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Original Research

Multi-omics analysis profile oral tumor module clusters to reveal the potential pathogenic mechanism

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Abstract: Oral tumors are malignant cancers caused by abnormal proliferation or pathological changes of soft or hard tissues in the oral cavity. Serious cases may pose a threat to life. However, its precancerous lesions remain unclear. This study is based on a comprehensive strategy to explore a multi-factor-driven oral cancer barrier module, which is an attempt to describe the pathogenesis of the disease and potential regulatory drugs from a global perspective. Functional disease modules were identified by constructing a protein-specific interaction network in patients' oral tissues. Then, comprehensive pathogenesis was explored through combination with analysis of functional and signaling pathway enrichment, prediction of key regulatory factors. It was found that these specifically expressed proteins and their interactions often play a pivotal part in oral tumors. This is reflected in the results of functional and pathway enrichment of modulating genes, which show that they are mainly involved in various immune responses, inflammatory reactions, oral plaque, and oral ulcer-related regulatory processes. This may represent the potential pathogenesis of oral tumors. On the predictive analysis of regulators, a series of ncRNAs (including miR-590, CRNDE and miR-340) and transcription factors (including E2F1, MYC and TP53) were identified that have potential important regulatory effects on oral tumors. These key regulators may manipulate a crucial part of the module sub-network and then work together to mediate the occurrence of oral tumors. On the comprehensive Multi-omics module analysis, the specific proteins and their interactions in patients' oral tissues were identified, while the prominent pivotal regulators were involved in the different pathogenic functions of oral tumors.

Key words: Oral tumors; Immune response, Pivotal regulators; Pathogenic mechanism; ncRNA; Transcription factor.

Introduction

Cancer is one of the most important causes of death in humans, which has many types (1-6). Oral tumors are common malignant tumors of head and neck caused by chronic infection of oral epithelial cells by pathogens (bacteria, fungi, viruses, etc.) in clinical medicine (7, 8). As the world's sixth most common cancer, it causes more than 200,000 new diagnoses each year. It can be divided into the following sub-sites: oral cancer, oral tongue squamous cell carcinoma (OTC), Oral Squamous Cell Carcinoma (OSCC), Oral and oropharyngeal cancer (OPC) and other different forms of development. Progress has been made in cancer diagnosis and therapy of oral cancer, but results are far below expectations, and overall mortality has remained unchanged over the past three decades (9-12). Therefore, there is an urgent need for finding an outstanding therapeutic target for oral cancer.

Many steps and factors will lead to the birth of oral cancer, which is a process disease. potential molecules, epigenetic and genetic alterations, together with environmental risk factors, will cause oral cancer. Alcohol, tobacco, consumption and human papillomavirus infection are all major contributors to the disease (13-15). Its potential pathogenic molecules and related functions have been reported in recent years. Carcinogenic E3 ligase of biological macromolecule targeting tumor suppressor protein can act as ubiquitin-mediated degradation and inhibit specific targeting oncogene products. It induces an imbalance between carcinogenic signal and tumor-suppressive pathway through imbalance, which leads to cell proliferation and metastasis of oral cancer and other malignant tumors (15). RACK1 gene was significantly expressed in the protein-protein interaction network (PPIs), and directly interacted with two subunits and 14 differential proteins (13). Jumonji-C domain-containing protein 5 (JMJD5) down-regulated and inhibited OSCC transfer and induced apoptosis through p53//NF-kappa B pathway (14). In addition, researchers such as Valverde have also found that Hedgehog (HC) molecules are significantly involved through autocrine and paracrine signaling pathways and are associated with angiogenesis (16). Taken together, these finding indicates that many pathogenic molecules and their regulatory protein-protein interaction (PPI) networks act a pivotal part in the occurrence and development of the oral tumor, but a systematic and comprehensive understanding of the role of these factors and the PPI networks in the occurrence and development of the oral tumor is lacking.

Epigenetic mutations, such as histone modifications,

DNA methylation, and non-coding RNA modifications (miRNAs) have been related to the etiology of oral cancer (17). Plenty of evidence showed that non-coding RNAs(ncRNAs) played significant regulatory parts in the cellular physiological process, such as long non-coding RNAs (lncRNAs) (18). For example, lncRNAs could weaken regulations of miRNAs on mRNAs by acting as miRNAs sponge (19, 20). LncRNAs related to cancer pathogenesis mainly exist in cellular macromolecules (including RNA, protein, chromatin) (21, 22). It is likely that ncRNAs modulation takes part in oral cancers occurrence and development. However, the regulatory mechanism of lncRNA-induced oral cancers is still unknown.

In addition, evidence shows that several genetic alterations in tumor genes governing normal cellular processes (EIF3E, GSTM1), proto-oncogenes (Myc), oncogenes (Ras) and suppressor genes (APC, p53) have been involved in oral cancers (23). However, the underlying synergistic mechanisms of these factors remain elusive.

Based on what described above, this study aims to explore ncRNA and transcription factors as pivotal regulators in oral tumors by analyzing the functional modules under the combined action of multiple factors, which is conducive to deepening the understanding of disease regulation.

Materials and Methods

Data resources

We first collected an array of gene expression profiles of oral tumors from the NCBI Gene Expression Omnibus database (24), which is named GSE74530. The data were obtained from the same patient's oral tumors and adjacent non-tumorous oral tissue samples of genes and their expression. Then, we downloaded data on all human protein interactions from the STRING V10 database. (25) to build the differentially targeted gene PPIs associated with microRNAs. which interacting genes or proteins could be retrieved by a common search tool STRING database. It is useful when we explore and interpret functional interactions in the life system. Finally, from the RAID v2.0 database, ncRNA-protein interaction pairs with a score of 0.5 and above were screened (26) to predict target genes. Meanwhile, the transcription factor target data of all humans in the TRRUST V2 database were downloaded and used (17).

Difference analysis

The R language limma package could be used in our research, so it allowed us to observe microRNA expression profile data and analyzed their differential expression. (18-20). First of all, background correction and standardization could be achieved with the background correct function. Next, we filtered out the control and the low expression probe through the method of the normal Between Array function quantile normalization. Finally, according to ImFit and eBayes functions and default parameters, we were able to identify differentially expressed genes(DEGs) that might be involved in the pathogenesis of oral cancer in the data set.

Recognition module based on PPIs

Modularized methods can effectively identify the degree of high interaction between pathogenic genes. Firstly, we constructed a differentially expressed PPIs for oral tumors by mapping the DEGs into PPIs and extracting interaction pairs involving only these genes. Secondly, we use Cytoscape (21) visualization method to observe gene interaction more intuitively. Then, the plug-in ClusterONE (22) with default parameters was used to recognize modules according to neighbor selection strategy and cohesion algorithm. At last, we conducted connectivity analysis among genes in light of modularization to sift the most interacting endogenous genes in the module.

Functional and pathway enrichment analysis (EA)

It is usually helpful to investigate the signaling pathways and functions of gene involvement to research the mechanisms of disease at the molecular level. In dysfunctional modules, genes enrichment is a fruitful way to probe into the potential mechanism of oral cancer. Hence, based on the R language Cluster Profiler package (23), we conducted gene EA of the module with Go function (p-value cut off = 0.05, q-value Cut off = 0.05) and KEGG pathway (p-value Cut off = 0.05, q-value Cut off = 0.05).

Pivot analysis and prediction of nRNA and TF in regulatory module

For each dysfunctional module, we could specify that pivot regulator so that the targeted regulators' number between each module and each regulator exceeded 2. Then, a hypergeometric test was used to calculate the significance of the interaction between the regulator and the module ($p < 0.01$). We predicted pivot analysis in this research based on target data of TF and ncRNA and associated with the Python program. Thus, pivot regulators of significant regulatory dysfunction modules were obtained.

Results

Construction of differentially expressed protein-protein interaction network

Potential genes connected with the occurrence and development of oral tumors can be identified by differential expression analysis. Thus, based on the expression profiles of oral cancer microarrays, we screened DEGs in oral cancer samples from patients with oral squamous cell adenocarcinoma and adjacent non-tumor tissue samples. A total of 5623 DEGs were obtained from the integration results. These genes may act a pivotal part in the pathogenesis of oral tumors.

To find the interaction between potential pathogenic genes of oral cancer, we mapped it into human PPIs and obtained a PPIs (DEPPIs) containing only differential genes (P-value < 0.05). These PPIs contain 729 gene nodes and 11014 edges. According to the principles of system biology and molecular biology, PPIs can definitely generalize the molecular mechanism of oral cancer to some degree.

High interaction module to characterize potential dysfunction of oral tumors

To research the pivot pathways involved in oral cancer, we proceeded with a modular analysis of PPIs associated with target genes. According to neighbor selection algorithms and cohesion, 18 functional modules were identified, including 867 related genes (Figure 1). Comparatively speaking, these interaction modules show a more significant interaction relationship, in which the basic molecular mechanism of oral cancer can be better characterized. Gene modules stand for an array of high-related genes. Genes may play similar biological functions in the same module or co-regulate certain biological processes. According to systems biology, it is possible to link individual gene functions and global network characteristics by searching for gene modules with potential functions. Besides, each module may be a pathway for the pathogenesis of oral tumors. Hence, the identification of gene functional modules is the kernel of targeted oral cancer research and also the pivot point to understand its molecular mechanism.

In view of the pathogenesis of oral cancer, to observe the function of module genes, we enriched and analysed the pathway and function of module genes. Results obtained 17056 biological processes, 2316 molecular functions, 1938 cell components and 902 KEGG pathways (Figure 2A, 2B). we found the module genes were remarkably enriched in diverse biological processes related to oral tumors, such as regulating DNA metabolism, active regulation of catabolic metabolism and T cell activation. Meanwhile, module genes also immensely participate in human T-cell leukemia virus 1 infection, microRNA in cancer and other oral cancer-related signaling pathways. Furthermore, we found that as many as 16 modules were remarkably enriched in the regulation of multi-biological processes according to statistical analysis, while 15 modules were notably trapped in the regulation of protein catabolism, protein complex assembly and dynamic balance of anatomical structure. Ten modular genes exist in KEGG pathways, such as human papillomavirus infection, human immunodeficiency virus 1 infection and viral carcinogenesis.

The modular introductory gene may be the core gene of oral cancer disease

The modular way has paved the way for a better perception of the fundamental molecular mechanism of oral cancer, however, 729 genes still could not accurately be involved in the dysfunction mechanism of oral cancer. Therefore, in order to find the decisive genes in the dysfunction module, we first established a PPI subnet of module genes. Next, based on the module subnet, we analysed the connectivity of nodes (Figure 3). We know that genes with very strong connectivity have distinct roles in a module, so in dysfunctional modules, we consider genes as intrinsic genes. Based on the connectivity's order, we found NUP107, the core gene of module 14, shows the most significant. It forcefully drives dysfunctional modules and targets other genes. Therefore, NUP107 is considered to play a key role in the potential pathogenesis of oral cancer. NUP160, NUP37 and NUP85 in the same module also have high connectivity, and their molecular roles are also worthy of attention.

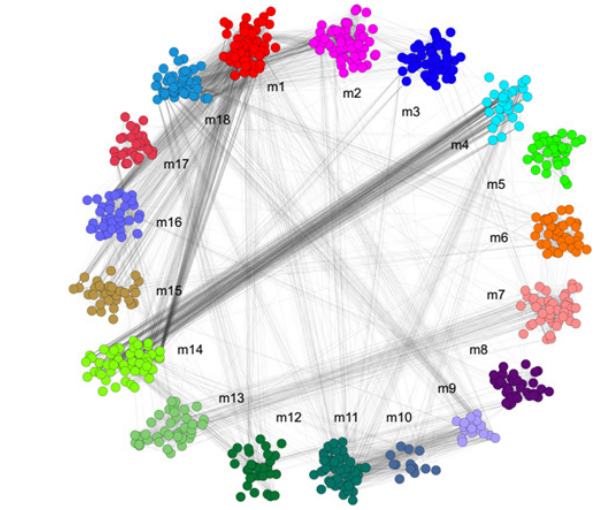

Figure 1. The potential dysfunction of oral tumors is characterized by a highly interactive module. Eighteen highly interacting modules of oral tumors were obtained by modular analysis. Different color dot groups show 18 genes of different modules.

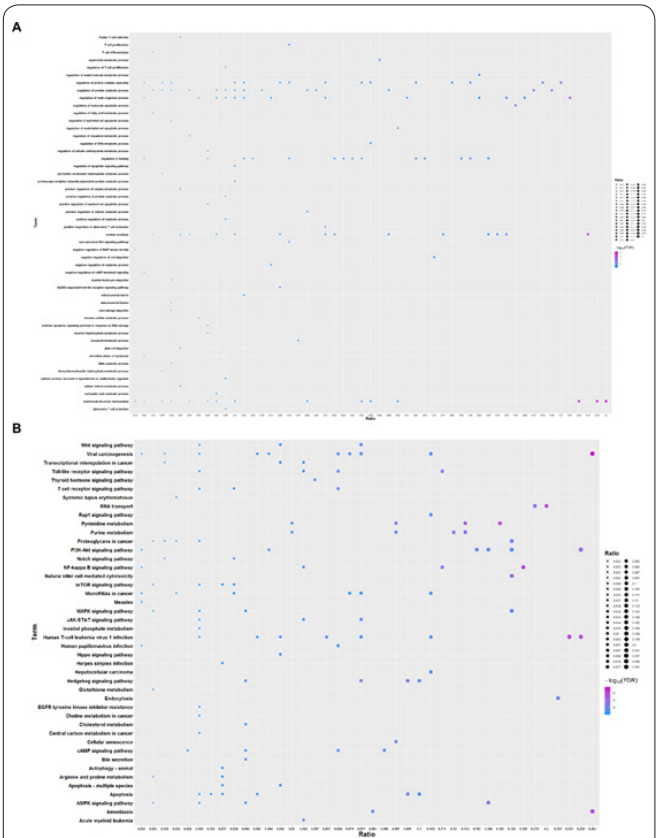

Figure 2. Functional and pathway EA of modular genes. A: GO functional EA of module genes (excerpts). From blue to purple, the enrichment increased significantly. In GO functional entry genes, the circle is positively associated with the proportion of module genes; B: KEGG pathway EA of modular genes (excerpts). From blue to purple, the enrichment expanded remarkably. The circle is positively associated with the proportion of module genes to KEGG pathway entry genes.

Modular ncRNA pivot mediates dysfunction of oral tumors

At either the transcriptional or post-transcriptional level, they have been considered to be important regulators of the occurrence and development of the disease. And ncRNA is regarded as a gene regulator. While, on

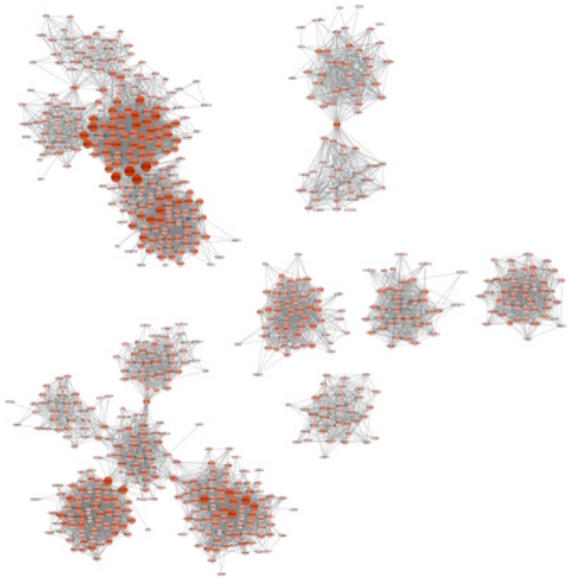

Figure 3. Highly interacting module-driven genes. From brown to dark red, the connectivity of module genes is larger. Each module is represented by each node group.

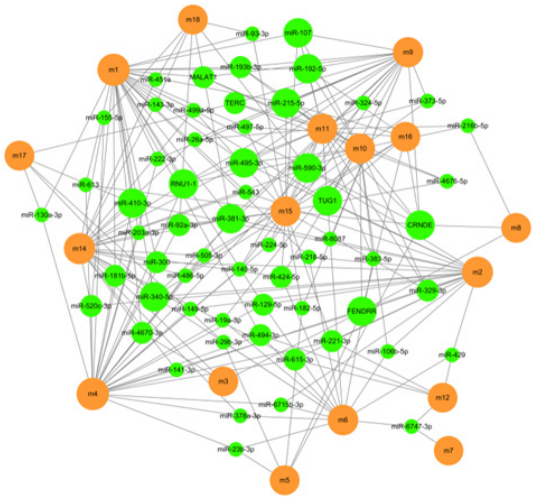

Figure 4. Regulation of ncRNA pivot regulator on dysfunction module. The orange circle stands for the module. The green circle symbolizes the ncRNA of the control module. The circle size represents the number of control modules. The circle is positively associated with the number of regulations.

the pathogenesis of oral cancer, the regulation of single or several ncRNAs has been proved by biologists, their comprehensive regulation of dysfunctional modules was few focused. Scientific prediction of ncRNA pivot regulators in dysfunctional modules is helpful to further investigate the transcriptional regulation mechanism of oral tumors. So, pivot analysis derived from the targeting relationship between ncRNA and module genes was carried out to research the module dysfunction caused by ncRNA regulators (Figure 4). Results showed 501 ncRNAs involved 788 ncRNA-module target pairs, which significantly regulated these functional modules related to oral cancer and affected disease occurrence and development. Besides, we statistically analyzed the number of pivot regulatory modules and we found microRNA micro590-3p significantly regulated nine functional modules, while CRNDE significantly regulated eight modules. The regulation of miR-340-5p and FENDRR in seven functional modules are obvious, and they

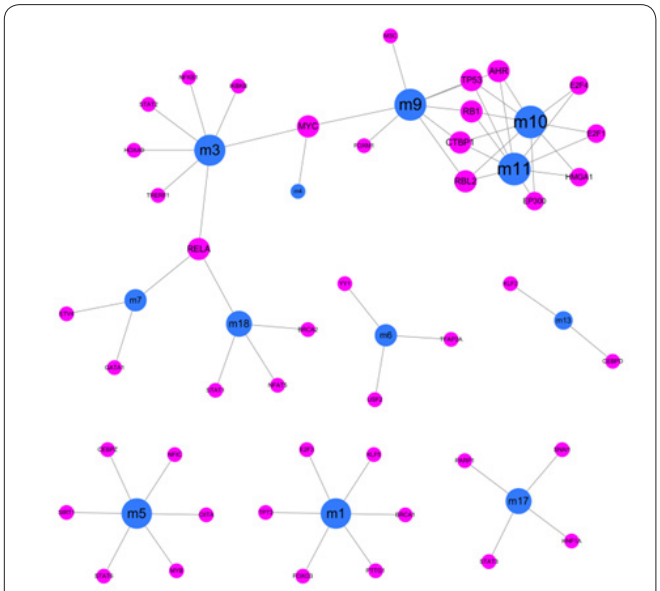

Figure 5. Regulation of TF pivot regulator on the dysfunction module. Blue circles represent modules. The purple circle indicates the transcription factor of the regulatory module. The circle size reflects the number of modules being regulated. The circle is positive correction with the number of regulations.

play a pivot part in the potential dysfunction mechanism of oral tumors. This module is also regulated by other ncRNAs with remarkable effects. We analyzed that this may be involved in the occurrence of oral cancer and is a potential pathogenic factor.

TF pivot driver module participates in the mechanism of oral cancer dysfunction

At the same time, transcription factors are as important as ncRNAs for the transcriptional regulation of genes. By studying multiple studies, we have seen that the disordered expression of transcription factors can cause a variety of diseases. The occurrence of oral tumors is also closely related to the dysfunction of transcription factors, which is thoroughly reflected in the regulation of dysfunctional modules. In accordance with the pivot analysis of transcription factors (Figure 5), we found 44 transcription factors that may be involved in the dysfunctional mechanism of oral tumors, involving 61 TF-module control regulatory pairs. Among them, TP53 mediates four modules, and MYC, CTBP1, AHR, RELA, RB1, RBL2 mediate 3 modules. The major transcription factors that cause and lead to the development of oral tumors are considered to be these. Other transcription factors, such as EP300 and E2F1, also show considerable regulatory effects on the module and contribute to the pathogenesis of oral tumors, and they may be potential dysfunctional molecules in oral tumors. Follow-up studies on the pathogenesis of oral cancer should focus on validating the mechanism of these dysfunctional molecules, which is also one of our future research priorities.

Discussion

The occurrence and development of oral cancer is a complex interaction between environment and genetics to understand the molecular mechanism of the pathogenesis of oral cancer (27). Oral cancers-associated genes,

ncRNAs, transcriptional factors, proteins and protein-protein interaction networks were analyzed. Observing the modules and nodes involved in the different networks, we identified that the foremost mediated factors at different landscapes, such as NUP107 at the gene level, 501 ncRNAs as a gene regulator, TP53 as transcriptional factors and KEGG pathway. In addition, we also revealed many significant factors and mediated factors involved in the occurrence and development of oral cancers. A systematic and comprehensive understanding of the underlying mechanism of the oral tumor occurrence and development by these studies and analyses. At the same time, it contributes to finding outstanding therapeutic targets by those findings.

Individual susceptibility is mediated by genetic factors and carcinogen exposure behavior (28). Although researchers have investigated the etiology of oral tumors on various sides, the individual potential molecular pathogenesis of oral tumors remains unclear. We synthesized multivariate analysis to explore the driving mechanism of oral cancer through dysfunctional module-mediated signaling pathways. We first verified that 16 modules were remarkably enriched in the regulation of multi-biological processes, which also confirmed the multifactorial mediation and complexity of oral tumors. It is noteworthy that 10 modules were found to be significantly involved in human papillomavirus infection and other pathways. High-risk HPV16 and HPV18 genotypes were identified in oral and oropharyngeal cancer molecules (29). Therefore, specified testing especially tests the activates KEGG pathway and infection with human papillomavirus (HPV), can be used as an important method for the diagnosis of oral cancers.

On the other hand, at the level of the genes, by constructing internal subnets of highly interactive modules, and based on connectivity analysis of each module subnet, the most closely related intrinsic genes can be explored. Similarly, the maximum connectivity of introductory genes represents that there're the most genes interacting with them in this module, to put it from another way, they are able to pull the whole body together. Globally, a minor abnormal expression of endogenous genes can also bring about dramatic changes. Among them, NUP107 has the highest connectivity and has been identified as the core gene in this study. At present, although there is no relevant research on oral cancer, the predicted results of this study and its potential role are enough to be defined as biomarkers for further exploration.

In addition, lncRNA plays a vital part in cell development, differentiation, migration and invasion. CRNDE plays an important role in the cell lines and tissues of tongue squamous cell carcinoma (TSCC), one of the common oral tumors. Overexpression of CRNDE enhances the proliferation and invasiveness of TSCC cells (30). The expression of microRNA-155-5p affects the progression of OSCC related to epithelial-mesenchymal transition (EMT) and can be used as a biomarker for predicting recurrence (31). Mi-193b-3p and Mi-218-5p are potential diagnostic biomarkers and therapeutic targets of OSCC, respectively (32, 33). In our study, CRNDE has a significant effect on eight dysfunctional modules. Mi-155-5p and Mi-193b-3p significantly regulate four modules. Mi-218-5p regulates three modules, while the other ncRNAs regulate different numbers of modules. Therefore, those ncRNAs as biomarkers are worthwhile taking into consideration. Then, controlling gene expression using new technologies is of particular importance (34).

In the end, transcriptional factors play a significant part in oral cancers progress. It has reported that TP53 mutation is very common and consistent in primary tumors and related local metastasis and recurrence, which provides a basis for further research on the use of TP53 mutation as a diagnostic biomarker in patients with diseases (35). Aromatic hydrocarbon receptor (AHR) is a nuclear transcription factor of the dioxin receptor, which involves a variety of cellular processes and plays a key role in the molecular domains of human oral cancer cells, and its gene expression has a complete correlation with cancer grade. It can be used as a prognostic marker for the treatment of oral cancer (36). In addition, it has been reported that the development of OSCC and the self-renewal of normal tissue-specific stem cells are closely associated with AHR. Knock-down expression of AHR can block the rapid migration of OSCC cancer cells, chemical resistance, and tumorigenesis under low adhesion conditions (37). Proto-oncogene (Myc) can interfere with proliferation and regulate the apoptosis of diseases. It mediates the development of oral cancer by regulating chromosome separation, DNA damage repair and other functions and pathways with tumor suppressor gene (APC, p53) and oncogene (Ras) (38, 39). It could also be used as a molecular target for effective treatment to diagnose local recurrence and treatment outcomes in patients with oral cancer (40). Revealing potential gene mutations in oral tumors can help to develop diagnostic biomarkers and improve diagnosis and post-treatment monitoring. Meanwhile, genome-wide association studies and prediction based on next-generation sequencing showed that candidate gene RB1 was hereditary to the pathogenesis of oral cancer (41). Both of them have a tumorigenic effect on oral epithelial cells (42). In our study, we found that TP53 is foremost and AHR, MYC, RB1 are significant TF factors. Naturally, we cannot ignore these key drivers because they play their respective regulatory roles in influencing the formation of oral cancer.

In summary, we can conclude that this study provides a systematic and comprehensive understanding of the follow-up study of the pathogenesis of oral tumors, and also provides a valuable reference for its clinical application and personalized clinical diagnosis of oral cancers.

This study found that oral cancer is a cancerous disease of oral tissue caused by many pathogenic factors, such as endogenous genes, ncRNA, transcription factors, and so on. Meanwhile, it also modulates the development of oral cancer by mediating the functions and pathways of the human breast cancer virus, which has a profound impact on the development of oral cancer. These results are useful both to explore the molecular and biological characteristics of oral cancer and to develop diagnostic markers, therapeutic targets and promote targeted therapy in the future.

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