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Exploring the role of Mir204/211 in HNSCC by the combination of bioinformatic analysis of ceRNA and transcription factor regulation

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ABSTRACT

Objectives: This study aimed to reveal the regulatory roles of microRNAs in head and neck squamous cell carcinoma (HNSCC) through comprehensive ceRNA, miRNA-transcription factor (TF)-hub gene network and survival analysis.

Materials and methods: Expression analysis was performed using the 'edgeR' package based on The Cancer Genome Atlas database. The ceRNA network was screened by intersecting prediction results from miRcode, miRTarBase, miRDB and TargetScan. GSE30784, GSE59102 and GSE107591 from the Gene Expression Omnibus repository were chosen for cross-validation. Hub genes were identified using a protein-protein interaction network constructed by Search Tool for the Retrieval of Interacting Genes. The Transcriptional Regulatory Relationships Unraveled by Sentence-based Text mining (TTRUST) was utilized to map the miRNA-TF-Hub gene network. Patient overall survival was analyzed using the 'survival' package in R. Structural and functional analysis of miR-204/211 was based on miRbase and RNAstructure.

Results: A ceRNA network of 178 lncRNAs, 19 miRNAs and 55 mRNAs was generated, and a TF regulatory network with 11 miRNAs, 11 TFs and 18 hub genes was constructed from the 52 hub genes identified through the protein–protein interaction (PPI) network. Survival analysis demonstrated that the dysregulated expression of 11 lncRNAs and 14 mRNAs was highly related to overall survival. Furthermore, miR-204 and miR-211 were significantly involved in the network with identical mature structures, indicating them as key miRNAs in HNSCC.

Conclusion: This study reveals the comprehensive molecular regulatory networks centralized by miRNAs in HNSCC and uncovers the crucial role of miR-204 and miR-211, which may become potential diagnostic and therapeutic targets.

Introduction

Head and neck squamous cell carcinoma (HNSCC) is the sixth leading cancer worldwide, with a high incidence of 500,000 newly diagnosed cases per year, a high rate of metastatic recurrence, and a five-year overall survival rate as low as approximately 50% [1–5]. Emerging targeted therapies, such as monoclonal antibodies against epidermal growth factor receptor (EGFR), tyrosine kinase inhibitors, the phosphoinositide 3-kinase (PI3K)/AKT/ mammalian target of rapamycin (mTOR) pathway pathway inhibitors, and immunotherapy agents such as anti-PD-1/PD-L1 antibodies and TLR-8 agonists, are under development, but with limited efficacy [6]. Therefore, an increased understanding of HNSCC pathogenesis at the molecular level is urgently needed to identify novel therapeutic strategies.

The gene expression network of cancer has been predicted to be intricate and fathomable, involving a large variety of players, such as transcription factors (TFs), microRNAs (miRNAs), long noncoding RNAs (lncRNAs) and coding genes. Among all factors, miRNAs predominately play direct roles through translation inhibition and mRNA degradation [7,8], while two or more miRNAs with similar sequences can frequently reside in clusters and function synergistically in the pathogenesis of various diseases [9–11]. Being responsible for oncogenesis, miRNAs

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possess great potential to be biomarkers in HNSCC and other cancers [8,9,12].

Several crosstalk mechanisms have gained ground. For example, miRNAs act as a bridge between lncRNAs and targeted mRNAs. Salmena *et al.* proposed the competing endogenous RNA (ceRNA) hypothesis: the same miRNA response elements (MRE) are shared among lncRNAs and mRNAs, enabling lncRNAs to interact with miRNAs as "sponges" and suppress their impact on mRNAs [13]. CeRNA networks have been verified in multiple cancers, including HNSCC [14–18]. Another factor at play is the impact of miRNAs on TFs. Compared with coding genes, a miRNA is twice as likely to choose a TF as its target [19], which entails indirect regulation on the transcriptional process besides its post-transcriptional role. Furthermore, a mathematical model quantified the maximal post-transcriptional regulatory power achievable by miRNA-mediated crosstalk in the case of ceRNA circuits. This model clarified that miRNA-mediated control could eclipse other regulation, such as direct transcriptional control via DNA-binding factors [20]. In conclusion, the current scenario indicates that besides its widely recognized noise-buffering role, miRNAs may indeed act as master regulators of gene expression.

Considering the intricate regulatory roles of miRNAs in affecting cell phenotype, a comprehensive and systematic analysis of their roles in lncRNA-miRNA-mRNA, miRNA-TF-mRNA triplet regulatory networks and related survival effects remains obscure. An integrated regulatory network centralized by miRNAs would contribute greatly to the understanding of the oncogenesis of HNSCC. Therefore, in our study, we explored The Cancer Genome Atlas database (TCGA) database and 3 related Gene Expression Omnibus (GEO) datasets to illustrate the pathogenesis of HNSCC. With multiple bioinformatic strategies, we established both a ceRNA network connecting lncRNAs, miRNAs, and mRNAs and a TF regulatory network connecting miRNAs, TFs and hub genes. We also conducted a closely related survival analysis illustrating the pathogenesis of HNSCC. As a result, we identified miR-204 and miR-211 as a promising cluster in the HNSCC pathological process, shedding light on new diagnostic and therapeutic approaches.

Materials and methods

Microarray data acquisition

Expression data on HNSCC from the TCGA database (https://cancergenome.nih.gov/) were downloaded on June 8, 2018. The RNA-seq data include 502 HNSCC samples and 44 normal samples, while the miRNA-seq data include 525 HNSCC samples and 44 normal samples. Our study adhered to the TCGA publication guidelines and data access policies (http://cancergenome.nih.gov/publications/publicationguidelines).

Differentially expressed gene analysis

Based on the data from TCGA, the Empirical Analysis of Digital Gene Expression Data in R (edgeR) package (http://bioconductor.org/packages/release/bioc/html/edgeR.html) was used to screen out differentially expressed mRNAs, miRNAs, and lncRNAs (DE mRNAs, DE miRNAs, DE lncRNAs).

The p value was calculated with t-tests and adjusted for multiple testing using the false discovery rate (FDR) method. A threshold was set for FDR < 0.05 and $|\log2\text{foldChange(FC)}| > 1.5$, 1, 1 individually for DE mRNAs, DE miRNAs, and DE lncRNAs, respectively. All analyses were performed with the R 3.5.0 framework.

Gene ontology and KEGG pathway analyses of DE mRNAs

Functional annotation for DE mRNAs was conducted based on Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analyses. All functional clustering was assessed by Enrichr (http://amp.pharm.mssm.edu/Enrichr/). Significant GO terms were identified with a cut-off of $p < 0.01$, while KEGG pathways annotated with $p < 0.05$.

Constructing the ceRNA network

To clarify the roles of miRNAs influenced by lncRNAs with ceRNA regulation, we built a ceRNA network. The regulatory relationship between DE lncRNAs and DE miRNAs pairs were predicted by miRcode (http://www.mircode.org). DE miRNA and target mRNA pairs were predicted by miRTarBase (http://mirtarbase.mbc.nctu.edu.tw), miRDB (http://www.mirdb.org/) and TargetScan (http://www.targetscan.org/mamm_31/). The regulation pairs with the same expression trends were filtered out. Then, the network was visualized by Cytoscape v3.6.1 software (http://www.cytoscape.org/).

Protein-Protein interaction network and DE miRNA-TF-Hub gene regulatory network construction

To screen out hub genes of HNSCC, we first filtered datasets in GEO (http://www.ncbi.nlm.nih.-gov/geo/) according to the following criteria for further validation: (1) cancer type: HNSCC and its subtypes; (2) resources: homo sapiens (both cancerous and noncancerous samples); (3) total sample quantity: > 30; (4) the value distribution of the datasets should meet the standard requirement. Then, GSE83519, GSE107591, GSE51985, GSE37991, GSE25099, GSE30784, GSE59102 were screened out. GEO2R (https://www.ncbi.nlm.nih.gov/geo/geo2r/) was applied to detect differentially expressed genes with cut-off criteria as an adjusted P value < 0.05 and $|\log FC| \geq 1$. We compared each dataset with our TCGA results and took 300 overlapping DE mRNAs as a threshold for further analysis. Finally, GSE107591, GSE30784 and GSE59102 were included in our work. Namely, GSE30784 includes 167 oral squamous cell carcinoma (OSCC) samples and 45 normal samples (GLP 570, Affymetrix Human Genome U133 Plus 2.0 Array). GSE59102 contains 29 larynx squamous cell carcinoma (LSCC) samples and 13 normal samples (GPL6480, Agilent-014850 Whole Human Genome Microarray 4x44K G4112F). GSE107591 has 24 HNSCC and 23 normal samples (GPL6244 Affymetrix Human Gene 1.0 ST Array). The common genes were then uploaded to the Search Tool for the Retrieval of Interacting Genes (STRING, https://string-db.org/), and the outcomes with a cut-off value with a minimum interaction score of 0.4 were visualized in Cytoscape 3.6.1 to construct a protein–protein interaction (PPI) network. Furthermore, the Molecular Complex Detection (MCODE) and Network Analyzer were used to screen out hub genes with a standard as both the MCODE Score and degree > 5.

Aiming to determine miRNAs' regulatory impact on mRNA expression besides the ceRNA network, we first screened out the transcription factors of the hub genes applying The Transcriptional Regulatory Relationships Unraveled by Sentence-based Text mining (TRRUST) (http://www.grnpedia.org/trrust/). Then, TFs, which are targets of DE miRNAs predicted by miRTarBase, miRDB and TargetScan, were selected for network analysis. DE miRNA-TF-Hub gene regulatory network was constructed and visualized by Cytoscape 3.6.1 based on the regulation pairs of TF and hub genes and the predicted target pairs between DE miRNAs and TFs.

Survival analysis

To identify the prognostic genes for HNSCC, we plotted the survival curves of samples from TCGA with clinical data of differentially expressed lncRNAs, miRNAs and mRNAs using the 'survival' package in R (https://cran.r-project.org/web/packages/survival/index.html). This univariate survival analysis was estimated based on Kaplan-Meier curve analysis to show the differences in patients' overall survival between the high-expression group and the low-expression group and the statistical significance; p-values less than 0.05 were considered significant.

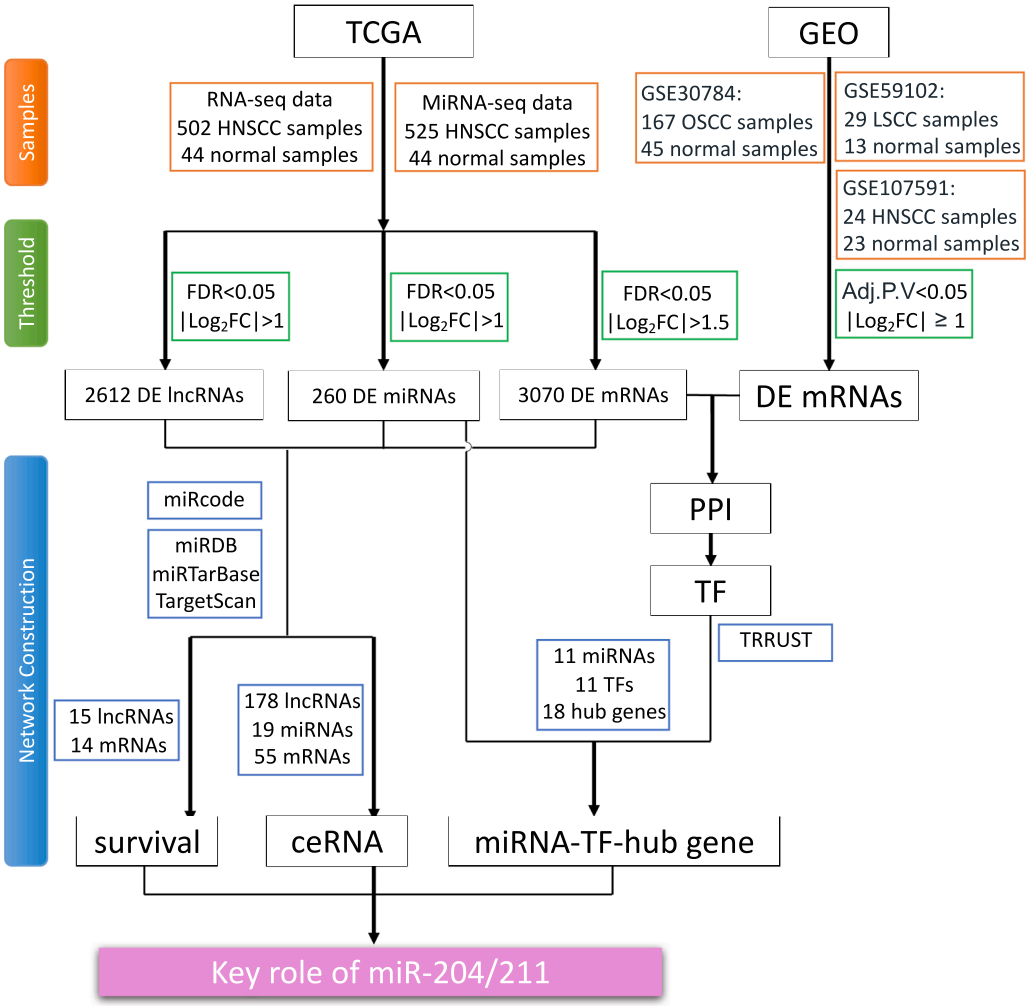

Fig. 1. Outline of the study.

Analysis of gene sequences, 2nd structure and functional annotation of miR-204/211.

Pre-miRNAs that have stem-loop sequences can generate two mature miRNAs from opposite arms. To determine the potentially functionally mature miRNA of miR-204/211, miRbase (http://www.mirbase.org/) was used for analysis. We used RNAstructure (http://rna.urmc.rochester.edu/RNAstructureWeb/) to predict the common 2nd structure of these two miRNAs.

Results

Identification of differentially expressed mRNAs, miRNAs and lncRNAs

The workflow of our study is shown in Fig. 1. We collected HNSCC-related expression data from TCGA, including RNA-seq data with 502 HNSCC samples and 44 normal samples as well as miRNA-seq data with 525 HNSCC samples and 44 normal samples. Volcano plots were used to assess differentially expressed genes. In total, 3070 DE mRNAs were screened out, including 1650 downregulated and 1420 upregulated mRNAs in cancerous samples. Of the 2612 DE lncRNAs, 771 were downregulated, and 1841 were upregulated. Of the 260 DE miRNAs, 99 were downregulated, and 161 were upregulated (Fig. 2).

Functional analysis of differentially expressed mRNAs

GO and KEGG pathway analyses were utilized to examine the pathological process in HNSCC. With a cut-off of $p < 0.01$, 147 separate GO terms were enriched in up-regulated mRNAs, while 154 were found in down-regulated mRNAs. Up-regulated DE mRNAs were most significantly enriched in GO terms related to “extracellular matrix”, while down-regulated DE mRNAs were related to “muscle” functions. Meanwhile, 21 and 42 KEGG pathways were respectively obtained for up-regulated and down-regulated DE mRNAs without overlap, using $p < 0.05$ as the cut-off value. Most up-regulated DE mRNAs fell into protein digestion and absorption and ligand-receptor interaction pathways, while down-regulated DE mRNAs significantly covered salivary secretion and metabolism-related pathways (Fig. 3). Up-regulated DE mRNAs were most significantly enriched in GO terms related to extracellular matrix, synaptic function and endoderm development; down-regulated DE mRNAs in HNSCC were related to the muscle system-related processes.

Construction of the ceRNA regulatory network

For a better understanding of DE miRNAs’ functions regulated by DE lncRNAs, a ceRNA regulatory network was constructed by intersecting the results of miRcode prediction and differential expression analysis.

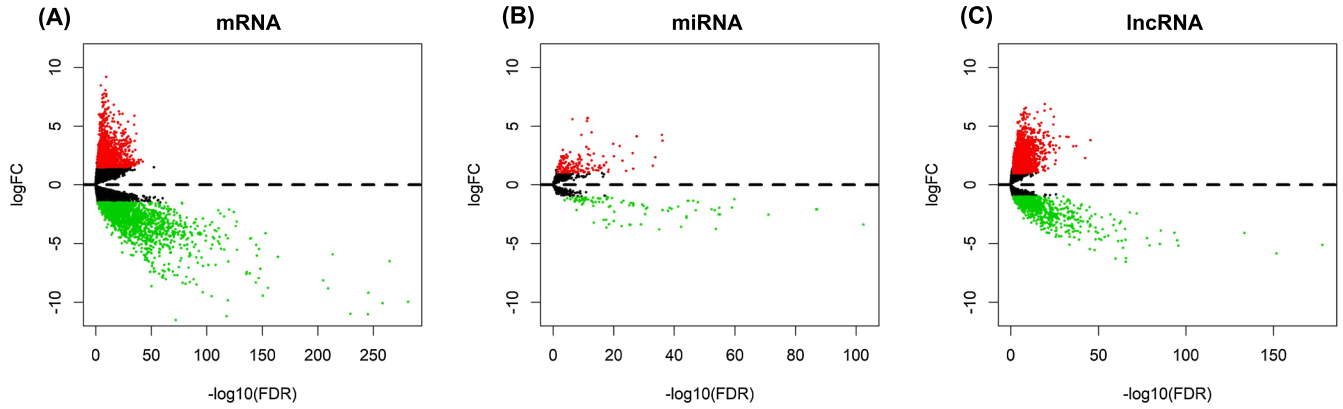

Fig. 2. Expression profiles of mRNAs (A), microRNAs (miRNAs) (B) and long non-coding RNAs (lncRNAs) (C) in HNSCC. Volcano plots show significantly changed mRNAs with log2-fold change ≥ 1.5 and significantly changed miRNAs and lncRNAs with log2-fold change ≥ 1. Significantly up-regulated expression is shown in red, while green indicates significantly down-regulated expression ($p < 0.05$; false discovery rate < 0.05). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

In all, 251 DE lncRNAs and 32 DE miRNAs combined with 1588 pairs of interaction relationships were first screened out. Furthermore, 93 targeted mRNAs were identified, through synthesizing miRDB, miRTarBase, TargetScan prediction and DE mRNA analysis. DE miRNAs with no targeted DE mRNA or positive relations between lncRNA-miRNA and miRNA-mRNA were then excluded. Finally, a network of 178 lncRNAs, 19 miRNAs and 55 mRNAs combined with 590 interaction relationships were built and visualized by Cytoscape v3.6.1 (Fig. 4). It was intriguing that miR-204 and miR-211 dominated the network's list of degree analysis, scoring 56 and 55, respectively, with their networks highly entwined. Namely, 46 of these DE mRNA targets, including PRDM2, TPPP and SP1, are shared, while the numbers of predicted targets of miR-204 and miR-211 were 68 and 50, respectively. Moreover, 77 out of 251 predicted lncRNAs, such as TTTY14, PART1, MIAT and HCG15, were connected to both miR-204 and 211.

Construction of the protein-protein interaction network and DE miRNA-TF-Hub gene regulatory network

To identify more reliable DE mRNAs with different platforms, we used GEO datasets for further validation. In all, 246 DE mRNAs whose expression levels significantly changed among the GSE30784, GSE59102, GSE10759 datasets and TCGA were used to construct a PPI network (Fig. 5A&B). With the results of the MCODE plug-in and Network Analysis of this PPI network in Cytoscape, 52 DE mRNAs with both MCODE Score and degree higher than 5 were chosen to be the hub genes. MELK, PLK1, CEP55, CDKN3, KIF18A and KIF4A were the genes with the highest scores.

To further explore how DE miRNAs regulate DE mRNAs in transcriptional processes, we built the DE miRNA-TF-Hub gene regulatory network. In all, 39 predicted TFs were screened out by the website, each with at least 2 target genes overlapping with the 52 hub genes. Among them, RELA, NF-κB and SP1 had the highest overlap score; 11 common

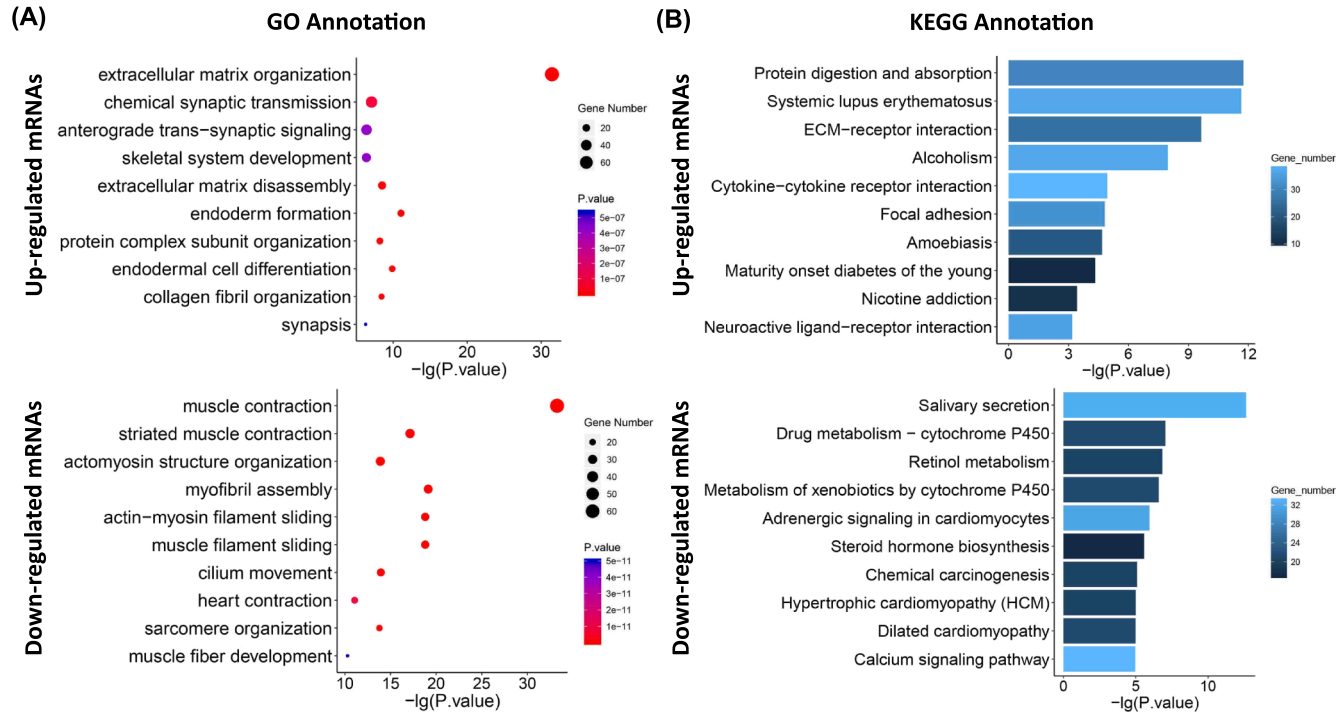

Fig. 3. Functional analysis of differential mRNAs in HNSCC. (A) Top 10 Gene Ontology (GO) annotations and (B) Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway annotations of up and down-regulated mRNAs were selected with top enrichment score (−log(p value)).

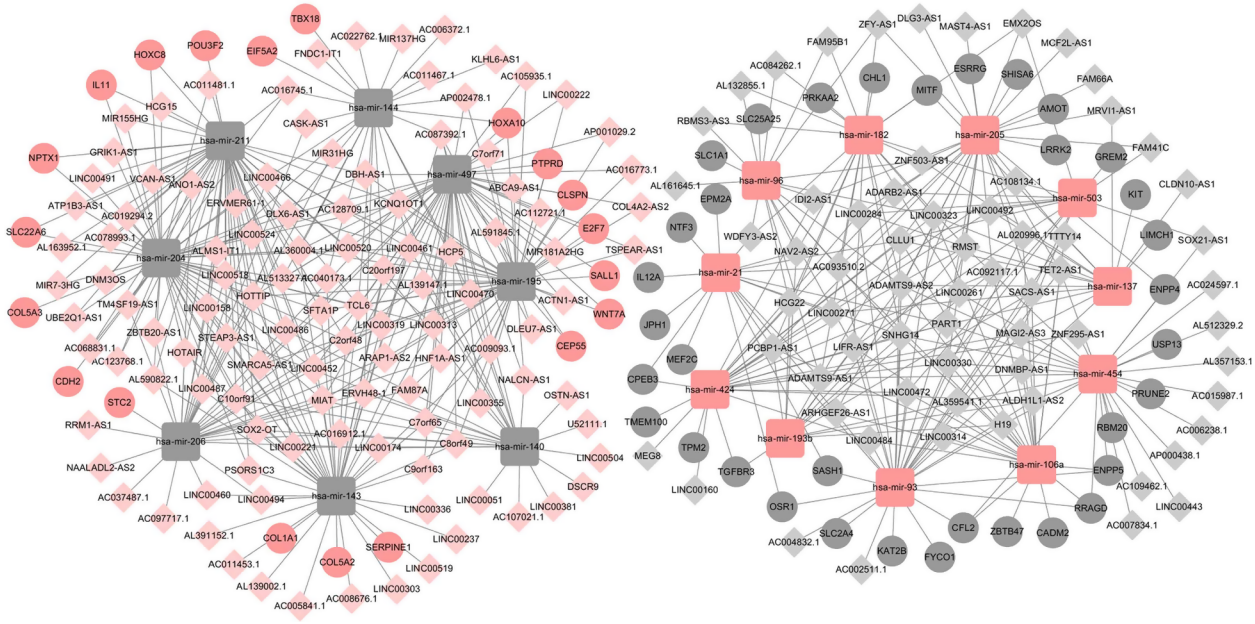

Fig. 4. The competence endogenous RNA (ceRNA) regulatory network of HNSCC pathogenesis. Rhombuses, squares and circles stand for lncRNAs, miRNAs and mRNAs, respectively. Up-regulated RNAs are shown in pink, while down-regulated ones are shown in gray. Gray lines represent regulatory relationships. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

TFs, listed in the predicted target mRNAs of DE miRNAs, which were identified by the 3 websites mentioned above, were selected for further analysis. The 18 hub genes and 11 DE miRNAs were then sorted in accordance with the 11 TFs. Coherently, these three components constitute the network of DE miRNA-TF-hub genes with 21 miRNA-TF pairs and 38 TF-hub gene pairs (Fig. 5C). The network presented elucidated another regulatory possibility of miRNA as tipping the expression level of TFs to control the progress of HNSCC. Specifically, SP1 remains the TF with highest degree in network analysis and is predicted to regulate hub genes such as PLAU, SERPINE1, TIMP1, MMP9, SPP, MYBL2, TNC, and FOXM1. Most strikingly, in this network, miR-204/211 appear to be SP1-predicted regulators, which demonstrates that their indirect

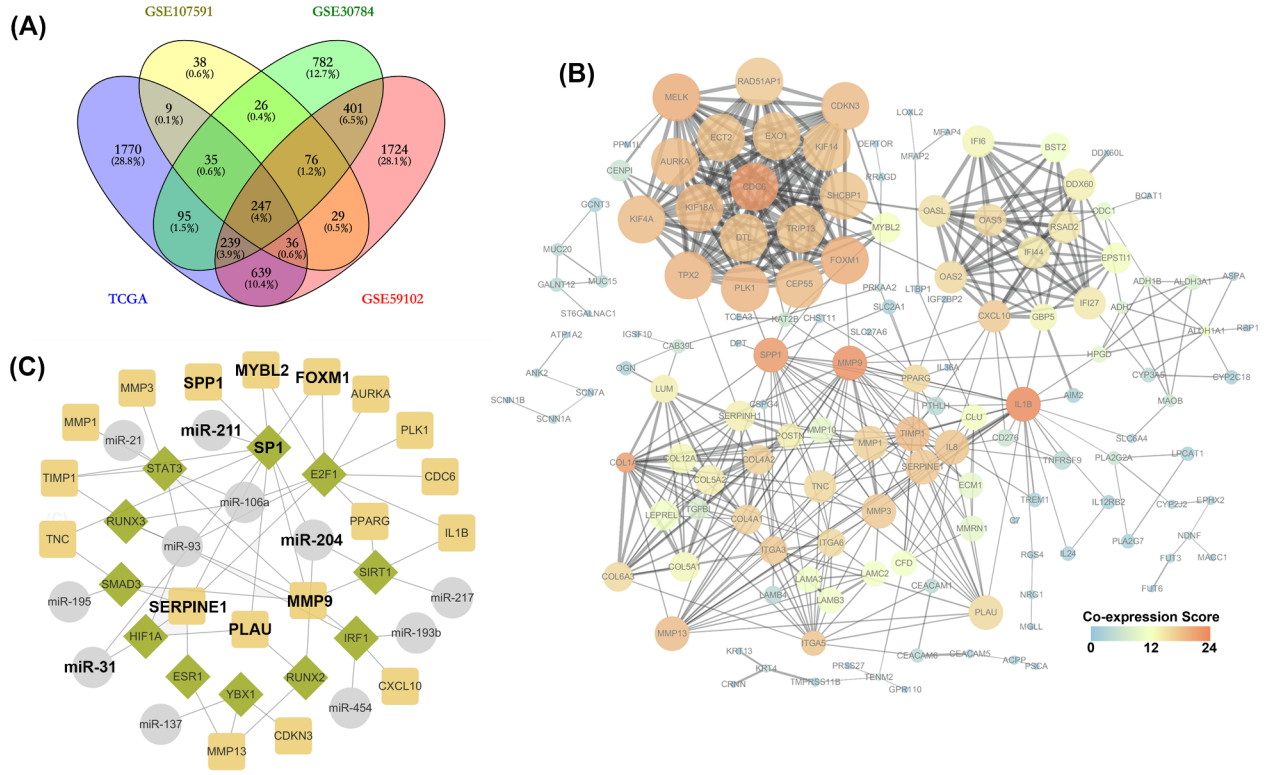

Fig. 5. Validation in GEO database (A). Venn diagram shows validated DE mRNA by GSE30784, GSE59102, GSE10759 datasets and TCGA. The DE miRNA-TF-Hub gene regulatory network, the SP-1 associated elements are highlighted by heavy symbols (B). Gray circles stand for DE miRNA, green rhombuses for TFs, and yellow squares represent hub genes. Protein-protein interaction (PPI) network (C). Node colors are mapped to co-expression scores. Node sizes are mapped to MCODE Score, with low values mapped to small sizes. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

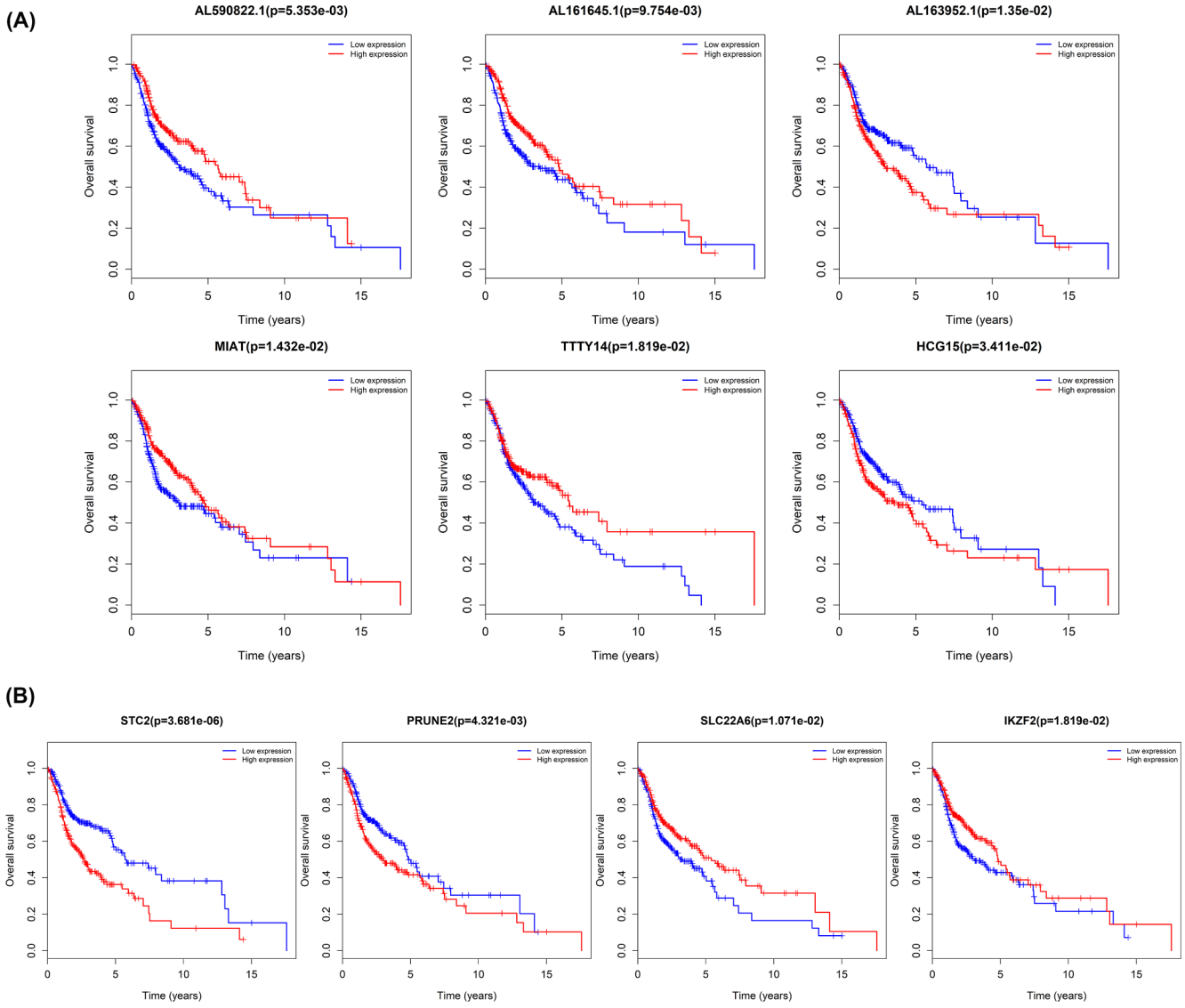

Fig. 6. Survival analysis of top miR-204/211-related DE RNAs in HNSCC. Plots show Kaplan-Meier survival curves of 6 DE lncRNA (A) and 4 DE mRNA (B) from 499 HNSCC patients' clinical data on TCGA. The median value of expression was set as cut-off point.

regulatory role through transcription factor on the bulk of hub genes. It is worth noting that besides miR-204/211, miR-106 and miR-193 have 3 and 5 predicted target TFs respectively, revealing their value for further study.

A compelling pattern of patient survival-related RNAs linked to miR-204/211

Survival analysis was based on profiles of 499 HNSCC patients in TCGA clinical datasets. Patients were divided into high-expression and low-expression groups according to the median value of each gene expression level. Kaplan-Meier survival curves showed that the expression levels of 15 DE lncRNAs and 14 DE mRNAs were co-related with overall survival. Among them, 6 out of 15 DE lncRNAs were directly related to miR-204/211, while 4 out of 14 DE mRNAs were predicted to be targeted by miR-204/211 (Fig. 6). Namely, the related lncRNAs were TTTY14, AL590822.1, AL163952.1, MIAT, HCG15, and AL161645.1, and the targeted mRNAs were IKZF2, SERP1, SLC22A6, and STC2. Meanwhile, PLAU and SERPINE1, two targeted hub genes of SP1, were predicted to be indirectly mediated by miR-204/211 and significant to OS. Therefore, we have provided an overview of the survival-related genes of HNSCC, some of which deserve further study. Furthermore, the genes in Fig. 6 interact with miR-204/211, which underlines the crucial role of the two miRNAs based on clinical data.

Mature sequences of hsa-miR-204-5p and hsa-miR-211-5p share the same seed sequence and may be a critical miRNA cluster in HNSCC

According to the central role of miR-204 and miR-211 in both ceRNA and TF regulatory networks, with large proportions of their related genes shown to be significant in a survival analysis, it is clear that both miR-204 and miR-211 play critical roles in the HNSCC pathological process. Although there are two mature miRNAs for each pre-miRNA, only one is abundant and plays a predominant role. Based on sequence alignment and miRbase database analysis, miR-204-5p and miR-211-5p were found to act on behalf of the two pre-miRNAs. Identical seed sequences of these two mature miRNAs were then revealed (Fig. 7). On the one hand, these results may explain the shared target genes of the two miRNAs; on the other hand, the identical structure may result in the entwined relationship of miR-204/211, which play an intricate and crucial role in HNSCC.

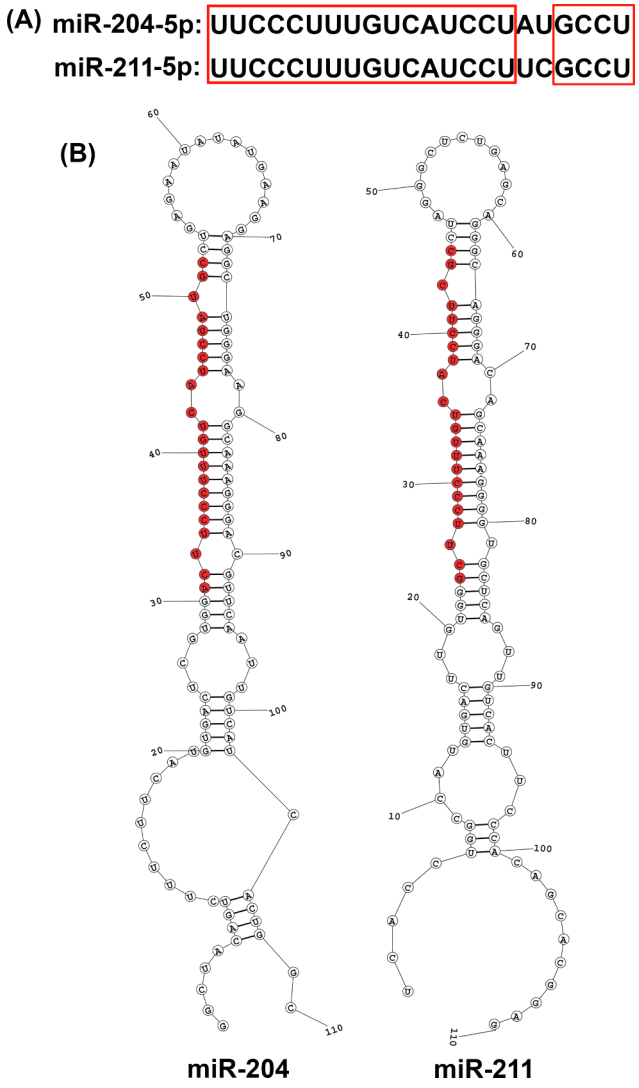

Fig. 7. Gene sequences and 2nd structure of miR-204 and miR-211 (A). Similar mature sequences of miR-204 and miR-211; the sequences in the red frame are common base seed sequences (B). Predicted 2nd structure of pre-miR-204/211. The mature miR-204/211-5p are indicated in red. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Discussion

Multiple studies have revealed the complex gene expression pattern during oncogenesis. In some circumstances, miRNA-mediated control may indeed act as a master regulator of gene expression [20]. Clarification of such interactive regulatory networks in cancers could provide new therapeutic opportunities. However, a comprehensive analysis based on the centric role of miRNAs in HNSCC remains unavailable. Therefore, we aimed to explore the effects of miRNAs in HNSCC pathogenesis focusing on both pre-transcriptional and post-transcriptional processes. Here, we conducted constructed a ceRNA network, unraveling the bridging role of miRNA between lncRNA and mRNA. An miRNA-TF-hub gene network showed the pre-transcription regulation by miRNA. In addition, survival analysis deepened our findings in the clinical context of HNSCC. Similar research studies were conducted in HNSCC or OSCC that focused on either the "sponging" role of lncRNA or the direct interaction of miRNAs on mRNAs without considering TFs [16,18,21]. In contrast, our study introduced the ceRNA network as a filter to sort out promising miRNAs and took other networks into consideration. Furthermore, we underscored the centric role of miRNA from both pre- and post-transcription perspectives, together with confirmatory survival analysis. Therefore, our study provides a new and plausible multidimensional approach for studies on cancer pathogenesis.

Notably, the role of miR-204/211 was highlighted in our networks. Not only did these miRNAs rank highly in the ceRNA network, they also both interacted with SP1, a crucial TF in HNSCC. Moreover, a compelling pattern of RNAs was related to overall patient survival and was also linked to miR-204/211. These findings led us to explore their secondary structures, where a high similarity in both pre- and mature miRNA forms was confirmed. Echoing our results, identical target genes and similar functions of miR-204/211 in cancer pathogenesis were revealed in other studies [16,22–25]. Besides, the host gene of miR-204, TRPM3, as well as miR-211's host gene TRPM1, show a decreasing trend in HNSCC, though without significance (data not shown). The two genes both encode members of the transient receptor potential cation channel subfamily. Intriguingly, TRPM3 was shown to be significantly altered in gingival-buccal oral squamous cell carcinoma and could function synergistically with miR-204 as suppressor of tumor growth, migration and invasion [26]. Therefore, such similarities between miR-204 and miR-211 could demonstrate their possible interplay in HNSCC progression.

Meanwhile, the differences between miR-204 and miR-211 were also noted in our ceRNA and DE miRNA-TF-Hub gene network of HNSCC. Specifically, we noted the differently targeted lncRNAs, mRNAs, TFs and hub genes (Figs. 4, 5B) by miR-204/211. Among them, a particular TF, RUNX2, was found to be regulated by miR-204 but not miR-211. The RUNX2-PTHLH axis was shown to stimulate tumor growth [27], while enforced RUNX2/INHBA expression promoted HNSCC metastasis [28]. Therefore, the negative regulation of miR-204 on RUNX2 displayed in our work could echo the tumor-suppressive role of miR-204 [29–32]. In contrast, miR-211 lacks this regulatory pathway, which could partly explain its opposing, oncogenic role in HNSCC [33–35], and is open for further exploration.

Furthermore, miR-204/211 both appeared to decline in HNSCC tissue compared with normal tissue in our work, in line with other bioinformatics and in vivo/in vitro studies [18,29-32,36], showing their possible tumor-suppressive function. In addition, in Chen's study on human samples, a reduced miR-211 expression was found in tumor samples compared with noncancerous tissue [34]. However, some research on transgenic mice or cell lines revealed miR-204 as suppressive and miR-211 as oncogenic in HNSCC [33–35], which appears to be a contradiction between bioinformatic prediction and clinical data. To the best of our knowledge, the heterogeneity of samples in large-scale bioinformatic analyses could help explain the divergence. For instance, Chen et al. found different results in their studies on human samples and mice tissue. The authors attributed such contradictions to the high miR-211 expression in submucosal tissue from humans, which confounded the comparison between those samples and epithelial-only tissue, as in mouse samples. Because the HNSCC and normal samples included in our study were from genetically different patients, and the samples were not confined to epithelial tissue, our bioinformatic prediction could differ from genetic- and type-controlled research, yet could be applied to a wider range of conditions. Thus, broadly considering bioinformatic analyses of heterogenetic samples and a large number of molecules could provide us with a general overview, while point-focused and tissue-specific in vitro/in vivo research help us obtain a deeper understanding. Therefore, a combination of bioinformatics and in vitro/in vivo studies could contribute to mutual validation, facilitation and inspiration in cancer research.

Conclusion

In summary, we established lncRNA-miRNA-mRNA and miRNA-TF-mRNA regulatory networks and then introduced survival analysis to reveal the possible role of miRNAs in HNSCC. On the one hand, the

promising centric standing of miR-204/211 that we found may shed light on improved diagnosis or targeted treatment of HNSCC; on the other hand, a comprehensive approach to pathogenesis analysis of other diseases is provided.

Declaration of Competing Interest

Jingyi Cai, Yeke Yu, Yuzi Xu, Hao Liu, Jiawei Shou, Liangkun You, Hanliang Jiang, XuFeng Han, Binbin Xie, Weidong Han declare that: we have no proprietary, financial, professional or other personal interest of any nature or kind in any product, service and/or company that could be construed as influencing the position presented in, or the review of, the manuscript entitled: Exploring the role of Mir204/211 in HNSCC by the combination of bioinformatic analysis of ceRNA and transcription factor regulation.

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