IDENTIFICATION OF POTENTIAL PATHOGENIC MECHANISMS BASED ON MULTIFACTORIAL MEDIATED DYSFUNCTION OF ORAL TUMORS

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ABSTRACT

An oral tumor is cancer formed by abnormal hyperplasia or lesions in soft and hard tissues in the mouth. The clinical manifestations are oral ulcers, and severe oral tumors can threaten life and health. At present, people do not understand the pathogenesis of multi-factor-mediated oral cancer. Therefore, based on a comprehensive strategy, we explore multi-factorially driven oral tumor pathogenesis and potential therapeutic targets. Based on the gene expression profile matrix of oral tumors, we constructed an interaction network for the specific expression of proteins in the oral tissues of patients, and thus identified a dysfunction module. Those specifically expressed proteins often play an important role in oral tumors. The internal drive gene in the protein subnet best characterizes the molecular mechanism of oral tumors, and it is a potential core molecule. Based on the predictor analysis of regulators, we identified a series of ncRNAs and transcription factors that have possible regulatory effects on oral tumors. These pivotal regulators are an essential component of the manipulation module network genes. Overall, based on a comprehensive functional block analysis, we identified proteins, and their interactions expressed explicitly in the patient's oral tissues. The regulation of potential drugs and the pharmacological effects of binding to biological targets also provide a valuable reference for drug developers to conduct drug relocation studies.

Keywords: *Oral tumor, dysfunction module, pivotal regulator, the pathogenic mechanism.*

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Introduction

Oral tumors are common malignant tumors in clinical medicine[1, 2], caused by pathogens (bacteria, fungi, viruses). The disease has diverse developmental characteristics including oral cancer, Oral Squamous Cell Carcinoma (OSCC), oral tongue squamous cell carcinoma (OTC), Oral and oropharyngeal cancer (OPC)[3-6]. In precancerous lesions, it is often accompanied by a pathological characterization of leukoplakia, erythema and lichen planus. At present, lichen planus is considered to be a potentially malignant condition[7]. Also, visible oral ulcers and masses are also early signs of oral cancer[8]. Clinically, oral cancer is mainly characterized by pain in the tongue and mandible, loose teeth, difficulty swallowing and abnormal sound[9, 10]. The severe danger of oral tumors has led many scholars to carry out experimental research on pathogenic mechanisms.

Genome-wide association analysis (GWAS) identified seven genes involved in oral and oropharyngeal cancer[11]. Also, smoking and drinking are the main risk factors, and other risk factors include papillomavirus infection and (HPV)-16 infection[12, 13]. Oral tumors occur mostly in middle-aged and older adults, but in recent years there has been

a downward trend in age, and the number of young and female patients has increased[8, 14]. The oncogenic E3 ligase of the biomacromolecule targets a tumor suppressor protein that has a ubiquitin-mediated degradation. Moreover, its inhibition of specific targeting of oncogene products. It induces an imbalance between oncogenic signaling and tumor suppressor pathway through dysregulation, which leads to cell proliferation and metastasis of malignant tumors such as oral cancer[15].

Jumonji-C domain-containing protein 5 (JMJD5) down-regulates and inhibits OSCC metastasis and induces apoptosis through the p53//NF-κB pathway[16]. The gene RACK1 is significantly expressed in protein interaction networks (PPIs), and the gene RACK1 interacts directly with two subunits and 14 differential proteins[17]. Also, researchers such as Valverde also found that Hedgehog (HC) molecules are significantly involved in autocrine and paracrine signaling pathways and are associated with tumor angiogenesis[18]. The critical long non-coding RNA (lncRNA) node was confirmed to be positively correlated with clinical stage, lymph node metastasis, distant metastasis, and survival status, which may be a potential key factor in the pathogenesis of OSCC[19]. It is noteworthy that lymph node metastasis and invasion are considered to be one of the causes of death in oral cancer. Silencing of critical regulatory genes CCND1, JUN, and SPP1OECM-1 in human oral cancer cell lines can promote oral cancer cell invasion[20].

The phospholipid inositol 3-kinase (P13-kinase) pathway is dysregulated in immune cells, and this phenomenon will affect the surgical treatment of patients with oral tongue squamous cell carcinoma (SCC). This finding confirms the effectiveness of immunosuppression in oral tumors and also helps guide the clinical development of P13-kinase immunosuppressive agents[21].

Studies have revealed the pathogenic mechanisms of oral tumors and the potential role of chronic immunosuppression, but this is far from a comprehensive understanding of the molecular mechanisms in oral tumors. Based on this, this study aims to explore the targeted regulation of ncRNA, transcription factors and other pivotal regulators of oral tumors by analyzing the functional modules under the combined action of multiple factors.

It is conducive to deepen the understanding of the pathogenesis of oral tumors and provide a new theoretical basis and research direction for its potential therapeutic targets.

Materials and methods

Data resource

We first collected a set of gene expression profiles for oral tumors from the NCBI Gene Expression Omnibus database (GEO Dataset)[41], numbered GSE74530. This data is derived from the genes and expression of oral tumor tissue and adjacent non-tumor vocal tissue samples from the same patient.

Furthermore, we downloaded all human protein-protein interaction data from the STRING v10 database[42] to construct differential miRNA-related targeting gene PPIs. We then screened the ncRNA-mRNA interaction pairs with a score >= 0.5 from the RAID v2.0 database[43] for use in predicting target genes. At the same time, all human transcription factor target data was downloaded and used in the TRRUST v2 database[44].

Difference analysis

Differential expression analysis of miRNA expression profile data in this study was performed using the R language limma package[45-47].

Initially, we used the background correction function to perform background correction and normalization of the data. Then, the control probe and the low expression probe were filtered out using the normalize Between Arrays function and the quarantine normalization method. Further, based on the lmFit and eBayes functions and using default parameters, the differentially expressed genes in the data set that are potentially involved in the pathogenesis of oral tumors are identified.

The protein-based network identification module

The modular approach is useful in identifying the high degree of interaction of disease-causing genes. We mapped the differentially expressed genes and assigned them into protein-protein interaction networks (PPIs).

Then, we extracted the interaction pairs of genes and constructed a differentially expressed PPIs for oral tumors. Secondly, the Cytoscape[48] visualization method is used to observe the interaction between genes more intuitively.

Then, we apply the plug-in ClusterONE[49] with default parameters for module identification based on cohesion algorithm and neighbor selection strategy. Finally, from modularity, we also performed inter-gene connectivity analysis to screen out the most interconnected internal drive genes in the module.

Functional and pathway enrichment analysis

In genes, the exploration of function and signaling pathways can help study the molecular mechanisms of disease. For dysfunctional modules, enrichment analysis of functions and pathways is an effective means of exploring the underlying mechanisms of oral tumors. Therefore, based on the R language Clusterprofiler package[50], we performed enrichment analysis on the gene of the module (PvalueCutoff = 0.05, qvalueCutoff = 0.05) and KEGG pathway (p-value cutoff = 0.05, qvalueCutoff = 0.05).

Verification of key genes by qPCR

Specifically, total RNA in whole blood was extracted, transcribed into cDNA using a reverse transcription kit, and qPCR reaction was carried out using the SYBR qPCR Detection Kit. The qPCR program begins the initial 3 minute denaturation step at 95 °C to activate the hot-start iTaqTM DNA polymerase. This was followed by 45 cycles of denaturation at 95 °C for 10 seconds and annealing and extension at 60 °C for 45 seconds. The internal reference gene is beta-actin.

Pivot analysis predicts nRNA and TF of the regulatory module

For each dysfunctional module, we specify that the pivot regulator refers to the number of targeted adjustments between each regulator and each module exceeding two. At the same time, we calculated the significance of the interaction between the regulator and the module based on the hypergeometric test (p-value<0.01). In this study, we used the target data of ncRNA and TF as the background and combined with the written python program to predict the pivot analysis. We obtained the pivotal regulator of the significant regulation of the dysfunction module.

Result

Construction of differentially expressed protein-protein action networks

Through differential expression analysis, we identified relevant potential genes that lead to the development and progression of oral tumors.

Therefore, we performed differential gene screening on oral tumor samples and adjacent non-tumor tissue samples from patients with oral squamous adenocarcinoma. A total of 5623 differentially expressed genes were obtained from the integration results, and these genes may have a significant effect on the pathogenesis of oral tumors. To observe the interaction between potential pathogenic genes of oral tumors, we mapped them into human protein-protein interaction networks (PPIs), and finally, obtained PPIs (Depp is) of differential genes (p-value<0.05).

This PPI contains 729 gene nodes and 11104 edges. Based on systems biology and molecular biology principles, we can conclude that PPIs explain the pathogenic factors of oral tumors.

Dysfunction module characterizes the potential dysfunction of oral tumors

To further explore the functions and pathways involved in oral tumors, we performed a modular analysis of target gene-related PPIs.

Based on the cohesive and neighbor selection algorithms, we identified 18 functional modules with 867 related genes (Figure 1). These interaction modules have a more significant interaction, which makes them more representative of the underlying molecular mechanisms of oral tumors. We believe that the gene function module is the core of the development of oral tumor research.

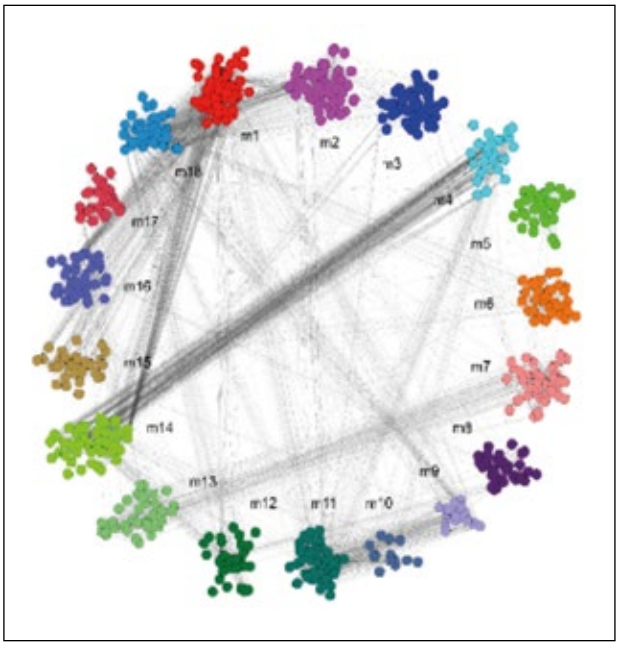

Figure 1: The high-interaction module characterizes the potential dysfunction of oral tumors. The 18 oral tumor height interaction modules obtained by modular analysis, the dot group of different colors represent the genes of 18 different modules.

To observe the function of the module gene in the pathogenesis of oral tumors, we performed functional and pathway enrichment analysis on the module genes. As a result, 17056 biological processes, 1938 cells, 2316 molecular functions,

and 902 KEGG pathways (Figure 2A, 2B) were obtained. It was observed that the genes in the module were significantly enriched in regulating DNA metabolism, active regulation of catabolic processes, and T cell activation.

At the same time, the modular gene is also considerably involved in viral infection in T cells and microRNAs in cancer.

Besides, based on statistical analysis, we found that up to 16 module genes significantly enriched the regulation of multiple biological processes, and 15 modular genes were significantly involved in regulating protein catabolism, controlling complex protein assembly and dynamic anatomical balance. There are 10 module genes involved in papillomavirus infection, human immunodeficiency virus one infection and viral carcinogenesis.

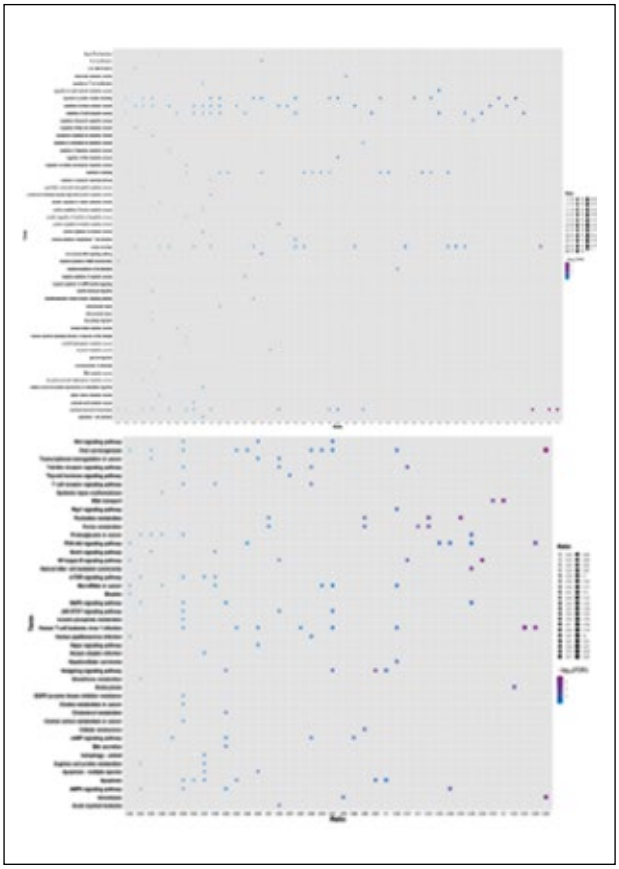

Figure 2: The result shows the function and pathway enrichment analysis of the module gene. A. GO function enrichment analysis of modular genes. The color increases from blue to purple, and the enrichment rises significantly. The larger the circle, the more significant the proportion of the gene in the module that accounts for the GO function. B. KEGG pathway enrichment analysis of modular genes. The color increases from blue to purple, and the enrichment rises significantly. The larger the circle, the more significant the proportion of the gene in the KEGG pathway entry.

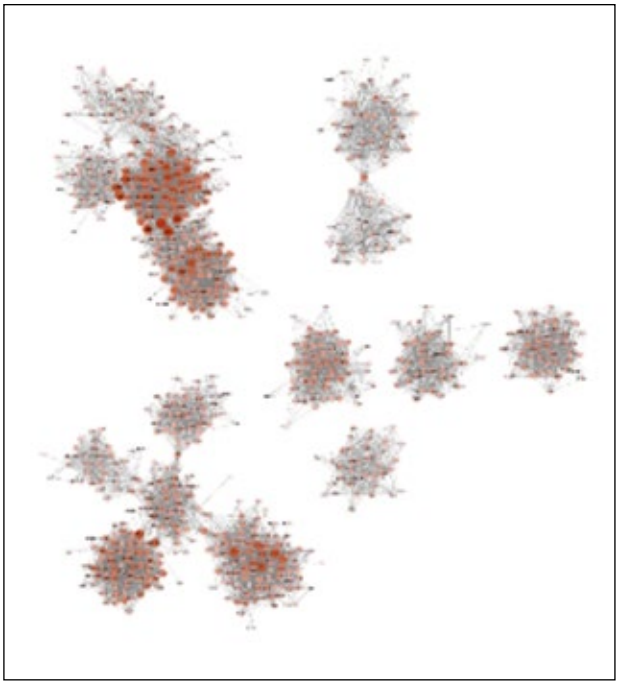

Figure 3: Highly interactive module internal drive genes. The color of the nodes from brownish red to deep red represents the connectivity of the module genes from small to large, and each node group represents each module.

Module internal drive gene may be the core gene of oral tumor disease

The modular approach has deepened our understanding of the underlying molecular mechanisms of oral tumors, but 729 genes still fail to represent the dysfunction mechanisms of oral tumors accurately.

Thus, to identify the genes that play a central role in the dysfunctional module, we first constructed a protein interaction subnet for the genes within the module. Then, based on this set of module subnets, we perform a connectivity analysis of the nodes (Figure 3). A gene with greater connectivity means more effective regulation in the module, so in one module, the most interconnected gene will be considered an internal drive gene for the dysfunctional module. Based on the ordering of connectivity, we found that the core gene NUP107 of module 14 is the most prominent, which actively targets other genes and drives dysfunction modules, and thus is considered to play an essential role in the potential pathogenesis of oral tumors.

The NUP160, NUP37, and NUP85 in the same module also have high connectivity, and their molecular effects are also worthy of attention.

Furthermore, the expression level of key genes was verified by qPCR (Figure 4). We found that the expression trend of key genes was consistent with the previous results.

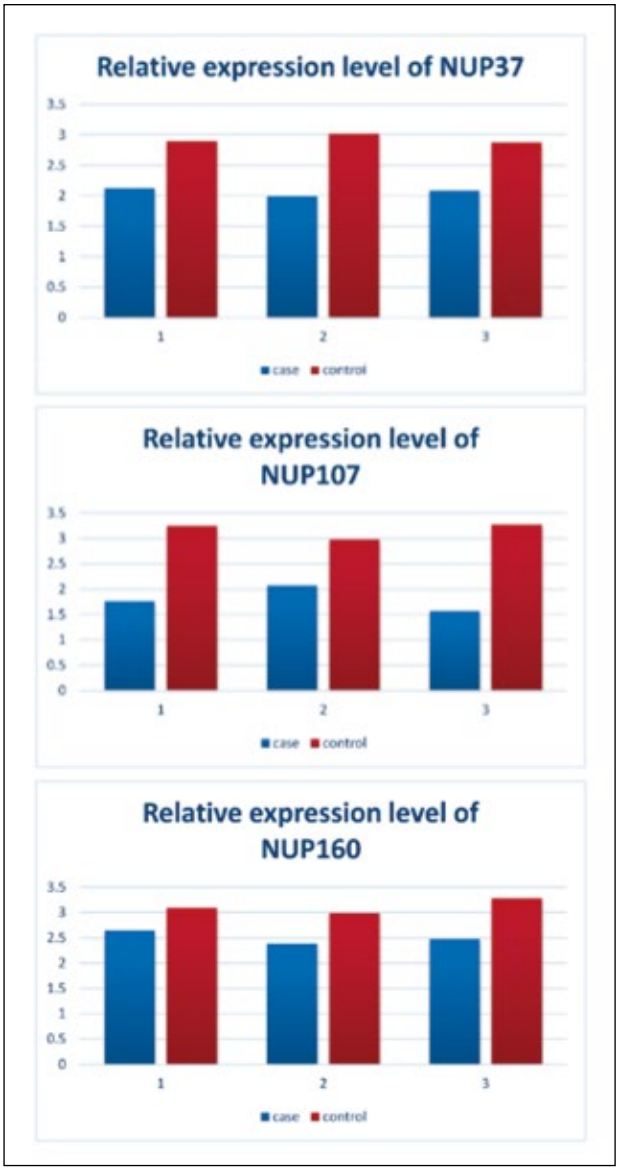

Figure 4: The relative expression level of NUP107, NUP160 and NUP37.

The module of ncRNA pivot mediates oral tumor dysfunction

In systemic genetics, transcription and post-transcriptional regulation of genes have long been recognized as a critical regulator of disease development and progression, while ncRNA is an identified gene regulator. Although biologists have confirmed the management of single or several ncRNAs on the pathogenesis of oral tumors, few studies have focused on their comprehensive regulation of dysfunctional module genes.

The scientific prediction of the ncRNA pivot regulator of the dysfunctional module is helpful for us to explore the transcriptional regulation mechanism of oral tumors further. To this end, based on the pivotal analysis of the targeting relationship between ncRNA and the module gene, we explored the ncRNA regulator that caused the module to be dysfunctional (Figure 5). The results showed that a total of 501 ncRNAs involved 788 ncRNA-module target pairs, significantly regulating these oral tumor-related functional modules, affecting the occurrence and development of the disease. Besides, statistical analysis of the number of pivot control modules, we found that microRNA miR-590-3p significantly regulates nine functional modules, and CRNDE has significant regulatory effects on eight modules.

The regulation of miR-340-5p and FENDRR, which are both microRNAs, is evident in the seven functional modules and plays a central role in the potential dysfunctional mechanisms of oral tumors. Other ncRNAs also show significant regulation of the module, which may be a possible pathogenic factor for oral tumors and play a potential role.

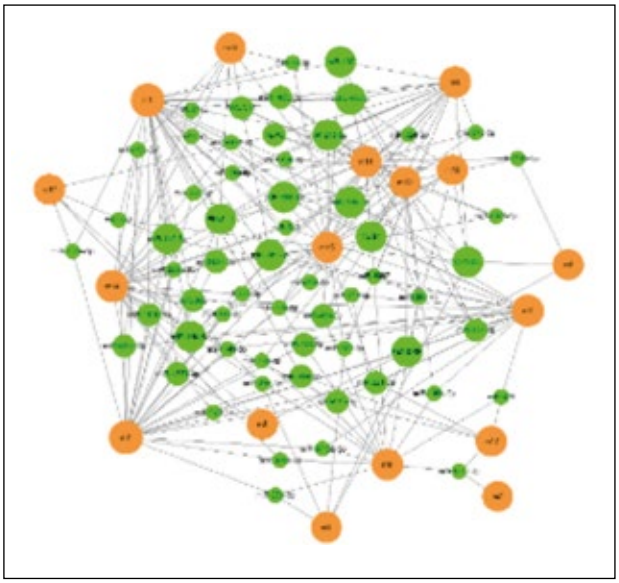

Figure 5: The regulation of ncRNA pivot regulators on dysfunction modules. The orange circle represents the module. The green circle represents the ncRNA of the regulatory module, and the size of the circle represents the number of modules that are regulated. The larger the circle, the more the number of controls.

TF pivot driver module participates in oral tumor dysfunction mechanism

In addition to ncRNA, transcription factors are equally important for transcriptional regulation of genes. Many studies have shown that the dysregulation of transcription factors may lead to various diseases. The occurrence of oral tumors is also inseparable from the imbalance of transcription factors, which is fully reflected in the regulation of dysfunctional modules. Based on the results of the transcription factor-related pivot analysis (Figure 6),

we identified 45 transcription factors that may be involved in the dysregulation of oral tumors, involving 62 TF-module regulatory pairs.

It is worth noting that for the statistical analysis of these TF-module regulatory pairs, we found that AHR, CTBP1, MYC, RB1, RBL2, TP53, and other modular factors significantly regulate three modules, while E2F1, E2F4, EP300, HMGA1 for 2 The module has a regulatory role.

Other transcription factors have also shown significant modulation of the module, contributing to the pathogenesis of oral tumors, and may be potential dysfunctional molecules in oral tumors. Further verification of the mechanism of action of these dysfunctional molecules has become the key to study the pathogenesis of oral tumors further and has become one of our future research directions.

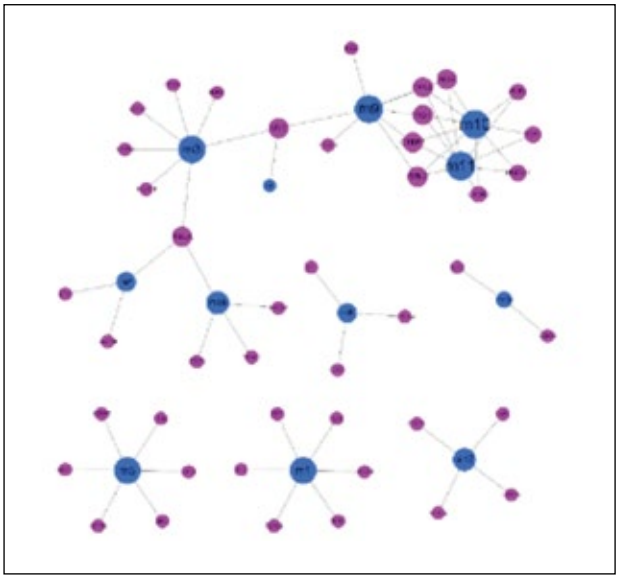

Figure 6: TF pivot regulator regulates dysfunction modules. The blue circle represents the module. The purple circle represents the transcription factor of the regulatory module. The size of the circle represents the number of modules that are regulated. The larger the circle, the more the number of controls.

Discussion

Oral tumors are recognized as multifactorial oral diseases, and their susceptibility is mediated by genetic factors and carcinogen exposure[22]. Although researchers have explored the etiology of oral tumors in various ways, the underlying molecular pathogenesis is still unclear. In this study, we combined multivariate analysis to investigate the driving mechanisms of oral tumors through signaling pathways mediated by dysfunctional modules. At the module level, we first noticed that 16 modules were significantly enriched to the regulation of multiple biological processes, which also confirmed the multifactorial mediation and complexity of oral tumors. Besides, ten modules were found to be significantly involved in pathways such as human papillomavirus infection. Papillomaviruses are DNA viruses that have a trend of disease[23]. High-risk HPV16 and HPV18 genotypes were identified in samples of oral and oropharyngeal cancer[24]. Therefore, saliva detection can be used as an essential method for diagnosing oral infection of human papillomavirus (HPV)[25].

On the other hand, at the molecular level, based on the connectivity analysis of each module subnet, we construct a protein network with a high degree of interaction, and at the same time explore the internal drive genes that are most closely related to the interaction genes. The maximum connectivity of the internal drive genes means that the genes that interact with them in this module are the most, that is, they have the effect of pulling the whole body. Among them, NUP107 had the highest connectivity and was identified as a core internal drive gene in this study. At present, although there is no research related to oral tumors, the expression level of the experimental verification results in this study is significantly lower than that of healthy controls.

Further, we also predicted that 788 ncRNAs participate in the development and progression of oral tumors through a mediator module. Among them, CRNDE showed a significant effect on the eight dysfunctional modules in the analysis results. miR-155-5p and miR-193b-3p significantly regulate four modules, and miR-218-5p has a regulatory impact on three modules. In tongue squamous cell carcinoma (TSCC) cell lines and tissues, CRNDE plays an important role, and its overexpression enhances the proliferation and invasion ability of TSCC cells[26]. Besides, the expression of miR-155-5p affects the progression of oral squamous cell carcinoma (OSCC), which is associated with epithelial-mesenchymal transition (EMT) and, at the same time, serves as a biomarker for predicting relapse[27]. Pathogenesis is regulated by mediating a variety of oral tumor pathogenesis, oncogenic miRNAs and tumor suppressor miRNAs[28].

The downstream target gene of miR-129-5p significantly regulates the estrogen signaling pathway. The p53 signaling pathway and the RIG-I-like receptor signaling pathway affect the conversion of oral leukoplakia (OLK) to OSCC[29]. While miR-193b-3p and miR-218-5p are potential diagnostic biomarkers

and therapeutic targets for OSCC, respectively[30, 31]. On the other hand, TF pivot analysis showed that 45 transcription factors were differentially expressed in different degrees and significantly regulated the oral tumor dysfunction module. The most significant pivot drivers AHR, MYC, RB1, and TP53, have a regulating effect on the three modules. Among them, the aromatic hydrocarbon receptor (AHR) is a nuclear transcription factor of the dioxin receptor, which is involved in various cellular processes and plays a key role in tumor molecule domains such as human oral cancer cells.

Moreover, there is a significant correlation between gene expression and cancer grade, which can be used as a prognostic marker for oral cancer[32]. Besides, AHR has been reported to be involved in the development of oral squamous cell carcinoma (OSCC) and normal tissue-specific stem cell self-renewal[33]. The proto-oncogene (Myc) affects the disease to interfere with proliferation and regulate apoptosis. Proto-oncogene (Myc) and tumor suppressor gene (APC, p53). Oncogenes (Ras) repair DNA damage by controlling chromosome segregation. Proto-oncogenes and oncogenes mediate the development of oral cancer[34, 35]. It can also be used as a molecular target for an effective treatment for local recurrence and prediction of treatment outcome in patients with oral cancer[36]. It reveals that potential genetic mutations in oral tumors can help diagnose biomarker development and improve diagnostic and post-treatment monitoring.

TP53 mutations are widespread and consistent in primary tumors and associated localized regional metastasis and recurrence, providing a basis for further investigation of the use of TP53 mutations as diagnostic biomarkers in disease patients[37]. At the same time, genome-wide association studies and next-generation sequencing-based methods predict that the candidate gene RB1 is inherited for oral cancer pathogenesis[38]. Both of them produce tumorigenic effects on oral epithelial cells[39], and more, they are also found in betel nut and tobacco compounds. RB1 is associated with aflatoxin B1 produced by the use of such substances, while TP53 is the significant component of arecoline[40]. The remaining transcription factors also have different degrees of regulation on oral tumors and need to be confirmed by experiments.

In summary, this study found that oral tumors are cancerous diseases of the oral tissues that are caused by various pathogenic factors such as endogenous genes, ncRNAs, and transcription factors. At the same time, it also has a profound impact on the development of oral tumors by regulating the development of diseases such as human mammary tumor virus and other pathways. The above analysis not only provides a new molecular basis for understanding the biological characteristics of oral tumors but also provides a useful resource for the development of oral tumor treatment in the future.

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