Candidate pathways and genes for nasopharyngeal carcinoma based on bioinformatics study

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Abstract: Purpose: To reveal the potential microRNAs (miRNAs), genes, pathways and regulatory network involved in the process of nasopharyngeal carcinoma (NPC) by using the method of bioinformatics. Methods: Gene expression profiles GSE12452 (31 NPC and 10 normal samples) and GSE53819 (18 NPC and 18 normal samples), as well as miRNA expression profiles GSE32960 (312 NPC and 18 normal samples) and GSE36682 (62 NPC and 6 normal samples) were obtained from Gene Expression Omnibus database. The differentially expressed genes (DEGs) and miRNAs (DEmiRNAs) between NPC and normal samples were identified by using t-test based on MATLAB software (FDR < 0.01), followed by pathway enrichment analysis based on DAVID software (P-value < 0.1). Then, DEmiRNA-DEG regulatory network was constructed. Results: A total of 1254 DEGs and 107 DEmiRNAs were identified, respectively. Then, 16 pathways (including cell cycle) and 32 pathways (including pathways in cancer) were enriched by DEGs and target genes of DEmiRNAs, respectively. Furthermore, DEmiRNA-DEG regulatory network was constructed, containing 12 DEmiRNAs (including has-miR-615-3P) and 180 DEGs (including MCM4 and CCNE2). Conclusion: has-miR-615-3p might take part in the pathogenetic process of NPC through regulating MCM4 which is enriched in cell cycle. The DEmiRNAs identified in the present study might serve as new biomarkers for NPC.

Keywords: Nasopharyngeal carcinoma, differentially expressed genes, microRNAs, pathway enrichment, regulatory network

Introduction

Nasopharyngeal carcinoma (NPC), one of the most common cancers originating in nasopharynx, is caused by various factors like virus, environmental influences, and heredity [1]. Previous studies indicate that NPC is associated with the infection of Epstein-Barr virus (EBV) [2], consumption of salted food [3], smoking, and alcohol consumption [4]. Although NPC can be treated by surgery, chemotherapy or radiotherapy [5], the morbidity and risk of NPC is increasing, causing a significant decline in health-related life quality. In 2010, NPC resulted in 65,000 deaths globally [6], and NPC is extremely common in China [3, 7]. However, the detailed biological mechanism in the development of NPC is still unclear [8].

Available data have suggested that polymorphisms of genes, including CYP2E1, XRCC1, and hOGG1, are involved in DNA damage or repair, which further participate in the process of NPC [9, 10]. Studies of families at high risk of NPC have suggested that there is a linkage between DNA in chromosomal 4 and NPC [11]. Besides of variation in DNA, the dysregulation of microRNAs (miRNAs) is also implicated in the development and progression of NPC: miR-18a promotes the malignant progression by impairing microRNA biogenesis in NPC [12]; miRNA-125a-5p increased p53 protein expression in HNE-1 cells and decreased Her2 protein expression in HNE-1 and HK-1 cells [13]; miR-BART7 is highly expressed and regularly secreted into the extracellular environment of NPC cells, which is also proved to be a biomarker for the diagnosis and treatment of NPC [14]. Therefore, miRNAs and target genes might play important roles in the process of NPC, requiring further studies.

Herein, microarray data of genes and miRNAs expression from GEO (Gene Expression Omnibus) database were used in the present
study. The differentially expressed genes (DEGs) and miRNAs (DEmiRNAs) were identified, followed by the KEGG (Kyoto Encyclopedia of Genes and Genomes) pathway enrichment analysis and DEmiRNA-DEG regulatory network analysis. This study might provide evidence for the candidate genes and miRNAs involved in NPC.

Materials and methods

Microarray data

Gene expression profiles GSE12452 [15] (platform: GPL570, Affymetrix Human Genome U133 Plus 2.0 Array) and GSE53819 (platform: GPL6480, Agilent-014850 Whole Human Genome Microarray 4 × 44K G4112F) were obtained from NCBI (National Center for Biotechnology Information) GEO database (http://www.ncbi.nlm.nih.gov/geo/). A total of 31 NPC and 10 normal nasopharyngeal specimens were included in GSE12452, while 18 NPC and 18 normal nasopharyngeal specimens were included in GSE53819.

MiRNA expression profiles GSE32960 [16] (platform: GPL14722, microRNA array) and GSE36682 (platform: GPL15311, Human miRNA database accessible through the internet, storing a huge amount of PPIs. Totally, 37443 MTIs (including 596 miRNAs and 12104 target genes) were downloaded from miRTarBase, and 37080 PPIs were downloaded from HPRD.

Identification of DEGs and DEmiRNAs

DEGs and DEmiRNAs were identified by using t-test based on MATLAB software [19]. The criterion for this analysis was false discovery rate (FDR) < 0.01. In this study, DEGs represent the genes differentially expressed between NPC and normal specimens in both of GSE12452 and GSE53819, and DEGs must have same change direction (up or down) in GSE12452 and GSE53819. Similarly, DEmiRNAs represent the miRNAs differentially expressed between NPC and normal specimens in both of GSE32960 and GSE36682, and DEmiRNAs must have same change direction (up or down) in GSE32960 and GSE36682.

Pathway enrichment analysis

The KEGG database [20] contains information of how molecules or genes are networked, which is complementary to most of the existing molecular biology databases that contain the

| Table 1. Top 10 up-regulated and down-regulated DEGs (or DEmiRNAs) |
|-----------------|----------------|----------------|----------------|
| Gene ID | Gene symbol | miRNA ID | miRNA symbol |
| Up-regulated | | | |
| 100 | ADA | hsa-miR-34c-5p | hsa-miR-34c-5p |
| 128 | ADH5 | hsa-miR-145 | hsa-miR-145 |
| 140 | ADORA3 | hsa-miR-768-3p | hsa-miR-768-3p |
| 191 | AHY | hsa-miR-200a | hsa-miR-200a |
| 204 | AK2 | hsa-miR-199a-3p | hsa-miR-199a-3p |
| 377 | ARF3 | hsa-let-7e | hsa-let-7e |
| 468 | ATF4 | hsa-miR-34b | hsa-miR-34b |
| 518 | ATP5G3 | hsa-miR-363 | hsa-miR-363 |
| 526 | ATP6V1B2 | hsa-miR-26a | hsa-miR-26a |
| 637 | BID | hsa-miR-203 | hsa-miR-203 |
| Down-regulated | | | |
| 18 | ABAT | hsa-miR-125b | hsa-miR-125b |
| 124 | ADH1A | hsa-miR-100 | hsa-miR-100 |
| 125 | ADH1B | hsa-miR-191 | hsa-miR-191 |
| 126 | ADH1C | hsa-miR-143 | hsa-miR-143 |
| 131 | ADH7 | hsa-miR-451 | hsa-miR-451 |
| 150 | ADRA2A | hsa-let-7d | hsa-let-7d |
| 203 | AK1 | hsa-miR-421 | hsa-miR-421 |
| 246 | ALOX15 | hsa-miR-29c | hsa-miR-29c |
| 267 | AMFR | hsa-miR-140-3p | hsa-miR-140-3p |
| 311 | ANXA11 | hsa-miR-26b | hsa-miR-26b |

DEGs: differentially expressed genes; DEmiRNAs: differentially expressed microRNAs.
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Identification of DEGs and DEmiRNAs

After DEGs screening, 1254 significant DEGs (FDR < 0.01) were found to exist in both of GSE12452 and GSE53819 and have same change direction in GSE12452 and GSE53819. Among these DEGs, 503 DEGs were significantly up-regulated, and 751 DEGs were significantly down-regulated in NPC specimens, compared with normal specimens. Furthermore, 107 significant DEmiRNAs (FDR < 0.01) were identified to exist in both of GSE32960 and GSE36682 and have same change direction in GSE32960 and GSE36682. Among these DEmiRNAs, 45 DEmiRNAs were significantly up-regulated, and 62 DEmiRNAs were significantly down-regulated. The top 10 up-regulated and down-regulated DEGs (or DEmiRNAs) were listed in Table 1.

KEGG pathways involved in NPC

The online software DAVID was used to identify significant KEGG pathways with P-value < 0.1. As a result, a total of 16 pathways were identified, each of which was involved in NPC. The top 10 pathways that were up-regulated and down-regulated in both datasets were listed in Table 1.
KEGG pathways enriched by the target genes of DEmiRNAs were listed in Table 3.

Construction of DEmiRNA-DEG regulatory network

The 1254 DEGs and 107 DEmiRNAs were mapped to MTI-PPI network, resulting in the construction of DEmiRNA-DEG regulatory network. This network contained 253 regulatory relationships, 41 PPIs, 180 DEGs, and 12 DEmiRNAs (Figure 1). The DEGs like ADRA2A and CTPS, as well as the DEmiRNAs like hsa-miR-615-3p, hsa-miR-296-3p, and hsa-miR-342-3p had high degree in this network. Furthermore, DEGs in this network were mainly enriched in KEGG pathways like cell cycle, p53 signaling pathway, and pathways in cancer. Especially, MCM4, CCNE2, CDC6, CCND2, HDAC2, CDK4, PCNA, MAD2L1, and E2F3 were significantly enriched in cell cycle. Among these genes, MCM4, CDC6, PCNA, and MAD2L1 were regulated by hsa-miR-615-3p, CCNE2, CDK4, and E2F3 were regulated by hsa-miR-34c-5p, and CCND2 and HDAC2 were regulated by hsa-miR-342-3p.

Discussion

NPC is one of the most common cancers originating in nasopharynx worldwide. Previous studies indicate that some genes and miRNAs...
play important roles in the process of NPC. In the present research, a series of bioinformatics analyses were performed based on two human nasopharyngeal gene expression profiles and two human nasopharyngeal miRNAs expression profiles. Consequently, 1254 DEGs were both existed in two gene expression profiles, and significantly enriched in 16 pathways. A total of 107 DEmiRNAs were both existed in two miRNAs expression profiles, and their target genes were significantly enriched in 32 pathways. Furthermore, the DEmiRNA-DEG regulatory network was constructed, involving 180 DEGs and 12 DEmiRNAs. Especially, MCM4, CCNE2, CDC6, CCND2, HDAC2, CDK4, PCNA, MAD2L1, and E2F3 in the regulatory network were significantly enriched in cell cycle.

MCM4 codes a member of highly conserved mini-chromosome maintenance proteins (MCM) that are essential for the initiation of eukaryotic genome replication [23]. Watanabe et al. have reported that MCM4 mutation can cause tumors in mouse through affecting the formation of MCM4/6/7 complex [24]. The partial MCM4 deficiency can result in natural killer cell deficiency and cancer [25-27]. CCNE2 (cyclin E2), which is encoded by the CCNE2 gene in humans, plays a critical role in the G1/S portion of cell cycle [28]. CCNE2 increased proportion of abnormal mitoses, micronuclei and chromosomal aberrations in cancer setting [29]. In the present study, the potential NPC-related genes including MCM4 and CCNE2 were enriched in cell cycle and p53 signaling pathway which are both associated with the pathogenesis of NPC [30-33]. Thus, the results of pathway enrichment analysis in the present study were consist with previous studies, and we speculated that the regulation of DEGs involved in these pathways might have a positive effect on NPC inhibition and treatment.

miRNAs are post-transcriptional regulators of gene expression with critical functions in health and disease [34]. Genome-wide analyses of radiation-associated miRNAs expression profile in NPC have shown the important relationship between miRNAs and NPC [35, 36]. In the present study, 9 DEGs (including MCM4, CCNE2, CDC6, CCND2, HDAC2, CDK4, PCNA, MAD2L1, and E2F3) involved in cell cycle were regulated by has-miR-615-3p, hsa-miR-34c-5p and has-miR-342-3p. These DEmiRNAs were estimated to regulate the process of NPC through targeting these DEGs. Thus, the DEmiRNAs identified in the present study might serve as new biomarkers for NPC.

In conclusion, the DEGs (including MCM4 and CCNE2) enriched in the biological pathways like cell cycle and p53 signaling pathway were found to be related with NPC. Furthermore, DEmiRNAs including has-miR-615-3p, hsa-miR-34c-5p and has-miR-342-3p might take part in the process of NPC. However, further studies were required to validate these predictions.

Disclosure of conflict of interest
None.

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