Cancer Genetics 233–234 (2019) 75–83

Cancer Genetics

ORIGINAL ARTICLE

Identification of eight meta-signature miRNAs as potential biomarkers for oropharyngeal cancers

Bo Li, Hee-moon Kyung*

Department of Orthodontics, Dental School, Kyungpook National University, 2177, Dalgubeoldae Ro, Jung Gu, Daegu 41940, Republic of Korea

Abstract

Background: Oropharyngeal Cancers (OC) is a commonly-seen disease with a high risk. The earlier studies of miRNAs on this disease were restricted by factors as sequencing platform, filtration conditions, causing the inconformity in the obtained result. We aimed to explore the miRNA biomarkers that can function as the predictive and therapeutic markers. Meta-analysis was performed on the currently obtained miRNA result and the functions of the target genes regulated by meta-signature miRNA were further investigated.

Material and methods: Seven representative miRNA datasets of OC were selected, and the meta-signature miRNAs were determined by overlap comparison. The corresponding target genes were predicted by TargetScan software. Then, functional enrichment and transcriptional factors analysis were performed on these target genes by DAVID (The Database for Annotation, Visualization, and Integrated Discovery) dataset and Tfacts dataset.

Results: Eight meta-signature miRNAs were identified, including seven were up-regulated and one down-regulated (hsa-miR-203a-5p). The up-regulated miRNAs were mainly enriched in pathways as GO:0000122-negative regulation of transcription from RNA polymerase II promoter, phosphatidylinositol phosphorylation, MAPK signaling pathway, and Ras signaling pathway, etc., while the down-regulated miRNAs were enriched in pathways as, response to reactive oxygen species, p53 signaling pathway, calcium signaling pathway, etc. A total of 124 transcription factors (TFs) were identified, 43 among were found to co-exist in both types of target genes.

Conclusion: Eight important miRNAs were identified by meta-analysis as well as the corresponding target genes and transcription factors. The potential functions were revealed, which will provide novel insights for the target treatment of OC.

Keywords Oropharyngeal cancer, miRNA, Function, Meta-analysis.

Introduction

Oropharyngeal cancer is a commonly seen cancer which is usually formed in the tissue of the throat. It is usually divided into two types, HPV-positive and HPV-negative cancer. HPV-positive is the type that patients with human papillomavirus infection are prone to be affected, and the other type HPV-negative cancer, whose occurrence is usually linked to alcohol or tobacco use. It usually develops into many different stages. The unhealthy habits as smoking and drinking alcohol can largely increase the risk of the patients with OC. Besides, patients with oropharyngeal cancer are prone to have an increased risk of another cancer in the head or neck, since it can easily spread to other parts of the body through tissue, blood, etc. Patients with OC are usually featured by some obvious symptoms, such as a lump in the back of the mouth, throat, or neck or having a sore throat. However, sometimes there are not any early signs or symptoms caused by oropharyngeal cancer, which thus makes the diagnosis of this disease using molecular methods quite necessary and critical. What is more, the clinical treatment for oropharyngeal cancer is restricted to factors as resection, radiotherapy, and chemotherapy, and the effect is not quite satisfactory. What is worse, patients are generally with poor prognosis after treatment Therefore, the research on the new molecular biomarker as prognostic and therapeutic target in clinical management may improve the prognostic status of the patients with OC.

* Corresponding author.
E-mail address: mkyung@knu.ac.kr

2210-7762/$ - see front matter © 2018 Published by Elsevier Inc.
https://doi.org/10.1016/j.cancergen.2018.10.004

MicroRNA (miRNA), a kind of non-coding small miRNAs with the length ranging from 18 nucleotides to 24 nucleotides, is becoming increasingly prevalent in medical research. MiRNAs are reported to be in close relation to the occurrence and development of many types of cancers and it can act as oncogenes or anti-oncogenes. Besides, they are reported to be involved in many biological processes as cell growth, apoptosis, cell proliferation and differentiation, since it can degrade the mRNAs of target genes or interfere the translation process. The dysregulation of miRNAs may be the cause of many kinds of diseases, which hence can provide theoretical evidence for target therapy of many types of cancers. Research on miRNAs has been successfully applied in various types of cancers, including glioma, lymphocytic leukemia, gastric cancer, colonic cancer, but as for oropharyngeal cancer, there has not been much research.

In this study, we aimed to provide the reliable information of miRNA associated with oropharyngeal cancer and to identify the corresponding target genes. A total of seven representative miRNA datasets of OC were selected and as a result, eight meta-signature miRNAs were identified. The target genes were predicted by TargetScan software. Besides, the functional enrichment and transcriptional factors analysis were combined used on these target genes, in the purpose for the identification of prognostic and predictive biomarkers for the survival of oropharyngeal cancer.

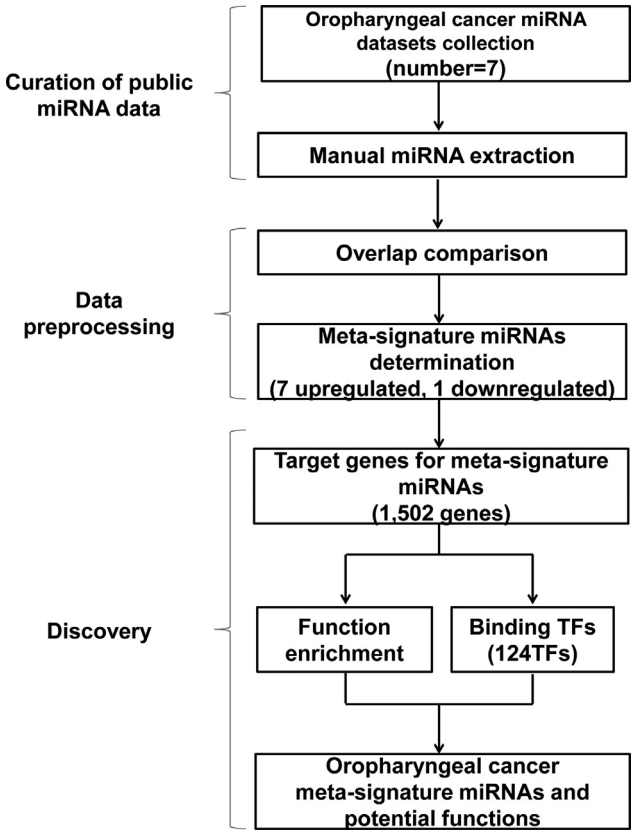

Fig. 1 Flow chart of this research.

Materials and methods

Identification of miRNA datasets and screening of differentially expressed miRNAs

Articles about oropharyngeal cancers were explored using the key words “Oropharyngeal Cancer” and “miRNA” on google website and the samples used in these studies were carefully explored and studied, including the aspects of sequencing samples, the control samples, the identification and verification method of the differentially expressed miRNAs, etc. The information of the differentially expressed miRNAs in detail was extracted and the detailed information of the up and down-regulated miRNAs of OC was also obtained by comparing with the control samples. The incidence of these up and down-regulated miRNAs appearing in conditional, separate datasets was counted and the criterion was set as that the miRNAs appeared in at least two separate datasets, according to the distribution situation of the differentially expressed miRNAs in the dataset. The flow chart was shown in Fig. 1.

Target genes prediction of differentially expressed miRNAs

Sequencing information of the differentially expressed miRNAs was explored from the miRBase dataset (http://www.mirbase.org/, version 21) and the sequencing information was arranged into fasta files. TargetScan software [1] was used to predict the corresponding target genes of the differentially expressed miRNAs. The maximum number of target genes of each miRNA was set as 300, and the other values used the default parameters.

Functional enrichment analysis of target genes of the differentially expressed miRNAs of OC

The predicted target genes of the differentially expressed miRNAs were mapped to DAVID datasets [2]. Gene ontology (GO) [3] and KEGG pathway [4, 5] analysis were performed on these genes. The top five mostly enriched biological terms shown by Go and KEGG analysis were extracted out, all with the p-value less than 0.05.

Transcription factors analysis of target genes of up and down regulated miRNAs

The target genes of up and down-regulated miRNAs were submitted to Tfacts datasets [6, 7] with the website of http://www.tfacts.org/. Four indicators, p-value, q-value, E-value and FDR, were used to predict the transcription factors regulating the expression of the target genes of meta-miRNAs. Only factors with value of both indicators among less than 0.05 were recognized as reliable factors. The transcriptional factors of both up and down-regulated were counted respectively. Besides, we also determined the common and specific transcription factors among the target genes regulated by up and down miRNAs.

Table 1 The basic feature of the differentially expressed miRNA datasets of OC.

Dataset	Acronym	Samples	Assay/sequencing type	Validated
(Kalfert et al., 2015)	**DK**	It comprised of 51 patients with HNSCC (23 oropharyngeal, 24 laryngeal and 4 hypopharyngeal carcinomas). Total RNA was extracted from tumor tissue and normal squamous epithelium using the miRNeasy FFPE Kit.	TagMan® MicroRNA assay	–
(Brito et al., 2016)	**BL**	Paraffin-embedded tissue samples from 35 oral cavity and oropharynx squamous cell carcinoma cases and 10 non-neoplastic samples from oral mucosa tissue adjacent to biopsies of inflammatory salivary glands were obtained from files from the Department of Pathology, AC Camargo Cancer Center, São Paulo, Brazil.	Real-time RT-PCR	–
(Ge et al., 2013)	**GG**	A total of 150 oropharyngeal SCC cases were included in this study, including 101 cases for training and 49 cases for validating a new miRNA-based prognostic model.	Real-time reverse transcriptase-polymerase chain reaction (RT-PCR)	–
(Hui et al., 2013)	**AB**	88 formalin-fixed and paraffin-embedded (FFPE) OPC biopsies (p16-positive: 56; p16-negative: 32).	TaqMan Low Density Array (TLDA) Human MicroRNA Panel v1.0 (Applied Biosystems)	–
(Lajer et al., 2011)	**CLa**	51 patients with OSCC/PSCC and 40 control patients.	Affymetrix miRNA array chips	qPCR
(Lajer et al., 2012)	**CLb**	31 tumour samples were selected for laser microdissection and 13 samples from patients with non-neoplastic tonsillar disease were included as controls.	Affymetrix GeneChip miRNA Array	qPCR
(Zhang et al., 2016)	**XZ**	The cohort consisted of 43 patients (39 males, 4 females) treated for tonsillar cancer at Royal Prince Alfred Hospital Sydney, Australia between 2002 and 2006.	A commercial LNA-modified oligonucleotides library (Exiqon, Denmark)	qRT–PCR

Results

The differentially expressed miRNAs screening

Meta-analysis method was used in this study to determine the critical up and down-regulated miRNAs of the currently obtained miRNA dataset of OC. The target genes were then predicted. The functional enrichment analysis and the transcriptional factors analysis were also performed. Seven separate miRNA expression datasets were determined based on the current result of the miRNAs expression profile of OC. The expression result of the miRNAs was shown clearly in these datasets compared with the control samples. Acronym of the authors was used for the numbering of these seven miRNAs expression datasets. The information in detail was listed below: (1) DK [8]; (2) BL [9]; (3) GG [10]; (4) AB [11]; (5) CLa [12]; (6) CLb [13]; (7) XZ [14]. The main character of the seven datasets used for analysis was shown in Table 1. There was some difference in the number, types of the samples, and the types of the microarrays. The distribution situation of the differentially expressed miRNAs in the seven datasets was analyzed by SVG model painting of Perl. There was a significant difference between the up and down-regulated miRNAs among different datasets (Fig. 2). The top three datasets were dataset AB (40, all up-regulated), dataset CLa (21, 2 up-regulated and 19 down-regulated), and dataset GG (13, 8 up-regulated and 5 down-regulated). There were the most up-regulated miRNAs (40) in AB dataset, and there were the most down-regulated miRNAs (19) in CLa dataset, followed by GG dataset (five down-regulated miRNAs) and BL dataset (three down-regulated miRNAs). There was no down-regulated miRNA in dataset AB and dataset XZ.

Screening and determination of the differentially expressed miRNAs

A total of 75 non-redundant miRNAs were identified in these seven separate datasets. There was a significant difference in the number and component of the differentially expressed miRNAs among different datasets, which made the meta-analysis on these datasets quite necessary (Fig. 2A). The differentially expressed miRNAs, supported by at least two datasets, was set as the reliable and meta-featured miRNAs, that is, meta-signature miRNA (Fig. 2B and C). As a result, a total of 8 meta-signature miRNAs were identified, among which seven was found to be up-regulated, that is, hsa-miR-106b-5p,hsa-miR-146a-5p, hsa-miR-146b-5p, hsa-miR-195-5p, hsa-miR-21-5p, hsa-miR-26b-5p, and hsa-miR-9-5p, only one, that is, hsa-miR-203a-5p, was found to be down-regulated. The specific location information was extracted from the miRBase dataset [15] (Table 2). The distribution of the chromosome of these seven miRNAs were dispersed, among which the fifth chromosome, (hsa-miR-146a-5p and hsa-miR-9-5p) and the seventeenth chromosome (hsa-miR-195-5p and hsa-miR-21-5p) included two meta-signature

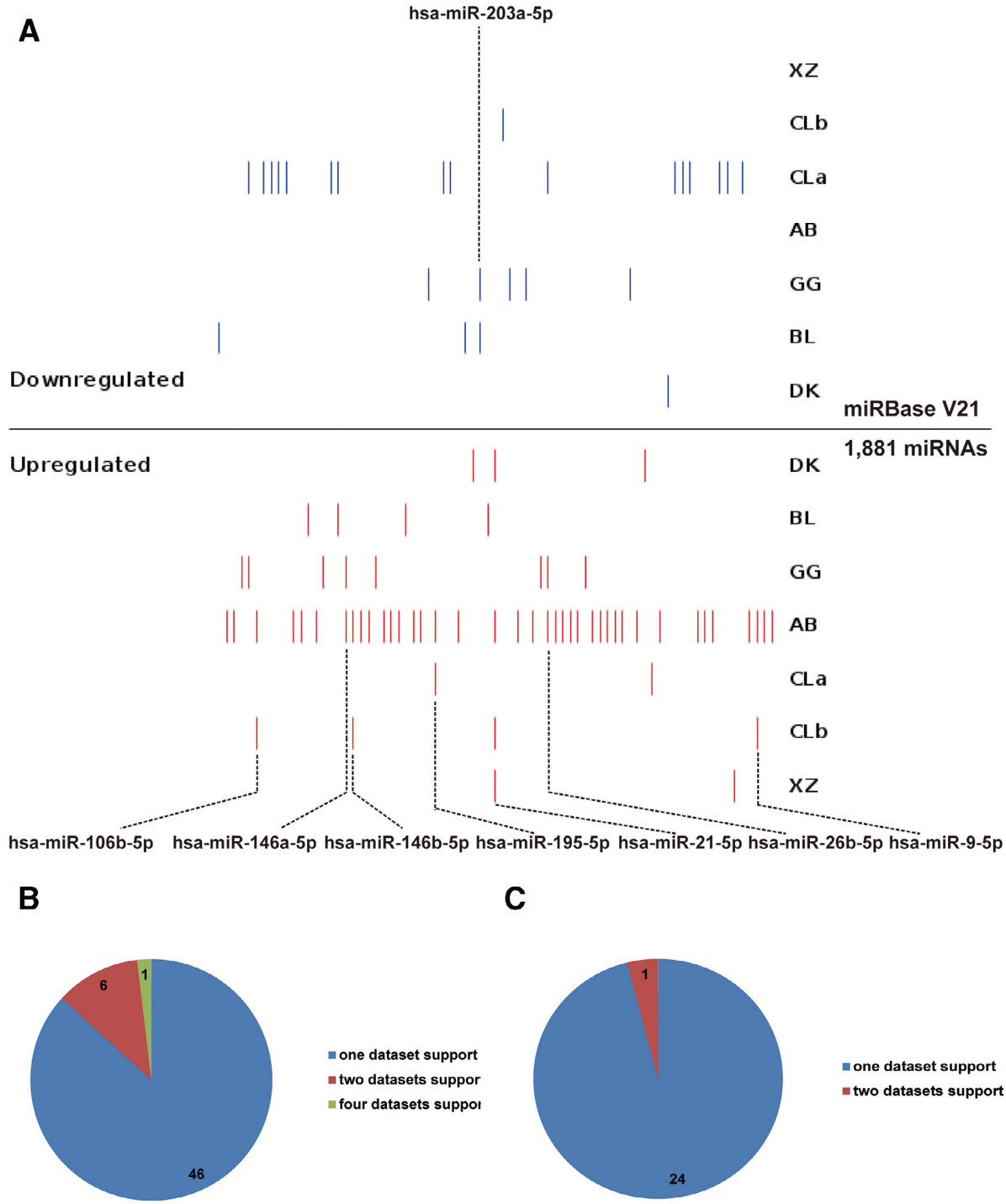

Fig. 2 The distribution of the differentially expressed miRNAs in the seven OC datasets. (A) The distribution of the differentially expressed miRNAs in different datasets. The red line represented the up-regulated miRNAs while the blue line represented the down-regulated miRNAs. The pattern indicated that there was great difference in the number and distribution of the differentially expressed miRNAs in different datasets. The eight miRNAs highlighted in this figure were miRNAs with meta feature. (B) The statistic of the up-regulated miRNAs in OC dataset. A total of 53 up-regulated miRNAs were obtained in the seven datasets, among which 46 miRNAs were supported by one dataset, and 6 miRNAs were supported by two datasets, one miRNA was supported by four datasets. (C) The statistic of the number of the down-regulated miRNAs in OC dataset. A total of 25 down-regulated miRNAs were obtained in the seven datasets, among which 24 were supported by one dataset and one miRNA was supported by two datasets. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Table 2 Meta-signature miRNA list of OC.

miRNA	Chr	Beg	End	Strand	Support datasets	Sequence
Upregulated						
hsa-miR-106b-5p	chr7	100094043	100094063	–	2	UAAAGUGCUGACAGUGCAGAU
hsa-miR-146a-5p	chr5	160485372	160485393	+	2	UGAGAACUGAAUUCCAUGGGUU
hsa-miR-146b-5p	chr10	102436520	102436541	+	2	UGAGAACUGAAUUCCAUAGGCU
hsa-miR-195-5p	chr17	7017667	7017687	–	2	UAGCAGCACAGAAAUAUUGGC
hsa-miR-21-5p	chr17	59841273	59841294	+	4	UAGCUUAUCAGACUGAUGUUGA
hsa-miR-26b-5p	chr2	218402657	218402677	+	2	UUCAAGUAAUUCAGGAUAGGU
hsa-miR-9-5p	chr5	88666902	88666924	–	2	UCUUUGGUUAUCUAGCUGUAUGA
Downregulated						
hsa-miR-203a-5p	chr14	104117431	104117455	+	2	AGUGGUUCUUAACAGUUCAACAGUU

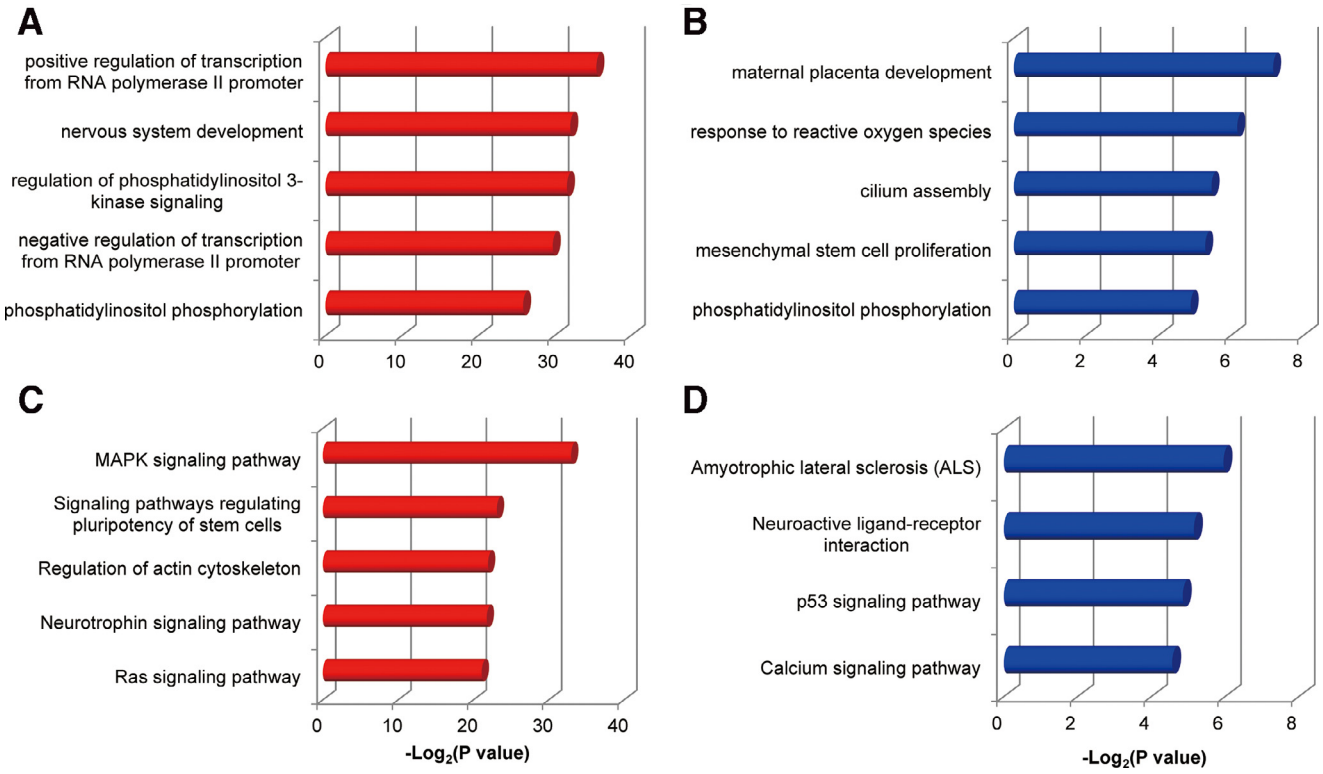

Fig. 3 Go and KEGG enrichment analysis of the target genes of the up and down-regulated meta-signature miRNAs. (A) Go enrichment analysis of target genes of the up-regulated meta-signature miRNAs. (B) Go enrichment analysis of target genes of the down-regulated meta-signature miRNAs. (C) KEGG enrichment analysis of target genes of the up-regulated meta-signature miRNAs. (D) KEGG enrichment analysis of target genes of the down-regulated meta-signature miRNAs.

miRNAs, while the second chromosome (hsa-miR-26b-5p), the seventh chromosome (hsa-miR-106b-5p), the tenth chromosome (hsa-miR-146b-5p) and the fourteenth chromosome (hsa-miR-203a-5p) included only one meta-signature miRNA, respectively.

Target genes prediction of the differentially expressed miRNAs

The target genes of the eight meta-signature miRNA were predicted by TargetScan software [16] (Supplementary Table 1). The number of the target genes was set as 300 at the most. The number of four meta-signature miRNA (hsa-miR-106b-5p, hsa-miR-21-5p, hsa-miR-9-5p, and hsa-miR-203a-5p) among was 300 and the number of the left four meta-signature miRNAs, that is, hsa-miR-146a-5p, hsa-miR-195-5p, hsa-miR-146b-5p, and hsa-miR-26b-5p was 235, 122, 40, and 23, respectively.

The functional enrichment analysis of the target genes regulated by the differentially expressed miRNAs

Go functional enrichment analysis was performed on the differentially expressed meta-signature miRNAs

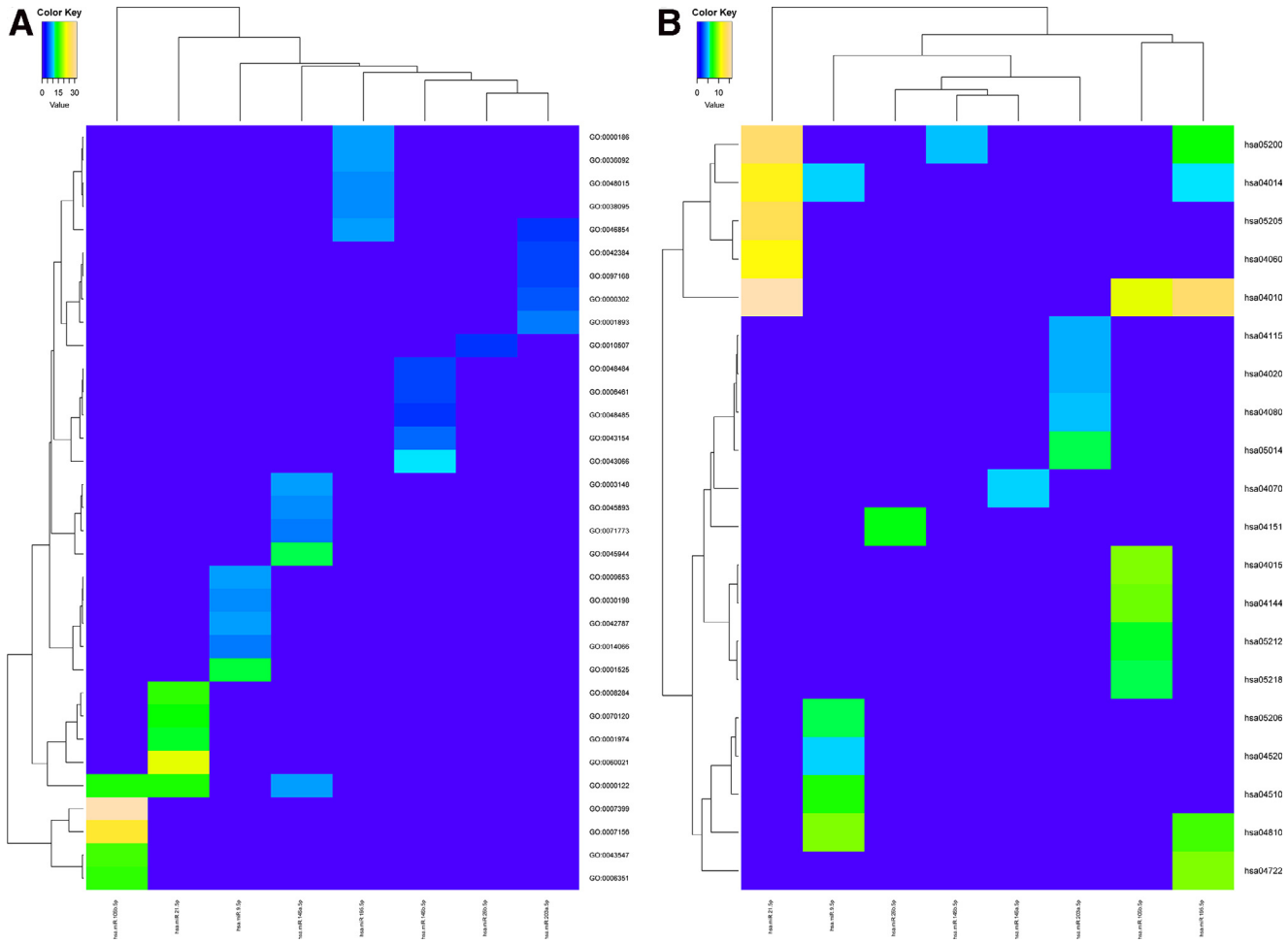

Fig. 4 Go and KEGG enrichment analysis of target genes of the meta-signature miRNAs. (A) Go enrichment analysis of target genes of meta-signature miRNAs. (B) KEGG enrichment analysis of target genes of meta-signature miRNAs.

(Supplementary Table 2**)**. The result showed that the target genes of the up-regulated meta-signature mRNAs were mainly enriched in biological processes, as GO:0045944-positive regulation of transcription from RNA polymerase II promoter, GO:0000122-negative regulation of transcription from RNA polymerase II promoter, GO:0014066-regulation of phosphatidylinositol 3-kinase signaling, GO:0046854-phosphatidylinositol phosphorylation, etc. (Fig. 3A), while the target genes of the down-regulated meta-signature mRNAs were mainly enriched in biological pathways as GO:0000302-response to reactive oxygen species, GO:0046854-phosphatidylinositol phosphorylation, etc. (Fig. 3B).

The result of KEGG enrichment analysis showed that the target genes of the up-regulated miRNAs were mainly enriched in pathways as hsa04010:MAPK signaling pathway, hsa04810: Regulation of actin cytoskeleton, hsa04014: Ras signaling pathway, etc. (Fig. 3B), while the target genes of the down-regulated meta-signature miRNAs were mainly enriched in pathways as hsa04115: p53 signaling pathway and hsa04020: Calcium signaling pathway (Fig. 3C).

Go and KEGG enrichment analysis was then performed on each meta-signature miRNAs, and we found that there was a significant difference in the enriched terms and the enriched degree among different meta-signature miRNAs (Fig. 4). The result of Go enrichment analysis showed that hsa-miR-106b-5p, hsa-miR-146a-5p, and hsa-miR-21-5p were mainly enriched in GO:0000122-negative regulation of transcription from RNA polymerase II promoter (Fig. 4A), indicating that it may have a great effect on the occurrence of OC. Besides, three pathways, hsa04010:MAPK signaling pathway, hsa04014: Ras signaling pathway and hsa05200: Pathways in cancer, were found to be mostly enriched in the target genes of three meta-signature miRNAs, and all had a higher enriched degree, indicating their potentially important role in the occurrence of OC (Fig. 4B).

Transcription factors analysis of the target genes of the differentially expressed miRNAs

Transcription factors genes of the up and down-regulated miRNAs were further analyzed (Supplementary Table 3). And we compared the difference between the two types of target genes. As a result, a total of 436 interactions were formed between the 104 transcription factor genes and the 166 target genes. Among the target genes of the down-regulated miRNAs, a total of 104 interactions were formed between the 63

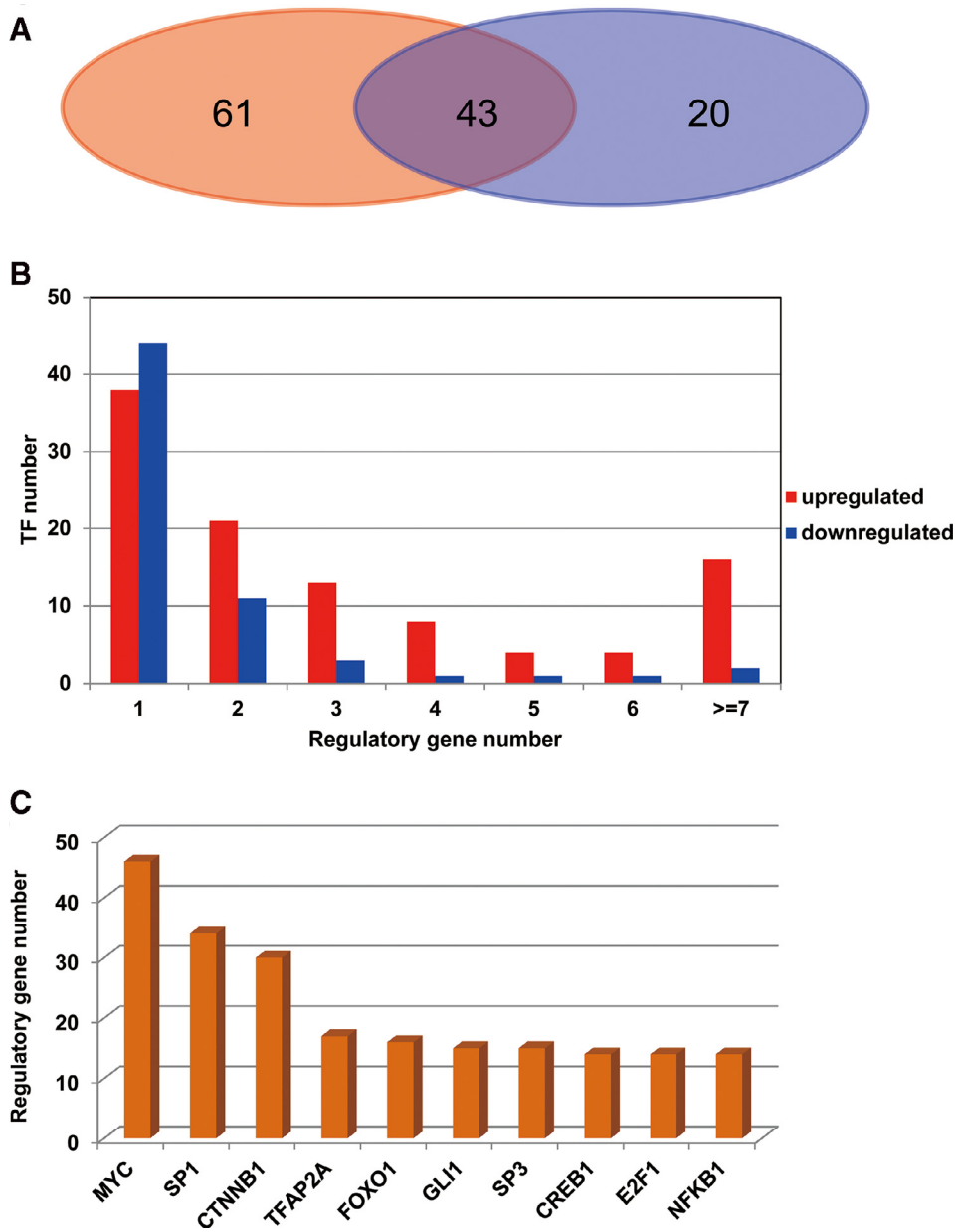

Fig. 5 The transcriptional factors analysis of the target genes of the up and down-regulated miRNAs. (A) The common and specific transcriptional factors of the target genes of the up and down-regulated meta-signature miRNA. The red background represented that the number of transcriptional factors of the target genes of the up-regulated meta-signature miRNA while the blue background represented that the number of transcriptional factors of the target genes of the down-regulated meta-signature miRNA. (B) The number statistics of the expressed genes regulated by the transcriptional factor genes. (C) The top 10 transcriptional genes that regulated the largest number of genes. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

transcription factor genes and the 26 target genes. There were a total of 124 transcription factor genes that regulated the target genes of meta-signature miRNA, and 43 genes among were co-existing in both types of target genes, accounting for 34.7% of all the transcription factor genes. A high rate of transcription factor genes only regulated the single target gene, which was shown in Fig. 5B. By analyzing the statistic data of the regulated target genes, we found that transcription factors as *MYC, SP1, CTNNB1, TFAP2A,* and *FOXO1* had a great regulatory effect on the target genes of miRNA (Fig. 5).

Discussion

In this study, a total of eight essential meta-signature miRNAs, that is, hsa-miR-106b-5p, hsa-miR-146a-5p, hsa-miR-146b-5p, hsa-miR-195-5p, hsa-miR-21-5p, hsa-miR-26b-5p, hsa-miR-9-5p and hsa-miR-203a-5p, were identified, as well as the corresponding target genes and the transcriptional factors, which had the potential to serve as the predictive and target therapeutic biomarkers for the oropharyngeal cancer in clinical management. Transcriptional factor as *MYC, SP1, CTNNB1, TFAP2A* and *FOXO1,* were found to play an

important role in the regulation process of the target genes of miRNAs. Besides, meta-analysis method was used in this research, which was quite necessary and reasonable for multi-dataset analysis, due to the multi-factorial differences caused by factors as platform, sample selection and filtration conditions, etc. Herein, the candidate miRNAs identified in this article were more convincing and feasible for further potential application in clinical practice.

It was reported that hsa-miR-106b was up-regulated in many types of cancers, including colonic cancer, gastric cancer, glioma, and its effect may be initiated by inhibiting the target genes. In this study, hsa-miR-106b was found to be up-regulated in patients with oropharyngeal cancer, we assumed that its role on the occurrence and development of OC may be played by affecting the expression status of the target genes. As for miR-146a, it was reported to have a critical role in the occurrence of prostate cancer cells apoptosis by the regulation of ROCK/Caspase 3 pathway [17]. According to the result in this research, hsa-miR-146a-5p was found to be up-regulated, which could be explained by that the target genes were involved in the cell apoptosis by the regulation of the actin cytoskeleton signaling pathway (hsa04810). Among the eight significantly differentially expressed meta-signature miRNAs, only hsa-miR-203a-5p was found to be down-regulated. Likewise, hsa-miR-203a was also reported to show the low expression levels in patients on the early stage of nasopharyngeal carcinoma [18], which can validate our findings of hsa-miR-203a-5p as the signature of oropharyngeal cancer somehow.

The result of Go functional enrichment analysis showed that the target genes of the up-regulated meta-signature mRNAs (hsa-miR-106b-5p, hsa-miR-146a-5p and hsa-miR-21-5p) were mostly enriched terms as GO:0045944-positive regulation of transcription from RNA polymerase II promoter, GO:0000122-negative regulation of transcription from RNA polymerase II promoter, GO:0014066-regulation of phosphatidylinositol 3-kinase signaling, GO:0046854-phosphatidylinositol phosphorylation, etc., while the target genes of the down-regulated meta-signature mRNAs were involved in processes as GO:0000302-response to reactive oxygen species, GO:0046854-phosphatidylinositol phosphorylation, etc. Patients with oropharyngeal cancer were mainly featured by the repeated injury and hyperplasia of oral mucosa and it finally led to the phenomenon of chromosomal abnormality and nucleus abnormality at the molecular level, the DNA content as well as increased abnormally. The enriched GO terms of regulation of transcription from RNA polymerase II promoter and phosphorylation may be involved in the dysregulation of DNA content in the tissue with OC. Therefore, we assumed that it may have a great effect on the occurrence of oropharyngeal cancer. Besides, as for the KEGG enrichment analysis, signaling pathways as MAPK signaling pathway (hsa04010:MAPK signaling pathway), Ras signaling pathway (hsa04014: Ras signaling pathway) and cancer signaling pathway (hsa05200: Pathways in cancer) were mostly enriched in target genes of the up-regulated meta-signature miRNAs, while those target genes of the down-regulated meta-signature miRNAs were mostly enriched in hsa04115: p53 signaling pathway and hsa04020: Calcium signaling pathway. The MAPK and Ras signaling pathways were known to play important roles in controlling several functions, such as cell growth and survival. Combined with the fact of the abnormal proliferation of cells in OC, their involvement in the development of OC may be associated with the cell cycle, etc. Besides, it was reported that the MAPK pathway comprises several key signaling components and phosphorylation events that play a role in tumorigenesis.

Ras mutations are commonly seen in approximately 30% of cancers, like colorectal cancer [19], hepatocellular carcinoma [20], interrupting the normal differentiation, proliferation and cell survival of the patients. In this study, the finding of the mostly enriched term MAPK and Ras signaling pathway may have a close association with the development of oropharyngeal disease in this way, and this result may provide insights for the further development on the agents targeting Ras signaling pathway, which can disrupt signaling through Ras and downstream proteins.

Conclusions

In summary, we identified eight meta-signature miRNAs to serve as predictive and prognostic biomarkers for the clinical management of oropharyngeal cancer. The more specific molecular mechanism of their involvement in this disease needs further research.

Competing interests

The authors declare that they have no competing interests.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.cancergen.2018.10.004.

References

[1] Bartel D. MicroRNAs: target recognition and regulatory functions. Cell 2009;136(2):215–33.
[2] Dennis G, et al. DAVID: database for annotation, visualization, and integrated discovery. Genome Biol 2003;4(5):P3.
[3] Ashburner M, et al. Gene ontology: tool for the unification of biology. The Gene Ontology Consortium. Nat. Genet 2000;25(1):25–9.
[4] Kanehisa M, Goto S. KEGG: Kyoto encyclopedia of genes and genomes. Nucleic Acids Res 2000;28(1):27–30.
[5] Kanehisa M, et al. The KEGG resource for deciphering the genome. Nucleic Acids Res 2004;32:D277–80 Database issue.
[6] Essaghir A, Demoulin J. A minimal connected network of transcription factors regulated in human tumors and its application to the quest for universal cancer biomarkers. PLoS ONE 2012;7(6):e39666.
[7] Essaghir A, et al. Transcription factor regulation can be accurately predicted from the presence of target gene signatures in microarray gene expression data. Nucleic Acids Res 2010;38(11):e120.
[8] Kalfert D, et al. MicroRNA profile in site-specific head and neck squamous cell cancer. Anticancer Res 2015;35(4):2455–63.
[9] Brito B, et al. Expression of stem cell-regulating miRNAs in oral cavity and oropharynx squamous cell carcinoma. J Oral Pathol Med 2016;45(9):647–54.

[10] Gao G, et al. A microRNA expression signature for the prognosis of oropharyngeal squamous cell carcinoma. Cancer 2013;119(1):72–80.
[11] Hui A, et al. Potentially prognostic miRNAs in HPV-associated oropharyngeal carcinoma. Clin Cancer Res 2013;19(8):2154–62.
[12] Lajer C, et al. Different miRNA signatures of oral and pharyngeal squamous cell carcinomas: a prospective translational study. Br J Cancer 2011;104(5):830–40.
[13] Lajer C, et al. The role of miRNAs in human papilloma virus (HPV)-associated cancers: bridging between HPV-related head and neck cancer and cervical cancer. Br J Cancer 2017;117(5):e2.
[14] Zhang X, et al. Regulation of the tumour suppressor PDCD4 by miR-499 and miR-21 in oropharyngeal cancers. BMC Cancer 2016;16:86.
[15] Griffiths-Jones S, et al. miRBase: tools for microRNA genomics. Nucleic Acids Res 2008;36:D154–8 Database issue.
[16] Lewis B, Burge C, Bartel D. Conserved seed pairing, often flanked by adenosines, indicates that thousands of human genes are microRNA targets. Cell 2005;120(1):15–20.
[17] Xu B, et al. Hsa-miR-146a-5p modulates androgen-independent prostate cancer cells apoptosis by targeting ROCK1. Prostate 2015;75(16):1896–903.
[18] Li S, et al. Distinctive microRNA expression in early stage nasopharyngeal carcinoma patients. J Cell Mol Med 2016;20(12):2259–68.
[19] Nandan M, Yang V. An update on the biology of RAS/RAF mutations in colorectal cancer. Curr Colorectal Cancer Rep 2011;7(2):113–20.
[20] Yang S, Liu G. Targeting the Ras/Raf/MEK/ERK pathway in hepatocellular carcinoma. Oncol Lett 2017;13(3):1041–7.