International Journal of Pediatric Otorhinolaryngology 79 (2015) 1727–1731

Contents lists available at ScienceDirect

International Journal of Pediatric Otorhinolaryngology

journal homepage: www.elsevier.com/locate/ijporl

Bioinformatics analysis of microRNA comprehensive regulatory network in congenital microtia

Gangqiang Wei *

Burn and Plastic Surgery Department, Tangshan Gongren Hospital, Tangshan 063000, Hebei, China

ARTICLE INFO

Article history:
Received 11 March 2015
Received in revised form 24 July 2015
Accepted 25 July 2015
Available online 4 August 2015

Keywords:
Microtia
MicroRNA
Target gene
Long non-coding RNAs
Regulatory network

ABSTRACT

Purpose: This study was aimed to reveal the involvement of miRNAs in the progression of microtia by bioinformatics analyses.
Methods: The data in this study came from the paper of Li et al. who analyzed the microRNA (miRNA) expression profiling between congenital microtia and normal controls. Based on the 11 identified differentially expressed miRNAs, we predicted the target genes, long non-coding RNAs (lncRNA) and transcription factors (TFs). Then we constructed the miRNAs-centered comprehensive regulatory network. In addition, we performed functional enrichment analysis to analyze the functions of target genes.
Results: From the miRNAs comprehensive regulatory network, we found that has-miR-203 regulated a large number of target genes and lncRNAs, including suppressor of cytokine signaling 3 (SOCS3) and metastasis associated in lung adenocarcinoma transcript 1 (MALAT1). The has-miR-185, has-miR-451 and has-miR-200c were regulated by a host of TFs including signal transducer and activator of transcription 1 (STAT1) and STAT2. Additionally, the target genes of hsa-miR-486-5p were mainly enriched in 17 Gene Ontology terms and target genes of has-miRNA-203 were enriched in 6 pathways.
Conclusions: The expression of has-miR-203, has-miR-200c and has-miR-451 were significantly different in microtia. Target gene of SOCS3, TFs of STAT1 and STAT2, and lncRNA of MALAT1 may play important roles in the development of the external ear.

1. Introduction

Microtia is a congenital malformation of the external ear, characterized by a small, abnormally shaped auricle [1,2]. This disease occurs in 1 out of about 8000–10,000 births with phenotypes ranging from minor deformities to complete absence of the external ear [3]. Microtia can be unilateral or bilateral, while unilateral microtia usually has normal hearing in the other ear [1].

The pathogenesis of microtia is currently unknown, but most researchers would agree that the etiology is multifactorial, including demographic and clinical factors, familial clustering, and chromosomal abnormalities [4–6]. At present, there are more than 18 different syndromes associated with microtia as a result of the chromosomal aberrations or single-gene defects. Accordingly, genetic factors seem to play important roles [7]. Tekin et al. [8], for instance, reported that fibroblast growth factor 3 could lead to some congenital malformations such as congenital deafness, inner ear agenesis and microtia. Coulbault et al. [9] suggested that the bone morphogenetic protein 5 maternal peptide gene acted as the predisposing genes of microtia. Additionally, homeobox A2 has previously been demonstrated to be linked to autosomal recessive bilateral microtia [2]. Importantly, Li et al. [7] attempted to reveal the involvement of microRNA (miRNA) in microtia pathogenesis by screening miRNAs expression profiling of microtia and found that the abnormal expression of miR-200c, miR-451 and miR-486-5p could be possible causes of microtia. Although progresses have been achieved about the pathogenesis of microtia, the genetic etiology has remained elusive.

In the present study, we took advantage of the data in the study of Li et al. [7] to analyze the miRNA expression profiling between 9 congenital microtia and 3 normal controls. Based on the identified differentially expressed miRNAs, we predicted the target genes, long non-coding RNAs (lncRNA) and transcription factors (TFs), and constructed the miRNAs-centered comprehensive regulatory network. In addition, we performed functional enrichment analysis to analyze the functions of the target genes. We

* Tel.:+86 0315 2907285; fax: +86 0315 2907285.
E-mail address: werffdsf@hotmail.com

http://dx.doi.org/10.1016/j.ijporl.2015.07.036
0165-5876/

aimed to further reveal the involvement of miRNAs in the progression of microtia.

2. Data and methods

2.1. Data source

The data in our study came from the study of Li et al. [7]. They analyzed the miRNA expression profile data between 9 congenital microtia and 3 normal controls. finally, 11 differentially expressed miRNAs including 6 up-regulated and 5 down-regulated ones were identified (Table 1). Based on this findings, we constructed the miRNA regulatory network to further identify the miRNA associated with congenital microtia.

2.2. Target gene prediction

The target genes of miRNAs were predicted by seven published algorithms, including miRanda (http://microrna.sanger.ac.uk) [10], MirTarget2 (http://nar.oxfordjournals.org/cgi/content/abstract/34/5/1646) [11], PicTar (http://pictar.bio.nyu.edu) [12], Probability of Interaction by Target Accessibility (PITA, http://genie.weizmann.ac.il/pubs/mir07) [13], TargetScan (http://targetscan.org) [14], miRecords (http://miRecords.umn.edu/miRecords) [15], Mirwalk (http://mirwalk.uni-hd.de/) [16]. The miRNA-gene pair would be reserved when it was predicted by not less than 4 of the algorithms above.

2.3. miRNA-lncRNA regulatory relationship prediction

StarBase v2.0 (http://starbase.sysu.edu.cn/) [17] database is used to systematically identify the RNA-RNA and RNA-protein interaction networks (miRNA-pseudogene, miRNA-lncRNA, miRNA-mRNA, miRNA-circRNA and protein-RNA) from 108 CLIP-Seq (CLASH, PAR-CLIP, iCLIP, HITS-CLIP) generated datasets.

In our study, all the interaction networks of miRNA-lncRNA were downloaded from starBase v2.0, and then, the sub-networks related to the 11 differentially expressed miRNAs were selected.

2.4. TF-miRNA regulatory relationship prediction

ChIPBase (http://deepbase.sysu.edu.cn/chipbase/) [18] is a database for annotating and discovering transcriptional regulatory relationships of miRNAs and lncRNAs and TF binding maps from ChIP-Seq data.

In the present study, the TF-miRNA interaction networks of TF binding sites ranging from the 5-kb region upstream and the 1-kb region downstream were downloaded from ChIPBase. Then, the sub-networks related to the 11 differentially expressed miRNAs were selected to be further analyzed.

2.5. Functional enrichment analysis

The Database for Annotation, Visualization and Integrated Discovery (DAVID, (http://david.niaid.nih.gov) [19] has been developed for systematically mapping a great number of genes to associated pathways and Gene Ontology (GO) terms.

We performed GO and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analyses to analyze the target genes using the DAVID online tool. The p-value <0.05 was considered as threshold.

3. Results

3.1. miRNA-TF-gene-lncRNA comprehensive regulatory network

Based on the predicted miRNA-gene, TF-miRNA and miRNA-lncRNA regulatory networks, we constructed the miRNA-centered comprehensive regulatory network (Fig. 1). From the network, we found that hsa-miR-486-5p and has-miR-203 regulated a large number of target genes including suppressor of cytokine signaling 3 (SOCS3). Besides, has-miR-16, has-miR-708 and has-miR-185 regulated many lncRNAs. Furthermore, has-miR-185, has-miR-451 and has-miR-200c were regulated by a host of TFs including signal transducer and activator of transcription 1 (STAT1) and STAT2.

3.2. Function enrichment analysis of has-miRNA-486-5p target genes

The target genes of hsa-miR-486-5p were performed GO enrichment analysis and the result was shown in Table 2. These target genes were mainly enriched in 17 GO terms, such as regulation of transcription, phosphorus metabolic process and phosphate metabolic process.

3.3. Pathway enrichment analysis of has-miRNA-203 target genes

The pathways enriched by the target genes of has-miRNA-203 were shown in Table 3. These target genes were mainly enriched in 6 pathways, such as adipocytokine signaling pathway, insulin signaling pathway and focal adhesion pathway.

3.4. lncRNAs targeted by multiple differentially expressed miRNAs

From the regulatory network, we found that some lncRNAs were regulated by multiple differentially expressed miRNAs. The lncRNAs which were regulated by at least 3 miRNAs were shown in Table 4, such as XIST, zinc finger protein 518A (ZNF518A, lincRNA) and metastasis associated in lung adenocarcinoma transcript 1 (MALAT1).

Table 1
Differentially expressed microRNAs list.

Name	State	Sequence	Fold change	p-Value	Chromosome location
hsa-miR-486-5p	Up	UCCUGUACUGAGCUGCCCCGAG	12.73	0.0029	chr8:41517959–41518026(−)
hsa-miR-451	Up	AAACCGUUACCAUUACUGAGUU	8.16	0.0091	chr17:27188387–27188458(−)
hsa-miR-140-3p	Up	UACCACAGGGUAGAACCACGG	7.87	0.0125	chr16:69966984–69967083(+)
hsa-miR-16	Up	UAGCAGCACGUAAAUAUUGGCG	4.64	0.0218	chr13:49521110–49521198(−)
hsa-miR-185	Up	UGGAGAGAAAGGCAGUUCCUGA	3.59	0.0342	chr22:20020662–20020743(+)
hsa-miR-126	Up	UCGUACCGUGAGUAAUAAUGCG	3.03	0.0481	chr9:139565054–139565138(+)
hsa-miR-708	Down	AAGGAGCUUACAAUCUAGCUGGG	0.49	0.0382	chr11:79113066–79113153(−)
hsa-miR-1308	Down	GCAUGGGUGGUUCAGUGG	0.31	0.0203	chrX:22080259–22080312(−)
hsa-miR-200c	Down	UAAUACUGCCGGGUAAUGAUGGA	0.025	0.0001	chr12:7072862–7072929(+)
hsa-miR-203	Down	GUGAAAUGUUUAGGACCACUAG	0.023	0.0001	chr14:104583742-104583851(+)
hsa-miR-205	Down	UCCUUCAUUCCACCGGAGUCUG	0.014	0.0001	chr1:209605478–209605587(+)