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Network analysis of microRNAs, transcription factors, target genes and host genes in human anaplastic astrocytoma

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Abstract. Numerous studies have investigated the roles played by various genes and microRNAs (miRNAs) in neoplasms, including anaplastic astrocytoma (AA). However, the specific regulatory mechanisms involving these genes and miRNAs remain unclear. In the present study, associated biological factors (miRNAs, transcription factors, target genes and host genes) from existing studies of human AA were combined methodically through the interactions between genes and miRNAs, as opposed to studying one or several. Three regulatory networks, including abnormally expressed, related and global networks were constructed with the aim of identifying significant gene and miRNA pathways. Each network is composed of three associations between miRNAs targeted at genes, transcription factors (TFs) regulating miRNAs and miRNAs located on their host genes. Among these, the abnormally expressed network, which involves the pathways of previously identified abnormally expressed genes and miRNAs, partially indicated the regulatory mechanism underlying AA. The network contains numerous abnormal regulation associations when AA emerges. By modifying the abnormally expressed network factors to a normal expression pattern, the faulty regulation may be corrected and tumorigenesis of AA may be prevented. Certain specific pathways are highlighted in AA, for example PTEN which is targeted by miR-21 and miR-106b, regulates miR-25 which in turn targets TP53. PTEN and miR-21 have been observed to form feedback loops. Furthermore, by comparing and analyzing the pathway predecessors and successors of abnormally expressed genes and miRNAs in three networks, similarities and differences of regulatory pathways may be identified and proposed. In summary, the present study aids in elucidating the occurrence, mechanism, prevention and treatment of AA. These results may aid further investigation into therapeutic approaches for this disease.

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Abbreviations: miRNA, microRNA; AA, anaplastic astrocytoma; TFs, transcription factors; targets, target genes; FBLs, feedback loops; NCBI, National Center for Biotechnology Information; TFBSs, transcription factor binding sites

Key words: anaplastic astrocytoma, network, microRNA, transcription factor, target gene, host gene

Introduction

Astrocytoma is a tumor of the astrocytic glial cells and the most common type of central nervous system (CNS) neoplasm, accounting for more than 60% of all primary brain tumors (1). Anaplastic astrocytoma (AA) is a high grade malignant glioma [World Health Organization (WHO) grade III] (2) of the CNS which develops from a low grade diffuse astrocytoma (DA; WHO grade II) and invariably progresses into lethal glioblastoma (WHO grade IV) (3). AA patients have an average survival period of three years (4). However, the treatment of AA is controversial, as there is no proven benefit of adjuvant chemotherapy or supplementary treatments, and novel chemotherapeutic approaches are required for the treatment of AA (5).

Transcription factors (TFs) are proteins that are able to activate or repress transcription by binding to specific DNA sequences (6,7). In molecular biology, TFs regulate the transcription of genes by promotion or suppression (6). MicroRNAs (miRNAs) are short noncoding RNAs that are implicated in tumorigenesis and function as tumor suppressors or oncogenes (8). TFs and miRNAs are prominent regulators for gene expression (7).

miRNAs post-transcriptionally modulate gene expression by negatively regulating the stability or translational efficiency of their target mRNAs (9). Numerous prior studies have investigated the associations between specific miRNAs and their target genes, and a number of databases are available for accessing information regarding miRNAs and their targets.

Host genes are genes that code miRNAs. Rodriguez *et al* demonstrated that miRNAs are transcribed in parallel with their host transcripts, and that the two transcription classes

of miRNAs ('exonic' and 'intronic') have been identified (10). Baskerville *et al* indicated that intronic miRNA and its host gene are closely associated (11). It has been suggested that miRNAs and their host genes together or separately could contribute to cancer progression (12).

To date, researchers have conducted numerous studies of AA and knowledge of genes and miRNAs can be obtained (13). Acquired data are classified as abnormally expressed data or related data according to the degree of correlation with AA. The abnormally expressed genes and miRNAs are fatal elements, as the abnormal expression of any one of them may cause the nosogenesis of AA. Related genes and miRNAs play auxiliary roles but not the vital ones. Abnormally expressed genes are defined as genes exhibiting mutation, upregulation, downregulation, overexpression, low-expression, SNP gene, loss of expression, differential expression and inactivation (14). Abnormally expressed miRNAs primarily exhibit upregulation, downregulation, overexpression, low-expression, differentially expression, mutation and deletion (15). Killela *et al* reported that exome sequencing of AA frequently identified mutations in IDH1, ATRX and TP53. Furthermore, they identified mutations of novel genes, particularly in the Notch pathway genes NOTCH1 and NOTCH2 (16). In the report of Guan *et al*, 16 miRNAs were found to be differentially expressed in AA (17).

The genes and miRNAs associated with AA can be found in scattered form, which makes it difficult to analyze the pathogeneses of AA systematically as experiments are conducted from different angles. However, it may be possible to construct a biological regulation network based on the existing associations between genes and miRNAs (18). In the present study, comprehensive genes and miRNAs associated with AA were collected manually from databases and previous studies in order to construct networks and identify the pathology. Three networks were established; abnormally expressed, related and global. Each network was combined with three associations between miRNAs and their target genes, TFs regulating miRNAs and miRNAs locating on their host genes. The abnormally expressed network is the core regulation network which contains the abnormally expressed genes and miRNAs and may indicates the underlying control mechanism in AA. Certain pathways in the abnormally expressed network were analyzed. When these pathways abnormally modulate, it may result in the development of AA. Notably, according to the proposed mechanism of TF binding, certain identified TFs were predicted using the P-match method (19). In addition, contrast tables of upstream and downstream of abnormally expressed genes, abnormally expressed miRNAs and predicted TFs were extracted to find the similarities and to determine differences between the three networks. Crucial elements and pathways were focused on to reveal the pathogeneses. The results may improve our understanding of the pathogenesis of AA, thus guiding future studies in identifying novel therapy strategies.

Materials and methods

Datasets of experimentally validated interactions between genes and miRNAs. The experimentally validated dataset of human miRNAs and their target genes was collected from the Tarbase (http://diana.imis.athena-innovation.gr/DianaTools/index.php?r=tarbase/index) (20), miRTarBase (http://mirtarbase.mbc.nctu.edu.tw/) (21) and miRecords (http://c1.accurascience.com/miRecords/) (22). The dataset was defined as set U_1.

The dataset of human TFs regulating miRNAs was acquired from TransmiR (http://www.cuilab.cn/transmir) (23) and was considered as set U_2.

The host genes of human miRNAs were extracted from miRBase (http://www.mirbase.org/) (24) and NCBI. The dataset of host genes including miRNAs was defined as set U_3.

Collection of abnormally expressed genes and miRNAs in AA. Genes and miRNAs are divided into two classes of abnormally expressed and related by the importance of their roles in AA, while abnormally expressed elements are involved in related elements. Abnormally expressed genes and miRNAs of AA were collected from published literature. There are numerous databases of abnormally expressed genes and miRNAs such as the NCBI SNP database (http://www.ncbi.nlm.nih.gov/SNP/), KEGG (www.genome.jp/kegg/pathway.html), Cancer GeneticsWeb (http://www.cancerindex.org/geneweb/clink30.htm) and mir2Disease (http://www.mir2disease.org/). However, the associated materials of AA were not found in these databases, thus indicating that the study of AA is lacking and requires further investigation.

The datasets of abnormally expressed genes and miRNAs were separately considered as set U_4 and U_5.

Collection of related genes and miRNAs in AA. All related miRNAs were found in pertinent literatures. Related genes which affect tumor growth, migration, radial therapy and clinical outcome of AA came from three data sources including published literature, GeneCards database (http://www.genecards.org/) (25) and P-match method. GeneCards is a database of human genes that provides concise genomic related information on all known and predicted human genes (25) and the first 100 genes associated with AA were selected. Furthermore, an algorithmic P-match method, which combines pattern matching and weight matrix approaches to identify transcription factor binding sites in DNA sequences (19), was used to predict TFs that were considered to be related genes. Prior to using the P-match method, 1,000 nt promoter region sequences of targets of abnormally expressed miRNAs were downloaded from UCSC database (http://genome.ucsc.edu/) (26). Subsequently, the P-match method was used to identify transcription factor binding sites (TFBSs) in 1,000 nt promoter region sequences and mapped TFBSs onto matrix library in TRANSFAC database (http://www.gene-regulation.com/pub/databases.html). Finally, the TFs of DNA sequence were acquired. The vertebrate matrix was used and restricted to high quality matrix criteria.

Datasets of related genes and miRNAs in AA were separately considered as set U_6 and U_7. Symbols of the genes in this paper were checked against the NCBI database (http://www.ncbi.nlm.nih.gov/gene).

Network construction at three levels. In this study, three networks of transcription processes related to AA were

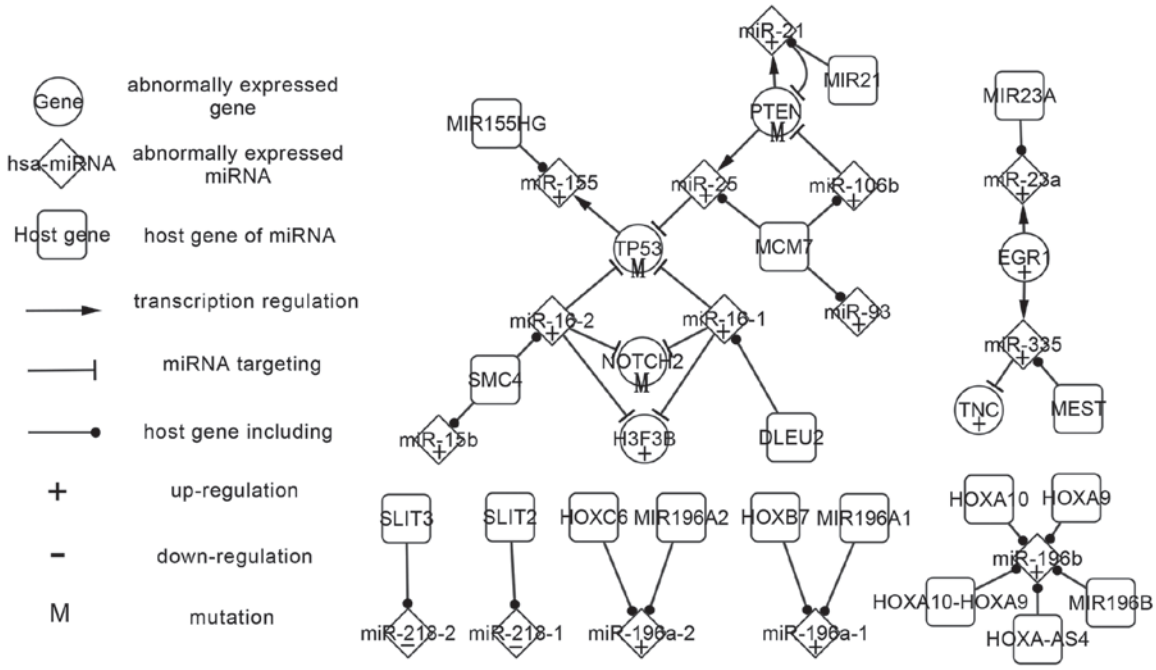

Figure 1. Network of abnormally expressed genes and miRNAs and their host genes in anaplastic astrocytoma (AA). All elements in the network are abnormally expressed, with the exception of host genes. The relations between genes and miRNAs partially reveal the underlying mechanism of AA. Notably, the changes of elements are investigated. miRNA, microRNA.

constructed, including abnormally expressed, related and global networks. Sets U_1, U_2 and U_3 were combined to derive the global network, thus indicating that the global network includes all the experimentally validated relations between miRNAs and their targets, TFs regulating miRNAs and miRNAs located on host genes.

The abnormally expressed network was derived by mapping abnormally expressed genes and miRNAs in addition to their relations from the global network. If a gene and miRNA pair contained separately in sets U_4 and U_5 was found in the global network to form a certain relation, the elements and the relation were included in the abnormally expressed network.

Datasets U_6 and U_7, that separately represent related genes and miRNAs, were organized into an AA-related network using the same method of referring to the relations in the global network. The related network is also a mapping of the global network and it includes the abnormally expressed network.

Finally, the software Cytoscape (version 3.0.0; http://www.cytoscape.org/) was used to realize network visualization, which assisted in analyzing the regulatory pathways graphically.

Results

Abnormally expressed network of AA. Using the methods and datasets mentioned above, three networks were successfully constructed. The abnormally expressed network was a core network that displays the regulatory mechanism of AA. The abnormally expressed network contains molecular elements that could be used in the diagnosis and treatment of AA, in addition to numerous crucial regulatory relations between the elements. This network indicates the pathogenesis of AA in terms of the regulatory relations between abnormally expressed genes and miRNAs.

The abnormally expressed network is composed of 15 abnormally expressed miRNAs, six abnormally expressed genes and 18 host genes, including three types of relations between miRNAs targeting genes, TFs regulating miRNAs and host genes, including miRNAs. Other abnormally expressed genes and miRNAs that do not exhibit regulatory relations with others were not included in this study, though each of these may have significance. The differences in these elements are presented in Fig. 1 according to the existing experiments. To investigate the changes in genes and miRNAs in abnormally expressed network contributes much to the network analysis.

Firstly, abnormally expressed TFs and miRNAs are analyzed. There are three TFs in Fig. 1; PTEN, TP53 and EGR1. PTEN and TP53 are crucial genes in AA. In patients with AA, PTEN and p53 mutations have been significantly associated with reduced and prolonged survival, respectively (27). The three TFs collectively regulate five abnormally expressed miRNAs (miR-21, miR-25, miR-155, miR-335 and miR-23a) and are targeted by a total of five miRNAs (miR-25, miR-16-1, miR-16-2, miR-21 and miR-106b). EGR1 regulates miR-335 and miR-23a, and is not itself targeted by any known miRNA. PTEN regulates miR-21 and miR-25 while being targeted by miR-21 and miR-106b. TP53 is targeted by miR-16-1, miR-16-2 and miR-25 while regulating miR-155. Genes that are targeted by miRNAs, but do not regulate any miRNAs, may be involved in AA. For example, TNC is the target gene of miR-335 and regulates no further miRNAs as successors. TNC may be involved in astrocytoma invasion, gliomagenesis, cell motility, proliferation and the neoplastic transformation of AA, and the upregulation of TNC may

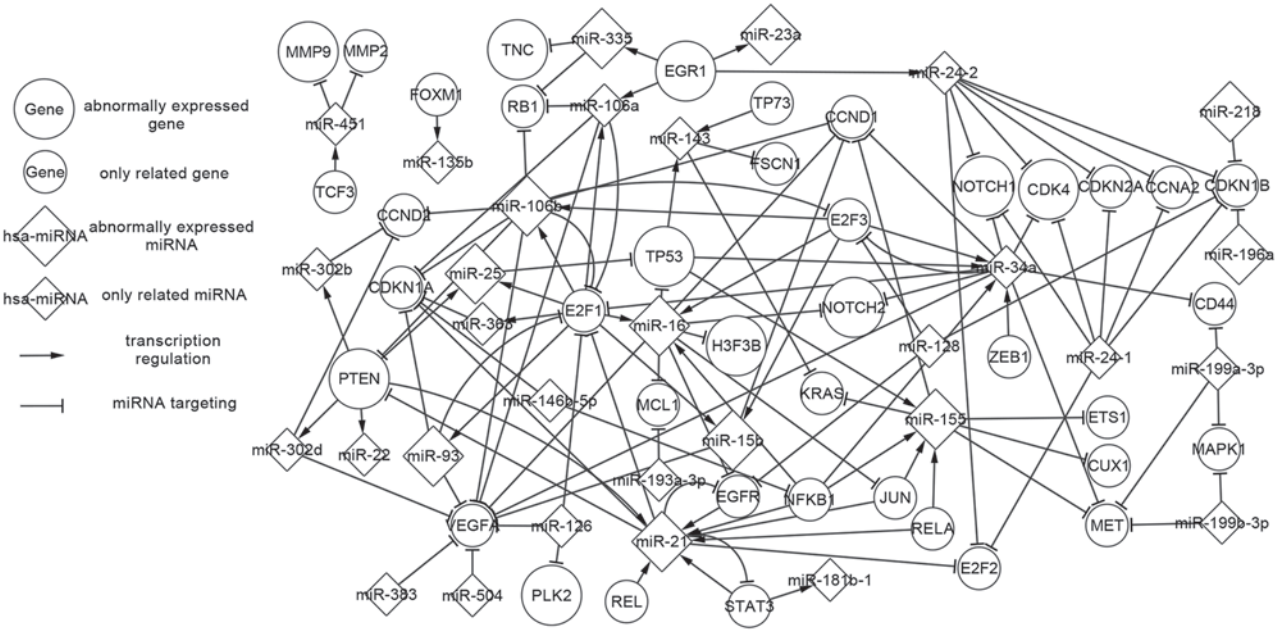

Figure 2. Related network of associated genes and miRNAs in anaplastic astrocytoma (AA). Larger nodes represent abnormally expressed genes and miRNAs, while the smaller nodes represent related genes and miRNAs, thus distinguishing between different genes and miRNAs. Host genes are omitted in this figure. miRNA, microRNA.

indicate the presence and metastasis of AA (28). PTEN and miR-21 form feedback loops (FBLs), via PTEN regulating the expression of miR-21, with miR-21 in turn targeting PTEN. Certain genes and miRNAs constitute a multistage regulation pathway, which proceeds as follows: miR-21 or miR-106b → PTEN → miR-25 → TP53 → miR-155. Any abnormal expression of the elements in this pathway may influence the successors and impact the carcinogenesis of AA.

Furthermore, miRNAs that exclusively targetted genes have been investigated. In Fig. 1, miR-16 (miR-16-1 and miR-16-2) and miR-106b belong to this category of miRNAs, which are not regulated by TFs. miR-16-1 and miR-16-2 target TP53, H3F3B and NOTCH2, indicating that the two miRNAs have some similarity of function. miR-106b targets PTEN and indirectly influences the successors. This type of miRNA may result in the abnormal regulatory network, functioning as start nodes.

Finally, host genes are included in the abnormally expressed network when the corresponding miRNAs are abnormally expressed. In Fig. 1, a host gene could contain multiple miRNAs, with different miRNAs being produced by a single host gene. miR-196b has five host genes, including HOXA9, HOXA10, HOXA-AS4, HOXA10-HOXA9 and MIR196B. MCM7 may be a crucial host gene in AA, as it includes three miRNAs (miR-25, miR-106b and miR-93). miR-25 and miR-106b function in the PTEN regulation pathway. miR21, which contains the key miRNA hsa-miR-21, is similarly important. miR-16-1 and miR-16-2 are from different host genes, DLEU2 and SMC4, though the two miRNAs have the same targets in AA.

Furthermore, investigation of the changes of abnormal genes and miRNAs may be of clinical relevance. The genes in Fig. 1 show abnormally expressed patterns of mutation, upregulation and downregulation when compared to normal tissues. The abnormally expressed patterns of miRNAs include up regulation and down regulation. The majority of the miRNAs serve oncogenic function in the AA abnormally expressed network. Modifying the abnormal expression of genes and miRNAs may correct faulty regulation and prevent tumorigenesis. EGR1, which is upregulated in AA (28), regulates miR-335. Furthermore, the upregulation of miR-335 (29) in AA may incorrectly target TNC and lead to the upregulation of TNC. The pathway indicates potential approaches for the therapy of AA by adjusting the abnormal expression in EGR1, miR-335 and TNC to normal expression levels. Mutation of PTEN (27) may lead to the upregulation of miR-25 (2), while mutation of TP53 (30) may result in the upregulation of miR-155 (2). In addition, Guan *et al* indicated that elevated miR-196 expression levels were significantly associated with poor survival in patients with AA (17). These findings suggest that the mutation, upregulation or downregulation of genes and miRNAs may contribute to tumorigenesis, and may have diagnostic and prognostic value. If the regulatory network were adjusted to a normal expression pattern, AA may hypothetically be prevented and cured. Further studies are required with the aim of correcting the abnormal expression to normal expression, based on the abnormally expressed network.

Related network of AA. The related network contains the abnormally expressed network, and also contains additional regulatory relations not present in the abnormally expressed network. Fig. 2 displays 40 genes, including 10 abnormally expressed genes and 30 related genes (not abnormally expressed), 30 miRNAs including 11 abnormally expressed miRNAs and 19 only related miRNAs. The abnormally expressed TFs are able to regulate additional related miRNAs, and abnormally expressed miRNAs could target at related genes. The additional related elements interact with abnormally

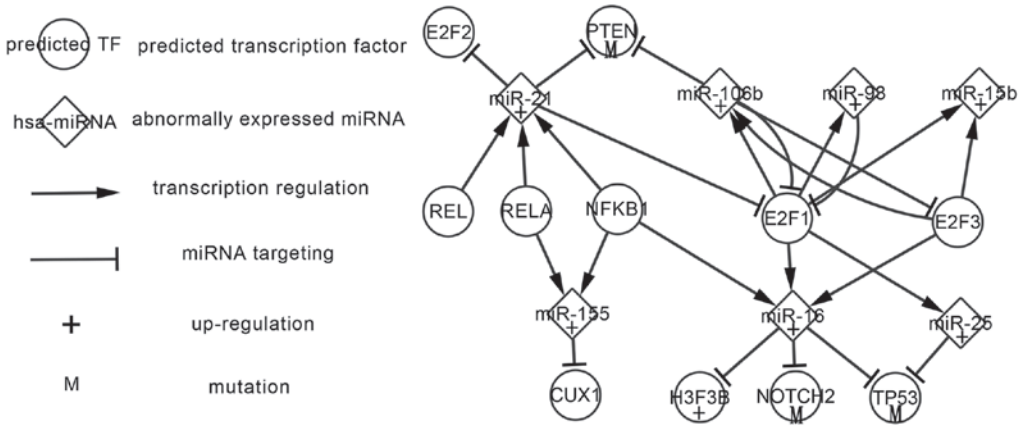

Figure 3. Transcriptional network of predicted TFs and abnormally expressed miRNAs. The validated changes of abnormally expressed genes and miRNAs are demonstrated. miRNA, microRNA; TF, transcription factor.

expressed elements as well as related elements. In the related network, EGR1, an abnormally expressed TF, regulates two more related miRNAs, including miR-106a and miR-24-2. TP73 is a related TF that shares a high degree of similarity with TP53 in its primary sequence and functions (30), and is involved in regulating a related miRNA miR-143. Related genes such as CCND1, CDKN1A, CDKN1B and VEGFA are targetted by abundant miRNAs. The AA-related network shows increased topology relations compared with the abnormally expressed network and aids in further understand of the pathogenesis in AA.

Global network of AA. The global network contains all of the regulatory relations between genes and miRNAs. It is an experimentally validated biological network in the human body. It is just used as a reference of the abnormally expressed network and related network.

Regulatory network of predicted TFs and abnormally expressed miRNAs. The predicted TFs were selected using the P-match method mentioned above. They are considered to be related genes and may be involved in the transcriptional regulation of AA. Fig. 3 displays regulatory relations between seven predicted TFs, seven abnormally expressed miRNAs and four abnormally expressed target genes in AA. A total of five predicted TFs including REL, RELA, NFKB1, E2F1 and E2F3 have the possibility to co-regulate seven abnormally expressed miRNAs, and these miRNAs target abnormally expressed genes and predicted TFs. Among the targets, PTEN, NOTCH2, H3F3B and TP53 are abnormally expressed genes and can be indirectly regulated by the predicted TFs. E2F1 and E2F3 show features in common; both regulate miR-16, miR-106b and miR-15b while being targeted by miR-106b. Furthermore, E2F1 regulates miR-25 and miR-93 and is targeted by miR-21 and miR-93 more strongly than E2F3. E2F1 and miR-106b, E2F1 and miR-93, E2F3 and miR-106b form FBLs. miR-21 is regulated by REL, RELA and NFKB1 and targets at E2F1, E2F2 and PTEN. RELA and NFKB1 co-regulate miR-155 and miR-21. Interactions between TFs and abnormally expressed miRNAs and targets may be involved in the regulatory mechanism underlying AA. Furthermore, the validated changes of abnormally expressed miRNAs and targets may aid in the investigation of predicted TFs in future studies.

Comparison and analysis about predecessors and successors of abnormally expressed genes. The abnormally expressed genes are crucial in pathogenesis of AA. Nodes were classified according to the regulatory relation of adjacent nodes in three level networks, thus comparing and analyzing the regulatory pathway of each abnormally expressed gene. A total of 43 abnormally expressed genes were classified into five classes. The first class of abnormally expressed genes has six types of adjacent nodes (three successors and three precursor nodes), including TP53 and PTEN. Between 50 and 60% of AAs manifested TP53 mutations (31), which has significant interactions between the TP53 and miRNAs in three networks.

The predecessors and successors of TP53 are presented in three levels in Table I. It shows that miR-16 and miR-25 target at TP53 and TP53 regulates miR-155 in the abnormally expressed network. In the related network, TP53 additionally regulates miR-143 and miR-34a. Experimentally validated miRNAs in global network support these regulation relations. The predecessors of TP53 may influence the successors by affecting TP53. Furthermore, TP53 is able to indirectly influence more elements downstream via successors. Similarly, TP53 can be controlled by other elements upstream through the predecessors. For example, PTEN has indirect influence on TP53 by regulating miR-25 in the abnormally expressed network.

The second class of abnormally expressed genes has three types of adjacent nodes (three successors), including EGR1, a TF in the abnormally expressed network.

The third class has three types of predecessor, including H3F3B, NOTCH2 and TNC. They only act as target genes in the abnormally expressed network.

The fourth class has two types of predecessor, including CDK4, MMP9, NOTCH1 and PLK2. They are only targets and show up in the related network.

The majority of abnormally expressed genes have no adjacent nodes. These isolated genes are not discussed in the present study.

Comparison and analysis of predecessors and successors of abnormally expressed miRNAs. The regulatory pathway

Table I. Regulatory relations between miRNAs and TP53.

A, miRNAs that target TP53

miRNA	Type of network
hsa-miR-16	Abnormally expressed, related, global
hsa-miR-25	Abnormally expressed, related, global
hsa-miR-125a-5p	Global
hsa-miR-125b	Global
hsa-miR-125b-5p	Global
hsa-miR-1285	Global
hsa-miR-15a	Global
hsa-miR-221	Global
hsa-miR-222	Global
hsa-miR-30d	Global
hsa-miR-380-5p	Global
hsa-miR-612	Global

B, miRNAs regulated by TP53

miRNA	Type of network
hsa-miR-155	Abnormally expressed, related, global
hsa-miR-143	Related, global
hsa-miR-34a	Related, global
hsa-miR-107	Global
hsa-miR-125b	Global
hsa-miR-145	Global
hsa-miR-192	Global
hsa-miR-194	Global
hsa-miR-200a	Global
hsa-miR-200b	Global
hsa-miR-200c	Global
hsa-miR-215	Global
hsa-miR-29	Global
hsa-miR-29a	Global
hsa-miR-29c	Global
hsa-miR-34	Global
hsa-miR-34b	Global
hsa-miR-34c	Global
hsa-miR-519d	Global

miRNA, microRNA.

of each abnormally expressed miRNA is analyzed using the same method. The 12 abnormally expressed miRNAs can be classified into seven types. The first class of abnormally expressed miRNAs has six adjacent nodes (three predecessors and three successors), including miR-25, miR-21 and miR-335. It has been well documented that miR-21 is overexpressed in a number of types of human cancer, and can function as an anti-apoptotic factor by downregulating the expression of tumor suppressor genes, such as PTEN (32).

In Table II, miR-21 is regulated by PTEN and targets PTEN in the abnormally expressed network. Notably, hsa-miR-21

Table II. Regulatory relations between genes and miR-21 in three network types.

A, Genes that target miR-21

Gene	Type of network
PTEN	Abnormally expressed, related, global
EGFR, JUN	Related, global
STAT3, NFKB1	Related, global
REL, RELA	Related, global
BMP6, BMPR1A	Global
DDX5, ESR1	Global
ETV5, FOXO3	Global
NFIB, RASGRF1	Global
REST, STAT4	Global
TCF4	Global

B, Genes that are targeted by miR-21

Gene	Type of network
PTEN	Abnormally expressed, related, global
CDKN1A, EGFR	Related, global
STAT3, E2F1	Related, global
E2F2	Related, global
ACTA2, ANKRD46	Global
APAF1, BASP1	Global
BCL2, BMPR2	Global
BTG2, CCR1	Global
CDC25A, CDK2AP1	Global
CDK6, CFL2	Global
DAXX, DERL1	Global
EIF2S1, EIF4A2	Global
ERBB2, FAM3C	Global
FAS, FMOD	Global
GLCCI1, HIPK3	Global
HNRNPK, ICAM1	Global
IL1B, IL6R	Global
ISCU, JAG1	Global
JMY, LRRFIP1	Global
MARCKS, MEF2C	Global
MSH2, MSH6	Global
MTAP, MYC	Global
NCAPG, NCOA3	Global
NFIB, NT-3	Global
PCBP1, PDCD4	Global
PDHA2, PELI1	Global
PLAT, PLOD3	Global
PPARA, PPIF	Global
PRRG4, PTX3	Global
RASA1, RASGRP1	Global
RECK, REST	Global
RHOB, RP2	Global
RPS7, RTN4	Global
SERPINB5, SESN1	Global

Table II. Continued.

B, Genes that are targeted by miR-21

Gene	Type of network
SGK3, SLC16A10	Global
SOCS5, SOX5	Global
SPATS2L, SPRY2	Global
TGFB1, TGFBI	Global
TGFBR2, TGFBR3	Global
TGFBR3, TGFBR3	Global
TGFBR3, TGIF1	Global
TIAM1, TIMP3	Global
TM9SF3, TNFAIP3	Global
TOPORS, TP53BP2	Global
TP63, TPM1	Global
WFS1, WIBG	Global

miR, microRNA.

and PTEN form FBLs. miR-21 is regulated by seven genes while targeting six genes in the AA-related network. Two pairs of FBLs are added; miR-21 and EGFR, miR-21 and STAT3. In the global network, miR-21 has 19 predecessors and 87 successors, which are necessary for identifying the regulatory relations. The second class of abnormally expressed miRNAs has three predecessors and two successors, including miR-155. The third class of abnormally expressed miRNAs has five adjacent nodes (three successors and two predecessors), including miR-106b and miR-16. The fourth class has two successors and two predecessors, including miR-15b and miR-93. The fifth class, miR-23a, has three predecessors and no gene targets. The sixth class has two predecessors; miR-196a and miR-218.

miR-196b has no gene interactions and is an isolated miRNA.

Comparison and analysis of predecessors and successors of predicted TFs. Finally, the pathway of each predicted TF was analyzed at three levels using the same method. Among the predicted TFs, E2F1 has the most abundant adjacent nodes.

In Table III, E2F1 is targeted by three miRNAs, while regulating five miRNAs in the abnormally expressed network. Six related miRNAs target E2F1 and seven related miRNAs are regulated by E2F1. miRNAs in the global network validated by experiments provide theoretical foundation for the research. In the comparison table, miR-106b and miR-93 form FBLs with E2F1 in the abnormally expressed network, while hsa-miR-106a is added to form FBLs with E2F1 in the related network.

Discussion

In the present study, a novel approach was developed for the study of the relationships between genes and miRNAs associated with AA, in order to clarify the underlying

Table III. Regulatory relations between miRNAs and E2F1 in three networks.

A, miRNAs that target E2F1

miRNA	Type of network
hsa-miR-106b	Abnormally expressed, related, global
hsa-miR-21	Abnormally expressed, related, global
hsa-miR-93	Abnormally expressed, related, global
hsa-miR-106a	Related, global
hsa-miR-126	Related, global
hsa-miR-34a	Related, global
hsa-let-7a	Global
hsa-miR-106a-5p	Global
hsa-miR-149*	Global
hsa-miR-17	Global
hsa-miR-17-5p	Global
hsa-miR-20	Global
hsa-miR-203a	Global
hsa-miR-20a	Global
hsa-miR-223	Global
hsa-miR-23b	Global
hsa-miR-330-3p	Global
hsa-miR-331-3p	Global
hsa-miR-34a	Global
hsa-miR-93	Global
hsa-miR-98	Global

B, miRNAs regulated by E2F1

miRNA	Type of network
hsa-miR-106b	Abnormally expressed, related, global
hsa-miR-15b	Abnormally expressed, related, global
hsa-miR-16	Abnormally expressed, related, global
hsa-miR-25	Abnormally expressed, related, global
hsa-miR-93	Abnormally expressed, related, global
hsa-miR-106a	Related, global
hsa-miR-363	Related, global
hsa-let-7a	Global
hsa-let-7i	Global
hsa-miR-106	Global
hsa-miR-15a	Global
hsa-miR-17	Global
hsa-miR-18a, -18b	Global
hsa-miR-195	Global
hsa-miR-223	Global
hsa-miR-19a, -19b	Global
hsa-miR-20, -20a, -20b	Global
hsa-miR-449, -449a	Global
hsa-miR-449b, -449c	Global
hsa-miR-91	Global
hsa-miR-92, -92a	Global

miRNA, microRNA.

pathology of AA. Based on the three types of experimentally validated relations, three networks were constructed hierarchically; abnormally expressed, related and global networks. Pathways were analyzed in order to identify the regulatory features. Specific regulatory relations have been validated in other cancer types; for example, miR-21 is overexpressed in numerous types of human cancer and is able to function as an anti-apoptotic factor by downregulating the expression of the tumor suppressor gene PTEN (32). In the AA abnormally expressed network, the pathway is extended to multistage regulation. miR-21 targets PTEN, while PTEN regulates miR-25 and miR-21, which form FBLs. Furthermore, miR-25 targets TP53, while TP53 regulates miR-155. The pathways, which include important transcriptional and targeting regulation, may contribute to future gene therapy development. The related network may also contribute to the improved understanding of the pathogenesis of AA. In addition, the predecessors and successors of abnormally expressed genes and miRNAs were compared and analyzed to identify the similarities and differences between the three networks. Furthermore, predicted TFs were investigated in the three networks. The present study may aid in elucidating the underlying pathogenesis of AA, by use of topological networks. In future, with the increasing study of AA, the occurrence, mechanism, improvement, metastasis and treatment of AA may be improved.

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