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Original Article

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

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	AHCY	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.

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 1K) were obtained from NCBI GEO database as well. A total of 312 NPC and 18 normal samples were included in GSE32960, while 62 NPC and 6 normal samples were included in GSE36682. For all expression profiles, data after normalization were downloaded.

Data of miRNA-target and protein-protein interaction (PPI)

The miRTarBase (http://mirtarbase.mbc.nctu.edu.tw) database has accumulated more than fifty thousand miRNA-target interactions (MTIs), which are collected by systematically manual literature mining [17]. The Human Protein Reference Database (HPRD) [18] is a protein 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 2. Top 10 pathways enriched by differentially expressed genes

Pathway ID	Pathway name	Total gene	*P*-value	Genes
hsa04110	Cell cycle	125	0.000758	MCM4, CCNE2, CDC6, CCND2, HDAC2, etc.
hsa00982	Drug metabolism	62	0.001483	GSTA1, GSTA3, CYP2B6, CYP2C8, MAOB, etc.
hsa05222	Small cell lung cancer	84	0.006000	CKS1B, COL4A2, E2F3, COL4A1, PTGS2, etc.
hsa00450	Selenoamino acid metabolism	26	0.021307	AHCY, GGT7, MAT2A, MARS2, PAPSS2, etc.
hsa00980	Metabolism of xenobiotics by cytochrome P450	60	0.033427	GSTA1, GSTA3, CYP2F1, CYP2B6, CYP2C8, etc.
hsa04640	Hematopoietic cell lineage	86	0.043065	CR1, CD19, TFRC, FCER2, MS4A1, etc.
hsa00230	Purine metabolism	153	0.057698	POLR2H, GDA, AK1, AK2, AK7, etc.
hsa04115	p53 signaling pathway	68	0.062786	CCNE2, BID, CDK1, TNFRSF10B, CCND2, etc.
hsa03410	Base excision repair	35	0.066446	POLD4, UNG, TDG, NEIL1, PCNA, etc.
hsa03050	Proteasome	47	0.073129	PSMA2, PSMA1, PSMD14, PSMA4, PSMB3, etc.

Table 3. Top 10 pathways enriched by the target genes of differentially expressed microRNAs

Pathway name	Total gene	*P*-value	Genes
Ribosome	87	2.70E-11	RPL13, RPL15, RPL27A, RPL36, RPS2, etc.
Spliceosome	126	3.44E-09	ISY1, SNRPD2, SF3A1, SF3B2, HSPA8, etc.
Lysine degradation	44	3.74E-05	SETDB1, DLST, EHMT1, SETD1A, SUV39H1, etc.
Cell cycle	125	1.09E-04	MCM4, CCNE2, CDK4, CCND2, HDAC2, etc.
Glycolysis/Gluconeogenesis	60	0.0013266	HK1, PGAM1, ALDOA, ALDOC, ALDH1B1, etc.
Pathways in cancer	328	0.0017572	HSP90AB1, E2F3, HRAS, FGF9, SPI1, etc.
Pancreatic cancer	72	0.0030168	E2F3, RALBP1, ERBB2, TGFBR1, SMAD4, etc.
Huntington's disease	180	0.0035936	ATP5E, ATP5B, CYC1, NDUFAB1, CYTB, etc.
Parkinson's disease	128	0.0048767	ATP5E, ND4, SLC25A5, ND5, ATP5B, etc.
Adherens junction	77	0.0058749	FGFR1, PARD3, ACTN4, ERBB2, TGFBR1, etc.

information of individual molecules or individual genes. Online software DAVID [21] consists of biological knowledgebase and analytic tools aimed at systematically extracting biological meaning from large gene lists. We used DAVID to identify significant KEGG pathways with *P*-value < 0.1.

Construction of DEmiRNA-DEG regulatory network

Based on the data of MTIs and PPIs, MTI-PPI network was constructed, and the identified DEGs and DEmiRNAs were mapped to MTI-PPI network. In this study, both direct regulation (miRNA-target) and indirect regulation (miRNA-target-gene with PPI) between DEmiRNAs and DEGs were remained. In doing so, DEmiRNA-DEG regulatory network was constructed, and then visualized by Cytoscape software [22].

Results

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 enriched by DEGs, e.g., cell cycle, p53 signaling pathway, and DNA replication. The top 10 pathways enriched by DEGs were listed in **Table 2**. According to MTIs, a total of 32 KEGG pathways were enriched by the target genes of DEmiRNAs, e.g., cell cycle, pathways in cancer, p53 signaling pathway, and focal adhesion. The top 10

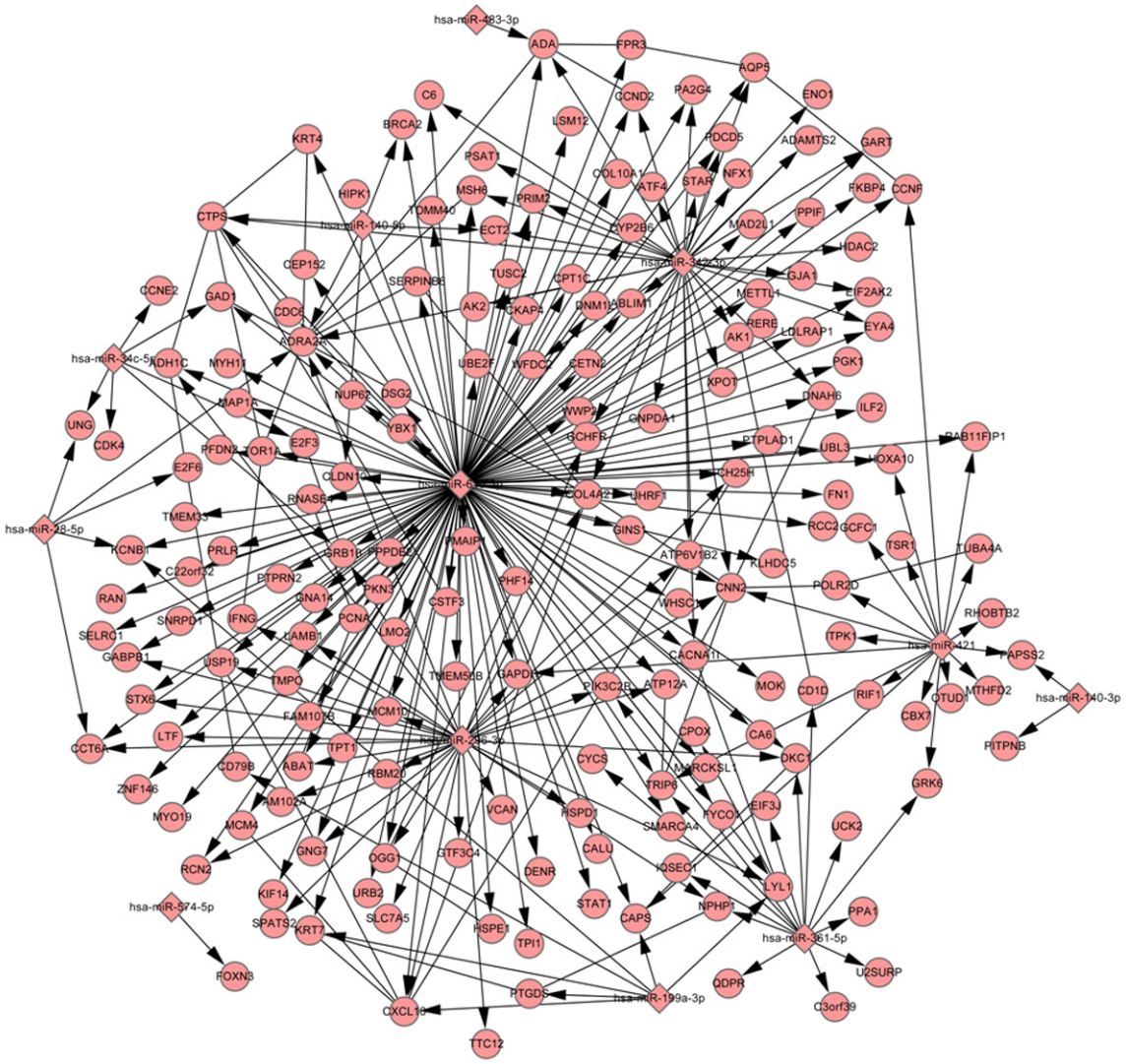

Figure 1. DEmiRNA-DEG regulatory network. DEmiRNA: differentially expressed microRNA; DEG: differentially expressed gene; diamond node represents DEmiRNA; circular node represents DEG; line with arrow represents the regulatory interaction between DEmiRNA and DEG; line without arrow represents protein-protein interaction between DEGs.

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 consistent 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 radio resistance-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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