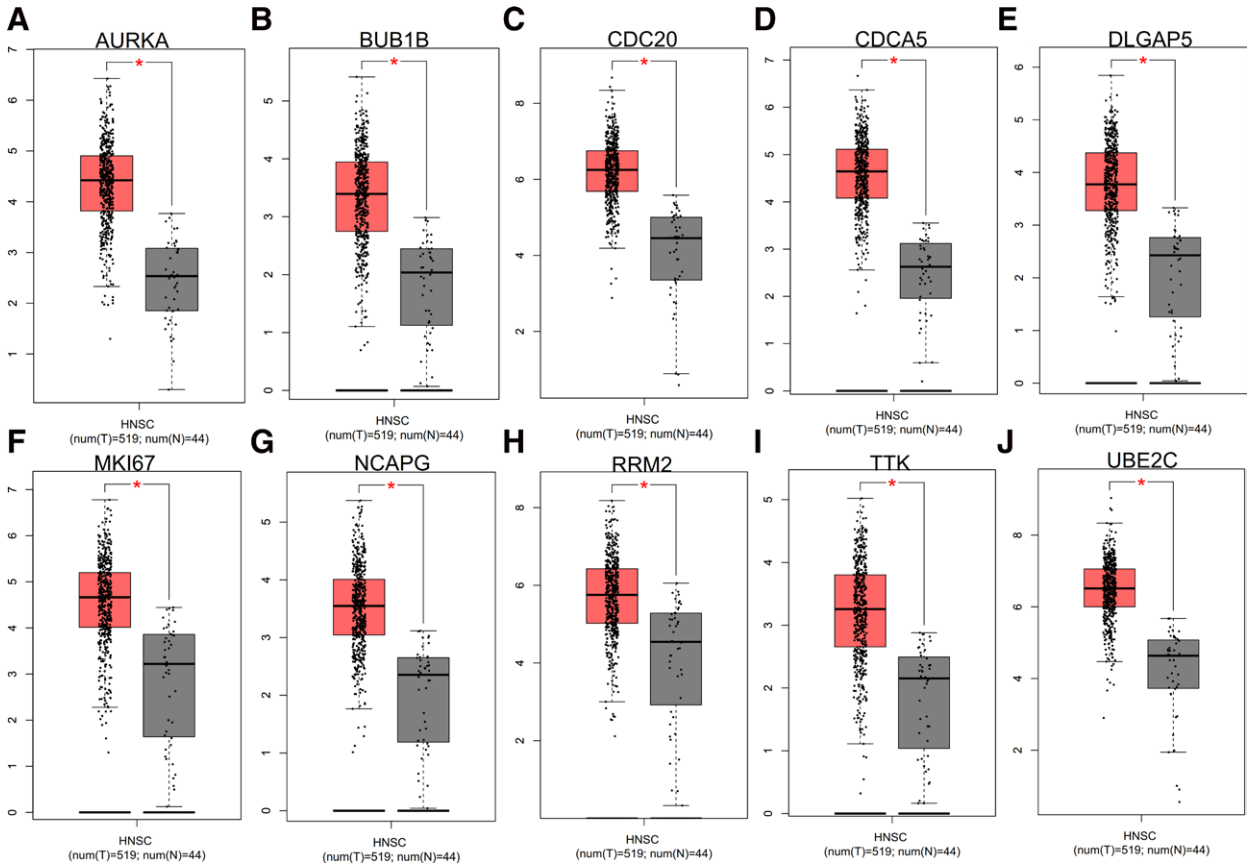

Figure 9. Survival analysis. (A) AURKA (B) BUB1B (C) CDC20 (D) CDCA5 (E) DLGAP5 (F) MKI67 (G) NCAPG (H) RRM2 (I) TTK (J) UBE2C.*$P < .05$.

8.3.1. CTD analysis. In this study, we entered the list of hub genes into the CTD website to find diseases related to core genes, which improved the understanding of gene-disease association. Ten genes (CDCA5, MKI67, CDC20, AURKA, BUB1B, TTK, DLGAP5, UBE2C, NCAPG, RRM2) were found to be related to breast tumors, peripheral nervous system diseases, colorectal tumors, esophageal squamous cell carcinoma, liver cancer, hepatocellular carcinoma, breast tumors, and ovarian tumors. (Fig. 10)

9.3.1. Prediction and functional annotation of miRNA associated with hub genes. In this study, we input the list of hub genes into Targetsacan to find relevant miRNAand improve the understanding of gene expression regulation (Table 1). We found that the related miRNA of RRM2 gene were hsa-let-7d-5p, hsa-let-7i-5p and hsa-let-7e-5p. The related miRNA of AURKA gene is hsa-Mir-490-3p. The related miRNA of TTK gene was hsa-miR-455-3p.1. The related miRNA of DLGAP5 gene was hsa-miR-409-5p. The related miRNA of NCAPG gene was hsa-miR-9-5p.

4. Discussion

Oral squamous cell carcinoma is the most common oral malignant tumor. The malignant transformation of oral squamous epithelium and the formation of malignant tumors are more complicated to treat.[11] Because the oral mucosa is close to the jaw, oral squamous cell carcinoma is easy to invade the jaw, involve the mandibular canal, injure the mandibular nerve, and cause referred pain. Moreover, it has great damage to the oral mucosa, which can be manifested as local ulceration, and in severe cases, it can affect life safety.[12] In-depth exploration of the molecular mechanism of oral squamous cell carcinoma is extremely important for the research of targeted drugs. The main result of this study is that MKI67 is highly expressed in oral squamous cell carcinoma, and MKI67 may be an oncogene in oral squamous cell carcinoma.

MKI67 is an important proliferative marker in pathology and is restricted to the accessory basal cell layer of normal stratified squamous mucosa. The only relevant feature of MKI67 is that it is not expressed in resting cells but is expressed when cells proliferate.[13] MKI67 is a nuclear and nucleolar protein that is only expressed in the active phase and cell cycle (G1, S, G2 and M phases), but not in the quiescent phase (G0 and early G1).[14] The expression level of MKI67 increased from the G1 phase to the mitotic phase and decreased rapidly thereafter. MKI67 protein expression could be assessed in nuclei in the G1/S/G2 phase and mitotic phase, but not in quiescent nuclei in the G0 phase.[15,16] Therefore, the expression of MKI67 can reflect the proliferative status of cells. MKI67 was expressed in the nucleus in all cell cycles except the G0 phase. There are several different methods for detecting MKI67 protein in tissue and cell cultures, most of which are specific to human cells.[17] MKI67 protein is degraded by the proteasome in G1 phase and is degraded upon cell cycle exit, and its mRNA is expressed in a cell cycle-dependent manner.[18,19] MKI67 protein was low expressed or absent in G0 and G1 phases and accumulated in S, G2 and M phases.[20] The low levels of MKI67 in the G0 and G1 phases depend on the time the cell spends in G0, and proteasomal degradation leads to loss of MKI67 at telophase of mitosis as well as in the G0 and G1 phases.[21] Cell cyclin-dependent expression is characteristic of MKI67 protein, and detection of MKI67 has been an indicator of cell proliferation.[22] Persistently low levels of MKI67 after entering G0 can be used as an indicator of the resting state. MKI67 can be detected at ribosomal

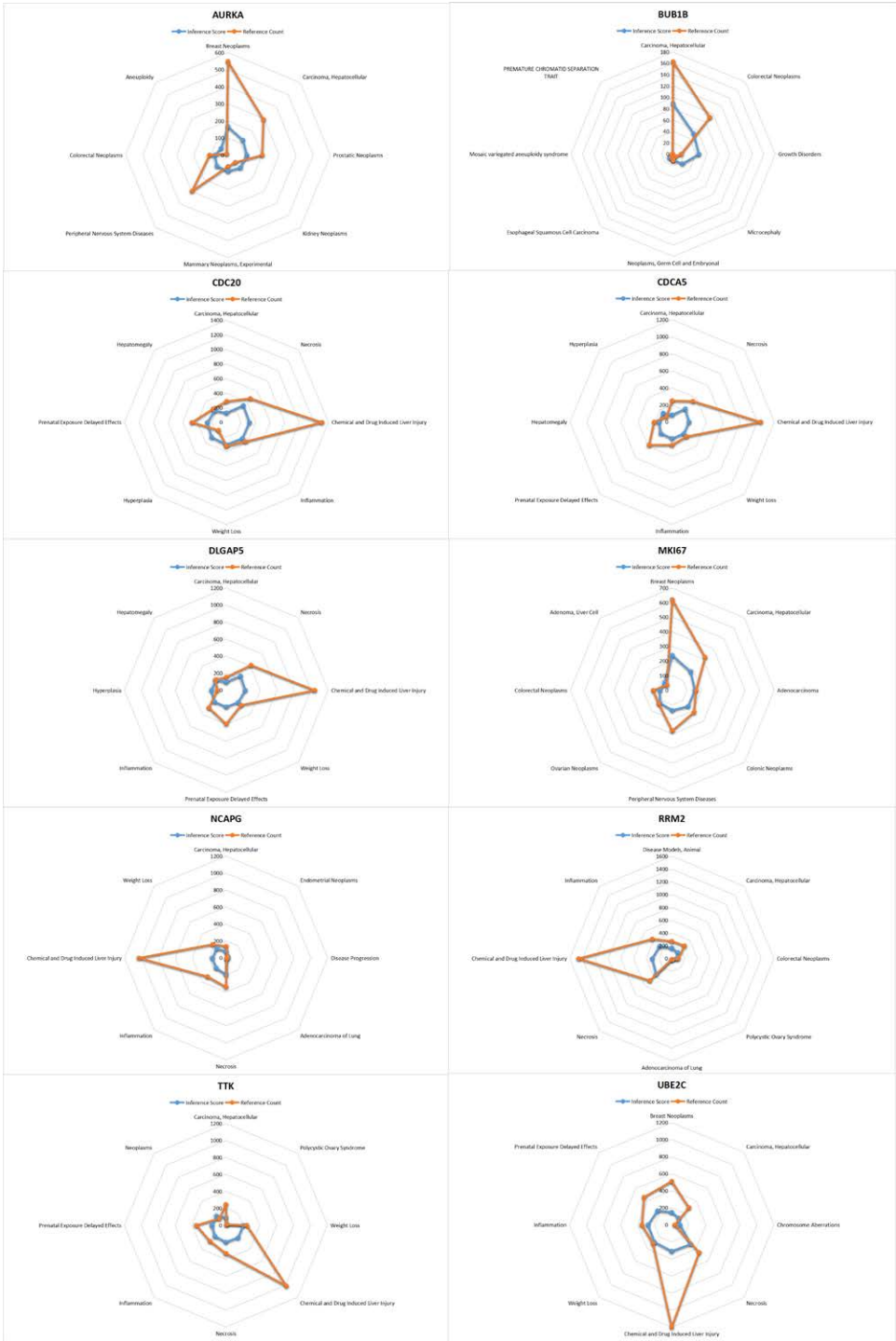

Figure 10. Analysis of CTD.10 hug genes (CDCA5, MKI67, CDC20, AURKA, BUB1B, TTK, DLGAP5, UBE2C, NCAPG, RRM2) were related to breast tumors, peripheral nervous system diseases, colorectal tumors, esophageal squamous cell carcinoma, liver cancer, hepatocellular carcinoma, breast tumors, and ovarian tumors. CTD = comparative toxicogenomics database.

RNA synthesis sites in quiescent cells.[23] Since MKI67 is degraded upon cell cycle exit, this may alter chromatin structure. MKI67 degradation may be involved in heterochromatin rearrangements observed during the onset of senescence. MKI67 is required for heterochromatin compaction, and its degradation may be involved in heterochromatin breakdown that occurs at the onset of aging. Heterochromatin reorganization caused by down-regulation of MKI67 expression does not affect cell cycle progression or cell proliferation, but may be involved in the remodeling of gene expression.

Cancer cells spend less time between mitosis and reentry into S-phase than normal cells. Thus, low MKI67 expression relative to undetectable expression in the G0 or G1 phase is an indicator of prognosis in malignant disease.[24] In cultured cells, MKI67 levels were highest in the G2 and mitotic phases.[25] MKI67 promotes attachment of chromosomes to the mitotic spindle and movement of individual chromosomes by covering the chromosome surface and forming a membrane-independent intracellular compartment.[26] By covering chromosomes and forming perichromosomal regions, MKI67

Table 1

A summary of miRNAs that regulate hub genes.

	Gene	miRNA		
1	RRM2	hsa-let-7d-5p	hsa-let-7i-5p	hsa-let-7e-5p
2	AURKA	hsa-miR-490-3p		
3	TTK	hsa-miR-455-3p.1		
4	DLGAP5	hsa-miR-409-5p		
5	NCAPG	hsa-miR-9-5p		
6	UBE2C	none		
7	CDCA5	none		
8	MKI67	none		
9	CDC20	none		
10	BUB1B	none		

miRNAs = micro ribose nucleic acids.

also contributes to heterochromatin organization.[21] MKI67 is involved in all active phases of the cell cycle (G1, S, G2, and mitotic phases) and is not expressed in quiescent (G0 phase) cells, making it a good marker for determining tumor growth fraction.[16] High expression of MKI67 is associated with high cell proliferation, and MKI67 is expressed in the nucleus throughout the cell cycle. Normally, MKI67 shows cortical nucleolar localization during interphase and is recruited to condensed chromosomes at mitosis.[27,28] The up-regulation of MKI67 is closely related to the degree of immune infiltration of B cells, CD4 + T cells, CD8 + T cells, neutrophils, neutrophils, macrophages, DCs and many functional T cells, and MKI67 plays an important role in tumor microenvironment (TME) and innate immunity.

MKI67 is a cell cycle regulatory protein, a marker of proliferating nuclei, and an indicator of tumor proliferation rate. With the increase of MKI67 expression level, the proliferation activity of tumor cells also increased. Excessive cell division and proliferation have been shown to be important components of malignant transformation.[29] MKI67 is highly expressed in cancer cells, which is positively correlated with advanced clinical stage, histological type, tumor differentiation degree, myometrial invasion and other invasive pathological factors, and negatively correlated with prognosis, which can be used as a predictor of cancer prognosis.[30,31] In addition, MKI67 has been extensively studied in retrospective articles as a candidate prognostic predictor of cancer proliferation.[32] A large body of evidence supports the role of MKI67 in cancer diagnosis.[33] Studies have shown that MKI67 is closely related to the prognosis, immune infiltration and T cell exhaustion of hepatocellular carcinoma.[34] MKI67 is up-regulated in uterine leiomyosarcoma and is a potential biomarker for its diagnosis.[29] MKI67 plays a key role in evaluating neoadjuvant endocrine therapy for hormone receptor-positive breast cancer.[35] The expression of MKI67 is up-regulated in patients with late TNM stage, poor tumor differentiation, serosal and surrounding organ invasion, lymph node metastasis and distant metastasis, suggesting that MKI67 can be used as an indicator to predict the progression of gastric cancer and identify high-risk patients, so as to optimize individualized treatment management and improve the prognosis of gastric cancer patients.[36] A recent study confirmed that specific MKI67 splice variants contribute to cancer progression by affecting the cell cycle.[37] MKI67 proliferation index can be used as a marker of recurrence time of intracranial meningiomas.[38]

MKI67 is a nuclear protein expressed in all proliferating vertebrate cells and is a widely used biomarker to estimate the proportion of dividing cells to grade tumors. MKI67 is important for positioning nucleolar material to the periphery of mitotic chromosomes and constructing perinuclear heterochromatin, and data suggest that MKI67 also has a critical role in cancer development.[39] Another aspect of MKI67 transcriptional regulation is the down-regulation of MKI67 by the tumor suppressor p53.[40] MKI67 is a target of the p53-p21-DREAM pathway, leading to cell cycle arrest.[41] MKI67 is a significant cancer marker, and the p53 tumor suppressor indirectly down-regulates the transcription of MKI67 gene. Mechanisms controlling MKI67 gene expression and MKI67 protein synthesis during cell cycle, DNA damage induction, and p53 activation.[8] MKI67 is closely related to the proliferation, growth and progression of tumor cells, and is widely used in routine clinicopathological research. MKI67 reflects the proliferation rate of tumor cells and is associated with the progression, metastasis and prognosis of many different malignancies.[42,43] MKI67 protein can be used as a tumor proliferation marker.[44] Therefore, it is speculated that MKI67 plays a key role in the progression of oral squamous cell carcinoma.

Despite the rigorous bioinformatics analysis in this paper, there are still some shortcomings. In this study, no animal experiments were performed to further verify its function. Therefore, in future research, we should conduct in-depth exploration in this aspect.

In conclusion, MKI67 is highly expressed in oral squamous cell carcinoma, and MKI67 may be an oncogene of oral squamous cell carcinoma and provide a new direction for the treatment of oral squamous cell carcinoma.

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