Network analysis of microRNAs, transcription factors, target genes and host genes in nasopharyngeal carcinoma

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Received January 21, 2015; Accepted February 17, 2016

DOI: 10.3892/ol.2016.4476

Abstract. Numerous studies on the morbidity of nasopharyngeal carcinoma (NPC) have identified several genes, microRNAs (miRNAs or miRs) and transcription factors (TFs) that influence the pathogenesis of NPC. However, summarizing all the regulatory networks involved in NPC is challenging. In the present study, the genes, miRNAs and TFs involved in NPC were considered as the nodes of the so-called regulatory network, and the associations between them were investigated. To clearly represent these associations, three regulatory networks were built seperately, namely, the differentially expressed network, the associated network and the global network. The differentially expressed network is the most important one of these three networks, since its nodes are differentially expressed genes whose mutations may lead to the development of NPC. Therefore, by modifying the aberrant expression of those genes that are differentially expressed in this network, their dysregulation may be corrected and the tumorigenesis of NPC may thus be prevented. Analysis of the aforementioned three networks highlighted the importance of certain pathways, such as self-adaptation pathways, in the development of NPC. For example, cyclin D1 (CCND1) was observed to regulate *Homo sapiens*-miR-20a, which in turn targeted CCND1. The present study conducted a systematic analysis of the pathogenesis of NPC through the three aforementioned regulatory networks, and provided a theoretical model for biologists. Future studies are required to evaluate the influence of the highlighted pathways in NPC.

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Abbreviations: miRNA, microRNA; TFs, transcription factors; targets, target genes; NCBI, National Center for Biotechnology Information; TFBSs, transcription factor binding sites

Key words: nasopharyngeal carcinoma, microRNA, transcription factor, network, host gene

Introduction

Nasopharyngeal carcinoma (NPC) is a non-lymphomatous, squamous-cell carcinoma that is particularly prevalent in Southeast Asia and North Africa (1,2). Previous studies have suggested that differentially expressed genes and microRNAs (miRNAs or miRs), and the corresponding genes and miRNAs associated with them, participate in the pathogenesis of NPC (1,3).

Transcription factors (TFs) and miRNAs are important regulators of gene expression (4). TFs are proteins capable of activating or repressing the transcription of numerous genes by binding to cis-regulatory elements located in the upstream region of these genes (5). TFs may regulate gene expression at the transcriptional level alone or in cooperation with other proteins (6).

miRNAs participate in various biological processes, including proliferation and mutation, by targeting particular genes (6,7). miRNAs may repress the translation of messenger (m)RNAs or degrade mRNAs, thus regulating gene expression at the post-transcriptional level (8).

Host genes are the genes where miRNAs are located (9). Rodriguez *et al* (9) indicated that miRNAs are transcribed in parallel with their host transcripts, and two different types of miRNAs (termed exonic and intronic) were identified by the authors. miRNAs and their host genes are closely associated, and usually function together in different biological processes (7).

Although numerous studies on NPC exist in the literature, the majority of studies conducted to date have only focused on one element (either a gene or a miRNA), thus impeding the systematic analysis of the nosogenesis of NPC (1,3,6). In the present study, the associations between all the elements that participate in NPC were investigated by building three networks, which clearly displayed the identified associations between the different NPC elements. The present study focused on the study of the associations existing between miRNAs located on host genes, genes regulating miRNAs and miRNAs targeting target genes. The differentially expressed genes and other elements analyzed in the present study were selected based on previous studies on NPC available in the literature and pertinent databases (1,10). Subsequently, three regulatory networks were constructed, which were termed differentially expressed

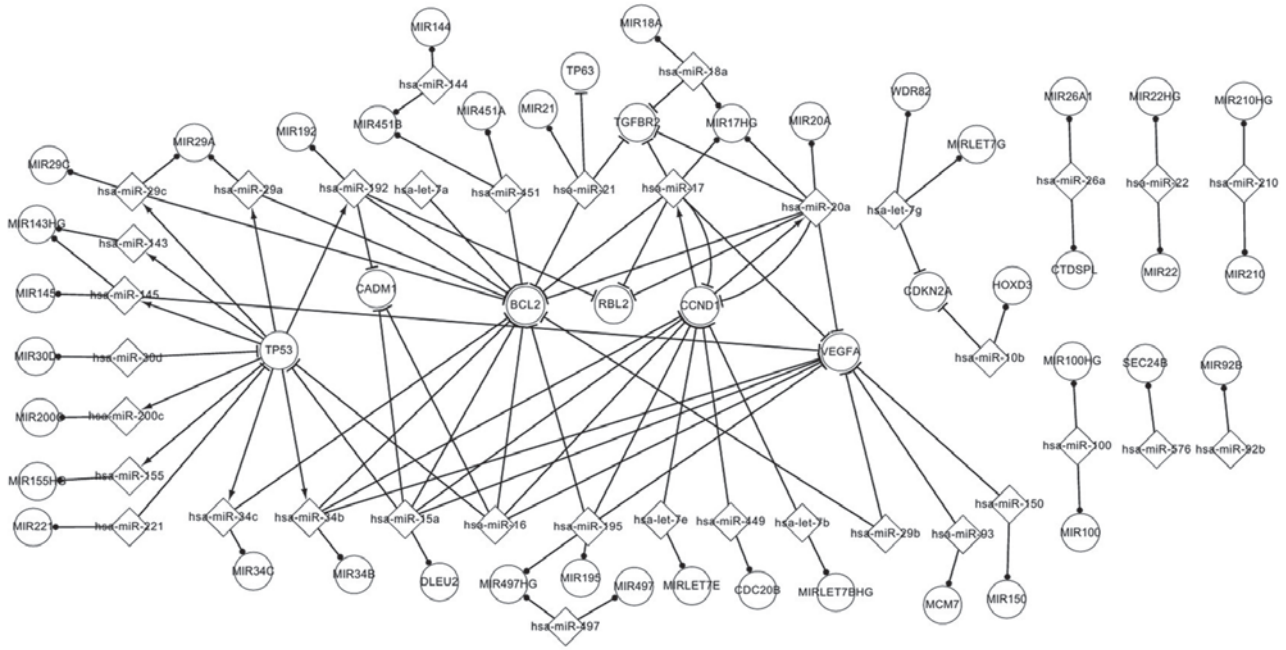

Figure 1. Differentially expressed network in nasopharyngeal carcinoma. MIR, microRNA; miR, microRNA; hsa, *Homo sapiens*; HG, host gene; TP63, tumor protein p63; WDR82, WD repeat domain 82; TGFBR2, transforming growth factor, beta receptor II; TP53, tumor protein p53; CADM1, cell adhesion molecule 1; BCL2, B-cell lymphoma 2; RBL2, retinoblastoma-like 2; CCDN1, cylin D1; VEGFA, vascular endothelial growth factor A; CDKN2A, cyclin-dependent kinase inhibitor 2A; HOXD3, homeobox D3; CTDSPL, carboxy-terminal domain, RNA polymerase II, polypeptide A small phosphatase-like; SEC24B, SEC24 family member B; DLEU2, deleted in lymphocytic leukemia 2; CDC20B, cell division cycle 20 homolog B; MCM7, minichromosome maintenance complex component 7.

network, associated network and global network, respectively. However, the global network was observed to be too complex to provide any useful information, since it was constructed by using almost all the elements involved in NPC that had been experimentally validated in previous studies. Therefore, the present study focused on the analysis of the pathways involving differentially expressed genes and relevant TFs. The associations between these elements were analyzed in order to identify the important molecules and signaling pathways involved in the development of NPC.

Materials and methods

Data identification and processing. The experimentally validated dataset of human miRNAs and their target genes used in the present study was obtained from TarBase 7.0 (http://diana.imis.athena-innovation.gr/DianaTools/index.php?r=tarbase/index) and miRTarBase (http://mirtarbase.mbc.nctu.edu.tw/). The names used in the present study to unify each gene and miRNA are available at the National Center for Biotechnology Information (NCBI) database (http://www.ncbi.nlm.nih.gov/gene/).

TransmiR (http://www.cuilab.cn/transmir) (10) was used to identify experimentally validated datasets of human TFs and their regulated miRNAs, while miRBase (http://www.mirbase.org/) (11) and the aforementioned NCBI database were used to identify host genes of human miRNAs. Differentially expressed genes in NPC were identified from CancerGenetics Web (http://www.cancerindex.org/geneweb/), NCBI dbSNP database (http://www.ncbi.nlm.nih.gov/projects/SNP/index.html) and previous studies on NPC available in the literature (12). NPC-associated genes were identified from the information contained in the GeneCards database (http://www.genecards.org/) (13) and previous studies on NPC published in the literature (1). Relevant TFs were extracted by the P-Match method (14). Of these, the present study only focused on those TFs that appeared in TransmiR, which were considered to be NPC-associated genes. The promoter region sequences (of 1,000 nt in length) of the targets of the differentially expressed genes were downloaded from the UCSC database (http://hgdownload.soe.ucsc.edu/downloads.html) (15). The P-Match method, which combines pattern matching and weight matrix approaches, was used to identify transcription factor binding sites (TFBSs) in the above 1,000-nt promoter region sequences, and mapped these TFBSs onto the promoter region of the target genes. Since P-Match uses the matrix library and sets of known TFBSs available at TRANSFAC® (http://www.gene-regulation.com/pub/databases.html), this method enables to search for multiple TFBSs. Furthermore, the standard matrix and restricted high quality criterion were used for the aforementioned matrix.

Differentially expressed miRNAs were identified from the information available at mir2Disease (http://www.mir2disease.org/) (3) and published studies on NPC, while NPC-associated miRNAs were mainly identified in the relevant literature (12).

Networks construction. In the present study, three regulatory networks of NPC were constructed, namely, the differentially expressed network, the NPC-associated network and the global network. All the regulatory associations between host genes, target genes, miRNAs and TFs were extracted and combined to construc the global regulatory network. The differentially expressed elements were extracted, and the associations between

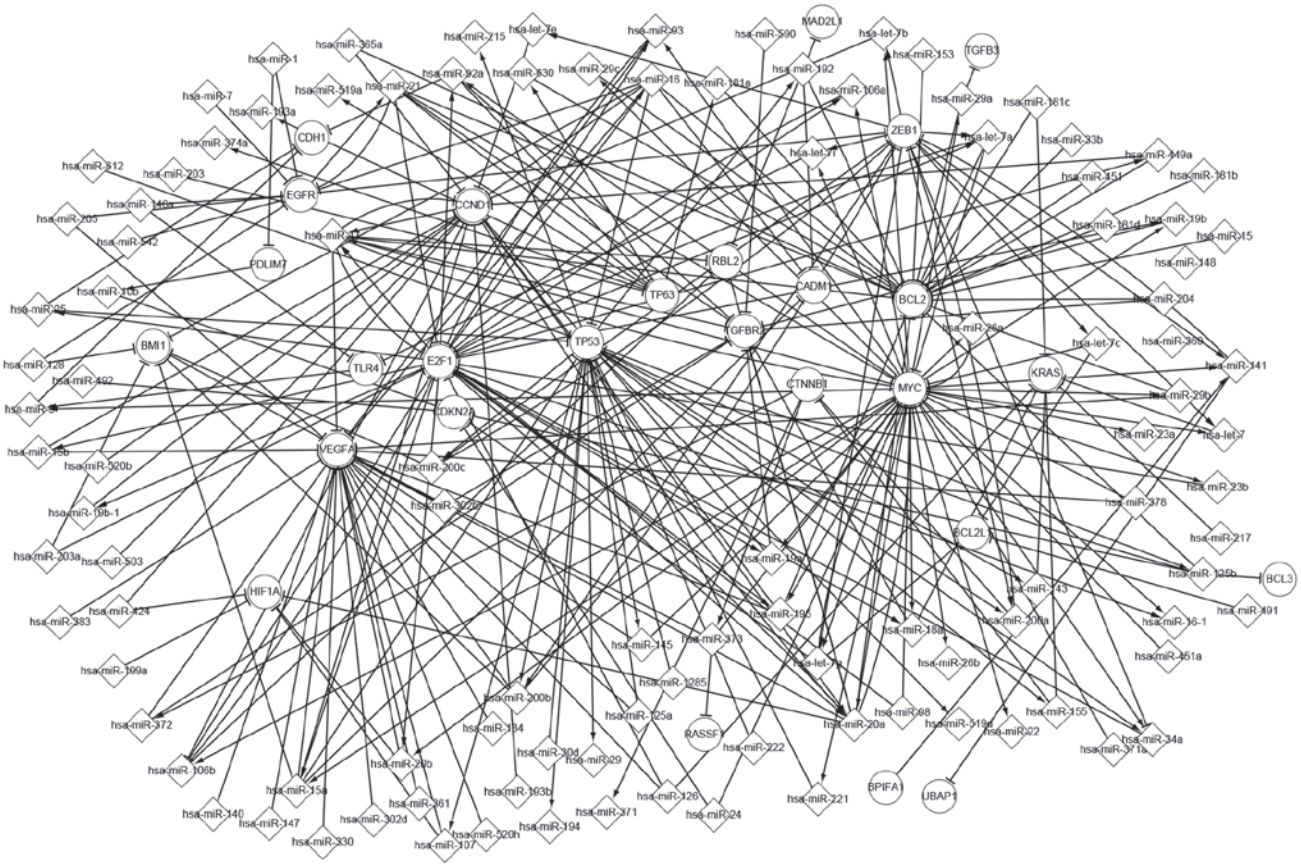

Figure 2. Associated network in nasopharyngeal carcinoma. MIR, microRNA; miR, microRNA; hsa, *Homo sapiens*; HG, host gene; MAD2L1, mitotic arrest deficient-like 1; TGFB3, transforming growth factor, beta 3; CDH1, cadherin 1; ZEB1, zinc finger E-box binding homeobox 1; EGFR, epidermal growth factor receptor; CCND1, cyclin D1; PDLIM7, PDZ and LIM domain 7 (enigma); RBL2, retinoblastoma-like 2; TP63, tumor protein p63; CADM1, cell adhesion molecule 1; BCL2, B-cell lymphoma 2; TGFBR2, transforming growth factor, beta receptor II; BMI1, B lymphoma Mo-MLV insertion region 1 homolog; TLR4, Toll-like receptor 4; E2F1, E2F transcription factor 1; CDKN2A, cyclin-dependent kinase inhibitor 2A; CTNNB1, catenin beta 1; MYC, v-Myc avian myelocytomatosis viral oncogene homolog; KRAS, Kirsten rat sarcoma viral oncogene homolog; BCL2L11, BCL2-like 11; HIF1A, hypoxia inducible factor 1, alpha subunit; RASSF, Ras association domain family; BPIFA1, bactericidal permeability-increasing fold containing family A, member 1; UBAP1, ubiquitin associated protein 1; BCL3, B-cell lymphoma 3.

them were selected from the global network in order to construct the differentially expressed network. The associated elements and the selected associations between them were extracted from the global network in order to construct the NPC-associated network.

Results

Differentially expressed network of NPC. Fig. 1 represents various significant regulatory pathways and elements involved in NPC. This differentially expressed network of NPC includes 2 TFs, 9 targets of miRNAs and 40 miRNAs with their host genes. All the elements are differentially expressed in NPC, with the exception of host genes. Three types of associations were identified in this network, including those existing between miRNAs and their target genes, between host genes and host miRNAs, and between genes that regulate miRNAs and these miRNAs. Particular pathways were also identified in this network. Notably, one host gene may host ≥1 miRNAs, and the miRNAs that target a particular host gene may also target other genes. For example, MIR195 hosts *Homo sapiens* (hsa)-miR-195, while hsa-miR-195 targets vascular endothelial growth factor A (VEGFA) and cyclin D1 (CCND1). In addition, one miRNA may locate in one or several genes. For example, hsa-miR-145 locates in MIR145 and MIR143 host gene (HG). Additional significant associations were identified. For example, CCND1 regulates both hsa-miR-20a and hsa-miR-17, while in turn these miRNAs target CCND1. Therefore, hsa-miR-20a and CCND1, and hsa-miR-17 and CCND1 exhibit a self-adaptation type of association. Hsa-miR-15a and hsa-miR-16 both target tumor protein p53 (TP53), cell adhesion molecule 1 (CADM1), B-cell lymphoma 2 (BCL2), VEGFA and CCND1, but are not regulated by any differentially expressed gene. VEGFA is targeted by 10 different miRNAs (namely, hsa-miR-145, hsa-miR-150, hsa-miR-15a, hsa-miR-16, hsa-miR-17, hsa-miR-195, hsa-miR-20a, hsa-miR-29b, hsa-miR-34b and hsa-miR-93), but does not regulate any miRNA. CADM1 is targeted by 3 miRNAs (hsa-miR-15a, hsa-miR-16 and hsa-miR-192), but does not regulate any miRNA. Retinoblastoma-like 2 (RBL2) is targeted by 3 miRNAs (hsa-miR-192, hsa-miR-17 and hsa-miR-20a), but does not regulate any miRNA. BCL2 is targeted by 14 miRNAs, but does not regulate any miRNA. This differentially expressed network partly explains the regulatory mechanism of NPC.

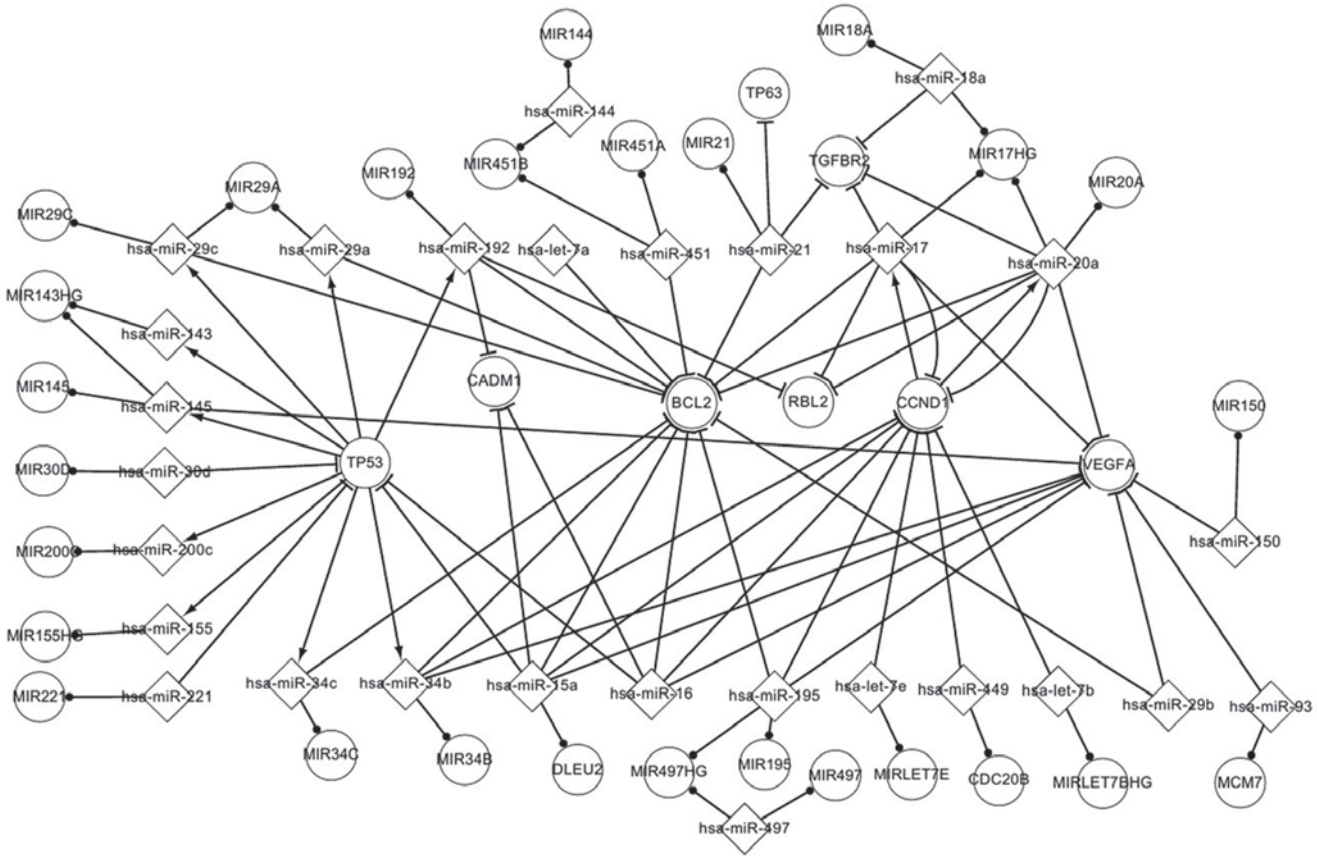

Figure 3. Important associations between host genes and their microRNAs in nasopharyngeal carcinoma. MIR, microRNA; miR, microRNA; hsa, *Homo sapiens*; HG, host gene; TP63, tumor protein p63; TGFBR2, transforming growth factor, beta receptor II; CADM1, cell adhesion molecule 1; BCL2, B-cell lymphoma 2; RBL2, retinoblastoma-like 2; CCND1, cyclin D1; VEGFA, vascular endothelial growth factor A; TP53, tumor protein p53; DLEU2, deleted in lymphocytic leukemia 2; CDC20B, cell division cycle 20 homolog B; MCM7, minichromosome maintenance complex component 7.

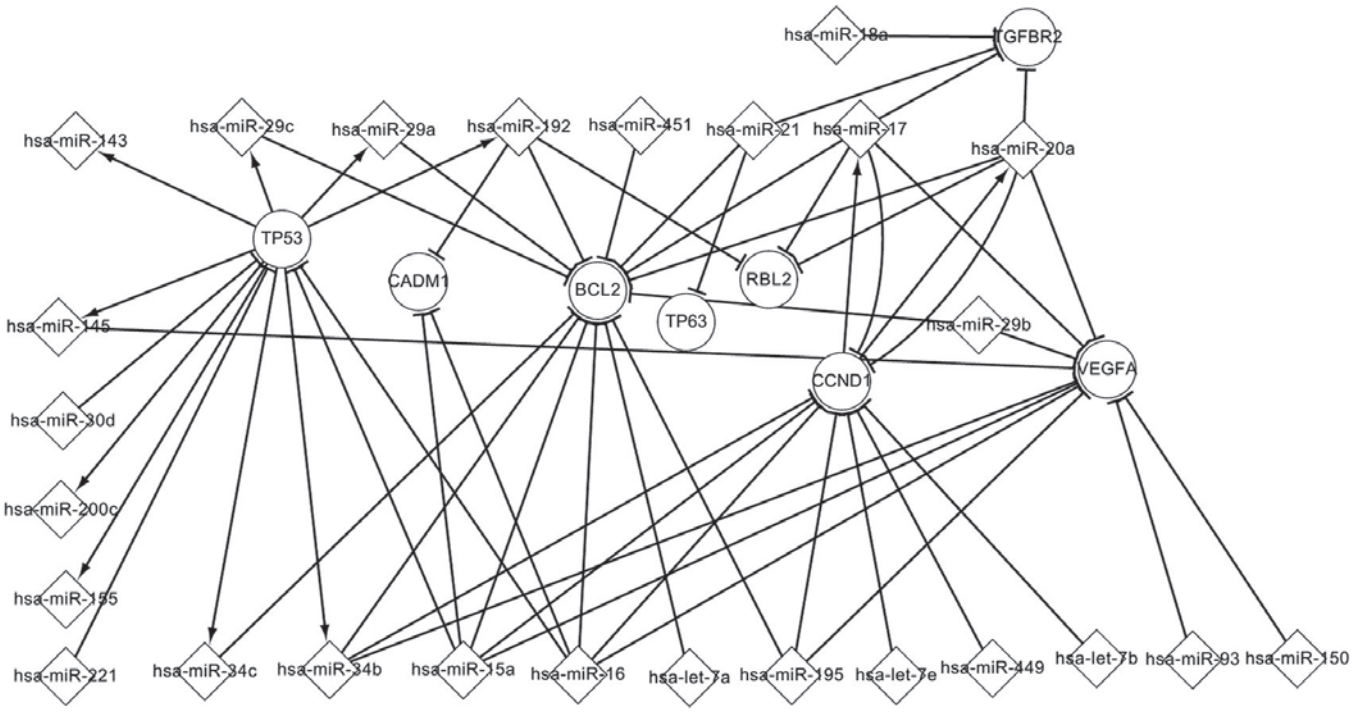

Figure 4. Transcriptional network of transcription factors and differentially expressed microRNAs in nasopharyngeal carcinoma. MIR, microRNA; miR, microRNA; hsa, *Homo sapiens*; HG, host gene; TGFBR2, transforming growth factor, beta receptor II; TP53, tumor protein p53; CADM1, cell adhesion molecule 1; BCL2, B-cell lymphoma 2; TP63, tumor protein p63; RBL2, retinoblastoma-like 2; CCND1, cyclin D1; VEGFA, vascular endothelial growth factor A.

Table I. Regulatory associations between miRNAs and the CCND1 gene.

miRNAs that target CCND1			miRNAs that are regulated by CCND1		
Differentially expressed network	Associated network	Global network	Differentially expressed network	Associated network	Global network
hsa-let-7b	hsa-let-7b	hsa-let-7b	hsa-miR-17	hsa-miR-17	hsa-miR-17
hsa-let-7e	hsa-let-7e	hsa-let-7e	hsa-miR-20a	hsa-miR-20a	hsa-miR-91
hsa-miR-15a	hsa-miR-106b	hsa-miR-106b			hsa-miR-20
hsa-miR-16	hsa-miR-15a	hsa-miR-15a			hsa-miR-20a
hsa-miR-17	hsa-miR-15b	hsa-miR-15b			
hsa-miR-195	hsa-miR-16	hsa-miR-16			
hsa-miR-20a	hsa-miR-17	hsa-miR-17			
hsa-miR-34b	hsa-miR-193b	hsa-miR-193b			
hsa-miR-449	hsa-miR-195	hsa-miR-195			
	hsa-miR-19b-1	hsa-miR-19b-1			
	hsa-miR-20a	hsa-miR-20a			
	hsa-miR-34b	hsa-miR-302a			
	hsa-miR-365a	hsa-miR-302c			
	hsa-miR-424	hsa-miR-322			
	hsa-miR-449	hsa-miR-34a			
	hsa-miR-503	hsa-miR-34b			
	hsa-miR-520b	hsa-miR-365a			
		hsa-miR-424			
		hsa-miR-449			
		hsa-miR-449a			
		hsa-miR-503			
		hsa-miR-520b			
		hsa-miR-91			

miR, microRNA; miRNA, microRNA; hsa, *Homo sapiens*; CCND1, cyclin D1.

Associated network of NPC. Since NPC-associated elements include differentially expressed elements, the above differentially expressed regulatory network forms part of the NPC-associated network. The associated network constructed in the present study reveals additional pathways involving genes and miRNAs, and may contribute to further understanding the pathogenesis of NPC. As depicted in Fig. 2, more signaling pathways are observed in the associated network than in the differentially expressed regulatory network. For instance, v-myc avian myelocytomatosis viral oncogene homolog (MYC) and hsa-miR-26a form a self-adaptation type of association. In addition, MYC regulates hsa-miR-29c and hsa-miR-29a, which both target BCL2.

Global network of NPC. The global network includes the differentially expressed network and the associated network, and contains more regulatory associations than the above two networks. The global network may be interpreted as an overall experimentally validated biological network of NPC in the human body. However, due to their complexity, the data associated with this network are not shown. Instead, the present study focused on the analysis of the differentially expressed and NPC-associated networks.

Host genes and their miRNAs in NPC. In the differentially expressed network, all the elements were differentially expressed, with the exception of host genes. To analyze the associations between these elements, it must be considered that the host genes are differentially expressed when their miRNAs are differentially expressed. These associations are represented in Fig. 3.

When the associations in the differentially expressed network were analyzed, significant features were noted. Thus, it was observed that one host gene may host several miRNAs that target one gene each or target one gene together. For example, MIR17HG hosts 3 miRNAs (hsa-miR-18a, hsa-miR-20a and hsa-miR-17), which together target transforming growth factor beta receptor II (TGFBR2). Deleted in lymphocytic leukemia 2 hosts hsa-miR-15a, which targets TP53, CADM1, BCL2, CCND1 and VEGFA. It has been previously suggested that the associations between host genes and their miRNAs may aid to understand the pathogenesis of NPC (3,7).

Transcriptional network of TFs and differentially expressed miRNAs. The association between differentially expressed miRNAs and TFs in NPC was analyzed in the present study.

Table II. Regulatory associations between hsa-miR-17 and its target genes.

Genes that regulate hsa-miR-17			Target genes of hsa-miR-17		
Differentially expressed network	Associated network	Global network	Differentially expressed network	Associated network	Global network
CCND1	CCND1	CCND1	BCL2	BCL2	APP, BCL2, BCL2L11
	MYC	E2F1	CCND1	CCND1	BMPR2, CCL1, CCND1
		MIR17HG	RBL2	RBL2	CCND2, CDKN1A, DNAJC27
		MYC	VEGFA	VEGFA	E2F1, E2F3, FBXO31
		MYCN	TGFBR2	TGFBR2	GPR137B, GPX2, ICAM1
		NFKB1		MYC	JAK1, MAP3K12, MAPK9
		NKX2-5			MEF2D, MUC17, MYC
		SPI1			NCOA3, NPAT, OBFC2A
		STAT5B			PKD2, PTEN, PTPRO
		TLX1			RB1, RBL1, RBL2
		TLX3			RUNX1, SELE, SMAD4
		TNF			SOD2, TGFBR2, THBS1
					TNFSF12, TXNRD2, VEGFA
					VIM, WEE1, YES1, ZNFX1

miR, microRNA; hsa, *Homo sapiens*; CCND1, cyclin D1; MYC, v-Myc avian myelocytomatosis viral oncogene homolog; E2F1, E2F transcription factor 1; HG, host gene; MYCN, v-Myc avian myelocytomatosis viral oncogene neuroblastoma derived homolog; NFKB1, nuclear factor of kappa light polypeptide gene enhancer in B-cells 1; NKX2-5, NK2 homeobox 5; SPI1, spleen focus forming virus proviral integration oncogene Spi1; STAT5B, signal transducer and activator of transcription 5B; TLX1, T-cell leukemia homeobox 1; TLX3, T-cell leukemia homeobox 3; TNF, tumor necrosis factor; BCL2, B-cell lymphoma 2; RBL2, retinoblastoma-like 2; VEGFA, vascular endothelial growth factor A; TGFBR2, transforming growth factor, beta receptor II; APP, amyloid precursor protein; BCL2L11, BCL2-like 11; BMPR2, bone morphogenetic protein receptor type II; CCL1, chemokine (C-C motif) ligand 1; CCND2, cyclin D2; CDKN1A, cyclin-dependent kinase inhibitor 1A; DNAJC27, DnaJ (Hsp40) homolog, subfamily C, member 27; E2F3, E2F transcription factor 3; FBXO31, F-box protein 31; GPR137B, G protein-coupled receptor 137B; GPX2, glutathione peroxidase 2; ICAM1, intercellular adhesion molecule 1; JAK1, Janus kinase 1; MAP3K12, mitogen-activated protein kinase kinase kinase 12; MAPK9, mitogen-activated protein kinase 9; MEF2D, myocyte enhancer factor 2D; MUC17, mucin 17; NCOA3, nuclear receptor coactivator 3; NPAT, nuclear protein, ataxia-telangiectasia locus; OBFC2A, oligonucleotide/oligosaccharide-binding fold-containing protein 2A; PKD2, polycystic kidney disease 2; PTEN, phosphatase and tensin homolog; PTPRO, protein tyrosine phosphatase receptor type O; RB1, retinoblastoma 1; RBL1, retinoblastoma-like 1; RBL2, retinoblastoma-like 2; RUNX1, runt-related transcription factor 1; SELE, selectin E; SMAD4, SMAD family member 4; SOD2, superoxide dismutase 2, mitochondrial; THBS1, thrombospondin 1; TNFSF12, tumor necrosis factor (ligand) superfamily, member 12; TXNRD2, thioredoxin reductase 2; VIM, vimentin; WEE1, WEE1 G2 checkpoint kinase; YES1, YES proto-oncogene 1, Src family tyrosine kinase; ZNFX1, zinc finger, NFX1-type containing 1.

Fig. 4 represents the regulatory associations existing between the relevant differentially expressed TFs and miRNAs in NPC. As observed in Fig. 4, these elements influence their successors by targeting or regulating them. For example, CCND1 regulates 2 miRNAs (hsa-miR-17 and hsa-miR-20a), and is targeted by 9 miRNAs (hsa-miR-34b, hsa-miR-15a, hsa-miR-16, hsa-miR-195, hsa-miR-7e, hsa-miR-449, hsa-miR-7b, hsa-miR-17 and hsa-miR-20a). Thus, hsa-miR-17 and CCND1, and hsa-miR-20a and CCND1, form a self-adaptation type of association. CCND1 regulates hsa-miR-20a, which targets BCL2, RBL2, CCND1 and VEGFA. TP53 is targeted by 4 miRNAs (hsa-miR-30d, hsa-miR-221, hsa-miR-15a and hsa-miR-16), and regulates 9 miRNAs (hsa-miR-192, hsa-miR-29a, hsa-miR-29c, hsa-miR-143, hsa-miR-145, hsa-miR-200c, hsa-miR-155, hsa-miR-34c and hsa-miR-34b). These miRNAs, alone or together, target other TFs, including hsa-miR-192, hsa-miR-15a and hsa-miR-16, which target CADM1; hsa-miR-29c, hsa-miR-29a, hsa-miR-192, hsa-miR-34c and hsa-miR-34b, which target BCL2; hsa-miR-192, which targets RBL2; hsa-miR-34b, which targets CCND1; and hsa-miR-34b, which targets VEGFA. Overall, these data suggest that TP53 and CCND1 participate in NPC. These associations between TFs and miRNAs may contribute to further understanding the pathogenesis of NPC.

Regulatory pathways involving differentially expressed genes. In order to describe the regulatory network of NPC more clearly, the upstream and downstream nodes of the important elements involved in NPC were extracted, including differentially expressed genes, differentially expressed miRNAs and relevant TFs identified by the P-Match method. Upon extraction of the successor and precursor nodes of the differentially expressed genes of NPC from the three constructed networks, the results obtained were listed in a table, and certain pathways were highlighted.

Table III. Regulatory associations between miRNAs and the ZEB1 gene.

miRNAs that target ZEB1			miRNAs that are regulated by ZEB1		
Differentially expressed network	Associated network	Global network	Differentially expressed network	Associated network	Global network
hsa-miR-200c	hsa-miR-141	hsa-miR-141	hsa-let-7a	hsa-let-7	hsa-let-7
	hsa-miR-200a	hsa-miR-200	hsa-let-7b	hsa-let-7a	hsa-let-7a
	hsa-miR-200b	hsa-miR-200a	hsa-let-7e	hsa-let-7b	hsa-let-7a-1
	hsa-miR-200c	hsa-miR-200a	hsa-miR-200c	hsa-let-7c	hsa-let-7a-2
	hsa-miR-205	hsa-miR-200a		hsa-let-7d	hsa-let-7a-3
		hsa-miR-200a-3p		hsa-let-7e	hsa-let-7b
		hsa-miR-200b		hsa-let-7g	hsa-let-7c
		hsa-miR-200b		hsa-let-7i	hsa-let-7d
		hsa-miR-200b		hsa-miR-141	hsa-let-7e
		hsa-miR-200b-3p		hsa-miR-200a	hsa-let-7f
		hsa-miR-200c		hsa-miR-200b	hsa-let-7f-1
		hsa-miR-200c		hsa-miR-200c	hsa-let-7f-2
		hsa-miR-200c		hsa-miR-34a	hsa-let-7g
		hsa-miR-200c-3p			hsa-let-7i
		hsa-miR-205			hsa-miR-141
		hsa-miR-205			hsa-miR-200a
		hsa-miR-429			hsa-miR-200b
					hsa-miR-200c
					hsa-miR-34
					hsa-miR-34a
					hsa-miR-34b

miR, microRNA; hsa, *Homo sapiens*; ZEB1, zinc finger E-box binding homeobox 1.

Of the TFs identified, including CCND1 and TP53, the present study only focused on CCND1 as a representative example. Notably, CCND1 and its target miRNAs form a self-adaptation type of association. In Table I, the precursors and successors of CCND1 in the differentially expressed, associated and global networks are listed. In the differentially expressed network, CCND1 regulates 2 miRNAs and is targeted by 9 miRNAs. In the associated network, CCND1 regulates 4 miRNAs and is targeted by 9 miRNAs. In the global network, CCND1 regulates 2 miRNAs and is targeted by 28 miRNAs. Notably, CCND1 exhibits a self-adaptation type of association with a number of miRNAs. For example, hsa-miR-20a and hsa-miR-17 target CCND1, while CCND1 regulates them. CCND1 can influence other genes via regulation of its miRNAs. For example, CCND1 regulates hsa-miR-17, which in turn targets RBL2 and VEGFA. In addition, CCND1 can also be influenced by other genes. For example, TP53 regulates hsa-miR-34b, which targets CCND1.

Regulatory pathways involving differentially expressed miRNAs. The pathways of differentially expressed miRNAs were analyzed as described above. As an example, the results obtained for hsa-miR-17 are discussed below.

Upon analysis of the three constructed networks, hsa-miR-17 was observed to display 6 types of adjacent nodes (3 predecessors and 3 successors). Other miRNAs were also identifed to exhibit 6 types of adjacent nodes, including hsa-miR-34a, hsa-miR-17, hsa-miR-192, hsa-miR-29a, hsa-miR-29c and hsa-miR-20a. The present study only focused on hsa-miR-17, as a representative example. In Table II, the precursors and successors of hsa-miR-17 identified in the differentially expressed, associated and global networks are listed. In the differentially expressed network, there was 1 gene regulating hsa-miR-17, which targeted 5 genes. In the associated network, there were 2 genes regulating hsa-miR-17, which targeted 6 genes. In the global network, there were 13 genes regulating hsa-miR-17, which targeted 43 genes. In addition, CCND1 and hsa-miR-17 formed a self-adaptation type of association, and hsa-miR-17 was capable of influencing other miRNAs via TFs. For example, hsa-miR-17 targeted CCND1, while CCND1 regulated hsa-miR-20a. Furthermore, hsa-miR-17 may also be influenced by other miRNAs. For example, hsa-miR-195 targets CCND1, which regulates hsa-miR-17.

Other miRNAs were identified to exhibit <6 types of adjacent nodes. For example, hsa-miR-29b displayed 5 types of adjacent nodes, and had no successors in the differentially expressed network. These miRNAs can be analyzed as described above.

Regulatory pathways involving relevant TFs. Relevant TFs, which were extracted with the P-Match method, were

analyzed in the associated network. A number of these TFs, including zinc finger E-box binding homeobox 1 (ZEB1) and E2F transcription factor 1 (E2F1), form self-adaptation associations with their miRNAs. Certain TFs have 3 types of successors (differentially expressed, associated and global), in addition to 3 types of predecessors. As a representative example, ZEB1 is discussed in the present study. In Table III, the 3 types of successors and 3 types of predecessors of ZEB1 are listed. ZEB1 is targeted by 1 differentially expressed miRNA (hsa-miR-200c), and regulates 4 differentially expressed miRNAs (hsa-let-7a, hsa-let-7b, hsa-let-7e and hsa-miR-200c). In addition, ZEB1 is targeted by 5 associated miRNAs, and regulates 13 associated miRNAs, while 17 miRNAs target ZEB1 and 21 miRNAs are regulated by ZEB1 in the global network. According to Table III, ZEB1 forms self-adaptation associations with hsa-miR-141, hsa-miR-200a, hsa-miR-200b and hsa-miR-200c in the associated network. Other TFs that exhibit <3 types of successors or predecessors in the associated network can be analyzed as described above.

Discussion

The signaling pathways identified in the differentially expressed network constructed in the present study suggest that the TP53 and CCND1 signaling pathways should be further investigated in connection with NPC in future studies, since CCND1 forms self-adaptation associations with hsa-miR-17 and hsa-miR-20a in the differentially expressed network. Furthermore, both hsa-miR-17 and hsa-miR20a target TGFBR2. In addition, TP53 was observed to regulate 9 differentially expressed miRNAs, and these miRNAs (alone or together) targeted CADM1, BCL2, RBL2, CCND1 and VEGFA. These significant associations may contribute to further understanding the mechanism of NPC pathogenesis. In addition, the TFs identified by the P-Match method suggest potential associations between differentially expressed miRNAs and TFs, which remain to be experimentally validated.

In conclusion, three regulatory networks were constructed in the present study, which revealed the associations between the different elements involved in NPC. Experimentally validated data regarding NPC was collected in order to build these networks. The associations observed in the three networks were analyzed, including various successors and precursors of a number of key elements involved in NPC. All the elements in the differentially expressed network were differentially expressed, which suggests that the tumorigenesis of NPC may be prevented by modifying the aberrant expression exhibited by these differentially expressed elements. In addition, the P-Match method was used to elaborate certain hypotheses, which may provide valuable information for future studies on experimentally validated data of NPC.

Acknowledgements

The present study was supported by grants from the National Natural Science Foundation of China (Beijing, China; grant no. 60973091) and the Science and Technology Development Plan of Jilin Province (Changchun, China; grant no. 20130101166JC).

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